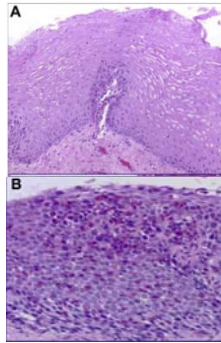


Chemical and Mechanical Stimuli Drive Fibroblast Activity: Implications for EoE Fibrosis

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Eosinophilic Esophagitis

- Chronic inflammatory disease characterized by eosinophil infiltration in the esophageal epithelium
- Unchecked inflammation leads to fibrosis
 - Stricture
 - Recurrent food impaction
 - Dysphagia
 - Dysmotility



Liacouras et al. JACI 2011

Fibrosis

- Uncontrolled fibroblast activation leads to excessive collagen deposition increasing tissue stiffness
- In EoE, adults typically present with symptoms of fibrotic disease, such as food impaction and stricture, whereas pediatric patients present with food refusal and emesis

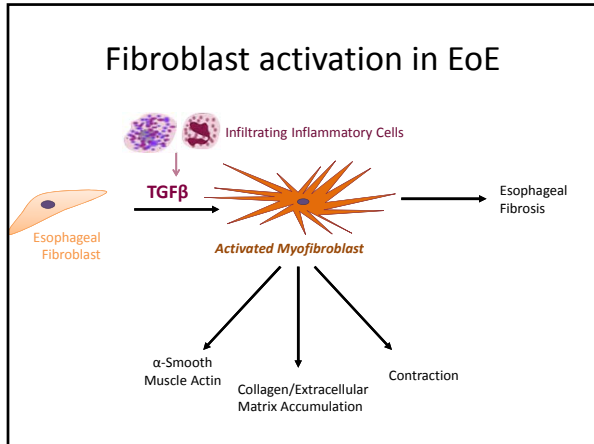


Normal esophagus

Trachealization

Stricture

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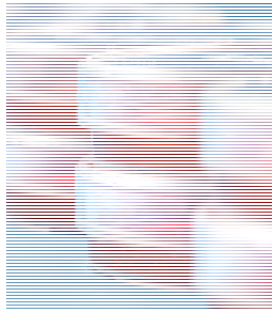
Aims

Understand how primary esophageal fibroblasts respond to changes in the chemical and mechanical microenvironment associated with EoE.

Determine if the behavior of these fibroblasts changes with patient age or disease state.

Methods

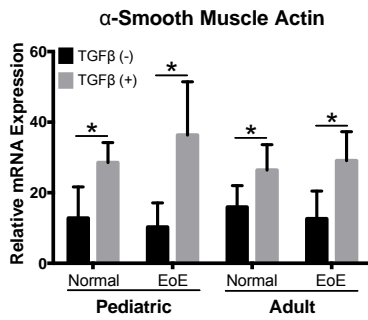
- Esophageal biopsy from patients with or without EoE
 - The Children’s Hospital of Philadelphia
 - Hospital of the University of Pennsylvania
- Isolated primary fibroblast cultures



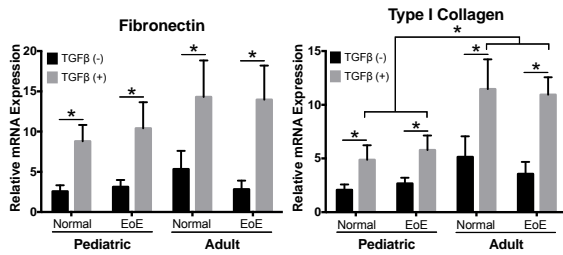
Patient Demographics

	Pediatric (n=14)		Adult (n=16)	
	Normal (n=6)	EoE (n=8)	Normal (n=4)	EoE (n=12)
Age (mean ± sd) ¹	10.9 ± 1.6	8.9 ± 1.0	35.5 ± 8.7	31.0 ± 3.1
Male (n, percent)	3 (50%)	7 (88%)	2 (50%)	8 (67%)
Sx dysphagia (n, %)	4 (57%)	2 (25%)	1 (25%)	6 (67%)
Sx regurgitation (n, %)	1 (14%)	3 (38%)	0 (0%)	0 (0%)
Hx impaction (n, %)	1 (14%)	2 (25%)	0 (0%)	7 (78%)
Hx stricture (n, %)	0 (0%)	1 (13%)	0 (0%)	4 (44%)

