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RESEARCH ARTICLE

Role of STIM1 in stretch-induced signaling in human airway smooth muscle

Yang Yao,[1,3] Mengning Zheng,[2,3] Niyati A. Borkar,[3] Michael A. Thompson,[3] Emily Y. Zhang,[3] Maunick Lefin Koloko Ngassie,[3,4,5] Shengyu Wang,[1] Christina M. Pabelick,[3,6] Elizabeth R. Vogel,[3] and Y. S. Prakash[3,6]

[1]*Department of Respiratory and Critical Care Medicine, The First Affiliated Hospital of Xi'an Medical University, Xi'an, People's Republic of China;* [2]*Department of Respiratory and Critical Care Medicine, Guizhou Province People's Hospital, Guiyang, People's Republic of China;* [3]*Department of Anesthesiology, Mayo Clinic, Rochester, Minnesota, United States;* [4]*Department of Pathology and Medical Biology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands;* [5]*Groningen Research Institute for Asthma and COPD, University Medical Center Groningen, University of Groningen, Groningen, Netherlands; and* [6]*Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, Minnesota, United States*

Abstract

Alteration in the normal mechanical forces of breathing can contribute to changes in contractility and remodeling characteristic of airway diseases, but the mechanisms that mediate these effects in airway cells are still under investigation. Airway smooth muscle (ASM) cells contribute to both contractility and extracellular matrix (ECM) remodeling. In this study, we explored ASM mechanisms activated by mechanical stretch, focusing on mechanosensitive piezo channels and the key Ca^{2+} regulatory protein stromal interaction molecule 1 (STIM1). Expression of Ca^{2+} regulatory proteins, including STIM1, Orai1, and caveolin-1, mechanosensitive ion channels Piezo-1 and Piezo-2, and NLRP3 inflammasomes were upregulated by 10% static stretch superimposed on 5% cyclic stretch. These effects were blunted by STIM1 siRNA. Histamine-induced $[Ca^{2+}]_i$ responses and inflammasome activation were similarly blunted by STIM1 knockdown. These data show that the effects of mechanical stretch in human ASM cells are mediated through STIM1, which activates multiple pathways, including Piezo channels and the inflammasome, leading to potential downstream changes in contractility and ECM remodeling.

NEW & NOTEWORTHY Mechanical forces on the airway can contribute to altered contractility and remodeling in airway diseases, but the mechanisms are not clearly understood. Using human airway smooth muscle cells exposed to cyclic forces with static stretch to mimic breathing and static pressure, we found that the effects of stretch are mediated through STIM1, resulting in the activation of multiple pathways, including Piezo channels and the inflammasome, with potential downstream influences on contractility and remodeling.

asthma; inflammasome; mechanobiology; mechanosensitivity; store-operated calcium entry

INTRODUCTION

In obstructive lung diseases such as asthma (1–3), both altered bronchial airway contractility and remodeling (increased cell proliferation and fibrosis) are well-known features. Although many cell types are involved (3–6), airway smooth muscle (ASM) cells play a key role through the alteration of intracellular Ca^{2+} ($[Ca^{2+}]_i$) responses (7–9) that can have downstream influences on both contractility and remodeling (10, 11). In this regard, the store-operated calcium entry (SOCE) pathway has emerged as particularly important for ASM-mediated remodeling (12), including the stromal interaction molecule (STIM1) (13) that is present in the sarcoendoplasmic reticulum (SR) and acts as a Ca^{2+} sensor (14), and the plasma membrane Ca^{2+} channel Orai1 that STIM1 interacts with to promote Ca^{2+} influx. Although its importance is established, the downstream mechanisms beyond Orai1 through which STIM1 could influence ASM are still under investigation.

An emerging aspect of airway biology is the recognition that mechanical forces play a role in remodeling (15, 16). For example, short-duration episodes of mechanical stress increase goblet cell number and mucin MUC5AC expression in bronchial epithelial cells (17). Patients with asthma who undergo repeated episodes of bronchoconstriction show pathological remodeling, such as goblet cell hyperplasia and increased airway collagen deposition (18). The assumption is that mechanosensitive pathways in airway cells transmit and transduce mechanical signals into intracellular pathways that promote remodeling. Piezo channels are an emerging family of mechanosensitive cation channels (19, 20) with permeability to Ca^{2+} (21). We recently showed that both

Correspondence: Y. S. Prakash (prakash.ys@mayo.edu).
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developing and adult human ASM express both Piezo1 and Piezo2 channels and that these channels contribute to Ca^{2+} responses to stretch in these cells (22). However, the mechanisms that link physical forces to intracellular signaling pathways in ASM, and the upstream pathways that regulate Piezo channels, are not fully understood. In this study, using adult human ASM cells, we tested the hypothesis that stretch effects involve STIM1, leading to downstream impact on mechanisms such as Piezo channels that ultimately contribute to altered contractility and remodeling.

METHODS

Human ASM Cells

The techniques for isolating human ASM cells have been previously described (23). Briefly, cells were enzymatically dissociated from third- to sixth-generation bronchi of noncancerous lung specimens collected incidentally during thoracic surgeries at Mayo Clinic (focal, noninfectious indications; typically, lobectomies and rarely pneumonectomies). The protocols were approved by the Mayo Clinic Institutional Review Board (IRB no. 08–002518). ASM cells were grown in Dulbecco's modified Eagle medium (DMEM)-F12 (Invitrogen) medium containing 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic (AbAm, Invitrogen) and growth-arrested in 1% serum for 24 h maintained at 37°C under 5% CO_2 before being exposed to experimental conditions.

