Immune-mediated mechanisms of (mal)adaptive renal tissue repair

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Chapter 1

General introduction and scope of the thesis

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This thesis aims to gain more insight into the role of the innate immune system in renal inflammatory diseases, focusing on the danger ligands S100A8/A9 (i.e. calprotectin) and the innate immune receptor TREM-1. Before elaborating on the evidences obtained during the last 5 years of research, I will briefly introduce the renal diseases I have been working on and the inflammatory mediators studied in this thesis.

Renal homeostasis and disease

The kidney's primary function is to maintain the volume and composition of body fluids. By excreting water and solutes through the urine, the kidney removes excess water and waste products. Moreover, the kidney also functions as a regulatory organ, by producing and secreting important hormones and controlling blood pressure. The functional unit of the kidney is the nephron, which consists of the glomerulus, the proximal tubule, the loop of Henle, the distal tubule and the collecting duct system.

Urine formation begins with blood filtration in the glomerular capillaries. The capillary endothelium, glomerular basement membrane and podocyte foot processes form the filtration barrier. The glomerulus is encased in Bowman's capsule, which connects the glomerulus to the proximal tubule. The proximal tubule is the powerhouse of the kidney; it reabsorbs the majority of the filtered water, Na+, Cl-, K+ and other solutes, in an energy-demanding manner. The key element in this process is represented by the Na+/K+ ATPase pump, present in the basolateral membrane of the proximal tubular epithelial cells (TECs).

These cells are equipped with a brush border on the apical membrane, whereas the basolateral membrane is densely packed with mitochondria, in order to meet the high energy requirement of the Na+/K+ ATPase pump. Due to their high metabolic demand, proximal TECs, especially those present in the S3 segment of the cortico-medullary area, represent the section of the nephron most susceptible to oxygen deprivation[1]. Along the nephron, the loop of Henle is responsible for the reabsorption of NaCl and water, whereas the distal segment of the nephron (distal tubules and collecting duct) makes the final adjustments in the composition and volume of the urine.

Once urine leaves the renal pelvis, it flows through the ureters and enters the urinary bladder, where it is stored. Renal function can be clinically assessed by evaluating the glomerular filtration rate (GFR) and the plasma levels of urea and creatinine. Renal diseases are associated with high morbidity and mortality[2,3]. Additionally, renal replacement therapies, which include dialysis and transplantation, although having been shown to extend life expectancy in patients with renal failure, still represent a financial burden for the healthcare system[4]. This, highlights the need for new avenues for therapeutic

intervention and the development of novel biomarkers, to provide specific treatments or earlier diagnosis. Renal diseases can manifest in two forms: acute or chronic.
Acute Kidney Injury (AKI) can be considered a reversible clinical condition, if not lethal. It is primarily characterized by rapid loss of renal function due to tubular destruction. AKI can be caused by transient ischemia, due to decreased or interrupted renal blood flow, followed by the reperfusion phase, such as during kidney transplant procedures. This is the so-called Ischemia/Reperfusion (IR)-induced acute kidney injury (AKI), which is one of the major causes of AKI[5,6]. Therefore, in our research, we studied experimental renal IR both in mice and in vitro, as a preclinical model of AKI. Conversely, chronic kidney disease (CKD) is a slower process, characterized by progressive loss of renal function due to the development of fibrosis. Tubular atrophy and interstitial fibrosis are the hallmarks of CKD and include tubular injury, chronic inflammation, myofibroblast activation and extracellular matrix deposition[7,8]. To investigate the roles of S100A8/A9 and TREM-1 in the development of progressive renal fibrosis, we used the well-established experimental model of Unilateral Ureteral Obstruction (UUO)[9].

