The characterization of α5-integrin expression on tubular epithelium during renal injury

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White LR, Blanchette JB, Ren L, Awn A, Trpkov K, Muruve DA. The characterization of α5-integrin expression on tubular epithelium during renal injury. Am J Physiol Renal Physiol 292: F567–F576, 2007. First published October 3, 2006; doi:10.1152/ajprenal.00212.2006.—The hallmark of progressive chronic kidney disease is the deposition of extracellular matrix proteins and tubulointerstitial fibrosis. Integrins mediate cell-extracellular matrix interaction and may play a role tubular epithelial injury. Murine primary tubular epithelial cells (TECs) express α5-integrin, a fibroblast marker and the natural receptor for fibronectin. Microscopy localized α5-integrin on E-cadherin-positive cells, confirming epithelial expression. The expression of α5-integrin increased in TECs grown on fibronectin and occurred in parallel with an upregulation of α-smooth muscle actin (αSMA), a marker of epithelial-mesenchymal transition (EMT). Exposure of TECs to transforming growth factor (TGF)-β also increased TEC α5-integrin expression in association with αSMA and EMT. Knock-down of α5-integrin expression with short interfering RNA attenuated the TGF-β induction of αSMA but did not alter morphologic EMT. Rather, α5-integrin was necessary for epithelial cell migration on fibronectin but not type IV collagen during cell spreading and epithelial wound healing in vitro. Immunohistochemistry revealed basolateral tubular epithelial α5-integrin expression in mouse kidneys after unilateral ureteric obstruction but not in contralateral control kidneys. In patient biopsies of nondiabetic kidney disease, α5-integrin expression was increased significantly in the renal interstitium. Focal basolateral staining was also detected in injured, but not in normal, tubular epithelium. In summary, these data show that TECs are induced to express α5-integrin during EMT and tubular epithelial injury in vitro and in vivo. These results increase our understanding of the biology of integrins during EMT and tubular injury in chronic kidney disease.

chronic kidney disease; epithelial mesenchymal transition

CHRONIC KIDNEY DISEASE LEADING TO END-STAGE RENAL DISEASE (ESRD) is a source of significant morbidity and cost to health care. The etiology of chronic kidney disease is diverse and includes conditions associated with diabetes and vascular and glomerular disease (25). The pathogenesis of chronic kidney disease is characterized by a gradual loss of renal function due to progressive deposition of extracellular matrix and tubulointerstitial fibrosis (2). The extent of tubulointerstitial fibrosis is a prognostic factor for the progression of chronic kidney disease to ESRD (19).

Tubular epithelial cells (TECs) play a vital role during chronic kidney disease progression by directly contributing to progressive fibrosis. Secreted growth factors (e.g., PDGF, FGF-2) and cytokines e.g., transforming growth factor (TGF)-β] activate TECs to secrete matrix proteins, such as fibronectin and collagen (11). Growth factors, cytokines, and extracellular matrix proteins also play a key role in mediating phenotypic transformation of TECs into myofibroblasts (epithelial-mesenchymal transition, or EMT), which is a key step in the development of progressive fibrosis in the kidney (17). TECs are also activated to secrete a variety of chemokines (e.g., MCP-1) and cytokines that facilitate the recruitment of leukocytes to the injured kidney (3) to create a cycle of inflammation and fibrosis that results in progressive chronic kidney disease.

The factors that regulate TEC phenotype during injury are incompletely understood. Although TGF-β is a central player, recent studies have provided further insight into the biology of the tubular epithelial cell in progressive kidney disease. The most abundant extracellular matrix protein in renal basement membranes is type IV collagen (18). The surrounding interstitium is principally made up of type I and type III collagens and fibronectin. Tubular epithelial cells are normally sheltered from the interstitial matrix and are not exposed to high levels of such alternative extracellular matrix proteins. During disease states, basement membrane integrity is compromised by increased local production of matrix metalloproteinases (17, 30). The resulting exposure to increased concentrations of fibrogenic matrix proteins such as type I collagen results in a profibrogenic TEC phenotype and the subsequent migration into the renal interstitium (29).

