Glucose-induced changes in integrins and matrix-related functions in cultured human glomerular epithelial cells

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Interactions of cells with matrix molecules are primarily mediated by the integrin superfamilies receptors (14, 15). Most integrins recognize more than one ECM protein, such as collagens, fibronectin, and laminin (33), and, on binding, they transduce signals to the cell interior via mechanisms such as protein phosphorylation (6). For example, tyrosine kinase p125FAK [focal adhesion kinase (FAK)] becomes phosphorylated and activated after ligand-induced integrin clustering. Integrin ligation regulates cell functions such as adhesion, migration, anchorage-dependent growth, and gene expression (6, 11). FAK may function as a key mediator for these events by integrating signals from integrins.

One function attributed to integrins is regulation of the expression of matrix metalloproteinases (MMPs)/matrixins, zinc-dependent endopeptidases linked to the degradation and remodeling of ECM (24, 25, 34, 35, 45). Examples are gelatinases A and B (72-kDa gelatinase [MMP-2] and 92-kDa gelatinase [MMP-9], respectively), which degrade collagen types IV, V, VII, and IX, gelatin, elastin, and fibronectin (27, 28). Gelatinases are synthesized and secreted as inactive forms (pro-MMPs), and their matrix-degrading activities are regulated by activators and inhibitors. Most cells produce and secrete specific tissue inhibitors of metalloproteinases, TIMP-1 and TIMP-2, which preferentially bind to MMP-9 and MMP-2, respectively, thus regulating their matrix-degrading activity (27, 28).

To elucidate mechanisms leading to GBM thickening in DN, we have used as a model T-SV40 immortalized human glomerular epithelial cells (HGEC) to study whether increased glucose concentrations affected integrin-mediated interactions of these cells with type IV collagen, thus contributing to differential expression of several factors controlling ECM synthesis and degradation. T-SV40-immortalized HGEC express differentiation markers on the surface of primary glomerular epithelial cells, interact with type IV collagen, and are therefore similar to their primary counterparts (8, 22). The integrin profile of HGEC was examined in normal (5 mM) and high (25 mM) glucose, and quantitative

THE GLOMERULAR BASEMENT MEMBRANE (GBM) underlying glomerular epithelial cells, an important component of the kidney permselective barrier, is thickened in diabetic nephropathy (DN). GBM thickening could be due to increased deposition (synthesis and accumulation) of the extracellular matrix (ECM) macromolecules such as collagen, fibronectin, laminin, and proteoglycans, which could be explained by an imbalance between matrix synthesis and degradation. Matrix synthesis and degradation are regulated in part by cell-matrix interactions (7, 39).

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changes in integrin expression were observed in the presence of increased glucose concentrations. These changes were accompanied by modulation of integrin-mediated interactions of HGEC with type IV collagen, a predominant component of the GBM. Furthermore, high glucose altered the expression and production of proteins involved in matrix degradation. In our experiments, the expression of MMP-2 was regulated in part by α5β1-integrin, which, on ligation, enhanced FAK phosphorylation and resulted in upregulation of MMP-2.

Our findings suggest that increased glucose concentrations altered normal matrix-related cell functions of HGEC and resulted in differential gene expression, possibly contributing to matrix accumulation. The observed changes could help explain the thickening of the GBM in DN.

MATERIALS AND METHODS

Cell culture. HGEC (8, 22) were cultured at 37°C in media compounded of DMEM-Ham’s F-12 containing 1% FCS, 15 mM HEPES, 2 mM glutamine, ITS (5 μg/ml insulin, 5 μg/ml transferrin, and 5 ng/ml sodium selenite), 50 mM dexamethasone, antibiotics, and 5 or 25 mM d-glucose. Cells were released from their tissue culture flasks for passaging or use in experiments by treatment with 0.05% trypsin in 1 mM EDTA. For experiments, cells were cultured for at least three passages. For zymography and Western blotting analysis of conditioned media, cells (2 × 10⁶) were cultured for 48 h. The medium was then replaced with 10 ml of serum-free medium, and cells were incubated for 24 h. At the end of the 24-h incubation period, the conditioned media were collected, supplemented to 1 mM Na₂EDTA and 0.02% sodium azide, and stored at −20°C for further use.

