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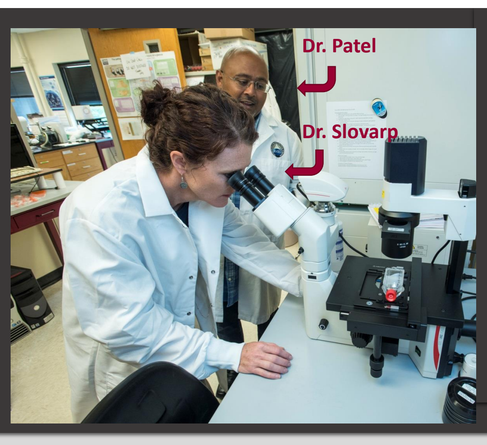
Investigating the Underlying Mechanisms Responsible for the Effectiveness of Behavioral Cough Suppression Therapy

Laurie Slovarp
University of Montana, Missoula

Jane Reynolds
University of Montana, Missoula, jr145900@umconnect.umt.edu

Sarah Popp
University of Montana, Missoula



Investigating the Underlying Mechanisms Responsible for the Effectiveness of Behavioral Cough Suppression Therapy

Laurie Slovarp, Ph.D., CCC-SLP, Sarjubhai Patel, Ph.D.,
Jane Reynolds, M.S., CCC-SLP, Sarah Popp, B.A., B.S.

Feature Article

Introduction

An estimated 20% of patients with CC do not respond to medical treatment and are said to have refractory chronic cough (RCC)[1-2]. Evidence suggests RCC is caused by hypersensitivity of sensory receptors in the airway epithelium known to regulate cough[2]. The primary sensory receptors are the transient receptor potential vanilloid (TRPV)[3-7]. These receptors can be found in the epithelial layer of the bronchus, larynx and nose[3-5] and are very plastic. Behavioral cough suppression therapy (BCST) has been shown to result in reduced cough sensitivity[7-9]; however, the underlying mechanism that results in reduced cough sensitivity is unknown.

Hypothesis

BCST works by stimulating neuroplasticity that results in a reduction in TRPV expression in the airway epithelium of patients with RCC?

Specific Aim

Quantify TRPV expression in human laryngeal epithelial cells (HLEC) pre and post BCST

Methods

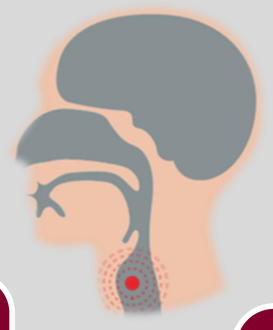
Baseline Testing
- Epiglottic biopsy
-Urge-to-cough Test
- Leicester cough questionnaire

Analyze TRPV expression via Western blot[1] analysis

Analyze TRPV mRNA expression via qPCR[2]

Patient completes 3-4 weeks of BCST

Repeat steps post-BCST



[1]Western blot analysis is used to directly measure the presence of specific proteins in tissue samples

[2]qPCR = quantitative polymerase chain reaction. Measures protein expression indirectly by measuring mRNA which precedes protein production

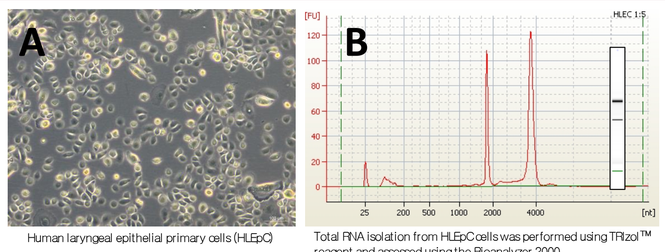
Urge-to-Cough (UTC) test

Urge-to-Cough Scale											
None	Just noticeable	Very weak	Weak	Moderate	Somewhat strong	Strong		Very strong		Very, Very Strong	Maximum (unable to avoid cough)
0	.5	1	2	3	4	5	6	7	8	9	10

Results

We are in the early stages of this study. We've perfected qPCR on human laryngeal epithelial primary cells (HLEpC) and measured TRPV receptors on two healthy human biopsies (see below). Two patients with RCC are currently enrolled but have not yet been analyzed

qPCR on HLEpC



Human laryngeal epithelial primary cells (HLEpC)

Total RNA isolation from HLEpC cells was performed using TRIzol™ reagent and assessed using the Bioanalyzer 2000.

C

HLEpC RNA Isolation Protocol		
RNA Area:	101.3431	Final Conc (ng/ul)
RNA Concentration: (1:5 dilution)	61.85025	309.25
rRNA Ratio [28s / 18s]:	1.645794	
RNA Integrity Number (RIN):	9.8 (B.02.08)	
Result Flagging Color:	16768991	
Result Flagging Label:	RIN: 9.80	

D

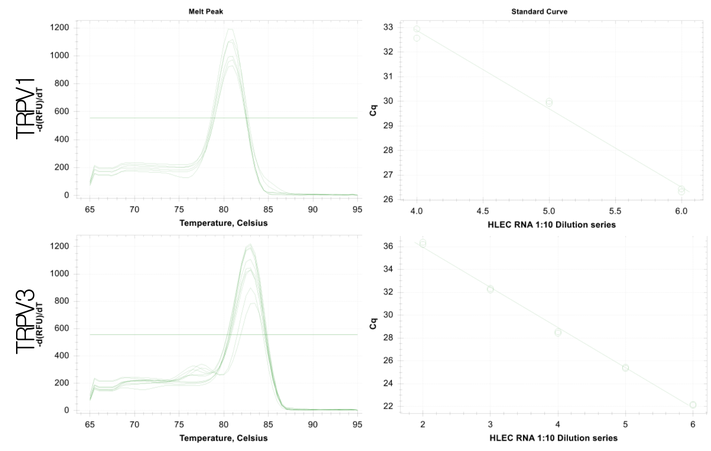
RT² qPCR Primer Assay	Gene	RefSeq Number
PPH08086F	TRPV1	NM_018727.5
PPH15769A	TRPV2	NM_016113.4
PPH21337A	TRPV3	NM_145068.3
PPH16107B	TRPV4	NM_021625.4
PPH15903A	TRPV5	NM_019841.6

A. Human laryngeal epithelial primary cells (HLEpC) were grown 25 cm^2 flasks (Corning) and maintained in BronchialLifeTM Epithelial Airway Complete Medium (Lifeline Cell Technology) in a humidified atmosphere of 5 % CO_2.

