Investigating DPP6 Expression and Cryptic Splicing in TDP-43 KO motor neurons

Introduction:

TAR DNA-binding protein-43 (TDP-43) is a crucial regulator of RNA splicing, playing a key role in neuronal health by ensuring proper RNA metabolism. Its mislocalisation and aggregation in the cytoplasm, a clinical hallmark of neurodegenerative diseases like amyotrophic lateral sclerosis (ALS), disrupt normal splicing and impair neuronal function (Kiernan et al., 2011; Suk and Rousseaux, 2020). Loss of TDP-43 function can lead to altered splicing of a plethora of genes such as STMN2, UNC13A, which impacts genes involved in axonal growth and neuronal excitability, respectively (Mackenzie and Rademakers, 2008).

Using proteomics and transcriptomics approach on TDP-43 knockout (KO) motor neurons (derived from human stem cells), our lab has identified Dipeptidyl aminopeptidase-like protein 6 (DPP6) – an auxiliary subunit of A-type potassium channels – to be downregulated, (Selvaraj lab -unpublished data). Noting DPP6 has been shown to modulating neuronal excitability and glutamate receptors in neurons (Malloy et al., 2022), necessitating further investigation. Bioinformatic studies also supported that DPP6 is mis-spliced leading to inclusion of cryptic exon between exon 5 and 6, hereby called “cryptic exon 4a”. However, it is not experimentally validated if DPP6 downregulation in TDP43 LOF MNs is mediated to mis-splicing of DPP6 leading to cryptic exons inclusion.

Against this background, in this project I sought to determine

1) Whether TDP43 LOF leads to mis-splicing of DPP6 mRNA
2) Does re-expression of TDP43 reverses mis-splicing of DPP6 mRNA

As a supplementary study and training, I assessed whether motor neurons derived from human stem cells are functional, by measuring action potential firing using patch-clamp electrophysiology.

Methods:

- RNA Extraction and cDNA Synthesis:
  Total RNA was extracted from iPSC-derived motor neurons using the RNeasy Mini Kit according to the manufacturer’s protocol. The RNA concentration and purity were determined using a NanoDrop machine. Complementary DNA (cDNA) was synthesized from 500 ng of total RNA.

- Quantitative PCR (qPCR):
  qPCR was performed to assess the expression of DPP6. We used forward and reverse primers of DPP6, STMN2 and 18S. 18S rRNA was used as endogenous controls. Relative gene expression was calculated using the 2^−ΔΔCt method.

- Protein Extraction and Western Blot: Proteins were extracted using RIPA buffer, and concentrations were measured with the BCA assay. Equal protein (20 µg) was run on SDS-PAGE, transferred to a nitrocellulose membrane, and blocked with 5% Milk. Membranes were incubated with anti-DPP6 and anti-GAPDH antibodies, followed by HRP-conjugated secondary antibodies. Bands were detected using ECL.

- Statistical Analysis: All experiments were performed in triplicate. Data were expressed as mean ± standard deviation (SD). Statistical analysis was conducted using GraphPad Prism 8 software.

Results:

As shown in Figure 1, we found that full-length DPP6 mRNA levels were lower in TDP-43 KO MNs compared to the control, and when TDP-43 was reexpressed, there was a trend towards recovery in DPP6 levels, suggestive of a reversible mechanism. However, more batches of cells are required for robust statistical analyses.

In Figure 2, our results showed much higher expression of DPP6-CE in TDP-43 KO MNs, suggesting that TDP-43 loss-of-function leads to cryptic splicing of DPP6. Re-expression of TDP-43 in the KO MNs led to modest decrease of the DPP6-CE.

