Identification of novel TMEM175 modulators using high-throughput automated patch-clamp and solid-supported membrane- (SSM-) based electrophysiology platforms

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1 Introduction

TMEM175 is a novel, constitutively active ion channel involved in regulating lysosomal pH and autophagy. Mutations in this gene impair normal lysosomal and mitochondrial function, thereby increasing aggregation of insoluble proteins such as phosphorylated α-synuclein, leading to symptoms typical of Parkinson’s Disease (PD). Consequently, TMEM175 demonstrates significant potential as a key player in the treatment of PD. The lack of specific pharmacological tools has hampered further investigation into the exact role of TMEM175 in normal lysosomal function and pathological processes. Here, the TMEM175 stable cell line was characterized using automated patch-clamp and SSM-based electrophysiology. We developed and executed robust, high-throughput, and high-content direct electrophysiological intracellular screening assays, with exceptionally high success rates.

2 TMEM175 high-throughput automated patch clamp screening

Application protocol. The application protocol consists of three additions of extracellular physiological solution for a minimum of 8 minutes, followed by an application of test compound for a minimum of three minutes. 2×C50 (50 µM) is then applied, before the channel is inhibited by a saturating concentration of 4-AP (1 mM).

A rapid and robust automated, high-throughput electrophysiology screening assay, SyncPatch 384, high-throughput automated electrophysiology system was used to screen a library of 10,000 small molecules at a single concentration of 10 µM. Average success rate (A) and 2’ factor (B) of the TMEM175 HTS assay. The screen yielded an average success rate of 82% [wells passing QC] and an average 2’ factor of 0.77.

3 TMEM175 activators and antagonists

SBD08533 CRC tested over several days

SBD08533 – A reproducible TMEM175 Activator. Representative current traces (A) and time courses (B) of the HTS activator SBD08533 against TMEM175. The application protocol consists of two additions of extracellular physiological solution for a minimum of 3 minutes each, followed by an application of SBD08533 for a minimum of two minutes. 2×C50 (50 µM) is then applied before the channel is inhibited by a saturating concentration of 4-AP (1 mM). Normalized concentration response curves (C & D) showing the reproducible activation by SBD08533 tested over several days.

4 Solid-supported membrane- (SSM-) based TMEM175 screening assay

New HTS technology for drug discovery on transporters and channels. The SURFER® F6SE is a solid-supported membrane (SSM-) based technology that allows measurement of slow and intracellular targets, not accessible in patch-clamp in a 96-well format. Parallel target activation in presence of different compound concentrations reveals IC50 and EC50 values. Lysosomes were purified from HEK293 cells overexpressing TMEM175, kindly provided by SB Drug Discovery. Lysosomes were assayed in SURFER® F6SE according to Nanion’s standards protocol. SD at IC50 concentration gradients were used to trigger K+ flux through TMEM175 at 0 mV and pH 7.6. SST conductivity assays are possible; native lysosomal pH gradients may be applied.

5 Conclusions

- TMEM175 stable cell line was characterized using the SyncPatch 384 automated patch clamp system and used to develop and execute a high-throughput electrophysiology screening assay.
- This screening campaign successfully identified a number of active compounds with the ability to modulate TMEM175 in a concentration-dependent manner with EC50/IC50 values in the low micromolar range.
- The successful development of a TMEM175 electrophysiology assay capable of identifying novel pharmacological tools will enable investigation of the role of this exciting target in normal physiology and in disease.
- An assay for TMEM175 on the SURFER® F6SE was developed and dose-dependent signal enhancement and inhibition of TMEM175 currents from purified lysosomal membranes of the stable cell line overexpressing TMEM175 were successfully characterized. This approach allows for stable and robust recordings from proteins residing in organellar membranes with a throughput of up to 10,000 data points per day.

Recent TMEM175 Literature Review Update: Following generation of this data an article was published (Ito et al. Cell 2022) in which TMEM175 channel is suggested as a therapeutic target for Parkinson’s disease. We are now developing a 4-AP assay to be used in conjunction with our 4-AP assay. Visit our bimonthly for more information.