Hindawi Publishing Corporation
Journal of Allergy
Volume 2012, Article ID 341282, 9 pages
doi:10.1155/2012/341282

Review Article

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

Chun Ming Teoh,[1] John Kit Chung Tam,[2] and Thai Tran[1]

*[1] Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, MD9,
2 Medical Drive, Singapore 117597*
[2] Department of Surgery, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 119228

Correspondence should be addressed to Thai Tran, tran_thai@nuhs.edu.sg

Received 29 April 2012; Accepted 24 July 2012

Academic Editor: Yassine Amrani

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. Studies have shown that the asthmatic airways can be completely occluded even with only 40% contraction of ASM cells following an asthma exacerbation to GPCR agonists, such as histamine, that induce muscle shortening [3, 4]. Therefore, ASM GPCRs are important targets for therapeutic agents in asthma treatment. However, there is increasing evidence to suggest that chronic use of β_2-adrenergic receptor agonists, which act on GPCRs, is associated with worsening of bronchoconstrictor response to airway spasmogen [5], loss of asthma control, and exacerbation of asthma symptoms [6, 7], as well as an increased incidence of asthma-related morbidity and mortality [8]. Moreover, glucocorticoids, which are used as first line therapy for the treatment of inflammation associated with asthma, decrease AHR only if introduced early in disease diagnosis [9, 10]. Even then, there are side effects associated with the use of glucocorticoid when used at high dose and over long periods [10, 11]. Thus, the current treatment for asthma is based on symptom relief only and the ultimate goal of treating asthma is to target the underlying mechanisms, which include AHR.

We and others have shown that integrins may influence signaling events that contribute to AHR [12–14]. However, the mechanism behind this regulation remains to be fully elucidated. Moreover, there is increasing evidence to show that GPCRs interact with integrins to regulate ASM signaling pathways that are important in asthma. The cellular signaling processes include the regulation of cell adhesion, calcium signaling, injury and remodeling, mechanotransduction signaling and synaptic plasticity [15–18]. 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.

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 elicit 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 Ca^{2+} [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 Ca^{2+} 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 G_α, G_β, and G_γ 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 G_q- and G_s-coupled signaling, with G_q being linked to ASM contraction signaling and G_s being linked to relaxation signaling [40–43]. Agonist binding causes the activation and association of GPCRs with G_q, which promotes GTP binding and dissociation of G_α from $G_{\beta\gamma}$ subunits. The dissociated G_q will then bind to effector phospholipase C, which then hydrolyses phosphoinositol 4,5-bisphosphate (PIP_2) into 1,2-diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3). The net effect of these events is to increase the levels of intracellular Ca^{2+} as well as to activate cell contractile machinery through Ca^{2+} 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 $G_{12/13}$ 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 $\beta1$ 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.

Integrin	Expression in ASM	Potential ligands	Change in expression in asthma (human)	Reference
$\alpha 1\beta 1$	Human, sheep, guinea pig	Collagen I, II, III, IV, laminin-111, fibronectin.	n.d.	[27–30]
$\alpha 2\beta 1$	Human, guinea pig	Collagen I, IV, laminin-111, tenascin.	n.d.	[27–29, 31, 32]
$\alpha 3\beta 1$	Human	Collagen I, fibronectin, laminin-211, laminin-221, laminin-322, laminin-511, laminin-521.	n.d.	[14, 27, 28]
$\alpha 4\beta 1$	Human, sheep	Fibronectin, osteopontin, VCAM-1.	↑	[27, 28, 30, 33]
$\alpha 5\beta 1$	Human, guinea pig	Fibronectin, osteopontin.	↑	[12, 27, 28, 32, 34, 35]
$\alpha 6\beta 1$	Human	Laminin-111, laminin-411, laminin-511, laminin-521.	n.d.	[14, 28]
$\alpha 6\beta 4$	Human	Laminin-322, laminin-511, laminin-521.	n.d.	[28]
$\alpha 7\beta 1$	Human	Laminin-111, laminin-211, laminin-221.	n.d.	[14]
$\alpha 8\beta 1$	Mouse	Fibronectin, tenascin, vitronectin	n.d.	[28]
$\alpha 9\beta 1$	Human, guinea pig, mouse	ADAMs 1, 2, 3, 9, 15, factor XIII, L1-Cell adhesion molecule, osteopontin, tenascin, VCAM-1, von Willebrands factor.	↓	[28, 36, 37]
$\alpha v\beta 1$	Human	Fibronectin.	↑	[28, 32]
$\alpha v\beta 3$	Human	Fibrinogen, fibronectin, GSP, laminin, osteopontin, thrombospondin, vitronectin, von Willebrands factor.	n.d.	[27, 28, 32]
$\alpha v\beta 5$	Human, mouse	Osteopontin, vitronectin	↑	[38]

n.d.: not determined.

