Focal adhesion signaling in acute renal failure

Alderliesten, M.C.

Citation

Alderliesten, M. C. (2009, February 11). *Focal adhesion signaling in acute renal failure*. LACDR, Division of Toxicology, Faculty of Science, Leiden University. Retrieved from https://hdl.handle.net/1887/13803

Version:	Corrected Publisher's Version
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/13803

Note: To cite this publication please use the final published version (if applicable).

Chapter 1

GENERAL INTRODUCTION TO ACUTE RENAL FAILURE AND RECOVERY

A General Introduction to Acute Renal Failure and Recovery

Acute renal failure (ARF) is an important clinical problem with a high mortality and morbidity. It affects 5% of hospitalized patients and has a mortality rate of approximately 50%[1]. One of the primary causes of ARF is ischemia/reperfusion (I/R), a drop in blood flow leading to inadequate supply of oxygen and nutrients to renal tissue which can be caused by, amongst others, surgery, organ transplantation and shock[1]. This initiates a complex and interrelated sequence of events that result in (sub)-lethal injury of renal cells. Although the kidney has the ability to recover completely, injury that is too severe can lead to chronic renal failure setting patients up for a life of weekly dialysis treatment[1;2]. Current therapy is limited to supportive measures and preventive strategies, none of which have been definitely shown to alter mortality [3;4]. Therefore fundamental insights into cellular and molecular biology of I/R induced renal injury are necessary to develop effective therapies that can prevent or reduce renal injury and thereby improve the quality of life of the patients.

Cell adhesions are important structures for cell function and are implicated in efficient and proper tissue and organ function. In this thesis the ***role of cell adhesion signaling during I/R-induced renal injury*** is studied. Therefore the different types of cell adhesion will be described before going into the pathophysiology of renal injury. In addition the molecular and cellular mechanisms of renal failure will be discussed. The focus of this introductory chapter is on the cell-extracellular matrix (ECM) interactions known as focal adhesions (FAs). Primarily the non-receptor protein tyrosine kinase, focal adhesion kinase (FAK) will be described, since FAK, an important component of the FAs, is involved in many signaling pathways implicated in renal I/R process and recovery of renal tissue.

Kidney physiology

The kidney has two functions, to rid the body of waste materials either ingested or produced by the metabolism and to control volume and composition of body fluids. The kidneys perform their important functions by filtering the plasma and removing substances from the filtrate at variable rate, depending on the needs of the body. Ultimately the kidneys clear unwanted substances from the filtrate by excreting them in urine while returning substances that are needed back in the bloodstream.

When the kidneys are bisected from top till bottom there are three major regions that can be identified, the outer cortex, the inner medulla and the papilla (Fig. 1A). Each human kidney consists of approximately one million nephrons which together form the functional unit of the kidney. Each nephron consists of a glomerulus, through which fluid is filtered from the blood and a long tubule in which the filtered fluid is converted into urine. The filtered fluid flows from the glomerulus into the Bowmans's capsule and then into the proximal tu-

bule. From the proximal tubule, fluid flows into the loop of Henle and via the macula densa it enters the distal tubule followed by the connecting tubule and the cortical collecting tubule that leads to the collecting ducts (Fig. 1B). A nephron can be considered as a series of functionally distinct segments each having unique permeability characteristics and fluid and solute transport capabilities. These specific characteristics are determined by the cyto-architecture of the epithelial cells and the manner in which the cells interact with each other and the ECM. These cell adhesions are involved in the restriction of permeability, establishment of epithelial cell polarity and normal transport and filtration of substances across the cell barrier [5].

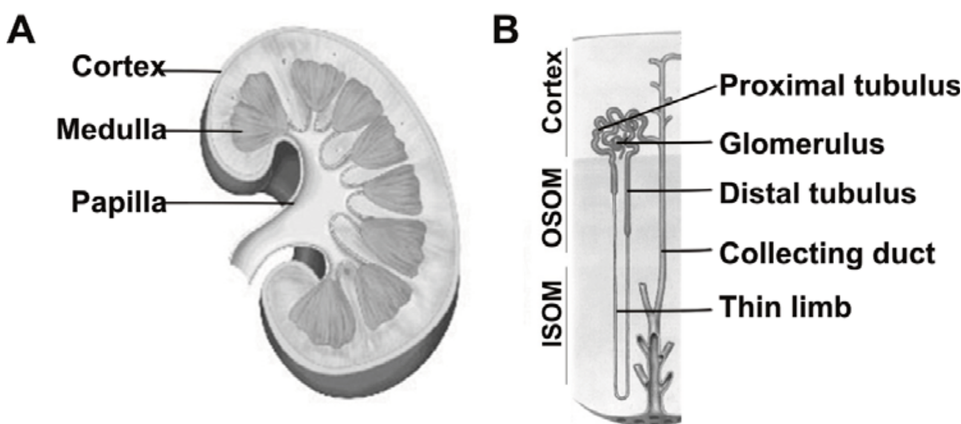

Figure 1. Illustration of a cross section of kidney and nephron. The kidney can be divided into three major regions i.e. cortex, medulla and papilla (A). The human kidney consists of approximately one million nephrons. A nephron contains a glomerulus, proximal tubule, descending and ascending thin limb, distal tubule and collecting duct (B).

Cell adhesions in the kidney

Most cell types, including the proximal tubular epithelial cells, require cell-cell and cell-ECM adhesion for proper cellular signaling and tissue function. Adherens and tight junctions mediate cell-cell adhesion, whereas cell adhesions to the ECM are known as FAs.

The tight junctions are also referred to as occluding junctions or *zonula occludens.* They are located near the apical surface at the lateral plasma membrane of the cell (Fig. 2) and act as a seal between cells that regulates diffusion of ions and solutes across the cell boundaries [6;7]. They also prevent lateral diffusion of plasma membrane proteins and lipids between the apical and basal-lateral plasma membrane domains and thus maintaining epithelial cell polarity. Occludin and ZO-1 are integral membrane proteins that interact with each other and anchor the junctional complex to the cytoskeletal elements of the

neighboring cells[6;7]. The tension of the actin cytoskeleton pulling on the tight junction is implicated in the regulation of paracellular permeability[6;8] (Fig. 3).

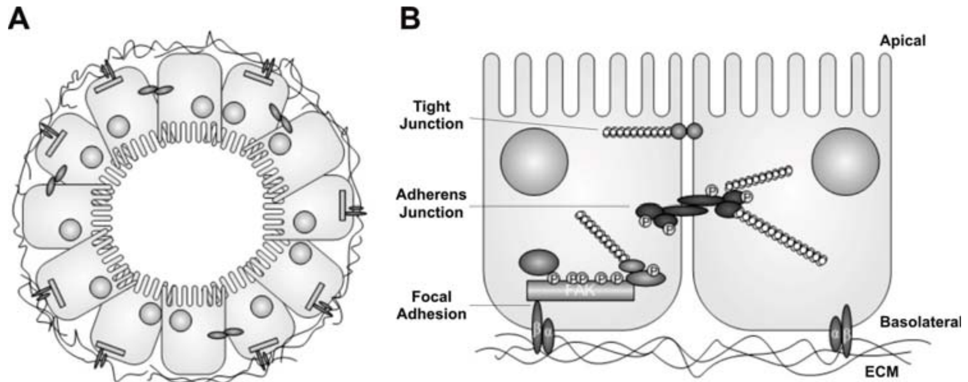

Figure 2. Schematic illustration of tubular epithelial cell adhesion. Renal tubules are consist of a single layer of epithelial cells (A). These epithelial cells are attached to each other via tight junctions and adherens junctions. Cell- extracellular matrix adhesion is mediated by focal adhesions. All cell adhesion complexes contain an number of structural and signaling molecules and are linked to the F-actin cytoskeleton (B).

