

Further Characterization of TRPV3 in the Lung

Research Topic Summary:

Air pollution is a major global health issue with particular local relevance. Wood and biomass smoke particulate matter (WBSPM) are known to be pneumotoxic. Exposure to WBSPM may increase susceptibility to respiratory infection and may exacerbate or cause a variety of respiratory diseases, specifically chronic obstructive pulmonary disorder and asthma. However, the molecular mechanisms by which WBSPM induce cytotoxicity are poorly understood. Previous work in the Reilly group has shown that the pneumotoxicity of many combustion-derived air toxicants such as cigarette smoke, diesel exhaust particles, and coal fly ash are mediated through the activation of transient receptor potential ion channels. This project will assess the both the expression of TRPV3 in the lung and the role of TRPV3 in the lung as a potential mediator of WBSPM toxicity.

Background:

Transient receptor potential (TRP) ion channels are a superfamily of nonselective calcium ion channels that are activated by a variety of physical and chemical stimuli. TRP channels serve a variety of biological roles, specifically in sensation of temperature, pain, and taste. A fairly well-known member of this family, transient receptor potential vanilloid-1 (TRPV1), is activated by both extremely hot temperatures as well as capsaicin, the compound found in chili peppers and pepper spray. The activation of TRPV1 in the lung by capsaicin induces the sensation of extreme heat and often results in the induction of many cytokines or even cell death on the cellular level, as well as inflammation and cough on the physiological level. However, unlike TRPV1, transient receptor potential vanilloid-3 (TRPV3) is a poorly understood member of the TRP ion channel family.

TRPV3 is activated thermally by innocuous warm temperatures and chemically by botanical compounds such as carvacrol, found in oregano. Current research has demonstrated that TRPV3 may play a role in cell proliferation and wound repair in the skin. A notable example of this relationship is found in Olmsted syndrome, wherein a gain-of-function mutation in the TRPV3 gene results in hyper-keratinization of the skin. Transient receptor potential vanilloid-3 (TRPV3) is known to be expressed at high levels in human keratinocytes (Peier et al., 2002). In the skin, TRPV3 plays some role in wound-healing and thermosensation, particularly in detecting innocuous warm temperatures (Miyamoto et al., 2011), as well as in inflammation (Nilius et al., 2013). Further, in oral epithelia, TRPV3 has been shown to play a role in wound healing. However, in the lung, the role of TRPV3 is not clear.

The chemical components of wood smoke include many xlenols and ethylphenols, which bear some structural similarity to potent agonists of TRPA1 and TRPV3, such as 2,4-di-tert-butylphenol and carvacrol, respectively. As such, wood smoke has been previously found to activate only TRPA1 and TRPV3, and no other TRP channels. Our group hypothesizes that TRPV3 is chemically activated by specific compounds in WBSPM, first resulting in inflammation and cytotoxicity, followed by some degree of adaptation to better withstand exposure to such toxicants.

Specific Aims and Timeline:

May-June: Fluorescent immunocytochemical assays (ICC).

Previously, qPCR and western blot showed the relative expression of TRPV3 in a variety of lung cells. The goal of ICC is to qualitatively demonstrate the subcellular localization of TRPV3 in the variety of lung cells. ICC represents a fundamental stepping stone for the ultimate goal of determining the tissue-level localization of TRPV3 expression in the lung.

May: qPCR of Various Mouse Lung Sections

These set of experiments will provide a general sense for where, at the tissue level, TRPV3 is enriched in the lung, for later immunohistochemical staining.

June-August: Mouse Lung Immunohistochemistry

Upon successful staining of TRPV3 in cultured lung cells and determining the regions of the lung where TRPV3 is most highly expressed transcriptionally, immunohistochemistry will be employed to further validate and demonstrate qualitatively that TRPV3 is also most highly expressed in these regions in terms of protein-expression.

May-August: Single Cell Isolation and Analysis

Individual cells will be collected and analyzed via qPCR and transcriptome analysis to assess TRPV3's potential role in wound-healing and cell proliferation, in the presence of a variety of different chemical and particle treatments.

Faculty Expertise

Dr. [REDACTED] is an expert in the field of TRP channels and of air pollution. His research program is unique and exciting in that it is the first to study TRPV3 in the context of air pollution toxicity. As far as I am aware, no other research laboratory at the University of Utah studies the molecular mechanisms of air pollution toxicity or the role of TRP channels in the lung.

Beyond his expertise in the field of pulmonary toxicology, I have worked under Dr. [REDACTED] for five semesters and have found him to be an outstanding mentor. He fosters an environment of collaboration, encouragement, and independence. Through Dr. [REDACTED], I have been afforded incredible opportunities both in learning new laboratory techniques as well as in allowing me to present our group's research at regional conferences.

Future Educational and Professional Goals:

In the future, I plan to attend either medical school and/or graduate school. Regardless of my ultimate decision, my interests lie in understanding the molecular mechanisms of a variety of disease. This project applies both biochemical knowledge and molecular biology techniques to assess the role and expression of TRPV3 in the lung and will supplement the theories learned in lectures with practical, laboratory skills and knowledge. More generally, this project will further my skills of analysis, comprehension, and scientific thinking.

References

Peier, A. M. et al. A heat-sensitive TRP channel expressed in keratinocytes. Science 296, 2046-2049 (2002).

Deering-Rice, Cassandra E. et al. Transient Receptor Potential Vanilloid-1 (TRPV1) Is a

Mediator of Lung Toxicity for Coal Fly Ash Particulate Material. *Molecular Pharmacology* 81.3 (2012): 411-419.

Miyamoto, T. et al. TRPV3 regulates nitric oxide synthase-independent nitric oxide synthesis in the skin. *Nat. Commun.* 2:369 doi: 10.1038/ncomms1371 (2011).

Nilius B, Biro T, Owsianik G. TRPV3: time to decipher a poorly understood family member ! *J Physiol.* 2013;592:295-304.

Li, Xiaolei et al. Overexpression of TRPV3 Correlates with Tumor Progression in Non- Small Cell Lung Cancer. Ed. William Chi-shing Cho. *International Journal of Molecular Sciences* 17.4 (2016): 437. PMC. Web. 27 June 2017.

Lo, I. C., Chan, H. C., Qi, Z., Ng, K. L., So, C. and Tsang, S. Y. (2016), TRPV3 Channel Negatively Regulates Cell Cycle Progression and Safeguards the Pluripotency of Embryonic Stem Cells. *J. Cell. Physiol.*, 231: 403-413. doi:10.1002/jcp.25086

Example