Regulation of integrin expression by Gα12

An additional potential mechanism modulating cell attachment

Tianqing Kong,1,* Daosong Xu,2 Mei Tran1 and Bradley M. Denker1
1Renal Division, Brigham and Women’s Hospital; Harvard Medical School; Boston, MA USA; 2Dana Farber Cancer Institute; Boston, MA USA

Integrins regulate cell attachment and migration through interactions with specific proteins in the extra-cellular matrix. Heterotrimeric G proteins are essential signal transduction proteins that intersect with integrin signaling to regulate fundamental cellular behaviors. Although integrin and G protein signaling often act in concert, how these mechanisms interact in epithelial cells has not been extensively studied. We recently reported Gα12 regulation of epithelial cell attachment and migration on collagen-I through α2β1 integrins (Kong et al. Mol Biol Cell 2009). Activated Gα12 inhibited α2β1 integrin functions through an inside-out signaling mechanism that involved Rho, Src and protein phosphatases without affecting α2 or β1 expression. Activated Gα12 prevented tubulogenesis in 3D-MDCK cell cultures and promoted the formation of cystic structures. Herein, we extend these findings to show Gα12-stimulated transcriptional changes in integrin expression that affect MDCK cell attachment. Based on results from a microarray with MDCK cells expressing constitutively active Gα12 (QLα12), we confirmed with real time PCR that expressing QLα12 led to a 4-fold inhibition of α6 mRNA expression. Cell surface expression and total α6 protein was reduced by FACS and immunofluorescence. QLα12 expressing MDCK cells also revealed less attachment to laminin-5, α6 integrin ligand. Taken together, G proteins regulate integrins through canonical signaling pathways and potentially regulate integrin expression levels to modulate cellular responses in a variety of pathophysiologic conditions including polycystic kidney disease.

Epithelial cells must maintain complex interactions with each other and the underlying matrix. Appropriate regulation of these cell-cell and cell-matrix interactions are essential for polarized functions and barrier formation, yet epithelial cells must also migrate and establish these connections during development and recovery from epithelial injury. Disregulation of epithelial cell attachment and adhesion contributes to metastatic potential in epithelial malignancies, and re-establishing an intact epithelial layer is essential for recovery from ischemic or toxic injury of epithelial organs such as the kidney and intestine. These processes are also important in hereditary diseases such as Autosomal Dominant Polycystic Kidney Disease (ADPKD), a genetic disease characterized by renal failure due to progressive growth of cystic structures originating within renal tubules. Although integrin functions and signaling interact with heterotrimeric G protein signaling, there were few studies linking these pathways in epithelial cells. We recently reported Gα12 regulation of MDCK cell attachment, invasion and migration on collagen-I, and we identified regulation of α2β1 integrin function through an inside-out signaling mechanism involving canonical pathways (Rho, Src and phosphatases). This Commentary & View will briefly summarize those findings and provide new data revealing potential regulation of integrin gene expression by activated G proteins. A brief discussion of possible implications

Key words: G proteins, kidney, extra-cellular matrix, collagen, laminin, gene expression

Abbreviations: ADPKD, autosomal dominant polycystic kidney disease

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*Correspondence to: Tianqing Kong; Email: tkong@rics.bwh.harvard.edu
Integrins are a large family of heterodimeric (α and β subunits) single transmembrane glycoproteins that mediate cell interactions with the matrix. There are 18α and 8β subunits, and they assemble into 24 distinct integrins (reviewed in ref. 1). They are ubiquitously expressed in all cell types, and in addition to interacting with the matrix, they also function as receptors to stimulate signaling pathways important for cell migration, proliferation, cell survival and cytoskeletal organization. The ligands for integrins are components of the extra-cellular matrix, and unique integrin dimers preferentially interact with specific components of the extra-cellular matrix. The extra-cellular matrix composition varies amongst tissues, and this diversity, combined with specificity of integrin dimers provides for a wide range of potential cell-matrix interactions. For example, α1β1, α2β1, α10β1 and α11β1 are collagen receptors and α3β1, α6β1 are laminin receptors. α3 and α6 can also dimerize with β4. The renal tubular basement membrane is predominantly collagen-I, a ligand for α2β1 integrins, while the glomerular basement membrane collagen is predominantly collagen IV, a ligand for α5β1 integrin. Integrin cytoplasmic domains interact with a complex containing talin, vinculin, paxillin and numerous adaptor proteins that link to multiple signaling pathways including FAK, Src, MAP kinase, PI-3 kinase and Rho pathways leading to the pleiotropic effects of integrin activation (reviewed in ref. 1).