The adherens junctions, also termed zonula adherens, are calcium dependent adhesion complexes located basal to the tight junctions[9;10] (Fig. 2). Cadherin cell-cell adhesion molecules organize a plaque structure that contains a variety of membrane-cytoskeletal proteins that link to the actin cytoskeleton and signal transduction proteins including growth factor receptors and several tyrosine kinases. Cadherins (e.g. epithelial E-cadherin, neural N-cadherin, placental P-cadherin and kidney K-cadherin) are transmembrane proteins with a single transmembrane-spanning region, an extracellular domain and an intracellular domain[11;12.]. The extracellular domains of cadherins of two adjacent cells bind, linking the cells in a zipperlike way[13;14]. Intracellular, cadherins interact with the actin cytoskeleton via catenins (e.g. α-catenin, β-catenin, plakoglobin and p120 catenin)[13]. Stability of the adherens junctions is regulated by for example tyrosine phosphorylation of the proteins located at the junction[15] (Fig. 3).

FAs are the closest contact between the cell and the ECM (Fig. 2). The formation of FAs is mediated by the integrin family of heterodimeric receptors. Integrins are composed of α and β subunits. There are 16 different α and 8 different β subunits that heterodimerize into at least 25 different receptors[16;17]. The heterodimers subsequently function as cellular receptors for ECM glycoproteins like fibronectin, laminin and collagen[17-19]. Integrins cluster upon interaction with their extracellular ligand providing binding places at their cytoplasmic tail for cytoskeletal, scaffolding and signaling molecules which leads to the

formation of FAs[17-19]. Signal transduction via FAs is implicated in many cellular processes like proliferation, migration, stress, survival and F-actin cytoskeleton organization[20;21]. The formation and remodeling of FAs is a dynamic process under regulation of protein tyrosine kinases. Recruitment of proteins like FAK and paxillin to the FAs precedes their tyrosine phosphorylation[22;23.] Thus, phosphorylation of these FA proteins and subsequent signaling happens primarily at these adhesion sites (Fig. 3)

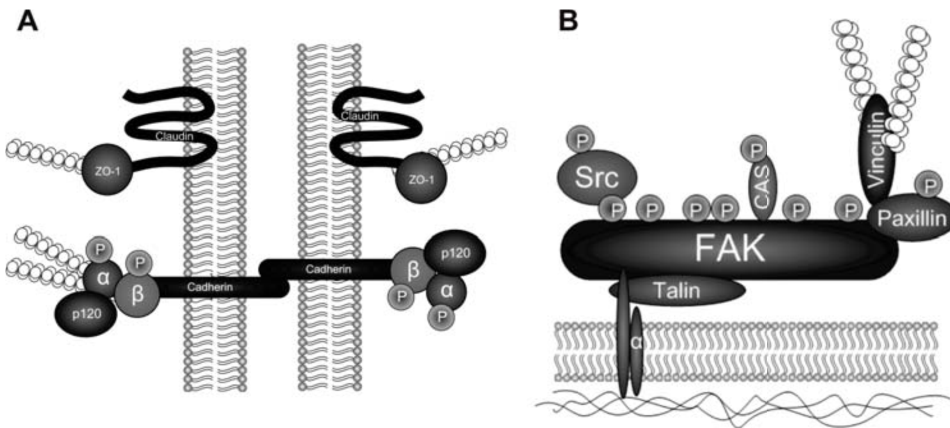

Figure 3. Cell-cell and cell-ECM adhesion complexes. The tight junctions consist of the transmembrane protein claudin and ZO-1 proteins that are connected to the F-actin cytoskeleton. The adherens junctions contain cadherins, which link two cells in a zipper-like way, and catenins that link the complex to the F-actin cytoskeleton (A). The cell-ECM interactions, FAs are formed by integrins and FA proteins like, FAK, Src and vinculin, talin, paxillin that can link the complex to the F-actin cytoskeleton (B).

Renal injury

Diseases of the kidney are among the most important causes of death and disability in many countries around the world. Most kidney diseases can be divided into two main categories: (1) ARF in which the kidney abruptly stops working entirely but may eventually recover normal function, and (2) chronic renal failure, in which progressive loss of function of nephrons gradually decreases kidney function. Chronic renal failure can easily occur when renal tissue is unable to completely recover after ARF[24-26].The causes of ARF can be divided into three main categories: pre-renal, intra-renal acute and post-renal ARF. Abnormalities that originate within the kidney and that abruptly diminish urine output fall into the category of intra-renal acute renal failure. This thesis will focus on intra-renal ARF, which has emerged as the most common and serious subtype in hospitalized patients. The most common causes of intra-renal ARF are I/R and poisons, toxins and medications that destroy the tubular epithelial cells [24-26].

ARF can lead to retention of extracellular fluid of water, waste products and electrolytes

in the blood. When the injury is severe excessive retention of potassium (hyperkalemia) can be fatal. Patients can develop metabolic acidosis because of the inability to excrete hydrogen ions, which is also lethal. In the most severe cases of ARF, complete anuria occurs. Incomplete restoration of kidney tissue and therefore renal function can lead to chronic acute renal failure and ultimately death[24-26].

I/R-induced renal injury and repair

During the initiating period of ischemia the most global effects of ATP depletion and failure of perfusion occur [2]. During the following postischemic phase, reperfusion allows both additional injury and recovery. After an ischemic insult total blood flow returns to normal conditions except in the outer medullary region, the so called outer stripe of the outer medulla (OSOM). This region has high energy demands and is marginally oxygenated under normal conditions, after ischemia the blood flow will be only 10% of normal flow[27]. The reduced blood flow and hypoxic conditions lead to ATP depletion and deprivation of vital nutrients in the nephron segments of the OSOM region. The S3 segment of the proximal tubule resides in this region and is extremely susceptible to I/R injury and ATP depletion because of their low glycolytic capacity to generate ATP under conditions of impaired oxidative phosphorylation[24;27;28].

Upon an I/R insult, depletion of ATP in renal cells activates many systems that directly cause disruption of the cytoskeleton and cell polarity and cell death or indirectly via activating other cellular systems such as endothelial cells and leukocytes resulting in general tubulointerstitial damage[29]. Hypoxia causes shut down of the Na^+, K^+-ATPase activity, subsequently the sodium concentration increases in the cells causing the Na^+,H^+ and Na^+/Ca^{2+} exchanger to pump out excessive sodium. Consequence is that intracellular pH decreases and intracellular calcium increases. Free intracellular calcium but also calcium-independent pathways can activate cysteine proteases like calpains and caspases, phospholipase A and nitric oxidase synthases that can break down the cytoskeleton or cytoskeleton binding proteins, damage cellular proteins and membranes[29;30]. Cell-cell and cell-ECM proteins are redistributed and many proteins are dephosphorylated[31;32]. Ischemia results in rapid loss of cytoskeletal integrity and cell polarity with mislocalization of adhesion molecules and other membrane molecules like Na^+, K^+-ATPase. The apical actin network is disrupted early after ischemia. The brush border of the proximal tubule cells disappears with shedding and internalization of the apical membrane proteins and blebbing of the apical membrane[33]. Mislocalization of adhesion molecules can lead to detachment of the tubular cells from the ECM and each other resulting in viable cells in the urine of patients and laboratory animals following ARF[34-36]. Denudation of the epithelial lining of the tubules can result in a decrease in glomerular filtrate

rate, a hallmark of ARF[37]. The overall processes of cellular injury, cell detachment and associated stress signaling lead to release of cytokines and other inflammatory mediators and recruitment of leukocytes and macrophages to the site of injury. This can contribute both to more cellular injury but also to the repair process[38].

