REVIEW

β1-integrins and glomerular injury

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Abstract : The renal glomerulus is composed of three types of glomerular cells (mesangial cell (MC), endothelial cell and podocyte) and extracellular matrix (ECM) consisting of the glomerular basement membrane (GBM) and mesangial matrix. It constitutes a highly specialized microcirculation in which the permeability characteristics of the capillary wall allow its unique filtration function. The proliferation of MCs, an increase of mesangial ECM and detachment podocyte from GBM are key biological features of progressive glomerulonephritis (GN), leading to glomerular scarring and dysfunction. Thus, the study of the molecular and cellular mechanisms responsible for pathological glomerular alterations may help to elucidate the pathogenesis of progressive glomerular diseases. A growing body of evidence indicates that β1 integrin family (β1 integrins), that mainly mediates cell adhesion to ECM, controls cell behaviors such as cell migration, proliferation, apoptosis and ECM assembly. In addition, a correlation between glomerular expression of β1 integrins and their ligand ECM components is observed in various human and experimental GN, suggesting that altered β1 integrins-mediated cell behaviors may contribute to the progression of GN. It is now becoming apparent that the expression of glomerular β1 integrins is not only critical for maintaining the glomerular capillary permeability but it modulates cell signaling pathways regulating the cell phenotypes involved in the progression of glomerular diseases. J. Med. Invest. 51 : 1-13, February, 2004

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INTRODUCTION

The adhesion between cells and the extracellular matrix (ECM) surrounding cells is essential to develop tissue and maintain its function ; it is also necessary to repair tissue and regenerate its function after being damaged (1). ECM is a super-high molecular complex comprised of cell adhesive glycoproteins such as collagen (COL), fibronectin (FN), laminin (LM), and proteoglycan (2). Cell adhesion to ECM are mainly mediated by the β1 integrin family (β1 integrins) expressed on cell surfaces. β1 integrins-induced signal at cell adhesion controls a wide variety of cell phenotypes, including cell proliferation, migration, differentiation, cell survival, ECM synthesis, degradation, and construction of ECM molecules (3, 4).

The glomerulus possesses a unique architecture that allows it to carry out its function of purifying the blood through filtration. This structure is established and maintained through the interactions of glomerular cells with ECM consisting of the glomerular basement membrane (GBM) and mesangial matrix. Glomerular cells interact with ECM through β1 integrins that mediate attachment to the ECM. Recent evidences have demonstrated that β1 integrins functions as a two-way street between the cell and the ECM. The ECM controls the cell proliferative, synthetic, and metabolic states, and responsiveness to the extracellular factors while events in the cell can affect β1 integrins-mediated ECM formation.

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Progressive form of glomerulonephritis (GN) is characterized with mesangial cell (MC) proliferation, accumulation of mesangial ECM and podocyte detachment from the GBM. Therefore, the molecular pathology of progressive GN can be studied with regard to the cell phenotypes controlled by β1 integrins that are expressed by glomerular cells. Specifically, unregulated ECM reorganization and remodeling by mesangial cell (MC)-β1 integrins may alter the morphology and function of the glomerular mesangium ; and there is the possibility that a vicious cycle may form in which these alterations may further modify MC function to induce even more pathological mesangial remodeling that finally leads to podocyte detachment and collapse of glomerular capillary ultrafiltration apparatus (5, 6). Currently, it is thought that excessive expression of fibrogenic mediators, including PDGF-BB, TGF-β, angiotensin II and endothelin are involved in the progression of chronic GN (7-9) ; and it is found that these mediators influence the expression of MC-β1 integrins and control its function (10, 11). In this brief review, we report comments on the role of β1 integrins that is responsible for glomerular cells-ECM adhesion. Moreover, we reviews recent investigations focussing on the expression of β1 integrins in MCs, its control by soluble mediators, and the role of β1 integrins in the progression of glomerular diseases.

Function and structure of β1 integrins

Integrins are non-covalently-bound heterodimeric cell adhesion molecules that link the ECM to the cytoskeleton. β1 integrin family (β1 integrins), the largest group of integrin family, is composed of a β1 and 1 of 12 α subunits (α1-α11 and αv) and function predominantly cell-ECM adhesion. Both subunits have a single hydrophobic transmembrane domain, cytoplasmic tails and extracellular domains. It transmit information from ECM context surrouding cell into cell (outside-in signaling), while the extracellular binding activity of integrins is regulated from the inside of the cells (inside-out signaling). Although inside-out signaling is the chief mechanism by which cells control integrin function, it has not been investigated in detail on nephrology field. Therefore, the present review concentrates on outside-in signaling, which has profound effects on many glomerular cell functions.

The combination of the α-and β1-subunits not only determines the specificity of ligand ECM compoments but also intracellular signaling properties affecting the cell phenotypes such as proliferation, differentiation, survival and ECM assembly (3, 12). As the cytoplasmic tails of integrins are very short and devoid of enzymatic activity, they transduce signals by associating with adaptor proteins (talin, paxillin, vinculin) that connect the integrin to the cytoskeleton and cytoplasmic kinases (Focal adhesion kinase (FAK), Src-kinase family, Phosphatidylinositol-3 kinase (PI-3K)). *In vitro*, as integrins bind to ECM compoments, they become clustered in the plane of the cell membrane and associate with a cytoskeletal and signaling complex that promotes the assembly of actin filaments. The organization of actin filaments into larger stress fibers, in turn, causes more integrin clustering, thus enhancing the bindng of ECM compoments and organization by integrins. As a result, ECM proteins, integrins, and cytoskeletal proteins assemble into large multiprotein aggregates, termed focal adhesion and ECM contacts(3). Importantly, integrins may transduce signals through their association with and clustering of growth factor receptors (4, 13). Integrin and growth factor signalings do not function independently, but extensive crosstalk takes place between integrin signaling and a large variety of growth factor signaling pathways (14).