Integrins consist of a large family of cell surface receptors that mediate cell-extracellular matrix, cell-cell interactions and adhesion (9). Integrins are heterodimers composed of α and β subunits and are the primary receptors for extracellular matrix proteins such as fibronectin and collagen. Integrin-extracellular matrix protein interaction results in cytoskeletal changes and signaling that affects various cellular processes, such as cell growth, differentiation, motility, and gene expression (7). The current understanding of TEC biology in conjunction with the known increase in extracellular matrix proteins in diseased kidneys suggest a possible role for integrins in the TEC phenotype observed during renal injury. The defined role for integrin-linked kinase (ILK) in EMT further supports this premise (15).

Identifying and understanding the biology of TEC integrins may reveal novel mechanisms that underlie progressive chronic kidney disease. Only a few studies have studied integrin expression and function on tubular epithelium during renal injury. β1 and αv integrin subunits are known to increase or

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change their distribution on tubular epithelium in acute and chronic renal disease (22, 32). α5-integrin (VLA-5) exists as a β1-integrin heterodimer and is the major fibronectin binding integrin (9, 26). In the kidney, α5-integrin is normally expressed on interstitial fibroblasts and endothelial cells but not on epithelium (22). In this study, we demonstrate for the first time that α5-integrin is induced on tubular epithelium in association with EMT in vitro and during renal injury in vivo. The expression of α5-integrin mediates epithelial cell migration, a significant event in tubular injury and EMT.

MATERIALS AND METHODS

Tubular epithelial cell culture. Primary TECs were isolated from the kidneys of C57BL/6 mice, as described previously (28). TECs were established on a substrate of type IV collagen (human placenta; Sigma, St. Louis, MO), type I collagen (bovine skin, Angiotech Biomaterials, Palo Alto, CA) or fibronectin (bovine plasma; Sigma) coated onto tissue culture plates at a concentration of 10 μg/cm² unless otherwise specified. TECs were maintained in DMEM/F12 containing 10% FCS, 1% penicillin-streptomycin, 125 ng/ml PGE1 (Calbiochem, San Diego, CA), 25 ng/ml EGF (Sigma), 1.8 μg/ml 3,3′-thiouracil (Sigma), 3.38 ng/ml hydrocortisone, and 2.5 mg/ml of an insulin-transferrin-sodium selenite supplement (I-T-SS) (Sigma). In all experiments, TECs were used at passage <10 to ensure epithelial phenotype. Mouse kidney-derived fibroblasts were grown in DMEM containing 10% FCS and used as controls in certain experiments. For TGF-β experiments, TECs were incubated with human TGF-β1 (Sigma) for 10 min at 37°C before plating.

Immunoblotting. TECs were lysed in hypotonic lysis buffer, and equivalent amounts of protein were separated on a 8% SDS-polyacrylamide gel, followed by transfer to a polyvinylidene difluoride membrane. Membranes were incubated with rabbit anti-α5-integrin (CD49e, Santa Cruz Biotechnology, Santa Cruz, CA), rat anti-CD29 (β1-integrin, clone 9EG7, BD Pharmingen, San Diego, CA), mouse anti-α-smooth muscle actin (αSMA, clone IA4, Sigma), rabbit anti-poly (ADP-ribose) polymerase (PARP) (Cell Signaling, Danvers, MA) or mouse anti-actin (Chemicon, Temecula, CA) primary antibodies. Appropriate peroxidase-conjugated secondary anti-IgGs were used, and proteins were visualized using the enhanced chemiluminescence detection system (Amersham Pharmacia). Protein expression was quantified based on the density ratio of α5-integrin or αSMA to actin control within the same sample using Quantity-One software (Bio-Rad, Hercules, CA).

Immunohistochemistry and immunofluorescence microscopy. TECs were cultured on coverslips coated with fibronectin, type I or type IV collagen, fixed with acetone/methanol (1:1) and incubated with appropriate primary antibodies. Mouse anti-pan cytokeratin (clone PCK-26, Sigma), mouse anti-E-cadherin (clone 36, BD Pharmingen), rat anti-zona occludens (ZO)-1 (clone R04-76, Chemicon), rat anti-CD49e (α5-integrin, clone 5H10–27, BD Pharmingen), rat mouse anti-actin (Chemicon, Temecula, CA) primary antibodies. Appropriate peroxidase-conjugated secondary anti-IgGs were used, and proteins were visualized using the enhanced chemiluminescence detection system (Amersham Pharmacia). Protein expression was quantified based on the density ratio of α5-integrin or αSMA to actin control within the same sample using Quantity-One software (Bio-Rad, Hercules, CA).

