TGF-β1 Evokes Human Airway Smooth Muscle Shortening and Hyperresponsiveness: A New Job Description?

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ABSTRACT

RATIONALE: The factors contributing to airway hyperresponsiveness (AHR), a defining characteristic of asthma, remain incompletely understood. Transforming growth factor beta 1 (TGF-β1), a cytokine elevated in bronchoalveolar lavage fluid from patients with asthma[1], modulates airway remodeling and inflammation in asthma. However, the effects of TGF-β1 on AHR in human airway smooth muscle (HASM) remain unknown. We hypothesize that TGF-β1 directly induces HASM shortening and AHR through Smad2/3 activation.

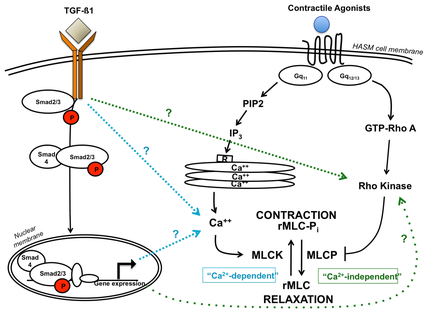
METHODS: Human precision-cut lung slices (hPCLS) were treated with TGF-β1 (100 ng/ml) acutely (0-5 min) or overnight. Bronchoconstriction was detected by analyzing changes in airway lumen area using a live-feed microscope and an Image Pro-Plus software macro. Isolated and cultured primary HASM cells were treated with TGF-β1 (1-100 ng/ml) for 1 min – 24 h and/or 10 μM carbachol for 10 min. HASM cell protein was isolated and changes in Smad2/3 and myosin light chain (MLC) phosphorylation were determined by immunoblot. Intracellular calcium ($[Ca^{2+}]_i$) was measured in HASM cells using the fluorescent dye Fluo-8. Additionally, HASM cell cytoskeletal stiffness was measured as a surrogate for contraction using magnetic twisting cytometry (MTC).

RESULTS: TGF-β1 (100 ng/ml) treatment induced acute (19%±3%) and chronic (36%) reductions in hPCLS airway lumen diameter compared to carbachol (74%±3%). Baseline and methacholine-induced HASM cell stiffness was markedly increased at both 4 h and 24 h following TGF-β1 (100 ng/ml) treatment. TGF-β1 (10 ng/ml) treatment induced a respective 2-fold and 1.5-fold increase in basal and carbachol-induced MLC phosphorylation in HASM cells, but had little effect on basal and carbachol-induced $[Ca^{2+}]_i$. Additionally, knockdown of Smad2/3 using siRNA decreased acute MLC phosphorylation by TGF-β1 (10 ng/ml) in HASM cells.

CONCLUSIONS: Our findings suggest that TGF-β1 contributes to asthma pathogenesis by directly evoking HASM shortening and AHR. TGF-β1 treatment had little effect on HASM cell $[Ca^{2+}]_i$, suggesting an alternative mechanism for TGF-β1-induced HASM shortening and AHR. The mechanism by which TGF-β1 induces HASM shortening and AHR may require activation of Smad2/3 signaling. Together, these data suggest a novel role for TGF-β1 and Smad2/3 in asthma pathogenesis.

TGF-ß1 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-ß1 - an important mediator of airway structural alterations[2] – signals through Smad transcription factor-dependent and -independent pathways.
- TGF-ß1 may modulate Ca^{2+}-dependent or Ca^{2+}-sensitization E-C coupling pathways in HASM to induce HASM cell shortening and AHR.



Hypothesis

TGF-ß1 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-ß1 Induces hPCLS Bronchoconstriction and AHR

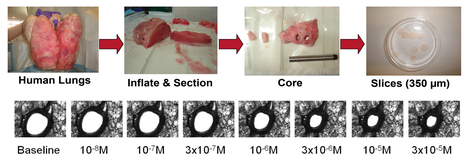
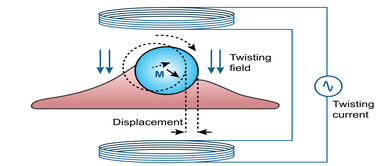
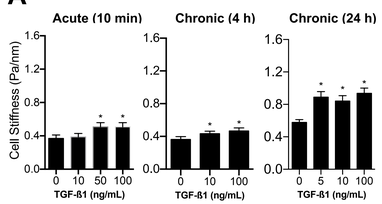


Figure 1. TGF-ß1 augments basal and carbachol (Cch)-induced hPCLS bronchoconstriction. A) TGF-ß1 (100 ng/mL) alone induces acute and chronic hPCLS bronchoconstriction (N=4-5 slices). **B)** TGF-ß1 (100 ng/mL) pretreatment augments Cch-induced hPCLS bronchoconstriction (N=6-9 slices) *$P \leq 0.05$