Role of β1 integrins in glomerular injury

Many histochemical and cell biological studies on rat and human glomeruli and cultured glomerular cells have demonstrated that glomerular cell types express multiple integrins (6, 15). The β1 integrins expressed on glomerular cells *in vivo* and *in vitro* and ECM components recognized by β1 integrins are summarized in Table 1. The expression of β1 integrins has been investigated in several experimental models of rat and human GN, and the results have suggested that they play roles in glomerular diseases. Moreover,

Table 1. β1 integrins expressed on glomerular cell types.

	Ligand-matrix component		
	Laminin	Fibronectin	Collagen
Glomerulus			
Endothelial cell	α1β1, α3β1	α5β1, αvβ1	α1β1, α2β1, α3β1
Mesangial cell	α1β1, α3β1	α5β1, α8β1	α1β1, α2β1, α3β1
Podocyte	α3β1	α3β1	α3β1

ablation of glomerular integrin function through the use of either function-blocking integrin antibody or an integrin gene knock-out strategy has shown that integrin expression by glomerular cells plays important roles in glomerular injury.

In a rat model of anti-Thy 1 antibody-induced GN in which TGF-β promotes mesangial ECM accumulation, coordinated increases in α1β1, α5β1 and α8β1 integrins and their ligands (i.e., FN, COL Ⅰ, Ⅳ, and LM) have been reported (16, 17). Semiquantitative immunohistochemical analysis in this model of GN showed that the level of glomerular TGF-β expression paralleled the level of the glomerular expression of β1 integrins (α1β1, α5β1 integrins) and their ECM ligands, suggesting that TGF-β may contribute to pathological mesangial ECM accumulation by enhancing α1β1, α5β1 integrin-mediated ECM assembly (16). In another model of GN, IRC-derived GN mice, which showed a marked accumulation of mesangial ECM components (FN, COL Ⅰ, Ⅳ, and LM) and increased TGF-β expression in diseased glomeruli, the increased expression of α1, α2, α5 and β1-subunits was noted (18). Additionally, our immunohistological study of human GN found significant increases in glomerular β1 integrins (α1β1, α5β1 integrins) in IgA nephropathy and lupus GN that paralleled both the severity of glomerular lesions such as ECM deposition and cellularity, and the glomerular synthesis of TGF-β (19). Positive correlations have been reported between the mesangial expression of α1β1, α5β1 integrins and mesangial expansion in patients with IgA nephropathy, Henoch-Schoenlein nephritis, and diabetic nephropathy (20-23).

Recently, we examined the effect of the administration of function-blocking mouse anti-α1 integrin antibody (anti-α1 Ab) in rats with anti-Thy-1 GN. We found that the *in vivo* application of anti-α1 Ab reduces MC-mediated pathological mesangial matrix remodeling, suggesting that α1β1 integrin is involved in the development of experimental GN characterized by MC proliferation and ECM accumulation (Fig. 1)(24). In an experiment similar to ours, Cook *et al*. reported that treatment with mouse anti-α1 Ab reduced glomerular and tubulointerstitial scarring in a rat model of crescentic GN (25). Another approach examining the role of α1β1 integrin in renal disease has been reported in double-knockout mouse at both the collagen α3 (IV) gene (Alport mouse) and the α1 integrin gene (26). The extensive expansion of the mesangial matrix observed in Alport mice was attenuated in age-