Cell Stretch

ASM cells were seeded onto Flexcell biaxial six-well culture plates (coated with type I collagen, BioFlex-I; Flexcell International Corp.) at a density of 20,000 cells per well. After the cells reached 60% to 70% confluence, the culture medium was replaced with serum-free medium. Cells were subjected to a sinusoidal 5% cyclic strain (15 cycles/min) mimicking breathing, without (control) versus with 10% static stretch mimicking additional static airway stretch from exposures like continuous positive airway pressure (CPAP) or positive end-expiratory pressure (PEEP) during mechanical ventilation. Stretch exposure was performed for 24 h using a Flexcell FX-4000 Tension System at 37°C in a humidified incubator with 5% CO_2. Interventions, such as siRNA, were performed in the Flexcell plates before stretch exposure.

Immunofluorescent Staining

ASM cells grown on four-chamber Lab-Teks (Nalgene Nunc International, Rochester, NY) were fixed in 4% paraformaldehyde for 10 min, then washed three times in PBS, permeabilized with 0.1% Triton-X for 5 min, washed again in PBS, and blocked for 60 min in 4% normal donkey serum. Samples were incubated in primary mouse anti-STIM1 (1:100, Abcam-ab57834, Cambridge, MA), rabbit anti-Piezo1 (1:100, Novus-NBP2-78537, Canada), or TBS overnight at 4°C. Samples were then washed in PBS and incubated for 2 h in Alexa 488-conjugated donkey anti-mouse (1:500; Invitrogen) or Alexa 555-conjugated donkey anti-mouse secondary antibodies (1:500). Cells were visualized on a Nikon Eclipse microscope with a Nikon C2 laser scanning confocal system.

Ca^{2+} Measurements

After stretch exposure, the flexible bottom membrane from the Flexcell system was cut and placed into 24-well plates. The ASM was then incubated in 5 μM fluo-4/AM (#F14217, Invitrogen) for 60 min at room temperature in Hanks balanced salt solution (HBSS; 2 mM Ca^{2+}, pH 7.4) and visualized using a Nikon Eclipse Ti imaging system with an LED fluorescence light source and a 16-bit high-sensitivity CCD camera, with an excitation wavelength of 488 nm and emission detected at 515 nm. Cells were initially perfused with HBSS (2.5 mM Ca^{2+} at room temperature), and baseline fluorescence levels were established before exposure to 10 μM histamine in HBSS to elicit $[Ca^{2+}]_i$ responses. The baseline, peak, and plateau values of these responses were measured. $[Ca^{2+}]_i$ calibrations for semiquantification of fluo-4 levels were done using previously described empirical procedures (24) involving sequential measurements of fluo-4 levels in permeabilized ASM cells exposed to media with known extracellular Ca^{2+} levels from 0 nM to 10 μM.

siRNA Knockdown

ASM cells were grown in six-well Flexcell plates until 50–60% confluent. Cells were then transfected with DharmaFECT Transfection reagent and appropriate scrambled control or STIM1 siRNA [Dharmacon, L-011785-00-0005 (20 nM)] for 72 h according to the manufacturer's protocol. The efficacy of siRNA transfection was verified with protein quantification using a JESS system.

shRNA Lentiviral Particle Transfection

ASM cells were cultured in six-well plates to approximately 50–60% confluence before transfection with 20-μL viral stock containing 1×10^5 infectious units of the virus for control and Piezo1 lentiviral particles. The samples were then exposed to 5% serum medium with polybrene (Santa Cruz Biotechnology; sc-134200) when cells reached 60–70% confluence. Lentiviral particles were added, and the cells were incubated overnight (Santa Cruz Biotechnology; Piezo 1-sc-93227-V and Control shRNA Lentiviral Particles-sc-108080). Medium containing 1% AbAm without polybrene was then added for 24 h, following which the medium was replaced with 10% serum containing 5 μg/mL puromycin for selection of cells stably transfected with shRNA. Cells were maintained for 48–72 h. Efficacy and successful shRNA transduction were verified by JESS.

JESS Analysis

ASM cells were harvested using standard techniques for protein isolation. A bicinchoninic acid (BCA) Protein Assay Kit (ProteinSimple, San Jose CA) was used to measure protein concentration. Protein expression was measured using the JESS capillary electrophoresis system (Protein Simple, San Jose, CA) according to the manufacturer's protocol (25, 26). Three microlitres of each sample were loaded onto the plate; 12–230 kDa and 66–440 kDa cartridges were used. The target proteins were immune-probed with anti-STIM1 (Abcam-ab57834, Cambridge, MA), anti-Orai1 (Novus-NBP1-77289, Canada), anti-Cav-1 (Abcam-ab211503, Cambridge, MA), anti-NLRP3 (Abcam-ab4207, Cambridge, MA), anti-ASC (Abcam-ab283684, Cambridge, MA), or anti-Caspase-1 (Abcam-ab207802, Cambridge, MA) diluted in antibody

buffer at a 1:50 ratio followed by horseradish peroxidase (HRP)-conjugated secondary antibodies. Ten microlitres of primary and secondary were loaded for each sample. The plate was spun down for 5 min at 1,000 *g* to remove bubbles. Capillaries and the plate were loaded into the JESS device. Protein separation, blocking, antibody incubation, and signal detection were conducted automatically via standard manufacturer protocol. The vendor-supplied Compass software was used for data analysis. The electropherogram data were digitally represented and normalized to total protein. Of note, the digital representation of protein expression was used for illustrative purposes only. Actual quantification of protein expression was done using the electropherogram signals with background signal subtraction.

Quantification of Cytokines Using ELISA

Supernatants from ASM cultures were collected and stored at −80°C until ready for use. Following the manufacturer's protocol, IL-1β (R&D Systems, Cat. No. DY210) was measured using an ELISA kit (R&D, Minneapolis, Minnesota). Briefly, cell supernatant was placed in a 96-well microplate, incubated for 1 h at 37°C with HRP-conjugated Streptavidin for 20 min, and then incubated with Ultra TMB substrate for 20 min at 37°C in the dark. After stopping the reaction by adding 2 M H_2SO_4, the optical density at 450 nm (OD450) was measured using a 96-well plate reader. The data were normalized to the total protein concentration using the BCA protein assay kit (Sigma Aldrich, Cat. No. BCA1). Three biological replicates were performed for ELISA analysis.