Antibodies. Rabbit anti-human polyclonal antibodies to integrin subunits, collagenase MMP-9, and TIMP-1 and TIMP-2, as well as monoclonal antibodies (MAbs) against α₂ (P1E6), α₁ (P1B5), α₅ (P1D6), α₂β₁ (Lm609), and β₁ (P5D2)-integrin subunits, were obtained from Chemicon International. Polyclonal antibody Ab45 against collagenase MMP-2 was provided by Dr. Stetler-Stevenson. Anti-human leukaocyte antigen (HLA) MAb (W6/32) was used as negative control (catalog no. HB95, American Type Culture Collection, Rockville, MD). Antitubulin MAb was purchased from Sigma. Rabbit polyclonal anti-FAK antibody specific for human pp125FAK and antiphosphotyrosine MAb (clone 4G10) were obtained from Upstate Biotechnology. Peroxidase-conjugated goat anti-rabbit immunoglobulins and sheep anti-mouse immunoglobulins were purchased from Amersham.

Culture of cells with MAbs. Cells were detached from confluent monolayer cultures, resuspended in culture medium (3 × 10⁶ cells/ml), preincubated with MAb at 50 μg/ml each for 30 min at 37°C, and then diluted 10-fold to a final concentration of 5 μg/ml each or preincubated without antibody and cultured for 24 h. The medium was then replaced with serum-free medium with or without 5 μg/ml of each MAb, and cells were incubated for 24 h. At the end of the 24-h incubation period, the conditioned media were collected for zymography, and the cells were harvested and counted.

Isolation of type IV collagen. Type IV collagen was isolated from the Engelbreth-Holm-Swarm tumor system using previously described techniques (19, 29). Protein concentration was determined using the method of Waddell (44).

Cell adhesion assay. Microtiter plates (96-well; Microlon 600, Greiner) were coated with 50 μl of increasing concentrations of type IV collagen (0.3–200 μg/ml PBS) and allowed to evaporate to dryness at 29°C. The remaining reactive sites were blocked with 0.2% BSA in PBS for 2 h at 37°C. Plates were then washed once with PBS and immediately used for experiments. HGEC were grown in 5 or 25 mM d-glucose in T-25 flasks until 75–80% confluency was reached and were metabolically labeled for 18 h with 0.15 mCi of [³⁵S]methionine (Amersham) per flask. Cells were washed twice with DMEM, and 50 μl (5,000 cells) of cell suspension in binding buffer (DMEM, 2 mg/ml BSA, and 25 mM HEPES, pH 7.5) were added to each well and allowed to adhere for 45 min at 37°C. The wells were then washed three times with binding buffer to remove nonadherent cells, and lysis buffer (0.5 N NaOH and 1% SDS) was added to each well. The lysate was transferred to scintillation vials and counted. For inhibition of cell adhesion to type IV collagen, cells were processed as for the adhesion assay. In competition experiments, the following criteria were selected to achieve optimal antibody effect: half-maximal binding of HGEC to type IV collagen by using 5 μg/ml type IV collagen and a short-term assay (30 min). After plates were coated with 50 μl of type IV collagen at 5 μg/ml, MAb to integrin subunit or MAb to HLA was added to each well (100 μl/well) at a final concentration of 10 μg/ml followed immediately by 50 μl of cell suspension in binding buffer (5,000 cells/well). Cells were allowed to adhere for 30 min at 37°C and processed as for the adhesion assays. The concentration of antibodies used in inhibition assays was above the saturating concentration as determined by flow cytometry. The data were normalized by expressing the binding in the absence of antibody as maximal (100%), and adhesion in the presence of antibodies is shown as percentage of binding in the absence of antibody. All assays were performed a minimum of three times in hexaplicate for each experimental condition.

Zymography. Gelatin zymography was performed as previously described (3). Briefly, aliquots of each sample of conditioned media were concentrated and subjected to SDS-PAGE under nonreducing conditions in 10% polyacrylamide gels containing 0.1% gelatin. The volume of conditioned medium loaded per lane was adjusted according to the cell number obtained at harvest. After electrophoresis, the gel was washed three times for 30 min each with 50 mM Tris-HCl, pH 7.5, 5 mM CaCl₂, 1 mM ZnCl₂, 2.5% Triton X-100, and 0.02% NaN₃ at room temperature, incubated in the same buffer without Triton X-100 for 48 h at 37°C, stained with Coomassie Brilliant blue R-250 for 3 h, and destained in water.