Although both apoptosis and necrosis are prominent features of ischemic acute renal failure, much of the injury relevant to failure of organ function is sublethal. Recovery relies on a sequence of events that include epithelial cell spreading and possibly migration to cover exposed areas of the basement membrane, cell dedifferentiation and proliferation to restore cell number, followed by differentiation which results in restoration of the functional integrity of the kidney[30;39] (Fig. 4).

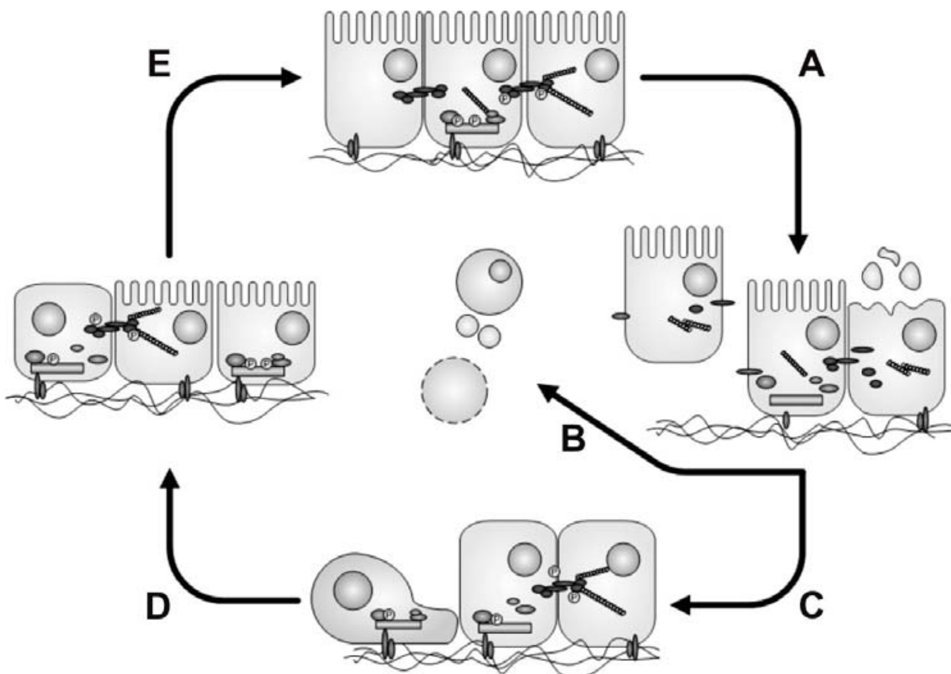

Figure 4. Working model of the proximal tubule during I/R-induced injury and repair. In the early phase of I/R the F-actin cytoskeleton is disrupted causing brush border loss. Furthermore signaling and adhesion proteins are dephosphorylated and often mislocalized which can lead to cell detachment (A). In addition these changes can lead to cell death by either necrosis or apoptosis (B). Remaining or sublethally injured cells recover during the late reperfusion phase. Proteins are rephosphorylated and dedifferentiation, migration and proliferation processes restore epithelial lining (C). Cells differentiate and restore their polarity (D) resulting in the restoration of the functionality of the proximal tubule (E).

Stress pathway activation during I/R

I/R injury or simulated I/R *in vitro* results in the activation of several cellular stress pathways, including mitogen-activated protein kinase (MAPK) and NFκB pathways and an inflammatory response that leads to the infiltration of immune cells in the damaged area[38].
The MAPK family kinases are serine-threonine protein kinases that mediate the response of cells to a wide variety of physiological and stress-related stimuli including I/R, reactive oxygen species and ATP depletion and convert the extracellular signal to intracellular signals that control the expression of genes. MAPKs have been implicated in post-I/R cell survival, necrosis and apoptosis[15;40]. Members of the MAPK family are p38, extracellular signal-regulated kinases (ERKs) and c-Jun NH2-terminal kinases (JNKs). MAPK kinases are known to localize at the FAs possibly implicating them in cell-ECM signaling during renal injury. JNK transduces signals to the nucleus in response to cellular stresses and is found markedly increased in activity in the proximal and tubular cells after I/R injury[41;42]. Inhibition of JNK activity during ischemia seems to ameliorate renal injury[43]. Another MAPK kinase activated after renal injury is p38. Activation of p38, just as the activation of JNK, may contribute to apoptosis by phosphorylation of various transcription factors or cellular proteins. Inhibition of p38 activity prevented apoptosis *in vitro* after simulated ischemia[44;45]. ERKs are activated downstream of tyrosine kinase receptors[46] and known to be activated after renal injury. The ERK pathway is known to play a role in cell survival signaling. Although there is some evidence suggesting that activation of ERK may contribute to injury and apoptotic cell death after renal injury[47], the precise role of ERK activation during I/R induced renal injury remains to be established.
The transcription factor NFκB can both induce and repress gene expression by binding to κB elements in the enhancers or promoters. NFκB exists in an inactive form in the cytoplasm due to binding to the inhibitory protein IκB4[8;49]. NFκB signaling can be activated by many stimuli including ischemia and reactive oxygen species produced during reperfusion[48;49]. Activation of NFκB typically involves phosphorylation of IκB by IκB kinase complex which results in IκB degradation. This releases NFκB and allows it to translocate to the nucleus. Genes regulated by NFκB include those controlling apoptosis, cell adhesion, proliferation, immune-response, inflammation, the cellular stress-response and tissue remodeling[48;49]. The outcome of NFκB activation depends on the nature and cellular context of its induction[48;49]. Inhibition of NFκB during I/R by NFκB decoy oligodeoxynucleotides is reported to be renoprotective[50]. During renal I/R NFκB activation and signaling is involved in the production of cytokines like TNFα and IL-1 causing upregulation of chemokines that are chemotactic and immunomodulatory to leukocytes. Furthermore NFκB can stimulate the expression of monocyte chemoattractant protein-1 and intercellular adhesion molecule-1 resulting in the infiltration of macrophages[45;51]. Importantly focal adhe-

sion kinase (FAK) is necessary for activation of NFκB in different cell types[52-54]. Whether FAK-mediated signaling is also involved in NFκB activation and immune response after renal I/R is yet unclear.