Tubular epithelial cells express various integrins in vivo and in vitro (13, 20–22, 33). To determine integrin subunit expression in TECs, immunoblotting experiments were performed. At baseline, both primary TECs and fibroblasts expressed similar levels of β1 integrin subunits (Fig. 1B). α5-integrin (VLA-5) exists as a heterodimer with β1-integrin and is expressed primarily on fibroblasts, monocytes, and endothelial cells but not on epithelium (4, 9, 12). As expected, fibroblasts expressed high levels of α5-integrin. Surprisingly, low levels of α5-integrin were also detected on TECs (passage 0) (Fig. 1B). To confirm that the α5-integrin detected by immunoblotting was not due to contaminating fibroblasts, immunofluorescence mi-

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Cytokeratin

ZO-1

E-cad

α5

α5 + E-cad

α5

Actin

A

B

C

TEC

Fibroblast

TEC

Fibroblast

TEC

Fibroblast

Fig. 1. Integrin expression in tubular epithelial cells. A: immunofluorescence microscopy for epithelial markers cytokeratin, zona occludens (ZO)-1, and E-cadherin in low passage tubular epithelial cells (TECs; passage 0) and kidney-derived fibroblasts (×60). B: integrin expression in TECs. Immunoblot for α5 and β1 integrin subunits in low passage TECs and kidney-derived fibroblasts. C: immunofluorescence microscopy for α5-integrin and E-cadherin in TECs (×60) and kidney-derived fibroblasts.

Fig. 2. α5-Integrin expression in tubular epithelial cells. A: immunoblot for α5-integrin expression in TECs isolated and grown on type I, type IV collagen, or fibronectin. B: immunofluorescence microscopy of α5-integrin and E-cadherin expression on TECs isolated and grown on type I, type IV collagen, or fibronectin (×60).

croscopy for α5-integrin and E-cadherin was performed. α5-integrin was expressed at low levels on E-cadherin-expressing cells (Fig. 1C). These results show that α5-integrin is expressed on primary low-passage TECs.

The regulation of α5-integrin expression in tubular epithelial cells. The discovery of α5-integrin expression on primary TECs suggested that this integrin might be induced during injury. The previous studies were performed on TECs grown on type IV collagen, the native collagen of tubular basement membrane. During renal injury, the renal tubular epithelium is exposed to increasing tissue levels of type I collagen and fibronectin, which is the primary ligand for α5β1 integrin. To examine the effect of different extracellular matrix proteins on α5-integrin expression, TECs were isolated and plated on type IV, type I collagen and fibronectin. Cells were allowed to reach confluence, and cell lysates were analyzed by immunoblotting. α5-Integrin expression was significantly increased on TECs grown on fibronectin compared with cells plated on type IV and type I collagen (Fig. 2A). The increased α5-integrin expression was not simply due to an increased number of fibroblasts, as immunofluorescence microscopy confirmed α5-integrin localized to E-cadherin-expressing cells grown on fibronectin (Fig. 2B). Next, to determine whether a change in the extracellular matrix could induce α5-integrin expression on established TEC cultures, cells were first isolated and grown on type IV collagen to establish a cell population with low basal levels of α5-integrin. TECs were then passaged and plated on type IV collagen, type I collagen, or fibronectin and analyzed for α5-integrin by immunoblotting (Fig. 3A). At 24 h, α5-integrin expression was significantly increased in cells transferred to fibronectin substratum compared with cells passaged onto type IV or type I collagen (type I collagen not shown). The induction of α5-integrin on TECs plated onto fibronectin also occurred in a dose-dependent manner. TECs grown on increasing concentrations of type I and type IV collagen (10–80 μg/cm²) did not increase α5-integrin expression. In
contrast, α5-integrin expression increased significantly following growth on fibronectin at 10, 40, and 80 μg/cm².