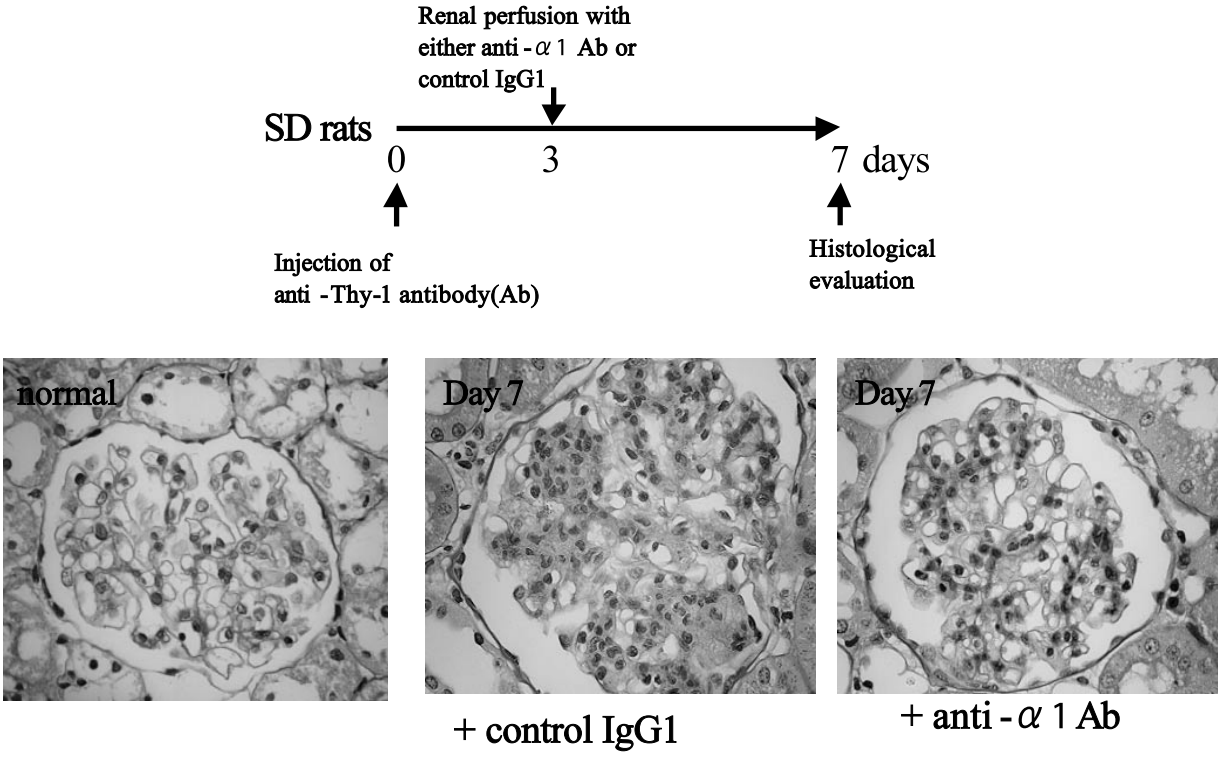

Figure. 1. Effect of mouse anti-α1 integrin monoclonal antibody (anti-α1 Ab) on rats with anti-Thy-1 GN. Rats injected with either mouse IgG1 or anti-α1 Ab in the left renal artery 3 days after induction of anti-Thy-1 GN. Micrographs show representative results of PAS staining of glomeruli from normal rat (normal) and day 7 anti-Thy-1 GN rats treated with either mouse IgG1 (+control IgG1) or anti-α1 Ab (+anti-α1 Ab) (Magnification ×200).

matched double-knockout mice. This close relationship between MC-α1β1 integrin expression and mesangial ECM expansion strongly supports the notion that α1β1 integrin plays a direct role in pathological mesangial remodeling (scar remodeling) in GN. Recently, Hartner *et al*. showed using α8-deficient mice treated with desoxy-corticosterone (DOCA)-salt which induced glomerular hypertension as well as mechanical stress of the glomerular mesangium that the expression of MC-α8 integrin helps to maintain the integrity of the glomerular capillary tuft (27). However, it has also been shown that MC-α8 integrin is not likely to be involved in the development of glomerulosclerosis in this mouse model of glomerular injury.

The normal glomerular capillary wall barrier to filtration of macromolecules is maintained by integrin-mediated glomerular podocyte-GBM interactions. This is demonstrated by the distribution of integrins along the surface of cells abutting the GBM and study of animal models of proteinuric glomerular injury. Damage on normal podocyte-GBM adhesion function induce abnormal proteinuria. Podocyte detachement and retraction from the GBM are important morphologic correlates of the onset of proteinuria in several non-antibody-mediated models of proteinuria, including puromycin-induced nephrosis, protein overload proteinuria, and subtotal nephrectomy (28, 29) and the areas of denuded GBM are probably critical sites of protein leakage into the urine (28, 30). Proteinuria may be preceded by podocyte cytoskeletal disaggregation and loss of actin and vinculin from tertiary podocytic processes (31), suggestive of disruption of focal adhesions. Two antibody-mediated models of proteinuria may be mediated by interference with normal podocyte adhesion. In these models, antibody injection produces transient proteinuria without evidence of involvement of mediator systems such as complement or leukocytes. In the first, non-complement-fixing anti-GBM antibodies produce proteinuria, which has been theorized to be due to antibody-induced changes in GBM structure or contamination with antibodies reactive with podocyte antigens (32). In the second, the F(ab)2 and F (ab)' fragments of anti-Fx1 A antibodies cause a transient complement and leukocyte independent lesion following binding to podocyte (33). Both anti-Fx1 A antibody and an anti-GBM antibody, which causes complement independent proteinuria, have been shown to have prominent reactivity with β1 integrins and to be capable of inhibiting podocyte attachment to substrate *in vitro* (34, 35). Thus, binding of antibodies to integrins on podocyte might lead to proteinuria by direct interference with podocyte-GBM interactions. Direct evidence of increased glomerular permeability to macromolecules by cross-linking of β1 integrins has been obtained using anti-β1 antibodies and isolated glomeruli in an *in vitro* system (36).

Focally decreased staining for α3β1 integrin on the glomerular capillary wall has been reported in membranous nephropathy, indicating the presumed importance of α3β1 in podocyte adhesion to the GBM (37, 38).

Role of β1 integrins in MC-induced ECM assembly

There is accumulating evidence that critical step of ECM assembly is not passively ocurred (self-assemble) but rather is actively mediated by β1-integrins (39-43). Integrins adhere to soluble secreted ECM components at the cell surface, and are constructing insoluble ECM networks.

