

IQGAP1-dependent scaffold suppresses RhoA and inhibits airway smooth muscle contraction

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The intracellular scaffold protein IQGAP1 supports protein complexes in conjunction with numerous binding partners involved in multiple cellular processes. Here, we determined that IQGAP1 modulates airway smooth muscle contractility. Compared with WT controls, at baseline as well as after immune sensitization and challenge, *Iqgap1*^{-/-} mice had higher airway responsiveness. Tracheal rings from *Iqgap1*^{-/-} mice generated greater agonist-induced contractile force, even after removal of the epithelium. RhoA, a regulator of airway smooth muscle contractility, was activated in airway smooth muscle lysates from *Iqgap1*^{-/-} mice. Likewise, knockdown of IQGAP1 in primary human airway smooth muscle cells increased RhoA activity. Immunoprecipitation studies indicated that IQGAP1 binds to both RhoA and p190A-RhoGAP, a GTPase-activating protein that normally inhibits RhoA activation. Proximity ligation assays in primary airway human smooth muscle cells and mouse tracheal sections revealed colocalization of p190A-RhoGAP and RhoA; however, these proteins did not colocalize in IQGAP1 knockdown cells or in *Iqgap1*^{-/-} trachea. Compared with healthy controls, human subjects with asthma had decreased IQGAP1 expression in airway biopsies. Together, these data demonstrate that IQGAP1 acts as a scaffold that colocalizes p190A-RhoGAP and RhoA, inactivating RhoA and suppressing airway smooth muscle contraction. Furthermore, our results suggest that IQGAP1 has the potential to modulate airway contraction severity in acute asthma.

Introduction

In asthma, smooth muscle contraction leads to airway narrowing and resistance to airflow. Better understanding of the mechanisms that regulate airway smooth muscle contractility is important for the development of novel therapies targeting airway obstruction. Smooth muscle contraction is enabled by phosphorylation of myosin light chain (MLC), which increases actin-myosin crossbridge cycling (1). MLC is subject to dephosphorylation by MLC phosphatase, and the active form of the GTPase RhoA, RhoA-GTP, induces phosphorylation and thus inactivation of MLC phosphatase, thereby supporting MLC phosphorylation and contractility.

IQGAP1 is a 190-kDa protein that serves as a scaffold for protein complexes in many cellular contexts, including actin cytoskeletal organization (2), MAPK signaling (3–5), and cell-cell adhesion (6). IQGAP1 does not possess GTPase activating activity (7). However, one of its demonstrated properties is that it binds Rho family GTPases, including RhoA (8, 9). We therefore considered the possibility that IQGAP1 determines RhoA-dependent airway smooth muscle contractility. In the present study, we described a role for IQGAP1 in suppressing RhoA activation in airway smooth muscle and found that IQGAP1 acted as a scaffold for colocalization of

RhoA with the RhoA inhibitory factor p190A-RhoGAP, thereby facilitating RhoA inactivation and providing a brake on airway smooth muscle contraction.

Results and Discussion

We tested *Iqgap1*^{-/-} mice (10) in an OVA sensitization model of airway hyperresponsiveness (11, 12). Both at baseline and with OVA challenge, *Iqgap1*^{-/-} mice had increased airway responsiveness compared with WT mice (Figure 1, A and B). No difference in airway inflammation was noted (Figure 1, C–E). We then tested isolated tracheal rings in a muscle bath system and found increased agonist-induced force of contraction in *Iqgap1*^{-/-} compared with WT mice (Figure 2A and Supplemental Figure 1A; supplemental material available online with this article; doi:10.1172/JCI76658DS1). To evaluate the relaxation response, contraction was induced in tracheal rings by methacholine administration followed by addition of isoproterenol. Rings from *Iqgap1*^{-/-} mice were again more contractile than WT rings, but had a parallel decrease in force with addition of isoproterenol (Supplemental Figure 1B). Given that IQGAP1 was expressed both in the epithelium and in smooth muscle in trachea (Figure 2B), we mechanically debrided the epithelium from the rings prior to testing and found increased smooth muscle contraction in samples stripped of epithelium (Figure 2C and Supplemental Figure 1C). This result supports the known relaxing effect of airway epithelium on airway smooth muscle contraction (13). However, removal of the epithelium did not abrogate the increased contraction of *Iqgap1*^{-/-} compared with WT mice, which suggests

