CRISPR screening of genes associated with neuronal pentraxin 2 gene expression in human iPSC-derived glutamatergic excitatory neurons

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Abstract

Neuronal pentraxin 2 (NPTX2/NP2/Narp), an immediate early gene enriched at the excitatory synapse of glutamatergic neurons and inhibitory parvalbumin interneurons, is critical in the dynamic regulation of homeostatic scaling of synaptic activity in response to stimuli (Tsui et al., 1996; Xu et al., 2003; Chang et al., 2010). Studies suggest that NPTX2 gene and protein expression is downregulated with aging and greatly reduced at the onset of cognitive impairment in Alzheimer's Disease (Hanson, 2017; Xiao et al., 2017). Reprogrammable human induced pluripotent stem cells (hiPSCs) derived from human adult cells alongside CRISPR gene editing technology present an innovative approach in investigating these complex cellular mechanisms (Romito and Cobellis., 2015). In this study, we aimed to utilize hiPSC-derived excitatory glutamatergic cortical neurons (engineered with opti-ox technology) programmed to constitutively express the Cas nuclease, generating CRISPR-ready excitatory glutamatergic neurons (bitbio®) to 1) Establish a protocol to quantify mRNA levels of NPTX2 using reverse-transcription quantitative polymerase chain reaction (RT-qPCR) readouts and validate a knockdown of *Nptx2* in these excitatory glutamatergic neurons and 2) Further develop a protocol for knockout of target genes associated with *Nptx2* by optimizing guide RNA delivery. We have established a RT-qPCR method to quantify mRNA levels of NPTX2 with siRNA-knockdown treated neurons compared to control. Three sgRNAs were designed for each *Nptx2*-correlated gene and delivered at Day 4 with a 72h incubation. As a positive control of CRISPR-ready iPSCs, *Sox11* sgRNA was delivered in parallel with gene-of-interests and showed 50% knockout efficiency, validating the functional CRISPR workflow. The expressions of *Arhgef7, Rimbp2*, and *Trim9* were significantly downregulated by the pre-designed sgRNAs, where also the *Nptx2* downregulations were observed. Findings in this study provide novel insights on the potential modulatory effects of these target genes on NPTX2 mRNA expression in excitatory glutamatergic neurons but may require further experimental optimization to elucidate robust effects of these target gene effects on *Nptx2* expression.

Methods

Experimental Procedure

hiPSC-derived excitatory glutamatergic neurons were seeded, stabilized, and maintained in 96-well plates for 7- or 14-day experimental endpoints. To manipulate gene expression, siRNA or sgRNA were delivered using RNAiMAX 72 hr prior to mRNA isolation and mRNA expression was measured using RT-qPCR. RT-qPCR results were analyzed using *Gapdh* as a housekeeping gene. All CRISPR designs and experiments were conducted using commercial kits and software.

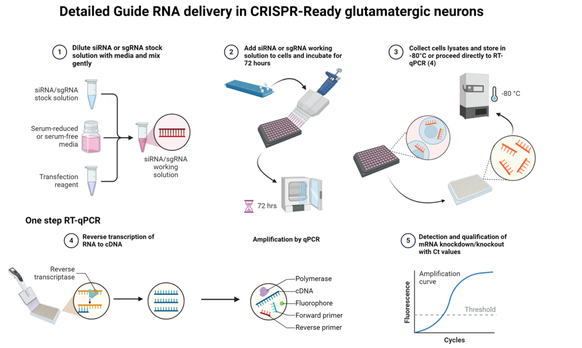

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Figure 1: Detailed protocol for RNA delivery in glutamatergic neurons. siRNA or sgRNA preparation and transfection in cell culture, cell lysate collection and One step RT-PCR processing and analysis.

Results

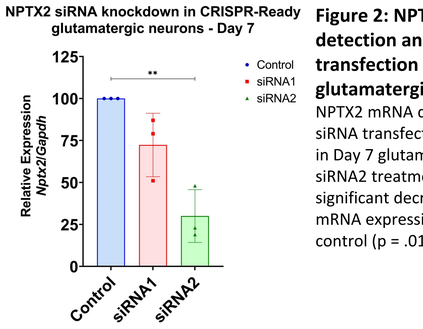

Figure 2: NPTX2 mRNA detection and siRNA transfection in Day 7 glutamatergic neurons. NPTX2 mRNA detection and siRNA transfection was validated in Day 7 glutamatergic neurons. siRNA2 treatment showed a significant decrease in NPTX2 mRNA expression compared to control (p = .010).

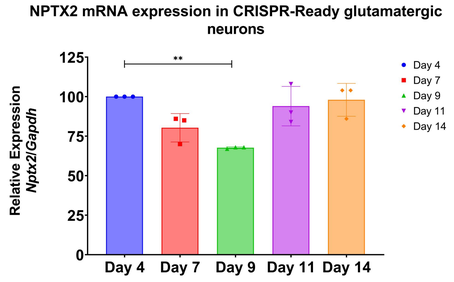

Figure 3: NPTX2 mRNA detection in glutamatergic neurons. NPTX2 mRNA was quantified across days of neuronal maturation (p=.002), showed a statistically significant decrease in NPTX2 mRNA expression between Day 4 and Day 9 (p=.003). NPTX2 mRNA expression on other days were similar to Day 4 and stabilized post Day 9.

