Influenza Infects Human Lung Microvascular Endothelium Leading to Microvascular Leak: Role of Apoptosis and Claudin-5

Susan Armstrong, Jayesh Tigdi, Changsen Wang, Theo Moraes, and Warren Lee

Institute of Medical Science, University of Toronto; Keenan Research Centre of the Li Ka Shing Knowledge Institute of St. Michael's Hospital (Toronto)
• Severe influenza infections
  – Acute lung injury
    • Increased pulmonary microvascular permeability
  – Respiratory failure

• 20% of patients die

• Therapy is limited

• Novel therapeutic approaches are needed
A Possible Role for Endothelium

• Endothelial cells express influenza receptors
• Receptor expression is stimulated by host response to the virus
• Whether influenza can infect lung endothelium and induce microvascular leak is unknown
Hypotheses

• Influenza infects lung microvascular endothelium and induces microvascular leak

• Blocking leak will improve outcome
Influenza Replicates in Lung Endothelium

VIRAL NUCLEIC ACID (RT PCR)
Measurement of Endothelial Permeability

- Transendothelial Electrical Resistance (TEER)
  
- Dextran flux

Add fluorescein-tagged dextran to upper chamber → Allow 40 min for dextran flux → Measure Fluorescence
Influenza Induces Endothelial Permeability

- **p < 0.05
- **p < 0.005

Permeability to dextran

**p < 0.01

Multiplicity of infection

Permeability to dextran

Control, Flu, ZVAD, Flu + ZVAD

* * *
Replication-deficient Influenza Induces Endothelial Permeability
Replication-deficient Influenza Causes Loss of Claudin-5

**CLAUDIN-5**
- **Control**
- **UV flu**

**VE-CADHERIN**
- **Control**
- **UV flu**

**MOI**
- **Control** 0.5  8
- **Claudin-5**
- **VE-cadherin**
- **GAPDH**

![Graph showing protein expression](image)

* *p < 0.001
Claudin-5 Overexpression Blocks UV-flu Induced Leak
Potential Mechanisms of Decreased Claudin-5 Expression

- Decreased production
- Increased degradation
  - Lysosomes
  - Proteosome
  - Cleavage by matrix metalloproteases (MMPs)
Influenza Does Not Decrease Claudin-5 Production

<table>
<thead>
<tr>
<th>UV flu</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>$2^{-22.6-18.4}$ = 0.053</td>
<td>$2^{-22.8-18.2}$ = 0.040</td>
</tr>
<tr>
<td>$2^{-24.7-20.1}$ = 0.042</td>
<td>$2^{-23.9-19.1}$ = 0.038</td>
</tr>
<tr>
<td>$2^{-23.9-19.5}$ = 0.048</td>
<td>$2^{-23.8-19.5}$ = 0.051</td>
</tr>
<tr>
<td>Mean ± SE = 0.047 ± 0.003</td>
<td>Mean ± SE = 0.043 ± 0.004</td>
</tr>
</tbody>
</table>

qPCR shows no difference
Claudin-5 is Not Degraded by Lysosomes
Claudin-5 is Not Degraded in the Proteosome
Claudin-5 is Cleaved Extracellularly by Matrix Metalloproteases
Formoterol Induces Claudin-5 Expression

Formoterol (mM) 0 0.01 0.1 1
Claudin-5
GAPDH

Claudin-5 expression

* \( p < 0.05 \)
** \( p < 0.005 \)
Formoterol Blocks Influenza-induced Loss of Claudin-5
Formoterol Blocks Influenza-induced Leak

* p < 0.05
Formoterol Attenuates Influenza-induced Lung Vascular Leak in Mice

\[ *p < 0.05 \]
\[ **p < 0.001 \]
Key Findings

• Human influenza replicates in lung microvascular endothelium

• Infection increases lung endothelial permeability
  – Endothelial apoptosis
  – Loss of claudin-5

• Enhancement of endothelial barrier integrity may represent a novel therapeutic approach for severe influenza
Acknowledgements

Dr. Warren Lee
• Changsen Wang
• Asela Gamage
• Jayesh Tigdi
• Carlo Dumpit

Thesis Committee
• Dr. Jane Batt
• Dr. Lisa Robinson
• Dr. Arthur Slutsky

Dr. Theo Moraes (HSC)
• Xiaoe Si

Funding
• McLaughlin Centre
• Ontario Thoracic Society
• PSI Foundation
• Early Researcher Award (Ontario)