Statistical Analysis

Statistical analysis was performed using unpaired *t* test or one- or two-way ANOVA followed by Tukey's post hoc multiple comparisons test using GraphPad Prism version 9.1.0 for Windows (Graph Pad Software Inc.). A value of $P < 0.05$ was considered statistically significant. A minimum of three individual patient samples were used for each experimental exposure. *n* represents the number of patient samples. Experiments were done with at least three technical replicates for each patient sample. Data are presented as means ± SE.

RESULTS

Localization of STIM1 and Piezo1 in Asthmatic ASM Cells

STIM1 was found to be primarily expressed within the cytoplasm (consistent with its SR localization) while

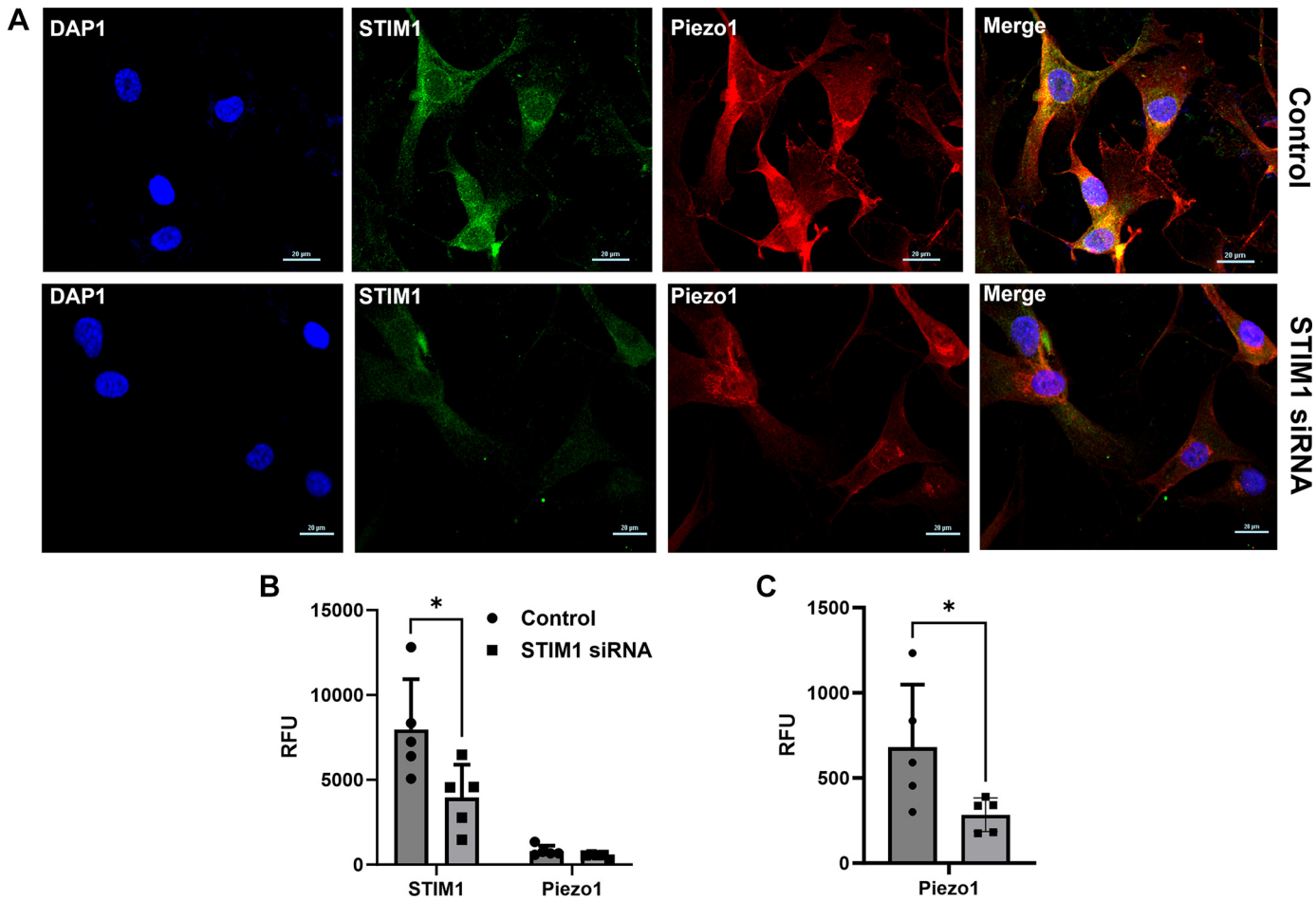

Figure 1. Immunofluorescence staining of stromal interaction molecule 1 (STIM1) and Piezo1 in human airway smooth muscle (ASM) cells. *A*: STIM1 (green) was primarily expressed in intracellular compartments while Piezo1 (red) was also present on plasma membrane. Knockdown of STIM1 using siRNA not only resulted in expected significant decrease in its expression (*B*) but also suppressed Piezo1, particularly on the plasma membrane (*C*). Data expressed as means ± SE. *$P < 0.05$; $n = 5$, where each *n* is representative of an individual patient cell line.