AKI and CKD are interconnected

Epidemiological studies show that the incidence of AKI is greatly increasing in hospitalized patients[10-12]. The risk factors for AKI and CKD are quite similar. It was previously believed that patients who recovered from AKI had benign long term renal outcomes. However, since 2008, multiple observational studies, with follow up in patients who suffered from AKI, have shown a strong association between AKI and subsequent development of CKD[13]. It appears that, although patients who suffered from acute tubular necrosis are able to regain stable renal function, they later develop chronic sequelae of AKI, with progression to advanced stages of CKD, thereby increasing the risk of developing end stage renal disease (ESRD)[14-17]. This indicates that AKI is not only linked to, but may also be a primary cause of, CKD and its progression.
Animal studies have described different pathways that enhance organ dysfunction leading to fibrosis, including maladaptive repair, impaired tubular regeneration or a combination of the two[18-20]. However, the appropriate first-line and follow-up treatment for patients who survive an episode of AKI, regardless of whether they have CKD, is unclear. In light of this, novel biomarkers may be helpful to identify specific targets or the most effective moment for intervention. More importantly, particular attention should be taken to identify risk factors for progression, as this is a key element in prevention.

(Mal)adaptive repair after AKI

Most of the pre-clinical models studied to evaluate the pathophysiological events mediating the progression of AKI to CKD have used ischemia/reperfusion (IR) injury[13].

Nephrotoxic models and reversible ureteral obstruction have also been described, with fibrosis as the primary end point. However, a better animal model should be developed, one that mimics several aspects of human AKI, including the comorbidities, which represent a great risk for progression.

During ischemia, the kidney experiences a shortage of oxygen and glucose due to hypoperfusion. This is generally followed by restoration of blood flow, which is accompanied by renal injury and the initiation of inflammatory responses[21,22]. Within the nephron, the cells most susceptible to oxygen deprivation and ATP depletion are the proximal TECs, because of their high metabolic rate. Hence, IR induces severe damage in TECs, which gives rise to an altered morphology and cell death by necrosis or apoptosis[23-25]. Damaged TECs do not respond passively to injury but, instead, participate in the activation of the inflammatory response. Necrosis induces the release of pro-inflammatory mediators by TECs. This first results in infiltration of granulocytes, followed by macrophages in the renal parenchyma, which aid in the digestion and removal of necrotic material[26,27]. M1 macrophages are the first players in the tubular injury response. Granulocytes and macrophages release pro-inflammatory cytokines, including TNFα, which further fuels cell death in TECs. Hence, in this so-called "injury phase" there is a vicious cycle in which injury-induced cell death enhances inflammation, thereby promoting further cell death.

Renal regeneration, accomplished by active tissue repair, depends on the presence of an appropriate microenviroment in which surviving TECs can re-differentiate and proliferate, in order to replace lost cells. During the initial repair, macrophages switch from an M1 to M2 phenotype, which plays a beneficial role in wound healing as M2 macrophages function as scavengers of cell debris and promote tubular regeneration[28-31]. M2 macrophages secrete growth factors and other molecules, often associated with fibrosis, which support tubular repair[32]. During repair, the surviving TECs upregulate different markers, many of which are associated with fibroblasts or fibrosis markers. This, together with their expression of proliferation marker Ki67, suggests that the de-differentiated pool of TECs is actively proliferating in order to re-establish tubular morphology and function[33-35].

The abovementioned mechanism is the so-called 'adaptive repair' after AKI, which leads to the regaining of normal renal function over the course of few days, without scarring. However, it also suggests that fibrosis functions as a kind of a scaffold to facilitate the proliferation and re-differentiation of TECs. The progression of AKI into CKD, is often the result of a maladaptive repair response. Recent evidences have unraveled the different mechanisms underlying this detrimental process.

