Integrins in Kidney Disease

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ABSTRACT

A major hallmark of chronic kidney injury is fibrosis, which is characterized by increased accumulation of extracellular matrix components that replace the damaged tissue. Normally, the synthesis and degradation of extracellular matrix components are finely regulated; however, when matrix replacement goes unchecked, there is unwanted and irreversible tissue scarring with consequent organ damage, organ failure, and, in certain cases, death. Many factors, including cell-matrix interactions, play a role in the development of renal fibrosis. Cell-matrix interactions are made possible by integrins, a family of transmembrane receptors that, upon binding to the extracellular matrix, activate intracellular signaling. Thus, they control various cell functions, including survival, proliferation, migration, and matrix homeostasis. Genetic mutations in humans and the development of animal models lacking integrins in selective parts of the kidney have improved our understanding of molecular mechanisms and pathways controlling matrix remodeling in kidney disease. Here we outline the major integrins involved in kidney disease and some of the major molecular mechanisms whereby integrins contribute to kidney fibrosis.


The kidneys are essential regulatory organs that play a critical role in the excretion of waste, fluids, electrolyte and acid-base homeostasis, BP control, and the production of various hormones. The adult human kidney consists of approximately 1 million filtering nephrons and a collecting system. Each nephron consists of a glomerulus, followed by a tubule that is divided into distinct segments. Nephrons are derived from the metanephric mesenchyme, and the collecting system derives from the ureteric bud. The glomerulus consists of a capillary network maintained in an open three-dimensional space by the mesangium and a glomerular filtration barrier composed of an inner fenestrated endothelial cell layer; an outer layer of visceral epithelial cells (podocytes); and the intervening glomerular basement membrane (GBM), composed of extracellular matrix (ECM) components. The Bowman capsule, which is also the beginning of the proximal tubule, surrounds the glomerulus. Cells that are both anatomically and functionally distinct form different segments of the renal tubule, from the proximal tubule to the distal collecting duct.

As with all organs, the interaction between cells and the surrounding ECM is required for normal kidney development and function. The principal cellular receptors that mediate cell-ECM interactions are integrins, heterodimeric transmembrane glycoproteins that consist of non-covalently associated α and β subunits. There are 18α and 8β subunits in mammals, which form 24 unique heterodimers with distinct specificities for different ECM components. Integrins are classified as collagen, laminin, and arginine-glycine-aspartic acid (RGD)–binding receptors. Each integrin subunit has a large extracellular domain that constitutes the ligand-binding domain, a single transmembrane domain, and a short cytoplasmic tail. A principal function of integrins is to anchor cells to the ECM, but they also signal bi-directionally across the plasma membrane. Thus, they control various cell functions, including cell proliferation, survival and migration, differentiation, and matrix homeostasis. Because the cytoplasmic tails of integrins lack enzymatic and actin-binding activity, they need to bind adaptor proteins for intracellular signal propagation.

Integrins play a critical role in kidney development, homeostasis, and renal disease. Because the role of integrins in kidney development was recently reviewed, we focus on their role in kidney disease. In particular, we highlight the contribution of various integrins in kidney disease based on their ligand specificity.

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THE LAMININ RECEPTORS

The principal laminin–binding receptors found in the kidney are integrins α3β1, α6β1, and α6β4. Integrin α3β1 is the most highly expressed integrin in the kidney and is found in both the glomerulus and the tubules. A definitive role for this integrin in normal kidney function comes from the finding that global integrin α3–null mice have abnormalities in both the collecting system and the glomerulus.3 Subsequent selective deletion of the integrin α3 subunit in the podocytes results in a severe developmental phenotype characterized by foot process effacement and severe proteinuria.4 Interestingly, a A349S missense mutation results in no surface expression of integrin α3 in podocytes and is related to podocyte loss. Mechanistically, CD151 localizes to the podocyte-GBM interface, where it interacts with integrin α3β1. CD151 shifts integrin α3β1 from focal adhesions into tetraspanin webs, allowing for tight adhesion of podocytes to the GBM. In the absence of CD151, this shift does not occur, resulting in a weak adhesion of podocytes to the GBM and consequent podocyte detachment and loss. Thus, it is likely that mutations in the integrin α3 subunit or CD151 result in glomerulosclerosis in humans, primarily by leading to increased podocyte loss because integrin α3β1 is the principal integrin on the podocyte and CD151 plays a critical role in facilitating firm adhesion of podocytes to the GBM (Figure 1).

