# TGF-β1 Induces HASM Cell Shortening and Airway Hyperresponsiveness Through a Smad3-Dependent Signaling Pathway

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## ABSTRACT

**RATIONALE:** The mechanisms governing airway hyperresponsiveness (AHR) in asthma are incompletely understood. Transforming growth factor beta 1 (TGF-β1) - a cytokine whose expression and activity is increased in airway structural cells and bronchoalveolar lavage fluid, respectively – plays essential roles in airway inflammation and remodeling in asthma. However, the role of TGF-β1 in AHR and human airway smooth muscle (HASM) cell shortening remains unclear. We hypothesize that TGF-β1 induces AHR in HASM cells through a Smad3-dependent signaling pathway.

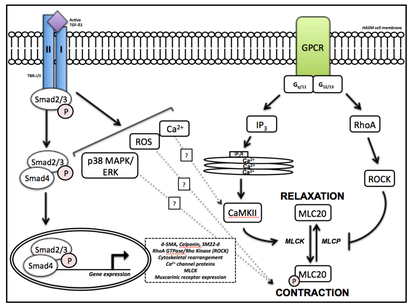
**METHODS:** Human precision-cut lung slices (hPCLS) were treated with TGF-β1 (100 ng/ml) overnight. Bronchoconstriction to the contractile agonist carbachol was detected by analyzing changes in airway lumen area using a live-feed microscope and an Image Pro-Plus software macro. Using HiPerFect reagent and a reverse transfection protocol, Smad3, Smad2, Smad4, or non-targeting siRNA was transfected into HASM cells prior to treatment and collection. Serum-starved HASM cells were treated with 10 ng/mL TGF-β1 for 24 h and/or carbachol (10 uM, 10 min). HASM cell protein was isolated and expression of phosphorylated myosin light chain (MLC) and MYPT1 was determined by immunoblot. Additionally, HASM cell contraction was measured by assessing alterations in cytoskeletal stiffness using magnetic twisting cytometry (MTC).

**RESULTS:** Overnight TGF-β1 treatment induced a 36% reduction in hPCLS airway lumen diameter over control. Following a carbachol dose response, TGF-β1 pretreated hPCLS exhibited significantly increased area under the curve, sensitivity as shown by EC50, and total bronchoconstriction. TGF-β1 treatment also significantly increased basal and agonist-induced HASM cell cytoskeletal stiffness, phosphorylated MLC expression, and phosphorylated MYPT1 expression. Interestingly, siRNA targeted against Smad3 – but not Smad2 - significantly attenuated MLC and MYPT1 phosphorylation by TGF-β1. Smad4 siRNA also decreased MLC and MYPT1 phosphorylation by TGF-β1 to a lesser extent.

**CONCLUSIONS:** Our data suggest that TGF-β1 contributes to HASM cell shortening and AHR in asthma through a Smad3-dependent mechanism. While Smad2 and Smad3 are closely related, Smad2 knockdown showed little effect on TGF-β1-induced HASM cell shortening. Together, these data provide a novel role for Smad3 activation in TGF-β1-induced AHR in asthma.

## TGF-β1 and Human Airway Smooth Muscle (HASM) Cell Excitation-Contraction (E-C) Coupling

- The link between AHR and airway structural alterations in asthma is poorly understood.
- TGF-β1 - an important mediator of airway structural alterations[2] – signals through Smad transcription factor-dependent and -independent pathways.
- TGF-β1 may modulate $Ca^{2+}$-dependent or $Ca^{2+}$-sensitization E-C coupling pathways in HASM to induce HASM cell shortening and AHR.




## HYPOTHESIS

**TGF-β1 signaling induces AHR by directly modifying $Ca^{2+}$-dependent or $Ca^{2+}$-sensitization pathways in HASM cell E-C coupling.**

*QR Code*

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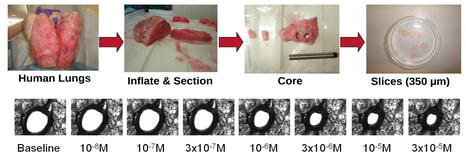
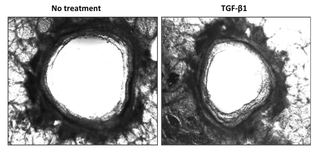
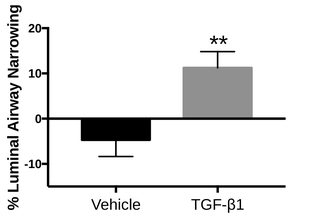
## TGF-β1 Induces hPCLS Bronchoconstriction and AHR




### A Chronic Bronchoconstriction




### B Carbachol-Induced Bronchoconstriction

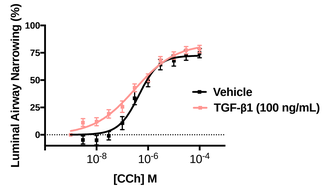






**Figure 1. TGF-β1 augments basal and carbachol (Cch)-induced hPCLS bronchoconstriction. A)** TGF-β1 (100 ng/mL, 18h) induces hPCLS bronchoconstriction (N=9 donors). **B)** TGF-β1 (100 ng/mL, 18h) augments Cch-induced hPCLS bronchoconstriction (N=9 donors) *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; ****$P \leq 0.0001$

