A TRiP through the mechanical world of TRPP channels

C.D. Cox, CA. Ng and B. Martinac, Victor Chang Cardiac Research Institute, Darlinghurst, NSW 2010, Australia.

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most prevalent lethal monogenic disorders affecting around 1: 500 live births. The disease is characterized by wide-spread cyst formation in the kidney and other organs. In excess of 50% of these patients will require renal replacement in their lifetimes with a huge associated healthcare bill. Greater than 80% of disease causing variants can be traced to the prototypical members of the polycystic kidney disease (PKD) protein family; PKD1 and PKD2 (TRPP1). However, the exact function of these proteins remains enigmatic. One suggestion is that these proteins form a mechanical complex localized to the cilia, cell-cell junctions and focal adhesions. Here we sought to robustly address the mechanosensitivity of the PKD2 protein family using electrophysiological techniques.

This family consists of three ubiquitously expressed members; PKD2, PKD2-L1 and PKD2-L2, that form part of the TRP channel superfamily. In order to probe their mechanosensitivity we created doxycycline inducible Flp-In stably expressing HEK293T cell lines. In the cell-attached configuration we could elicit large robust mechanically-activated PKD2-L1 currents over pressure ranges similar to those that gate the bona fide mechanically-gated channel Piezo1. The activity in cell-attached patches was highly dependent on the presence of divalent cations. PKD2-L1 is known to activate in response to rises in intracellular Ca^{2+} and then subsequently desensitize. We found that multiple responses to mechanical stimuli was only possible in the absence of extracellular Ca^{2+}. When 1 mM Ca^{2+} or Ba2+ was present currents rapidly and irreversibly desensitized. Activity could still be elicited in the presence of BAPTA or EGTA in the extracellular solution indicating that the response was not mediated by external Ca^{2+} influx. The activity was boosted by the addition of cytochalasin D (10 μM) or GsMTx-4 (5 μM) and inhibited by colchicine (10 μM). We also identify a number of residues that reside within a hydrophobic lipid-filled cavity that determines the mechanical response of PKD-2L1.

These data suggest that PKD2-L1 integrates mechanical force and that both the microtubule cytoskeleton and lipid bilayer play an important role in this process. Given the lack of a phenotype of PKD2-L1 knockout mice it is thus essential to see if the prototypical member of this family, PKD2, acts as a physiologically relevant mechanosensor.