A cell adhesion assay showed that human and rat MCs utilize β1 integrins to adhere to FN, COL I ,IV and LM. MCs primarily use the abundantly expressed α1β1 integrin to bind to COL I ,IVand LM while α2β1 integrin expressed on MC, another COL and LM receptor, plays a minor role in cell binding to COL I and IV(44, 45). The α5β1 and α8β1 integrins mediate MC adhesion to FN. Cultured MCs (α8-/-) obtained from α8 knock-out mice showed a marked inhibition of cell adhesion to FN, suggesting that α8β1 integrin is a major FN receptor (45, 46). Culture studies of MCs demonstrated that β1 integrins can be organized in focal adhesions that mediate attachment and spreading on ECM molecules, and transduce signal from ECM. On COL I, MCs organized α1β1, α2β1 and α3β1 integrins, on LM, MCs organized α1β1 and α3β1, on FN, MCs organized α3β1, α5β1 and α8β1 (17, 47).

Regarding β1 integrins-mediated ECM assembly, it has been confirmed using FN assembly assay that FN secreted from cells is assembled into insoluble matrix outside of cells via the function of α5β1 integrin expressed on the cell surface (40, 41). On the other hand, the collagen gel contraction assay, a three-dimensional cell culture method, is useful for investigating the function of collagen-binding integrins in collagen matrix assembly (reorganization) and scar remodeling seen in damaged organs, including the skin, lung, liver and kidney (48-51). This culture system is composed of several phases including cell adhesion to collagen fibers and cell migration, followed by assembly of the surrounding collagen fibers into more dense collagen fibrils within the collagen lattice (10, 11). Thus, the process of gel contraction seems to be similar to mesangial collagen matrix remodeling or sclerosis

in GN, since abnormal mesangial assembly of COL I, III and IV, dense collagenous matrix deposition, and disfiguring scarring of mesangium are characteristic features of mesangial sclerosis in progressive glomerular diseases (50). Using this assay, we demonstrated that MC-α1β1 integrin is essential for collagen-dependent adhesion/migration and is thereby involved in collagen matrix reorganization (11).

TGF-β and PDGF-BB have been recognized to be key mediators in the development of glomerular scarring in experimental and human kidney diseases (7, 8). Both factors stimulated FN assembly through the increased cell-surface expression of α5β1 integrin (52-54). Recently, connective tissue growth factor (CTGF), a downstream mediator of TGF-β, has been shown to play a role in TGF-β-induced FN fibril formation by upregulating active α5β1 integrin in human MCs(55). Both growth factors also enhanced MC-α1β1 integrin-dependent collagen matrix reorganization (11). The ability of TGF-β to stimulate collagen matrix reorganization is dependent on increased α1β1 integrin expression, which leads to an increased number of sites for MCs to adhere to collagen I, whereas PDGF-BB enhancement has been shown to depend on increased α1β1 integrin-mediated MC migratory activity. Indeed, we found that the overexpression of α1β1 integrin is associated with the enhanced ability of MC to perform collagen matrix remodeling (50). Furthermore, considering that CTGF has been shown to enhance α1β1 integrin-dependent human MC adhesion and migration, CTGF may induce α1β1 integrin-mediated collagen matrix reorganization by MCs (56). Angiotensin II (Ang II) and endothelin-1 (ET) are potent vasoconstricting peptides that have been implicated in fibrosis in various organs, including kidney (9, 57). Ang II stimulates cardiac fibroblasts-induced collagen matrix remodeling by enhancing β1 integrin expression (10). We reported that ET promotes collagen matrix reorganization by enhancing MC-α1β1 integrin-dependent migration and MMP-2 activity (58). Interestingly, recent reports have demonstrated that the process of collagen fibril formation is greatly influenced by cell-derived FN polymerization via cell-surface α5β1 integrin, suggesting that *in vivo* collagen matrix deposition is dynamically integrated with FN assembly (42, 43). Taken together, enhanced MC-β1 integrins-mediated ECM organization induced by many soluble nephritogenic factors may contribute to the abnormal mesangial remodeling observed in progressive GN.

β1 integrins-mediated signaling pathways regulate cell proliferation, survival and ECM remodeling by MCs

Recent cell biological studies have revealed that β1 integrins, together with receptors for soluble mediator molecules, play a crucial role in regulating cell proliferation (4, 59). In general, the cell cycle is controlled by cyclin-dependent kinases (CDKs). These proteins are expressed constitutively, but their activity is enhanced by binding to cyclins and inhibited by the action of CDK inhibitors (CKIs)(60). CKIs, including p21 Waf-1, p27 Kip1, and members of the INK4 family of CDK inhibitors, negatively regulate the cell cycle by inhibiting the formation or activation of cyclin-CDK complexes (61). Progression through the G1 phase is controlled by CDK4 and CDK6, which interact with cyclin D1, and by CDK2, which binds to cyclin E. The CKIs p21 cip1 and p27 kip1 inactivate CDK2. CDKs are responsible for the phosphorylation of Rb, which induces other cell cycle proteins including cyclin A. The association of cyclin A with CDK2 then initiates the G1/S transition. Integrin-mediated cell adhesion regulates the G1 phase of the cell cycle (4). Integrins cooperate with receptor tyrosine kinases to stimulate cyclin D1 expression and suppress CKI levels, and thereby support the cyclin E-CDK2 activity that drives the transition to S-phase (62). The enhanced and sustained extracellular signal regulated kinase (ERK) activity in adherent cells explains in part the supportive role of integrins in cyclin D1 transcription (63, 64). Many pathways that connect integrins to ERK activation have been implicated in cell proliferation, such as the assembly of protein complexes including Src, FAK, and p130 Cas (65) or including Fyn, caveolin, and Shc (Wary *et al*.,1998). Additionally, RhoA and Rac activity have been implicated in integrin-mediated control of the levels of cyclin D1 and CKI (67, 68).

