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IQGAP1 Mediates α-Smooth Muscle Actin Expression and Enhances Contractility of Lung Fibroblasts

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Abstract

Contractility is a predominant feature of myofibroblasts and a key process in fibrotic tissue remodeling. We previously demonstrated that lung fibroblasts from scleroderma patients with interstitial lung disease express a robust contractile phenotype and contain large amounts of the IQ motif containing GTPase activating protein (IQGAP1). Here we show the contribution of IQGAP1 to two major features of lung myofibroblasts: α-smooth muscle actin (SMA) expression and collagen gel contraction. We found that IQGAP1 mediates SMA expression and contractile activity of scleroderma lung myofibroblasts as well as normal lung fibroblasts stimulated *in vitro* with thrombin, TGFβ1, or CTGF. We show that IQGAP1 co-immunoprecipitates with SMA and co-localizes with monomeric SMA, but not with the highly organized SMA in scleroderma lung myofibroblasts. In contrast, IQGAP1 binds directly to polymerized β- and γ-actin isoforms suggesting distinct mechanisms of α-SMA and β-/γ-actin polymerization in scleroderma lung myofibroblasts. We conclude that the primary role of IQGAP1 in promoting contractility of the lung parenchyma and contributing to restrictive lung disease is through the regulation of SMA expression and organization in lung fibroblasts.

Keywords: IQGAP1; SMA; Thrombin; Contractility scleroderma lung myofibroblasts

Introduction

Scleroderma associated interstitial lung disease (SSc-ILD) is an irreversible and progressive disease process often leading to respiratory failure and death [1,2]. The pathology of SSc-ILD demonstrates excessive deposition of collagen and other extracellular matrix proteins within the pulmonary interstitium, ultimately leading to obliteration of airspaces and progressive loss of lung function [3]. The understanding of mediators that orchestrate this aberrant tissue repair, as well as their longitudinal expression, will allow the development of much needed novel interventions to treat this major complication of SSc.

Previously, we found IQ motif containing GTPase activating protein (IQGAP1) to be consistently elevated in lung fibroblasts isolated from SSc patients [4]. We also found that IQGAP1 expression could be induced in normal lung fibroblasts when exposed to connective tissue growth factor (CTGF, CCN2); moreover, suppression of IQGAP1 by RNA interference decreases the migration of scleroderma and CTGF-induced normal lung fibroblasts [4].

IQGAP1 is a ubiquitous 189 kDa molecule that contains five protein-interacting domains. Calponin homology domain binds to F-actin and mediates IQGAP1-induced actin polymerization *in vitro* [5]. The WW domain is an interaction module for proline-rich ligands characterized by 2 conserved tryptophan residues [6]. The IQ domain is a tandem repeat of four IQ motifs that bind calmodulin and myosin light chain proteins [7,8]. The GAP-related domain mediates the binding of the CDC42 and Rac1 GTPases [6]. The RasGAP carboxyl terminus domain interacts with microtubule-binding protein CLIP170 necessary for binding β-catenin and E-cadherin [9]. Through interaction with its target proteins, IQGAP1 participates in multiple cellular functions including adhesion, migration, and integration of complex signaling pathways within the cell [10].

During the development of SSc-ILD, lung fibroblasts undergo specific phenotypic modulation and develop cytoskeletal features similar to those of smooth muscle cells. These phenotypically altered, activated fibroblasts, or myofibroblasts, express the highly contractile protein SMA and appear to be the principal cells responsible for tissue remodeling, collagen deposition, and the restrictive nature of the lung parenchyma associated with SSc-ILD [11-13]. IQGAP1 is known to regulate total actin polymerization [14]; however, effects of IQGAP1 on SMA nave not been reported.

The present study was undertaken to investigate the potential regulation of SMA by IQGAP1.

Material and Methods

Cell culture and transfection

Lung fibroblasts were derived from SSc patients and from age-, race-, and sex-matched normal subjects who died from non-pulmonary causes as previously described [15,16] in accordance with regulations of the Institutional Review Board of the Medical University of South Carolina. IQGAP1 in pcDNA3 (generously provided by Dr. David Sacks, Harvard Medical School) was transfected into normal lung fibroblasts using Effectene reagent (Qiagen, Valencia, CA) as previously described [4]. Empty pcDNA3 was used as a control. Amount of DNA used for transfection was 4 μg per 100-mm dish, 1 μg/well for 6-well plates, and 0.2 μg/well for 24-well plates. IQGAP1 siRNA (150 pmol per 100-mm dish, 75 pmol/well for 6-well plates, and 15 pmol/well for 24-well plates) (Santa Cruz Biotechnology, Santa Cruz, CA) was transfected into cells according to the manufacturer's instructions [4]. Scrambled siRNA containing a sequence that does not lead to the specific degradation of any known cellular mRNA was used as a control. Transfection efficiency was routinely tested in 48 hours after transfection by immunoblotting with anti-IQGAP1 antibody (Santa Cruz Biotechnology, Santa Cruz, CA).