Heterotrimeric G proteins (four major families named for the Gt subunit (Gtα, Gtα/i/o, Gtαq and Gt12/13)) are ubiquitously expressed and signal through seven transmembrane G-protein-coupled receptors (GPCRs). Activation of a GPCR leads to a conformational change in Gtα and dissociation of bound GDP. GTP is normally present in higher concentrations than GDP, thus favoring GTP binding to the Gtα subunit. GTP binding activates Gtα and leads to dissociation of Gt from Gβγ and permits interactions with downstream effectors until the system is reset by the hydrolysis of GTP to GDP on Gtα. Integrins and G proteins converge on numerous signaling pathways including Rho and non-receptor tyrosine kinases such as Src. In hematopoietic cells and fibroblasts, direct links from G protein signaling to integrin function have been described. In platelets, integrin mediated aggregation was stimulated with thrombin, ADP, epinephrine, thromboxane and other agonists that utilize numerous G protein coupled pathways. Costimulation of Gt12/13 and Gt3 pathways (with thromboxane A2 and ADP) led to irreversible integrin (αIIbβ3)-mediated aggregation. In splenic B cells, lysosphatidic acid (LPA) regulated integrin mediated adhesion through Gt3 and Gt12/13 pathways. The leading edge of migrating leukocytes utilized Gt3 stimulated production of 3′-phosphoinositool lipids and activated Rac, while the trailing edge used Gt12/13 regulation of Rho, and both systems coordinate differential regulation of the actin cytoskeleton in the presence of chemoattractant. In fibroblasts, Gt12/13 were required for directed cell migration in wound healing assays, and cells lacking Gt12/13 failed to localize active Rho or its effector mDia at the wound edge.

Gt12/13 family of G proteins regulate numerous cellular functions and have well described roles in regulating Rho signaling and the actin cytoskeleton through direct interaction with the Rho exchange proteins p115RhoGEF and PDZ-RhoGEF. Gt12/13 have important cellular functions regulating cell growth, transformation, apoptosis and polarity (reviewed in ref. 9). Increased Gt12/13 protein levels were described in prostate and breast cancer, which was associated with increased invasion and metastasis but not proliferation. Gt12/13 also regulate cell-cell adhesion through interactions with adherens junction protein, E-cadherin, and Gt12 regulates tight junction assembly through Src tyrosine phosphorylation of tight junction proteins including the Gt12 binding protein ZO-1. Taken together with the interaction of G protein and integrin signaling in fibroblasts and hematopoietic cells, it was likely that G proteins also overlap with integrin signaling pathways in epithelia.

We recently examined the role of Gt12 in regulating MDCK cell attachment to different substrates, invasion, wound healing and tubulogenesis. Using well characterized inducible MDCK cell lines overexpressing wild type Gt12 and the GTPase deficient and constitutively active Gt12 (QLα12), we found evidence for Gt12 dependent cell attachment to collagen-I, the major ligand for α2β1 integrins. We identified impaired invasion of MDCK cells and impaired recovery from wound healing with Gt12 activation (QLα12 expression) by activation of endogenous Gt12 with thrombin. There was Gt12-dependent loss of focal adhesions and activation of both Rho and Src signaling. Examination of FAK and paxillin phosphorylation with and without Gt12 activation revealed an inside-out signaling pathway that also required an unidentified protein tyrosine phosphatase. In 3D tubulogenesis assays that were composed of collagen-I, activated Gt12 impaired tubulogenesis and led to the formation of cyst-like structures, and this effect was blocked in Gt12-silenced cells.

Although these studies revealed an important role for Gt12-mediated attachment to collagen-I and numerous consequences for epithelial cell function, our studies also suggested Gt12 regulation of other integrin families. For example, we found decreased attachment to matrigel and laminin-1 suggesting regulation of α6, αβ1 and α7 integrins. We had examined cell surface protein expression for α1, α2, α3 and β1 integrins and found no difference with QLα12 expression. Although there could be overlapping signaling pathways with other integrin families, we reasoned that there might be Gt12-regulated gene expression of other integrins that account for some of the Gt12-dependent interactions with other matrix components. To address this possibility, we performed a microarray (in triplicate) with QLα12-MDCK cells with and without QLα12 protein expression for 72 h. We identified significant downregulation of integrin α6, β8 and several other proteins potentially involved in cell adhesion (claudin 16 and cadherin 5). α6 integrin was reduced 2.2-fold and was seen with two independent probes. Both α6 and β8 integrins form heterodimers with integrin αv and serve as receptors for fibronectin. However, we did not detect significant interactions of control MDCK cells with

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fibronectin perhaps due low endogenous levels of αv integrin in renal epithelia.

Activated Gq12 leading to downregulated α6 integrin expression was first confirmed by reverse-transcription PCR (Fig. 1A). We next quantified α6 mRNA relative to actin by real time PCR (Fig. 1B) and found about a 4-fold reduction with QLα12 expression. Efforts to confirm reduced α6 integrin protein expression blot of total lysates was unsuccessful due to lack of anti-α6 antibodies that can detect α6 protein in MDCK cells by western. To gain insights into protein expression in MDCK cells, we examined cell surface expression of α6 by flow cytometry, and a significant reduction was observed (Fig. 1C) in QLα12-induced MDCK cells versus the control. In addition, immunofluorescent microscopy was performed on MDCK cells +/- QLα12 expression and images obtained under identical conditions (Fig. 1D). Semi-quantification of the fluorescent signal (using Image J 1.38, Wayne Rasband) showed about 2.5-fold more α6 integrin in control MDCK cells (-QLα12) versus +QLα12 expressing.