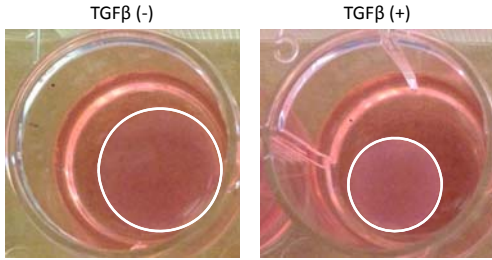
TGFβ enhances activation of fibroblasts *in vitro*

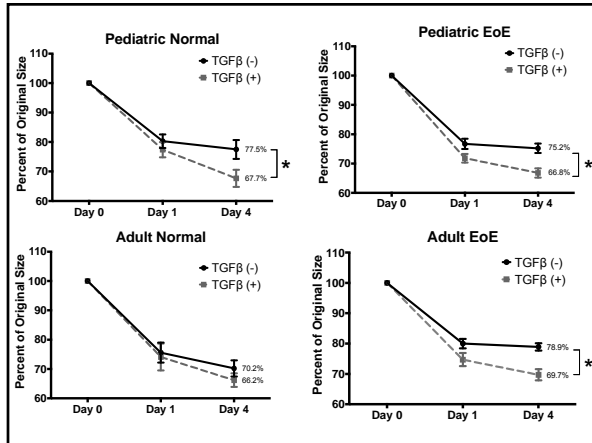


TGFβ-enhanced expression of ECM components



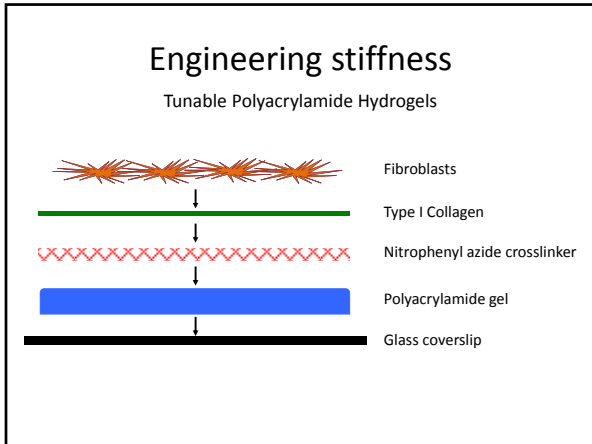
Fibroblast contraction assay

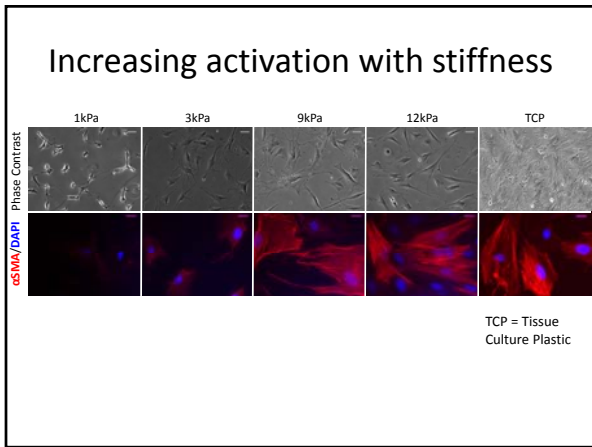


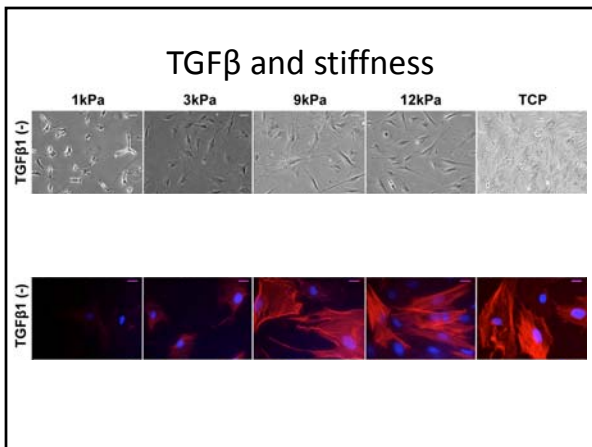


Summary

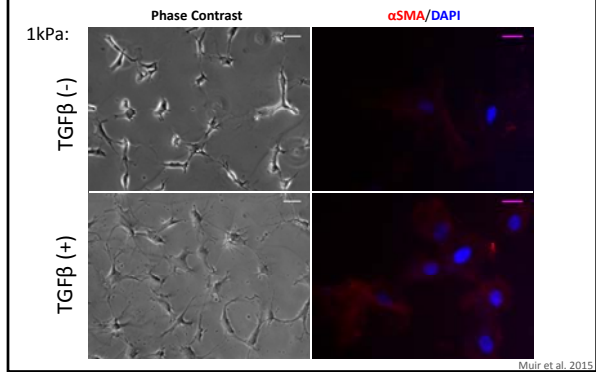