One of the main discoveries was that TECs can undergo cell cycle arrest in G2/M and senescence[18,36]. This is similar to what occurs in the aged kidney, which suffers nephron loss due to a failure in TEC re-differentiation and tubular senescence[37-39]. The mechanisms involved in this aberrant wound healing response that leads to CKD include: (a) altered growth factor expression, mainly pro-fibrotic, such as TGFβ and CTGF, secreted from G2/M-arrested TECs[40]. These pro-fibrotic factors stimulate (b) fibroblast activation and proliferation, which in turn leads to (c) increased interstitial collagen deposition. The failure to re-differentiate induces (d) chronic expression of KIM-1 on surviving TECs and increases (e) tubular loss[41]. Injured TECs retain pro-inflammatory capacities, thus, as a defense mechanism they stimulate (e) chronic inflammatory infiltration. As we mentioned earlier, macrophages play an important role in tubular repair[42]. However, when tubular injury is persistent, M2 macrophages accumulate in the renal parenchyma and gain a pro-fibrotic role, by secreting large amounts of pro-fibrotic factors, such as TGFβ[43]. Interestingly, kidney progression towards CKD, displays many characteristics associated with natural ageing, including the abovementioned pathological responses, but most importantly, tubular senescence[44,45]. Senescence is the result of a cellular stress response that results in a permanent growth arrest after acute or chronic triggers. Senescent cells are not inactive but, instead, retain the ability to propagate their senescent phenotype through a peculiar secretome. This so called "senescence-associated secretory phenotype" (SASP) is a mix of pro-inflammatory and fibrotic mediators, which can further promote chronic inflammation and fibrosis[46,47]. There is growing evidence that senescence promotes tissue degeneration during ageing and in several pathophysiological contexts[48], including multiple kidney diseases[49]. Accelerated senescence may promote pathologic alterations in kidneys, including AKI-CKD progression and shorten healthy lifespan[50].

The proximal tubule is the target of many injuries which drive the development of renal fibrosis. Proximal TECs require proper mitochondrial function in order to regulate a plethora of metabolic functions and signaling pathways[51]. Impaired mitochondrial homeostasis in TECs, is often associated with enhanced oxidative stress and accumulation of ROS, which is cytotoxic and may induce cell cycle arrest[52,53]. Recently, it has been described that mitochondrial dysfunction can also induce a senescent state termed MiDAS (mitochondrial dysfunction-associated secretory phenotype). This senescent state causes a mitotic arrest[54], suggesting that energy metabolism is an important factor in cell cycle arrest and senescence.
These evidences highlight the fact that new metabolic mechanisms should be investigated in order to prevent tubular senescence and reduce the risk of developing chronic disease after acute injury.

Inflammatory mediators in tissue injury

The Danger Theory elegantly describes that ischemia or trauma trigger an inflammatory response in different organs, just as a pathogen would[55]. Injured cells release damage-associated molecular patterns (DAMPs), which are recognized by Pattern Recognition Receptor (PRRs) that, in turn, activate an inflammatory response. Both ligands and receptors that activate the innate immune response have been shown to play a role in the pathophysiology of renal diseases.

In this thesis, we focused our attention on one class of DAMPs, the protein complex S100A8/A9, also known as calprotectin, and the innate immune receptor TREM-1. We explored the contribution of these two components of the innate immune system in the pathogenesis of acute and chronic kidney diseases.

S100A8/A9 protein complex

The S100 proteins were discovered in 1965 and identified in bovine brain fractions as proteins of the nervous system[56,57]. The genes encoding the majority of S100 proteins are located in the chromosomal region 1q21. In humans, the S100 family belongs to the Ca^{2+}-binding protein superfamily, that comprises circa 25 members. The majority of these proteins, including the S100A8/A9 complex, form heterodimers in vitro and in vivo, which confer protein function.

S100A8 and S100A9 are primarily expressed in myeloid cells, specifically neutrophils and monocyte/macrophages. However, increasing evidences describe their expression in epithelial cells, under inflammatory conditions[58].

Two forms are described for the complex protein: a secreted form and an intracellular form, which represents circa 60% of the cytosolic portion in neutrophils but is less abundant in monocytes. The secreted form can occur during translocation from the cytosol to the plasma membrane (active way), or as an extracellular release from the cytosol, as a result of tissue damage and cellular necrosis. In this last case, S100A8/A9 acts as DAMPs and modulate the inflammatory response by promoting innate immune cell recruitment to the site of injury. Moreover, the S100A8/A9 protein complex signals through Toll Like Receptor (TLR)-4 and Receptor of Advanced glycated end products (RAGE), to promote effector functions of the inflammatory response[59-61].

Additionally, extracellular S100A8/A9 can induce loss of integrity and cell death in endothelial cells and has an apoptotic effect in many cancer cell lines.

Elevated levels of the protein are present in sera and fluids of patients with several inflammatory conditions; it is, indeed, used as a reliable biomarker for arthritis, inflammatory bowel diseases, renal allograft rejection and cardiovascular diseases.

