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


Eosinophilic Esophagitis

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

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
Fibroblast activation in EoE

- Infiltrating Inflammatory Cells
- TGFβ
- Activated Myofibroblast
- Collagen/Extracellular Matrix Accumulation
- Contraction
- Esophageal Fibrosis

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

<table>
<thead>
<tr>
<th></th>
<th>Pediatric (n=14)</th>
<th>Adult (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal (n=6)</td>
<td>EoE (n=8)</td>
</tr>
<tr>
<td></td>
<td>Normal (n=4)</td>
<td>EoE (n=12)</td>
</tr>
<tr>
<td>Age (mean ± sd)</td>
<td>10.9 ± 1.6</td>
<td>8.9 ± 1.0</td>
</tr>
<tr>
<td>Male (n, percent)</td>
<td>3 (50%)</td>
<td>7 (88%)</td>
</tr>
<tr>
<td>Sx dysphagia (n, %)</td>
<td>4 (25%)</td>
<td>2 (25%)</td>
</tr>
<tr>
<td>Sx regurgitation (n, %)</td>
<td>1 (14%)</td>
<td>3 (38%)</td>
</tr>
<tr>
<td>Hx impaction (n, %)</td>
<td>1 (14%)</td>
<td>2 (25%)</td>
</tr>
<tr>
<td>Hx stricture (n, %)</td>
<td>0 (0%)</td>
<td>1 (13%)</td>
</tr>
</tbody>
</table>

TGFβ enhances activation of fibroblasts in vitro

α-Smooth Muscle Actin

*relative mRNA Expression*

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>EoE</th>
<th>Pediatric</th>
<th>Normal</th>
<th>EoE</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGFβ (−)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGFβ (+)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TGFβ-enhanced expression of ECM components

*relative mRNA Expression*
Fibroblast contraction assay

TGFβ (-)  TGFβ (+)

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pediatric Normal</td>
<td>77.5%</td>
<td>67.7%</td>
<td></td>
</tr>
<tr>
<td>Adult Normal</td>
<td>70.2%</td>
<td>66.2%</td>
<td></td>
</tr>
<tr>
<td>Pediatric EoE</td>
<td>75.2%</td>
<td>66.8%</td>
<td></td>
</tr>
<tr>
<td>Adult EoE</td>
<td>78.9%</td>
<td>69.7%</td>
<td></td>
</tr>
</tbody>
</table>

Summary

TGFβ enhances fibroblast activation, extracellular matrix expression, and fibroblast contractility independent of patient age and phenotype.
Engineering stiffness
Tunable Polyacrylamide Hydrogels

Fibroblasts
Type I Collagen
Nitrophenyl azide crosslinker
Polyacrylamide gel
Glass coverslip

Increasing activation with stiffness

TCP = Tissue Culture Plastic

TGFβ and stiffness
Effects of TGFβ dependent on stiffness

1kPa:

TGFβ (-)

TGFβ (+)

Muir et al. 2015

Effects of TGFβ dependent on stiffness

12kPa:

TGFβ (-)

TGFβ (+)

Muir et al. 2015

pSMAD3 nuclear localization dependent on matrix stiffness and TGFβ-activation

1kPa 3kPa 9kPa 12kPa TCP

TGFβ (+)
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 TGFβ 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.