Review Article

Integrin and GPCR Crosstalk in the Regulation of ASM Contraction Signaling in Asthma

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Airway hyperresponsiveness (AHR) is one of the cardinal features of asthma. Contraction of airway smooth muscle (ASM) cells that line the airway wall is thought to influence aspects of AHR, resulting in excessive narrowing or occlusion of the airway. ASM contraction is primarily controlled by agonists that bind G protein-coupled receptor (GPCR), which are expressed on ASM. Integrins also play a role in regulating ASM contraction signaling. As therapies for asthma are based on symptom relief, better understanding of the crosstalk between GPCRs and integrins holds good promise for the design of more effective therapies that target the underlying cellular and molecular mechanism that governs AHR. In this paper, we will review current knowledge about integrins and GPCRs in their regulation of ASM contraction signaling and discuss the emerging concept of crosstalk between the two and the implication of this crosstalk on the development of agents that target AHR.

1. Introduction

Airway hyperresponsiveness (AHR) is the exaggerated response to relatively low concentrations of constricting agents (such as methacholine or histamine) or indirectly acting stimuli (such as cold air, respiratory infections or allergens, exercise, or cigarette smoke) that is observed in asthmatic subjects [1]. Contraction of airway smooth muscle (ASM) cells that line the airway wall is thought to influence aspects of AHR, culminating in the generation of force and excessive narrowing or occlusion of the airway [2]. ASM contraction is primarily controlled by agonists that bind G protein-coupled receptors (GPCR), which are expressed on ASM. Integrins also play a role in regulating ASM contraction signaling. As therapies for asthma are based on symptom relief, better understanding of the crosstalk between GPCRs and integrins holds good promise for the design of more effective therapies that target the underlying cellular and molecular mechanism that governs AHR. In this paper, we will review current knowledge about integrins and GPCRs in their regulation of ASM contraction signaling and discuss the emerging concept of crosstalk between the two and the implication of this crosstalk on the development of agents that target AHR.
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2. Integrins and ASM Contraction Signaling

Integrins are heterodimeric transmembrane proteins comprising one α and β chain. The expression of different integrins in ASM, their potential ligands and change in expression in asthma are detailed in Table 1. Integrin activation via ECM protein binding leads to the formation of a complex called focal adhesion, which consists of many structural proteins such as vinculin, talin, α-actinin, and paxillin [19–21]. Integrins can signal through the cell membrane in both directions: inside-out signaling and outside-in signaling. The extracellular binding activity of integrins is regulated from the inside of the cell (inside-out signaling), while the binding of ECM proteins such as laminin elicits signals that are transmitted into the cell (outside-in signaling) [22]. It is through these signaling activation events that integrins regulate cell attachment, survival, proliferation, cell spreading, differentiation, cytoskeleton reorganization, cell shape, cell migration, gene expression, tumorigenicity, intracellular pH, and increase in concentration of cytosolic Ca²⁺ [23].

Activation of integrins by either contractile or mechanical stimuli can result in two signaling events to cause ASM cell contraction. Firstly, integrin activation causes the phosphorylation of focal adhesion kinase (FAK) and association with paxillin, leading to reorganization of the cytoskeleton [24–26]. Secondly, integrin activation will also increase intracellular Ca²⁺ concentration causing the phosphorylation of myosin light chain kinase (MLCK) and activation of myosin ATPase activity, and crossbridge cycling [24–26].