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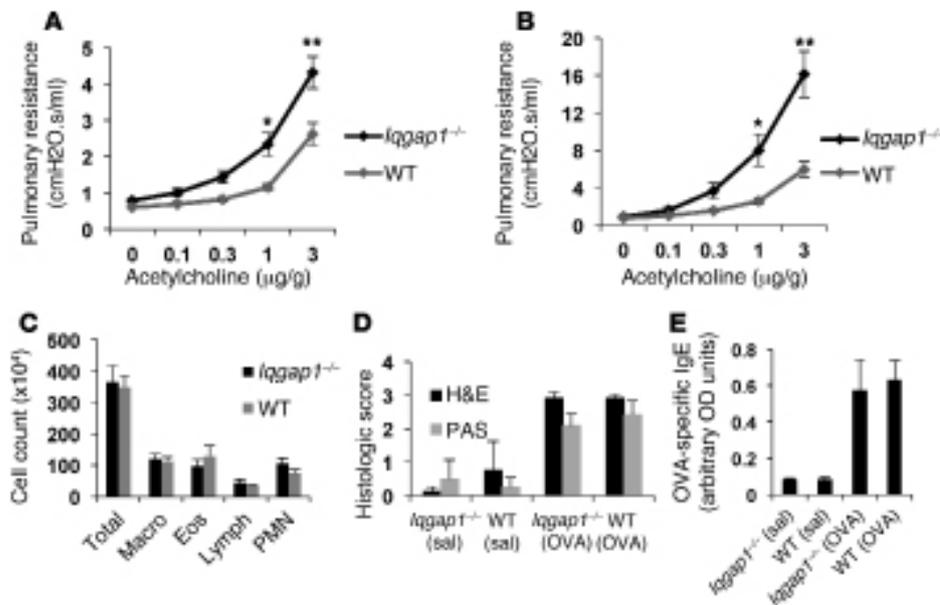


Figure 1. Airway responsiveness and inflammation in *Iqgap1*^{-/-} mice. (A) Pulmonary resistance in anesthetized, mechanically ventilated *Iqgap1*^{-/-} and WT mice treated with increasing doses of i.v. acetylcholine ($n = 7-8$ per group). * $P \leq 0.01$, ** $P \leq 0.001$ vs. WT. (B) Pulmonary resistance in OVA-sensitized *Iqgap1*^{-/-} and WT mice ($n = 10-11$ per group). * $P \leq 0.01$, ** $P \leq 0.001$ vs. WT. (C) Bronchoalveolar lavage cell count and differential in OVA-sensitized *Iqgap1*^{-/-} and WT mice ($n = 3-6$ per group). (D) Inflammation in *Iqgap1*^{-/-} and WT lung sections, quantified by scoring of periodic acid-Schiff (PAS) and H&E stain ($n = 4-6$ per group). (E) Serum OVA-specific IgE, measured by ELISA ($n = 4-6$ per group).

that IQGAP1 normally inhibits airway contraction through a direct effect within the airway smooth muscle. No difference in quantity of smooth muscle was found by cross-sectional immunofluorescence (Supplemental Figure 2A), which suggests that the increased contractile force in *Iqgap1*^{-/-} mice was not attributable to airway smooth muscle hypertrophy. We also found no difference between *Iqgap1*^{-/-} and WT mice in expression of the M3 muscarinic receptor (the receptor for methacholine, one of the agonists used in tracheal ring contraction studies; Supplemental Figure 2B). In human airway smooth muscle cells with and without IQGAP1 knockdown, methacholine-induced inositol 1-phosphate accumulation (a measure of M3 muscarinic receptor pathway activity) was likewise equivalent (Supplemental Figure 2C and Supplemental Figure 3A).

The small GTPase RhoA increases smooth muscle contractility, and IQGAP1 has previously been shown to bind RhoA (9). Consistent with a pattern of increased RhoA activation, MLC phosphatase phosphorylation and MLC phosphorylation were both increased in *Iqgap1*^{-/-} compared with WT samples (Figure 2D). RhoA-GTP, the active form, was increased in *Iqgap1*^{-/-} tracheal smooth muscle and in human airway smooth muscle cells with shRNA-mediated IQGAP1 knockdown (Figure 2, E and F, and Supplemental Figure 3A). These data suggest that IQGAP1 normally blunts airway smooth muscle contractility by inhibiting RhoA activation.