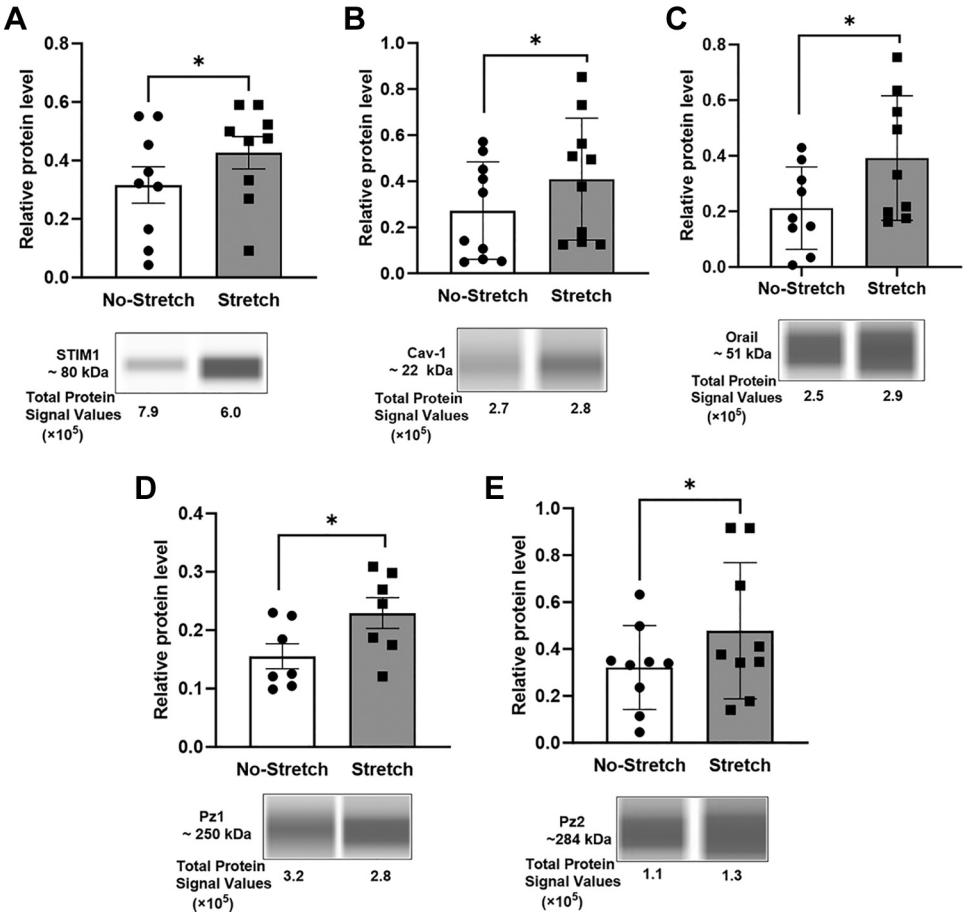

Figure 2. Mechanical stretch alters expression of calcium regulatory proteins in human airway smooth muscle (ASM). Cyclical stretch (mimicking breathing) with superimposed static stretch (see METHODS) increased expression of stromal interaction molecule 1 (STIM1) (*A*), the plasma membrane proteins caveolin-1 (Cav-1) (*B*) and Orai1 (*C*) as well as both isoforms of the mechanosensitive ion channel Piezo (*D* and *E*). Digital representations of electropherograms from ProteinSimple capillary electrophoresis systems. Data expressed as means ± SE. $^*P < 0.05$; $n \geq 5$, where each n is representative of an individual patient cell line.

Piezo1 was also found in the plasma membrane (Fig. 1*A*). Knockdown of STIM1 via siRNA was confirmed by the reduction of observed fluorescence in the cytoplasm (Fig. 1, *A* and *B*). Interestingly, the reduction in STIM1 expression also suppressed plasma membrane expression of Piezo1 (Fig. 1, *A* and *B*).

Effect of Stretch on Regulatory Protein Expression

We and others have previously demonstrated that there are microdomains in ASM that regulate Ca^{2+} involving STIM1, Orai1, and the plasma membrane protein caveolin-1 (Cav-1), which is critical to the formation of caveolae within which

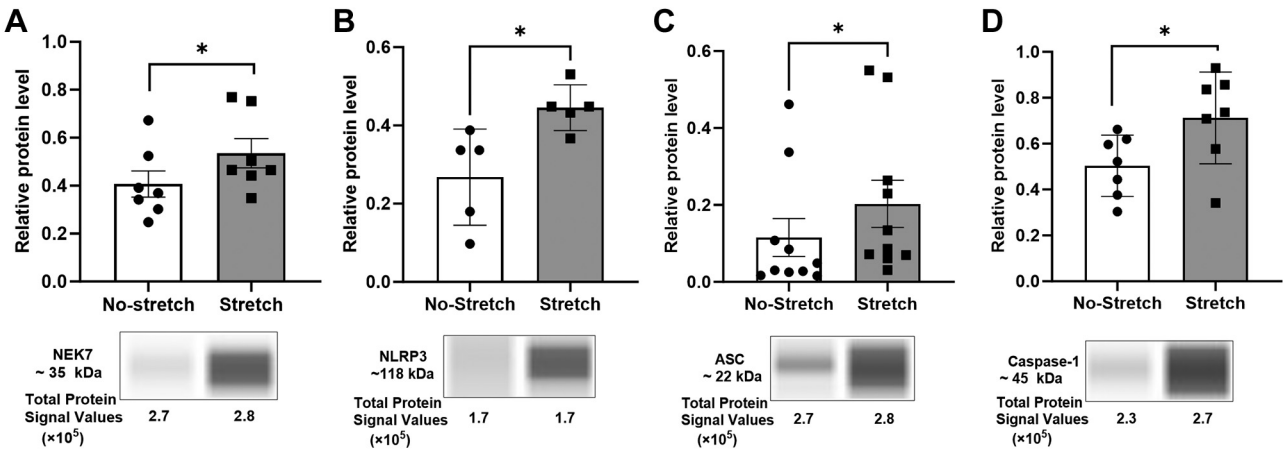

Figure 3. Effect of stretch on inflammasome in airway smooth muscle (ASM) cells. Stretch activated multiple elements of the inflammasome pathway including NEK7 (*A*), NLRP3 (*B*), ASC (*C*), and Caspase-1 (*D*). Digital representations of electropherograms from ProteinSimple capillary electrophoresis systems. Data expressed as means ± SE. $^*P < 0.05$, $n \geq 5$, where each n is representative of an individual patient cell line.

