Cryo-EM Enabled Drug Discovery Targeting Aquaporin-4 in Brain Edema

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Aquaporin-4 (AQP4) is a membrane-integrated water channel, abundantly expressed in astrocytes of the central nervous system (CNS) that is essential for maintaining brain water homeostasis (1, 2). Due to its ability to facilitate bidirectional water permeability across the plasma membrane of astrocytes, AQP4 plays a central role in the development and progression of cytotoxic edema (3). However, due to the high degree of sequence and structural conservation among aquaporin (AQP) family members, direct pharmacological inhibition carries a substantial risk of off-target inhibition of other AQPs which may lead to systemic adverse effects. We previously showed that Calmodulin (CaM), a Ca^{2+}-dependent signaling protein, directly binds AQP4 governing its astrocytic membrane localization and disrupting the AQP4-CaM interaction with trifluoperazine (TFP) effectively ablated cytotoxic edema in spinal cord injured rats (2). We screened a series of TFP derivatives using a cell swelling assay and identified several compounds that modulate the interaction between AQP4 and calmodulin (CaM). Notably, one compound completely abolished CaM binding to AQP4, as confirmed by microscale thermophoresis. We are currently working on solving the structure of AQP4 in complex with CaM by single particle cryoEM to guide the rational design of a new generation of compounds which will be evaluated for their ability to inhibit function and disrupt CaM binding.

In parallel, we are pursuing structural studies of AQP4 on its own in complex with small compounds that has shown to have effect on its water permeability. We have also initiated fragment-based screening using using weak affinity chromatography (WAC™) using nanodisc-reconstituted AQP4 that has been immobilized on silica via biotin conjugation (through MSP biotinylation), supporting further hit optimization.

Optimized compounds will be re-evaluated using biochemical and cellular assays to assess off-target effects and potential liabilities. The most promising candidates will then be advanced for further pharmacological evaluation.

Reference:

1. M. K. Oklinski et al., Immunolocalization of Water Channel Proteins AQP1 and AQP4 in Rat Spinal Cord. J Histochem Cytochem 62, 598-611 (2014).

2. P. Kitchen et al., Targeting Aquaporin-4 Subcellular Localization to Treat Central Nervous System Edema. Cell 181, 784-799 e719 (2020).

3. N. N. Haj-Yasein et al., Glial-conditional deletion of aquaporin-4 (Aqp4) reduces blood-brain water uptake and confers barrier function on perivascular astrocyte endfeet. Proc Natl Acad Sci U S A 108, 17815-17820 (2011).