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Preparation of cell extracts and immunoblotting

Normal and SSc lung fibroblasts were washed with ice-cold PBS and lysed with ice-cold lysis buffer (10 mM Tris, 10 mM EDTA, 1% Nonidet P-40, 0.5% deoxycholate, 0.1% SDS, pH=7.4). Protein concentration was determined by BCA™ protein assay as described previously [17]. For each sample, 40 μg of protein was denatured, subjected to SDS-polyacrylamide gel electrophoresis and analyzed by immunoblotting with appropriate antibodies. The immunoblots were then stripped and re-blotted with anti-β-actin antibody as a loading control.

Collagen gel contraction assays

Collagen lattices were prepared with type I collagen from rat tail tendon adjusted to a final value of 2.5 mg/ml with 0.01% acetic acid. Lung fibroblasts at a concentration 2.5×10^5 cells/ml were suspended in collagen (1.25 mg/ml of collagen) and aliquoted into 24 well plates (300 μl/well). Then collagen lattices were polymerized for 45 minutes in a humidified 10% CO_2 atmosphere at 37°C; 500 μl/well DMEM containing 10% FCS was added for 4 hours, followed by overnight incubation in serum-free medium. To initiate collagen gel contraction polymerized gels were gently released from the underlying culture dish and then fibroblasts were stimulated with thrombin, TGFβ1 or CTGF. To determine the degree of collagen gel contraction, pictures were taken after 24 hours of stimulation using a digital camera. Measurement of the diameter of each gel in mm was recorded as the average values of the major and minor axes as previously described [15,17]. Calculation of gel contraction was presented as difference between diameters of wells and contracted gels. In some experiments, collagen gels were collected, digested with collagenase and analyzed by Western blot using anti-SMA, anti-IQGAP1, and anti-β-actin antibodies.

Luciferase assay

Lung fibroblasts were cultured in 24-well plates and transfected with human SMA promoter luciferase reporter construct (generously provided by Dr. Gerard Elberg, University of Oklahoma Health Sciences Center) using Effectene Transfection Reagent. To investigate effects of IQGAP1, cells were co-transfected either with IQGAP1 pcDNA3 or pcDNA3, or with IQGAP1 siRNA or control siRNA. In all experiments, GFP plasmid was co-transfected in order to standardize for transfection efficiency. The cells were incubated with or without TGFβ1 for 24 h and lysed in Passive Lysis Buffer according to the Promega luciferase assay system protocol. The luciferase activity of the cell lysates was measured with luciferase substrate using a luminometer. Data were expressed as relative firefly luciferase signal normalized by the GFP signal for each individual analysis. Each sample was analyzed in triplicate.

Immunofluorescent studies

Human lung fibroblasts were cultured to sub-confluence on glass slides, serum starved overnight, fixed with 4% formaldehyde, and blocked with PBS containing 5% BSA, 0.1% Triton, and 0.0004% sodium azide. Cells were incubated overnight with anti-SMA antibody (Sigma-Aldrich, St. Louis, MO) and/or anti-IQGAP1 followed by Alexa Fluor 555 conjugated secondary antibody (Life Technology, Carlsbad, CA). Next, slides were washed with PBS and incubated with Alexa Fluor 488 phalloidin (Life Technology, Carlsbad, CA) for 30 minutes. Finally, slides were mounted with ProLong Gold antifade reagent with DAPI and visualized under Olympus FV10i laser scanning confocal or Zeiss Axio Imager M2 microscope system.

Statistical analysis

Statistical analyses were performed using analysis of variance models followed by *post hoc* testing or nonparametric test as appropriate with KaleidaGraph 4.0 (Synergy Software, Reading, PA). All results were considered significant if $p<0.05$.

Results

IQGAP1 and contractile activity of lung fibroblasts

We previously reported that thrombin induces collagen gel contraction by normal lung fibroblasts in a dose-dependent manner with a maximal effect at 0.5 units/ml [11]. To determine whether IQGAP1 is involved in thrombin-induced contraction of normal lung fibroblasts, we knocked down IQGAP1 expression using siRNA. Transfection with IQGAP1 siRNA resulted in a significant reduction of IQGAP1 protein (Figure 1), and the contractile activity of thrombin-stimulated lung fibroblasts decreased by more than 30% (Figure 1A). We observed no significant differences in collagen gel contractions when lung fibroblasts were cultured in serum-free medium without thrombin using either IQGAP1siRNA or scrambled siRNA (Figure 1A).