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

ECM/integrin ligands	Potential crosstalk with G proteins	Disease	Reference
Cyr61	$G_{12/13}$	↑ in breast and endometrial cancers	[44]
RGD sequence in $P2Y_2$ receptor	G_0	n.d.	[45]
Laminin-111	G_i, and G_s	↑ in asthma	[46–48]
Fibronectin	G_q and $G_{12/13}$	↑ in asthma	[47–50]
Collagen I	G_q	↑ in asthma	[47, 48, 51]
Collagen V	G_i and G_s	↑ in asthma	[46–48]

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_o 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 μ 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 pathophysiological 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-β_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-$\beta1$ integrins in conjunction with the actin cytoskeleton have the ability to reduce $\beta1$-adrenergic receptor-induced L-type Ca^{2+} and enhance β_2-adrenergic receptor-induced L-type Ca^{2+} current in the same cell [63]. Recently, the same group shows that β_1-integrin-induced activation of the FAK/PI3K/Akt pathway can inhibit β_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 β_2-adrenergic receptor signaling to β_1-adrenergic receptor signaling, and this may be mediated in part via $\beta1$ integrin-induced FAK/PI3K/Akt pathway.

As for atrial myocytes, β_2-adrenergic receptor stimulation of Ca^{2+} current is shown to be enhanced by β_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 β_2-adrenergic signaling, which depends more on G_i/ERK/$cPLA_2$/AA signaling (contraction) instead of G_s/AC/cAMP/PKA signaling (relaxation). Cheng and coworkers also elegantly show the relationship between β_1 integrin and β-adrenergic receptor regulation of L-type Ca^{2+} current in neonatal rat ventricular myocytes [66]. Overexpression of β_1 integrin impedes β-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 β-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 β_2-adrenergic receptor leads to the activation of G_i rather than G_s. The activation of G_i and decreased G_s signaling translate into low AC activity and thus decreased cAMP accumulation [46]. Altered phosphorylation states of the β_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_i 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_α 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