TGFβ enhances fibroblast activation, extracellular matrix expression, and fibroblast contractility independent of patient age and phenotype.



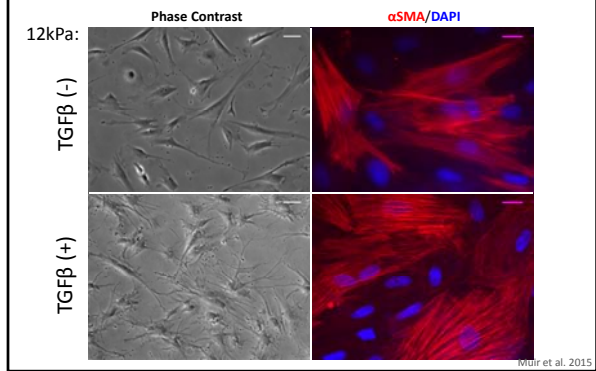




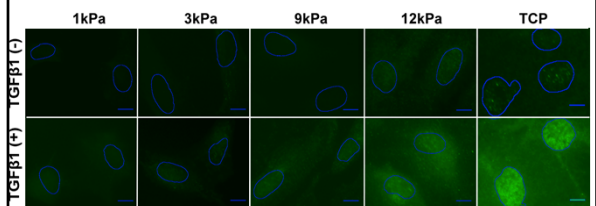
Effects of TGFβ dependent on stiffness



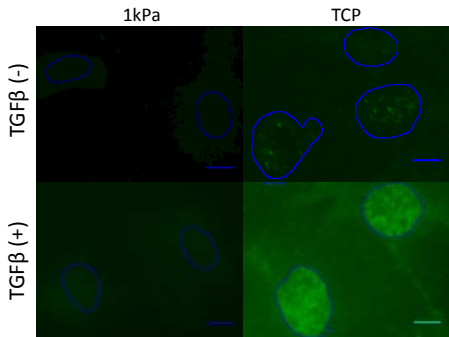
Effects of TGFβ dependent on stiffness



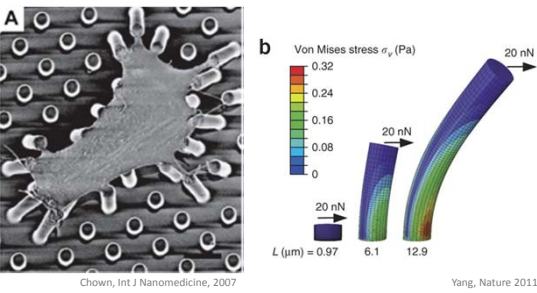
pSMAD3 nuclear localization dependent on matrix stiffness and TGFβ-activation