To investigate effects of IQGAP1 on contractile activity of lung fibroblasts in more detail, we transfected cells with IQGAP1 pcDNA3. The level of IQGAP1 in transfected normal lung fibroblasts approximately tripled as compared to cells transfected with vector alone (Figure 1D). Interestingly, we noted enhanced expression of IQGAP1 when thrombin was added to lung fibroblasts. Contractile activity of normal lung fibroblasts transfected with IQGAP1 increased from 2.77 ± 0.86 mm to 4.31 ± 1.09 mm, a difference that approached statistical significance ($p=0.06$). Overexpression of IQGAP1 further promoted thrombin-induced collagen gel contraction increasing thrombin-induced contractile activity of lung fibroblasts from 8.67 ± 1.56 mm in control cells to 10.58 ± 1.71 mm in IQGAP1 pcDNA3-transfected cells (Figure 1B).

Contractile activity of lung fibroblasts is dependent on the expression of SMA [7,8]. We observed that thrombin increases the transcriptional activity of the SMA promoter 3.32-fold in normal lung fibroblasts (Figure 2). When cells were transfected with IQGAP1siRNA, activity of the SMA promoter induced by thrombin was reduced by nearly 1.7-fold and was not affected by either scrambled or IQGAP1siRNA in resting cells not activated with thrombin. Thrombin increased the transcriptional activity of the SMA promoter approximately 4-fold in lung fibroblasts transfected with IQGAP1 pcDNA3; cells transfected with IQGAP1 pcDNA3 but not stimulated with thrombin were characterized by a modest, 1.8-fold increase of the transcriptional activity of SMA promoter (Figure 2).

Regulation of thrombin-induced SMA protein expression by IQGAP1 was studied by immunoblotting. We found that depletion of IQGAP1 by siRNA has little or no effect on the basal expression of SMA, but significantly reduces (2.6-fold, $p<0.01$) thrombin-induced SMA expression (Figure 3). Expression of SMA in lung fibroblasts transfected with IQGAP1 was notably greater than in lung fibroblasts transfected with vector. Thrombin increased the expression of SMA 6.8-fold in lung fibroblasts transfected with vector and 9.6-fold in lung fibroblasts transfected with IQGAP1 (Figure 3).

Other profibrotic agents, such as TGFβ1 and CTGF, are known to induce SMA and a contractile phenotype in isolated lung fibroblasts [13,18]. To determine whether IQGAP1 is involved in TGFβ1- and CTGF-mediated contractile activity, we knocked down IQGAP1 expression and observed that depletion of IQGAP1 decreased both TGFβ1- and CTGF-induced collagen gel contraction by 47.66% and 35.5%, respectively (Figure 4A). TGFβ1- and CTGF-induced expression of SMA was also reduced in cells transfected with IQGAP1siRNA (Figure 4B).

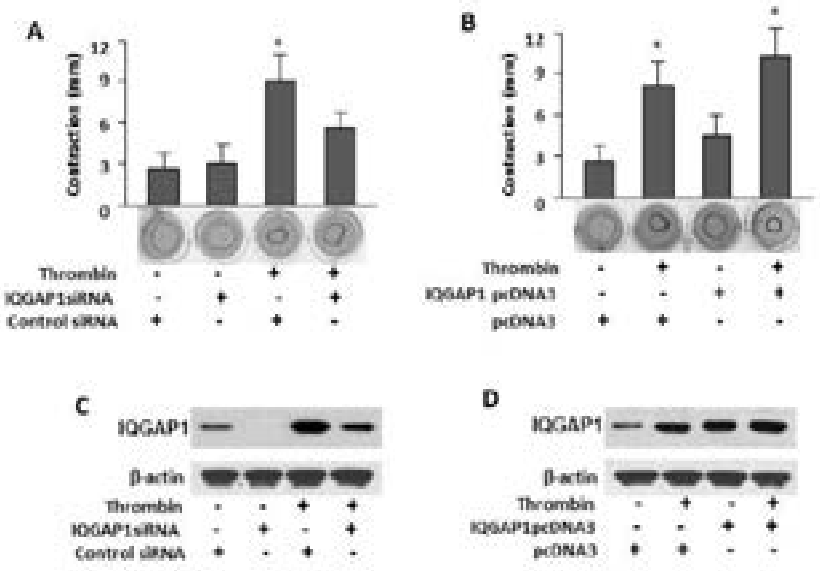

Figure 1: IQGAP1 mediates contractile activity of thrombin-stimulated lung fibroblasts. A and B: Normal lung fibroblasts were transfected with IQGAP1 siRNA or scrambled siRNA (A) and with IQGAP1 pcDNA3 or empty vector (B) As detailed under Material and Methods. Cells were cultured within 1.5 mg/ml collagen in 24 well plates in DMEM with 2% FBS overnight followed by incubation with and without 0.5 U/ml thrombin for 24 hours. C and D: Transfection efficiency was routinely tested by immunoblotting with anti-IQGAP1 antibody; anti-β-actin antibody was used as a loading control. Experiment was repeated 3 times, and representative results are presented. The asterisk represents statistically significant differences between cells stimulated with thrombin versus unstimulated cells ($p<0.05$).

