Structural and Functional insights on TMEM120A membrane protein

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Abstract

TMEM120A (TACAN) has been proposed to function as a mechanosensitive ion channel involved in the detection of painful mechanical stimuli. However, subsequent studies have disputed this hypothesis, showing that TACAN does not display mechanosensitive activity when expressed in HEK293 or CHO cells, nor in giant Unilamellar vesicle (GUV) patch-clamp assays. More recent data suggest that human TACAN (hTACAN) may represent a distinct class of ion channel or membrane-associated regulator. Molecular dynamics and electrophysiological studies indicate that residue M207 acts as a gate within the ion conduction pathway, with its mutation to alanine (M207A) enhancing ion permeability. Structural rearrangements of transmembrane helices TM3 and TM4, involving residues F223 and M207, may underlie conformational transitions leading to channel opening.

An unpublished lipidomics study from our laboratory (Mohebali, 2021) demonstrated that TACAN interacts with phosphatidic acid via residue R152, suggesting a potential lipid-dependent regulatory mechanism. This project aims to determine whether TMEM120A functions as a mechanosensitive ion channel or as a modulator of other ion channels using electrophysiology, biophysical assays, and cryo-EM analysis of mutants M207A and F223A.

We optimized purification protocols for both TMEM120A mutants, selecting from nine workflows. TMEM120A-M207A was expressed using pcDNA3.1 and pCAG vectors and purified in SEC buffer containing 0.00015% LMNG and 0.00003% CHS. Initial cryo-EM data (3000 micrographs) confirmed particle presence in ~10% of picked particles, prompting adoption of the pCAG vector for higher yield.

Electrophysiological experiments revealed that wild-type TMEM120A and M207A increased voltage-dependent currents, whereas F223A reduced them. These currents were abolished by K^+ channel blockers, indicating a modulatory role rather than direct ion conduction. TMEM120A likely regulates endogenous ion channels such as Kv2.1 and Kv2.2 in a mutation-dependent manner. Ongoing work focuses on high-resolution cryo-EM structure determination and solid-state NMR studies to elucidate TMEM120A's lipid interactions and structural mechanisms.