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General introduction
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INTRODUCTION

Acute renal failure (ARF) refers to a sudden loss of kidney function in a short period and is clinically characterized by an abrupt and sustained drop in glomerular filtration rate (GFR). High incidence of hospitalization and mortality makes it a frequent and significant clinical problem, particularly in the intensive care unit (ICU). It has been reported that up to 25% of hospitalized patients suffer from ARF and the mortality rate of these is 15-60% even after dialysis.

Ischemia and toxin-induced tubular necrosis accounts for more than 80% of cases of ARF. In both cases, primary tubular damage occurs in the proximal tubules, and is associated with disruption of normal cell adhesion and reorganization of the actin cytoskeleton network of epithelial cells.

Little progress has been made over the past two decades in preventing ARF or reducing mortality. This study aims to contribute to a better understanding of the pathophysiology, biochemistry, cell biology and molecular biology associated with ARF, and is essential for accelerating the progress in developing efficient therapeutic strategies to prevent ARF. The human data on the pathology of ARF are still scarce, therefore several animal models of acute tubular necrosis and cellular models of renal cell injury are developed and utilized for laboratory study. In particular, proximal tubular epithelial cells (PTEC) have received much attention on cell adhesion-associated signalling pathways, which contributes potential therapeutic targets towards prevention of ARF.

In this chapter, cellular pathological alterations of PTECs during both I/R injury and toxin-induced nephrotoxicity are described, in the context of cell adhesion, stress, survival/death and several associated signalling pathways. The potential of regulating proximal tubular cell adhesion is discussed, mainly focusing on genetic modulation of FAK signalling and pharmacological activation of cAMP-Epac-Rap signalling, the two central topics of this thesis.

Renal physiology

The kidneys function to maintain the haemostatic balance of body fluids via filtration of blood, regulation of composition of blood (ions, pH, water, osmotic pressure), absorption of nutrients, secretion/elimination of wastes and production of urine. The kidney also secretes hormones including renin and erythropoietin.

Macroscopically, the kidney is divided into cortex and medulla (further divided into outer medulla and inner medulla). The human kidney contains separated cone-shaped medullary pyramids, while the mouse medullary area is a continuous region. Each human kidney contains about one million nephrons, the basic structural and functional unit of the kidney, which is composed of the glomerulus and a tubule (Figure 1).

The glomerulus is a capillary network surrounded by a capsule, called the Bowman's capsule, which is continuous with the tubular lumen. The endothelial cells, glomerular basement membrane and podocytes compose the blood-urine filtration barrier, through which approximately 1/5 of all plasma is filtered into the Bowman's capsule. The remainder of the blood rejoins the main bloodstream via the efferent circulation, while glomerular filtrate (primary urine) is further processed along the nephron.
The tubule is formed by tubular epithelia and mainly involved in fluid reabsorption towards peritubular capillaries. The tubule is divided into several segments according to different localization and functional characteristics. The proximal tubule is structured with tall simple cuboidal proximal tubular epithelial cells (hereafter termed as PTECs) and reabsorbs 70% to 80% of filtered sodium ions and water. It also serves to selectively reabsorb other ions and macromolecules. It is subdivided into S1, S2 and lastly the S3 segment, which meets the loop of Henle, an intermediate tubule connecting proximal and distal tubules, in the corticomedullary area. Further reabsorption of ions and water occurs in the distal tubule before the fluid enters the collecting duct to form urine.

**Cell adhesions in PTECs**

Of all the cell types in the nephron, PTECs carry out the most reabsorption. This vectorial transport across the cell from lumen to blood relies on the high polarity of PTECs and membrane-localized enzymes, ion channels, transporters and receptors. The epithelial cell polarity is established by biochemical and functional differences between apical and basolateral membrane domains.

Under physiologic conditions, PTECs connect to adjacent cells through cell-cell junctions and attach to the basement membrane of the tubular wall through cell extracellular matrix (ECM) adhesions (Figure 2A). These cell adhesions are mainly regulated by cytoskeleton-membrane interactions mediated through the F-actin cytoskeleton and can be promoted by Epac-Rap signalling pathway.
Cell-cell junctions

Two important protein complexes that contribute to intracellular adhesion at epithelial cell-cell junctions are tight junctions and adherens junctions (Figure 2B). These complexes contain trans-membrane proteins with adhesive function and also cytosolic proteins that link to the cytoskeleton and help establish and maintain normal epithelial cell polarity.\(^6,7\)

Tight junctions locate directly apical to adherens junctions on the lateral plasma membrane of PTECs. Tight junctions contribute to the establishment of a barrier to lateral diffusion of intrinsic membrane proteins and outer leaflet phospholipids, and to paracellular transport of ions and solutes, thus maintaining proper trans-epithelial resistance (TER) and para-cellular permeability of PTECs.\(^7\) The tight junction is associated with the actin cytoskeleton, and several proteins are structural components of this complex. The first identified component of the tight junctions, zona occludens-1 (ZO-1), has a large C-terminal proline-rich domain that binds actin microfilaments directly.\(^8\) Two relatives of ZO-1,
designated as ZO-2 and ZO-3 are also localized at tight junctions. These cytosolic proteins, together with others, directly bind to the carboxyl termini of transmembrane components of tight junctions, such as occludin, claudins and JAM, via their PDZ domains or GUK domains.

Adherens junctions are calcium-dependent adhesion complexes located directly basal to tight junctions. The cadherin/catenin complex serves as the key structural components that link two cells in a zipper-like manner and are also connected to actin filaments and other signalling proteins. The C-terminal intracellular domain of the cadherin binds β- or γ-catenin, which in turn binds α-catenin to mediate actin cytoskeleton linkages, while the N-terminal extracellular sequence is unique to each cadherin for self-recognition.

The most abundant cadherins present in adult kidney are classical cadherins including N- and E-cadherin, and the atypical kidney-specific cadherin known as Ksp-cadherin. The classical cadherins are key molecules that maintain the tubular cell-cell adhesion along the nephron, and are differentially expressed and localized in various segments of the nephron: N-cadherin is predominantly expressed in the proximal tubule, whereas E-cadherin predominantly present in the other tubular segments of the nephron. Ksp-cadherin is structurally distinct from classic cadherins with a highly truncated cytoplasmic tail that lacks catenin-binding domain. It is exclusively expressed in tubular epithelial cells and localized in the lateral membrane where it may function in maintaining renal tubular integrity.

It should be emphasized that adherens junctions and tight junctions are structurally and functionally linked: α-catenin binds the actin binding protein ZO-1. Thus tight junction involves E-cadherin mediated cell-cell interactions, and cadherins also contribute to the formation of tight junctions and epithelial barrier.