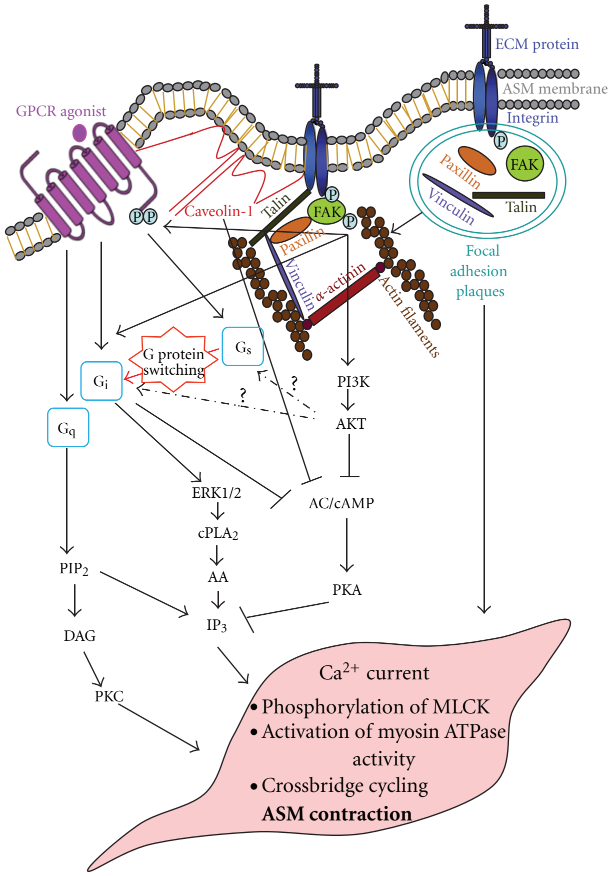

Figure 1: Schematic diagram showing the proposed crosstalk between integrins and GPCRs in ASM cell contraction signaling. Integrin activation is achieved via the formation of focal adhesion plaques leading to cytoskeleton reorganization, which is essential for actin polymerisation and recruitment of linker proteins for tension development. Integrin activation causes the phosphorylation of FAK and activation of downstream signaling events leading to ASM contraction. Integrin activation will also increase intracellular Ca^{2+} concentration to cause phosphorylation of MLCK and activation of myosin ATPase activity and crossbridge cycling. GPCR-induced ASM contraction signaling can be enhanced either by inhibition of cAMP/AC activity that regulates ASM relaxation signaling, or by activation of Ca^{2+} current that is necessary for ASM contraction signaling. Activation of integrins can attenuate GPCR-induced AC activity via the FAK/PI3K/Akt pathway. cAMP accumulation and AC activity can be decreased by integrin activation via G protein switching, in which G_i is activated instead of G_s. Altered phosphorylation of GPCR by integrins is thought to underlie G protein switching in ASM cell. Caveolin-1 that binds integrin has been shown to regulate GPCR signaling. Caveolae which are rich in caveolin-1 function as negative regulators of cAMP accumulation in ASM cell. GPCR stimulation of Ca^{2+} can be enhanced by integrin via inhibition of cAMP/PKA and activation of the G_i/ERK1/2/$cPLA_2$/AA signaling. AA: arachidonic acid; AC: adenyl cyclase; AKT: protein kinase B; ASM: airway smooth muscle; cAMP: cyclic adenosine monophosphate; cPLA2: cytosolic phospholipase A2; DAG: diacylglycerol; ECM: extracellular matrix; ERK1/2: extracellular signal regulated kinase1/2; FAK: focal adhesion kinase; GPCR: G protein-coupled receptor; IP_3: inositol 3,4,5-triphosphate; PIP_2: phosphoinositol 4,5-bisphosphate; PI3K: phosphatidylinositol 3′-kinase; PKA: protein kinase A; PKC: protein kinas C.

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 Ca^{2+}-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 Ca^{2+}. The addition of RGD peptide at millimolar range elicited increased levels of intracellular Ca^{2+} concentration [18]. This activation of ryanodine-sensitive Ca^{2+} store and lysosome-like organelles by RGD peptide [18, 84] suggests important role of integrin-dependent Ca^{2+} signaling in regulating smooth muscle contraction. In support, $\alpha_7\beta_1$ integrin has been implicated to regulate transient elevation of intracellular-free Ca^{2+} concentration from both IP_3 evoked Ca^{2+} release from intracellular stores and extracellular Ca^{2+} influx through voltage-gated L-type Ca^{2+} 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 $\alpha v\beta 5$ 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.

Acknowledgments

T. Tran is supported by the Singapore Ministry of Health's National Medical Research Council (NMRC) under its Individual Research Grant scheme, NMRC block vote, and Deputy President (Research and Technology) scheme. J. K. C. Tam is supported by the Singapore Ministry of Health's NMRC under its NMRC block vote and Deputy President (Research and Technology) scheme.

References

[1] N. C. Thomson, "Neurogenic and myogenic mechanisms of nonspecific bronchial hyperresponsiveness," *European Journal of Respiratory Diseases. Supplement*, vol. 128, no. 1, pp. 206–212, 1983.

[2] S. An, T. R. Bai, J. H. T. Bates et al., "Airway smooth muscle dynamics: a common pathway of airway obstruction in asthma," *European Respiratory Journal*, vol. 29, no. 5, pp. 834–860, 2007.

[3] A. J. Woolcock, C. M. Salome, and K. Yan, "The shape of the dose-response curve to histamine in asthmatic and normal subjects," *American Review of Respiratory Disease*, vol. 130, no. 1, pp. 71–75, 1984.

[4] A. L. James, P. D. Pare, and J. C. Hogg, "The mechanics of airway narrowing in asthma," *American Review of Respiratory Disease*, vol. 139, no. 1, pp. 242–246, 1989.

[5] W. O. Spitzer, S. Suissa, P. Ernst et al., "The use of beta-agonists and the risk of death and near death from asthma," *The New England Journal of Medicine*, vol. 326, no. 8, pp. 501–506, 1992.

[6] J. M. Drazen, E. Israel, H. A. Boushey et al., "Comparison of regularly scheduled with as-needed use of albuterol in mild asthma," *The New England Journal of Medicine*, vol. 335, no. 12, pp. 841–847, 1996.

[7] D. R. Taylor, G. Town, and G. Herbison, "Asthma control during long term treatment with regular inhaled salbutamol and salmeterol," *Thorax*, vol. 53, no. 9, pp. 744–752, 1998.