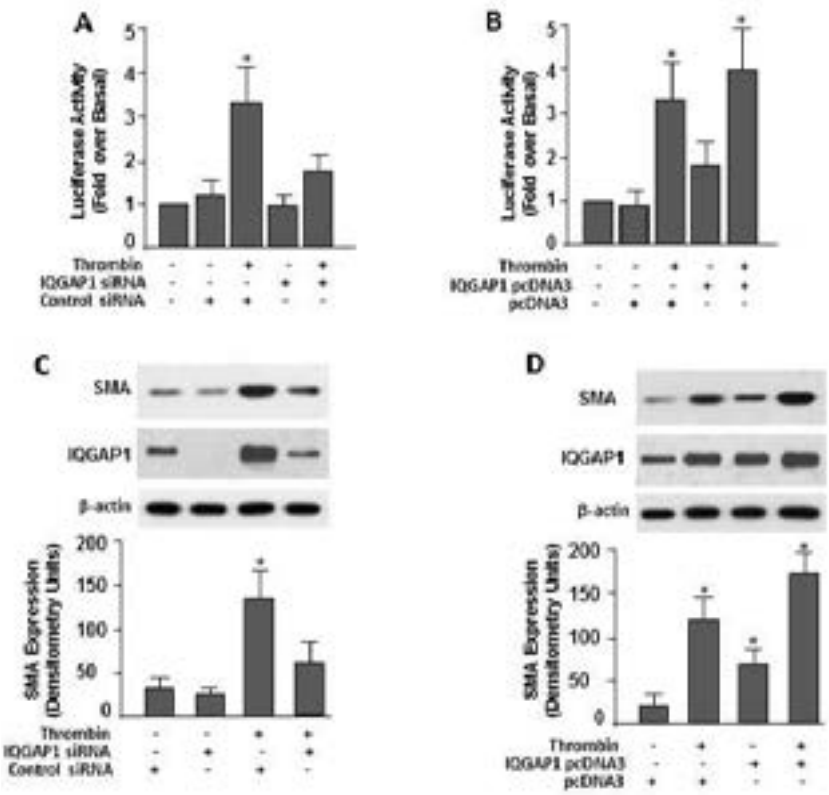

Figure 2: Thrombin induces transcriptional activity of SMA promoter in IQGAP1-dependent manner. A and B: Normal lung fibroblasts were co-transfected with SMA promoter luciferase reporter construct and with IQGAP1 siRNA or scrambled siRNA (A) and with SMA promoter luciferase reporter construct and IQGAP1 pcDNA3 or empty vector (B). Cells were incubated with and without 0.5 U/ml thrombin for 24 hours and subjected to luciferase assay as detailed under Material and Methods. Data are expressed as relative luciferase signal normalized by the green fluorescent protein signal for each individual analysis. The asterisk represents statistically significant differences between cells stimulated with thrombin versus unstimulated cells ($p<0.05$). C and D: Transfection efficiency was routinely tested by immunoblotting with anti-IQGAP1 antibody; anti-β-actin antibody was used as a loading control.

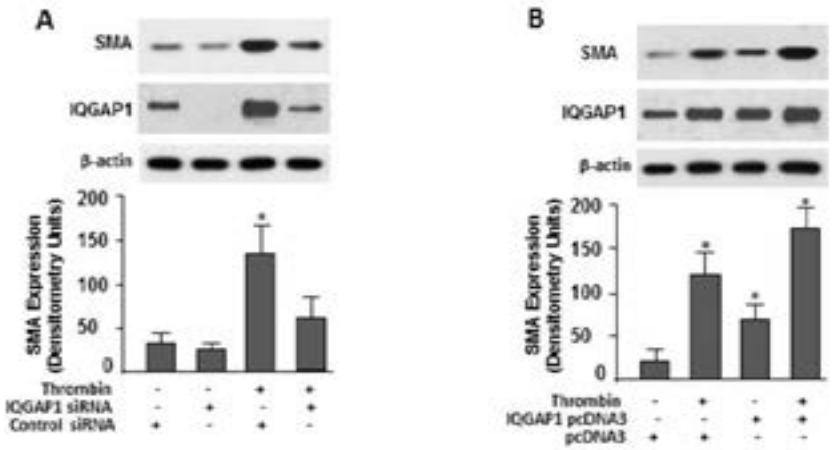

Figure 3: Thrombin induces SMA expression in lung fibroblasts in IQGAP1-dependent manner. Normal lung fibroblasts, cultured in 6-well plates, were transfected with IQGAP1 siRNA or scrambled siRNA (A) and with IQGAP1 pcDNA3 or empty vector (B). Cells were stimulated with and without 0.5 U/ml thrombin for 24 hours and subjected to immunoblotting with anti-SMA, anti-IQGAP1, and anti-β-actin antibodies. Representative immunoblots from 3 independent experiments are presented. SMA immunoblots were analyzed by scanning densitometry using NIH Image software; values are the mean and SD from 3 independent experiments. The asterisk represents statistically significant differences versus siRNA-transfected (A) and pcDNA3-transfected (B) unstimulated cells ($p<0.05$).

