Characterization of human iPSC-derived neurons as an in vitro model to investigate Sigma-2 receptor functions.

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INTRODUCTION

Induced pluripotent stem cells (iPSCs) have become a widely used tool to study brain molecular functions. iPSC-derived neurons are a physiologically relevant model to study human neurodegenerative diseases. Alzheimer's disease (AD) is one of the most prevalent neurodegenerative diseases afflicting millions worldwide, characterized by the presence of amyloid pathology including toxic amyloid-beta (Aβ) oligomers in the brain (1,2). The Aβ oligomer receptor is a protein complex that is comprised of cellular prion protein (PrP^c), and binds to TMEM97, also known as the sigma-2 receptor (S2R) (3). On neurons, binding of Aβ oligomers to their receptors lead to synaptotoxicity, resulting in synapse loss and cognitive decline. The S2R is expressed in multiple cell types susceptible to degeneration, including neurons (4). In preclinical and clinical studies, we have demonstrated that S2R small molecule modulators, including our lead investigational therapeutic CT1812, can displace Aβ oligomers from their receptors on neuronal synapses and restore neuronal function (Fig.1) (1,2). Here, we characterized human glutamatergic iPSC-derived neurons as a model to further investigate S2R complex mechanism of action.

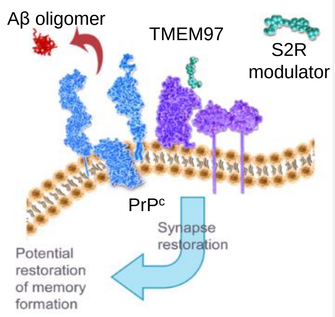
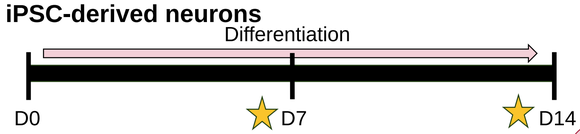


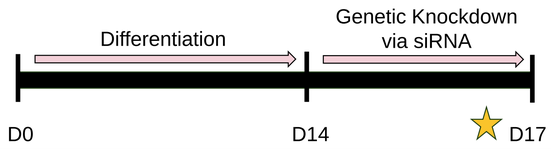
Figure 1

METHODS

Aim 1: Characterize TMEM97 and PrP^c levels in differentiated glutamatergic iPSC-derived neurons



Aim 2: Validate genetic knockdown



Endpoints:
- mRNA and protein expression
  - qRT-PCR
  - Western Blot
  - Immunofluorescence

