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In vitro models in studying nephropharmacology

Renal fibrosis in precision-cut kidney slices

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ABSTRACT

Chronic kidney disease (CKD) is associated with renal fibrosis, a pathological process that is characterized by excessive accumulation of extracellular matrix proteins resulting in loss of organ architecture and function. Currently, renal transplantation and dialysis are the sole treatment options for advanced CKD, yet these therapies have limited impact on fibrogenesis. Even though antifibrotic therapies are being developed, the search for effective antifibrotic drugs is being hampered by the lack of appropriate cell and animal models to study renal fibrosis. *In vitro* models lack cellular heterogeneity whereas *in vivo* models do not fully reflect human pathology. Precision-cut tissue slices, prepared from human or rodent tissue, provide a unique *ex vivo* model system that captures the complexity of organs, and they are widely used for ADME/Tox drug testing. Moreover, precision-cut kidney slices (PCKS) have been recently established as a useful model to study renal fibrosis. This review summarizes the currently available models for renal fibrosis, describes the wide array of possibilities with PCKS and shows its role in the search for antifibrotic drugs.

1. Introduction

The kidneys play an important role in maintaining total body homeostasis and the complexity of this task is reflected by the unique architecture of the organ, which consists of glomeruli, the tubular system, interstitium and vasculature. Injury caused by trauma, infection, ischemia or systemic disease can lead to injury at any of these sites and this will often result in a disruption of the physiological balance between extracellular matrix (ECM) production and degradation (Decleves and Sharma, 2014; Mutsaers et al., 2015). When ECM production is favored, damaged renal tissue will be replaced by acellular and collagen-rich scar tissue thereby diminishing the functionality of the kidney. These lesions in the kidney are characterized by glomerulosclerosis, tubular atrophy, tubulointerstitial fibrosis and intima hyperplasia (Boor et al., 2010), more generally termed renal fibrosis. The pathogenesis of fibrosis is extremely complex involving a variety of resident and circulating cells as well as several molecular signaling pathways (Boor et al., 2010). Transforming growth factor β (TGF-β), platelet derived growth factor (PDGF) and connective tissue growth factor have been identified as quintessential players in the fibrotic process in most organs (Eddy, 2014; Kok et al., 2014). After the initial injury, these pathways synergize with each other to promote fibrosis and activate profibrotic cells (Wang et al., 2011). (Myo)fibroblasts, *i.e.* ECM-producing cells, make up the majority of the renal interstitial cell population (Zeisberg and Kalluri, 2015) and they are key players in the fibrotic process. However, molecular markers to discern mesenchymal stromal cells, resident fibroblasts and activated fibroblasts remain elusive, hampering the study of these cells and their specific roles in health and disease (Thedieck et al., 2007; de Almeida et al., 2016). They can however be recognized by their spindle-shaped morphology and their abundance of rough endoplasmic reticulum. Resident fibroblasts can acquire the phenotype of profibrotic myofibroblasts as a response to epithelial or endothelial injury resulting in an increased synthesis of ECM (mostly collagen I, III and fibronectin; (Liu, 2011; Zeisberg and Neilson, 2010)). Other precursor populations of pathological myofibroblasts have been identified over the last years including pericytes, bone-marrow-derived cells and cells originating from either epithelial- or endothelial-to-mesenchymal transition (EMT and EndoMT; Mack and Yanagita, 2015; Falke et al., 2015; LeBleu et al., 2013; Kramann et al., 2013). However, the origin of renal myofibroblasts remains debated, and there exists considerable controversy as to the role of cellular transition in the ontogeny of these cells (Kriz et al., 2011). Yet, consensus is arising that renal myofibroblasts arise from FOXD1-lineage cells, which give rise to stromal and mural cells of the kidney (Duffield, 2014).

Renal fibrosis is the final deleterious outcome of several chronic kidney diseases (CKD). Currently, the incidence of CKD is rising,

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which has a substantial impact on health care budgets due to the high prevalence of morbidity and mortality associated with CKD (Couser et al., 2011; Jha et al., 2013). Unfortunately, the sole treatment options for advanced CKD, *i.e.* end-stage renal disease (ESRD), are dialysis and transplantation. Since the fibrotic process is highly intertwined with the development and progression of CKD many therapeutic options have been investigated in hopes of slowing down or even reversing fibrosis (Cernaro et al., 2014). Although several studies have been successful at the pre-clinical level, only limited advances have been made at this time in the translation of these findings to the level of patient treatment (Lee et al., 2015; Mutsaers et al., 2015).

The search for antifibrotic drugs is impeded by the lack of appropriate cell and animal models to study human renal fibrosis. *In vitro* studies lack cellular heterogeneity, which is a prerequisite to mimic the multicellular character of fibrosis, while results from animal experiments often do not match the human situation, and differ per strain within a given species (Inoue et al., 2015). Precision-cut kidney slices (PCKS) might be an extremely useful model to elucidate the process of renal fibrogenesis and to accelerate the search for effective antifibrotics. This review summarizes the currently available models for renal fibrosis, describes the wide array of possibilities with PCKS and shows its role in the search for antifibrotic drugs.

