Histopathological and functional characterization of nephrectomy-induced renal repair

Background and Aim

Acute kidney injury (AKI) and chronic kidney disease (CKD) are important renal pathologies with an increasing incidence worldwide. It has been demonstrated that AKI is an underestimated, yet important risk factor for the development of CKD. The pathophysiology of ischemic AKI is complex, with various cellular factors contributing to injury. The most prominent effects of renal ischemia are seen in the proximal tubules, where it is causing epithelial cell death, accompanied by tubulointerstitial inflammation, which contribute to tubulointerstitial fibrosis. It is currently thought that maladaptive renal regeneration/repair causes the fibrotic outcome after injury. A strong correlation was observed between G2M cell cycle arrest (i.e. defective proliferation) in proximal tubular cells and the development of fibrosis. Because of the enormous complexity of the cell-biological and physiological characteristics of the kidney, animal models play an essential role in such fundamental studies. In mice models, it has been established that the fibrotic outcome of a single injured kidney can be greatly reduced by removal of the healthy contralateral kidney. The mechanisms of nephrectomy-induced renal repair aren’t fully elucidated, yet may provide interesting clues on how to address the inherent regeneration capacity of the kidney.

OBJECTIVES. Our general goal is to study the natural progression from AKI to CKD as well as the biological phenomenon that promotes repair after AKI and attenuates development of CKD. Here, we aim to perform a further histopathological and functional validation and characterization of our model (Fig. 1).

Materials and Methods

Experimental setup (Fig. 1)

• 8 weeks old, B6D2F1 transgenic male mice, genetically labelled proximal epithelial cells
• AKI induced by ischemia/reperfusion through clamping of the renal blood flow for 21 minutes in the left kidney.
• Nephrectomy of the healthy kidney at day 3 after ischemic injury (or sham operation)
• Tissue for analysis will be collected at day 4 and day 18 after nephrectomy. A substantial amount of tissue from these time points is already available in the lab.

Chemical tissue staining

• PAS staining is performed for the assessment of the general morphometry of kidney sections. From these sections, different cortical and medullary parameters (glomerular size-frequency distribution, distance from the glomeruli to the kidney’s outer perimeter, interglomerular distance, amount of luminal debris) will be evaluated.
• Jones staining will be performed to increase the resolution of tubular basement membranes allowing visual evaluation of the tubular circumference.

Immunological tissue staining

Histological sections will be prepared to assess the anatomical pathology and to perform immunohistochemical- and fluorescent staining for certain antigens, involved in:
• Cell proliferation (Ki67)
• Renal hypotrophy (phosphorylated ribosomal protein S6 (p-RPS6)
• G0M cell arrest (phosphorylated Histone 3)
• Apoptosis (cleaved caspase-3)

Renal function analysis

Tubular injury marker, neutrophil gelatinase-associated lipocalin (NGAL) and creatinine will be measured in serum and urine.

Clone size frequency analysis

□ To determine the efficiency of proliferation
□ Inclusion in this work will depend on the availability of the mice

References


Further steps:

• Cutting and staining of all available tissue sections
• Acquiring additional tissue from different time points
• Image segmentation analysis by ‘Voxiovision’ and ‘Image’ software

Figure 1. Study setup of two pathological courses

(A) Two pathological courses of ischemic-induced kidney injury with and without contralateral nephrectomy.
(B) Collected kidneys at week 3 after I/R induced injury. Injured kidney shows signs of degeneration due to fibrosis compared to the right healthy kidney.
(C) Left kidney 6 weeks after nephrectomy of the right healthy kidney. Renal degeneration was greatly reduced after nephrectomy was performed compared to the degenerated, fibrotic kidney in B.

Figure 2. Transgenic breeding scheme

R26RCreRed fluorescent gene is present in all body cells. Yet, expression is blocked by a STOP-cassette.

Expression of Cre-recombinase specifically in proximal epithelial cells. Cre-recombinase stays in cytoplasm.

F1 Combination of all parental characteristics

F1 after tamoxifen Constitutive-expression of t/3f-EGFP in proximal epithelial cells.

Figure 3. Immunological staining with rabbit anti-phosphorylated RPS6 antibody on kidney sections after contralateral nephrectomy on day 3. (A) Chemical staining with avidin-biotin complex through peroxidation of DAB+ by Horse Radish Peroxidase. Secondary immune response performed with biotinylated anti-rabbit IgG. (B) Fluorescent staining with secondary immune response performed with Alexa Fluor 555 donkey anti-rabbit IgG. Original magnification x200.

Figure 4. Immunological staining with rabbit anti-cleaved caspase-3 antibody. (A) Chemical staining with avidin-biotin complex through peroxidation of DAB+ by Horse Radish Peroxidase. Secondary immune response performed with biotinylated anti-rabbit IgG on kidney sections after contralateral nephrectomy at day 3. (B) Red fluorescent staining with secondary immune response performed with Alexa Fluor 555 donkey anti-rabbit IgG on kidney sections without contralateral nephrectomy. Green: enhanced background for tubular visualization. Blue: Hoechst DNA stain. Original magnification x200.