Cell-ECM adhesions

Cell-ECM adhesion is mediated via engagement of integrins with their extracellular ligands and subsequent assembly of an intracellular multiprotein complex termed the focal adhesion (FA) complex (Figure 2C). Integrins are a superfamily of non-covalently bound, heterodimeric transmembrane glycoproteins consisting of unrelated α and β subunits. Among all the subunits, β1 integrins are the most prominent integrins involved in PTEC-mediated cell-ECM interactions. FAs are prominently present at the basolateral membrane of PTECs and connected to the F-actin stress fibers in vivo. The complex consists of a large number of both cytoskeletal and signal transduction (adapter) proteins, including talin, vinculin, paxillin and focal adhesion kinase (FAK), and is physiologically rich in tyrosine phosphorylated proteins including PY397-FAK and PY118-paxillin.

FAK is a ubiquitously expressed non-receptor protein tyrosine kinase that localizes at FAs, and is structurally composed with three domains: N-terminal FERM-containing domain, central kinase domain and C-terminal domain containing focal adhesion targeting (FAT) sequence (Figure 3). The N-terminal Band 4.1, ezrin, radixin and moesin homology domain (FERM) can either positively or negatively regulate FAK. The intramolecular interaction between the FERM domain and the kinase domain prevents FAK autophosphorylation at tyrosine residue Y397 and subsequent FAK activation. Once integrins cluster, the FERM domain binds to the cytoplasmic tail of β-integrin, thus lifting an autoinhibition of FAK to allow the autophosphorylation. The tyrosine residue Y397 serves as a binding site for the
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SH2-domain of Src kinase, which is activated through tyrosine phosphorylation at Y416. Together the FAK/Src kinase complex phosphorylates downstream targets such as the signalling adapter protein paxillin and other tyrosine residues of FAK that all have their own function and influence on FA dynamics and downstream signalling. For instance, the phosphorylation of Y576/Y577 in the kinase domain results in increased FAK kinase activity. The phosphorylation at Y861 facilitates SH3 domain-mediated binding of p130Cas to the C-terminal proline-rich domains of FAK. PY861-FAK is also involved in cell migration and in c-Jun N-terminal kinase (JNK)-mediated expression of matrix metalloproteinase 9 (MMP9). The FERM domain also interacts with the Arp2/3 complex to enhance actin assembly, thus linking integrin signalling directly with the actin polymerization machinery.

F-actin cytoskeleton

The actin cytoskeleton is known to play fundamental roles in surface membrane formation and stability, junctional complex formation and regulation, and cell-extracellular membrane attachment.

Firstly, the actin cytoskeleton plays a vital role in the junction formation and contributes to the cell polarity in epithelial cells. During epithelial sheet formation, the initial cluster of cadherin/catenin complexes from opposing membranes (termed punctum) is formed upon contact of two neighboring cells and anchored to the actin cytoskeleton. More puncta
assemble at the edge of the contact and generate a zipper-like structure. With the actin-based dynamic movement, these zippers merge to seal the membranes into epithelial sheets\textsuperscript{28, 29}. Since cadherins play an important role as a precursor for the establishment of tight junctions and gap junctions, the actin-cadherin/catenin movements also influence the formation and maturation of these cell-cell junctions\textsuperscript{11}.

Secondly, the actin cytoskeleton mediates membrane-associated Na\textsuperscript{+}, K\textsuperscript{+}-ATPase stabilization, cell adhesion molecule attachment, and the attachment to ECM via integrins\textsuperscript{25}. The localization of β1 integrin at the cell-ECM contacts is also regulated by the integrity of the F-actin cytoskeletal network. Actin microfilaments are cross-linked by α-actinin and linked to the membrane-located integrin heterodimers by a protein complex that includes talin, vinculin, and actin microfilament capping proteins, thus in turn regulate cell-ECM adhesion. The assembly of F-actin stress fibers is associated with the formation of focal adhesions in various cell types, whereas the disruption of stress fibers precedes the impaired cell-ECM adhesion caused by ATP depletion in PTECs\textsuperscript{30, 31} and the complete loss of FA organization in ischemic rat kidneys\textsuperscript{20}.

Finally, F-actin filaments bundle together via actin-bundling proteins to mediate amplification of the apical surface membrane via microvilli. The actin bundle attaches to the surface membrane by the actin-binding proteins myosin I and ezrin\textsuperscript{32}.

The F-actin cytoskeleton dynamics (assembly and contraction) is tightly regulated by Rho family of small GTPases, including RhoA, Rac1 and Cdc42 that promote stress fiber, lamellipodia and filopodia formation, respectively\textsuperscript{33}. By directing actin dynamics, these small GTPases mediate the spatio-temporal adherens junction formation and therefore influence of cell-cell adhesion in epithelia\textsuperscript{34}. Not surprisingly, the Rho family GTPases also play a major role in cell-ECM adhesion signalling with an extensive interaction with the integrins and FA proteins\textsuperscript{35}.

The small GTPases Rap1 and Rap2 play a role in both cell-cell junction formation\textsuperscript{36} and cell-ECM adhesion\textsuperscript{37}, most likely through the stabilization of actin-cadherin/catenin complex during epithelial sheet formation.

Both the Rho family GTPases and the Rap GTPases belong to the superfamily of Ras-like small GTPases, which function as molecular switches, cycling between GTP- and GDP-bound forms\textsuperscript{38}. The switch is activated by GEFs-mediated binding of GTP, which causes a conformational change of the GTPase allowing the access to downstream effectors. It also results in the protein translocation from cytoplasm to the membrane surface where active effectors locate, and consequently initiates signalling cascades. The switch can be turned off through hydrolysis of GTP by the intrinsic GTPase activity which is stimulated by GTPase activating proteins (GAPs)\textsuperscript{39}. Consequently, the interaction between GTPase and its effectors is abolished and the signalling cascade is terminated.

c\textsuperscript{AMP}-Epac-Rap signalling

Cyclic adenosine monophosphate (cAMP) was the first identified second messenger\textsuperscript{40}. It is synthesized from ATP by a plasma membrane-bound enzyme adenylate cyclase (AC), and is rapidly hydrolyzed by cAMP phosphodiesterases (PDEs) into adenosine 5'-monophosphate (5'-AMP) (Figure 4A). Cyclic AMP functions ubiquitously, regulating many physiological processes including gene transcription, neuronal functions, cardiac muscle contraction,
vascular relaxation, and cell proliferation. Protein kinase A (PKA) was thought to be responsible for all the cAMP mediated effects, until the identification of another cAMP target, exchange protein directly activated by cAMP (Epac).