Figure 1. Effect of QLα12 on α6 mRNA, Surface Expression and Attachment to Laminin-5. (A) Reverse transcription. Total RNA was purified from MDCK cells with (+) and without (-) QLα12 expression for 72 h using standard methods. 5 μg of total RNA was reverse transcribed and equal amounts of cDNA amplified for β-actin and α6. The oligonucleotide primer sequences used for β-actin and α6 were as follows: β-actin: sense: 5’-CGC TAG TAG ATA AGC GCT C-3’; antisense, 5’-GCT TGC TGA TCC ACA TCT GCT G-3’; α6: sense, 5’-GTG ACA TGT GCT CAC CGA TAT G-3’; antisense, 3’-CTC TCC ACC AAC TTC ATA AGG C-5’. (B) Real time PCR for α6 mRNA in MDCK cells +/- QLα12 expression. Real time PCR was performed on a LightCycler apparatus (Roche Applied Science) using the QuantiTect SYBR Green PCR Kit (QIAGEN). The primer sets are the same as above. The analysis of β-actin was used to control for variations in the quantity of starting template. (C) Membrane expression of α6 integrin. QLα12-MDCK cells were cultured +/- dox for 72 h and then surface labeled with antibodies to α6 integrins (rat monoclonal from Abcam). Cells were then analyzed by FACS and sorted by intensity for specific integrin staining. The position along the X-axis reflects the amount of surface labeling and was significantly lower in the presence of QLα12 expression (+-QLα12, blue line). (D) Immunofluorescent staining of α6 integrin in MDCK cells +/- QLα12 expression. Cells were cultured to 60-80% confluence and fixed with Paraformaldehyde (3.7%) in PBS, washed and blocked with 5% goat-serum, 1% BSA in PBS for 1 hr at RT. After washing, α6-FITC (Rat monoclonal[GoH3]; Abcam at 1:50 dilution) was added for 1 h. Images were obtained using NIKON TE-2000-E microscope with 40X oil lenses. Size bar = 20 μM under identical conditions. There was 2.5-fold more fluorescent signal in -QLα12 versus +QLα12 MDCK cells (quantified in Image J). (E) QLα12 expression results in less attachment to laminin-5. Plasticware was precoated with laminin-5 (1:100). Detached cells were added to each well of a coated 96 well plate incubated at 37°C for 40–50 min. Each well was washed with PBS and fluorescence measured using a Spectra Max Plus 384 (Molecular Devices) fluorimeter. N = 4, and the experiment was repeated twice. Results are expressed as the amount of fluorescence in arbitrary units.
MDCK cells. We had previously found that QLα12-expressed MDCK cells had reduced adhesion to laminin-1 (an α6β1 integrin ligand) and Matrigel (composed of mostly laminin-1). We also found reduced adhesion to laminin-5 (a major ligand for α6β4 integrins) with QLα12-but not wild type Gtα12-expressing MDCK cells (shown in Supplement ref. 14). We repeated the attachment of MDCK cells +/- QLα12 expression on laminin-5, and these results are shown in (Fig. 1E). There was a significant decrease in attachment of QLα12-expressing MDCK cells compared with the control. The decreased attachment to laminin-5 is consistent with loss of α6 protein expression on the cell surface. Taken together, these findings reveal that activation of G proteins in vivo from inflammatory mediators, vasoactive hormones or in malignancy may lead to changes in gene expression for specific integrins. This would provide and additional mechanism of G protein regulation of integrins.

An example of human disease where these mechanisms may be particularly important is ADPKD. Mutations in these mechanisms may be particularly of G protein regulation of integrins.would provide an additional mechanism for specific integrins. This expression for these findings reveal that activation of G proteins in vivo from inflammatory mediators, vasoactive hormones or in malignancy may lead to changes in gene expression for specific integrins. This would provide and additional mechanism of G protein regulation of integrins.

In conclusion, we find that similar to other cell types, the interaction of G protein and integrin signaling in epithelia is complex and likely to involve numerous G protein coupled pathways and integrins. In addition to traditional signaling mechanisms by integrin interactions with extra-cellular matrix components, we have identified inside-out signaling through Gtα12 to regulate integrin function. Furthermore, the interactions are even more complex with the observation that stimulating G protein signaling can alter gene expression of several integrins that lead to changes in cell function. There are many pathophysiologic conditions where G proteins and integrins are activated and include inflammation, ischemic injury and malignancy. In each of these conditions, there is the potential for adverse signaling and regulation of integrin expression that contribute to disease. The challenges are to identify which G proteins and specific integrins are responsible, in order to provide novel therapeutic targets to modulate these pathways and alter the course of disease.

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