Orai1 and other proteins reside (24, 27, 28). Data in other cell types show that Piezo channels are also expressed within similar lipid rafts (29), suggesting the potential importance of microdomains in mechanosensitivity. We found that mechanical stretch significantly increased STIM1, Cav-1, and Orai1 in ASM cells compared with control (Fig. 2, *A–C*, $P < 0.05$). The expression of Piezo1and Piezo2 were also increased by stretch compared with the control (Fig. 2, *D* and *E*, $P < 0.05$).

Effect of Stretch on Inflammasome

There are emerging data that downstream effects of stretch involve activation of the inflammasome (30, 31), which may explain the longer-term influence of even brief exposure to changes in mechanical forces and the proinflammatory effect of stretch. We examined the role of mechanical stretch on airway inflammation, focusing on NEK7 and NLRP3 inflammasome (NLRP3, ASC, and Caspase-1). Stretch increased the expression of NEK7, NLRP3, ASC, and Caspase-1 significantly compared with the control (Fig. 3, $P < 0.05$).

Role of STIM1 in Stretch Effects

To better understand the role of STIM1 in the response to stretch, we knocked down STIM1 with siRNA (Fig. 4*A*, $P < 0.05$). STIM1 siRNA blunted the effects of stretch on STIM1 expression (Fig. 4*B*). Interestingly, silencing Piezo1 with shRNA blunted baseline STIM1 expression and prevented the previously observed increase in STIM1 expression with stretch exposure (Fig. 4*C*, $P < 0.05$). Silencing STIM1 also significantly abrogated stretch-induced changes in Orai1 and Cav-1 expression (Fig. 4, *D* and *E*, $P < 0.05$) as well as Piezo1 and Piezo2 expression (Fig. 4, *F* and *G*, $P < 0.05$). The baseline expression of NEK7 and NLRP3 inflammasome were also decreased with STIM1 siRNA (Fig. 5, *A–D*, $P < 0.05$). Importantly, stretch-induced increases in the NLRP3 inflammasome were prevented by STIM1 siRNA (Fig. 5, *A–D*, $P < 0.05$). Consistently, the level of IL-β in the stretch group was increased compared with the control group, but this increased expression was also suppressed by STIM1 siRNA (Fig. 5*E*, $P < 0.05$).

Ca^{2+}-related proteins were upregulated in ASM cells after stretch, an effect blunted by STIM1 siRNA. To help further understand the link between the two, we examined $[Ca^{2+}]_i$ responses in ASM cells treated with stretch, which were significantly higher than the control group. siRNA knockdown of STIM1 blunted these increased $[Ca^{2+}]_i$ responses in the stretch group (Fig. 6; $P < 0.05$).