Epac is a guanine nucleotide exchange factor (GEF) which interacts with the Rap GTPase and induces the exchange of GDP for GTP. Two main members Epac1 and Epac2 both consist of a regulatory domain and a catalytic part. The activity of Epac depends on the binding of cAMP to the regulatory domain, while the CDC25 homology domain in the catalytic part mediates guanine nucleotide exchange activity towards the small GTPase Rap1 and Rap2. Recent studies on the crystal structure of Epac proteins suggested that cAMP binding induces conformational changes of Epac proteins, thus lifting an autoinhibition of Epac to allow the binding and thus activation of Rap.

At the mRNA level, Epac1 is widely expressed and enriched in human kidney, ovary, brain, and skeletal muscles while Epac2 is mainly expressed in the brain and adrenal gland. At the protein level, different anchoring mechanisms regulate the differential subcellular distribution of Epac proteins and thus often discriminate between Epac1 and Epac2. Epac1 localizes to several cellular compartments, reflecting various functions assigned to Epac1. The Epac-Rap pathway increases endothelial barrier function in vitro by promoting maturation of VE cadherin-mediated cell-cell adhesions whereas inhibition of

![Signal transduction of cAMP-Epac-Rap pathway.](image)

(A) The second messenger molecule cAMP is generated from ATP by a transmembrane enzyme adenylyl cyclase (AC) upon stimulation of Gs-coupled receptors (GPCR), and is rapidly hydrolyzed by cAMP phosphodiesterases (PDEs) into adenosine 5'-monophosphate (5'-AMP). Cellular effects are elicited by cAMP via protein kinase A (PKA), cAMP-gated ion channels and Epac. (B) The binding of cAMP or analogues to Epac allowing the exchange of GTP for GDP on Rap GTPases. The GTP-bound forms of Rap activate multiple cellular functions.
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Rap1 activity prevents cadherin-based cell adhesion. Epac1 has also been implicated in the cAMP-dependent inhibition of the apical membrane protein Na+/H+ exchanger (NHE3) activity in mouse proximal tubules—an effect which would appear to be independent of cell adhesion. Recent studies demonstrated that cAMP induces Rap activation via Epac1 by controlling both its GEF activity and targeting to the plasma membrane. In addition, ezrin-radixin-moesin (ERM) proteins function as plasma membrane anchors for Epac1 and are required for coupling cAMP-Epac1-Rap1 signalling to integrin-mediated cell adhesion.

The Rap family includes Rap1A, Rap1B, Rap2A and Rap2B, sharing more than 70% amino acid sequence homology. Common second messengers like calcium, diacylglycerol and cAMP frequently mediate the activation of Rap1 through Epac or other GEFs, in response to a large variety of stimuli. Rap has been observed at many membrane compartments in the cell, such as the plasma membrane. Thus the differential distribution of the upstream GEFs (sometimes RapGAPs) may be responsible for the cellular localization of Rap activation. For instance, cAMP-induced translocation of Epac1 to the plasma membrane determines locally Rap activation and contributes to Rap-mediated cell-ECM adhesion. The Rap1 pathway has been implicated in a number of physiological processes including integrin-mediated cell adhesion and E-/VE-cadherin mediated cell-cell junction formation (Figure 4B).

Pathophysiology of ARF

Several factors contribute to the susceptibility of the kidney to exogenous or endogenous injury that causes ARF. The kidney receives 1/4 of the cardiac output. It possesses the largest luminal membrane surface area by weight among all the organs and multiple enzyme systems which result in high biotransformation and reabsorption capacity. The high metabolic activity leads to high oxygen consumption/delivery ratio, especially in the outer medulla where a baseline medullary hypoxia exists.

Causes of ARF can be broadly divided into three categories: pre-renal causes lead to a decrease in clearance by compromised renal perfusion with intact renal tubular and glomerular function. Post-renal causes are related to obstruction of the urinary outflow tract. Intra-renal causes, also termed intrinsic causes, involve both structural and functional injury in blood vessels, glomeruli, interstitium or tubules. The major cause of intrinsic renal failure is acute tubular necrosis, which accounts for more than 80% of the cases and most often results from ischemic or toxic insults to the kidney. Renal tubular cell injury is a hallmark of this process.

Ischemic and nephrotoxic ARF share many pathophysiologic features. The proximal tubule is considered the major target upon both insults, due to its high level of reabsorptive capacity and non-homogeneous renal parenchymal oxygenation. First, proximal tubules carry out the majority of reabsorption in the kidney and consume a high amount of oxygen, while the oxygen supply is limited particularly at the cortico-medullary area where physiologic medullary hypoxia normally exists. Second, proximal tubules have little capacity for anaerobic glycolysis; hence, they have little capacity to withstand hypoxia due to cessation of renal blood flow and oxygen delivery as occurs during ischemia. Not surprisingly, ischemic tubular injury predominantly occurs among tubular segments in the cortico-medullary region, namely medullary thick ascending limbs (MTALs) and S3 (straight) segments of the
proximal tubule in the outer medulla and medullary rays. Third, disproportionate transport processes in renal tubular epithelial cells result in high intracellular concentrations of circulating chemicals and thus particularly high susceptibility of these cells towards toxicant-mediated injury. Last, Na+/K+-ATPases are abundantly expressed at the basolateral membrane of PTECs, adjacent to mitochondria, which supply essential ATP. In many cases mitochondria are a critical target of toxicants which ultimately leads to cell injury due to the dependence of cellular functions on aerobic metabolism.

Ischemic injury

Ischemic ARF can be the result of a generalized or localized reduction in renal blood flow (renal hypoperfusion) that occurs under many clinical conditions. It is a reversible process in most cases if the underlying factors causing the renal hypoperfusion are corrected. However irreversible cortical necrosis can occur when the ischemia is too severe.

One of the primary events during ischemic injury is mitochondrial dysfunction leading to ATP depletion, which initiates a number of metabolic alterations in PTECs. Cell adhesions, either cell-ECM adhesions or cell-cell junctions, are tightly controlled by proper integrity of the actin cytoskeleton network, which requires ATP. Thus disruption of the actin cytoskeleton and loss of cell adhesion is one of the early responses of the tubular epithelium, preceding tubular epithelial cell death and inflammatory cell influx (Figure 5). Early after the ischemic period basolateral-apical protein polarity is disturbed, with mislocalisation of integrins and the Na+/K+-ATPase from the basal surface to the apical surface, and the microvilli brush border is lost in conjunction with loss of cytoskeletal integrity. Loss of cell adhesions also correlates with loss of cell function, pro-apoptotic signalling and cell death. As a result of severe injury, both viable and dead epithelial cells with compromised cell adhesions slough into the lumen, causing cast formation and tubular obstruction, while the denuded tubular wall contributes to the “backleak” of the glomerular filtrate. These alterations eventually lead to a reduction of glomerular filtration rate (GFR) and loss of renal function.