## TGF-β1 Induces HASM Cell Shortening Through a ROCK-Dependent, RhoA-Independent Pathway

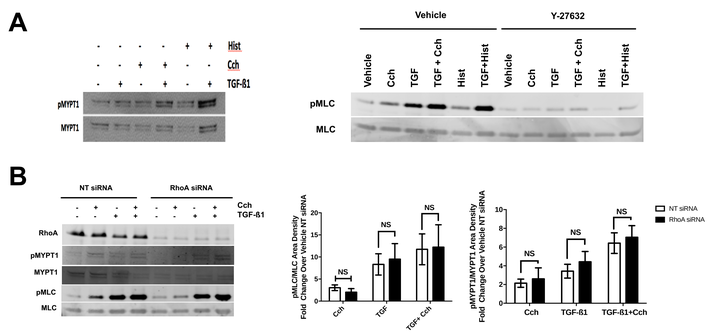
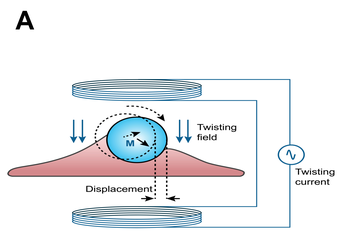
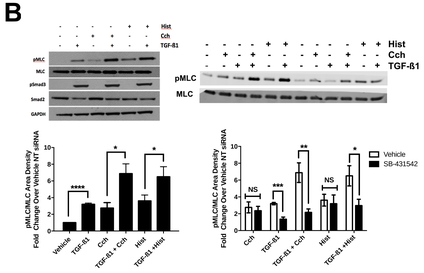
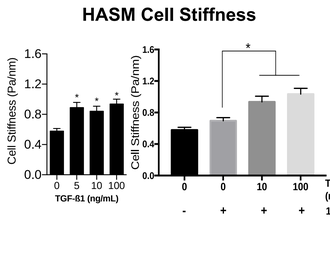



**Figure 3. Rho kinase (ROCK) inhibition prevents TGF-β1-induced MLC phosphorylation and AHR. A)** HASM cells were treated with TGF-β1 (10 ng/mL, 24 h) +/- Cch (10 μM), histamine (1 μM), and/or ROCK inhibitor Y-27632 (10 μM, 15 min). **B)** Non-targeting siRNA or RhoA siRNA-transfected HASM cells were treated with TGF-β1 (10 ng/mL, 24 h) +/- Cch (20 μM) (N=5+/-SEM).

## TGF-β1 Augments HASM Cell Shortening via TβR-I Activation



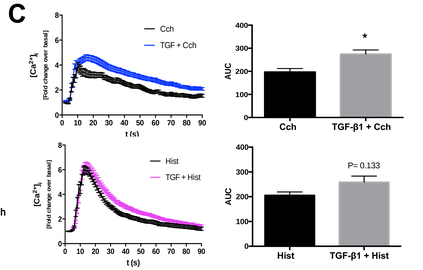




**Figure 2. TGF-β1 augments HASM cell shortening via TβR-I activation. A)** Methacholine (Mch)-induced stiffness following TGF-β1 (18 h) treatment (N=194-477 individual HASM cells). **B)** MLC phosphorylation in TGF-β1 (10 ng/mL, 18h)-treated HASM cells +/- Cch (10 μM, 10min), histamine (1 μM, 10 min), and/or SB-431542 (5 μM, 1h). N=5 +/-SEM. **C)** $[Ca^{2+}]_i$ in Cch (10 μm) or histamine (1 μM)-stimulated HASM cells following TGF-β1 (10 ng/mL) pretreatment (N=3 donors, two replicates each +/- SEM).

## Smad3 Knockdown Decreases TGF-β1-Induced HASM Cell Shortening and ROCK Activation

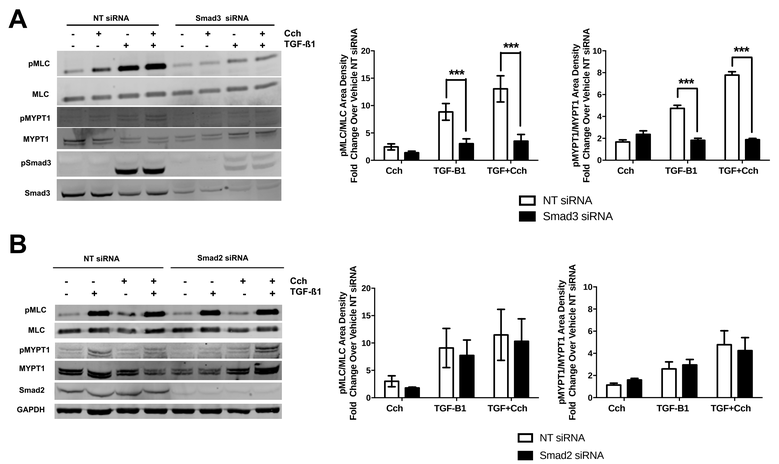



**Figure 4. Smad3 knockdown decreases TGF-β1-induced MLC phosphorylation and AHR.** HASM cells were transfected with siRNA against **A)** Smad3 or **B)** Smad2 and stimulated with TGF-β1 (10 ng/mL) or vehicle control for 18 h +/- Cch (10 μM, 10 min). (N=3-6; +/- SEM)

## Summary

- TGF-β1 induces hPCLS bronchoconstriction and AHR
- TGF-β1 augments basal and agonist-induced HASM cell shortening and MLC phosphorylation via TβR-I
- TGF-β1 induces HASM cell shortening through a ROCK-dependent, RhoA-independent pathway
- Smad3 knockdown decreases TGF-β1-induced HASM cell shortening and ROCK activation

## Significance

- TGF-β1 may induce AHR through Smad3-dependent ROCK activation.
- TGF-β1 release following repeated airway injury-repair may lead to increased bronchomotor tone and sustained airway hyperresponsiveness.
- Further elucidation of this pathway may lead to the development of novel therapeutic targets for moderate and severe persistent asthma.

## References

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