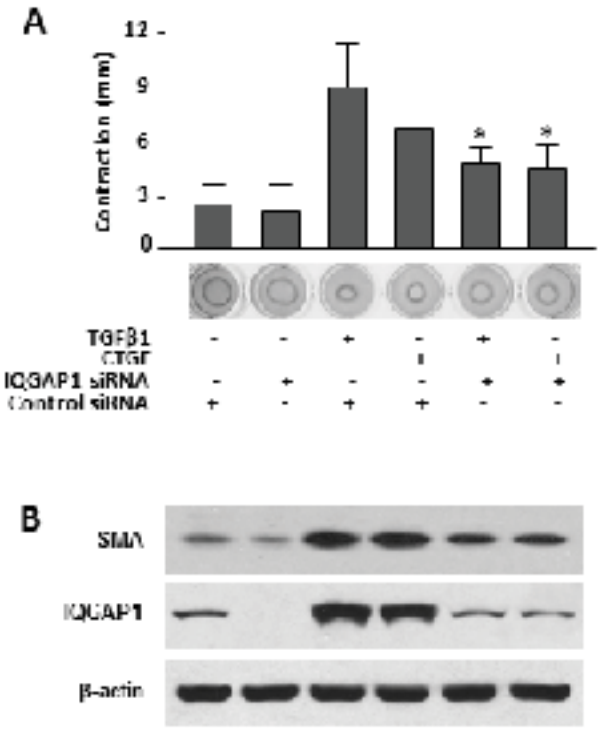

Figure 4: IQGAP1 mediates TGFβ- and CTGF-induced collagen gel contraction and SMA expression in lung fibroblasts. Normal lung fibroblasts were transfected with IQGAP1 siRNA or scrambled siRNA, cultured within 1.5 mg/ml collagen gel in 24 well plates or in 6-well plates without collagen and stimulated with and without TGFβ1 (2.5 ng/ml) or CTGF (50 ng/ml) for 24 hours. A: Illustrative collagen gel contraction image and graphs are presented. Data represent mean values ± SD from three experiments, each performed in duplicate. B: Representative immunoblots with anti-SMA, anti-IQGAP1, and anti-β-actin antibodies from three independent experiments are presented.

Previously, we reported that scleroderma lung fibroblasts innately express large amounts of highly organized SMA and can contract collagen gels without exogenous treatment [11]. To investigate if IQGAP1 influences the contractile activity of scleroderma lung fibroblasts, we studied collagen gel contraction in three different scleroderma cell lines transfected either with IQGAP1siRNA scrambled IQGAP1siRNA. We observed that scleroderma cells transfected with scrambled siRNA contracted gels from 15 mm in diameter to less than 5 mm in diameter, whereas all three cell lines transfected with IQGAP1siRNA contracted gels significantly ($p<0.05$) less (Figure 5A). The level of SMA expression in scleroderma lung fibroblasts transfected with IQGAP1siRNA was significantly ($p<0.05$) reduced as compared to cells transfected with control siRNA (Figure 5B).

Association of IQGAP1 and SMA in lung fibroblasts

IQGAP1 is known to interact with polymerized filamentous (F)-actin [5], so we hypothesized that IQGAP1 would interact with polymerized or organized SMA in lung fibroblasts. Normal lung fibroblasts were transfected with IQGAP1 and stimulated with thrombin to induce SMA expression and organization. Cell lysates were immunoprecipitated with anti-IQGAP1 antibody and immunoblotted with anti-SMA antibody. We could not co-immunoprecipitate IQGAP1 and SMA in cells transfected with empty vector and not stimulated with thrombin. However, in lung fibroblasts transfected with IQGAP1, we could co-immunoprecipitate IQGAP1 and SMA regardless with or without thrombin treatment (Figure 6A).