TEC exposure to abnormal matrix, such as type I collagen, can induce changes consistent with EMT (29). To determine whether TECs grown on increasing concentrations of fibronectin underwent a phenotypic change consistent with EMT, cell lysates were analyzed for αSMA expression (17). Similar to the effect observed with α5-integrin, expression of αSMA increased in TECs grown on increasing concentrations of fibronectin (Fig. 3A). Although immunofluorescence micros-

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**Fig. 3.** Induction of α5-integrin expression in TECs. A: immunoblot of α5-integrin and αSMA expression in TECs passaged onto increasing concentrations of fibronectin or type IV collagen substratum (10, 40, and 80 μg/cm²) *P < 0.005; n = 3. B: immunofluorescence microscopy of α5-integrin and E-cadherin expression in TECs passaged onto fibronectin substratum (10 μg/cm²) (×60).

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**Fig. 4.** Effect of transforming growth factor (TGF)-β on α5-integrin expression in tubular epithelial cells. A: immunoblot of α5-integrin and αSMA expression in TECs exposed to TGF-β at 24 and 48 h (*P < 0.05, n = 3). B: immunofluorescence microscopy of α5-integrin and E-cadherin expression in TECs exposed to TGF-β at 48 h (×60).
copy confirmed that $\alpha_5$-integrin expression was epithelial localized on cells expressing E-cadherin, obvious phenotypic change consistent with EMT could not be detected on morphologic grounds (Fig. 3B).

TGF-β is a major fibrogenic factor involved in the initiation and progression of chronic kidney disease. TGF-β plays a significant role modulating TEC phenotype during renal injury and mediates EMT (6). To determine whether TGF-β affected the expression of $\alpha_5$-integrin, TECs were passaged and plated back onto type IV collagen-coated plates in the presence or absence of TGF-β (10 ng/ml). TECs were grown for 24 and 48 h and analyzed for $\alpha_5$-integrin and $\alpha$SMA expression by immunoblotting. Compared with untreated TECs, TGF-β increased $\alpha_5$-integrin expression (Fig. 4A). Consistent with the known effect of TGF-β on TEC phenotype and EMT, $\alpha$SMA was also significantly increased in TECs at 24 and 48 h. Microscopically, TGF-β-treated but not control TECs became elongated and assumed the spindelike morphology of myofibroblasts (14). Immunofluorescence microscopy demonstrated that TGF-β induced $\alpha_5$-integrin expression on E-cadherin-positive cells (Fig. 4B). However, compared with control TECs, E-cadherin staining was increasingly disorganized following incubation with TGF-β, consistent with the observed myofibroblastic morphologic change (Fig. 4B). Taken together, these results demonstrate that $\alpha_5$-integrin is induced on TECs following exposure to TGF-β and its primary extracellular matrix ligand, fibronectin, in a dose-dependent manner. The induction of $\alpha_5$-integrin occurs in conjunction with increased $\alpha$SMA expression and EMT.

Role of $\alpha_5$-integrin in EMT and tubular epithelial cell motility. The fibronectin and TGF-β induction of $\alpha_5$-integrin on tubular epithelium suggested a functional role for this integrin in renal tubular cell injury. To first determine whether $\alpha_5$-integrin was required for TGF-β-induced EMT, studies were performed using siRNA (Fig. 5). TECs were transfected with siRNA targeting $\alpha_5$-integrin or control GAPDH. At 24 h following transfection, cells were exposed to TGF-β (10 ng/ml), as described above, and followed for 24 and 48 h. Cells were harvested and analyzed morphologically by light microscopy and for $\alpha$SMA and $\alpha_5$-integrin expression by immunoblotting. Compared with cells treated with control siRNA, $\alpha_5$-integrin siRNA effectively reduced TGF-β-induced $\alpha_5$-integrin expression at 24 and 48 h following stimulation with TGF-β (Fig. 5A). Although the $\alpha_5$-integrin knock-down was not complete at
48 h, protein levels were similar to control cells at baseline. Reduced α5-integrin expression was associated with an attenuation of αSMA upregulation in response to TGF-β. However, morphologically, no differences in EMT were discernible, as both control and α5-integrin siRNA-treated TECs transitioned to a myofibroblastic phenotype at 48 h of TGF-β stimulation (Fig. 5B). These data show an association between α5-integrin and αSMA expression and suggest that α5-integrin might contribute but is not necessary for TGF-β-induced EMT.