Since IQGAP1 is known to act as a protein scaffold (4, 5, 14), we reasoned that IQGAP1 might bind a RhoGAP and RhoA simultaneously, thus enhancing the inhibitory effect of the RhoGAP on RhoA activation. One RhoGAP in particular, p190A-RhoGAP, is well characterized in multiple cell types (15-17), and we found

p190A-RhoGAP knockdown to increase RhoA activation in human airway smooth muscle cells (Figure 2F and Supplemental Figure 3B). We wished to study the functional role of p190A-RhoGAP further, but noted that deficiency of p190A-RhoGAP in mice causes perinatal lethality due to CNS defects (18) and that shRNA targeting of p190A-RhoGAP caused minimal protein knockdown within the 3 days in which isolated tracheal rings remain viable for testing (Supplemental Figure 3B), presumably as a result of the relative stability of p190A-RhoGAP protein. Therefore, we pursued further studies in primary human airway smooth muscle cells, since we observed a congruent inhibitory effect of IQGAP1 on RhoA activation in these clinically and physiologically relevant cells (Figure 2F).

First, we found that IQGAP1 co-immunoprecipitated with p190A-RhoGAP and RhoA in airway smooth muscle cell lysates (Figure 3, A and B). The associations between IQGAP1 and p190A-RhoGAP and between IQGAP1 and RhoA were confirmed by proximity ligation assay (PLA; Figure 3C). We then reasoned that if IQGAP1 serves as a scaffold for p190A-RhoGAP and RhoA, its deficiency should decrease the association between p190A-RhoGAP and RhoA. Using PLA to test this hypothesis, we found decreased numbers of PLA foci for p190A-RhoGAP and RhoA in cells with IQGAP1 knockdown and in *Iqgap1*^{-/-} tracheal smooth muscle (Figure 3, D and E).

To explore the potential relevance of these findings to clinical airway diseases, we quantified IQGAP1 in lysates of airway biopsies acquired previously as part of a study of the effect of ozone exposure on both asthmatic patients and healthy controls (19). In airway biopsy lysates from asthmatic patients, α -smooth muscle actin (α -SMA) was increased; since the preponderant