There have been a few reports regarding cell cycle regulation of MC from the perspective of cell-ECM interaction. To analyze the possible involvement of collagen-binding integrins in COL I-derived growth signals, Schocklmann *et al* recently examined how two structurally distinct forms of COL I, monomer versus polymerized fibrils, affected the proliferation and expression of G1-phase regulators in MCs, using a two-dimensional cell culture (69). The adhesion of MCs to monomer or polymerized collagen was equally well. However, in contrast to a control substratum of plastic or monomer COL I, polymerized COL I completely prevented the serum-induced increase in DNA synthesis and MC replication. The inhibitory effect of polymerized COL I was characterized by the rapid down-

regulation of cyclins D1 and E and the lack of serum-mediated suppression of CKI p27Kip1. They speculated that polymerized COL I fibrils specifically regulate early α1β1 or α2β1 integrin signaling, which leads to the inhibition of MC proliferation. Of note, overexpression of α1β1 integrin in cultured MCs showed decreased cell mitogenicity and increased expression of the CKI p27Kip1, suggesting that α1β1 integrin expression itself play a role in the regulation of MC growth (50).

Abnormal mesangial ECM remodeling by MCs is a prominent cell biological feature of progressive GN. It is characterized by an increased incorporation of normal mesangial ECM components such as FN, LM and COL IV and/or pathological ECM components of COL I and III into nephritic glomeruli (6, 16). Therefore, identification of the integrin signaling molecules that are involved in abnormal MC-induced ECM remodeling may provide us with a selective target for the pharmacological inhibition of pathological mesangial remodeling. To investigate the mechanism of MC-mediated collagen matrix remodeling, we studied the cell signaling pathways of MC that participate in the regulation of α 1 β 1 integrin-mediated collagen gel contraction. In this study, we found that activation of ERK is critical for the α1β1 integrin-dependent MC migration needed for collagen matrix reorganization (Fig. 2, 3)(70). Furthermore, we found that PDGF-BB, a representative fibrogenic growth factor in GN, enhances α1β1 integrin-mediated collagen matrix reorganization through synergistic activation of the ERK/AP-1 pathway that is crucial for MC migration (71). Recently, Zent *et al*. found that reactive oxygen species (ROS) stimulate MC-induced collagen matrix reorganization accompanied by the tyrosine phosphorylation of several proteins, among which FAK is prominent (72). Interestingly, as demonstrated using FAK-deficient and FAK-reconstituted fibroblasts, FAK positively contributes to ERK activity required for PDGF-BB-stimulated migration of smooth muscle cells (73).

A growing body of evidences suggests that FN assembly is regulated by α5β1 integrin-mediated signaling (39). Interaction of FN with α5β1 integrin results in the activation of FAK, which then binds to the signaling proteins Src and PI-3K (74, 75). The asso-