RESULTS

TMEM97 and PrP^c are expressed in differentiated iPSC-derived neurons

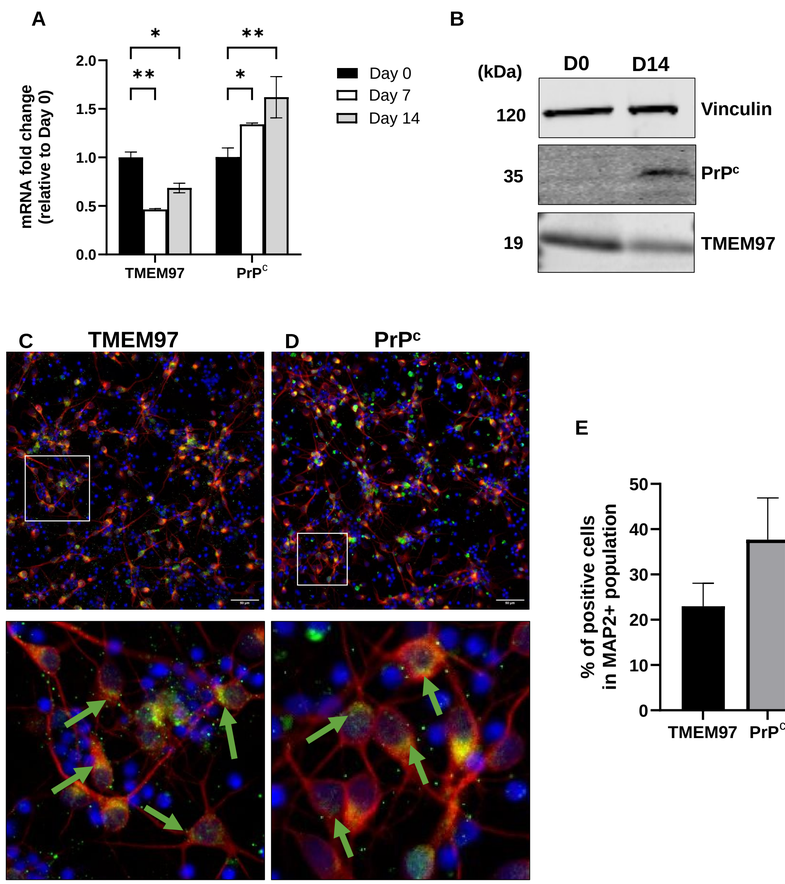


Figure 2. Expression levels of TMEM97 and PrP^c in iPSC-derived neurons after 7- and 14-days differentiation measured by (A) qRT-PCR (n=2, data shown as mean ± SD, 2-way ANOVA * p<0.05, ** p<0.01) and (B) western blot (n=2; data normalized to housekeeping gene, vinculin). Assessment of TMEM97 and PrP^c expression levels via Immunofluorescence at DIV 14. Representative microscopy images of iPSC-derived neurons stained for (C) TMEM97 (green) and (D) PrP^c. (green), neuronal processes (MAP2, red) and DAPI (blue). (E) Percentage of $TMEM97^+$ or PrP^{c+} cells within $MAP2^+$ iPSC-derived neuron population (n=4, data shown as mean ± SD).

TMEM97 mRNA and protein levels after genetic knockdown

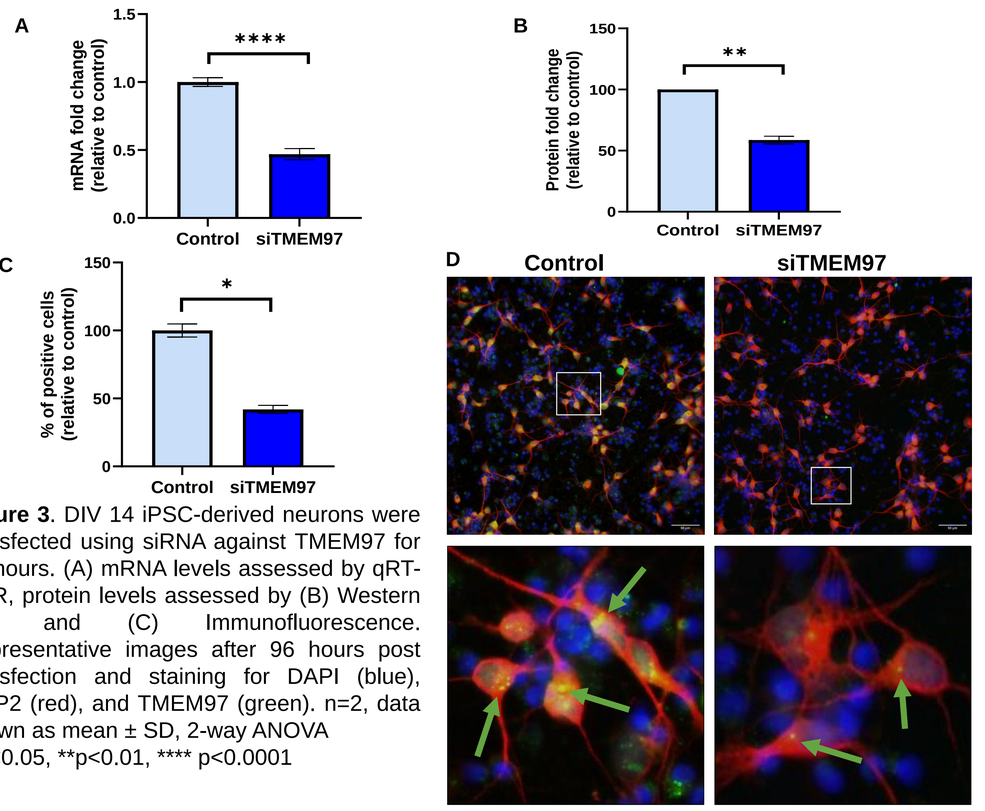


Figure 3. DIV 14 iPSC-derived neurons were transfected using siRNA against TMEM97 for 96-hours. (A) mRNA levels assessed by qRT-PCR, protein levels assessed by (B) Western blot and (C) Immunofluorescence. Representative images after 96 hours post transfection and staining for DAPI (blue), MAP2 (red), and TMEM97 (green). n=2, data shown as mean ± SD, 2-way ANOVA
* p<0.05, **p<0.01, **** p<0.0001