We observed a strong interaction of IQGAP1 with SMA in lung fibroblasts derived from scleroderma patients with lung fibrosis. Co-immunoprecipitation of IQGAP1 and SMA in scleroderma cells occurred independent of thrombin, CTGF or TGFβ (Figure 6B). To investigate whether SMA polymerization is necessary for SMA/IQGAP1 interaction, we used an inhibitor of actin organization, cytochalasin D. Surprisingly, we found that disruption of actin organization by cytochalasin D led to enhanced SMA/IQGAP1 interaction in scleroderma lung fibroblasts (Figure 6C). We confirmed by immunofluorescence that IQGAP1 co-localizes with monomeric SMA but not with highly organized SMA in Figure 6D. Importantly, IQGAP1 co-localizes with F-actin in scleroderma lung fibroblasts

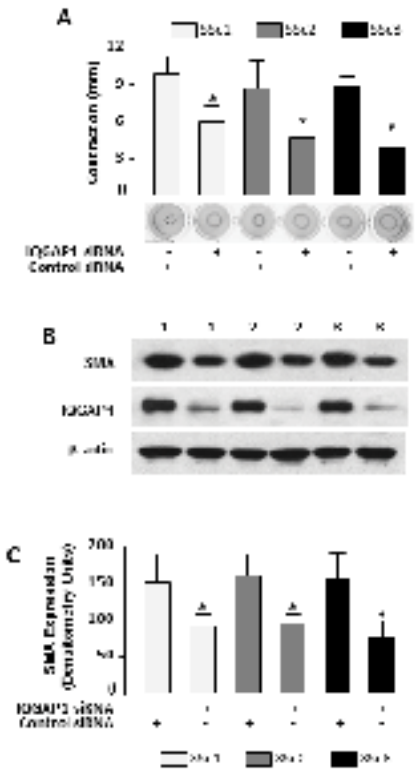

Figure 5: IQGAP1 siRNA reduces SMA expression and contractile activity of SSc lung fibroblasts. Scleroderma lung fibroblasts were transfected with IQGAP1 siRNA or scrambled siRNA and cultured within 1.5 mg/ml collagen gel in 24-well plates or in 6-well plates without collagen. A: Collagen gel contraction was measured in 48 hours after siRNA transfection. Data represent mean values ± SD from four experiments. B: representative immunoblots with anti-SMA, anti-IQGAP1, and anti-β-actin antibodies from four independent experiments are presented. C: SMA immunoblots were analyzed by scanning densitometry using NIH Image software; values are the mean and SD from four independent experiments. The asterisk represents statistically significant differences versus unstimulated cells ($p<0.05$).

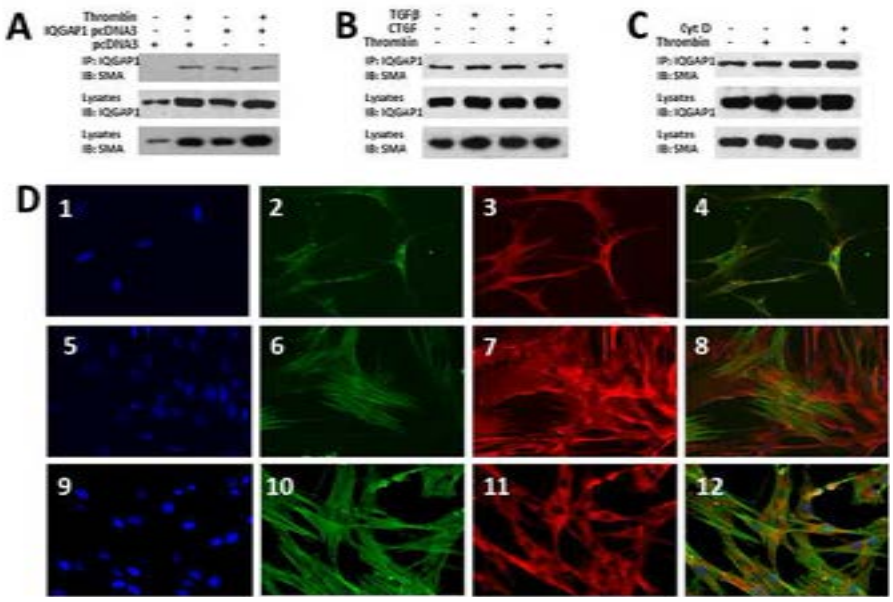

Figure 6: Co-localization pattern of IQGAP1 and SMA in lung fibroblasts. A: IQGAP1 co-immunoprecipitates with SMA in thrombin-induced normal lung fibroblasts. Normal lung fibroblasts were transfected with IQGAP1 pcDNA3 or empty vector followed by incubation with and without 0.5 U/ml thrombin for 24 hours. Co-immunoprecipitation was performed as detailed under Material and Methods. B: Co-immunoprecipitation of IQGAP1 with SMA in scleroderma lung fibroblasts. Cells were incubated with and without TGFβ1, CTGF, and thrombin for 24 hours, collected, and subjected to immunoprecipitation. C: Cytochalasin D (Cyt D) enhances co-immunoprecipitation of IQGAP1 with SMA in scleroderma lung fibroblasts. D: IQGAP1 colocalizes with monomeric but not with highly organized SMA and with F-actin in scleroderma lung fibroblasts.