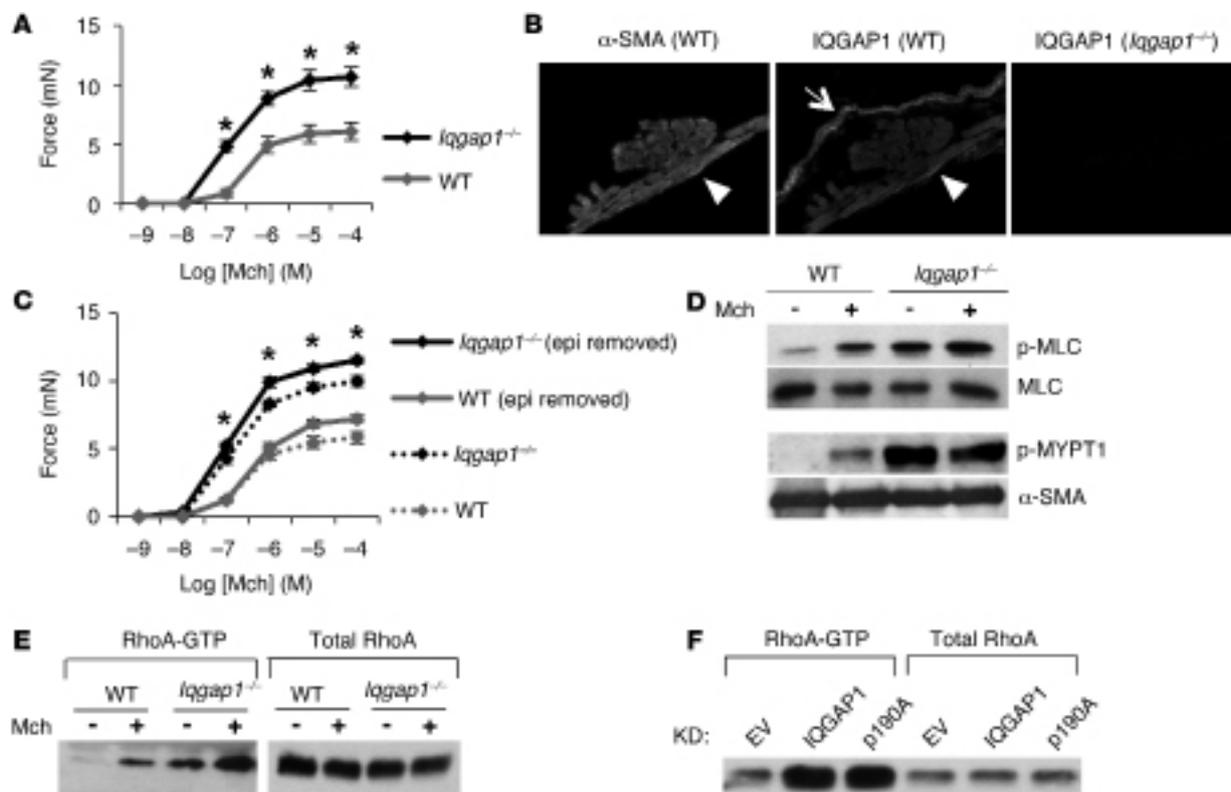


Figure 2. IQGAP1 suppresses airway smooth muscle contraction. (A) Force of contraction for *lqgap1*^{-/-} and WT tracheal rings in response to methacholine (Mch; $n = 6$ per group). * $P \leq 0.001$ vs. WT. (B) Tracheal immunofluorescence for IQGAP1 and α -SMA. IQGAP1 staining was seen in both the epithelium (arrow) and the smooth muscle (arrowheads). Original magnification, $\times 10$. (C) Force of contraction in response to methacholine for *lqgap1*^{-/-} and WT tracheal rings with and without epithelium removed ($n = 6$ per group). * $P \leq 0.001$ vs. respective WT. (D) Western blots with lysates of posterior trachea from *lqgap1*^{-/-} and WT mice. p-MLC, phosphorylated MLC; p-MYPT1, phosphorylated MLC phosphatase-1. (E) GST-rhotekin bead pull-down of RhoA-GTP (RhoA active form) with lysates of posterior trachea from *lqgap1*^{-/-} and WT mice. Results in D and E are representative of at least 3 individual experiments. (F) GST-rhotekin bead pull-down of RhoA-GTP with lysates of human airway smooth muscle cells with and without knockdown (KD) of IQGAP1 or p190A-RhoGAP. EV, empty vector.

fraction of α -SMA in these biopsies is present in airway smooth muscle, with relatively minor contributions from fibroblasts and vascular smooth muscle, this result is consistent with an expected increase in smooth muscle mass in these patients. When normalized to this increased α -SMA expression, IQGAP1 signal was significantly decreased in asthmatic patients compared with healthy controls (Figure 3F). Although we cannot absolutely exclude contributions from other α -SMA-expressing cells, this finding is consistent with a relative reduction in IQGAP1 expression in airway smooth muscle of asthmatic patients compared with controls, which could contribute to increased airway narrowing in asthma.

Herein, we demonstrated the importance of IQGAP1 as a signaling scaffold in multiple contexts. Together, our findings suggest a role for this scaffolding function in airway smooth muscle: IQGAP1 serves as a brake on RhoA activation by colocalizing RhoA with the inactivating enzyme p190A-RhoGAP. Future studies will further investigate the role of IQGAP1 expression in modulating the severity of airway contraction in acute asthma.

Methods

Further information can be found in Supplemental Methods.

Statistics. Data were evaluated with SigmaStat software by repeated-measures ANOVA or 2-tailed Student's *t* test. A *P* value of 0.05 or less was considered statistically significant. When differences found by ANOVA were statistically significant, further testing to account for multiple comparisons was performed by Tukey test. Unless otherwise indicated, data are presented as mean \pm SEM.

Study approval. All experiments using mice were approved by the Institutional Animal Care and Use Committee of UCSF. All experiments using human tissue were approved by the UCSF Committee on Human Research. Informed consent was obtained from all human subjects in accordance with the UCSF Committee on Human Research.