[8] S. Suissa, P. Ernst, J. F. Boivin et al., "A cohort analysis of excess mortality in asthma and the use of inhaled beta-agonists," *American Journal of Respiratory and Critical Care Medicine*, vol. 149, no. 3, article 1, pp. 604–610, 1994.

[9] T. Haahtela, M. Järvinen, T. Kava et al., "Effects of reducing or discontinuing inhaled budesonide in patients with mild asthma," *The New England Journal of Medicine*, vol. 331, no. 11, pp. 700–705, 1994.

[10] E. R. Sher, D. Y. M. Leung, W. Surs et al., "Steroid-resistant asthma. Cellular mechanisms contributing to inadequate response to glucocorticoid therapy," *The Journal of Clinical Investigation*, vol. 93, no. 1, pp. 33–39, 1994.

[11] H. Schäcke, W. D. Döcke, and K. Asadullah, "Mechanisms involved in the side effects of glucocorticoids," *Pharmacology and Therapeutics*, vol. 96, no. 1, pp. 23–43, 2002.

[12] B. G. J. Dekkers, I. S. T. Bos, R. Gosens, A. J. Halayko, J. Zaagsma, and H. Meurs, "The integrin-blocking peptide RGDS inhibits airway smooth muscle remodeling in a guinea pig model of allergic asthma," *American Journal of Respiratory and Critical Care Medicine*, vol. 181, no. 6, pp. 556–565, 2010.

[13] T. Tran, K. D. McNeill, W. T. Gerthoffer, H. Unruh, and A. J. Halayko, "Endogenous laminin is required for human airway smooth muscle cell maturation," *Respiratory Research*, vol. 7, article 117, 2006.

[14] T. Tran, K. Ens-Blackie, E. S. Rector et al., "Laminin-binding integrin $\alpha7$ is required for contractile phenotype expression by human airway myocytes," *American Journal of Respiratory Cell and Molecular Biology*, vol. 37, no. 6, pp. 668–680, 2007.

[15] B. E. Slack and M. S. Siniaia, "Adhesion-dependent redistribution of MAP kinase and MEK promotes muscarinic receptor-mediated signaling to the nucleus," *Journal of Cellular Biochemistry*, vol. 95, no. 2, pp. 366–378, 2005.

[16] F. J. Alenghat, J. D. Tytell, C. K. Thodeti, A. Derrien, and D. E. Ingber, "Mechanical control of cAMP signaling through integrins is mediated by the heterotrimeric Gαs protein," *Journal of Cellular Biochemistry*, vol. 106, no. 4, pp. 529–538, 2009.

[17] K. A. Berg, G. Zardeneta, K. M. Hargreaves, W. P. Clarke, and S. B. Milam, "Integrins regulate opioid receptor signaling in trigeminal ganglion neurons," *Neuroscience*, vol. 144, no. 3, pp. 889–897, 2007.

[18] W. L. Chan, N. H. Holstein-Rathlou, and K. P. Yip, "Integrin mobilizes intracellular Ca^{2+} in renal vascular smooth muscle cells," *American Journal of Physiology—Cell Physiology*, vol. 280, no. 3, pp. C593–C603, 2001.

[19] K. Burridge and M. Chrzanowska-Wodnicka, "Focal adhesions, contractility, and signaling," *Annual Review of Cell and Developmental Biology*, vol. 12, pp. 463–519, 1996.

[20] C. Brakebusch and R. Fässler, "The integrin-actin connection, an eternal love affair," *The EMBO Journal*, vol. 22, no. 10, pp. 2324–2333, 2003.

[21] D. R. Critchley, "Focal adhesions—the cytoskeletal connection," *Current Opinion in Cell Biology*, vol. 12, no. 1, pp. 133–139, 2000.

[22] F. G. Giancotti and E. Ruoslahti, "Integrin signaling," *Science*, vol. 285, no. 5430, pp. 1028–1032, 1999.

[23] R. S. Ross, "Molecular and mechanical synergy: cross-talk between integrins and growth factor receptors," *Cardiovascular Research*, vol. 63, no. 3, pp. 381–390, 2004.

[24] S. J. Gunst, D. D. Tang, and A. Opazo Saez, "Cytoskeletal remodeling of the airway smooth muscle cell: a mechanism for adaptation to mechanical forces in the lung," *Respiratory Physiology and Neurobiology*, vol. 137, no. 2-3, pp. 151–168, 2003.