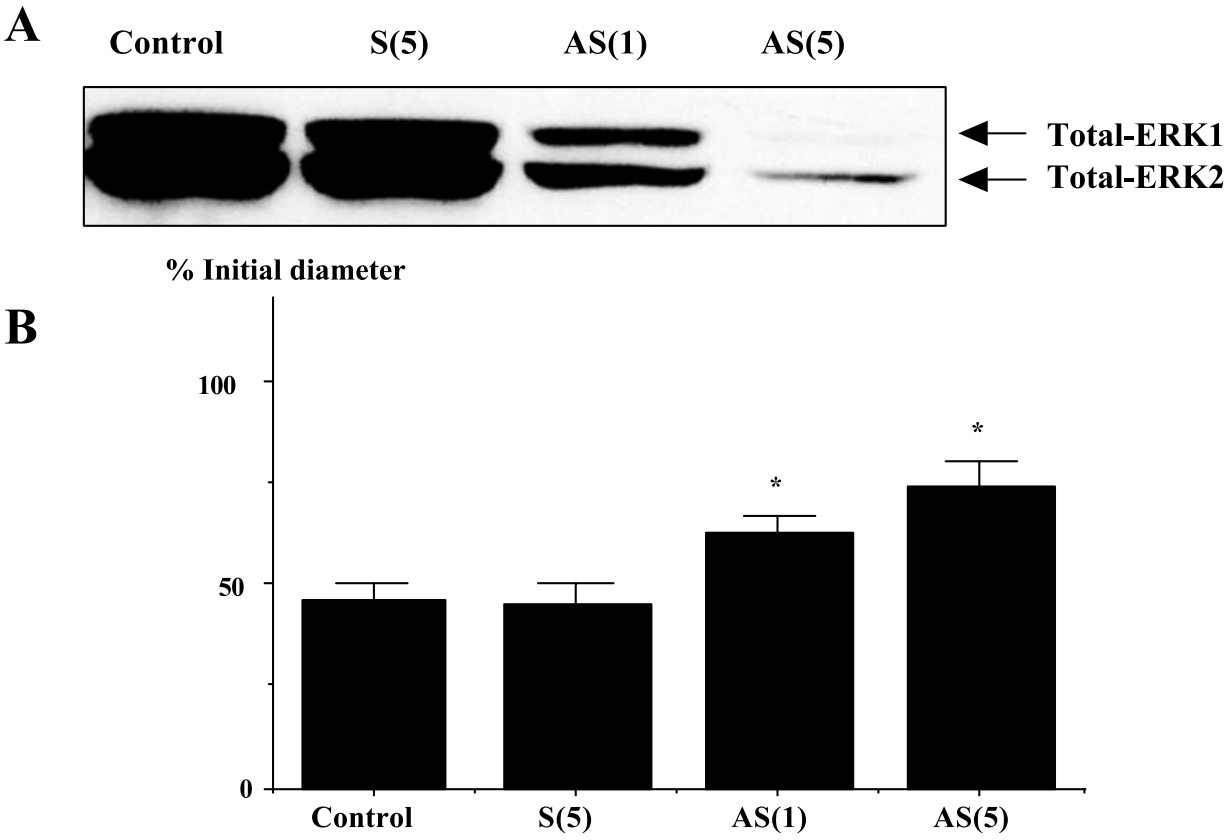

Figure. 2. The effect of ERK 1/2-specific antisense oligodeoxy-nucleotides (ODNs) on ERK 1/2 protein and collagen gel contraction by MCs (A, B). A. MCs were pretreated with the indicated concentrations (1,5 uM) of antisense ODNs (AS) or with control sense (S)(5uM) or without (vehicle control). Total cell lysates (10 ug) isolated from 4h collagen gel cultures were subjected to Western blot analysis for ERK 1/2 protein with anti-total ERK 1/2 antibody. B. MCs were subjected to 24h collagen gel contraction after exposure to AS (5uM), S (5uM) or without (vehicle control). The degree of gel contraction by MCs was compared with that by untreated, vehicle control cells (Control) (*P<0.01 versus control).