Because integrin α3β1 is a major laminin receptor, it is not surprising that patients carrying integrin α3 subunit mutations have many features of patients with Pierson syndrome. This autosomal recessive disease is caused by mutations in LAMB2, which encodes the laminin β2 chain, the principal laminin subunit in the GBM.13–15 Patients with Pierson syndrome and mice lacking laminin β2 present with glomerular injury characterized by diffuse mesangial sclerosis and nephrotic syndrome. Taken together, the diseases associated with mutations in integrin α3β1, CD151, and the laminin β2 suggest a key role for laminins and their principal receptors in normal glomerular function.

Laminins and their receptors are also highly expressed in the kidney tubules; however, their role in tubulointerstitial disease is poorly studied. On the basis of the observation that patients with integrin α3 mutations have generalized renal fibrosis, including the tubulointerstitial fibrosis, this receptor probably plays a critical role in regulating tubulointerstitial fibrosis as well. This possibility can be investigated by selectively deleting the integrin α3 subunit in specific nephron segments. It is also highly likely that α6-containing integrins are associated with fibrotic kidney disease because integrin α6β4 plays a key role in regulating tight adhesion of cells to basement membranes. In this regard, a patient presenting with FSGS and a mutation in the integrin β4 subunit has been described.18

Although mutations in the integrin α3 subunit that cause human kidney disease were described only recently, patients with nonsense mutations in the tetraspanin protein CD151 (which binds to integrin α3β1 and regulates its function) were previously found to have glomerulosclerosis.11 The mechanisms for these glomerular abnormalities are well defined using laminin-binding integrin and CD151-null mice.4,12 CD151-null mice present with FSGS, similar to that observed in humans carrying nonsense mutations in CD151. The degree of glomerulosclerosis in these mice is similar to that of mice lacking the integrin α3 subunit in podocytes and is related to podocyte loss. Mechanistically, CD151 localizes to the podocyte-GBM interface, where it interacts with integrin α3β1. CD151 shifts integrin α3β1 from focal adhesions into tetraspanin webs, allowing for tight adhesion of podocytes to the GBM. In the absence of CD151, this shift does not occur, resulting in a weak adhesion of podocytes to the GBM and consequent podocyte detachment and loss. Thus, it is likely that mutations in the integrin α3 subunit or CD151 result in glomerulosclerosis in humans, primarily by leading to increased podocyte loss because integrin α3β1 is the principal integrin on the podocyte and CD151 plays a critical role in facilitating firm adhesion of podocytes to the GBM (Figure 1).

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Studies in mice null for the integrin α6

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Figure 1. Integrin α3β1 is required for podocyte adhesion to the glomerular basement membrane. (A) CD151/integrin α3β1 interactions play an important role in determining strong adhesion of podocytes to the GBM, thus protecting the glomerulus from stress-mediated injury. (B) Loss of CD151 or loss and/or mutations of the integrin α3 subunit leads to podocyte detachment and loss and consequent glomerular damage.
and/or β4 subunit in the kidney need to be conducted to define the contribution of integrins α6β1 and α6β4 in kidney repair after injury.

THE COLLAGEN RECEPTORS

The two major collagen receptors, integrins α1β1 and α2β1, are widely expressed in the kidney as well as in leukocytes, which play a role in inflammatory forms of renal injury. Integrin α1β1 primarily binds collagen type IV, a major constituent of basement membranes, whereas integrin α2β1 binds collagen type I, whose expression is upregulated in renal disease. Neither of these integrins affects renal development because integrin α1- and α2-null mice do not show any obvious kidney phenotypes. No human diseases are directly attributed to mutations in these integrins; however, a large body of evidence suggests that their expression is altered in the course of human kidney disease and that they modulate renal fibrosis in rodents.

The first evidence that collagen-binding receptors regulate renal fibrosis comes from studies demonstrating that anti-integrin α1 antibodies reduce scarring in rat models of glomerular injury by inhibiting leukocyte function and the immune response. It was subsequently shown that integrin α1-null mice have decreased glomerular fibrosis in a mouse model of Alport syndrome. In contrast to these studies, there is overwhelming evidence that integrin α1–null mice have an exacerbation of glomerulosclerosis, suggesting that maneuvers to increase ligand/integrin binding that promote integrin α1β1 activation are beneficial in the setting of renal injury.