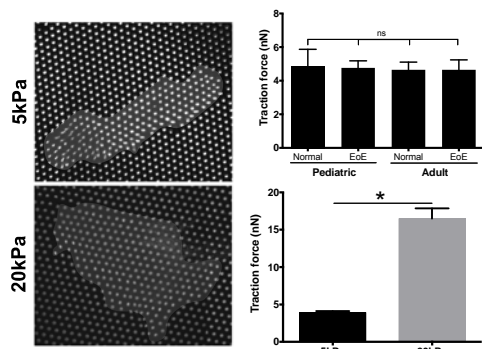
pSMAD3 nuclear localization dependent on matrix stiffness and TGFβ activation

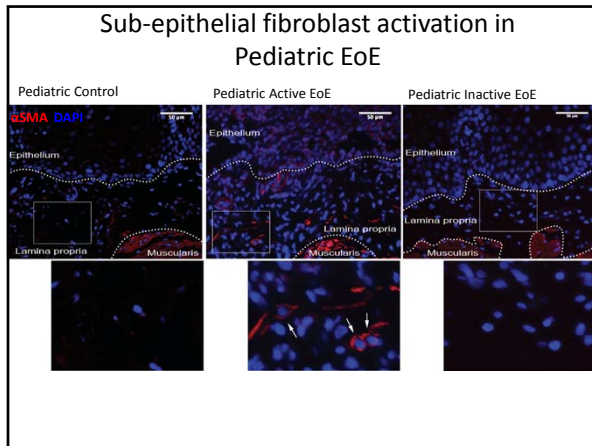


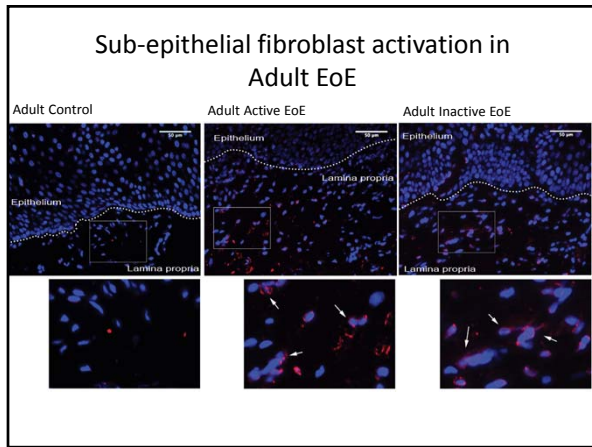
Traction Forces of Fibroblasts



Traction force increases with stiffness







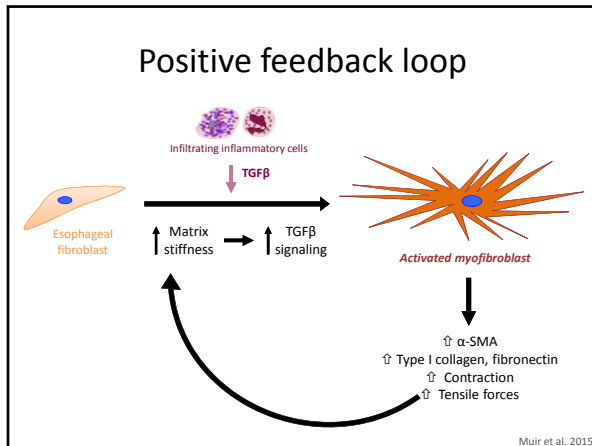
Summary

Esophageal fibroblasts are mechano- and chemo-sensitive.

There exists a requisite stiffness for TGF β induced fibroblast activation.

Fibroblasts have enhanced traction forces on matrices of increased stiffness

In vivo fibroblast activation may be irreversible after esophageal stiffness has occurred.



It is critical that therapies target fibrosis early in the development of Eosinophilic Esophagitis to prevent the potentially irreversible cellular consequences of fibrotic remodeling.

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