Cell-ECM signaling during renal injury

As described previously renal cell injury induced by I/R or chemical anoxia is associated with loss of cell-ECM interactions and reorganization of the F-actin cytoskeleton. These changes can eventually lead to detachment of viable and dead cells and loss of renal tubular function. However these changes also have an effect on the following recovery after the insult. In this section we discuss the most important findings that implicate cell-ECM interactions and cytoskeletal organization in I/R induced renal cell injury and recovery.
During ischemia *in vivo* or ATP depletion *in vitro* many cytoskeleton-associated components are either denatured or form large macromolecular aggregates[55;56]. In isolated proximal tubule cells, primary cultures of proximal tubule cells and cultures of epithelial cell lines, ATP depletion causes fragmentation of microvillar actin, dissociation of cortical actin and disassembly of basal stress fibers[33;57;58]. The basal F-actin network plays an integral part in cell-ECM adhesion and actin stress fibers are an important component of the basal F-actin network in renal tubular epithelial cells. Disruption of the basal actin network during ischemia or ATP depletion alters cell-ECM interaction[33;57;58]. The F-actin stress fibers are connected to FAs and are crucial for cell-ECM adhesion because the actin cytoskeleton stabilizes these adhesions by anchoring to the cytosolic domain of integrins. Using an *in vitro* oxidative stress model, Gailit *etal.* demonstrated as one of the first, redistribution of the integrin adhesion molecules[59]. They showed that the α3-integrin subunit relocalizes from the basolateral membrane to the apical membrane; providing evidence for FA disruption caused by renal oxidative stress *in vitro*[59]. Zuk *etal.* found a few years later using a unilateral I/R rat model that the ß1-integrin subunit translocates from the basolateral to the lateral membranes of tubules in the S3 segment of the proximal tubule, but not the apical membrane. During reperfusion more fibronectin was found which coincided with an increase in ß1 integrins predominantly located basally[60]. Preventing ß1-integrins from deactivation during I/R with HUTS-21 antibody, that recognizes an activation dependent epitope of ß1 integrins, preserves renal function[61]. These findings suggest a role for cell-ECM adhesions in both the initial injury phase (ischemia) and the following phase of reperfusion injury and recovery. However, although these studies show that the integrin mediated adhesion is perturbed during renal injury both *in vivo* and *in vitro*, only very little studies show how these pertubations may affect downstream FA formation and adhesion related signaling. The role of FAK, an important member and regulator of the FAs, is not well understood as well.

Focal Adhesion Kinase (FAK)

FAK is a non-receptor protein tyrosine kinase discovered in 1992 as a substrate of the Src family kinases[62;63]. It acts both as a scaffolding protein and a kinase at the FAs and is implicated in many signaling pathways involved in cell adhesion, cell spreading, proliferation, cytoskeleton organization and cellular stress.

FAK structure

FAK is comprised of several regions crucial for its function: the amino (N)-terminal domain that mediates interaction with membrane proteins, a centrally located kinase domain, three proline rich regions and a carboxyl (C)-terminal with the focal adhesion targeting (FAT) domain (Fig. 5).

The N-terminus is composed of an autophosphorylation site, tyrosine residue 397 (Y397), and a FERM domain named after its homology with band 4.1 and ezrin, radixin, moesin family proteins[64]. The FERM domain interacts with the ß-subunit integrins as well as some growth factor receptors (EGFR, PDGFR) [47;65]. The FERM domain also interacts with the kinase domain thereby inhibiting the function of the kinase domain[47;65;66]. Clustering of integrins enables binding of the FERM domain to the ß-integrin subunits. Subsequently

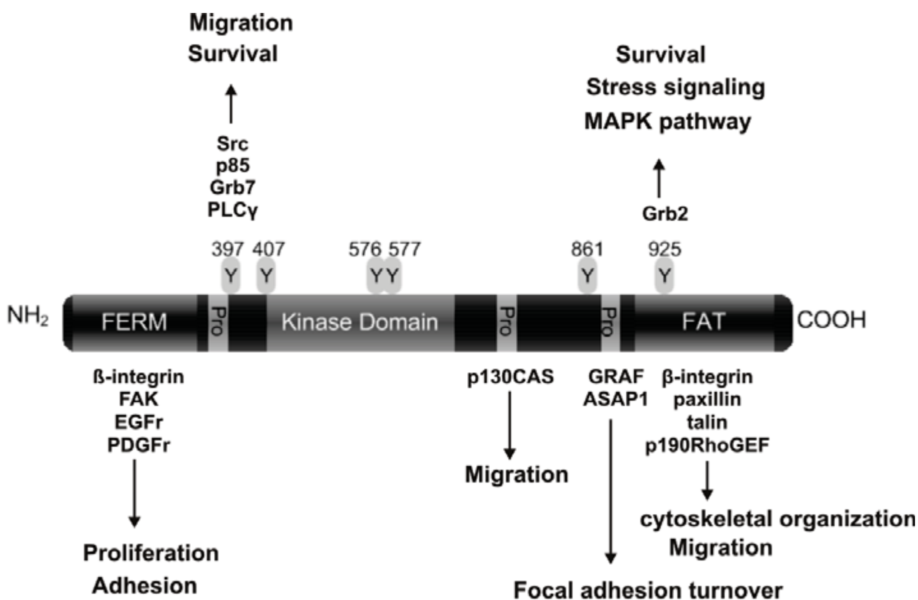

Figure 5. Structural features of FAK. FAK consists of a FERM domain at the N-terminus, a centrally located kinase domain, a FAT domain at the C-terminus and three proline rich regions. The FERM domain binds to integrins and growth factor receptors as well as its own kinase domain. The FAT domain is necessary for focal adhesion targeting directly or via binding partners paxillin and talin. The tyrosine phosphorylation sites provide binding sites as well. PY397 binds Src kinase but also Grb7 and p85 unit of PI3kinase and PY925 binds Grb2. PY407 is involved in negative regulation of FAK activity and PY576 and PY577 located in the kinase domain are necessary for full kinase activity.

FAK unfolds releasing the kinase domain allowing autophosphorylation and activation[67]. The C-terminus contains the FAT domain that is able to directly interact with the integrins but also provides binding sites for scaffolding and cytoskeleton binding proteins paxillin and talin that can recruit FAK to the integrins[68]. The FAT domain also interacts with p190RhoGEF, a Rho specific GDP/GTP exchange factor further involving FAK in cytoskeletal organization. Between the kinase domain and the FAT domain reside the proline rich domains that interact with SH3 domain containing proteins like p130CAS, GRAF (GTPase regulator) and ASAP1 (Arf-GAP). FAK localization and activition can be regulated by different mechanisms including dephosphorylation by phosphatases, inhibition by proteins like FIP200 but also interaction with other proteins and kinases like Src[69;70].

FAK activation, phosphorylation and regulation

Alluded to the above, clustering of integrins causes a conformational change in FAK that alters the interaction of the FERM domain with the kinase domain. FAK is autophosphorylated on tyrosine 397 providing a high affinity binding site for Src kinase[47;71]. Src is recruited to tyrosine phosphorylated residue 397 where it phosphorylates Y576/Y577 in the kinase domain thereby increasing kinase activity of FAK[72;73]. The FAK-Src signaling complex can phosphorylate paxillin bound to FAK as well as talin, and the CAS family proteins. Other tyrosine residues of FAK that are phosphorylated by Src are Y407, Y861 and Y925 and provide binding places for many SH2 domain containing proteins[74].

Tyrosine phosphorylation of FAK and the recruitment of other FA proteins to FAK results in the formation and turnover of the focal adhesions. The downstream signaling from FAK depends on its tyrosine phosphorylation status and the binding of specific proteins. PY397 binds besides Src several other signaling proteins including the p85 subunit of phosphoinositide 3 (PI3)-kinase, Grb7 and PLCγ[75-77]. PI3-kinase is activated after interaction with PY397, this leads to activation of the PKB/Akt survival pathway. Binding of Grb7 leads to migration signaling and is involved in adhesion. PY925 provides a binding place for Grb2 and mSOS (Ras GTP exchange factor) linking FAK to the Ras and MAPK pathway[69;78]. FAK phosphorylation at Y861 facilitates SH3 domain-mediated binding of p130Cas to the C-terminal proline-rich domains of FAK[79]. Tyrosine phosphorylated P130CAS, paxillin and FAK are involved in migration and spreading of cells. Phosphorylation of Y925 is associated with integrin adhesion and E-cadherin deregulation during Src-induced epithelial-mesenchymal transition[80] and with Erk activation[69;78]. Phosphorylation of Y925 also causes FAK loss from the FAs[81]. Phosphorylation of Y407 negatively regulates the enzymatic activity of FAK and supresses cell adhesion, spreading, proliferation and migration[82].