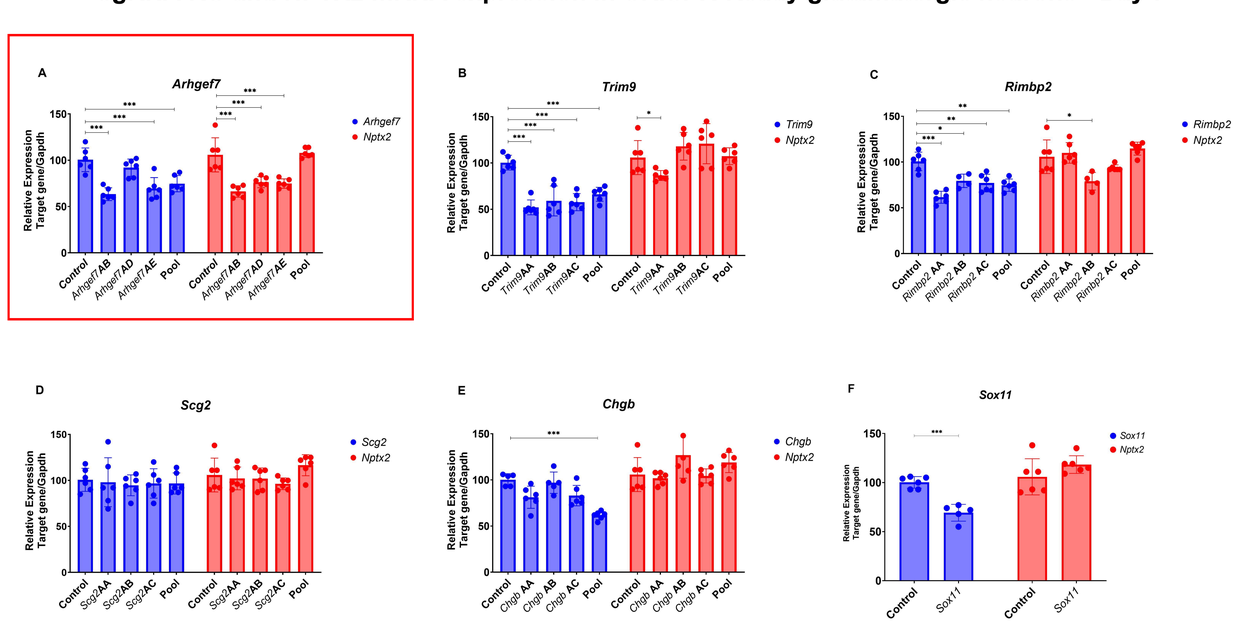

Figure 4: Target gene(s) sgRNA transfection and NPTX2 mRNA expression in Day 7 glutamatergic neurons. Effects of sgRNA treatment of target gene and NPTX2 mRNA expression levels in **A)** *Arhgef7*, **B)** *Trim9*, **C)** *Rimbp2*, **D)** *Scg2,* **E)** *Chgb* and control sgRNA delivery **F)** *Sox11* transfected cells compared to their non-target sgRNA controls (p<.001). A statistically significant reduction of NPTX2 mRNA expression levels was observed in *Arhgef7* (**AB,AD,AE**), *Trim9* (**AA**), *Rimbp2* (**AB**) transfected cells compared to non-target sgRNA controls. sgRNA treatment *of Scg2, Chgb,* and *Sox11* did not show any significant effects on NPTX2 mRNA expression compared to non-target sgRNA control, respectively.

Discussion

- This experiment establishes these CRISPR Ready neurons as a useful tool for understanding the molecular pathways involved with NPTX2 gene expression.
- Quantification of NPTX2 mRNA expression and probe detection validated in human iPSC-derived glutamatergic excitatory neurons.
  - NPTX2 siRNA transfection showed a significant knockdown of NPTX2 mRNA in human iPSC-derived glutamatergic excitatory neurons.
- Quantification of NPTX2 mRNA expression across days established in human iPSC-derived glutamatergic excitatory neurons (Day 4 to Day 14).
  - Day 9 NPTX2 mRNA expression was significantly reduced when compared to Day 4.
- Established a sgRNA delivery protocol in human iPSC-derived glutamatergic excitatory neurons.
  - sgRNA transfection of target genes of interest: *Arhgef7*, *Trim9,* and *Rimbp2* showed a significant decrease in gene expression (≤50% knockout efficacy) compared to non-target sgRNA controls.
  - Predesigned sgRNA transfections of *Arhgef7* (AB,AD,AE), *Trim9* (AA),and *Rimbp2* (AB) showed a statistically significant downregulation of NPTX2 mRNA expression compared to non-target sgRNA control.
  - *Sox11* sgRNA showed a significant decrease in *Sox11* expression (~50% knockout efficiency) compared to non-target sgRNA control in Day 7 glutamatergic neuron.
- Findings in this study provide novel insight on the potential modulatory effects of these target genes on NPTX2 mRNA expression in excitatory glutamatergic neurons but may require further experimental optimization to elucidate robust effects of these target gene effects on *Nptx2* expression.
- Future directions: Because of the relationship between Arhgef7-Nptx2 mRNA expression, follow up experiments with droplet RNA sequencing will be conducted to allow isolation of highly transfected cells for more sensitive measurements of the Arhgef7-Nptx2 interaction.

References

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Acknowledgements

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