In contrast to integrin α1β1, integrin α2β1 is a positive regulator of collagen synthesis and reactive oxygen species production. This is consistent with recent evidence that genetic deletion of the integrin α2 subunit or selective chemical antagonism of integrin α2β1 protects mice from the development of glomerular fibrosis after adriamycin administration or partial renal ablation. A possible mechanism whereby integrin α2β1 promotes collagen synthesis and subsequent glomerular injury is by activation of Stat3 in mesangial cells. Another novel mechanism whereby integrin α2β1 has been shown to promote ERK-mediated collagen synthesis is its ability to act as a receptor for IgA1 in mesangial cells, suggesting that various pathways control integrin α2β1–mediated collagen production. Although these studies propose that integrin α2β1 induces glomerular fibrosis, integrin α2–null mice seem to develop mild proteinuria and glomerular damage at 6 months of age because of increased expression of the profibrotic TGF-β and connective tissue growth factor. This effect appears to be strain specific because mild glomerular disease is observed only in 6-month-old C57/Black6 but not BALB/C or 129Sv integrin α2–null mice (Pozzi, unpublished observation). Despite these contradictory results, it appears that targeting integrin α2β1 with specific small molecule inhibitors might be beneficial for the treatment of renal fibrosis. To determine whether and at what stage of renal injury this receptor should be targeted, it is important to define how integrin α2β1 controls collagen synthesis. In this regard, integrin α1β1 negatively regulates integrin α2β1–mediated signaling, suggesting that concomitant activation of integrin α1β1 and inhibition of integrin α2β1 might be the optimal manner in which to modulate signaling by these very important regulators of collagen synthesis (Figure 2).

THE RGD-BINDING INTEGRINS

Two β1 integrins (α5β1, α8β1), five αV integrins (αvβ1, αvβ3, αvβ5, αvβ6, and αvβ8), and αIIbβ3 (found only on platelets) bind to ligands containing an RGD-active site primarily found in fibronectin and vitronectin. The fibronectin receptor integrin α5β1 does not play a major role in kidney disease; however, to the best of our knowledge, mice selectively lacking the integrin α5 subunit in the kidneys have not been generated. By contrast, constitutive deletion of the integrin α8 subunit leads to renal agenesis or dysgenesis due to loss of binding to nephrinectin, a major integrin α8β1 ligand (reviewed in detail by Mathew et al.). However, when crossed onto certain backgrounds, integrin α8–null mice develop kidneys, thus allowing us to determine the role of integrin α8β1 in renal disease. In this context, integrin α8–null mice present with abnormalities of the mesangium characterized by hypercellularity and altered glomerular capillaries, most likely due to impaired ability to protect the glomerulus from mechanical stress, including hypertension. In addition, there is evidence that a polymorphism within the promoter of the integrin α8 subunit regulates the progression of polycystic kidney disease. Altogether these findings suggest that mutations or loss of function of the α8 subunit could
render humans more susceptible to stress-induced glomerular damage or polycystic kidney disease.

The αv subunit is ubiquitously expressed in the adult kidney; however, the αvβ heterodimers present in the different nephron segments are unclear. There are two distinct forms of αv integrins: those that primarily interact with the RGD present in fibronectin and vitronectin (αvβ1, αvβ3, and αvβ5) and those that bind to the RGD motif of the TGF-β-binding latency-associated peptide (αvβ6 and αvβ8). Although the integrin αv subunit has never been selectively deleted in any cell types within the kidney, some indirect evidence suggests that integrin αvβ3 plays a role in regulating the glomerular filtration barrier (reviewed in detail by Reiser et al.⁴⁰,⁴¹). In this context, activation of integrin αvβ3 by the urokinase plasminogen receptor (uPAR) promotes podocyte injury in a lipopolysaccharide-mediated renal injury model in mice.⁴² More recently, research showed that a soluble form of uPAR, namely suPAR, interacts with and activates integrin αvβ3 in podocytes, leading to FSGS in humans.⁴³ Although these studies suggest a highly novel mechanism whereby integrin αvβ3 might contribute to FSGS, the conclusion that activation of this integrin in podocytes is deleterious was primarily made by using conformational antibodies able to detect activated integrin αvβ3 and by blocking integrin function with blocking antibodies or cyclic RGD. Thus, to conclusively determine the role of this integrin in podocyte homeostasis, it is important to define how the glomerulus responds to injury in the absence of integrin αvβ3. It is also critical to clarify how this integrin alters the response of the podocytes to the signals from uPAR. Although activation of small GTPases is a plausible explanation,⁴² the mechanism whereby this occurs has not been defined. The picture is also complicated by the fact that integrin αvβ3 function or affinity can be modulated by multiple factors, including integrin α3β1, which is the principal receptor whereby podocytes interact with the GBM.⁴⁴ Thus, more mechanistic studies are required to investigate how integrin αvβ3 regulates the glomerular filtration barrier and whether it does in fact play a direct role in glomerular diseases in humans. Some of the factors involved in activation and inhibition of integrin αvβ3 in podocytes are illustrated in Figure 3A.