In the prior studies, diminished TEC spreading on fibronectin, but not collagen IV was observed in cells transfected with α5-integrin siRNA compared with controls (Fig. 5C). This manifested as diminished cellular confluency at 24 h in α5-integrin siRNA transfectants that was not due to increased cellular apoptosis compared with control siRNA transfectants, as determined by PARP cleavage (Fig. 5D). Interestingly, the addition of TGF-β-induced equivalent levels of PARP cleavage in both control and α5-integrin siRNA-treated cells. These observations implied that α5-integrin mediates TEC motility, an important consequence of EMT (8). To test this possibility, an in vitro epithelial cell migration assay was used (Fig. 6). Confluent layers of TECs were scratched, and wound healing was determined. On type IV collagen and fibronectin substratum, TEC wound healing occurred within 24 h. Not unexpectedly, TEC expression of α5-integrin was increased as early as 6 h along the wound margins and increased over the time of wound healing, as determined by immunofluorescence microscopy and immunoblotting (Fig. 6A–D). Next, to determine whether α5-integrin-mediated cell migration during wound healing, α5-integrin siRNA and blocking α5-integrin antibodies were used. Transfection of TECs with α5-integrin siRNA prevented protein induction during wound healing at 24 h compared with control siRNA-treated cells (Fig. 6E). The siRNA-mediated reduction in α5-integrin expression was sufficient to significantly delay wound healing in cells grown on fibronectin (Fig. 6F). Similarly, antibody-mediated α5-integrin blockade also prevented TEC migration and wound healing on fibronectin at 24 h compared with untreated and isotype control-treated TECs (Fig. 6G). Interestingly, neither blocking α5-integrin antibodies nor siRNA impacted wound healing on type IV collagen (data not shown). However, because type IV collagen is not a ligand for α5-integrin, this observation is not entirely unexpected. Furthermore, increased PARP cleavage was not observed in α5-integrin siRNA and antibody-treated cells supporting the conclusion that the reduced wound healing was not due to increased apoptosis (data not shown). Therefore, these results show that during injury, α5-integrin is necessary for TEC migration on fibronectin but not type IV collagen.

α5-Integrin expression on tubular epithelium during renal injury in vivo. The previous experiments detail the expression and function of α5-integrin in tubular epithelium in vitro. To evaluate whether these observations were relevant in vivo, tubular α5-integrin expression was determined in the kidneys of mice following unilateral ureteric obstruction. C57BL/6 mice underwent ligation of the left lower ureter, and mice were killed at 1 to 6 wk. The ligated and contralateral control kidneys were harvested and analyzed for α5-integrin expression by immunohistochemistry. No primary and isotype control experiments were negative, confirming the specificity of the antibody used (Fig. 7A). In sham and contralateral control kidneys, α5-integrin expression was seen primarily within the interstitium, and in the vascular and glomerular endothelium. Consistent with previous reports, no tubular α5-integrin expression was seen (Fig. 7B). In ligated kidneys, α5-integrin was expressed in the same areas as in the control kidneys but
showed increased interstitial reactivity, consistent with the increase in interstitial fibroblasts and interstitial fibrosis seen in this model. In contrast to control kidneys, basolateral $\alpha_5$-integrin was focally expressed on tubular epithelium at 3 and 6 wk following the induction of hydronephrosis (Fig. 7, C and D). The results observed using immunohistochemistry were in keeping with total $\alpha_5$-integrin protein levels that were increased in ligated kidneys over the 6 wk experimental period (Fig. 7E). These data show that $\alpha_5$-integrin is upregulated and expressed on tubular epithelium following ureteric obstruction and subsequent renal injury in mice.