Given its high expression during inflammation in many organs, blocking S100A8/A9

represents an innovative approach to modulate the local inflammatory response, while avoiding systemic effects.

*Triggering receptor expressed on myeloid cells-1 (adapted from **Chapter 4**)*

The TREM-family comprises both activating and inhibitory receptors. Among the family members, TREM-1 represents the most widely studied activating receptor. Activation of TREM-1 is known to trigger and amplify inflammation, especially through synergism with TLR signaling.

Experiments performed by Colonna and colleagues where the first to show that TREM-1 is mainly expressed on myeloid cells, such as monocytes/macrophages and granulocytes. However, ongoing research shows that during inflammation, TREM-1 is also detected on parenchymal cell types, such as bronchial, corneal, gastric epithelial cells and hepatic endothelial cells[62-65]. TREM-1 is present in 2 forms: as a membrane-bound receptor and as a soluble protein. Membrane TREM-1 associates with the adaptor molecule TYROBP (TYRO protein tyrosine kinase-binding protein, more frequently called DAP12: DNAX activating protein of 12 kDa)[66] for signal transduction. TREM-1 activation stimulates the production of pro-inflammatory cytokines, chemokines and cell-surface molecules. In addition, TREM activation can promote mitochondrial integrity and cell survival by inactivating pro-apoptotic factors and inhibiting CytoC (Cytochrome-C) release from mitochondria[67,68]. Besides the membrane receptor, a soluble form of TREM-1 (sTREM-1) has been described. Although the origin and function of sTREM-1 is still elusive, its relevance as both a biomarker and a therapeutic target is high in both sterile and infectious disease settings. During infection or inflammation, sTREM-1 can be detected in biological fluids. In sterile inflammation, increased sTREM-1 levels have been reported during renal IR, in chronic kidney disease patients on hemodialysis, myocardial infarction, inflammatory bowel diseases, acute gouty inflammation and rheumatoid arthritis. The biological relevance of sTREM-1 in sterile inflammation is still unclear in contrast to its significance as a predictor of infection and inflammation in infectious diseases. In many inflammatory conditions, TREM-1 intervention shows a beneficial effect in murine studies, however, the precise mechanism underlying this protection is unknown, and the mechanism-of-action should be further investigated before proposing TREM-1 as a target for intervention therapy[69].

Outline of the thesis

The general aim of this thesis is to increase our knowledge into the immunopathogenesis of renal sterile inflammatory diseases, with a focus on the innate immune response activation by the danger protein S100A8/A9 and the receptor TREM-1. The different research topics covered in this thesis are illustrated in Figure 1.