Total protein extraction. Cells were lysed in a buffer containing 1% Triton X-100, 1 mM CaCl₂, a cocktail of protease inhibitors (catalog no. P8340, Sigma), 1 mM phenylmethylsulfonyl fluoride, and 1 mM N-ethylmaleimide in PBS for 30 min at 4°C. Insoluble material was removed by centrifugation, and the supernatant was stored at −20°C. Protein estimation was performed by the method of Bradford (Pierce).

Electrophoresis and immunoblotting. Electrophoresis in the presence of SDS was performed on 7.5% or 10% polyacrylamide gels under nonreducing conditions in 10% polyacrylamide gels containing 0.1% gelatin. The volume of conditioned medium loaded per lane was adjusted according to the cell number obtained at harvest. After electrophoresis, the gel was washed three times for 30 min each with 50 mM Tris-HCl, pH 7.5, 5 mM CaCl₂, 1 mM ZnCl₂, 2.5% Triton X-100, and 0.02% NaN₃ at room temperature, incubated in the same buffer without Triton X-100 for 48 h at 37°C, stained with Coomassie Brilliant blue R-250 for 3 h, and destained in water.

Electrophoresis and immunoblotting. Electrophoresis in the presence of SDS was performed on 7.5% or 10% polyacrylamide gels under reducing or nonreducing conditions. The resolved proteins were subsequently electrotransferred to Hybond-ECL nitrocellulose membrane (Amersham). Blots were saturated for 2 h at room temperature with 5% nonfat milk in Tris-buffered saline-0.1% Tween 20 and incubated overnight at 4°C with the appropriate dilutions of polyclonal antibodies in the same buffer without Tween 20. Incubations with peroxidase-conjugated goat anti-rabbit immunoglobulins or sheep anti-mouse immunoglobulins and detection of bound peroxidase activity were carried out as described for
the enhanced chemiluminescence blotting detection system (Amersham).

Immunoprecipitation of FAK. Confluent HGEC cultured in 5 mM d-glucose were serum starved for 24 h before detachment with trypsin. Cells were washed twice with DMEM, suspended in DMEM containing 25 mM HEPES and 2 mg/ml BSA, and incubated in suspension at 37°C for 45 min to allow kinases to become quiescent. Then 3 × 10⁶ cells were incubated in suspension at 37°C for 90 min with or without 10 μg/ml of each anti-α5- and anti-β1-integrin MAb. At the end of the incubation period, cells were washed twice with cold PBS and lysed in a modified RIPA buffer containing 50 mM Tris·HCl, pH 7.4, 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM Na₂EDTA, 1 mM phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate, 1 mM sodium pyrophosphate, 50 mM NaF, and a cocktail of protease inhibitors. Then 500 µg of protein from each cell lysate were incubated overnight at 4°C with 2 µg of a rabbit polyclonal antibody specific for human pp125FAK. Immune complexes were precipitated for 2 h at 4°C with protein A-Sepharose and washed three times in ice-cold modified RIPA buffer. Immune complexes were extracted into boiling Laemmli sample buffer containing 10% β-mercaptoethanol, electrophoresed on 7.5% polyacrylamide gels, and electrotransferred to nitrocellulose membrane. A monoclonal antiphosphotyrosine antibody was used to analyze Western blots for phosphotyrosine-containing proteins. The immunoblots were stripped in Re-Blot Plus Western blot stripping solution (catalog no. 2500, Chemicon) as recommended by the manufacturer, and then the proteins were immunoblotted with anti-FAK antibody to determine whether equal amounts of FAK were loaded per lane.

Statistical analysis. Values are means ± SD. In the assays of adhesion and inhibition of adhesion, the means of groups were compared using one-way ANOVA with post hoc testing using the Newman-Keuls test as appropriate. Results from images of Western blots and zymograms were analyzed using Student’s t-test and one-way ANOVA. Both tests gave similar results. P < 0.05 was considered statistically significant.