3. GPCR and ASM Contraction Signaling

GPCR spans the cell membrane seven times and transduces extracellular stimuli from the binding of cell surface ligands into intracellular second messengers. These second messengers are known as the heterotrimeric guanine nucleotide-binding protein (G proteins), which consists of Gα, Gβ, and Gγ subunits [39]. G proteins bind to the intracellular domain of GPCR and transmit signals that are important in ASM cellular functions. These functions include regulation of ASM proliferation and secretion of cytokines, chemokines, eicosanoids, or growth factors that orchestrate airway inflammation and remodeling [40]. GPCRs are also implicated to play important role in ASM cell contraction. The regulation of ASM tone is mediated by a balance between Gαq and Gq-coupled signaling, with Gq being linked to ASM contraction signaling and Gα being linked to relaxation signaling [40–43]. Agonist binding causes the activation and association of GPCRs with Gq/11, which promotes GTP binding and dissociation of Gα from Gβγ subunits. The dissociated Gq will then bind to effector phospholipase C, which then hydrolyses phosphoinositol 4,5-bisphosphate (PIP2) into 1,2-diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP₃). The net effect of these events is to increase the levels of intracellular Ca²⁺ as well as to activate cell contractile machinery through Ca²⁺ and protein kinase C-(PKC-) dependent mechanisms [40]. Activated PKC is able to phosphorylate a number of substrates which include calponin [41]. Phosphorylated calponin loses its ability to inhibit actomyosin ATPase, which is required for ASM cell contraction [41].

4. Evidence for Integrin and GPCR Crosstalk

There is emerging interest in crosstalk between integrins and GPCRs (Table 2). For example, muscarinic agonists that bind G12/13 protein can induce FAK activation and autophosphorylation in Swiss 3T3 cells, a fibroblast cell line, which is associated with integrin engagement signaling [52]. Arg-Gly-Asp (RGD) is a consensus amino acid sequence found in ECM proteins that is recognized by integrins. It is found that muscarinic-induced FAK activation can be blocked by RGD peptide, suggesting crosstalk between GPCRs and integrins [52]. Similar observations have been observed for other GPCR agonists such as gastrin, endothelin, lysophosphatidic acid (LPA), angiotensin II, and bombesin [23, 53–56]. For example, stimulation of Swiss 3T3 cells with bombesin or endothelin results in FAK and paxillin phosphorylation and accompanied formation of focal adhesion plaques. This study suggests the formation of focal adhesion plaques as a common signal transduction pathway that mediates GPCR and integrin crosstalk. As for endothelial cells, angiotensin II is able to induce FAK and paxillin phosphorylation which results in augmented cell migration necessary for blood vessel repair and wound healing. This suggests a critical role for integrins in the angiogenic effect of angiotensin II via FAK activation. Taken together, the existence of distinct pathways leading to FAK activation suggests the possibility of synergistic interaction between GPCRs and integrin receptors. One of the key signaling events following integrin ligation is the activation of FAK. FAK activation recruits phosphatidylinositol-3-kinase (PI3K), leading to the activation of Akt that regulates cellular processes such as survival, proliferation, and contraction signaling [22]. Integrin-GPCR crosstalk is also linked with the activation of the mitogen activated protein kinase (MAPK) signaling pathway [55]. Lysophosphatidic acid and thrombin receptors alone can activate MAPK in PC12 cells and this was blocked by RGD peptide and cytochalasin D, which is an actin depolymerising agent involved in the remodeling of the cytoskeleton [55]. This data suggests important crosstalk between integrins and GPCRs in regulating MAPK signaling. Amin and coworkers show that β1 integrin plays a crucial role in negating the apoptotic effects of β-adrenergic receptor stimulation in cardiac myocytes via the involvement of FAK and PI3K/Akt pathways [57]. Furthermore, a nonreceptor tyrosine kinase, PYK2, is able to link GPCRs to focal adhesion-dependent ERK activation to provide a point of convergence between signaling pathways triggered by integrins and certain GPCR
Table 1: Expression of different integrins in ASM, their potential ligands and change in expression in asthma.