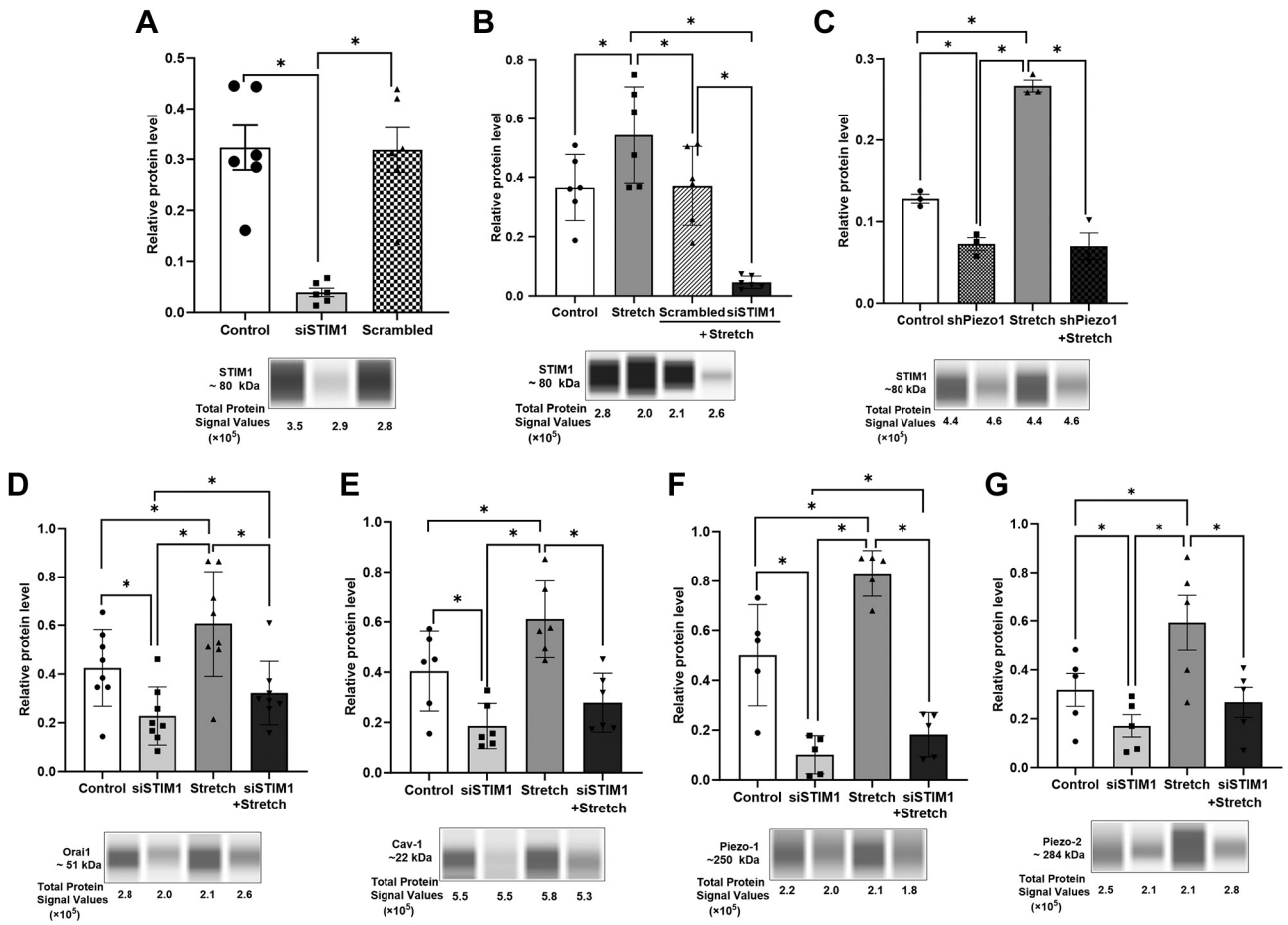

Figure 4. Stromal interaction molecule 1 (STIM1) mediates stretch effects in human airway smooth muscle (ASM). Knockdown of STIM1 using siRNA (*A*) decreased baseline STIM1 expression and blunted the stretch-induced increase in STIM1 expression (*B*). Interestingly, shRNA knockdown of Piezo1 blunted STIM1 changes in response to stretch (*C*). STIM1 siRNA further blunted stretch-induced changes in Orai1 (*D*), Cav-1 (*E*), Piezo1 (*F*), and Piezo2 (*G*). Digital representations of electropherograms from ProteinSimple capillary electrophoresis systems. Data expressed as means ± SE. *$P < 0.05$ vs. nonstretch. $n = 3$ for shRNA, $n \geq 5$ for remainder. Each n is representative of an individual patient cell line.

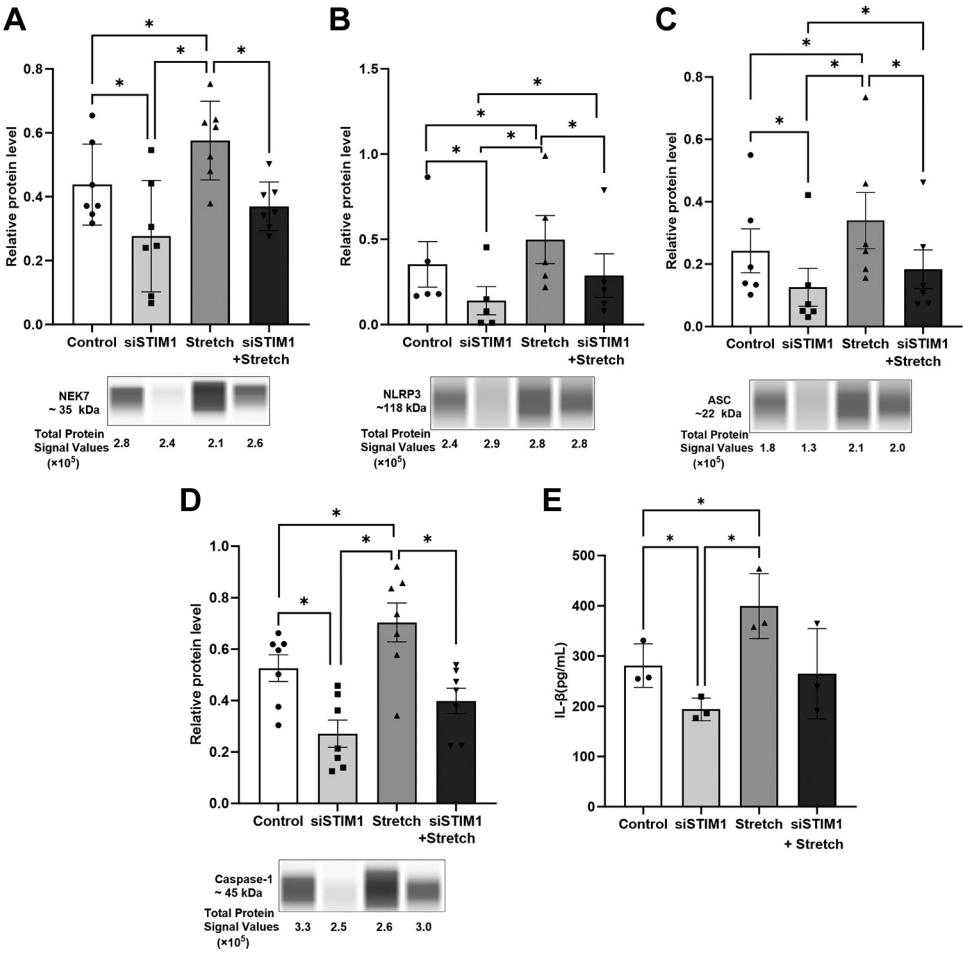

Figure 5. Stromal interaction molecule 1 (STIM1) mediates stretch activation of the inflammasome. Knockdown of STIM1 using siRNA blunted baseline and stretch-induced expression of inflammasome elements (*A–D*) and secretion of IL-1β (*E*). Digital representations of electropherograms from ProteinSimple capillary electrophoresis systems. Data expressed as means ± SE. *$P < 0.05$ vs. non-stretch. $n = 3$ for ELISA, $n \geq 5$ for remainder. Each *n* is representative of an individual patient cell line.

