Studying The potential of TMEM120A membrane protein as a voltage gated Ion channel

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Abstract

TMEM120A, also known as TACAN, has recently been proposed as a mechanosensitive ion channel. TACAN has been implicated in the in vivo detection of painful mechanical stimuli. However, subsequent studies indicated that TACAN does not exhibit mechanosensitive ion channel activity when expressed in HEK293 or CHO cells, nor does it display mechanosensitive channel activity in giant liposome vesicle patch experiments. More recent findings suggest that human TACAN (hTACAN) may represent a fundamentally unique type of ion channel. Molecular dynamics simulations and electrophysiological experiments have indicated that in wild type hTACAN, the M207 residue appears to function as a gate that obstructs the hypothesized ion conduction pathway. Mutation of M207 to alanine (M207A) increases hTACAN's permeability, suggesting the involvement of additional unidentified mechanisms. For instance, the rotation of helices TM3 and TM4 could cause the side chains of the hydrophobic residues F223 and M207 to shift, thereby opening the channel. The objective of this project is to investigate whether TACAN acts as a mechanosensitive ion channel or as an ion channel regulatory protein. We aim to achieve this through the following Three objectives:1. Establish the expression and purification of human TMEM120A wild-type and mutants (M207/A & F223/A) in mammalian cells. 2.Recharacterize the potential of TACAN as a voltage-gated ion channel. 3.Study the interaction of TACAN with mechanosensitive ion channel inhibitors (GdCl3 & GSMTX4) and activators (Jedi1 & YODA1). This year, we successfully expressed, purified, and scaled up the purification of TMEM120A wild-type and mutants (M207/A & F223/A) in HEK293SF cells. Additionally, our binding assays using MST and BLI revealed a strong binding affinity of TMEM120A wild-type and mutants to GSMTX4 (an MSC inhibitor) and a low binding affinity to Jedi1 (an MSC activator). Cryo-EM data collected for the TMEM120A(M207/A) mutant in 0.001% LMNG showed an extra density for the protein protruding outside the detergent micelle near the C-terminus, indicating the need for further optimization. Finally, both MD simulations and whole-cell patch-clamp results confirmed that TMEM120A wild-type and the M207/A mutant function as voltage-gated ion channels. We are currently exploring the channel selectivity through additional experiments focused on chloride selectivity, pH dependence, and mechanosensitivity using HEK293 PIEZO1 KO cells. Next, our efforts will focus on obtaining the active state structures of TMEM120A wild-type and mutants through Cryo-EM, following the elucidation of the gating mechanism of the TMEM120A ion channel.