$\alpha_5$-Integrin expression in human kidney disease. To assess the pattern of $\alpha_5$-integrin expression in humans, immunohistochemistry was performed on banked frozen kidney biopsies. Frozen kidney specimens from patients with a variety of non-diabetic kidney diseases and established tubulointerstitial disease were stained for $\alpha_5$-integrin using immunohistochemistry. Twelve biopsies were studied with the following diagnoses: IgA nephropathy, membranous nephropathy, pauci-immune glomerulonephritis, hypertensive/ischemic nephrosclerosis (one case each), and lupus nephritis, thin basement membrane disease with focal segmental glomerulosclerosis, interstitial nephritis, focal segmental glomerulosclerosis (two cases each). Normal kidney tissues from radical nephrectomy specimens were used as controls. In normal kidney biopsies. Control sections stained with isotype control antibodies or secondary antibodies alone stained negative, confirming the specificity of the anti-$\alpha_5$-integrin antibody (Fig. 8D). In diseased kidney biopsy specimens, $\alpha_5$-integrin expression changed significantly (Fig. 8, E–G). Again, the majority of staining occurred on endothelial cells as in normal kidney. Consistent with previous reports (22), $\alpha_5$-integrin expression also increased substantially on the inflammatory cells and within the interstitium of kidneys with chronic tubulointerstitial disease and fibrosis. In 9 of 12 patient biopsies, focal tubular $\alpha_5$-integrin staining was also detected. $\alpha_5$-Integrin was induced only in the tubular epithelium in areas of tubulointerstitial disease and occurred primarily on the basolateral cell surface (Fig. 8, E–G). No tubular epithelial cell staining was seen in biopsies where tubules and interstitium were normal, suggesting that $\alpha_5$-integrin is expressed in the epithelium only during tubular injury (Fig. 8H). Interestingly, $\alpha_5$-integrin staining was pronounced in the media and the areas of fibrointimal thickening of atherosclerotic arteries compared with normals (Fig. 8I). Taken together, these data show that $\alpha_5$-integrin is induced in tubular epithelium of patients with kidney disease and tubulointerstitial injury.

**DISCUSSION**

An increased understanding of the biology of chronic kidney disease is essential to develop new therapies that will prevent progression to ESRD. The microenvironment is significantly
altered during renal injury, and it is likely that cell surface integrins play a role in defining cellular phenotype in chronic kidney disease. In this study, we show for the first time that TECs can be induced to express \( \alpha_5 \beta_1 \)-integrin. The induction of \( \alpha_5 \beta_1 \)-integrin occurred in response to the extracellular matrix protein fibronectin and TGF-\( \beta \) that are increased in chronic kidney disease and tubulointerstitial fibrosis. Importantly, \( \alpha_5 \beta_1 \)-integrin expression occurred during EMT and was necessary for cell migration and wound healing on fibronectin, an observation that is consistent with the current model of EMT and renal fibrosis. Finally, we demonstrate \( \alpha_5 \beta_1 \)-integrin expression on injured renal tubules in a mouse model of renal injury and in human kidney biopsies.

The principle integrin receptor for fibronectin is the combination of the \( \alpha_5 \) and \( \beta_1 \)-integrin monomers (\( \alpha_5 \beta_1 \)). \( \alpha_5 \beta_1 \)-Integrin is known to be expressed on numerous cell types, including monocytes, endothelial cells, and fibroblasts. Some studies have demonstrated \( \alpha_5 \beta_1 \)-integrin expression in cultured TECs, but this could not be confirmed by studies in vivo. An early study looking at the distribution of \( \alpha_5 \beta_1 \)-integrin subunits in the cortex of normal human kidney samples showed consistent staining of \( \alpha_5 \beta_1 \)-integrin only in the arterioles and a lack of expression in the tubular epithelium (24). Similarly, Roy-Chaudhury and colleagues (8, 22) examined integrin expression in kidney biopsies from patients with a variety of diseases and reported that \( \alpha_5 \beta_1 \)-integrin was limited to the interstitium and vascular endothelium of the kidney (8, 22). In this study, we demonstrate inducible epithelial expression of \( \alpha_5 \beta_1 \)-integrin under injurious conditions in vitro and in diseased kidney biopsies in vivo. Our study demonstrates that little or no \( \alpha_5 \beta_1 \)-integrin exists in the normal tubular epithelium in vitro and in vivo, consistent with prior studies. However, in disease states, \( \alpha_5 \beta_1 \)-integrin expression is induced on tubular epithelium. We note that the expression of \( \alpha_5 \beta_1 \)-integrin in patient biopsies is sporadic and correlates with the severity of tubulointerstitial disease. Furthermore, widespread epithelial \( \alpha_5 \beta_1 \)-integrin expression would not be expected given the indolent nature of many chronic kidney diseases. Thus focal tubular \( \alpha_5 \beta_1 \)-integrin expression may have been overlooked or below the limits of detection in prior studies. Focal expression of \( \alpha_5 \beta_1 \)-integrin in human kidney biopsies has, in fact, been observed in patients with nondiabetic tubulointerstitial disease (Roy-Chaudury P., personal communication).