DISCUSSION

Mechanical forces can contribute to airway remodeling and airway hyperreactivity in asthma (32, 33). Therefore, understanding how stretch stimuli are sensed and what the downstream responses to stretch are will be key to developing future alleviation strategies for airway diseases. In this study, we used human ASM cells as a model to explore the potential role of microdomain pathways that are involved in $[Ca^{2+}]_i$ responses and other downstream mechanisms relevant to contractility and remodeling in the airway. We found that superimposed static stretch increased expression of store-operated Ca^{2+} entry (SOCE) mechanisms, namely, the SR Ca^{2+} sensor STIM1, the plasma membrane protein Orai1, and the caveolar protein Cav-1. These mechanisms form a Ca^{2+} regulatory microdomain in ASM (34, 35) and contribute to asthma pathophysiology (8, 36). Furthermore, stretch increased the expression of the mechanosensitive plasma membrane ion channels Piezo-1 and Piezo-2. Beyond these Ca^{2+} pathways, stretch activated the NLRP3 inflammasome pathway in ASM. Interestingly, the effects of stretch on Orai1, Piezo channels, and the inflammasome appear to be mediated, at least in part, through STIM1 with knockdown of STIM1 resulting in the abrogation of the observed stretch-induced changes in protein expression and inflammasome activation. Overall, these data highlight links between mechanical forces

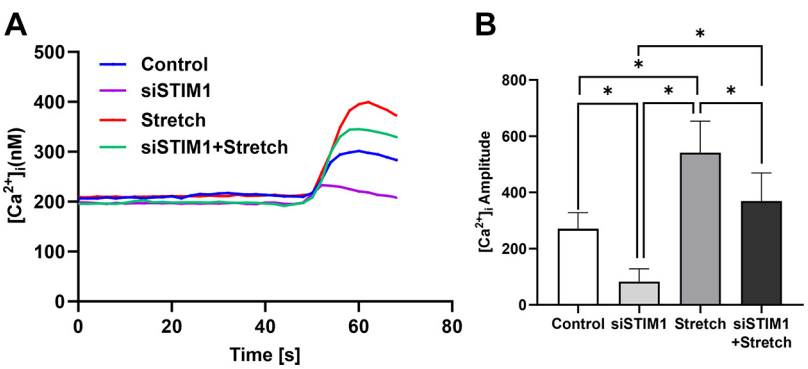

Figure 6. Stromal interaction molecule 1 (STIM1) modulates stretch-enhanced $[Ca^{2+}]_i$ responses in airway smooth muscles (ASM) cells. Representative tracings (*A*) and summaries (*B*) of $[Ca^{2+}]_i$ responses of human ASMs to 10 μM histamine following 24 h of stretch in the context of pretreatment with STIM1 siRNA, which blunted stretch effects. Data expressed as means ± SE. *$P < 0.05$ vs. non-stretch. $n \geq 5$, where each n is representative of an individual patient cell line.

and microdomain elements such as STIM1 that have effects on Ca^{2+} and inflammatory pathways relevant to asthma pathophysiology.

Asthma is a chronic inflammatory disease in which airway contractility and remodeling play important roles (37). In the context of a remodeled airway with increased cell proliferation and fibrosis, enhanced airway constriction results in greater mechanical forces on cells of the bronchial airway, including the epithelium and ASM. These effects can, in turn, alter the secretory and other functions of these cells (38). Accordingly, airway mechanobiology in asthma is considered an important aspect to explore to understand the impact of mechanical forces on cellular structure and behavior, and the interaction between intracellular components or between intracellular and extracellular components in the context of mechanical stimuli (39, 40). In turn, mechanosensitive pathways have the potential ability to influence cellular behavior to modulate both contractility and remodeling (41). However, the mechanisms that link mechanical forces to structural or functional changes in the airway are only now being understood.

Although discovered relatively recently, the Piezo channel family is rapidly gaining interest given the ubiquity of their expression and function in multiple organs and cell types, with potential pleiotropic effects that are still being explored (42). Piezo1 and Piezo2 are encoded by the Fam38A and Fam38B genes, respectively, on different chromosomes (19, 43), with the proteins forming trimeric propeller-like structures with an extracellular domain, and peripheral regions acting as force sensors to regulate the opening of the central pore (44). These channels are considered nonspecific cation channels, and their Ca^{2+} permeability makes them interesting for understanding potential roles in airway contractility or other signaling that involves Ca^{2+}. Piezo channels have been shown to be responsive to external forces, including shear, pressure, and tension, and are considered important for mechanotransduction under normal and pathological conditions (45). Although both Piezo1 and Piezo2 are present in the lung (46), their function is still not well understood. Piezo1 regulates lung epithelial homeostasis and repair (47) and can promote surfactant secretion in alveolar epithelial cells in response to stretch (48). We recently showed that both isoforms are present in developing and adult human ASM (22), albeit to different extents, and that Piezo channels can mediate Ca^{2+} responses in ASM in response to stretch. However, the upstream mechanisms that regulate Piezo expression are not well understood.

A key aspect of mechanical force effects is their influence on the cellular plasma membrane and both intracellular and extracellular elements that interact with it. Here, several Ca^{2+} regulatory pathways exist either within the plasma membrane or interact physically or functionally with it. Accordingly, mechanosensitive channels such as Piezo could interact with a number of mechanisms within a microdomain. Such cross talk between mechanosensitive channels and Ca^{2+} regulatory proteins has been shown recently in the context of cardiovascular systems (49). In this regard, given the relative novelty of Piezo channels, there is little known regarding interactions between Piezo channels and Ca^{2+} proteins in the airway. Nonetheless, SERCA2 has been found to interact with Piezo1 at plasma membrane-ER junctions (50) and is thought to modulate Piezo1-induced Ca^{2+} responses to stretch (50). Conversely, Piezo1 can modulate SR Ca^{2+} release dynamics in the cardiovascular system (51) via the inositol trisphosphate receptor (IP3R), and by modulating the generation of cAMP, thus affecting Ca^{2+} reuptake by the SR (51). In this broader context, the current study suggests a different element of microdomain interactions involving Piezo1. Here, we found that the SR Ca^{2+} sensor STIM1 is a regulator of Piezo expression and a mediator/modulator of stretch effects in ASM.