[25] S. J. Gunst and D. D. Tang, "The contractile apparatus and mechanical properties of airway smooth muscle," *European Respiratory Journal*, vol. 15, no. 3, pp. 600–616, 2000.

[26] W. Zhang and S. J. Gunst, "Interactions of airway smooth muscle cells with their tissue matrix implications for contraction," *Proceedings of the American Thoracic Society*, vol. 5, no. 1, pp. 32–39, 2008.

[27] T. T. B. Nguyen, J. P. T. Ward, and S. J. Hirst, "β1-integrins mediate enhancement of airway smooth muscle proliferation by collagen and fibronectin," *American Journal of Respiratory and Critical Care Medicine*, vol. 171, no. 3, pp. 217–223, 2005.

[28] D. J. Fernandes, J. V. Bonnacci, and A. G. Stewart, "Extracellular matrix, integrins, and masenchymal cell function in the airways," *Current Drug Targets*, vol. 7, no. 5, pp. 567–577, 2006.

[29] B. Bazán-Perkins, E. Sánchez-Guerrero, M. H. Vargas et al., "β1-integrins shedding in a guinea-pig model of chronic asthma with remodelled airways," *Clinical and Experimental Allergy*, vol. 39, no. 5, pp. 740–751, 2009.

[30] W. M. Abraham, A. Ahmed, I. Serebriakov et al., "A monoclonal antibody to alpha1beta1 blocks antigen-induced airway responses in sheep," *American Journal of Respiratory and Critical Care Medicine*, vol. 169, no. 1, pp. 97–104, 2004.

[31] J. V. Bonacci, M. Schuliga, T. Harris, and A. G. Stewart, "Collagen impairs glucocorticoid actions in airway smooth muscle through integrin signalling," *British Journal of Pharmacology*, vol. 149, no. 4, pp. 365–373, 2006.

[32] Q. Peng, D. Lai, T. T. B. Nguyen, V. Chan, T. Matsuda, and S. J. Hirst, "Multiple β1 integrins mediate enhancement of human airway smooth muscle cytokine secretion by fibronectin and type I collagen," *The Journal of Immunology*, vol. 174, no. 4, pp. 2258–2264, 2005.

[33] J. P. Abonia, J. Hallgren, T. Jones et al., "Alpha-4 integrins and VCAM-1, but not MAdCAM-1, are essential for recruitment of mast cell progenitors to the inflamed lung," *Blood*, vol. 108, no. 5, pp. 1588–1594, 2006.

[34] L. M. Moir, J. K. Burgess, and J. L. Black, "Transforming growth factor β1 increases fibronectin deposition through integrin receptor $\alpha5\beta1$ on human airway smooth muscle," *Journal of Allergy and Clinical Immunology*, vol. 121, no. 4, pp. 1034–1039.e4, 2008.

[35] A. M. Freyer, S. R. Johnson, and I. P. Hall, "Effects of growth factors and extracellular matrix on survival of human airway smooth muscle cells," *American Journal of Respiratory Cell and Molecular Biology*, vol. 25, no. 5, pp. 569–576, 2001.

[36] E. L. Palmer, C. Ruegg, R. Ferrando, R. Pytela, and D. Sheppard, "Sequence and tissue distribution of the integrin α9 subunit, a novel partner of β1 that is widely distributed in epithelia and muscle," *Journal of Cell Biology*, vol. 123, no. 5, pp. 1289–1297, 1993.

[37] C. Chen, M. Kudo, F. Rutaganira et al., "Integrin $\alpha9\beta1$ in airway smooth muscle suppresses exaggerated airway narrowing," *The Journal of Clinical Investigation*, vol. 122, no. 8, pp. 2916–2927, 2012.

[38] A. L. Tatler, A. E. John, L. Jolly et al., "Integrin $\alpha v\beta5$-mediated TGF-β activation by airway smooth muscle cells in asthma," *The Journal of Immunology*, vol. 187, no. 11, pp. 6094–6107, 2011.

[39] H. Gong, B. Shen, P. Flevaris et al., "G protein subunit Gα13 binds to integrin αIIbβ3 and mediates integrin "outside-in" signaling," *Science*, vol. 327, no. 5963, pp. 340–343, 2010.

[40] C. K. Billington and R. B. Penn, "Signaling and regulation of G protein-coupled receptors in airway smooth muscle," *Respiratory Research*, vol. 4, no. 1, article 2, 2003.

