Regulation of tensile homeostasis in the trabecular meshwork

Oleg Yarishkin, Monika Lakk and David Krizaj
Department of Ophthalmology & Visual Sciences, University of Utah School of Medicine, Salt Lake City, UT

GLAUCOMA RESEARCH FOUNDATION

INTRODUCTION

The hydraulic conductivity of the conventional outflow pathway is defined in part by the pressure-dependence of the trabecular meshwork (TM). The objective of this project was to characterize the roles of mechanosensing TREK-1, TRPV4 and TRPM4 channels in setting the tensile homeostasis of TM cells, TM resistance and IOP dynamics. We investigated how these channels transduce the effects of pressure and tensile stretch, and how their activation translates into changes in intracellular calcium levels, cytoskeletal reorganization and TM-ECM interactions.

DESIGN & METHODS

•*Cell culture and mechanical stress stimuli:* human TM cells (hTM) and primary human TM cells (pTM) of juxtacanalicular or corneoscleral origin isolated from healthy or OAG donors were incubated in the dedicated medium (ScienCell Laboratories) at 37 ºC and 5% CO_2.

•*Electrophysiology.* Whole-cell transmembrane currents in hTM cells were elicited by 1s RAMP pulses from -100 mV to 100 mV. The data were sampled at 10 kHz and filtered at 5 kHz. All experiments were conducted at a room temperature of 20–22^{0}C. Pressure steps were delivered by high-speed pressure clamp (see image).

•*Calcium imaging.* Intracellular calcium concentration $[Ca^{2+}]_i$ was measured in cells loaded with the ratiometric indicator Fura-2. Cells were stimulated with pharmacological agonists/antagonists of mechanosensitive channels.

•*Immunohistochemistry.* Cells were fixed using 4% FPA and immunostained with Phalloidin-conjugated Alexa Fluor 488, TRPV4, TREK1, TRPM4, zyxin, FAK, talin-1 and vinculin antibodies.

•*Tensile stretch*. Cells were stimulated with biaxial strain (0.5 Hz) in the presence/absence of Y27632 (1, 5, 10 uM, Life Sciences) for indicated durations (1, 3, 5 or 7 hours). Control cells were cultured under the same conditions without stretch.

Quantitative Real-Time PCR, Western blots, Immunocytochemistry: standard methods were used.

The permeability of TM monolayers was measured with ECIS (electric cell-substrate impedance sensing)

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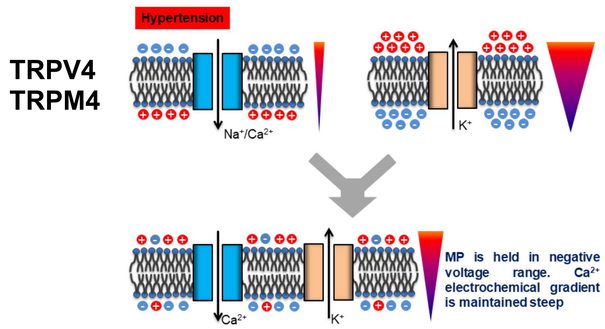
Statistical analysis: Statistical comparisons were performed using one-way ANOVA test followed by post-hoc Tukey's multiple comparison of means (Origin 8.0).

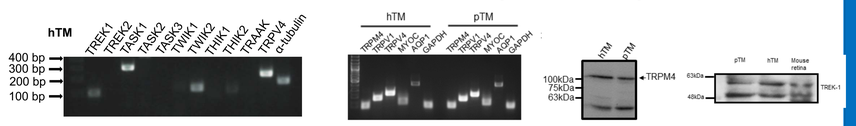
TENSILE HOMEOSTASIS



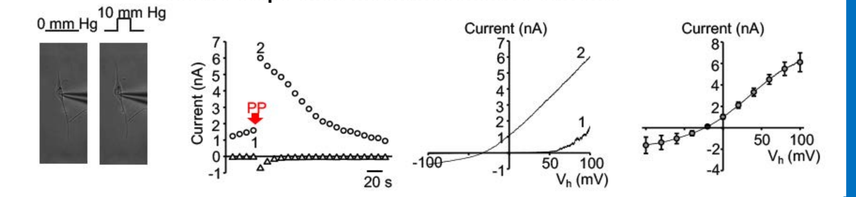
The hypothesis rests on the idea that tensile homeostasis and membrane potential (MP) of TM cells are maintained by concomitant activation of opposing channels that depolarize (TRPV4 + TRPM4) and hyperpolarize (TREK-1) the cells in response to pressure. TRPM4, a Ca-activated Na channel modulates the periodicity of TRPV4-mediated Ca oscillations.

RESULTS

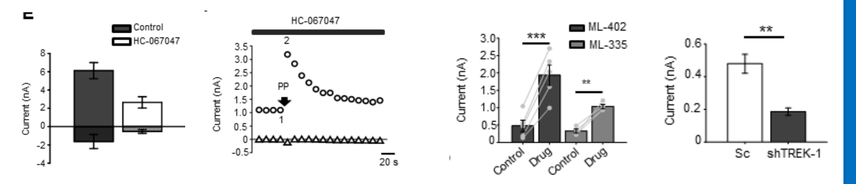
Mechanosensitive channel mRNA and protein are expressed in TM



Pressure steps elicit mechanosensitive currents



Mechanosensitive currents have TRPV4 and TREK-1 components



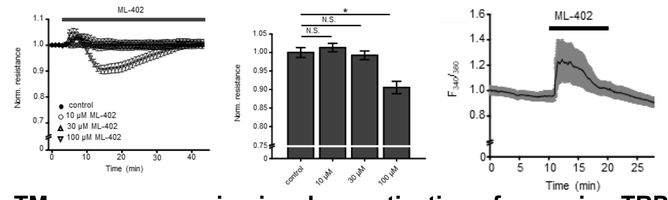
The TREK-1 antagonist HC067047 reduces the pressure-activated current, leaving a large outward conductance.

The pressure-activated outward current is mimicked by TREK1 agonists (ML-402) and inhibited by TREK1 shRNAs.

CONCLUSIONS

TREK-1 activation modulates TM calcium and permeability

ECIS and imaging: TREK1 agonists regulates [Ca2+]i and monolayer permeability



- **TM pressure sensing involves activation of opposing TRPV4 and TREK-1 channels**
- **TREK-1 maintains the resting potential, controls the driving force for Ca influx and cell-ECM interactions.**
- **TRPV4-TRPM4 interactions drive Ca oscillations and regulate cell volume**
- **The TM is a highly sophisticated pressure transducer that uses multiple mechanosensors to regulate function. In healthy eyes these sensors adapt the cells to large and/or sustained pressure stimuli; this ability may be lost in glaucoma.**

NEXT STEPS

The identification of TM mechanosensors is the first step in the determination of their role in IOP sensing in healthy and glaucomatous eyes. The next step will be to define the mechanothresholds of each channel, and how they interact in response to brief and chronic stimulation. Then, we will assess how TM mechanosensing and IOP regulation is impacted in the eyes of TRPV4-/-, TREK-1-/- and TRPM4-/- mice under normal and 'glaucoma' (elevated IOP) conditions. Finally, we will design ocular-permeable compounds that target these channels to manipulate intraocular pressure.

ACKNOWLEDGEMENTS

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