Normal basement membranes are complex networks of proteins that constitute the immediate microenvironment within which cells reside and affect cellular adhesion, migration, and differentiation. Renal tubular basement membranes are composed of type IV collagen and differ from the interstitial matrix, which is loosely organized and composed primarily of type I and type III collagen and fibronectin. During chronic kidney disease, type I collagen and fibronectin are found increasingly deposited in kidneys (10, 30). The induction of \( \alpha_5 \beta_1 \)-integrin in TECs following increasing exposure to its primary ligand is therefore not surprising. In contrast, TEC growth on type I collagen did not induce \( \alpha_5 \beta_1 \)-integrin, which may be related to the use of \( \alpha_1 \beta_1 \) and \( \alpha_2 \beta_1 \) as the principal integrin receptors for this matrix protein (5). The growth of TECs on fibronectin induced the expression of \( \alpha_{	ext{SMA}} \) but was not...
insufficient to induce obvious morphological change microscopically. TGF-β on the other hand, an important mediator of renal fibrosis and EMT, induced epithelial α5-integrin expression and the morphologic and biochemical changes indicative of EMT. The TEC expression of α5-integrin in this setting is consistent with the expected expression pattern of α5-integrin that includes mesenchymal cells such as fibroblasts. The siRNA-mediated downregulation of α5-integrin had an impact of the induction of αSMA, but similar to the observations seen during TEC growth on fibronectin, the reduction in α5-integrin was insufficient to block TGF-β-induced EMT morphologically. These results suggest that α5-integrin might play a role in EMT but only as part of a complex biological process that includes other factors. In this regard, studies that examine myocyte and adipocyte differentiation demonstrate a requirement for differentially regulated integrins in association with cytokines and growth factors to fully mediate cellular phenotypes (16, 23). Thus, in tubular epithelium, it is also likely that several factors, including the regulation of other integrin subunits are necessary to coordinate EMT. This is exemplified by studies that show the induction of EMT when TECs are grown on type I collagen, but not on native type IV collagen (29). Furthermore, ILK, which was demonstrated to be downstream of TGF-β, is also an essential mediator of EMT (15).

Numerous signaling pathways implicated in the EMT are activated downstream of integrins, such as ERK and phosphatidylinositol 3-kinase (1). The induction of α5-integrin likely mediates several phenotypic changes observed during epithelial cell injury. Our results show that increased α5-integrin expression in TECs is required for TEC migration on fibronectin. This observation is consistent with the proposed model of EMT in the kidney (6). It is probable that in the presence of an intact basement membrane, α5-integrin plays a little role in wound healing and repair, as supported by our data. However, upon disruption of the tubular basement membrane and the onset of EMT during injury, TEC exposure to a fibronectin-rich interstitium promotes cell migration into this compartment. Our data support the notion that α5-integrin is a critical mediator of this step of EMT. Future studies are required to fully characterize the impact of α5 and other integrins on the EMT and tubular epithelial cell injury.

Our findings highlight the plasticity of the tubular epithelium during renal injury and provide new insight into the role of integrins in renal injury and fibrosis. The induction of α5-integrin represents a novel marker and potential therapeutic target that characterizes and likely contributes to tubular injury, EMT, and progressive kidney disease.

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