Reperfusion injury and inflammation

The return of renal blood flow (reperfusion) after a period of ischemia restores oxygen in the kidney. There is a rapid burst of oxidant formation with the restoration of oxygen, which can cause marked tissue injury and pronounced inflammation during reperfusion.

Inflammation has a major role in the pathophysiology of ARF resulting from ischemia; endothelial and epithelial cells as well as leucocytes and T cells contribute to this inflammatory response. The inflammatory cells are recruited during reperfusion and release chemokines and cytokines that further increase the inflammatory cascade.

Neutrophils are the first inflammatory cells recruited into ischemic tissue and the major source of the oxidants in the kidney during reperfusion. After extravasation and chemotaxis, neutrophils release reactive oxygen species (ROS), proteases, phospholipases and other enzymes that cause injury to tubular cells. These substances, together with leukotriene B4, platelet-activating factor, and vasoconstrictive substances such as endothelin, can both
Figure 5. Diagram of proximal tubular alterations during acute renal injury. After ischemic or nephrotoxic injury, morphological alterations occur in proximal tubular epithelium, including loss of cell polarity, disruption of junction integrity and cell-matrix adhesion, and redistribution of membrane proteins (e.g. Na⁺/K⁺-ATPase and β1 integrins) to the apical surface. These changes result in cell death via necrosis or apoptosis. Both dead and viable cells slough into the tubular lumen, causing cast formation and luminal obstruction that further compromise renal function. (Modified from Schnellmann 2.)

increase vascular permeability and upregulate the expression of adhesion molecules that promote further inflammation 75, 76.

One of the adhesion molecules expressed on endothelial cells, intercellular adhesion molecule 1 (ICAM-1) interacts with CD11a/CD18 and CD11b/CD18 on neutrophils, facilitating the adhesion of neutrophils to endothelial cells 77, thus initiates leukocyte infiltration which is augmented by pro-inflammatory and chemotactic cytokines generated by ischemic tubular cells. These leukocytes obstruct the microcirculation and release cytotoxic cytokines, ROS, and proteolytic enzymes, which further damage the tissue 76.

Cell death and recovery

In contrast to most other organs, such as heart and brain where ischemia results in permanent cell loss and tissue damage, the kidney is able to completely and efficiently restore its structure and function after ischemic injury 1, 3. It is believed that the rate of organ functional recovery relates directly to the severity of cell injury during the initiation phase, since this process is dependent on the repair of sublethally injured and regeneration of non-injured cells 78.

The fate of an injured proximal tubule cell after an ischemic episode depends on the extent and duration of the ischemia. Cell death can occur immediately via necrosis or in a
more programmed fashion (apoptosis) hours to days after the injury. This phase is associated with expression of stress response genes and accumulation of mononuclear cells. Fortunately, most of the proximal tubular cells from the outer cortex are sub-lethally injured during ischemia and recover either in a direct fashion or via an intermediate undifferentiated cellular pathway. Again, the severity of the injury determines the route taken by a particular cell. Growth factors could play a part in determining the fate of the epithelial cells and might contribute to the generation of signals that result in neutrophil and monocyte infiltration. Alterations in cell cycling have also been shown to be involved in renal ischemic injury. Upregulation of p21, which inhibits cell cycling, appears to allow cellular repair and regeneration, whereas homozygous p21 knockout mice demonstrate enhanced cell necrosis in response to an ischemic insult.

Postischemic recovery duplicates certain aspects of normal renal development. Spreading and dedifferentiation of viable cells occur during the recovery via the intermediate undifferentiated cellular pathway. These poorly differentiated epithelial cells are thought to represent a population of stem cells residing in the kidney. The event following this dedifferentiation stage is a pronounced increase in proliferation and differentiation of the surviving proximal tubule cells. Regenerated tubular cells reestablish polarity and eventually produce a normal proximal tubular epithelium. Growth factors, including epidermal growth factor (EGF), hepatocyte growth factor (HGF), insulin-like growth factor 1 (IGF1), could have an important role in this response.

Nephrotoxic ARF

Chemicals with very diverse structures and sources, including drugs, natural products, industrial chemicals and environmental pollutants, account for a large number of cases of ARF. Aminoglycoside antibiotics, radiographic contrast media, anticancer drugs, conventional nonselective nonsteroidal anti-inflammatory drugs (NSAIDs), and angiotensin-converting enzyme (ACE) inhibitors are most frequently implicated in drug-induced nephrotoxicity. Because of the heterogeneous distribution of toxin accumulation that relates to selective sites of tubular reabsorption, these nephrotoxicants act at different nephron segments and involve in various alterations of renal function. Thus, the mechanisms to cause ARF may vary with different toxicants, and are generally categorized based on the target sites that are injured.

The disruption of haemodynamics and direct tubular epithelial cell damage are the most common mechanisms of toxicant-induced ARF. Blood flow to the glomerulus is tightly regulated by vasoconstriction and vasodilation of the afferent and efferent blood vessels, to maintain glomerular pressures. Several chemicals can disrupt this delicate homeostasis, such as NSAIDS, which suppress prostaglandin-mediated vasodilation of the afferent arteriole or ACE inhibitors, which suppress vasoconstriction of the efferent arteriole. Radiocontrast agents that are filtered in the glomerulus, but are not reabsorbed, are sensed in the macula densa cells of the distal tubule and activate an afferent vasoconstriction mechanism which normally functions to prevent excessive loss of salt. Cyclosporine causes renal injury via various mechanisms, including vasoconstriction through disruption of sympathetic control. These compound classes all result in reduced glomerular pressure and thus ultrafiltration of blood and also compromised renal perfusion.
Table 1. Exogenous and endogenous chemicals that cause ARF