FAK activation and phosphorylation during acute renal failure

Phosphorylation of proteins on their tyrosine residues by protein tyrosine kinases plays an important role in the regulation of cellular signaling processes. Only a few studies describe renal injury-induced integrin redistribution in the context of altered phosphorylation of FA-associated proteins and altered downstream FA signaling. In previous studies by van de Water *etal*. It was shown that treatment of renal cells with nephrotoxicants like dichloro-vinylcysteine (DCVC) caused loss of cell-ECM contacts which was preceded by tyrosine dephosphorylation of FAK and dissociation of FAK from the FAs[83]. In addition, inactivation of FAK kinase activity using dominant negative acting deletion mutants delayed formation of FAs and potentiated the onset of apoptosis caused by DCVC[84]. Furthermore, *in vitro* studies by Weinberg *etal.* showed reversible dephosphorylation of known FA proteins like FAK and paxillin during hypoxia in freshly isolated rabbit proximal tubules[85]. Liu *etal*. report that cytoskeleton associated proteins vinculin, paxillin and talin decrease during chemical anoxia *in vitro*[86]. However none of these results are supported by *in vivo* data. Therefore the relevance of the data as well as the implications for the understanding of the role of cell-ECM adhesion during I/R remain elusive due to a lack of *in vivo* data. Many questions remain and should be studied to come to a full understanding of the molecular and cellular mechanisms of ARF and the role of cell-ECM adhesions in this process.

Conditional genetic mouse models for renal failure

Transgenic technologies in mice have become invaluable experimental tools to identify the *in vivo* function of proteins. However, the conventional knockout approach, gene disruption by homologous recombination in embryonic stem cells, produces animals in which all cells have been affected. This can result in embryonic lethality and because genes are generally expressed in more than one cell type the knockout phenotype can be to complex to use for functional studies of the protein of interest in a specific tissue or organ because its pathology can be confounded by other organs or neighboring cells.

To overcome these limitations conditional gene targeting techniques have been developed allowing gene inactivation under specific conditions. The Cre recombinase/loxP system is most widely used to obtain an inducible tissue specific knockout mouse model[87]. For this two mouse lines are necessary. One mouse line has the gene of interest flanked by two loxP sites. LoxP sites are short sequences of 34 bp containing two 13-bp inverted repeats flanking an 8-bp asymmetric spacer. The second mouse line expresses Cre recombinase driven by a core promoter of a tissue-specific gene. Cre recombinase can delete the gene of interest by recombination of the two LoxP sites, thereby excising (part of the) gene residing between the sites. Crossing these two lines generates offspring where

the gene of interest is removed only in the tissue where the promoter that drives Cre is active. An advantage of the Cre/loxP system is that it is possible to determine the moment of gene inactivation, making it possible to delay that moment to adulthood, by using an inducible variant of Cre recombinase. In the tamoxifen-inducible system developed by Feil *etal*. and Inra *etal*. the Cre gene is fused to a mutant form of the ligand binding domain of the estrogen receptor (ER)[88;89]. These mutant form is insensitive to the natural ligand (17ß-estradiol) at normal physiological conditions but responsive to the synthetic estrogen antagonist tamoxifen (TAM) or 4-hydroxy-tamoxifen (4-OHT)[88-91]. In the absence of TAM or 4-OHT the Cre-ER hybrid protein (Cre-ER^{T2}) is sequestered in the cytoplasm of the cell and Cre-mediated recombination is prevented because this happens in the nucleus. Binding of the estrogen antagonist permits Cre-ER^{T2} translocation to the nucleus allowing temporally controlled inactivation of the floxed target gene by excision.
To study the function of FAK in I/R-induced injury we used a proximal tubule specific inducible FAK knockout mouse model for several reasons. We chose an inducible FAK knockout model because, (1) homozygous FAK knockout mice die after gastrulation around embryonic day 8.5 (E8.5) with severe mesodermal effects[92;93]. (2) Previous studies show that tissue specific deletion of FAK in keratinocytes or neurons does not result in embryonic or perinatal lethality. This prompted us to use the Cre/LoxP system to knockout FAK in kidney tissue. Furthermore (3) we wanted to investigate the role of FAK during I/R-induced renal injury in adult mice. Therefore we preferred an inducible knockout system where we could knockout FAK just before subjecting the kidneys to injury to prevent or reduce the contribution of developmental defects or compensatory signaling pathways that may affect induced injury or recovery. A requirement for the knockout model is a cell specific recombination because the kidney consists of many different cell types and the primary site of injury is the PTCs.
For our *in vivo* models we used a proximal tubule specific inducible Cre-recombinase mouse line γGT-Cre-ER^{T2} (Dworniczak *etal.* 2007) which is crossed with mice where the second kinase domain exon of FAK is flanked by 2 loxP sites ($FAK^{loxP/loxP}$) (Beggs *etal.* 2003) to obtain an inducible proximal tubular cell specific fak knockout mouse ($FAK^{loxP/loxP}$//γGT-Cre-ER^{T2})[94;95]. Disruption of this exon following cre-mediated recombination results in premature translational stop codon resulting in ablation of FAK protein expression[94]. In the proximal tubule specific inducible Cre-recombinase mouse line transcription of the Cre-ER^{T2} gene is driven by the truncated promoter of the mouse γ-glutamyl transpeptidase type II (γGT) gene. Cre mediated recombination is inducible by TAM or 4-OHT and restricted to the renal cells of the S3 segments of the proximal tubules[95].
We also used the Cre/LoxP technique to conditionally knockout FAK *in vitro*. We isolated renal cells from $FAK^{loxP/loxP}$//Rosa-Cre-ER^{T2} mice. Because the Rosa-promoter is ubiqui-

tously active, addition of 4-OHT results in the recombination of the *fak* gene in all cells.
To study the role of FAK during I/R we used well established *in vivo* and *in vitro* approaches. *In vivo* we used a unilateral I/R method by subjecting rats or mice to renal artery clamping or renal pedicle clamping respectively, followed by reperfusion. *In vitro* we chose to subject mouse primary renal cells to chemical anoxia, using a glycolysis inhibitor, 2-deoxyglucose, and an inhibitor of the respiratory chain, antimycin A. This approach results in rapid but reversible ATP depletion.