TMEM97 and PrP^c colocalize in iPSC-derived neurons

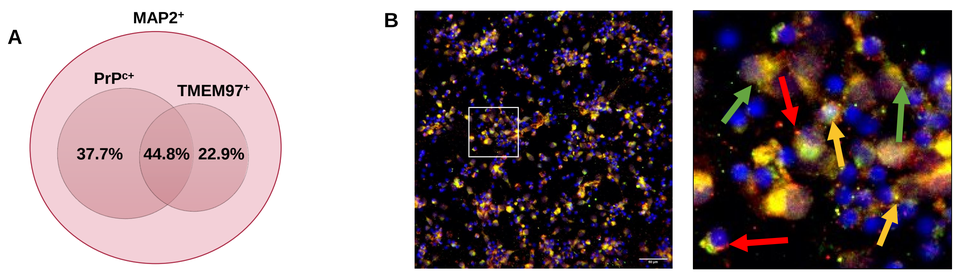
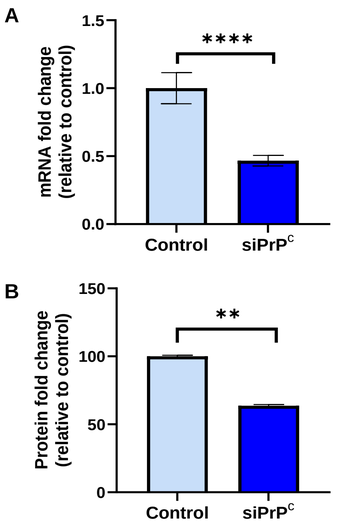


Figure 5. TMEM97 and PrP^c colocalization in iPSC-derived neurons. (A) Venn diagram visualizing % of $TMEM97^+$ and PrP^{c+} cells within $MAP2^+$ population and % of colocalization of $TMEM97^+$ and PrP^{c+}. (B) Representative images of iPSC-derived neurons stained for TMEM97 (red) and PrP^c (green) and arrows indicating proteins and colocalization (yellow). n=2.

PrP^c mRNA and protein levels after genetic knockdown



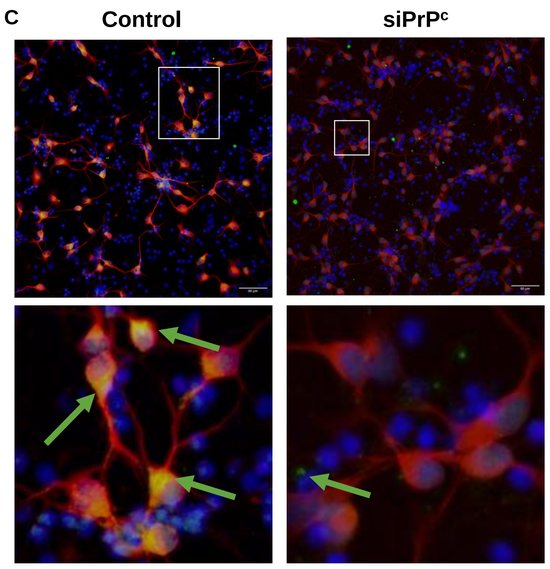


Figure 4. DIV 14 iPSC-derived neurons transfected for 96-hours using siRNA against *PRNP* (PrP^c). (A) mRNA levels of PrP^c assessed by qRT-PCR, (B) protein levels detected by immunofluorescence. (C) Representative images after 96 hours post transfection and staining for DAPI (blue), MAP2 (red), and PrP^c (green). n=2, data shown as mean ± SD, 2-way ANOVA ** p<0.01, **** p<0.0001.

CONCLUSIONS

Fully characterized human iPSC-derived neurons for S2R expression can be used as a model to investigate S2R mechanism of action as well as a screening tool to identify best in class S2R modulators to move forward as therapeutics for AD

References:
1. Izzo et al. (2014) Alzheimer's therapeutics targeting Amyloid Beta 1–42 oligomers I: Abeta 42 oligomer binding to specific neuronal receptors is displaced by drug candidates that improve cognitive deficits (doi:10.1371/journal.pone.0111898)
2. Izzo et al. (2014) Alzheimer's therapeutics targeting Amyloid Beta 1–42 oligomers II: Sigma-2/PGRMC1 receptors mediate Abeta 42 oligomer binding and synaptotoxicity (doi:10.1371/journal.pone.0111899)
3. Colom-Cadena et al. (2024) Transmembrane protein 97 is a potential synaptic amyloid beta receptor in human Alzheimer's disease. (https://doi.org/10.7488/ds/7664)
4. Lizama et al. (2023). Sigma-2 receptors – From basic biology to therapeutic target: A focus on age-related degenerative diseases (https://doi.org/10.3390/ ijms24076251)

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