<table>
<thead>
<tr>
<th>Category</th>
<th>Chemicals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotics</td>
<td>Aminoglycosides, amphotericin B, cephalosporins, penicillins, sulfonamides, polymixins, tetracycline</td>
</tr>
<tr>
<td>Chemotherapeutic agents</td>
<td>Cisplatin, Adriamycin, mitomycin C</td>
</tr>
<tr>
<td>Radiocontrast agents</td>
<td>Diatrizoate, Iothalamate, metrizamide</td>
</tr>
<tr>
<td>Immunosuppressive agents</td>
<td>Cyclosporine A, FK506</td>
</tr>
<tr>
<td>Antiviral agents</td>
<td>Acyclovir, foscarnet</td>
</tr>
<tr>
<td>Heavy metals</td>
<td>Gold, cadmium, mercury, lead, arsenic</td>
</tr>
<tr>
<td>Organic solvents</td>
<td>Ethylene glycol, carbon tetrachloride</td>
</tr>
<tr>
<td>Vasoactive agents</td>
<td>NSAIDs, ACE inhibitors, angiotensin receptor antagonists</td>
</tr>
<tr>
<td>Other drugs</td>
<td>Acetaminophen, penicillamine, lithium</td>
</tr>
<tr>
<td>Endogenous compounds</td>
<td>Hemoglobin, calcium, cystine</td>
</tr>
</tbody>
</table>

NSAIDs: nonsteroidal anti-inflammatory drugs; ACE: angiotensin-converting enzyme

Table 2. Mechanisms and associated toxicants that cause ARF

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Toxicants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduction in renal blood flow through alteration of intrarenal hemodynamics</td>
<td>NSAIDs, ACE inhibitors, radiocontrast agents, amphotericin B, cyclosporine, FK506</td>
</tr>
<tr>
<td>Direct tubular epithelial cell damage</td>
<td>Aminoglycosides, cisplatin, radiocontrast agents, amphotericin B, pentamidine, cyclosporine, FK506, foscarnet, heavy metals, organic solvents, DCVC</td>
</tr>
<tr>
<td>Allergic interstitial nephritis</td>
<td>NSAIDs, cyclooxygenase-2 inhibitors, cephalosporins, cadmium chloride, penicillins, ciprofloxacin, proton pump inhibitors, loop diuretics</td>
</tr>
<tr>
<td>Intratubular obstruction by precipitation of tissue degradation products or drugs and/or their metabolites</td>
<td>Acyclovir, indinavir, sulfonamides, foscarnet, methotrexate, triamterene</td>
</tr>
<tr>
<td>Heme pigment-induced toxicity (rhabdomyolysis)</td>
<td>Cocaine, ethanol, lovastatin (in combination with cyclosporine)</td>
</tr>
<tr>
<td>Renal vasculitis and thrombosis (hemolytic-uremic syndrome)</td>
<td>Mitomycin, cyclosporine, FK506, cocaine, gemcitabine, penicillamine, quinine</td>
</tr>
<tr>
<td>Glomerular dysfunction with proteinuria</td>
<td>Gold, Lithium, interferon-α, penicillamine, NSAIDs</td>
</tr>
</tbody>
</table>

DCVC: dichlorovinyl-L-cysteine
In addition to their ischemic effect, many nephrotoxicants damage tubules directly, such as amphotericin B and radiocontrast agents. Similarly to I/R injury, the tubular epithelial cells of the kidney are particularly vulnerable to toxicant-mediated injury due to their disproportionate exposure to circulating chemicals and transport processes that result in high intracellular concentrations. In general, nephrotoxicants mediate cell injury either through covalent or noncovalent binding to critical macromolecules that results in altered activity of these molecules, or through a direct increase in cellular ROS that results in oxidative damage to critical macromolecules. The amphipathic structure of amphotericin B facilitates its binding to sterols in the cell membranes and produces aqueous pores that increase tubular permeability and cell death. Radiocontrast agents increase ROS and lipid peroxidation in the cells that contribute to cell death. Aminoglycosides-induced proximal tubular epithelial cell damage is initiated by the cationic charge-mediated binding to the brush border membrane, followed by intracellular transport and concentration in lysosomes. The release of lysosomal enzymes, phospholipids and aminoglycosides into the cytosol after disruption of overloaded lysosomes can injure cellular functions and lead rapidly to cell death. Platin-containing compounds cause tubular damage predominantly in proximal tubules, as the result of impairment of cell energetics, possibly by binding to proximal tubular cellular proteins and sulfhydryl groups with disruption of cell enzyme activity and uncoupling of oxidative phosphorylation.

Cisplatin-induced nephrotoxicity

Cisplatin has been widely used in the past decades for clinical treatment of a variety of solid tumors. The clinical use of cisplatin has decreased in recent years because of its toxic side effects to healthy tissue in particular the kidney as well as the availability of alternative anti-cancer drugs. ARF appears within several days of a single administration of cisplatin, with direct tubular nephrotoxicity (glycosuria, hypomagnesemia, hypokalemia) as a predominant pathophysiologic process. Cisplatin is absorbed by and accumulates within cells in the proximal tubule (especially the S3 segment), where it is metabolized to more nephrotoxic reactive intermediates that cause cell injury. Cisplatin causes various biochemical and cellular perturbations in renal cells, including DNA cross-linking, decreased capacity of several transport systems such as Na+/K+-ATPase activity, reduced activity of antioxidant enzymes such as manganese superoxide dismutase, glutathione peroxidase, and glutathione transferase in association with lipid peroxidation, mitochondrial dysfunction, cytoskeletal reorganization and changes in cell adhesion.

The cytotoxicity can culminate in apoptosis and necrosis depending on the concentration of cisplatin and the antioxidant status of cells. In general, apoptosis occurs at low levels of drug exposure, whereas necrosis requires higher doses. This makes the study of apoptosis particularly relevant for the clinical usage of cisplatin as well as other drugs, since drugs are assumed to be used at doses and for durations of treatment that do not cause necrosis in the experimental animal. Several subcellular and molecular pathways are involved in cisplatin-induced apoptosis of PTECs, including activation of Bad and Bax, collapse of the mitochondrial membrane potential, inhibition of complexes I to IV of the respiratory chain, release of cytochrome c and release of Omi from mitochondria to the cytoplasm and degradation of XIAP. These signals are mostly associated with the mitochondrial...
pathway and are under tight control of Bcl-2 family members. The apoptosis then follows the common degradation pathway, which involves the activation of caspase-9 and caspase-3 followed by caspase substrate cleavage, nuclear condensation, and DNA fragmentation. Similar to ischemic injury, cisplatin-induced apoptosis is induced by oxidative stress in PTECs and activation of stress signal transduction cascades. In addition, cisplatin causes the formation of F-actin stress fibers and loss of cell junctions in PTECs preceding the activation of caspases and the onset of apoptosis.

**Cellular alterations during ARF**

Cellular responses during ARF concerns biochemical, signalling and structural changes in tubular cells that eventually lead to cell injury and death, the hallmark of both ischemic and nephrotoxic ARF.