[41] J. Pohl, S. J. Winder, B. G. Allen, M. P. Walsh, J. R. Sellers, and W. T. Gerthoffer, "Phosphorylation of calponin in airway smooth muscle," *American Journal of Physiology—Lung Cellular and Molecular Physiology*, vol. 272, no. 1, part 1, pp. L115–L123, 1997.

[42] H. Hakonarson and M. M. Grunstein, "Regulation of second messengers associated with airway smooth muscle contraction and relaxation," *American Journal of Respiratory and Critical Care Medicine*, vol. 158, no. 5, part 3, pp. S115–S122, 1998.

[43] M. A. Giembycz and D. Raeburn, "Current concepts on mechanisms of force generation and maintenance in airways smooth muscle," *Pulmonary Pharmacology*, vol. 5, no. 4, pp. 279–297, 1992.

[44] C. T. Walsh, D. Stupack, and J. H. Brown, "G protien-coupled receptors go extracellular: RhoA integrates the integrins," *Molecular Interventions*, vol. 8, no. 4, pp. 165–173, 2008.

[45] L. Erb, J. Liu, J. Ockerhausen et al., "An RGD sequence in the P2Y2 receptor interacts with $\alpha v\beta3$ integrins and is required for Go-mediated signal transduction," *Journal of Cell Biology*, vol. 152, no. 3, pp. 491–501, 2001.

[46] A. M. Freyer, C. K. Billington, R. B. Penn, and I. P. Hall, "Extracellular matrix modulates β2-adrenergic receptor signaling in human airway smooth muscle cells," *American Journal of Respiratory Cell and Molecular Biology*, vol. 31, no. 4, pp. 440–445, 2004.

[47] K. Parameswaran, A. Willems-Widyastuti, V. K. T. Alagappan, K. Radford, A. R. Kranenburg, and H. S. Sharma, "Role of extracellular matrix and its regulators in human airway smooth muscle biology," *Cell Biochemistry and Biophysics*, vol. 44, no. 1, pp. 139–146, 2006.

[48] W. R. Roche, J. H. Williams, R. Beasley, and S. T. Holgate, "Subepithelial fibrosis in the bronchi of asthmatics," *The Lancet*, vol. 1, no. 8637, pp. 520–524, 1989.

[49] M. L. Toews, E. E. Ustinova, and H. D. Schultz, "Lysophosphatidic acid enhances contractility of isolated airway smooth muscle," *Journal of Applied Physiology*, vol. 83, no. 4, pp. 1216–1222, 1997.

[50] J. M. Hartney, C. E. Gustafson, R. P. Bowler, R. Pelanda, and R. M. Torres, "Thromboxane receptor signaling is required for fibronectin-induced matrix metalloproteinase 9 production by human and murine macrophages and is attenuated by the Arhgef1 molecule," *The Journal of Biological Chemistry*, vol. 286, no. 52, pp. 44521–44531, 2011.

[51] S. Haag, S. Matthiesen, U. R. Juergens, and K. Racké, "Muscarinic receptors mediate stimulation of collagen synthesis in human lung fibroblasts," *European Respiratory Journal*, vol. 32, no. 3, pp. 555–562, 2008.

[52] E. Rozengurt, "Signal transduction pathways in the mitogenic response to G protein-coupled neuropeptide receptor agonists," *Journal of Cellular Physiology*, vol. 177, no. 4, pp. 507–517, 1998.

[53] M. Montiel, E. Pérez de la Blanca, and E. Jiménez, "Angiotensin II induces focal adhesion kinase/paxillin phosphorylation and cell migration in human umbilical vein endothelial cells," *Biochemical and Biophysical Research Communications*, vol. 327, no. 4, pp. 971–978, 2005.

[54] B. E. Slack, "Tyrosine phosphorylation of paxillin and focal adhesion kinase by activation of muscarinic m3 receptors is dependent on integrin engagement by the extracellular matrix," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 13, pp. 7281–7286, 1998.

[55] S. M. Short, J. L. Boyer, and R. L. Juliano, "Integrins regulate the linkage between upstream and downstream events in G protein-coupled receptor signaling to mitogen-activated protein kinase," *The Journal of Biological Chemistry*, vol. 275, no. 17, pp. 12970–12977, 2000.

[56] J. Sinnett-Smith, I. Zachary, A. M. Valverde, and E. Rozengurt, "Bombesin stimulation of p125 focal adhesion kinase tyrosine phosphorylation. Role of protein kinase C, Ca^{2+} mobilization, and the actin cytoskeleton," *The Journal of Biological Chemistry*, vol. 268, no. 19, pp. 14261–14268, 1993.

