Claudin-7 dysregulation in pediatric EoE: A role for TGF-β1 in esophageal epithelial barrier dysfunction

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I have no disclosures.

Eosinophilic Esophagitis: Histopathologic Features
Barrier in EoE: Ultrastructural Features


Barrier in EoE: Electrophysiological Assessment


Eosinophilic Esophagitis: Molecular Features

Makris et al, J Immunol 2002
Blanchard et al, JACI 2007
Rothenberg et al, JACI 2010
Aceves, Allergy 2010
Established Roles of TGF-β1 in EoE

TGF-β1
Epithelium
Mast cells
Eosinophils

Barrier Function?

TGF-β’s & Other Epithelial Barriers

Hypothesis
TGF-β1 regulates esophageal epithelial barrier function
Aims

1. Determine whether TGF-β1 alters esophageal epithelial barrier function \textit{in vitro}

1. Determine the molecular target(s) through which TGF-β1 may regulate esophageal epithelial barrier

Materials & Methods

- EPC2-hTERT cells \(\rightarrow\) 3D Air Liquid Interface (ALI)

- rhTGF-β1 5, 10 ng/mL

- 5-7 Days

- In vitro Barrier Function Assays on 3D-ALI
  - Barrier: Trans-epithelial electrical resistance (TEER)

  - Paracellular Permeability: 3kDa FITC Flux

  - Real time RT-PCR on 3D-ALI
    - Epithelial Junction Transmembrane Components
TGF-β1: Decreased Barrier & Increased Paracellular Permeability

![Graph showing TEER (Ohm cm²) and 37-kDa FITC Flux (μg/ml/min) for Control, 5 ng/ml, and 10 ng/ml TGF-β1.](image)

N=3; *p<0.05

TGF-β1 Disrupts Epithelial Structure

![Images of TGF-β1 10 ng/mL and Control.](image)

TGF-β1 Decreases Tight Junction CLDN7 Expression

![Graph showing Relative mRNA and Protein Expression for Control, 5 ng/ml, and 10 ng/ml TGF-β1.](image)

(N=3; *p<0.05, **p<0.01)
TGF-β1 Effects on Epithelial Junctions

CLDN7 Expression in EoE Patients

Subject Characteristics

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean Age (yrs)</th>
<th>Symptoms</th>
<th>Mean Eos / HPF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active EoE</td>
<td>10</td>
<td>10.1</td>
<td>Vomiting-4 Abdominal pain-6</td>
<td>46</td>
</tr>
<tr>
<td>Inactive EoE</td>
<td>6</td>
<td>9.1</td>
<td>None</td>
<td>2.8</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>11.7</td>
<td>Abdominal pain-5 Diarrhea-3 Vomiting-2</td>
<td>0</td>
</tr>
</tbody>
</table>

CLDN7 is Decreased in Active EoE

<table>
<thead>
<tr>
<th>Relative mRNA expression (18S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>0.9</td>
</tr>
</tbody>
</table>

N=3; *p<0.05, **p<0.01
Independent of TGF-β1, can attenuated CLDN7 expression be sufficient to decrease barrier function in esophageal epithelial cells?

**Generation of CLDN7 Knock-down Cells**

![Graph showing relative mRNA and protein expression levels for Control, CLDN7 shRNA, and shRNA groups.](image)

**CLDN7 KD: Decreased Barrier & Increased Paracellular Permeability**

![Graphs showing TEER and 3kDa FITC Flux for Control, CLDN7 shRNA, and shRNA groups.](image)
Summary

TGF-β1 → Barrier → CLDN7

Normal → Active EoE

Conclusion

TGF-β1 participates in esophageal epithelial barrier dysfunction

Clinical Relevance

The findings of these studies implicate the utility of TGF-β1 targeting therapies in preserving &/or restoring esophageal epithelial barrier function
Acknowledgements

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Thank You!!!