RESULTS

Expression of integrins by cultured HGEC in 5 and 25 mM glucose. Integrins may play a role in altered matrix synthesis and degradation in diabetic conditions. Therefore, we examined and compared the expression of the main integrin subunits of HGEC grown in 5 and 25 mM glucose. Western blot analysis (Fig. 1) demonstrated that α3-, β1-, and α2-integrins were decreased by 30–35%, whereas the protein levels of α5-, αv-, and β3-integrins were increased by 90%, 60%, and 30%, respectively, in HGEC grown in 25 mM glucose compared with cells grown in 5 mM glucose. These data were also confirmed by flow cytometry (data not shown). The observed effects were specifically due to d-glucose and not to osmotic effect, because Western blot analysis of cells grown in 25 mM L-glucose revealed no change in the expression of integrin subunits compared with cells grown in 5 mM d-glucose. Representative blots of the main α3- and β1-integrins are shown in Fig. 1C.

HGEC adhesion to type IV collagen in the presence of 5 and 25 mM glucose. The previous experiment indicated that the presence of increased glucose concentrations resulted in up- or downregulation of different integrin subunits of HGEC. This change could be accompanied by changes in cell adhesion to the GBM and its individual components. Therefore, we examined integrin-mediated adhesion of HGEC to type IV collagen by solid-phase binding assays. Type IV collagen binding of HGEC grown in 25 mM glucose was decreased by ~15–45% compared with cells grown in 5 mM glucose, depending on the concentration of type IV collagen used (Fig. 2). The observed differences were statistically significant (P < 0.05) in all but the highest concentration of type IV collagen.

Integrins mediating the binding of HGEC to type IV collagen in 5 and 25 mM glucose. For this experiment, adhesion of HGEC to type IV collagen in the presence of various inhibiting anti-integrin monoclonal antibodies was examined. The extent of inhibition of adhesion varied depending on the type of antibody used against different integrin subunits and glucose concentration. More specifically, anti-β3-integrin antibodies (P5D2) almost completely inhibited the adhesion of HGEC to

![Fig. 1. Western blot analysis of integrin expression in human glomerular epithelial cells (HGEC) cultured in low and high glucose. A: total protein was extracted from cells cultured in 5 mM (L) or 25 mM (H) d-glucose. Total protein (20 µg) was analyzed on 7.5% SDS-PAGE under nonreducing conditions and immunoblotted with the appropriate dilution of polyclonal antibodies against integrin subunits. Blots were stripped and reprobed with antitubulin antibody to verify protein loads, to which all quantitative data were normalized. B: quantification of protein content of each integrin subunit by scanning densitometry. Value of each control (open bar) is set at 100%. Values are means ± SD of 3 independent experiments. *P < 0.05 vs. 5 mM (t-test and 1-way ANOVA). C: Western blot of α3- and β1-integrin expression in HGEC cultured in 5 mM d-glucose (L) or 25 mM L-glucose (H).](http://ajprenal.physiology.org/DownloadedFrom/)
Fig. 2. Adhesion of HGEC to solid-phase type IV collagen. [35S]methionine-labeled HGEC cultured in media containing 5 or 25 mM d-glucose were seeded in 96-well plates (5,000 cells/well) coated with increasing concentrations of type IV collagen and allowed to adhere for 45 min at 37°C. Nonadherent cells were washed off, adherent cells were lysed, and radioactivity was quantitated. Bound counts are expressed as percentage of total counts to give percent adhesion. Values are means ± SD of 6 replicates and were analyzed using 1-way ANOVA and Newman-Keuls test. *P < 0.05 vs. 25 mM (1-way ANOVA).

Type IV collagen in 5 and 25 mM glucose (Fig. 3). In 5 mM glucose, antibodies Lm609 and P1D6 against the αvβ3- and αvβ5-integrins, respectively, resulted in ~14% and ~36% inhibition of maximal adhesion (adhesion in 5 mM glucose in the absence of antibody), respectively (Fig. 3A), whereas in 25 mM glucose, ~60% and ~55% inhibition of maximal adhesion (adhesion in 25 mM glucose in the absence of antibody), respectively, was observed (Fig. 3B). In 5 mM glucose, antibody P1E6 against the α2β1-subunit resulted in 40% inhibition of maximal adhesion (Fig. 3A), whereas in 25 mM glucose, only ~13% inhibition of maximal adhesion was observed (Fig. 3B). MAb P1B5 against α3-integrin caused HGEC aggregation and was not effective in blocking adhesion. However, using the F(ab) fragment of anti-α3-integrin MAb (P1B5), we previously documented that α3-integrin participated in the binding of HGEC to type IV collagen (22). There was no inhibition of adhesion by MAbs against HLA (negative control).