<table>
<thead>
<tr>
<th>Integrin</th>
<th>Expression in ASM</th>
<th>Potential ligands</th>
<th>Change in expression in asthma (human)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1β1</td>
<td>Human, sheep, guinea pig</td>
<td>Collagen I, II, III, IV, laminin-111, fibronectin.</td>
<td>n.d.</td>
<td>[27–30]</td>
</tr>
<tr>
<td>α3β1</td>
<td>Human</td>
<td>n.d.</td>
<td>[14, 27, 28]</td>
<td></td>
</tr>
<tr>
<td>α4β1</td>
<td>Human, sheep</td>
<td>Fibronectin, osteopontin, VCAM-1.</td>
<td>↑</td>
<td>[27, 28, 30, 33]</td>
</tr>
<tr>
<td>α5β1</td>
<td>Human, guinea pig</td>
<td>Fibronectin, osteopontin.</td>
<td>↑</td>
<td>[12, 27, 28, 32, 34, 35]</td>
</tr>
<tr>
<td>α6β1</td>
<td>Human</td>
<td>Laminin-111, laminin-411, laminin-511, laminin-521.</td>
<td>n.d.</td>
<td>[14, 28]</td>
</tr>
<tr>
<td>α6β4</td>
<td>Human</td>
<td>Laminin-322, laminin-511, laminin-521.</td>
<td>n.d.</td>
<td>[28]</td>
</tr>
<tr>
<td>α7β1</td>
<td>Human</td>
<td>Laminin-111, laminin-211, laminin-221.</td>
<td>n.d.</td>
<td>[14]</td>
</tr>
<tr>
<td>α8β1</td>
<td>Mouse</td>
<td>Fibronectin, tenascin, vitronectin ADAMs 1, 2, 3, 9, 15, factor XIII, L1-Cell adhesion molecule, osteopontin, tenasin, VCAM-1, von Willebrand factor.</td>
<td>n.d.</td>
<td>[28]</td>
</tr>
<tr>
<td>α9β1</td>
<td>Human, guinea pig, mouse</td>
<td></td>
<td>↑</td>
<td>[28, 36, 37]</td>
</tr>
<tr>
<td>αvβ1</td>
<td>Human</td>
<td>Fibronectin. Fibrinogen, fibronectin, GSP, laminin, osteopontin, thrombospondin, vitronectin, von Willebrand factor.</td>
<td>↑</td>
<td>[28, 32]</td>
</tr>
<tr>
<td>αvβ3</td>
<td>Human</td>
<td>n.d.</td>
<td>[27, 28, 32]</td>
<td></td>
</tr>
<tr>
<td>αvβ5</td>
<td>Human, mouse</td>
<td>Osteopontin, vitronectin</td>
<td>↑</td>
<td>[38]</td>
</tr>
</tbody>
</table>

n.d.: not determined.

Table 2: Expression of ECM/ligands/integrin ligands, their potential crosstalk with G proteins and change in expression in disease.

<table>
<thead>
<tr>
<th>ECM/integrin ligands</th>
<th>Potential crosstalk with G proteins</th>
<th>Disease</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyr61</td>
<td>G_{12/13}</td>
<td>↑ in breast and endometrial cancers</td>
<td>[44]</td>
</tr>
<tr>
<td>RGD sequence in P2Y_{2} receptor</td>
<td>G_{0}</td>
<td>n.d.</td>
<td>[45]</td>
</tr>
<tr>
<td>Laminin-111</td>
<td>G_{i} and G_{s}</td>
<td>↑ in asthma</td>
<td>[46–48]</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>G_{q} and G_{12/13}</td>
<td>↑ in asthma</td>
<td>[47–50]</td>
</tr>
<tr>
<td>Collagen I</td>
<td>G_{q}</td>
<td>↑ in asthma</td>
<td>[47, 48, 51]</td>
</tr>
<tr>
<td>Collagen V</td>
<td>G_{i} and G_{s}</td>
<td>↑ in asthma</td>
<td>[46–48]</td>
</tr>
</tbody>
</table>

n.d.: not determined.

agonists (histamine) in HEK 293 (human embryonic kidney cell line) and HeLa Cells [58]. In another study, Short and coworkers show that the regulation of MAPK activity by integrins and P2Y class of G_{q/11}-coupled receptors in human endothelial cells may involve activation of calcium and PKC [55]. Collectively, these studies support a role for integrin and GPCR crosstalk in physiological processes; however, integrin-GPCR interaction may be context-dependent given that different signaling mechanisms have been put forward.

