SUMMARY

The kidneys play an important role in preserving tissue homeostasis. Ischemia/reperfusion (I/R) injury and toxin-induced nephrotoxicity are two major causes of acute renal injury that lead to a rapid loss of kidney function and ultimately acute renal failure (ARF). This is most commonly the result of damage to cells of the proximal tubules. These cells carry out the majority of solute absorption, making them vulnerable to toxicants, which are also taken up and may be concentrated. Their high metabolic activity also makes proximal tubular epithelial cells (PTECs) vulnerable to deficiencies in blood perfusion, for example due to vascular injury. Proper cell adhesions, including cell-matrix adhesion and cell-cell interactions, are important for the structural integrity of the tubules and also generate intracellular signals necessary for normal PTEC function and survival. The earliest stages of renal injury are associated with perturbations in cell adhesion and reorganization of the associated actin cytoskeleton. The goal of the research presented in this thesis is to identify key molecules and pathways involved in cell adhesion alterations during acute renal injury, and to develop novel strategies for ARF therapy based on cell adhesion modulation.

Ischemia/reperfusion injury involves disruption of integrin-mediated cell adhesion and activation of the ERK pathway. How the dynamics of focal adhesion (FA) organization and phosphorylation during I/R are related to ERK activation was investigated in a rat unilateral renal I/R model (chapter 2). Firstly, protein tyrosine-rich FAs were present at the basolateral membrane of proximal tubules under in vivo conditions, enriched in FAK and paxillin and connected to basolateral F-actin stress fibers. Secondly, I/R caused a reversible protein dephosphorylation, which coincided with a dynamic disruption/re-structuring of FAs and the F-actin network. Thirdly, reperfusion caused activation of the MEK/ERK pathway, which preceded FA protein phosphorylation. Inhibition of this pathway by pre-treatment of rats with a specific MEK inhibitor, U0126, attenuated protein dephosphorylation and re-phosphorylation and dissolution of FAs in conjunction with decreased renal injury. These data support a model whereby ERK activation enhanced protein tyrosine phosphorylation during I/R, thereby driving the dynamic dissolution and re-structuring of FAs and the F-actin cytoskeleton during reperfusion and renal injury. Inhibition of the MEK/ERK pathway and/or a specific protein tyrosine kinase during the ischemic period may therefore be a potential therapeutic means to protect against renal failure caused by ischemic insults.

FAK is a non-receptor protein tyrosine kinase that localizes at FAs and its tyrosine phosphorylation during renal I/R injury is involved in the regulation of FA turnover in response to oxidative stress. First, the role of FAK in renal I/R injury was investigated in a novel conditional proximal tubule-specific FAK deletion mouse model (chapter 3). The selective and conditional deletion of fak in mouse proximal tubules protected unilateral I/R-induced renal injury by attenuating tubular damage – an effect also reflected by reduced expression of Kidney Injury Molecule-1 (KIM-1), which was independent of the post-ischemic inflammatory response. Second, primary cultured mouse renal cells were used to determine the effect of FAK deficiency on cellular responses to oxidative stress in vitro. The conditional FAK deletion did not affect cell survival but impaired the recovery of FAs after oxidative stress. This was associated with reduced activation of the stress kinase JNK and the subsequent phosphorylation of paxillin at serine 178, which is required for FA turnover.
Summary

These data support a model whereby FAK is required for recruitment of JNK to FAs leading to JNK-mediated phosphorylation of paxillin at Ser178 thereby facilitating FA turnover during oxidative stress. This FAK/JNK/paxillin linkage could potentially be the basis for future targets for therapeutic intervention in ARF.

Renal I/R injury is associated with the loss of tubular epithelial cell-cell and cell-matrix interactions which contribute to renal failure. The Epac-Rap signalling pathway is a potent regulator of cell-cell and cell-matrix adhesion. Activation of the Epac-Rap signalling pathway showed a reduction in both cellular stress and ischemia-induced kidney failure (chapter 4). Pharmacological activation of Epac-Rap signalling using an Epac-selective cAMP analogue preserved adherens junctions and FA complexes during in vitro hypoxia, maintaining the barrier function of the epithelial monolayer. In vivo Epac activation reduced renal failure in a mouse bilateral I/R model, accompanied by decreased expression of the tubular cell stress marker clusterin-α, and lateral expression of β-catenin after ischemia indicative of sustained tubular barrier function. These findings emphasize the undervalued importance of maintaining tubular epithelial cell adhesion in renal ischemia and propose both enhancement of tubular epithelial cell adhesion in general and specifically activation of the Epac-Rap signalling pathway as novel therapeutic strategies for reducing renal failure during early I/R injury.

Nephrotoxicity is the principal dose-limiting factor for cisplatin chemotherapy. Cisplatin-induced nephrotoxicity shows a similar pattern of injury to I/R, being primarily associated with pathological alterations of proximal tubular epithelial cells, including disruption of cell adhesions and induction of apoptosis. In a model of cisplatin-induced renal cell injury, cAMP signalling protected mouse proximal tubular epithelial cells against cisplatin-induced cytotoxicity via activation of the Epac-Rap signalling pathway (chapter 5). The preservation of the intercellular junctions and anti-apoptotic effects were both abrogated by silencing Epac-Rap signalling and were independent of protein kinase A. Importantly, Epac expression is absent in a number of cell lines from human cancers that are routinely treated with cisplatin. Therefore, activation of Epac-Rap signalling pathway has the potential to protect against nephrotoxicity without compromising the therapeutic value of cisplatin as an anti-cancer drug, for tumors that do not express Epac. These data identify Epac-Rap signalling as a cAMP-dependent cytoprotective pathway and the activation of Epac-Rap signalling pathway represents a potential strategy for reducing nephrotoxicity associated with cisplatin treatment, and as a result, broadens the therapeutic window of this widely used chemotherapy agent.

ATP depletion is the central biochemical event during renal ischemic injury. A detailed transcriptomics study was performed using oligonucleotide microarray analysis to investigate the genes that are involved in ATP depletion-mediated stress responses of primary rat PTECs during metabolic inhibition-induce hypoxic injury and regeneration (chapter 6). Gene transcription was significantly altered after ATP depletion, specifically involving changes in genes that participated in transmembrane transport, tissue development, cellular metabolism and homeostasis-associated processes, suggesting tissue remodelling and regeneration after ATP depletion-associated hypoxic renal injury. Genes which were differentially regulated by mild, sub-lethal injury are predicted to be associated with stress response pathways involved in recovery from injury, while genes that were differentially regulated by severe, lethal injury are predicted to be associated with the induction of cell death pathways. Using this approach,
several tissue homeostasis and development-associated pathways and genes were identified by gene ontology analysis. This study identified candidate genes and pathways that are associated with and may also play an important role in ATP depletion-mediated stress responses in the context of renal ischemic injury and regeneration. This may lead to novel targets and strategies to protect from or promote renal tissue repair following ischemic injury.