(Figure 6D) suggesting that IQGAP1 binds directly to polymerized β- and γ-actin isoforms but not to the polymerized SMA isoform.

Discussion

Increased contractility of lung parenchyma in pulmonary fibrosis associated with scleroderma and other interstitial lung diseases has been well recognized [11-13,19]. However, the factors orchestrating the contractile capacity of fibrotic lung are not completely understood. In this study, we demonstrate IQGAP1 to be an important contributor to the contractile forces leading to increased lung stiffness in SSc-ILD and *in vivo* and *in vitro* models of lung fibrosis.

IQGAP1 is a highly conserved scaffold protein that coordinates multiple signaling pathways including those involved in cytoskeletal rearrangement [20-22]. Here we present IQGAP1 as an important regulator of SMA expression and contractile forces in scleroderma lung myofibroblasts and normal lung fibroblasts stimulated with either thrombin, TGFβ1, or CTGF.

Contractile properties of myofibroblasts mostly depend on well-formed cytoskeletal microfilaments comprised of SMA stress fibers [12,13]. Forces generated by the contractile activity of myofibroblasts are transmitted through the large fibronexus adhesion complexes to the ECM [23]. Lung myofibroblasts cultured in collagen gel recognize extracellular collagen fibers as ECM and might serve as models of a stiffened lung parenchyma in pulmonary fibrosis [24]. Contraction of floating collagen gels resembles the initial phase of wound contraction and reflects the induction of the myofibroblast phenotype by various growth factors [21,22]. In contrast, attached or fixed collagen gels serve as a model of the late phase of excessive scarring observed in contractures and reflect the direct ability of proteins to enhance contraction of already formed SMA through mechanical stress [24,25]. In the current study, we present effects of IQGAP1on the floating collagen gel contractions. We demonstrate that depletion of IQGAP1 by siRNA results in reduction of the floating collagen gel contractions in scleroderma lung fibroblasts and in normal lung fibroblasts induced by profibrotic stimuli such as thrombin, TGFβ1 and CTGF. Correspondingly, downregulation of IQGAP1 by siRNA decreases thrombin-induced transcriptional activity of the SMA promoter and expression of SMA protein.

One of the most important observations made in this study is that IQGAP1 interacts with monomeric but not with a polymerized SMA. The actin cytoskeleton of myofibroblasts consists of SMA, β-actin and γ-actin isoforms, presented in cells in either a monomeric state (G-actin) or a polymeric state (F-actin). G-actin, also called cytosolic actin, localizes mainly in cell cytosol, while bundles of polymerized F-actin form the actin stress fiber network [26,27]. The β-actin and γ-actin isoforms are ubiquitous and remain the primary components of actin filaments in most cells including fibroblasts [26,27]. The CH domain of IQGAP1 was believed to bind F-actin of any actin isoform [5]. In this publication, we present data suggesting that IQGAP1 does not interact with F-actin of the SMA isoform. We propose that as a scaffolding protein IQGAP1 shuttles monomeric SMA facilitating its assembly through a mechanism distinct from β- and γ-actin polymerization in lung fibroblasts (Figure 7). Further studies are necessary to delineate such mechanisms. Our current study suggests that shuttling monomeric SMA results in the increased rate of SMA polymerization and organization in lung fibroblasts.

Funding

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Figure 7: Proposed role of IQGAP1 in actin polymerization. IQGAP1 binds and shuttles β- and γ- G-actin isoforms either as monomeric G-actins and as polymerized F-actins. In contrast, IQGAP1 binds SMA only as a monomeric G-actin isoform. Fast transportation of the monomeric SMA by IQGAP1 results in the increased rate of SMA polymerization and organization in lung fibroblasts.