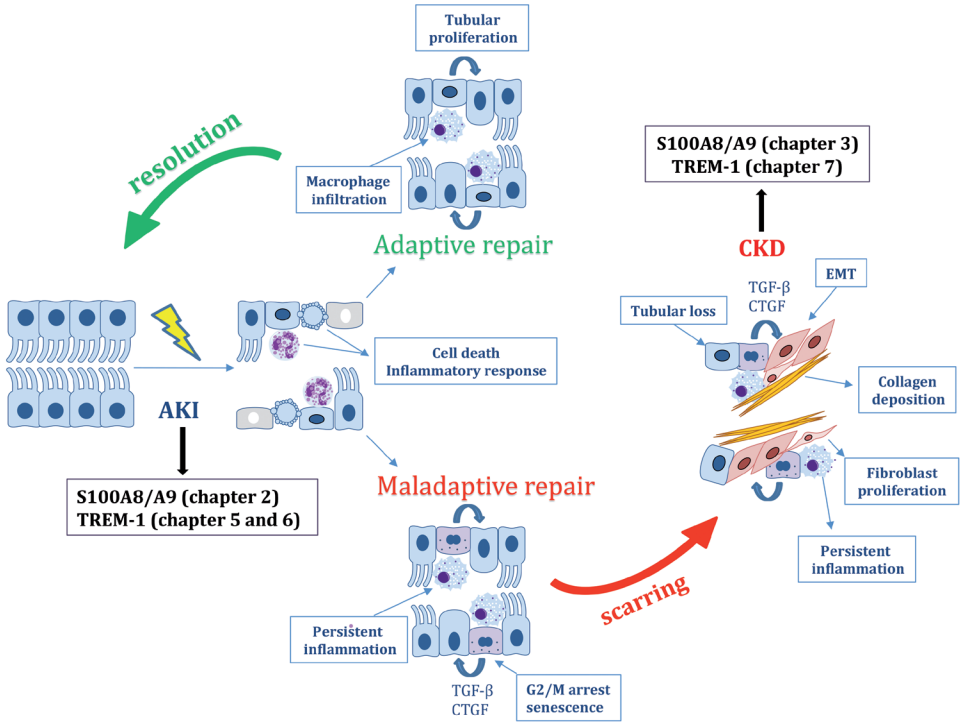

Figure 1: Thesis outline and simplified overview of maladaptive repair mechanisms post-AKI. The various research questions are depicted in black. AKI (acute kidney injury), TREM-1 (triggering receptor expressed on myeloid cells-1), TGFβ (transforming growth factor β), CTGF (connective tissue growth factor),EMT (epithelial mesenchymal transition) and CKD (chronic kidney disease).

In **Chapter 2,** the role of S100A8/A9 in the pathogenesis of IR-induced AKI was investigated. Mice lacking the S100A9 protein were subjected to IR and the injury and repair processes were evaluated. To increase our insight into S100A8/A9 signaling in the development of CKD, we used a murine model of obstructive nephropathy to induce tubulointerstitial fibrosis in WT and S100A9 KO animals in **Chapter 3**.

TREM-1 belongs to a family of receptors which are expressed on myeloid cells and is well-known to be activated during infection. However, increasing evidences describe its role during sterile inflammation. Hence, in **Chapter 4,** we reviewed the current evidences and understanding of TREM-1's role, specifically in sterile inflammatory diseases.

In **Chapter 5** we contributed to this knowledge by describing the role of TREM-1 in a pre-clinical model of AKI and in human kidney transplant patients. We subjected WT mice to renal IR injury and used different TREM-1 inhibitors to modulate its function, in order to evaluate the inflammatory response and renal damage in the acute phase of injury after ischemia. In transplanted kidney patients, we evaluated whether TREM-1 Single Nucleotide Variants (SNVs) were associated with any pathological consequences after transplantation.

An extensive research into the role of TREM-1 in the pathology of ischemic acute disease was performed in **Chapter 6,** where we used TREM-1/3 KO animals and subjected them to renal IR. In this study we set out to dissect the role of TREM-1 in kidney regeneration after injury.

Renal fibrosis can be a consequence of impaired renal regeneration, thus, in **Chapter 7,** we evaluated the specific role of TREM-1 signaling in a chronic model of renal injury. We investigated the roles of both TREM-1 role and DAP12, the signaling adapter molecule for TREM-1, in the development of renal fibrosis caused by unilateral ureteral obstruction.

Finally, in **Chapter 8**, we discuss our findings.