The expression of ECM proteins can be regulated by GPCR ligands. For example, thrombin, sphingosine-1-phosphate, and LPA that signal through G_{12/13} and Rho A activation can increase the expression of the ECM protein Cyr61 (CCN1) in fibroblast, smooth muscle cells, and prostatic epithelial cells, respectively [44]. Cyr61 subsequently binds to integrin and activates downstream signaling pathways that regulate cell migration, survival, and proliferation. The engagement of integrin signaling pathway via GPCR ligands provide a means to amplify and sustain GPCR signaling in normal and pathophysiological cellular functions. In the asthma context, exaggerated GPCR signaling in AHR may contribute to increased expression of ECM proteins in the airway. The activation of integrins by these ECM proteins may thus amplify and sustain GPCR signaling to contribute
to excessive bronchoconstriction that is observed in asthma exacerbations.

Activated integrins organize supramolecular complexes consisting of cytoskeletal domains and associated receptors and signaling molecules that may contribute to the formation of specialized lipid microdomains, which are referred to as “lipid rafts” [59]. Until now, there is no evidence for integrin-mediated activation of heterotrimeric G protein signaling cascade outside lipid rafts. However, there is some evidence to show that ligation of integrins within supramolecular complexes can lead to activation of GPCRs. CD47, an integrin associated protein, forms complexes with \( \alpha_{V}\beta_{3} \) integrin and activates G\(_{i}\) signaling [60]. Integrin association is also required for activation of G\(_{i}\) signaling by the P2Y\(_{2}\) receptor [45]. Recently, Berg and colleagues show that the relative amount of activated integrins at focal adhesion sites may govern signaling by \( \mu \) opioid receptor, perhaps by altering interactions with G proteins [17]. Moreover, Lin and coworkers show that integrin ligation can trigger AMPA receptor-dependent Ca\(^{2+}\) influx and intracellular Ca\(^{2+}\) store release [61]. Taken together, crosstalk between integrins and GPCRs is relevant to ASM cells and possible in the asthma pathophysiologic processes.

There are currently limited studies regarding the involvement of both integrins and GPCRs in the regulation of ASM cell contraction in healthy and asthmatic condition (Figure 1). However, crosstalk between integrins and GPCRs in contraction signaling is evident in other cell types. In the context of cardiac muscle contraction signaling, Wang and colleagues show that laminin binding-\( \beta_{1} \) integrins in association with the actin cytoskeleton are able to attenuate adenylate cyclase (AC) activity. This in turn inhibits cholinergic regulation of L-type Ca\(^{2+}\) current in cardiac muscle contraction [62]. Subsequently, they also show that laminin binding-\( \beta_{1} \) integrins in conjunction with the actin cytoskeleton have the ability to reduce \( \beta_{1} \)-adrenergic receptor-induced L-type Ca\(^{2+}\) current and enhance \( \beta_{2} \)-adrenergic receptor-induced L-type Ca\(^{2+}\) current in the same cell [63]. Recently, the same group shows that \( \beta_{1} \)-integrin-activated signaling of the FAK/PI3K/Akt pathway can inhibit \( \beta_{1} \)-adrenergic receptor-mediated stimulation of L-type Ca\(^{2+}\) current in cardiac muscle contraction [64]. This study suggests that increased deposition of ECM proteins such as laminin in a failing heart may favor \( \beta_{2} \)-adrenergic receptor signaling to \( \beta_{1} \)-adrenergic receptor signaling, and this may be mediated in part via \( \beta_{1} \) integrin-induced FAK/PI3K/Akt pathway.