**Biochemical perturbations**

Renal ischemia due to generalized or localized drop of blood flow results in a lower oxygen level that impairs normal mitochondrial function and subsequent biological processes. ATP depletion, as a result of mitochondrial dysfunction, is the central biochemical event during renal ischemic injury. The breakdown of ATP generates adenosine, inosine, and hypoxanthine, which further contribute to tubular injury and ROS formation. Oxidant formation occurs during reperfusion after a period of ischemia with the restoration of oxygen, or can be directly induced by nephrotoxins such as cisplatin. Several in vivo studies show that antioxidants or ROS scavengers protect against functional tissue damage in either ischemic or nephrotoxic renal injury, suggesting a role of oxygen free radicals in the pathogenesis of ARF. However, there is little compelling evidence to support the use of ROS scavengers in patients with ARF.

The loss of cellular ATP also leads to disruption of cellular ion homeostasis with decreased cellular K⁺ content and increased Na⁺ content, causing increased cytosolic free Ca²⁺ concentrations in early or late phase of cell injury. The increased intracellular calcium is able to activate proteases (calpains and caspases) and phospholipases (phospholipase A₂) that contribute to cell death, break down the cytoskeleton, and interfere with mitochondrial energy metabolism, thus causing tubular injury in addition to vasoconstriction.

**Activation of stress signalling pathways**

Protein kinase activities, in particular mitogen-activated protein kinases (MAPK) cascades, are important stress signalling mediators in ischemic or nephrotoxic injury as well as during the recovery after these insults. The family of MAPKs consists of extracellular signal-regulated kinases (ERKs), JNK and p38 MAPK. Renal ischemia/reperfusion causes activation of all three kinases, possibly as a direct consequence of oxidative stress. A variety of nephrotoxicants can also cause the activation of JNK and p38. These kinases localize amongst other sites at focal adhesions, which raise the possibility that the disturbance in cell adhesions during acute renal cell injury is in part a result of activation of these kinases.
The ERK pathway plays a role in cell growth and differentiation, giving cells a survival advantage, while it may also contribute to injury and apoptotic cell death, and is thus proposed as a determinant of the cell survival/cell death balance. There is growing evidence suggesting that activation of ERK in renal cells is involved in injury and apoptosis rather than contributing to cell survival. ERK is reported to localize at FAs and control adhesive structures by local activation in response to different stimuli including growth factors, which suggests a possible link between ERK activation and FA restructuring during I/R. Recent studies demonstrate that ERK is activated at FAs where it binds to paxillin and mediates FA disassembly and turnover together with FAK, paxillin and Src-kinase, contributing to cell and tissue injury during renal I/R.

JNK and p38 are particularly responsive to cellular stress conditions, which also occur in the kidney after acute renal cell injury. Previous studies using either antisense or pharmacological inhibitors suggest that cell death is promoted by a sustained activation of JNK and p38 after PTEC injury, whereas the protection against renal cell injury is due to reduced JNK and p38 activation. Both JNK and p38 determine the biological outcome of the cellular stress response via affecting gene expression and ultimately protein expression of various transcription factors, or alternatively, causing post-translational modification of other cellular proteins. For example, JNK can phosphorylate the anti-apoptotic protein Bcl-2, thereby inhibiting anti-apoptotic activity and sensitizing cells towards apoptosis. p38 MAPK activates the MAPKAP kinase 2, which phosphorylates other downstream targets, including small heat shock proteins such as Hsp27. Hsp27 was identified as the most prominent early modified protein in the cellular stress response after model nephrotoxicant treatment of renal epithelial cells. Both p38 activity and the phosphorylation of Hsp27 are implicated in the protection of renal epithelial cells against a reorganization of focal adhesions, activation of caspases, and the onset of apoptosis caused by nephrotoxic cellular stress. Furthermore, p38 MAPK plays an important role in the activation of tubular cells upon various stimuli to produce pro-inflammatory chemokines and cytokines, which eventually leads to renal fibrosis. Intracellular delivery of the p38 MAPK inhibitor SB202190 in renal tubular cells by administration of SB202190-LZM conjugate in a rat renal I/R model demonstrated a reduction of intra-renal p38 MAPK phosphorylation and α-smooth muscle actin protein expression, suggesting a potential strategy for the treatment of renal fibrosis.

Reorganization of F-actin cytoskeleton

Actin cytoskeletal disruption is a hallmark of ischemic injury in vivo and ATP depletion in a number of cell types, including renal epithelial cells. In rat kidneys, mild ischemia caused reorganization of F-actin network with a loss of stress fibers and a reduction in F-actin at the microvilli, which was overall recovered after 24 hours of reperfusion. However, severe ischemia resulted in marked changes in F-actin arrangement with a complete loss of F-actin containing microvilli, and the reperfusion time for completely regenerating the renal tubular F-actin structure was up to two weeks.

The F-actin cytoskeleton reorganization also occurs in cultured kidney epithelial cells in response to ATP depletion, with the disruption of stress fibers, cortical F-actin and apical actin bundles. This was followed by the loss of cell adhesion and associated with Rho
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inactivation during ischemia, whereas over-expression of active RhoA prevented disruption of stress fibers and cortical F-actin during ATP depletion and enhanced the rate of stress fiber reassembly during recovery. This corresponds with decreased myosin light chain phosphorylation in MDCK cells subjected to chemical anoxia-induced ATP depletion and suggests a role of Rho GTPases in the pathological process.

Recently, Caron et al. demonstrated a modified expression and localization pattern of Rac1, RhoA and Cdc42 in the proximal tubular fractions after renal I/R, which is correlated with alterations in β-actin and α-tubulin expression and distribution. Another study using the PTEC cell line confirmed a principal role for RhoA in cytoskeletal reorganization during ischemia and demonstrated that the activity of Rho GTPases can be maintained even at low GTP concentrations. To test whether blockade of the Rho signalling within kidneys can be beneficial in reducing renal injury, the Rho kinase inhibitor Y27632 was conjugated to the tubular-targeted drug carrier lysozyme (LZM) and applied in a rat renal I/R model. The treatment inhibited the activation of Rho kinase within tubular cells and attenuated tubular damage induced by I/R injury.