References

1. Liu, B.-C., Tang, T.-T., Lv, L.-L. & Lan, H.-Y. Renal tubule injury: a driving force toward chronic kidney disease. Kidney Int. **93**, 568–579 (2018).
2. Levin, A. et al. Global kidney health 2017 and beyond: a roadmap for closing gaps in care, research, and policy. Lancet **390**, 1888–1917 (2017).
3. Vanmassenhove, J., Kielstein, J., Jörres, A. & Biesen, W. Van. Management of patients at risk of acute kidney injury. Lancet **389**, 2139–2151 (2017).
4. Vanholder, R., Lameire, N., Annemans, L. & Van Biesen, W. Cost of renal replacement: how to help as many as possible while keeping expenses reasonable? Nephrol. Dial. Transplant. **31**, 1251–1261 (2016).
5. Sharfuddin, A. A. & Molitoris, B. A. Pathophysiology of ischemic acute kidney injury. Nat. Rev. Nephrol. **7**, 189–200 (2011).
6. Zuk, A. & Bonventre, J. V. Acute Kidney Injury. Annu. Rev. Med. **67**, 293–307 (2016).
7. Duffield, J. S. Cellular and molecular mechanisms in kidney fibrosis. J. Clin. Invest. **124**, 2299–306 (2014).
8. Zhou, D. & Liu, Y. Renal fibrosis in 2015: Understanding the mechanisms of kidney fibrosis. Nat. Rev. Nephrol. **12**, 68–70 (2016).
9. Chevalier, R. L., Forbes, M. S. & Thornhill, B. A. Ureteral obstruction as a model of renal interstitial fibrosis and obstructive nephropathy. Kidney Int **75**, 1145–1152 (2009).
10. Ishani, A. et al. Acute kidney injury increases risk of ESRD among elderly. J. Am. Soc. Nephrol. **20**, 223–8 (2009).
11. Xue, J. L. et al. Incidence and mortality of acute renal failure in Medicare beneficiaries, 1992 to 2001. J. Am. Soc. Nephrol. **17**, 1135–42 (2006).
12. Chawla, L. S., Eggers, P. W., Star, R. A. & Kimmel, P. L. Acute Kidney Injury and Chronic Kidney Disease as Interconnected Syndromes. N. Engl. J. Med. **371**, 58–66 (2014).
13. Basile, D. P. et al. Progression after AKI: Understanding Maladaptive Repair Processes to Predict and Identify Therapeutic Treatments. J. Am. Soc. Nephrol. **27**, 687–97 (2016).
14. Rifkin, D. E., Coca, S. G. & Kalantar-Zadeh, K. Does AKI truly lead to CKD? J. Am. Soc. Nephrol. **23**, 979–84 (2012).
15. Chawla, L. S. & Kimmel, P. L. Acute kidney injury and chronic kidney disease: an integrated clinical syndrome. Kidney Int **82**, 516–524 (2012).
16. Coca, S. G., Singanamala, S. & Parikh, C. R. Chronic kidney disease after acute kidney injury: a systematic review and meta-analysis. Kidney Int. **81**, 442–448 (2012).
17. Thakar, C. V, Christianson, A., Himmelfarb, J. & Leonard, A. C. Acute kidney injury episodes and chronic kidney disease risk in diabetes mellitus. Clin. J. Am. Soc. Nephrol. **6**, 2567–72 (2011).
18. Yang, L., Besschetnova, T. Y., Brooks, C. R., Shah, J. V & Bonventre, J. V. Epithelial cell cycle arrest in G2/M mediates kidney fibrosis after injury. Nat. Med. **16**, 535–543 (2010).
19. Grgic, I. et al. Targeted proximal tubule injury triggers interstitial fibrosis and glomerulosclerosis. Kidney Int. **82**, 172–183 (2012).