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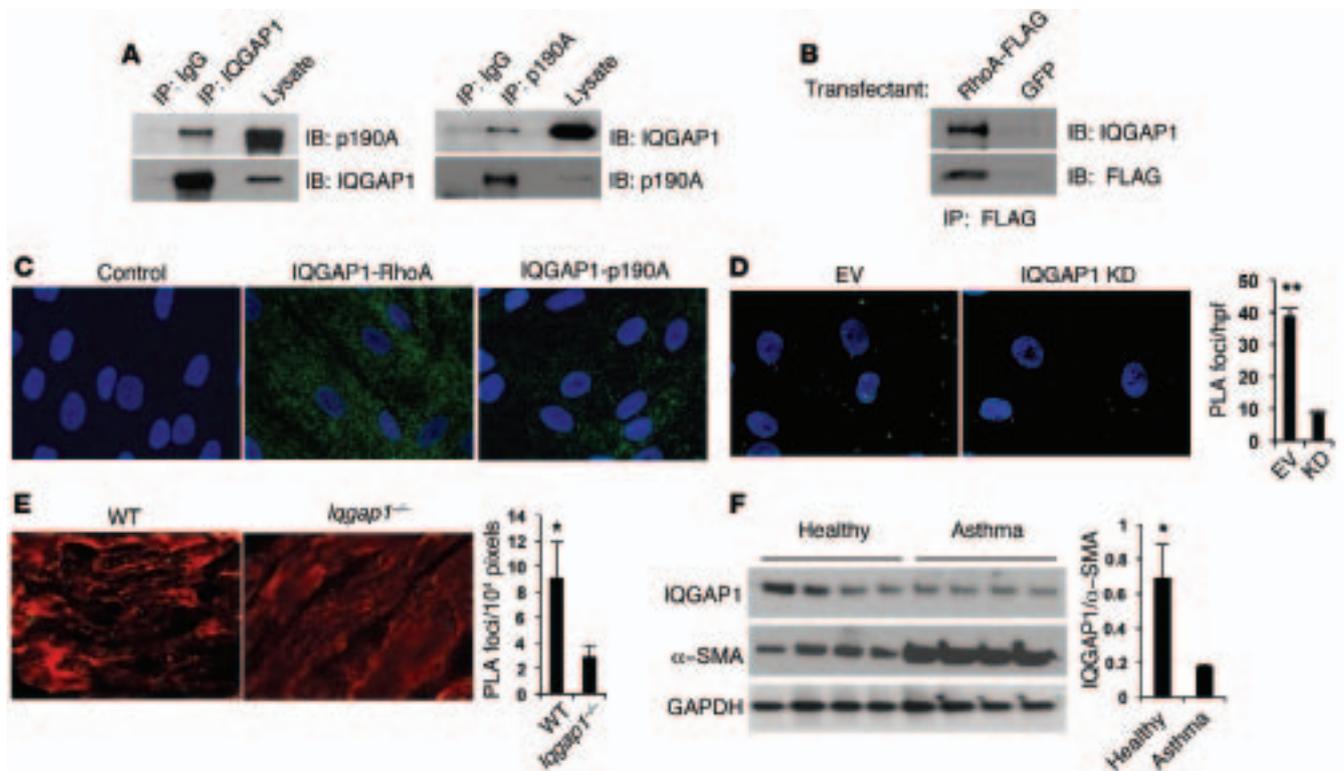


Figure 3. IQGAP1 acts as a scaffold that colocalizes p190A-RhoGAP and RhoA. (A) Co-immunoprecipitation of IQGAP1 and p190A-RhoGAP in lysates of human airway smooth muscle. (B) Co-immunoprecipitation of IQGAP1 and FLAG in lysates of human airway smooth muscle transfected with Rho-FLAG or GFP control. (C) Association of IQGAP1 with both RhoA and p190A-RhoGAP was also demonstrated by PLA in cultured human airway smooth muscle cells. In the control condition, PLA probes were applied without primary antibody. Nuclei were stained with DAPI. Original magnification, $\times 63$. (D) Spatial association of p190A-RhoGAP and RhoA, tested by PLA in cultured human airway smooth muscle cells with and without IQGAP1 knockdown and seeded at equal density. Nuclei were stained with DAPI. Number of PLA foci per high-power field (hpf) was calculated for 10 images per condition. $**P \leq 0.001$. Original magnification, $\times 63$. (E) Spatial association of p190A-RhoGAP and RhoA, tested by PLA in 10- μm mouse tracheal sections. Smooth muscle was stained with anti- α -SMA antibody (red). Number of PLA foci per unit area of smooth muscle is also shown ($n = 4$ per group). $*P \leq 0.05$. Original magnification, $\times 63$. (F) Immunoblots for IQGAP1, α -SMA, and GAPDH were performed for lysates of airway biopsies from separate healthy control and asthmatic human subjects. Bands were measured by densitometry, and IQGAP1 was quantified normalized to α -SMA ($n = 4$ per group). $*P \leq 0.05$.

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