2. Models of kidney fibrosis

2.1. In vitro models of renal fibrosis

Cell culture is a simple, cost-efficient and potential high-throughput method to study fibrosis (Desrochers et al., 2014). Consequently, a myriad of studies use primary or immortalized human cells to elucidate the different aspects of fibrogenesis. In general, either fibroblasts or proximal tubular epithelial cells, *e.g.* HK-2 cells, are used. Unfortunately, some of these cells do not fully reflect normal renal physiology (Jenkinson et al., 2012; Mutsaers et al., 2011). Moreover, this experimental method fails to replicate the multicellular feature of fibrosis as well as the complex 3D architecture of the kidney (Desrochers et al., 2014). To tackle the latter, 3D tissue engineered disease models are being developed using, amongst others, hydrogels, decellularized kidneys or organ-on-a-chip technology (Desrochers et al., 2014). However, these models face many technical hurdles and advancement is also dependent on the possibility to obtain functionally relevant and genetically accurate renal cell lines (Desrochers et al., 2014). Additionally, in order to study cell-cell interactions and to minimize the gap between cell culture and the *in vivo* situation, co-culture models have been developed (Dixon et al., 2014). Still, at this time, the usefulness of cell culture models to study renal fibrosis are limited, yet they are very helpful to understand single cell behavior in this pathological process.

2.2. In vivo models of renal fibrosis

Our current understanding of renal fibrosis is for a large part derived from animal studies. In the last decades, a wide variety of models have been established to study renal fibrosis using surgical interventions or the administration of toxic substances to initiate fibrogenesis. The three most widely used surgical models are: unilateral ureteral obstruction (UUO; (Klein et al., 2011; Chevalier et al., 2009)), subtotal nephrectomy (Ma and Fogo, 2003) and renal ischemia and reperfusion (Eddy et al., 2012). Chemically, renal fibrosis can be induced by antiserum (Tam et al., 1999), adriamycin (Lee and Harris, 2011), angiotensin II (Flamant et al., 2006; Liu et al., 2012) and a myriad of other compounds. Furthermore, fibrosis can also be induced by a high salt diet resulting in hypertension. In addition, a genetically modified mouse strain, COL4A3$^{-/-}$, with a phenotype resembling Alport syndrome, is associated with renal fibrosis (Gross et al., 2010). Unfortunately, the major disadvantage of all these models is that the obtained results differ per strain (Inoue et al., 2015), and the experiments are associated with considerable discomfort for the animals. Still, animal models have the advantage over cell culture since they allow for the study of renal fibrosis on a systemic level, yet translation to the human situation remains difficult.

2.3. PCKS as an ex vivo model of renal fibrosis

PCKS provide a unique *ex vivo/in vitro* model in which cellular heterogeneity and organ architecture is maintained. This makes the model very useful to elucidate multicellular pathological processes including fibrosis. Recently, Poosti et al. successfully used murine PCKS to study renal fibrosis as well as the antifibrotic effect of interferon γ (IFNγ) and a IFNγ conjugate targeted to the PDGF-β receptor (PPB-PEG-IFNγ). To improve the application of PCKS to study human disease, Stribos et al. (2015) prepared slices from human tissue obtained from tumor nephrectomies and provided an in-depth characterization of human PCKS demonstrating that the slices maintained their renal phenotype during long-term culture, and that the model can be used to study the early onset of renal fibrosis. Thus, PCKS could serve as a translational model bridging the gap between *in vitro* studies and clinical trials.

3. Precision-cut kidney slices

3.1. History of precision-cut tissue slices

The use of tissue slices for (patho)physiological research dates back to 1923, when Otto Warburg invented the use of slices to study cancer cell metabolism (Warburg, 1923; Koppenol et al., 2011). The technique was however not well optimized and the tissue slices were hand-cut with a razor blade. Decades later, Carlos Krumdieck developed a semiautomatic slicing machine leading to a revival of the popularity of the tissue slices technique in 1980 (Krumdieck et al., 1980). Because of the technical improvement of the methodology it is now possible to make accurate and reproducible tissue slices from (solid) organs from a variety of species including humans. To this day, the quality of precision-cut tissue slices (PCTS) is being improved by optimizing preparation and incubation settings (de Graaf et al., 2010), also in the field of tumor biology (Davies et al., 2015) for which Otto Warburg initially developed the method. All in all, the technique has made great progress and PCTS are now widely used to study drug metabolism (Niu et al., 2015; Elferink et al., 2011; van Midwoud et al., 2011a, 2011b), drug toxicity (Niu et al., 2014; Hadi et al., 2013; Iswandana et al., 2016; Elferink et al., 2008), drug efficacy (Westra et al., 2014a; Westra et al., 2016) and fibrosis (Pham et al., 2015; Westra et al., 2014b; Poosti et al., 2015; Stribos et al., 2015).

3.2. Precision-cut kidney slices: the technical aspects

The preparation of PCKS is illustrated in Fig. 1. In short, human renal tissue is obtained from kidneys that had to be surgically removed due to a renal cell carcinoma. Subsequently, cores are made with a biopsy punch using only macroscopically healthy parts of the kidney. For murine PCKS, kidneys are collected via a terminal procedure performed under isoflurane/O_2 anesthesia. From here on, the method to prepare either murine or human PCKS is identical. PCKS are obtained using the Krumdieck tissue slicer and the slices are subsequently cultured in William's E