FA dynamics

FAs are dynamic structures that vary in size and content of FA associated proteins. During I/R injury, extensive remodeling of FAs occurs, concurrently with F-actin cytoskeleton reorganization. β1 integrins, the major mediator of cell-ECM interactions, are lost from the PTEC basolateral membrane region after ischemia, and return to this side during reperfusion. Tyrosine phosphorylation of proteins at the FAs declines directly after the ischemic insult, which coincided with a disruption of the FA structures and the F-actin cytoskeleton network. During reperfusion, levels of protein tyrosine phosphorylation increase in association with an increase in FA size and F-actin stress fiber formation.

Different FA proteins show differential phosphorylation patterns during reperfusion. FAK is rephosphorylated during the reperfusion period in a both temporal and tyrosine residue site-specific manner. The auto-phosphorylation of FAK at Y397 phosphorylates Src kinase at Y416. Other tyrosine residues of FAK, as well as other FA proteins are respectively phosphorylated by Src kinase. Paxillin can be tyrosine phosphorylated at Y31 and Y118, which is required for efficient FA turnover and cell motility. During the reperfusion period, increased PY397-FAK, PY576/577-FAK and PY416-Src were found directly associated with increased PY118-paxillin and FA remodeling.

Disruption of cell-cell adhesion

During ARF, backleak occurs when the para-cellular space between cells is open for the flux of glomerular filtrate to leak back into the extracellular space and into the blood stream. This is believed to occur through functional and morphologic changes in tight junction integrity. ATP depletion in vitro models have shown transient disruption and reassembly of tight junctions. Staining of renal epithelial cells with antibodies that bind to ZO-1 illustrates the disruption of tight junctions during ATP depletion. In untreated Mardin-Darby canine kidney (MDCK) cells, ZO-1 is located at the periphery of cells at cell contact sites, forming a continuous linear contour. In ATP-depleted cells, however, the staining pattern is
discontinuous and large gaps are observable. Tight junctions also control TER. When the TER falls to zero with ATP depletion, this suggests that tight junction structural integrity has been compromised.

I/R in proximal tubules as well as ATP depletion in MDCK cells also cause E-cadherin/β-catenin complex disruption by selective degradation of E-cadherin. ATP depletion of proximal tubular cells resulted in loss of cell-cell interaction, which was associated with hyperphosphorylation of β-catenin and plakoglobin (γ-catenin). This phosphorylation was enhanced by vanadate, a tyrosine phosphatase inhibitor, and inhibited by genistein, a general tyrosine kinase inhibitor. Therefore, the function of both existing and assembling cell-cell adhesions is regulated by tyrosine phosphorylation.

**Proximal tubular cell adhesion modulation**

*Conditional FAK knockout in mouse proximal tubules*

FAK localises at FAs and is hyperphosphorylated during renal ischemia-reperfusion injury. These findings suggest that FAK plays a role in FA regulation and the proximal tubular epithelial stress response following ischemia. To assess the unknown role of FAK in the pathogenesis of ischemic renal injury, transgenic and gene knockout technologies have been used to inactive the *fak* gene either in animal tissue or in cultured cells.

Conventional gene knockout approaches by homologous recombination in embryonic stem cells produce genetic FAK knockout mice that display embryonic lethality (E8.5). Deletion of FAK in adulthood results in a complex phenotype due to its ubiquitous expression pattern. Non-specific knockdown following systemic administration of small interfering RNA targeting FAK in mice resulted in tissue insulin resistance. To circumvent these limitations conditional knockout strategies have been designed to inactivate *fak* gene in a spatiotemporally regulated manner.

Two main approaches are in use today to exert temporal control over gene expression. The tetracycline-dependent system activates transcription of the recombinase in a spatiotemporal manner. The tamoxifen-inducible estrogen-based system exclusively activates Cre recombinase which is constitutively expressed in the desired subset of cells under control of cell-specific promoters. In this system, a hybrid gene is constructed with the Cre gene fused to a mutant form of the ligand-binding domain of the estrogen receptor (ER). The mutant of the binding domain is insensitive to the natural ligand (17β-estradiol) at normal physiological conditions, but responsive to the synthetic estrogen antagonist tamoxifen (TAM) or 4-hydroxy-tamoxifen (4-OHT). Among currently available mutants, Cre-ERT2 with a triple mutation increases OHT affinity 10-fold and shows the best efficiency with respect to nuclear translocation and recombinase activity. In the absence of TAM or 4-OHT, the fusion protein Cre-ERT2 is confined to the cytoplasm and Cre-mediated recombination is prevented because this is a nuclear event. However in the presence of the estrogen antagonist, Cre-ERT2 is translocated to the nucleus, where it catalyzes recombination of the floxed target DNA sequences. More than ten different transgenic mouse lines expressing Cre recombinase in specific renal cells (including e.g. podocytes, collecting duct cells and proximal tubule cells) have been reported and they have been used to inactivate several genes important for kidney development and/or function.
Both I/R injury and nephrotoxic injury predominantly affect cells of the proximal tubules, therefore the role of FAK specifically in this segment of the nephron is to be investigated. To obtain a proximal tubular FAK knockout mouse line, the Cre recombinase/loxP (Cre/loxP) system is utilized with a core promoter of proximal tubule-specific \( \gamma \)-glutamyltranspeptidase type II (\( \gamma \text{GT} \)) gene. Two different mouse lines, FAK\( ^{\text{loxP/loxP}} \) and \( \gamma \text{GT-Cre-ER}^{\text{T2}} \), are generated separately. In the FAK\( ^{\text{loxP/loxP}} \) mouse line the second exon of the \( fak \) gene is flanked by two loxP sites using homologous recombination techniques in embryonic stem cells. The loxP sites are short sequence elements (34 bp) containing two 13-bp inverted repeats flanking an 8-bp asymmetric spacer. In the \( \gamma \text{GT-Cre-ER}^{\text{T2}} \) mouse line, generated by standard oocyte injection techniques, Cre recombinase expression is driven by a truncated promoter of the mouse \( \gamma \text{GT} \) gene, which has previously been proven to confer specific expression of \( \beta \)-galactosidase to the renal proximal tubules. Crossing these two transgenic mouse lines yields FAK\( ^{\text{loxP/loxP}}//\gamma \text{GT-Cre-ER}^{\text{T2}} \) offspring in which Cre recombinase is solely expressed in S3 segment of proximal tubule where it excises the floxed \( fak \) gene fragment and results in proximal tubular FAK knockout (Figure 6). Using these approaches FAK can be deleted in proximal tubule cells \textit{in vitro} and \textit{in vivo} and its specific role in I/R injury can be investigated.