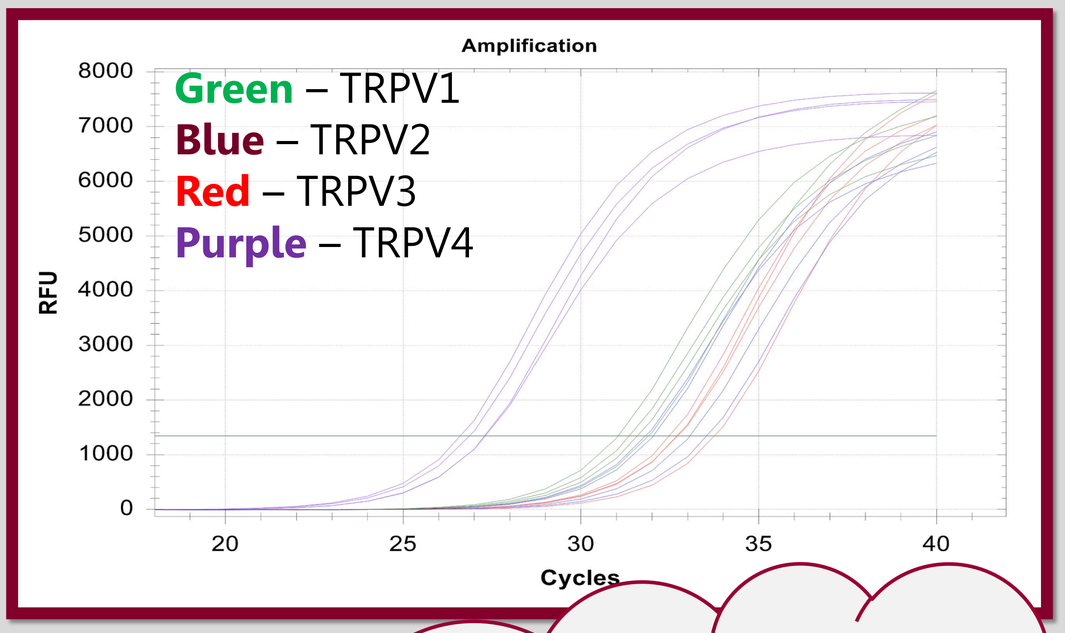
B. Total RNA was isolated from HELC Cells (Lifeline Cell Technology, FC-0045) using TRIzol Reagent (Thermo Fisher Scientific) according to manufacturers protocol. The RNA Integrity Number (RIN) and concentration was confirmed using the Agilent Bioanalyzer 2000 and spectrophotometric quantification respectively. RNA met the following criteria: RIN>9.5, 260/280>1.8, 260/230> 1.8.

C. Bioanalyser 2000 results for HLEpC total RNA .

D. TRPV 1-5 qPCR –RNA samples and no reverse transcription controls were reverse transcribed using RT² 1st Strand Kit (Qiagen) in accordance with the manufacturers protocol. Following reverse transcription, sample volume was adjusted to 100×l with nuclease free H_2O. 5×l aliquot of each sample was used in subsequent qPCR reactions. RT² qPCR Primer Assay (Qiagen, Frederick, MD). Efficiency for RT² qPCR primers as reported by the manufacturer to be >90%. A seven step, 10-fold limiting serial dilution was performed to determine limits of detection. The 25 l qPCR reaction contained 1x Syber Green Mastermix (Qiagen) and gene specific primers at a final concentration of 400nM each primer. Real-time PCR reactions were performed on the Bio-Rad CFX96 for 95°C for 10 min, 95°C for 15s, 60°C for 1 min, for 40 cycles, with fluorescence detection occurring after the annealing step. Dissociation curve analysis was performed following the PCR reaction for 95°C 1min, 55°C 30s, 95°C 30s with detection occurring during the ramp step from 55°C to 95°C.



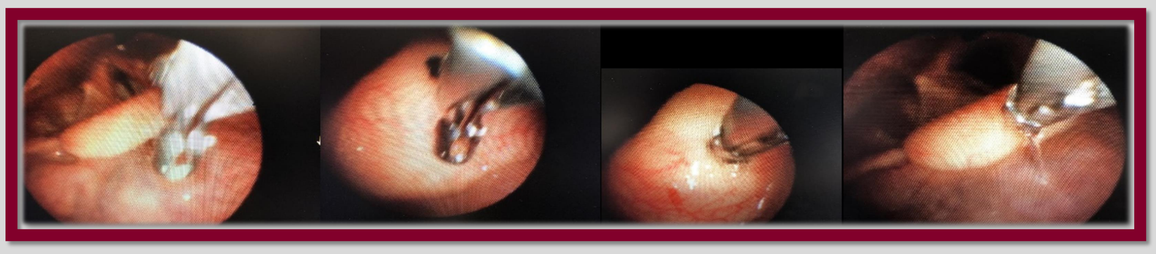
qPCR on Healthy HLEC



Implications

Explaining the mechanism of the effect of BCST may increase its application in the clinic as well as open doors to other potential treatments for RCC.

Epiglottic Biopsy



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