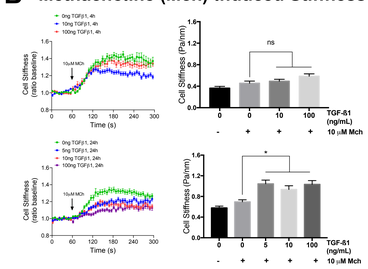
TGF-ß1 Augments Basal and Agonist-Induced HASM Cell Shortening



A Basal Stiffness



B Methacholine (Mch)-Induced Stiffness



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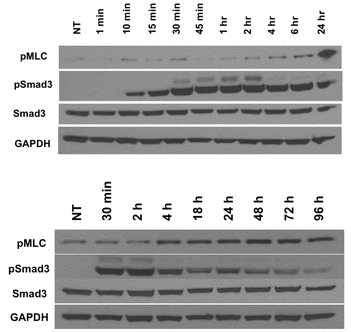

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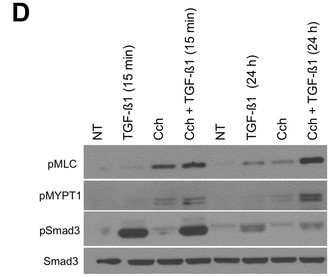

Figure 2. TGF-ß1-induced HASM cell shortening analyzed by MTC and immunoblot. A) TGF-ß1 induces acute (N=227-245) and chronic (4 h, N= 298-367; 24 h, N=477) HASM shortening. **B)** TGF-ß1 augments total Mch-induced HASM cell stiffness (4 h, N= 298-367; 24 h, N = 194-477). **C)** TGF-ß1 (10 ng/mL) induces time-dependent MLC phosphorylation (pMLC) in HASM cells and **D)** augments Cch-induced (10 μM, 15 min) pMLC and ROCK activation (pMYPT1). *$P \leq 0.05$

TGF-β1 Has Little Effect on Basal, but Augments Agonist-induced Intracellular $[Ca^{2+}]_i$ Mobilization in HASM Cells

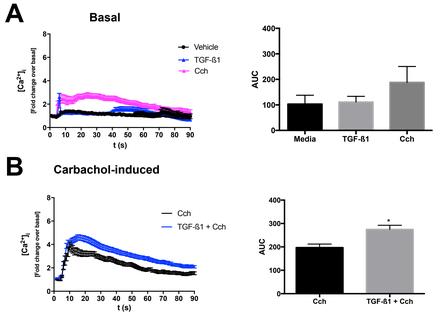

Figure 3. $[Ca^{2+}]_i$ mobilization in HASM cells. HASM cells were stimulated acutely or overnight (24 h) with 10 ng/mL TGF-ß1 or vehicle. A) $[Ca^{2+}]_i$ following treatment with TGF-ß1 or vehicle control. B) $[Ca^{2+}]_i$ in Cch-stimulated (10 μm) HASM cells following overnight TGF-ß1 pretreatment. Three donors; two replicates per condition.. *P ≤0.05

Smad2/3 Knockdown Decreases TGF-β1-induced HASM Cell Shortening and ROCK Activation

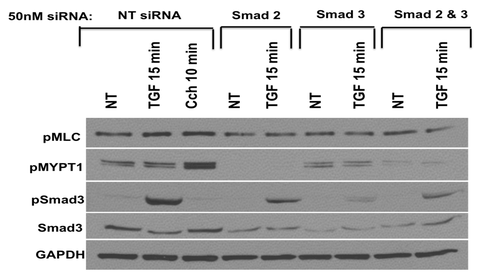

Figure 4. Smad siRNA inhibition in HASM cells. HASM cells were transfected with siRNA against Smad2, Smad3, or non-targeting control and stimulated acutely (15 min) with TGF-ß1 (10 ng/mL) or vehicle control. Following treatment, HASM cells were lysed with RIPA and subject to immunoblot.

Summary

- TGF-ß1 induces basal and agonist-induced hPCLS bronchoconstriction
- TGF-ß1 augments basal and agonist-induced HASM cell shortening, MLC phosphorylation, and ROCK activation
- TGF-ß1 augments agonist-induced $[Ca^{2+}]_i$ mobilization with little effects on basal $[Ca^{2+}]_i$
- Smad2/3 knockdown decreases TGF-ß1-induced MLC phosphorylation and ROCk activation in HASM cells

Significance

- TGF-ß1 may induce AHR through Smad-dependent ROCK activation.
- TGF-ß1 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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