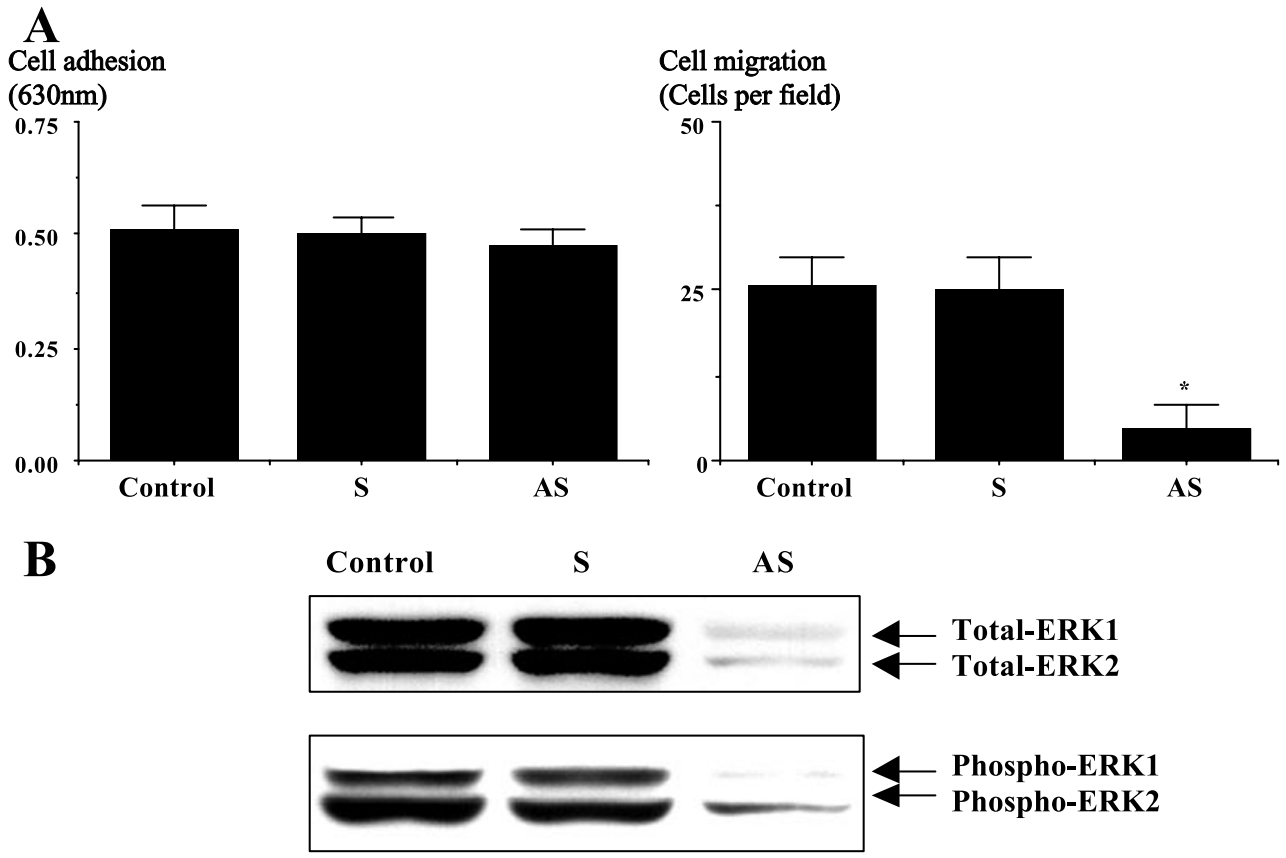

Figure. 3. The effect of ERK 1/2-specific antisense oligodeoxy-nucleotides (ODNs) on $\alpha 1\beta 1$ integrin-dependent MC adhesion and migration to collagen type I. A. MCs pretreated with either antisense ODNs (AS)(5uM) or with control sense (S)(5uM) or without (vehicle control) were subjected to adhesion and migration assays. B. In parallel to the MC adhesion assay, cell lysates (10ug) prepared from MC cultures treated with either AS, S or none (vehicle control) for 50 min were subjected to Western blot analysis with the anti-total ERK 1/2 antibody or anti-phospho-specific ERK 1/2 antibody.

ciation of Src with FAK is believed to be important for the reciprocal activation of those two kinases. Indeed, it has been reported that FAK-null cells show a dramatically reduced ability to assemble FN matrix, although their ability to attach to immobilized FN is only slightly impaired (76). Subsequently, Wierzbicka-Patynowski *et al*. showed using two different cell systems that the inhibition of Src by mutation of Src family kinase genes in SYF cells or with Src-specific inhibitors in CHOα5 cells significantly reduces FN matrix assembly (39). A further reduction in matrix assembly was seen with the concomitant inhibition of PI3-kinase activity. Therefore, they proposed that a subset of signaling molecules (Src, PI-3K) activated by FAK are essential for the proper initiation of FN matrix assembly. Analogous studies need to be performed for MCs to elucidate how MC-$\alpha 5\beta 1$ integrin may regulate FN assembly through an intracellular signaling pathway.

Integrin-linked kinase (ILK) is a cytoplasmic protein serine/threonine kinase that was identified based on its interaction with the $\beta 1$ integrin cytoplasmic domain (77). It is capable of interacting with several components of cell-matrix contact sites, including integrins ($\beta 1$, $\beta 3$ integrins), LIM protein PINCH, calponin homology domain-containing ILK binding protein CH-ILKBP, affixin, and paxillin (78). The PINCH-and CH-ILKBP-binding sites have been mapped to two separate domains (the N-and C-terminal domains, respectively) of ILK (79). Guo and Wu showed that ILK forms a tertiary complex with PINCH and CH-ILKBP in rat mesangial cells, which are co-clustered at fibrillar adhesions sites that are involved in FN matrix deposition (79). They demonstrated that the inhibition of PINCH-ILK-CH-ILKBP complex formation significantly reduced FN matrix deposition and inhibited cell proliferation, suggesting that the PINCH-ILK-CH-ILKBP complex is critically involved in the regulation of mesangial FN matrix deposition and cell proliferation in GN. Since glomerular mesangial ILK expression has been demonstrated to be increased in the expanded mesangium in patients with diabetic nephropathy and the ILK expression in MCs can be increased by hyperglycemia (80), the PINCH-ILK-CH-ILKBP complex may be a useful target in the therapeutic control of