References

1. Bogatkevich GS (2012) Lung Involvement in Scleroderma. Rheumatol Curr Res S1- e001.
2. Morales-Cárdenas A, Pérez-Madrid C, Arias L, Ojeda P, Mahecha MP, et al. (2016) Pulmonary involvement in systemic sclerosis. Autoimmun Rev 15: 1094-1108.
3. Akter T, Silver RM, Bogatkevich GS (2014) Recent advances in understanding the pathogenesis of scleroderma-interstitial lung disease. Curr Rheumatol Rep: 16: 411.
4. Bogatkevich GS, Ludwicka-Bradley A, Singleton CB, Bethard JR, Silver RM (2008) Proteomic analysis of CTGF-activated lung fibroblasts: identification of IQGAP1 as a key player in lung fibroblast migration. Am J Physiol: Lung Cell Mol Physiol 295: L603-611.
5. Mateer SC, Morris LE, Cromer DA, Bensenor LB, Bloom GS (2004) Actin filament binding by a monomeric IQGAP1 fragment with a single calponin homology domain. Cell Motility Cytoskel 58: 231-241.
6. Macias MJ, Wiesner S, Sudol M (2002) WW and SH3 domains, two different scaffolds to recognize proline-rich ligands. FEBS Letters 513: 30-37.
7. Ho YD, Joyal JL, Li Z, Sacks DB (1999) IQGAP1 integrates Ca2+/calmodulin and Cdc42 signaling. J Biol Chem 274: 464-470.
8. Weissbach L, Bernards A, Herion DW (1998) Binding of myosin essential light chain to the cytoskeleton-associated protein IQGAP1. Biochem Biophys Res Com 251: 269-276.
9. Fukata M, Watanabe T, Noritake J, Nakagawa M, Yamaga M, et al. (2002) Rac1 and Cdc42 capture microtubules through IQGAP1 and CLIP-170. Cell 109: 873-885.
10. Brown MD, Sacks DB (2006) IQGAP1 in cellular signaling: bridging the GAP. Trends Cell Biol 16: 242-249.
11. Bogatkevich GS, Tourkina E, Silver RM, Ludwicka-Bradley A (2001) Thrombin differentiates normal lung fibroblasts to a myofibroblast phenotype via the proteolytically activated receptor-1 and a protein kinase C-dependent pathway. J Biol Chemistry 276: 45184-45192.
12. Bogatkevich GS, Tourkina E, Abrams CS, Harley RA, Silver RM, et al. (2003) Contractile activity and smooth muscle alpha-actin organization in thrombin-induced human lung myofibroblasts. Am J Physiol: Lung Cell Mol Physiol 285: L334-343.
13. Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA (2002) Myofibroblasts and mechano-regulation of connective tissue remodelling. Nat Rev Mol Cell Biol 3: 349-363.
14. Brandt DT, Grosse R (2007) Get to grips: steering local actin dynamics with IQGAPs. EMBO reports 8: 1019-1023.
15. Bogatkevich GS, Ludwicka-Bradley A, Silver RM (2009) Dabigatran, a direct thrombin inhibitor, demonstrates antifibrotic effects on lung fibroblasts. Arthritis and Rheum 60: 3455-3464.
16. Atanelishvili I, Shirai Y, Akter T, Noguchi A, Ash KT, et al. (2016) D1398G Variant of MET Is Associated with Impaired Signaling of Hepatocyte Growth Factor in Alveolar Epithelial Cells and Lung Fibroblasts. PloS one 11: e0162357.
17. Bogatkevich GC, Akter T, Ludwicka-Bradley A, Silver RM (2012) Inhibitory effects of the dual endothelin receptor blocker bosentan on thrombin-activated lung fibroblasts isolated from scleroderma patients. Rheumatol Curr Res S1-005.

18. Bogatkevich GS, Ludwicka-Bradley A, Paul J. Nietert, Silver RM (2006) Scleroderma lung fibroblasts: contractility and connective tissue growth factor. In Tissue Repair, Contraction and the Myofibroblast (Gabbiani G, Chaponnier C, Desmouliere A, eds), Landes Bioscience, Georgetown, TX; 25-31.

19. Marinelli JP, Levin DL, Vassallo R, Carter RE, Hubmayr RD, et al. (2017) Quantitative assessment of lung stiffness in patients with interstitial lung disease using MR elastography. J Magn Reson Imaging 46: 365-374.

20. Briggs MW, Sacks DB (2003) IQGAP proteins are integral components of cytoskeletal regulation. EMBO reports 4: 571-574.

21. Samson EB, Tsao DS, Zimak J, McLaughlin RT, Trenton NJ, et al. (2017) The coordinating role of IQGAP1 in the regulation of local, endosome-specific actin networks. Biol Open 6: 785-799.

22. Tian Y, Gawlak G, Shah AS, Higginbotham K, Tian X, et al. (2015) Hepatocyte growth factor-induced Asef-IQGAP1 complex controls cytoskeletal remodeling and endothelial barrier. J biol chem 290: 4097-4109.

23. Gabbiani G (2003) The myofibroblast in wound healing and fibrocontractive diseases. J Pathol 200: 500-503.

24. Grinnell F (1999) Signal transduction pathways activated during fibroblast contraction of collagen matrices. Curr Top Pathol 93: 61-73.

25. Shi-Wen X, Chen Y, Denton CP, Eastwood M, Renzoni EA, et al. (2004) Endothelin-1 promotes myofibroblast induction through the ETA receptor via a rac/phosphoinositide 3-kinase/Akt-dependent pathway and is essential for the enhanced contractile phenotype of fibrotic fibroblasts. Mol Biol Cell 15: 2707-2719.

26. Herman IM (1993) Actin isoforms. Cur Opinion Cell Biol 5: 48-55.

27. Khaitlina SY (2001) Functional specificity of actin isoforms. Int Rev Cyt 202: 35-98.