[57] P. Amin, M. Singh, and K. Singh, "β-Adrenergic receptor-stimulated cardiac myocyte apoptosis: role of β1 integrins," *Journal of Signal Transduction*, vol. 2011, Article ID 179057, 9 pages, 2011.

[58] V. Litvak, D. Tian, Y. D. Shaul, and S. Lev, "Targeting of PYK2 to focal adhesions as a cellular mechanism for convergence between integrins and G protein-coupled receptor signaling cascades," *The Journal of Biological Chemistry*, vol. 275, no. 42, pp. 32736–32746, 2000.

[59] B. Leitinger and N. Hogg, "The involvement of lipid rafts in the regulation of integrin function," *Journal of Cell Science*, vol. 115, no. 5, pp. 963–972, 2002.

[60] J. E. Brittain, K. J. Mlinar, C. S. Anderson, E. P. Orringer, and L. V. Parise, "Activation of sickle red blood cell adhesion via integrin-associated protein/CD47-induced signal transduction," *The Journal of Clinical Investigation*, vol. 107, no. 12, pp. 1555–1562, 2001.

[61] C. Y. Lin, L. G. W. Hilgenberg, M. A. Smith, G. Lynch, and C. M. Gall, "Integrin regulation of cytoplasmic calcium in excitatory neurons depends upon glutamate receptors and release from intracellular stores," *Molecular and Cellular Neuroscience*, vol. 37, no. 4, pp. 770–780, 2008.

[62] Y. G. Wang, A. M. Samarel, and S. L. Lipsius, "Laminin acts via β1 integrin signalling to alter cholinergic regulation of L-type Ca^{2+} current in cat atrial myocytes," *Journal of Physiology*, vol. 526, no. 1, pp. 57–68, 2000.

[63] Y. G. Wang, A. M. Samarel, and S. L. Lipsius, "Laminin binding to β1-integrins selectively alters β1- and β2-adrenoceptor signalling in cat atrial myocytes," *Journal of Physiology*, vol. 527, no. 1, pp. 3–9, 2000.

[64] Y. G. Wang, X. Ji, M. Pabbidi, A. M. Samarel, and S. L. Lipsius, "Laminin acts via focal adhesion kinase/phosphatidylinositol-3′ kinase/protein kinase B to down-regulate β1-adrenergic receptor signalling in cat atrial myocytes," *Journal of Physiology*, vol. 587, no. 3, pp. 541–550, 2009.

[65] M. R. Pabbidi, X. Ji, A. M. Samarel, and S. L. Lipsius, "Laminin enhances β2-adrenergic receptor stimulation of L-type Ca^{2+} current via cytosolic phospholipase A2 signalling in cat atrial myocytes," *Journal of Physiology*, vol. 587, no. 20, pp. 4785–4797, 2009.

[66] Q. Cheng, R. S. Ross, and K. B. Walsh, "Overexpression of the integrin β1A subunit and the β1A cytoplasmic domain modifies the β-adrenergic regulation of the cardiac L-type Ca^{2+}current," *Journal of Molecular and Cellular Cardiology*, vol. 36, no. 6, pp. 809–819, 2004.

[67] Y. Daaka, L. M. Luttrell, and R. J. Lefkowitz, "Switching of the coupling of the β2-adrenergic receptor to different g proteins by protein kinase A," *Nature*, vol. 390, no. 6655, pp. 88–91, 1997.

[68] L. Moro, M. Venturino, C. Bozzo et al., "Integrins induce activation of EGF receptor: role in MAP kinase induction and adhesion-dependent cell survival," *The EMBO Journal*, vol. 17, no. 22, pp. 6622–6632, 1998.

[69] C. K. Billington, I. P. Hall, S. J. Mundell et al., "Inflammatory and contractile agents sensitize specific adenylyl cyclase isoforms in human airway smooth muscle," *American Journal of Respiratory Cell and Molecular Biology*, vol. 21, no. 5, pp. 597–606, 1999.

[70] D. Xu, C. Isaacs, I. P. Hall, and C. W. Emala, "Human airway smooth muscle expresses 7 isoforms of adenylyl cyclase: a dominant role for isoform V," *American Journal of Physiology—Lung Cellular and Molecular Physiology*, vol. 281, no. 4, pp. L832–L843, 2001.