Aim and outline of the thesis

The elucidation of the molecular and cellular mechanisms of ischemic ARF are very important in finding new strategies to reduce or prevent renal injury. Several studies show perturbations in cell-ECM adhesions by changes in integrin localization during I/R *in vivo*, however the potential presence of FAs and subsequent signaling remain not well studied especially under *in vivo* conditions. FAK is an important FA protein with a tyrosine kinase as well as a scaffolding function. Likewise it is involved in many downstream signaling pathways leading to very distinct biological processes. However these downstream pathways have not been well studied in context of renal failure. Therefore the general goal of the work presented in this thesis was to investigate ***the role of FAK and FA signaling during I/R.***
In ***chapter 2*** a unilateral renal I/R rat model was used to, firstly show the presence of tyrosine phosphorylated protein rich focal adhesions *in vivo*. Secondly to show that FAs and the F-actin network are disrupted directly after ischemia and rebuild during reperfusion. This process depends on tyrosine phosphorylation. FAK phosphorylation occured on different tyrosine residues during the reperfusion, implicating a role for FAK in activating different signaling pathways, during the course of the reperfusion process.
The MAPK kinase family member ERK is activated directly after ischemia. ERK is known to be involved in FA signaling but the role of ERK phosphorylation during I/R has not been investigated in light of FA disruption. Therefore in ***chapter 3*** the role of ERK signaling pathway during I/R induced renal injury *in vivo* was studied using a pharmacological inhibitor of MEK, U0126. Inhibition of ERK prevented the changes in FA protein phosphorylation after ischemia and diminished renal injury.
Establishing the presence of FAs in renal tubular cells and the altered FAK phosphorylation during I/R led to the question what role FAK plays during I/R. Therefore in ***chapter 4*** we used an inducible proximal tubule cell specific FAK knockout model to further investigate the role of FAK in I/R-induced injury and repair. We show that PTC specific FAK knockout mice are significantly less susceptible to unilateral I/R induced

injury compared to their wildtype littermates as is shown by histopathological markers and a well known biomarker of I/R induced renal injury, kidney injury molecule-1 (KIM-1). This coincides with less infiltration of immune cells into the injured tissue. Studies on how FAK is involved in the stress signaling during renal cell injury and recovery are limited. In ***chapter 5*** we studied FAK signaling under normal and simulated I/R *in vitro* in primary cultured mouse renal cells. Conditional knockout of FAK was used to study the role of FAK. Under normal circumstances FAK knockout renal cells show no major differences in morphology, proliferation and migration. However FAK knockout cells have increased FA size, aberrantly formed stress fibers and impaired spreading. During recovery from chemical-induced ATP depletion, FAK deleted cells show impaired recovery of the FAs and stress fibers. Concluding, in ***chapter 6***, the results of the studies described in this thesis and their implications for future research are discussed.

References

1. Hoste EA, Schurgers M: Epidemiology of acute kidney injury: how big is the problem? Crit Care Med 2008, 36:S146-S151.
2. Weinberg JM: The cell biology of ischemic renal injury. Kidney Int 1991, 39:476-500.
3. Marshall MR: Current status of dosing and quantification of acute renal replacement therapy. Part 2: dosing paradigms and clinical implementation. Nephrology (Carlton) 2006, 11:181-191.
4. Schiffl H: Dosing pattern of renal replacement therapy in acute renal failure: current status and future directions. Eur J Med Res 2006, 11:178-182.
5. Fish EM, Molitoris BA: Alterations in epithelial polarity and the pathogenesis of disease states. N Engl J Med 1994, 330:1580-1588.
6. Gonzalez-Mariscal L, Betanzos A, Nava P, Jaramillo BE: Tight junction proteins. Prog Biophys Mol Biol 2003, 81:1-44.
7. Turksen K, Troy TC: Barriers built on claudins. J Cell Sci 2004, 117:2435-2447.
8. Raschperger E, Thyberg J, Pettersson S, Philipson L, Fuxe J, Pettersson RF: The coxsa. Exp Cell Res 2006, 312:1566-1580.
9. Fanning AS, Mitic LL, Anderson JM: Transmembrane proteins in the tight junction barrier. J Am Soc Nephrol 1999, 10:1337-1345.
10. Kovacs EM, Yap AS: Cell-cell contact: cooperating clusters of actin and cadherin. Curr Biol 2008, 18:R667-R669.
11. Mah SP, Saueressig H, Goulding M, Kintner C, Dressler GR: Kidney development in cadherin-6 mutants: delayed mesenchyme-to-epithelial conversion and loss of nephrons. Dev Biol 2000, 223:38-53.
12. Rabb HA: Cell adhesion molecules and the kidney. Am J Kidney Dis 1994, 23:155-166.
13. Pappas DJ, Rimm DL: Direct interaction of the C-terminal domain of alpha-catenin and F-actin is necessary for stabilized cell-cell adhesion. Cell Commun Adhes 2006, 13:151-170.
14. Patel S, Mason RM, Suzuki J, Imaizumi A, Kamimura T, Zhang Z: Inhibitory effect of statins on renal epithelial-to-mesenchymal transition. Am J Nephrol 2006, 26:381-387.
15. Behrens J, Vakaet L, Friis R, Winterhager E, Van Roy F, Mareel MM, Birchmeier W: Loss of epithelial differentiation and gain of invasiveness correlates with tyrosine phosphorylation of the E-cadherin/beta-catenin complex in cells transformed with a temperature-sensitive v-SRC gene. J Cell Biol 1993, 120:757-766.
16. Hehlgans S, Haase M, Cordes N: Signalling via integrins: implications for cell survival and anticancer strategies. Biochim Biophys Acta 2007, 1775:163-180.

17. Reddig PJ, Juliano RL: Clinging to life: cell to matrix adhesion and cell survival. Cancer Metastasis Rev 2005, 24:425-439.
18. Kagami S, Kondo S, Urushihara M, Loster K, Reutter W, Saijo T, Kitamura A, Kobayashi S, Kuroda Y: Overexpression of alpha1beta1 integrin directly affects rat mesangial cell behavior. Kidney Int 2000, 58:1088-1097.
19. Sepulveda JL, Gkretsi V, Wu C: Assembly and signaling of adhesion complexes. Curr Top Dev Biol 2005, 68:183-225.
20. Burridge K, Chrzanowska-Wodnicka M, Zhong C: Focal adhesion assembly. Trends Cell Biol 1997, 7:342-347.
21. Giancotti FG, Ruoslahti E: Integrin signaling. Science 1999, 285:1028-1032.
22. Horwitz AR, Parsons JT: Cell migration--movin' on. Science 1999, 286:1102-1103.
23. Kirchner J, Kam Z, Tzur G, Bershadsky AD, Geiger B: Live-cell monitoring of tyrosine phosphorylation in focal adhesions following microtubule disruption. J Cell Sci 2003, 116:975-986.
24. Bonventre JV: Mechanisms of ischemic acute renal failure. Kidney Int 1993, 43:1160-1178.
25. Sheridan AM, Bonventre JV: Pathophysiology of ischemic acute renal failure. Contrib Nephrol 2001,7-21.
26. Thadhani R, Pascual M, Bonventre JV: Acute renal failure. N Engl J Med 1996, 334:1448-1460.
27. Vetterlein F, Petho A, Schmidt G: Distribution of capillary blood flow in rat kidney during postischemic renal failure. Am J Physiol 1986, 251:H510-H519.
28. Bonventre JV, Weinberg JM: Recent advances in the pathophysiology of ischemic acute renal failure. J Am Soc Nephrol 2003, 14:2199-2210.
29. Padanilam BJ: Cell death induced by acute renal injury: a perspective on the contributions of apoptosis and necrosis. Am J Physiol Renal Physiol 2003, 284:F608-F627.
30. Devarajan P: Cellular and molecular derangements in acute tubular necrosis. Curr Opin Pediatr 2005, 17:193-199.
31. Goligorsky MS, Lieberthal W, Racusen L, Simon EE: Integrin receptors in renal tubular epithelium: new insights into pathophysiology of acute renal failure. Am J Physiol 1993, 264:F1-F8.
32. Weinberg JM, Venkatachalam MA, Roeser NF, Senter RA, Nissim I: Energetic determinants of tyrosine phosphorylation of focal adhesion proteins during hypoxia/reoxygenation of kidney proximal tubules. Am J Pathol 2001, 158:2153-2164.
33. Molitoris BA: Actin cytoskeleton in ischemic acute renal failure. Kidney Int 2004, 66:871-883.
34. Racusen LC, Fivush BA, Li YL, Slatnik I, Solez K: Dissociation of tubular cell

detachment and tubular cell death in clinical and experimental "acute tubular necrosis". Lab Invest 1991, 64:546-556.

