ID: 125 **ROLE OF TRPM2 IN SPHINGOLIPID-MEDIATED RADIATION-INDUCED LUNG INJURY**

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Rationale We previously reported sphingolipid signaling is an important mediator of radiation-induced lung injury (RILI) although the mechanisms underlying these effects have not been fully defined. A potential molecule of interest in this regard in TRPM2 (transient receptor potential (melastatin) 2), an oxidant sensitive, non-selective cation channel expressed in the lung endothelium that is known to regulate endothelial cell (EC) permeability and cellular responses to radiation injury. Thus, we hypothesized that TRPM2 is an important regulator of RILI-mediated by sphingolipids.

Methods To assess the role of TRPM2 on endothelial cell barrier regulation, human pulmonary artery EC were grown to confluence overlying gold-plated microelectrodes for real-time measurements of transendothelial electrical resistance (TER) reflective of barrier integrity via an Electrical Cell-sensing Impedance System (ECIS, Applied Biophysics, Troy, NY). Cells were transfected with TRPM2 siRNA (100 mM, 3 d) or non-specific siRNA prior to treatment with sphingosine 1-phosphate (1 mM), known to induce barrier enhancement. In separate experiments, prior to S1P stimulation EC were treated with either DPQ or 3-AB, both inhibitors of poly-ADP-ribose polymerase (PARP), an enzyme that mediates TRPM2 channel opening. Subsequently, to assess the role of TRPM2 to RILI in a previously characterized *in vivo* model, female TRPM2-/- mice were subjected to 20 Gy single dose thoracic radiation with body weights measured every 2 weeks and bronchalveolar lavage fluid collected for measurement of protein levels at 6 wks.

Results TRPM2-silenced EC demonstrated a significant attenuation of S1P-induced barrier enhancement as measured by TER. In addition, barrier enhancement by S1P was also significantly attenuated in cells treated with either DPQ or 3-AB. In our murine RILI model, body weights increased at 2 weeks in wildtype radiated mice while weights were decreased in TRPM2−/− mice consistent with an increased injury response in these animals. Similarly, at 6 weeks TRPM2−/− mice were found to have a significant increase in BAL protein levels compared to TRPM2−/− control animals (1.54 fold change, $p<0.022$) while there was not a significant increase noted in RILI-challenged wildtype mice.

Conclusion Our data confirm TRPM2 as an important mediator of EC barrier regulation of S1P. Moreover, we found TRPM2−/− mice were more susceptible to RILI, a model of inflammatory lung injury mediated by sphingolipid signaling. Our findings suggest that modulation of TRPM2 effecting downstream sphingolipid signaling may represent a novel therapeutic strategy for some patients with inflammatory lung diseases including RILI.