As for atrial myocytes, \( \beta_{2} \)-adrenergic receptor stimulation of Ca\(^{2+}\) current is shown to be enhanced by \( \beta_{1} \) integrin via inhibition of cAMP/PKA and activation of G\(_{i} \)/ERK/cPLA\(_{2}\)/AA signaling [65]. This study suggests that increased ECM protein deposition in atrial diseases such as atrial fibrosis and/or hypertrophy may enhance \( \beta_{2} \)-adrenergic signaling, which depends more on G\(_{i} \)/ERK/cPLA\(_{2}\)/AA signaling (contraction) instead of G\(_{q} \)/cAMP/PKA signaling (relaxation). Cheng and coworkers also elegantly show the relationship between \( \beta_{1} \) integrin and \( \beta \)-adrenergic receptor regulation of L-type Ca\(^{2+}\) current in neonatal rat ventricular myocytes [66]. Overexpression of \( \beta_{1} \) integrin impedes \( \beta \)-adrenergic receptor-induced Ca\(^{2+}\) current via inhibition of AC/cAMP activity [66]. Similar observation is also obtained in adult cat atrial myocytes [62]. These findings suggest an important role for integrin and \( \beta \)-adrenergic receptor crosstalk in a diseased heart in which it is associated with chronic overload of pressure, increased ECM proteins and integrin receptors. Remodeling of GPCR receptor functions in asthma may occur too as there is increased deposition of ECM proteins and altered expression of integrins in the asthmatic airways. Collectively, these studies suggest that integrin activation might play a role in GPCR-induced muscle contraction of the airways.

In the context of ASM cell physiology there is only one study that links ECM proteins to GPCR-induced relaxation signaling [46]. Exposure of ASM cells to laminin decreases cAMP accumulation and AC activity [46]. The decrease in cAMP accumulation and AC activity could be due to a phenomenon known as “G protein switching” [46]. “G protein switching” occurs when agonists binding to the \( \beta_{2} \)-adrenergic receptor leads to the activation of G\(_{i}\) rather than G\(_{q}\). The activation of G\(_{i}\) and decreased G\(_{q}\) signaling translate into low AC activity and thus decreased cAMP accumulation [46]. Altered phosphorylation states of the \( \beta_{2} \)-adrenergic receptor may be the underlying cause of G protein switching [67]. Since integrins are able to phosphorylate cell surface receptors, it is thought to play a role in G protein switching [68]. Human ASM cells predominantly express AC isoforms V and VI. These isoforms can be inhibited by Ca\(^{2+}\) and G\(_{q}\) signaling but stimulated by PKC [69, 70]. As integrin activation leads to PKC activation and Ca\(^{2+}\) release and influx, it suggests that integrins may modulate AC activity. This would explain the decrease in AC activity of human ASM cells cultured on laminin [71, 72]. This finding is important given that cAMP and AC are regulators of ASM relaxation signaling and this is the first study to implicate that integrins may regulate ASM tone. However, the involvement of GPCR crosstalk with integrins in healthy and asthmatic ASM was not directly investigated in this study and future studies in this area are warranted.