[71] M. D. Sjaastad, R. S. Lewis, and W. J. Nelson, "Mechanisms of integrin-mediated calcium signaling in MDCK cells: regulation of adhesion by IP3- and store-independent calcium influx," *Molecular Biology of the Cell*, vol. 7, no. 7, pp. 1025–1041, 1996.

[72] J. S. Chun, M. J. Ha, and B. S. Jacobson, "Differential translocation of protein kinase C ε during HeLa cell adhesion to a gelatin substratum," *The Journal of Biological Chemistry*, vol. 271, no. 22, pp. 13008–13012, 1996.

[73] S. F. Steinberg and L. L. Brunton, "Compartmentation of G protein-coupled signaling pathways in cardiac myocytes," *Annual Review of Pharmacology and Toxicology*, vol. 41, pp. 751–773, 2001.

[74] J. M. Lewis and M. A. Schwartz, "Integrins regulate the association and phosphorylation of paxillin by c-Abl," *The Journal of Biological Chemistry*, vol. 273, no. 23, pp. 14225–14230, 1998.

[75] A. J. Halayko, T. Tran, and R. Gosens, "Phenotype and functional plasticity of airway smooth muscle: role of caveolae and caveolins," *Proceedings of the American Thoracic Society*, vol. 5, no. 1, pp. 80–88, 2008.

[76] R. S. Ostrom, C. Gregorian, R. M. Drenan, Y. Xiang, J. W. Regan, and P. A. Insel, "Receptor number and caveolar co-localization determine receptor coupling efficiency to adenylyl cyclase," *The Journal of Biological Chemistry*, vol. 276, no. 45, pp. 42063–42069, 2001.

[77] M. Chun, U. K. Liyanage, M. P. Lisanti, and H. F. Lodish, "Signal transduction of a G protein-coupled receptor in caveolae: colocalization of endothelin and its receptor with caveolin," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, no. 24, pp. 11728–11732, 1994.

[78] T. Sabourin, L. Bastien, D. R. Bachvarov, and F. Marceau, "Agonist-induced translocation of the kinin B1 receptor to caveolae-related rafts," *Molecular Pharmacology*, vol. 61, no. 3, pp. 546–553, 2002.

[79] R. Gosens, G. L. Stelmack, G. Dueck et al., "Caveolae facilitate muscarinic receptor-mediated intracellular Ca^{2+} mobilization and contraction in airway smooth muscle," *American Journal of Physiology—Lung Cellular and Molecular Physiology*, vol. 293, no. 6, pp. L1406–L1418, 2007.

[80] K. K. Wary, A. Mariotti, C. Zurzolo, and F. G. Giancotti, "A requirement for caveolin-1 and associated kinase Fyn in integrin signaling and anchorage-dependent cell growth," *Cell*, vol. 94, no. 5, pp. 625–634, 1998.

[81] V. O. Rybin, X. Xu, M. P. Lisanti, and S. F. Steinberg, "Differential targeting of β-adrenergic receptor subtypes and adenylyl cyclase to cardiomyocyte caveolae: a mechanism to functionally regulate the cAMP signaling pathway," *The Journal of Biological Chemistry*, vol. 275, no. 52, pp. 41447–41457, 2000.

[82] A. Bergdahl and K. Swärd, "Caveolae-associated signalling in smooth muscle," *Canadian Journal of Physiology and Pharmacology*, vol. 82, no. 5, pp. 289–299, 2004.

[83] A. J. Halayko and G. L. Stelmack, "The association of caveolae, actin, and the dystrophin-glycoprotein complex: a role in smooth muscle phenotype and function?" *Canadian Journal of Physiology and Pharmacology*, vol. 83, no. 10, pp. 877–891, 2005.

[84] A. Umesh, M. A. Thompson, E. N. Chini, K. P. Yip, and J. S. K. Sham, "Integrin ligands mobilize Ca^{2+} from ryanodine receptor-gated stores and lysosome-related acidic organelles

in pulmonary arterial smooth muscle cells," *The Journal of Biological Chemistry*, vol. 281, no. 45, pp. 34312–34323, 2006.

[85] M. S. Kwon, C. S. Park, K. R. Choi et al., "Calreticulin couples calcium release and calcium influx in integrin-mediated calcium signaling," *Molecular Biology of the Cell*, vol. 11, no. 4, pp. 1433–1443, 2000.

[86] C. L. Grainge, L. C. K. Lau, J. A. Ward et al., "Effect of bronchoconstriction on airway remodeling in asthma," *The New England Journal of Medicine*, vol. 364, no. 21, pp. 2006–2015, 2011.