20. Ferenbach, D. A. & Bonventre, J. V. Mechanisms of maladaptive repair after AKI leading to accelerated kidney ageing and CKD. Nat. Publ. Gr. **11**, 264–276 (2015).
21. Kumar, S. Cellular and molecular pathways of renal repair after acute kidney injury. Kidney Int. **93**, 27–40 (2018).
22. Bonventre, J. & Yang, L. Cellular pathophysiology of ischemic acute kidney injury. J. Clin. Invest. **121**, (2011).
23. Kers, J., Leemans, J. C. & Linkermann, A. An Overview of Pathways of Regulated Necrosis in Acute Kidney Injury. Semin. Nephrol. **36**, 139–152 (2016).
24. Havasi, A. & Borkan, S. C. Apoptosis and acute kidney injury. Kidney Int. **80**, 29–40 (2011).
25. Linkermann, A. et al. Regulated cell death in AKI. J. Am. Soc. Nephrol. **25**, 2689–701 (2014).
26. Clements, M. et al. Differential Ly6C Expression after Renal Ischemia-Reperfusion Identifies Unique Macrophage Populations. J. Am. Soc. Nephrol. ASN.2014111138- (2015). doi:10.1681/ASN.2014111138
27. Sachet, M., Liang, Y. Y. & Oehler, R. The immune response to secondary necrotic cells. Apoptosis **22**, 1189–1204 (2017).
28. Zhang, M.-Z. et al. CSF-1 signaling mediates recovery from acute kidney injury. J. Clin. Invest. **122**, 4519–32 (2012).
29. Kinsey, G. R. Macrophage dynamics in AKI to CKD progression. J. Am. Soc. Nephrol. **25**, 209–11 (2014).
30. Lee, S. et al. Distinct macrophage phenotypes contribute to kidney injury and repair. J. Am. Soc. Nephrol. **22**, 317–26 (2011).
31. Lin, S.-L. et al. Macrophage Wnt7b is critical for kidney repair and regeneration. Proc. Natl. Acad. Sci. U. S. A. **107**, 4194–9 (2010).
32. Vinuesa, E. et al. Macrophage involvement in the kidney repair phase after ischaemia/reperfusion injury. J. Pathol. **214**, 104–113 (2008).
33. Kusaba, T., Lalli, M., Kramann, R., Kobayashi, A. & Humphreys, B. D. Differentiated kidney epithelial cells repair injured proximal tubule. Proc. Natl. Acad. Sci. U. S. A. **111**, 1527–32 (2014).
34. Guan, Q., Nguan, C. Y. C. & Du, C. Expression of Transforming Growth Factor-β1 Limits Renal Ischemia-Reperfusion Injury. Transplantation **89**, 1320–1327 (2010).
35. Berger, K. et al. Origin of regenerating tubular cells after acute kidney injury. Proc. Natl. Acad. Sci. **111**, 1533–1538 (2014).
36. Krishnamurthy, J. et al. Ink4a/Arf expression is a biomarker of aging. J. Clin. Invest. **114**, 1299–1307 (2004).
37. Berkenkamp, B. et al. In Vivo and In Vitro Analysis of Age-Associated Changes and Somatic Cellular Senescence in Renal Epithelial Cells. PLoS One **9**, e88071 (2014).
38. Chkhotua, A. B. et al. Increased expression of p16(INK4a) and p27(Kip1) cyclin-dependent kinase inhibitor genes in aging human kidney and chronic allograft nephropathy. Am. J. Kidney Dis. **41**, 1303–13 (2003).

39. Baisantry, A. et al. Autophagy Induces Prosenescent Changes in Proximal Tubular S3 Segments. 1–8 (2015). doi:10.1681/ASN.2014111059
40. Qi, W. et al. Integrated actions of transforming growth factor-β 1 and connective tissue growth factor in renal fibrosis. Am. J. Physiol. Physiol. **288**, F800–F809 (2005).
41. Humphreys, B. D. et al. Chronic epithelial kidney injury molecule-1 expression causes murine kidney fibrosis. J. Clin. Invest. **123**, 4023–4035 (2013).
42. Lin, S. L. et al. Macrophage Wnt7b is critical for kidney repair and regeneration. Proc. Natl. Acad. Sci. U. S. A **107**, 4194–4199 (2010).
43. Braga, T. T., Agudelo, J. S. H. & Camara, N. O. S. Macrophages During the Fibrotic Process: M2 as Friend and Foe. Front. Immunol. **6**, 602 (2015).
44. Luo, C. et al. Wnt9a Promotes Renal Fibrosis by Accelerating Cellular Senescence in Tubular Epithelial Cells. J. Am. Soc. Nephrol. **29**, 1238–1256 (2018).
45. Clements, M. E., Chaber, C. J., Ledbetter, S. R. & Zuk, A. Increased Cellular Senescence and Vascular Rarefaction Exacerbate the Progression of Kidney Fibrosis in Aged Mice Following Transient Ischemic Injury. PLoS One **8**, e70464 (2013).
46. Gewin, L. & Zent, R. How does TGF-β mediate tubulointerstitial fibrosis? Semin. Nephrol. **32**, 228–35 (2012).
47. Rodier, F. et al. Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. Nat. Cell Biol. **11**, 973–979 (2009).
48. Muñoz-Espín, D. & Serrano, M. Cellular senescence: from physiology to pathology. Nat. Rev. Mol. Cell Biol. **15**, 482–496 (2014).
49. Valentijn, F. A., Falke, L. L., Nguyen, T. Q. & Goldschmeding, R. Cellular senescence in the aging and diseased kidney. J. Cell Commun. Signal. **12**, 69–82 (2018).
50. Sturmlechner, I., Durik, M., Sieben, C. J., Baker, D. J. & van Deursen, J. M. Cellular senescence in renal ageing and disease. Nat. Rev. Nephrol. **13**, 77–89 (2017).
51. Bhargava, P. & Schnellmann, R. G. Mitochondrial energetics in the kidney. Nat. Rev. Nephrol. **13**, 629–646 (2017).
52. Brooks, C., Wei, Q., Cho, S.-G. & Dong, Z. Regulation of mitochondrial dynamics in acute kidney injury in cell culture and rodent models. J. Clin. Invest. **119**, 1275–1285 (2009).
53. Small, D. M. et al. Oxidative stress and cell senescence combine to cause maximal renal tubular epithelial cell dysfunction and loss in an in vitro model of kidney disease. Nephron. Exp. Nephrol. **122**, 123–30 (2012).
54. Wiley, C. D. et al. Mitochondrial Dysfunction Induces Senescence with a Distinct Secretory Phenotype. Cell Metab. **23**, 303–314 (2016).
55. Matzinger, P. Tolerance, Danger, and the Extended Family. Annu. Rev. Immunol. **12**, 991–1045 (1994).
56. Moore, B. W. A soluble protein characteristic of the nervous system. Biochem. Biophys. Res. Commun. **19**, 739–44 (1965).