Integrins αvβ6 and αvβ8 regulate the levels of free and active TGF-β in tissues by binding to the LAP/TGF-β complex. Although neither of these integrins has been shown to play a role in human disease, they alter kidney function in rodents. Integrin αvβ6 contributes to unilateral ureter obstruction–mediated renal fibrosis by facilitating TGF-β release and activation, and loss of the integrin β6 subunit protects mice from this form of injury.⁴⁵ Similarly, integrin β6–null mice are protected from glomerulosclerosis in an Alport model.⁴⁶ In contrast to the integrin β6–null mice, which have no developmental phenotype, the integrin β8–null mice demonstrate subtle renal abnormalities characterized by azotemia, albuminuria, and mild podocyte foot process effacement.⁴⁷ The proposed mechanism for these findings is that in the glomerulus, integrin αvβ8 binds the LAP/TGF-β complex, keeping the TGF-β in an inactive state, and in the absence of integrin αvβ8 increased levels of bioactive TGF-β lead to glomerular damage. Thus, integrins αvβ6 and αvβ8 appear to have opposite effects in kidney injury, with αvβ6 increasing and αvβ8 decreasing the amount of active TGF-β (Figure 3B).

CONCLUSIONS

It is clear that multiple different integrins play a role in renal disease, and it is not surprising that there is no common theme as to how integrins regulate kidney disease—they are differentially expressed in different cell types in the kidney, bind to a diverse set of ligands, and regulate multiple different signaling pathways. It has only been in the last year that strong direct evidence has shown that mutations in integrins cause kidney disease in humans. Nevertheless, a large body of in vivo work in mice and in vitro studies in isolated kidney cells emphasizes the potential role of integrins in kidney disease in humans, in particular kidney fibrosis. Thus, several integrin-specific topics related to kidney disease should be explored further. Although it is clear that integrin α3β1 is vital for the normal function of

Figure 2. Collagen binding integrins exert opposing effects in the regulation of glomerular homeostasis. In mesangial cells, integrin α2β1 binding to collagen leads to activation of intracellular signaling (i.e., ERK or Stat3) resulting in increased collagen production. In contrast, activation of integrin α1β1, directly or via negative regulation of integrin α2β1 or epidermal growth factor receptor signaling, leads to reduced production of profibrotic signaling. ROS, reactive oxygen species.
the glomerular filtration barrier, the role of the other laminin-binding integrins, such as α6β1 and α6β4, in the injured glomerulus is unknown. In addition, the roles of the laminin receptors in tubulointerstitial disease are unclear.

There is overwhelming evidence in mouse models that the collagen-binding receptors integrins α1β1 and α2β1 regulate renal fibrosis. Thus, it would be helpful to determine whether mutations in these integrins are indeed associated with fibrosis in different human kidney diseases. In addition, further efforts should be placed on targeting these collagen receptors to prevent fibrosis; modulating their function will probably not cause major adverse effects because they are not required for normal development. Similarly, targeting integrin αvβ6 with small molecule inhibitors might be an effective way of dampening TGF-β signaling, which has been implicated in numerous fibrotic kidney diseases. Finally, more work is needed to define how activation of integrin αvβ3 is required for the development of uPAR- and suPAR-dependent FSGS because this exciting and provocative finding has major implications for human disease.

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REFERENCES


