A Yoda1-based Approach to Investigate Piezo1 Channels in Red Blood Cells Using Automated Patch Clamp Technology

Maria Giustina Rotordam

Piezo1 is a mechanosensitive ion channel supposed to regulate the volume and maintain the structural integrity in Red Blood Cells (RBCs), as gain-of-function mutations in this channel are associated to the RBC disease Hereditary Xerocytosis (Zarychanski et al., 2012; Bae et al., 2013). Piezo1 is activated by several mechanical forces, including stretching, poking and shear stress and allows Ca2+ and other cations to enter the cell generating an electrical response. In 2015, it has been discovered that Piezo1 is sensitive to a small molecule, Yoda1 (Syeda et al., 2015), which keeps the channel open and affects its inactivation kinetics. This finding has created new possibilities to elucidate Piezo1 gating mechanism and explore its functional significance in physiological and pathophysiological conditions.

Here, we present a patient with a novel PIEZO1 mutation (R2110W) and a patch clamp based high-throughput screening assay for Piezo1 activity. We established a protocol to detect functional Piezo1 mutations upon chemical stimulation by Yoda1, yet we were not able to stimulate the channel via mechanical force, i.e. pressure steps and shear-stress.

The assay was first developed on Neuro2A (N2A), a neuroblastoma cell-line endogenously expressing Piezo1 channels (kindly provided by Max-Delbrück Center, Berlin), due to larger abundance of Piezo1 channels in these cells. Initial experiments were performed on the Patchliner (Nanion Technologies GmbH, Munich), a medium-throughput automated patch clamp system able to record up to 8 cells at a time. Currents were elicited using a voltage ramp ranging from -100 to +80 mV for 300 ms, the holding potential was set to -60 mV. A significantly increased whole-cell current was observed upon 10 μM Yoda1 application in half of the recorded cells and the resulting Yoda1-induced currents were inhibited by 30 μM gadolinium chloride, a non-specific blocker of stretch-activated channels. The assay was then implemented on the SyncroPatch 384PE (Nanion Technologies GmbH, Munich), capable of recording up to 384 cells in parallel under identical experimental conditions, thus allowing for reliable statistical analysis. Yoda1 responding cells were selected based on strict quality control (QC) criteria, i.e. the seal resistance stability over time. In one example NPC-384 chip 140 out of 384 N2A cells (37%) passed the QC criteria and 85 cells (60% of the valid cells) were considered as Yoda1 responders. Finally, we investigated Piezo1 electrophysiological properties in healthy and patient RBCs carrying the novel PIEZO1 R2110W mutation. Similar to N2A cells, RBCs currents were analysed and divided into Yoda1 responders and non-responders according to our QC criteria. The increase in whole-cell currents induced by Yoda1 application was significantly higher in patient compared to control RBCs, which was also reflected by a higher number of Yoda1 responders compared to control. Residue R2110W is structurally located in a gating sensitive area of the channel protein suggesting a gain-of-function. This would be in line with previously described mutations in PIEZO1 (Albuisson et al.,
2013) and the mild form of anaemia observed in the patient. Furthermore, we could exclude any involvement of Gardos channels in the Yoda1-induced currents by comparing measurements in the presence and absence of the specific Gardos channel inhibitor TRAM-34.

Altogether, our work demonstrates that high-throughput patch clamping can provide a robust assay to study functional Piezo1 impairments in primary RBCs without expressing the mutated channel protein in a heterologous expression system. Our approach may be used to detect other channelopathies not only in RBCs and may serve as routine screening assay for diseases related to ion channel dysfunctions in general, complementary to gene sequencing.