35. Racusen LC, Solez K: Ideas in pathology. Exfoliation of renal tubular cells. Mod Pathol 1991, 4:368-370.
36. Racusen LC: Tubular injury in human kidneys: pathologic findings and pathogenic mechanisms. Clin Investig 1993, 71:858-860.
37. Kwon O, Nelson WJ, Sibley R, Huie P, Scandling JD, Dafoe D, Alfrey E, Myers BD: Backleak, tight junctions, and. J Clin Invest 1998, 101:2054-2064.
38. Bonventre JV, Zuk A: Ischemic acute renal failure: an inflammatory disease? Kidney Int 2004, 66:480-485.
39. Bonventre JV: Dedifferentiation and proliferation of surviving epithelial cells in acute renal failure. J Am Soc Nephrol 2003, 14 Suppl 1:S55-S61.
40. Pombo CM, Bonventre JV, Avruch J, Woodgett JR, Kyriakis JM, Force T: The stress-activated protein kinases are major c-Jun amino-terminal kinases activated by ischemia and reperfusion. J Biol Chem 1994, 269:26546-26551.
41. Kunduzova OR, Bianchi P, Pizzinat N, Escourrou G, Seguelas MH, Parini A, Cambon C: Regulation of JNK/ERK activation, cell apoptosis, and tissue regeneration by monoamine oxidases after renal ischemia-reperfusion. FASEB J 2002, 16:1129-1131.
42. Park KM, Chen A, Bonventre JV: Prevention of kidney ischemia/reperfusion-induced functional injury and JNK, p38, and MAPK kinase activation by remote ischemic pretreatment. J Biol Chem 2001, 276:11870-11876.
43. DiMari J, Megyesi J, Udvarhelyi N, Price P, Davis R, Safirstein R: N-acetyl cysteine ameliorates ischemic renal failure. Am J Physiol 1997, 272:F292-F298.
44. Meldrum KK, Meldrum DR, Hile KL, Yerkes EB, Ayala A, Cain MP, Rink RC, Casale AJ, Kaefer MA: p38 MAPK mediates renal tubular cell TNF-alpha production and TNF-alpha-dependent apoptosis during simulated ischemia. Am J Physiol Cell Physiol 2001, 281:C563-C570.
45. Meldrum KK, Hile K, Meldrum DR, Crone JA, Gearhart JP, Burnett AL: Simulated ischemia induces renal tubular cell apoptosis through a nuclear factor-kappaB dependent mechanism. J Urol 2002, 168:248-252.
46. Pearson MA, O'Farrell AM, Dexter TM, Whetton AD, Owen-Lynch PJ, Heyworth CM: Investigation of the molecular mechanisms underlying growth factor synergy: the role of ERK 2 activation in synergy. Growth Factors 1998, 15:293-306.
47. Schaller MD, Hildebrand JD, Shannon JD, Fox JW, Vines RR, Parsons JT: Autophosphorylation of the focal adhesion kinase, pp125FAK, directs SH2-dependent binding of pp60src. Mol Cell Biol 1994, 14:1680-1688.

48. Hayden MS, Ghosh S: Signaling to NF-kappaB. Genes Dev 2004, 18:2195-2224.
49. Hayden MS, Ghosh S: Shared principles in NF-kappaB signaling. Cell 2008, 132:344-362.
50. Cao CC, Ding XQ, Ou ZL, Liu CF, Li P, Wang L, Zhu CF: *In vivo* transfection of NF-kappaB decoy oligodeoxynucleotides attenuate renal ischemia/reperfusion injury in rats. Kidney Int 2004, 65:834-845.
51. Guijarro C, Egido J: Transcription factor-kappa B (NF-kappa B) and renal disease. Kidney Int 2001, 59:415-424.
52. Funakoshi-Tago M, Sonoda Y, Tanaka S, Hashimoto K, Tago K, Tominaga S, Kasahara T: Tumor necrosis factor-induced nuclear factor kappaB activation is impaired in focal adhesion kinase-deficient fibroblasts. J Biol Chem 2003, 278:29359-29365.
53. Huang D, Khoe M, Befekadu M, Chung S, Takata Y, Ilic D, Bryer-Ash M: Focal adhesion kinase mediates cell survival via NF-kappaB and ERK signaling pathways. Am J Physiol Cell Physiol 2007, 292:C1339-C1352.
54. Zhang HM, Keledjian KM, Rao JN, Zou T, Liu L, Marasa BS, Wang SR, Ru L, Strauch ED, Wang JY: Induced focal adhesion kinase expression suppresses apoptosis by activating NF-kappaB signaling in intestinal epithelial cells. Am J Physiol Cell Physiol 2006, 290:C1310-C1320.
55. Sogabe K, Roeser NF, Davis JA, Nurko S, Venkatachalam MA, Weinberg JM: Calcium dependence of integrity of the actin cytoskeleton of proximal tubule cell microvilli. Am J Physiol 1996, 271:F292-F303.
56. Shelden EA, Weinberg JM, Sorenson DR, Edwards CA, Pollock FM: Site-specific alteration of actin assembly visualized in living renal epithelial cells during ATP depletion. J Am Soc Nephrol 2002, 13:2667-2680.
57. Lieberthal W, Levine JS: Mechanisms of apoptosis and its potential role in renal tubular epithelial cell injury. Am J Physiol 1996, 271:F477-F488.
58. Lieberthal W, Koh JS, Levine JS: Necrosis and apoptosis in acute renal failure. Semin Nephrol 1998, 18:505-518.
59. Gailit J, Colflesh D, Rabiner I, Simone J, Goligorsky MS: Redistribution and dysfunction of integrins in cultured renal epithelial cells exposed to oxidative stress. Am J Physiol 1993, 264:F149-F157.
60. Zuk A, Bonventre JV, Brown D, Matlin KS: Polarity, integrin, and extracellular matrix dynamics in the postischemic rat kidney. Am J Physiol 1998, 275:C711-C731.
61. Molina A, Ubeda M, Escribese MM, Garcia-Bermejo L, Sancho D, de Lema GP, Liano F, Cabanas C, Sanchez-Madrid F, Mampaso F: Renal ischemia/reperfusion injury: functional tissue preservation by anti-activated {beta}1 integrin therapy. J Am Soc Nephrol 2005, 16:374-382.