57. Pruenster, M., Vogl, T., Roth, J. & Sperandio, M. S100A8/A9: From basic science to clinical application. Pharmacol. Ther. **167**, 120–131 (2016).
58. Donato, R. et al. Functions of S100 proteins. Curr. Mol. Med. **13**, 24–57 (2013).
59. Vogl, T. et al. Mrp8 and Mrp14 are endogenous activators of Toll-like receptor 4, promoting lethal, endotoxin-induced shock. Nat Med **13**, 1042–1049 (2007).
60. Ehlermann, P. et al. Increased proinflammatory endothelial response to S100A8/A9 after preactivation through advanced glycation end products. Cardiovasc. Diabetol. **5**, 6 (2006).
61. Narumi, K. et al. Proinflammatory Proteins S100A8/S100A9 Activate NK Cells via Interaction with RAGE. J. Immunol. **194**, 5539–48 (2015).
62. Barrow, a. D. et al. Cutting Edge: TREM-Like Transcript-1, a Platelet Immunoreceptor Tyrosine-Based Inhibition Motif Encoding Costimulatory Immunoreceptor that Enhances, Rather than Inhibits, Calcium Signaling via SHP-2. J. Immunol. **172**, 5838–5842 (2004).
63. Rigo, I. et al. Induction of triggering receptor expressed on myeloid cells (TREM-1) in airway epithelial cells by 1,25(OH) 2 vitamin D 3. Innate Immun. **18**, 250–257 (2012).
64. Schmausser, B. et al. Triggering receptor expressed on myeloid cells-1 (TREM-1) expression on gastric epithelium: implication for a role of TREM-1 in Helicobacter pylori infection. Clin Exp Immunol **152**, 88–94 (2008).
65. Chen, L. C., Laskin, J. D., Gordon, M. K. & Laskin, D. L. Regulation of TREM expression in hepatic macrophages and endothelial cells during acute endotoxemia. Exp Mol Pathol **84**, 145–155 (2008).
66. Colonna, M. & Facchetti, F. TREM-1 (triggering receptor expressed on myeloid cells): a new player in acute inflammatory responses. J. Infect. Dis. **187** Suppl, S397-401 (2003).
67. Yuan, Z. et al. Triggering receptor expressed on myeloid cells 1 (TREM-1)-mediated Bcl-2 induction prolongs macrophage survival. J Biol. Chem **289**, 15118–15129 (2014).
68. Yuan, Z. et al. TREM-1-accentuated lung injury via miR-155 is inhibited by LP17 nanomedicine. Am. J Physiol Lung Cell Mol. Physiol **310**, L426–L438 (2016).
69. Tammaro, A. et al. TREM-1 and its potential ligands in non-infectious diseases: from biology to clinical perspectives. Pharmacol. Ther. **177**, (2017).