CALHM1-Mediated ATP Release and CBF Modulation in Airway Epithelial Cells

Alan D. Workman
Dr. Noam A. Cohen, MD., Ph.D.
Department of Otorhinolaryngology
University of Pennsylvania
Philadelphia Veterans Administration Medical Center
The Role of Respiratory Cilia

- Ciliated cells make up 80% of the upper respiratory mucosa

- Function in mucociliary clearance

- Dysfunctional cilia lead to disease states
Ciliary Beat Frequency Modulation

- Increased ATP release acts on purinergic receptors to increase Ca2+ concentration in the cells.
- A higher intracellular Ca2+ concentration directly precipitates the rise in ciliary beat frequency (CBF).

Winters SL, Davis CW, and Boucher RC, 2007
Mechanostimulation and CBF

• Mechanosensors on cells provide feedback regulation; CBF is adjusted for local loads of debris and mucus
• Physical force from breathing, sneezing, coughing, liquid shear stress, or hypotonicity can result in ATP release (and subsequent rise in CBF)
• Pannexins seem to play a prominent role as the channels that mediate this ATP release
• Pannexin channel blockers or knockdowns do not completely abolish the ATP response of a ciliated cell to stimulation
CALHM1

• ATP channel
• Originally identified in association with Alzheimer’s Disease
• Necessary for bitter, sweet, and umami taste
• Has not yet been localized outside of the tongue and brain

Hypothesis: CALHM1 ATP release plays a role in the dynamic modulation of CBF
Experimental Model

- Nasal septal epithelial cells were obtained from CALHM1 knockout and wild type mice
- Cells were grown at an air-liquid interface
Methods: CBF Imaging

• A high-speed digital video camera captured image data
• This data was processed with computer software that is specialized to quantify CBF
• Software reports a beat frequency that is the arithmetic mean of all of the cilia frequencies in the video field.
• CBF was quantified at 15 second intervals
CBF Video
Experimental Paradigm: The “Sneeze”

- Apparatus delivered a 50 ms air puff to the apical surface of the culture
- This level of stimulation is consistent with the pressures and timing of a sneeze
55mmHg Air Puff

**Wild Type**

**CALHM1 KO**

<table>
<thead>
<tr>
<th>Time (S)</th>
<th>CBF Increase (%)</th>
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<tbody>
<tr>
<td>-50</td>
<td>100</td>
</tr>
<tr>
<td>0</td>
<td>110</td>
</tr>
<tr>
<td>50</td>
<td>120</td>
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<tr>
<td>100</td>
<td>130</td>
</tr>
<tr>
<td>150</td>
<td>140</td>
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Wild Type: CBF increase significantly higher than CALHM1 KO.

**Wild Type**

**CALHM1 KO**

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**Wild Type** has significantly higher CBF increase compared to **CALHM1 KO** (p < 0.01).
Apical ATP Release

![Graph showing ATP release comparison between Wild Type and CALHM1 KO.]
CBF Changes After Addition of ATP

![Graph showing CBF Increase (%)]

- Wild Type
- CALHM1 KO
CBF Changes Following an Air Puff of Doubled Strength
Conclusions

• Following mechanical stimulation, CBF increase was significantly lower in CALHM1 knockout cultures than in wild type cultures.
• Apical ATP release was commensurately lower in the CALHM1 knockout.
• Exogenous ATP increases CBF of both cultures equally.
• Air puff of doubled strength results in a similar initial increase, likely due to the recruitment of additional channels, such as pannexins.
Clinical Implications

• CALHM1 in the airway has important implications for diseases of mucociliary clearance

• CALHM1 could serve as a therapeutic target

• Prior work shows that human airway tissue from patients with chronic rhinosinusitis shows a blunted CBF response to mechanical stimulation

• CALHM1 transducing mechanical signals in novel tissues
Current Work and Future Directions

• RT PCR for the CALHM1 mRNA transcript in the knockout and wild type cells
• Trials with a pannexin knockout mouse underway
• Personal plans
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