Expression of matrixins (MMP-2 and MMP-9) and their inhibitors (TIMP-1 and TIMP-2) in 5 and 25 mM glucose. We first compared proteolytic activities of conditioned media from HGEC grown in 5 or 25 mM glucose with gelatin zymography. Enzymatic activity was detected at two major bands corresponding to 92-kDa proenzyme and 68-kDa active form of MMP-2) compared with media from control cells grown in 5 mM glucose (Fig. 4A). Densitometric analysis indicated that media from HGEC grown in 25 mM glucose contained 70% less of the 72/68-kDa form of MMP-2 than media from cells grown in 5 mM glucose. There were no significant changes in the total amount of the 92/88-kDa form of MMP-9 (Fig. 4B).

We then examined and compared the protein levels of MMPs expressed by HGEC grown in 5 and 25 mM glucose. Western blotting of conditioned media from HGEC grown in 25 mM glucose demonstrated an ~70% decrease in MMP-2 total protein levels (bands corresponding to 72-kDa proenzyme and 68-kDa active form of MMP-2) compared with media from control cells grown in 5 mM glucose (Fig. 5, A and B). Additionally, the latent (92 kDa) and activated (88 kDa) forms of MMP-9 were detected in media from HGEC, but protein levels were not affected by high-glucose treatment (Fig. 5, A and B).

Furthermore, expression of TIMPs was determined by Western blotting in conditioned media from HGEC grown in 5 and 25 mM glucose. TIMP-2, a single 22-kDa immunoreactive band, was increased by 2.5-fold in high-glucose media compared with control media. In contrast, the 28-kDa TIMP-1 immunoreactive band underwent a modest decrease (~30%) compared with the control (Fig. 5, C and D).

Effect of ligation of αvβ3- and αvβ5-integrins on MMP-2 expression in 5 and 25 mM glucose. This experiment was performed to examine the possible role of αvβ3, the main integrin dimer in HGEC (22), in the expression of MMPs in low- and high-glucose conditions. HGEC grown in 5 or 25 mM glucose were cultured in the absence or presence of MAbs against α3-
and β1-integrins (see MATERIALS AND METHODS). Alternatively, antibodies against α3β3-, α5-, and α2-integrins were used for the same experiment.

The presence of MMP-2 in conditioned media was examined by gelatin zymography. In HGEC grown in 5 mM glucose, ligation of α3β1-integrin with anti-α3- and anti-β1-integrin MAbs simultaneously resulted in a ∼2.5-fold increase in the amount of secreted MMP-2 compared with untreated cells (Fig. 6, A and B), whereas ligation of integrin subunits with anti-α3- or anti-β1-integrin MAb caused a modest increase (40–45%) in MMP-2 secretion compared with control untreated cells (Fig. 6, C and D). This observation suggested a cooperative effect of the two subunits. In comparison, ligation of α3β3-integrin, another abundant integrin dimer of HGEC, with specific anti-α3β3-integrin MAbs, had no effect on the amount of secreted MMP-2 (Fig. 6, A and B). Moreover, ligation of α5- or α2-integrin with specific anti-integrin MAbs had no effect on the secretion of MMP-2 (data not shown).

When ligation of α3β1-integrin was performed by specific antibodies in HGEC grown in 25 mM glucose, the amount of MMP-2 released in the media was substantially less than in cells grown in 5 mM glucose in the presence of the same antibodies (Fig. 6, A and B). In 25 mM glucose, the amount of MMP-2 in HGEC media in the presence of anti-α3- and anti-β1-integrin antibodies was similar to the amount secreted by control cells, which were grown in 5 mM glucose in the absence of antibodies. Again, treatment of HGEC grown in 25 mM glucose with anti-α3- or anti-β1-integrin antibody alone caused a modest increase (30–35%) in MMP-2 levels compared with cells grown in 25 mM glucose in the absence of antibodies (Fig. 6, C and D).