Pharmacological activation of Epac-Rap signalling

Since the discovery of Epac as an alternative cAMP target, various cAMP analogues have been developed with selective high-affinity binding to the cAMP-binding domain of either Epac or PKA, to distinguish between Epac-mediated signalling events and PKA-mediated events. The Epac-selective cAMP analogue 8-pCPT-2’-O-Me-cAMP is generated by replacing the 2’-hydroxyl group which is absolutely required for high-affinity binding of cAMP to PKA with the 2’-O-Me group which enhances efficient binding to and activation of

![Figure 6. Tamoxifen-inducible Cre/loxP-mediated proximal tubular fak knockout.](image-url)
Epac, and is able to selectively activate Epac without influencing the PKA pathway when used in the μM concentration range \(^1\) (Figure 7). A recent modification to 8-pCPT-2’-O-Me-cAMP by introducing an acetoxymethyl-ester (8-pCPT-2’-O-Me-cAMP-AM) was found to greatly enhance the in vitro biological activity of the compound presumably by facilitating cellular uptake without affecting its intracellular efficacy \(^1\). These have been used to demonstrate that Epac-Rap signalling controls many physiological processes, particularly adhesion-associated processes including migration and survival \(^5\).

The selectivity of 8-pCPT-2’-O-Me-cAMP for Epac1 vs Epac2 has not been compared directly. However, it is likely that 8-pCPT-2’-O-Me-cAMP shows a differential selectivity for these two isoforms. Probably an important determinant of which Epac isoform will be activated by these analogues will be the expression level of the proteins in the cell or organ concerned.

Epac-associated cell adhesion and survival in the kidney

In the kidney, Epac is most highly expressed by endothelial and epithelial cells \(^4\), \(^1\). Both Epac1 and Epac2 are expressed by all three segments of the proximal tubules and predominantly localized in the brush border, suggesting a functional role here for Epac-Rap signalling \(^5\), \(^1\).

Among the important cellular responses to cAMP-induced Epac-Rap signalling are enhanced integrin-mediated adhesion to the extracellular matrix \(^3\) and the preservation of cell-cell contacts \(^5\). Given the fact that the loss of proximal tubular cell adhesion is one of the early events observed during either ischemic or nephrotoxic ARF and correlated with following cellular and tissue injury \(^2\), \(^1\), the activation of Epac-Rap signalling in the kidney may prevent or alleviate renal injury during ARF. Stokman et al. \(^1\) recently reported that activation of Epac-Rap signalling via intrarenal administration of 8-pCPT-2’-O-Me-cAMP during renal ischemia protects against kidney failure in a mouse model.

Both cell-ECM as well as cell-cell interactions are vitally important in providing the renal epithelial cells with survival cues to prevent the onset of apoptosis; these are largely mediated
through the activation of the phosphoinositide-3 kinase/protein kinase B pathway \(^{162}\). The activated form of Akt/PKB phosphorylates Bad, which then associates with the chaperone protein 14-3-3 and becomes unable to exert its pro-apoptotic function \(^{163}\). A protective effect of cyclic AMP and inducers of cAMP against cisplatin-induced nephrotoxicity has been previously described \(^{164-166}\). Some studies have attributed this protective effect to activation of the archetypal cAMP target PKA \(^{165}\). The role for Epac in mediating anti-apoptotic effects of cAMP in various tissues is beginning to emerge; in cardiac myocytes, inhibitors of phosphodiesterase 4 (PDE4), which increase cAMP levels, were found to inhibit NO-mediated apoptosis – an effect that was mediated by Epac \(^{167}\). A different study (also in cardiac myocytes), showed that super-activation of adenylate cyclase via the β-adrenergic receptor enhanced apoptosis \(^{168}\). A recent study using Epac1 knockout mice demonstrated that this pro-apoptotic effect was indeed mediated by Epac1 \(^{169}\). However, whether Epac mediates the anti-apoptotic effect of cAMP in renal cells, particular PTECs, during ARF still remains unknown.

**Concluding remarks**

Proper cell adhesion provides PTECs in the proximal tubule with environmental signals necessary to maintain normal cellular processes that are required for kidney function, whereas perturbation of cell adhesion in association with reorganization of F-actin cytoskeleton occurs at early stage of renal disease such as ARF. Increasing evidence has emerged to support an essential role of FA signalling in the cellular stress response during renal injury, mediated by changes in the dynamics of adhesion complexes and their associated signalling proteins such as FAK and paxillin, and the stress kinases, ERK, JNK and p38. However, further study is still required to define the roles of these adhesion-related molecules and pathways in the progression of renal cell and tissue injury during ARF. This will contribute to a better understanding of disease pathophysiology and may help in identifying targets for the development of new therapeutic strategies.

**AIM AND OUTLINE OF THIS THESIS**

The general aim 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 (I/R) injury involves disruption of focal adhesion (FA) complex 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** describes a model that 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. Therefore the inhibition of ERK pathway represents a potential therapeutic means to protect against I/R injury.

The role of focal adhesion kinase (FAK) during I/R injury as well as cellular oxidative stress was investigated by using a conditional proximal tubule-specific FAK knockout mouse model. **Chapter 3** demonstrates an essential interplay between FAK, JNK and control of FA
dynamics as well as a role for these proteins in the stress response during progression of I/R-induced acute renal injury. This FAK/JNK/paxillin linkage could potentially be the basis for future targets for therapeutic intervention in ARF.

The Epac-Rap signalling pathway is a potent regulator of cell-cell and cell-matrix adhesion. Chapter 4 presents a study where the pharmacological activation of the Epac-Rap signalling pathway showed both in vivo and in vitro protective effects on ARF-associated renal cell injury. This suggests Epac-Rap signalling as a novel therapeutic target for enhancing tubular epithelial cell adhesion thereby reducing renal failure during early I/R injury.

The Epac-Rap signalling-mediated reno-protective effect was further examined in a model of cisplatin-induced renal cell injury. Chapter 5 presents a pharmacological approach by which activated Epac-Rap signalling has the potential to protect against nephrotoxicity without compromising the therapeutic value of cisplatin as an anti-cancer drug. This study identifies Epac-Rap signalling as a cAMP-dependent cytoprotective pathway and confirms the potential of enhancing tubular epithelial cell adhesion for reducing renal injury.

To identify genes that are involved in ATP depletion-mediated stress responses during ischemic injury, a transcriptomics study was performed using oligonucleotide microarray analysis. Chapter 6 provides an overview of genes and pathways that are involved in induction of cell death as well as regeneration from injury in primary cultured rat PTECs subjected to metabolic inhibition-induced hypoxic injury. This may lead to novel targets for protecting against ischemic injury or promoting regeneration during the recovery.

Chapter 7 discusses the findings of studies in this thesis and their implications for future research.

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