The role of STIM1 in store-operated Ca^{2+} entry (SOCE) is well established. Functioning as a luminal Ca^{2+} sensor, STIM1 is activated by decreased SR Ca^{2+} (e.g., in response to agonist stimulation) and translocates toward the plasma membrane (creating puncta) (52), and interacting with plasma membrane channel Orai1 to activate SOCE. Here, the caveolar protein Cav-1 facilitates SOCE by virtue of Orai1 being located within caveolae, and the membrane invaginations of caveolae promote plasma membrane-ER interactions (53). However, STIM1 may have other effects beyond SOCE, particularly in the context of long-term impact. STIM1 has also been implicated in the pathogenesis and development of asthma (7, 8). Enhanced expression of STIM1 protein is associated with airway remodeling in asthma (9) which represents a longer-term effect unlikely to be purely via SOCE. ASM-mediated airway hyperresponsiveness and airway remodeling are blunted in STIM1 knockout mice (10). Our results show that mechanical stimulation promotes STIM1 expression that in turn mediates changes in expression of other proteins within the SOCE microdomain including Orai1 and Cav-1, but also pathways not associated with SOCE including Piezo itself and the inflammasome. Importantly, we show that in the context of stretch, the increased expression of Piezo1 and Piezo2 is also mediated by STIM1. Furthermore, our study demonstrates that knockdown of Piezo1 blunts STIM1 expression, suggesting a bidirectional regulation of these mechanisms. There is currently no information on such "upstream" regulation of either protein, but this suggests that the relationship between stretch and downstream impacts involving Ca^{2+} (for

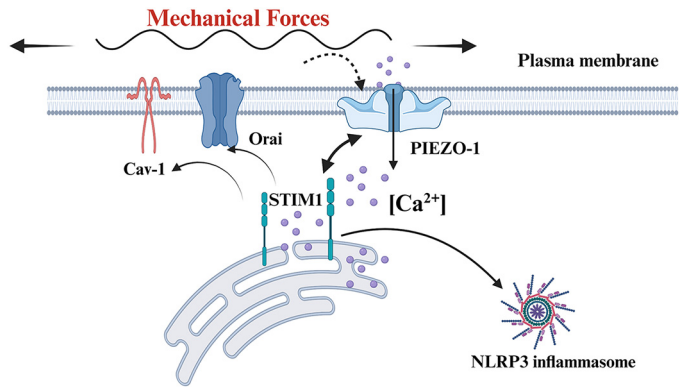

Figure 7. Role of stromal interaction molecule 1 (STIM1) in stretch effects in airway smooth muscle. Mechanical forces induce changes in expression and function of STIM1 that can further modulate expression and function of plasma membrane proteins such as Orai1, caveolin-1, and the mechanosensitive Piezo channels, overall altering Ca^{2+} regulation. STIM1 can further mediate stretch-induced activation of the inflammasome pathway. [Image created with a licensed version of BioRender.com.]

example) is not straightforward and could involve interplay between intracellular pathways such as STIM1 and truly mechanosensitive mechanisms such as Piezo channels in the plasma membrane. Whether such interactions are mediated by Ca^{2+} (a common factor in this case) or other signaling mechanisms remains to be established.

Our study additionally found that stretch activates NLRP3 in human ASM. There is now increasing evidence that mechanical forces can activate the inflammasome, for example, in the context of lung injury and mechanical ventilation (54, 55). Interestingly, there is also emerging evidence that Piezo1 contributes to NLRP3 activation in nucleus pulposus cells of intravertebral disks (56). Whether these effects involve Ca^{2+} per se is not clear, but other studies have linked Ca^{2+} signaling to the activation of NLRP3 and the inflammasome (57, 58). Although there may be multiple sources of elevated Ca^{2+} in ASM, including Ca^{2+} release from the SR and Ca^{2+} influx, whether SOCE plays a role in inflammasome is not clear. For example, in macrophages and dendritic cells, it is Ca^{2+} elevation but not necessarily SOCE that is important (59), while in epithelial cells, influenza-virus induced inflammasome activation involves STIM1 (60). There is currently no data linking mechanical forces, STIM, Piezo, calcium, and the inflammasome in ASM. In this study, we found that NLRP3 is activated under stretch and importantly, such effects are blunted by STIM1 siRNA, providing the first links between mechanical forces and inflammasome in ASM. The downstream impact of such activation remains to be established, but our observation of increased IL1β in the context of stretch and its blunting by STIM1 siRNA suggests modulation of inflammation that could have a downstream impact on remodeling.

In conclusion, the present data show that STIM1 plays an important role beyond $[Ca^{2+}]_i$ signaling in ASM, acting as a regulator of mechanical force effects by modulating the expression and function of important Ca^{2+} microdomain pathways, including Piezo channels and proinflammatory mechanisms. Furthermore, STIM1 interactions with plasma membrane mechanisms beyond Orai1, such as Piezo1 channels, could thus have complex effects on ASM structure and function. We propose that with mechanical stretch, there is activation of Piezo channels and other pathways leading to Ca^{2+} influx, as well as intracellular Ca^{2+} depletion and STIM1/Orai1 activation, with downstream effects on Ca^{2+} and also the inflammasome. STIM1, in turn, can then enhance expression of the proteins in the SR-plasma membrane microdomain and further contribute to inflammasome pathways (Fig. 7). What remains to be determined is how such interactions are altered in the context of diseases such as asthma, where SOCE mechanisms are known to play a role, and where mechanical forces may modulate enhanced airway contractility and remodeling.

DATA AVAILABILITY

Data will be made available upon reasonable request.

ACKNOWLEDGMENTS

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

C.M.P., E.R.V., and Y.S.P. conceived and designed research; Y.Y., M.Z., N.A.B., and M.A.T. performed experiments; Y.Y. and M.Z. analyzed data; Y.Y. and M.A.T. interpreted results of experiments; Y.Y. and N.A .B. prepared figures; Y.Y. drafted manuscript; Y.Y., N.A.B., C.M.P., E.R.V., and Y.S.P. edited and revised manuscript; Y.Y., M.Z., N.A.B., M.A.T., E.Y.Z., M.L.K.N., S.W., C.M.P., E.R.V., and Y.S.P. approved final version of manuscript.

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