Phosphorylation of FAK in HGEC treated with MAbs against α3- and β1-integrins. FAK has been described to be a component of the signaling pathway that mediates regulation of the expression of MMPs by integrins. Therefore, we examined tyrosine phosphorylation of pp125FAK in control (untreated) HGEC and HGEC treated with MAbs against α3- and β1-integrins simultaneously (Fig. 7). In cells treated with anti-α3- and anti-β1-integrin MAbs, there was a ∼30% increase in phosphotyrosine of pp125FAK compared with control.
untreated cells. To ensure equal amounts of protein loading, blots were stripped and reprobed with polyclonal anti-FAK antibodies.

DISCUSSION

We have provided evidence that increased glucose concentrations modulate integrin expression and integrin-related functions of cultured T-SV40-immortalized HGEC, which were shown to be similar to their primary counterparts (8, 22). The observed effects were specifically due to D-glucose and not to osmotic effect. We and others previously showed that L-glucose, ribitol, or mannitol had no effect (2, 30). We previously reported that/H92513/H92521 is the main integrin present at high density on the surface of/H1101197% of HGEC grown in the presence of 5 mM glucose (22). This dimer can mediate the binding of HGEC to collagen and laminin components of the GBM (22, 40). In addition, HGEC expressed α5-, α2-, and αβ3-integrins, which have been described to mediate cell binding to collagens, fibronectin, and fibrinogen (15).

In HGEC cultured in the presence of 25 mM glucose, we observed a significant decrease in α3β1 integrin when examined with immunoelectron microscopy; this decrease was thought to represent an early event that precedes the onset of DN (31, 32). Therefore, the reported findings in podocytes in diabetic conditions in situ corroborate our observations related to the effects of high glucose in cultured HGEC, insofar as the expression of α3β1 integrin was concerned. The mechanisms by which hyperglycemia alter the expression of integrins are not clearly understood. In kidneys, transforming growth factor-β1 (TGF-β1) has been reported to be a strong regulator of the expression of integrins (38). TGF-β1 has been demonstrated to suppress the expression of α3β1 integrin in glomeruli from nephrotic rats (18), and an overexpression of TGF-β1 has also been reported to occur in diabetic rat kidneys as early as 2–3 days after induction of hyperglycemia (37). Therefore, one mechanism of altered integrin expression could involve overexpression of TGF-β1.

Furthermore, our data provide evidence that glucose-induced changes in integrin expression were accompanied by altered binding to GBM components, such as type IV collagen. Type IV collagen was selected for the study of interactions with HGEC, because it is the predominant glycoprotein of the GBM (8, 22). We observed that high glucose decreased the number of HGEC bound to solid-phase type IV collagen. This binding was mediated in part by different integrins depending on glucose concentration. For example, there was substantial inhibition of HGEC binding to

![Fig. 6. Effects of anti-integrin MAbs on MMP-2 expression and secretion in HGEC. Cells were cultured in 5 or 25 mM D-glucose medium with or without 5 μg/ml of each anti-integrin MAb for 24 h at 37°C. Medium was then replaced with serum-free medium with or without 5 μg/ml of each MAb, and cells were cultured for 24 h. A and C: aliquots of each sample of conditioned media were analyzed by gelatin zymography (volume of conditioned medium loaded per lane was adjusted according to cell number obtained on harvest). B and D: results from gelatin zymography were analyzed by scanning densitometry. Values are means ± SD of 3 independent experiments. *P < 0.05 vs. no MAb (t-test and 1-way ANOVA).](http://ajprenal.physiology.org/)

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glucose-dependent switch of integrin subunits that bind HGEc to type IV collagen. Anti-β₁-integrin MAb resulted in nearly complete inhibition of adhesion to type IV collagen in either glucose concentration, as expected. The data indicate that β₁ is a predominant integrin; indeed, by fluorescein-activated cell sorter analysis, it is present at a high density on the surface of 84% of primary HGEc and 97% of immortalized HGEc (22). This subunit associates with different α-subunits, thus serving for multiple binding events to matrix components.