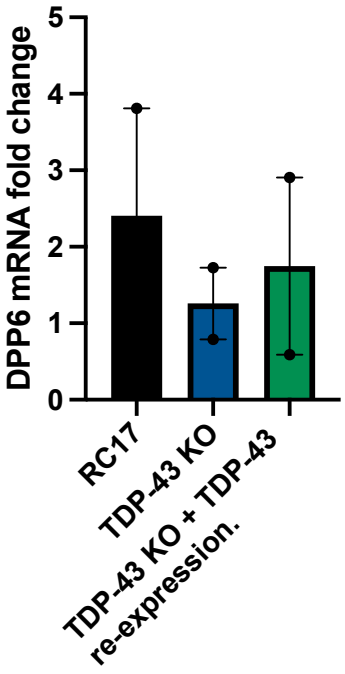

Figure 1: DPP6 mRNA levels are reduced in TDP-43 KO MNs and there is slight recovery after TDP-43 re-expression

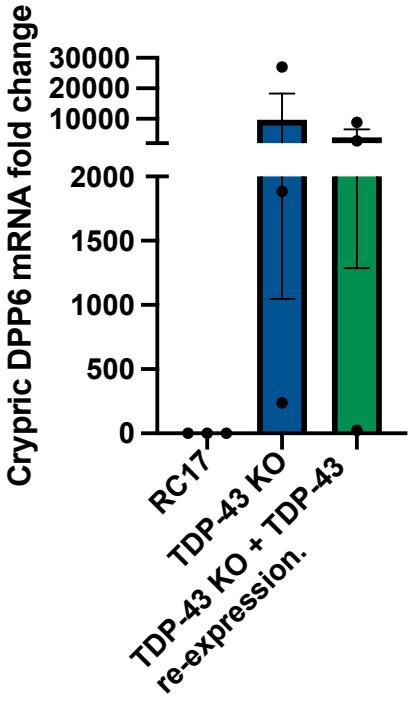

Figure 2: Cryptic splice sites increase significantly in TDP-43 KO MNs with a slight decrease after TDP-43 re-expression.

Furthermore, electrophysiological recordings confirmed the functionality of 3-week-old motor neurons (MNs) in culture. Whole-cell patch-clamp experiments showed that MNs reliably fired sustained action potentials in response to step-current pulses (-10 to +65 pA, 500 ms), indicating proper neuronal excitability (figure 3).

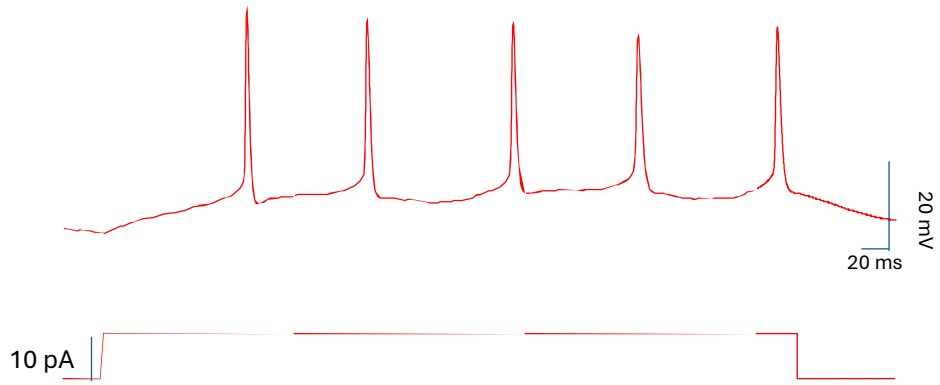

Figure 3: Representative whole-cell current-clamp trace from a 3-week-old motor neuron, displaying sustained action potential firing upon current injection

Benefits and future insights:

Joining the Selvaraj lab provided invaluable training and mentorship from my supervisor and other lab members. Their insights and expertise in molecular neuroscience enhanced my learning experience.

I received training in RNA extraction, qPCR, Western blotting, and patch-clamp techniques. These skills were fundamental for my ongoing project and have prepared me for more complex experimental designs in the future.

This project has made me aware of the importance of advanced electrophysiological techniques. I would like to further develop my skills in patch-clamp methods to better analyse neuronal activity and its relationship with gene expression changes in future studies.

I'm happy to say that I achieved both my personal and research goals during this project. The findings have opened new pathways for further exploration, and I feel more confident in my technical abilities than ever before. Beyond the scientific aspects, this project has increased my interest in neuroscience and neurodegenerative diseases. Collaborating with others in the lab has also broadened my network, offering me connections within the field.

References:

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