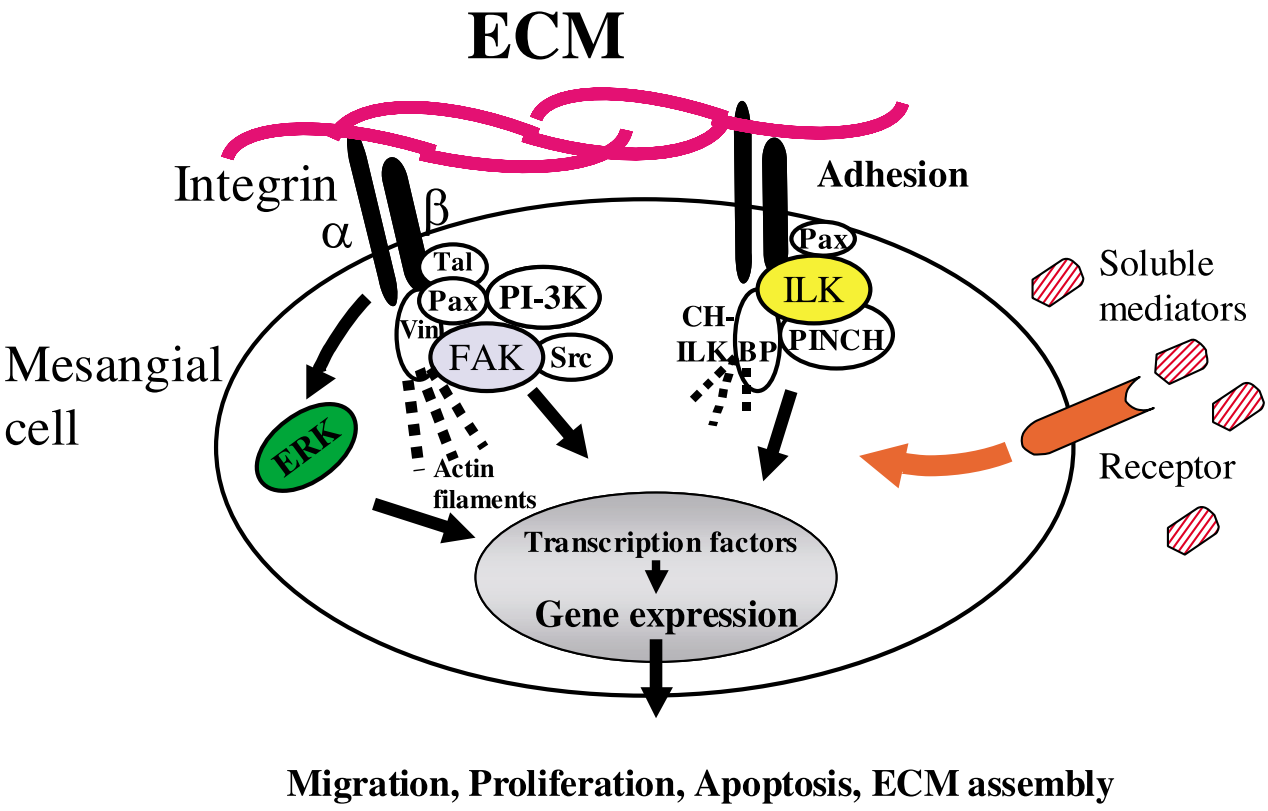

Figure. 4. Integrin control of mesangial cell behaviors. Cooperative activation of signaling molecules by β1-integrins and soluble mediator recepters control gene expression required for cell migration, proliferation, apoptosis and extracellular matrix (ECM) assembly. Pax, paxillin ; Tal, talin ; Vin, vinculin ; FAX, focal adhesion kinase ; PI-3 K, phosphatidylinositol-3 kinase ; ERK, extracellular-signal-regulated kinase ; ILK, integrin-linked kinase ; PINCH, particularly interesting new cystein-histidine rich protein ; CH-ILKBP, calponin homology domain-containing ILK-binding protein.

pathological processes involving abnormal cell proliferation and FN matrix deposition. Recently, we found that mesangial ILK expression and activity are upregulated in an experimental rat model of progressive GN, suggesting that ILK contributes to the progression of chronic GN (81).

While early mesangial injury is often associated with mesangial hypercellularity, advanced glomerulosclerosis is characterized by the massive accumulation of mesangial ECM and mesangial hypocellularity which appears to be irreversible even when the initial disease process subsides. Although the mechanisms of mesangial hypocellularity in glomerulosclerosis are not fully understood, several studies have suggested that the apoptosis of MCs is involved in the development of hypocellular lesions (82). Apoptosis is a regulated form of cell death that is crucial for maintaining an appropriate number of cells in tissue organization, as well as undesirable loss of cells in scar formation (82). Sugiyama *et al*. showed that in glomerulosclerosis there is an increase in MC apoptosis (83). Subsequently, they reported using β1 integrin antisense oligonucleotides that β1 integrin-mediated MC-ECM interaction regulates MC apoptosis (survival)(84). Moreover, Mooney *et al.* found in rat cultured MCs that the normal mesangial matrix proteins COL IV and LM promote MC survival by inhibiting apoptosis, via a β1 integrin-dependent but RDG (Arg-Gly-Glu) ligation-independent mechanism (85). In contrast, COL I, which is not expressed in normal glomeruli but overexpressed in sclerosing glomeruli, did not promote MC survival. Several mechanisms have been proposed to account for the β1 integrin-mediated suppression of apoptosis, including the upregulation of Bcl-2 expression, suppression of the proapoptotic enzyme IL-1β converting enzyme, and activation of MAP kinase and PI-3 K (86-88). Taken together, β1 integrins-mediated signaling pathways may regulate MC behaviors, such as cell migration, proliferation, ECM build-up ability and survival (apoptosis) in glomerular injury (Fig. 4). The same signaling molecules, such as FAK, ERK, PI-3K, are used to produce integrin-dependent, distinct MC phenotypes. Since many of these signaling pathways appear to interact, much more work is needed for identify the critical steps (molecules) that determine any specific β1 integrins-mediated MC phenotype.

CONCLUSION

The appropriate interaction between glomerular cells and ECM components is essential for maintaining the normal glomerular structure and function. Thus, disturbance in normal cell-ECM interactions may greatly influence the glomerular pathology in GN and the actual biology of glomerular cells, and determine the fate whether glomerular injury will progress or subside. The critical molecules controlling the glomerular cells-ECM interaction are the β1 integrins. β1 integrins are essential for glomerular cell adhesion and induce signals for migration, proliferation, survival and ECM assembly in close association with receptor signalings for soluble mediator molecules. To ascertain the cause of cell proliferation and abnormal ECM accumulation in nephritic glomeruli and the glomerular alteration of constituents in progressive GN, many researchers have been studied the regulation of gene expression for cell proliferatiom and ECM components induced by soluble regulatory molecules that act through a paracrine and/or autocrine mechanisms. To elucidate the cause of glomerulosclerosis and its pathophysiology, it is obiously needed to clarify the mechanism of remodeling to normal tissue in some cases, and the construction of irreversible sclerotic lesions in other cases, under the direct, three-dimensional interaction between glomerular cells and insoluble ECM components after glomerular injury. Clearly, analysis of β1 integrins-mediated signaling pathways and subsequent transcriptional regulations in these glomerular alterations is particularly important subject for future research. Studies from this perspective might result in the development of a new method of treatment for chronic, progressive GN, for which there is no conclusive treatment at present.

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