GPCR signaling has been shown to be highly compartmentalized and disruption of this subcellular organization may affect GPCR function [73]. Integrin clustering is a crucial step towards the formation of focal adhesion. Focal adhesion is able to recruit various proteins that are involved in cell signaling cascades which include G proteins in GPCR signaling [74]. Contractile human ASM cells exhibit omega-shaped plasma invaginations known as caveolae (developed from lipid rafts that bind caveolin-1 protein) [75]. Caveolae associate preferentially with signaling proteins that have roles in controlling smooth muscle contraction signaling, for example, G\(_{q}\) protein, members of the Rho small GTPase family, and PKC [75]. Depending on the type of GPCR, upon ligand binding, receptors may remain, exit or translocate into caveolae [76–78]. Muscarinic M\(_{3}\) and histamine H\(_{1}\) receptors have been found within the caveolae enriched membrane fraction of human ASM [75]. Moreover, muscarinic M\(_{3}\) receptors and G\(_{q}\) protein cofractionate in caveolin-1 enriched ASM cell membranes [79]. Caveolin-1 is able to bind to integrin α-subunits and has been shown
to regulate GPCR-mediated signaling [80, 81]. Caveolin-1 links integrin α-subunit to tyrosine kinase Fyn which then recruits Shc and Grb2. This sequence of events couples integrins to downstream signaling pathways such as Ras-ERK pathway. Caveolae function as negative regulators of cAMP accumulation. This suggests that integrin signaling regulated by caveolin-1 may serve as important modifier of GPCR signaling such as cAMP signaling in asthma. Caveolae are found in close proximity to peripheral sarcoplasmic reticulum and mitochondria, suggesting that caveolae may play a role in the spacial coordination of Ca2+-handling channels and organelles, which are implicated in ASM contraction signaling [82]. In addition, caveolae are anchored to the dystrophin glycoprotein complex (DGC). The DGC in turn links to ECM protein, laminin. This linkage is thought to help maintain membrane integrity [75, 83]. Collectively, these studies support the notion that caveolae may mediate ASM contractile response by aiding integrin and GPCR crosstalk signaling in asthma.

Integrins have also been implicated to regulate vascular smooth muscle cell contraction by mobilizing intracellular Ca2+. The addition of RGD peptide at millimolar range elicited increased levels of intracellular Ca2+ concentration [18]. This activation of ryanodine-sensitive Ca2+ store and lysosome-like organelles by RGD peptide [18, 84] suggests important role of integrin-dependent Ca2+ signaling in regulating smooth muscle contraction. In support, α2β1 integrin has been implicated to regulate transient elevation of intracellular-free Ca2+ concentration from both IP3 evoked Ca2+ release from intracellular stores and extracellular Ca2+ influx through voltage-gated L-type Ca2+ channels in skeletal muscle cell [85].

Lastly, it is worth noting that GPCR agonists may promote ECM protein production, either directly, or indirectly by promoting autocrine TGFβ release. TGFβ is linked to thickening of ASM layer and deposition of collagen. Tatler and colleagues show that GPCR agonists, LPA and methacholine, induced TGFβ activation via integrin αvβ5 by ASM cells [38]. In support, Grainge and colleagues provide evidence that repeated bronchoconstriction with methacholine increases TGFβ immunoreactivity within the airway epithelium and increases the thickness of the subepithelial collagen layer, which is indicative of an acute alteration in airway collagen dynamics [86]. These studies provide alternative means of crosstalk between GPCRs and integrins, and one that could amplify direct GPCR/integrin interactions.

5. Concluding Remarks

In summary, integrins may play a role in regulating GPCR-induced ASM cell contraction signaling in asthma. This finding may offer explanations for increased contractility of ASM cells in asthma in which ECM proteins and their binding receptor integrins are highly expressed. Thus, integrins may be an interesting therapeutic target to inhibit ASM contraction signaling in asthma. However, the development of integrin antagonists has proven to be challenging. The role of integrins in asthma is complex as multiple...
integrins may participate to exert asthma symptoms, making it difficult to specifically target integrins that are involved in ASM contraction signaling. Perhaps targeting “linker proteins” that link the crosstalk between integrins and GPCRs in ASM contraction signaling is a possible therapeutic strategy for the treatment of AHR in asthma. One such possible target is caveolin-1 that may regulate integrins and GPCRs crosstalk. Other possible targets may be those which participate in G protein switching that are induced by integrin activation. Nonetheless, further understanding of the mechanisms behind integrin and GPCR crosstalk in ASM cell contraction signaling will enhance the development of more tailored therapy in the future for asthma treatment where AHR is a feature.

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