62. Hanks SK, Calalb MB, Harper MC, Patel SK: Focal adhesion protein-tyrosine kinase phosphorylated in response to cell attachment to fibronectin. Proc Natl Acad Sci U S A 1992, 89:8487-8491.
63. Schaller MD, Borgman CA, Cobb BS, Vines RR, Reynolds AB, Parsons JT: pp125-FAK a structurally distinctive protein-tyrosine kinase associated with focal adhesions. Proc Natl Acad Sci U S A 1992, 89:5192-5196.
64. Girault JA, Labesse G, Mornon JP, Callebaut I: The N-termini of FAK and JAKs contain divergent band 4.1 domains. Trends Biochem Sci 1999, 24:54-57.
65. Dunty JM, Gabarra-Niecko V, King ML, Ceccarelli DF, Eck MJ, Schaller MD: FERM domain interaction promotes FAK signaling. Mol Cell Biol 2004, 24:5353-5368.
66. Cooper LA, Shen TL, Guan JL: Regulation of focal adhesion kinase by its amino-terminal domain through an autoinhibitory interaction. Mol Cell Biol 2003, 23:8030-8041.
67. Calalb MB, Polte TR, Hanks SK: Tyrosine phosphorylation of focal adhesion kinase at sites in the catalytic domain regulates kinase activity: a role for Src family kinases. Mol Cell Biol 1995, 15:954-963.
68. Martin KH, Boerner SA, Parsons JT: Regulation of focal adhesion targeting and inhibitory functions of the FAK related protein FRNK using a novel estrogen receptor "switch". Cell Motil Cytoskeleton 2002, 51:76-88.
69. Schlaepfer DD, Hanks SK, Hunter T, van der GP: Integrin-mediated signal transduction linked to Ras pathway by GRB2 binding to focal adhesion kinase. Nature 1994, 372:786-791.
70. Schlaepfer DD, Hauck CR, Sieg DJ: Signaling through focal adhesion kinase. Prog Biophys Mol Biol 1999, 71:435-478.
71. Xing Z, Chen HC, Nowlen JK, Taylor SJ, Shalloway D, Guan JL: Direct interaction of v-Src with the focal adhesion kinase mediated by the Src SH2 domain. Mol Biol Cell 1994, 5:413-421.
72. Hanks SK, Ryzhova L, Shin NY, Brabek J: Focal adhesion kinase signaling activities and their implications in the control of cell survival and motility. Front Biosci 2003, 8:d982-d996.
73. Owen JD, Ruest PJ, Fry DW, Hanks SK: Induced focal adhesion kinase (FAK) expression in FAK-null cells enhances cell spreading and migration requiring both. Mol Cell Biol 1999, 19:4806-4818.
74. Schaller MD, Hildebrand JD, Parsons JT: Complex formation with focal adhesion kinase: A mechanism to regulate activity and subcellular localization of Src kinases. Mol Biol Cell 1999, 10:3489-3505.
75. Akagi T, Murata K, Shishido T, Hanafusa H: v-Crk activates the phosphoinositide

3-kinase/AKT pathway by utilizing focal adhesion kinase and H-Ras. Mol Cell Biol 2002, 22:7015-7023.

76. Chen HC, Guan JL: Association of focal adhesion kinase with its potential substrate phosphatidylinositol 3-kinase. Proc Natl Acad Sci U S A 1994, 91:10148-10152.
77. Chen HC, Appeddu PA, Isoda H, Guan JL: Phosphorylation of tyrosine 397 in focal adhesion kinase is required for binding phosphatidylinositol 3-kinase. J Biol Chem 1996, 271:26329-26334.
78. Schlaepfer DD, Hunter T: Evidence for *in vivo* phosphorylation of the Grb2 SH2-domain binding site on focal adhesion kinase by Src-family protein-tyrosine kinases. Mol Cell Biol 1996, 16:5623-5633.
79. Lim Y, Han I, Jeon J, Park H, Bahk YY, Oh ES: Phosphorylation of focal adhesion kinase at tyrosine 861 is crucial for Ras transformation of fibroblasts. J Biol Chem 2004, 279:29060-29065.
80. Brunton VG, Avizienyte E, Fincham VJ, Serrels B, Metcalf CA, III, Sawyer TK, Frame MC: Identification of Src-specific phosphorylation site on focal adhesion kinase: dissection of the role of Src SH2 and catalytic functions and their consequences for tumor cell behavior. Cancer Res 2005, 65:1335-1342.
81. Katz BZ, Miyamoto S, Teramoto H, Zohar M, Krylov D, Vinson C, Gutkind JS, Yamada KM: Direct transmembrane clustering and cytoplasmic dimerization of focal adhesion kinase initiates its tyrosine phosphorylation. Biochim Biophys Acta 2002, 1592:141-152.
82. Lim Y, Park H, Jeon J, Han I, Kim J, Jho EH, Oh ES: Focal adhesion kinase is negatively regulated by phosphorylation at tyrosine 407. J Biol Chem 2007, 282:10398-10404.
83. van de WB, Nagelkerke JF, Stevens JL: Dephosphorylation of focal adhesion kinase (FAK) and loss of focal contacts precede caspase-mediated cleavage of FAK during apoptosis in renal epithelial cells. J Biol Chem 1999, 274:13328-13337.
84. van de WB, Houtepen F, Huigsloot M, Tijdens IB: Suppression of chemically induced apoptosis but not necrosis of renal proximal tubular epithelial (LLC-PK1) cells by focal adhesion kinase (FAK). Role of FAK in maintaining focal adhesion organization after acute renal cell injury. J Biol Chem 2001, 276:36183-36193.
85. Weinberg JM, Venkatachalam MA, Roeser NF, Senter RA, Nissim I: Energetic determinants of tyrosine phosphorylation of focal adhesion proteins during hypoxia/reoxygenation of kidney proximal tubules. Am J Pathol 2001, 158:2153-2164.
86. Liu X, Schnellmann RG: Calpain mediates progressive plasma membrane permeability and proteolysis of cytoskeleton-associated paxillin, talin, and vinculin during renal cell death. J Pharmacol Exp Ther 2003, 304:63-70.

87. Branda CS, Dymecki SM: Talking about a revolution: The impact of site-specific recombinases on genetic analyses in mice. Dev Cell 2004, 6:7-28.
88. Feil R, Brocard J, Mascrez B, LeMeur M, Metzger D, Chambon P: Ligand-activated site-specific recombination in mice. Proc Natl Acad Sci U S A 1996, 93:10887-10890.
89. Indra AK, Warot X, Brocard J, Bornert JM, Xiao JH, Chambon P, Metzger D: Temporally-controlled site-specific mutagenesis in the basal layer of the epidermis: comparison of the recombinase activity of the tamoxifen-inducible Cre-ER(T) and Cre-ER(^{T2}) recombinases. Nucleic Acids Res 1999, 27:4324-4327.
90. Danielian PS, White R, Hoare SA, Fawell SE, Parker MG: Identification of residues in the estrogen receptor that confer differential sensitivity to estrogen and hydroxytamoxifen. Mol Endocrinol 1993, 7:232-240.
91. Littlewood TD, Hancock DC, Danielian PS, Parker MG, Evan GI: A modified oestrogen receptor ligand-binding domain as an improved switch for the regulation of heterologous proteins. Nucleic Acids Res 1995, 23:1686-1690.
92. Furuta Y, Ilic D, Kanazawa S, Takeda N, Yamamoto T, Aizawa S: Mesodermal defect in late phase of gastrulation by a targeted mutation of focal adhesion kinase, FAK. Oncogene 1995, 11:1989-1995.
93. Ilic D, Furuta Y, Kanazawa S, Takeda N, Sobue K, Nakatsuji N, Nomura S, Fujimoto J, Okada M, Yamamoto T: Reduced cell motility and enhanced focal adhesion contact formation in cells from FAK-deficient mice. Nature 1995, 377:539-544.
94. Beggs HE, Schahin-Reed D, Zang K, Goebbels S, Nave KA, Gorski J, Jones KR, Sretavan D, Reichardt LF: FAK deficiency in cells contributing to the basal lamina results in cortical abnormalities resembling congenital muscular dystrophies. Neuron 2003, 40:501-514.
95. Dworniczak B, Skryabin B, Tchinda J, Heuck S, Seesing FJ, Metzger D, Chambon P, Horst J, Pennekamp P: Inducible Cre/loxP recombination in the mouse proximal tubule. Nephron Exp Nephrol 2007, 106:e11-e20.