In summary, different integrins in part mediate HGEc binding to type IV collagen, depending on glucose concentration. In low glucose, α₂ and α₅-integrins participated in the binding, whereas, in high glucose, the α₅β₁- and α₅-subunits were preferentially used. If similar changes occur in situ, in diabetic conditions, then altered interactions with matrix components could be anticipated to alter integrin-mediated signaling and various aspects of integrin-regulated cell functions, including protein phosphorylation (20, 21) and gene expression (3, 6, 11, 17).

For example, the expression and/or activation of several MMPs was shown to be regulated by integrins in different cell types (1, 9, 13, 24, 25, 34, 35, 45, 47). Our experiments indicate that changes in integrin expression in HGEc grown in 25 mM glucose were accompanied by changes in the expression and/or activity of MMP-2. In vivo, β₁-integrin associates with different α-subunits serving for multiple binding and specific signaling events. Because α₃β₁-integrin is the main subunit expressed by HGEc (22) and, moreover, there was decreased expression of α₃β₁-integrin and MMP-2 in 25 mM glucose compared with the control (5 mM glucose), we examined the hypothesis that α₃β₁-integrin was involved in regulation of MMP-2 expression. In HGEc grown in 5 mM glucose, ligation of the α₃β₁-integrin dimer with anti-α₃ and anti-β₁-integrin MAbs simultaneously resulted in a 2.5-fold increase in secreted MMP-2 compared with the untreated control, whereas MMP-9 remained unchanged (data not shown). The effect of α₁- or β₁-integrin ligation was more modest, resulting in a 40–45% increase of MMP-2 secretion. Antibodies against α₅β₁-integrin, which was increased in high glucose, had no effect on the expression of MMP-2. Furthermore, ligation of other integrins, such as α₅ or α₂, with specific anti-integrin MAbs had no effect on the secretion of MMP-2 (data not shown). Upregulation of MMP-2 by ligation of α₃β₁-integrin was also observed in HGEc cultured in 25 mM glucose, albeit to a lesser extent. Glucose-induced decrease of the expression of α₃β₁-integrin could possibly account for this difference.

Our findings suggest then that α₃β₁-integrin in part regulates the expression of MMP-2 in HGEc. Several other reports showed that ligation of α₃β₁-integrin with MAbs resulted in upregulation of MMP-2 production by tumor cells (5, 41, 43) or induced the activated form of MMP-2 and enhanced pro-MMP-2 secretion by rhabdomyosarcoma cells (23).
The mechanism by which αβ₃-integrin enhances the expression of MMP-2 remains to be substantiated. It has been previously documented that a major signaling pathway linking integrins to the regulation of the expression of MMPs involves FAK, mitogen-activated protein kinase, and transcription factor AP-1 (21, 42).

In our experiments, we observed increased FAK phosphorylation in HGEC as a response to αβ₃-integrin ligation, a finding that indicates the possible involvement of the above-mentioned pathway in regulation of the expression of MMP-2 in this cell type. This hypothesis is also in accord with a recent report showing that integrin interactions with their ligand can transduce stimulatory signals (through FAK-Src-type kinases) for MMP-2 and MMP-9 expression in human T lymphocytes (10). However, there might be alternative or additional regulatory mechanisms. Whatever the mechanism, the observed decreased expression of MMP-2 could result in impaired degradation of basement membrane components.

We additionally observed a substantial increase in the expression of TIMP-2, the specific inhibitor of MMP-2, a finding that suggests further impairment of matrix degradation, leading to matrix accumulation. The modest decrease in TIMP-1, the specific inhibitor of MMP-9, could be compensatory.

In situ, even a small decrease in MMP-2 and/or increase in TIMP-2 protein levels could be anticipated to impair the balance between ECM synthesis and degradation, resulting in matrix accumulation. We and others documented that increased glucose concentrations resulted in altered expression of matrix, MMPs, and TIMPs in cultured mesangial cells (3, 26) and also in situ in kidneys of streptozotocin-diabetic rats at early stages of diabetes in the absence of albuminuria (46).

Collectively, our data indicate that glucose-induced modulation of integrin expression was accompanied by functional changes in HGEC in vitro, which in turn could contribute to altered interactions with type IV collagen, a major component of the GBM and, in addition, decreased degradation of matrix proteins. The combined long-term effect could be microalbuminuria due, at least in part, to altered HGEC binding to the GBM components and matrix accumulation, which, if present in situ, could help explain the thickening of the GBM in DN.

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REFERENCES


