Poster #1446

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Development of *in vitro* neuronal cytotoxicity models for neurodegenerative disease gene therapy R&D

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TDP-43 and neurodegenerative disease

Globally, neurological disorders such as Alzheimer's Disease, Parkinson's Disease, Amyotrophic Lateral Sclerosis (ALS), and Frontal Temporal Dementia (FTD) are the second leading cause of death, with millions of new diagnoses each year. ALS alone affects 1 in 50,000 people per year worldwide, calling for increased demand in efficient therapies. Cytoplasmic mislocalization and subsequent accumulation and aggregation of transactive response DNA-binding protein 43 kDa (TDP-43) is a hallmark of ALS and, in most cases, represents a reliable post-mortem diagnostic marker (see Figure 1A). Multiple studies have demonstrated that overexpression of TDP-43 in animal models results in neurodegeneration and can be used as a disease model for ALS and other neurological disorders involving TDP-43 (see Figure 1B). While valuable, *in vivo* TDP-43 disease models are hindered by their low throughput capabilities. Here, we propose two cellular models of TDP-43 induced cytotoxicity mediated by adeno-associated virus (AAV) transduction.

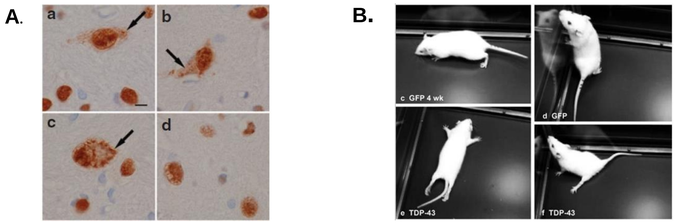

Figure 1. TDP-43 overexpression *in vivo* as a disease model for ALS. (A) Rat neurons overexpressing TDP-43 through AAV9 transduction show cytoplasmic-localized TDP-43 that appears both diffuse (a-c) and granular (d) by staining. Rat neurons treated with the control vector show only nuclear TDP-43 staining. Figure from Tatom et al., *Mol Ther.,* 2009. **(B)** Rats received an intravenous injection of AAV9-TDP-43 or AAV9-GFP and motor function was monitored over time. Four weeks after injection, TDP-43 injected rats showed significant deficits in rearing abilities and motor function (e, f) while the GFP-injected rats exhibited normal motor function and rearing capabilities (c, d). Figure from Wang et al., *Mol Ther.,* 2010.

Efficient transduction of primary cortical mouse neurons by AAV9

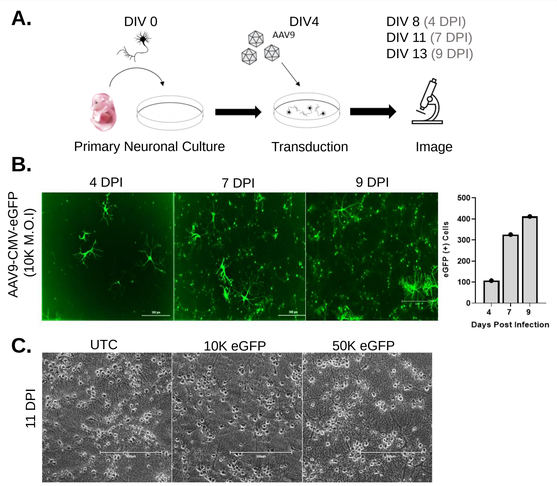

Figure 2. Complete transduction of primary neurons with AAV9 without toxicity. (A) On day 4 *in vitro*, primary mouse cortical neurons were transduced with AAV9-CMV-eGFP at 10,000 or 50,000 multiplicity of infection (MOI). **(B)** Quantification of GFP-positive neurons at 4, 7, and 9 days post infection (DPI) at 10,000 MOI. **(C)** After 11 DPI, no neuronal toxicity was observed in bright field images at either AAV9-CMV-eGFP dose, 10,000 or 50,000 MOI.

TDP-43 overexpression causes cytotoxicity in primary cortical mouse neurons

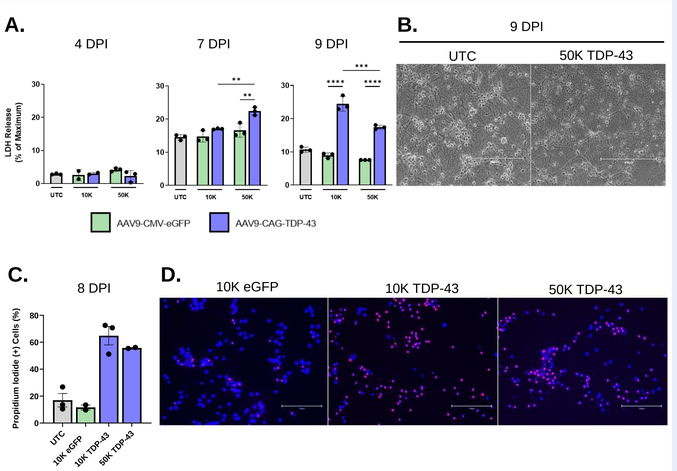

Figure 3. AAV9-mediated TDP-43 overexpression causes primary neuronal cell death. (A) At DIV 4, primary mouse cortical neurons were transduced with either AAV9-CMV-eGFP or AAV9-CAG-TDP-43 at 10,000 or 50,000 MOI. LDH activity was measured at 4, 7, and 9 DPI and normalized to the maximum cell death condition. (**B**) Brightfield images show morphological changes in neurons after 9 days of TDP-43 overexpression. (**C, D**) Dead cells were directly quantified using propidium iodide staining at 8 DPI. TDP-43 overexpression in primary cortical neurons leads to ~3-fold increase in dead cells compared to either control.

Development of a human cytotoxicity model

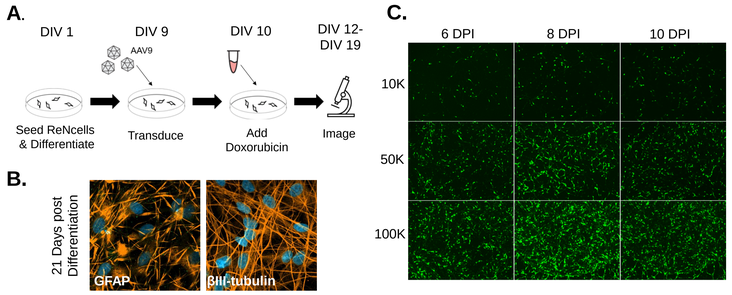

Figure 4. Using ReNcells, an immortalized human neural progenitor cell line, as a human cellular model for TDP-43 overexpression. (A) ReNcells were seeded, differentiated, then transduced with AAV9-CMV-eGFP at a dose of 10,000, 50,000, or 100,000 MOI. On 1 DPI, cells were treated with Doxorubicin (40 nM) to increase transduction efficiency. **(B)** ReNcells were differentiated using an established neuronal differentiation protocol (Boyer et al., 2012) and characterized by immunocytochemistry and transcriptomic analysis (data not shown). After 21 days of differentiation, cells express astrocytic markers such as GFAP and neuronal markers such as βIII-tubulin. **(C)** AAV9-CMV-eGFP transduces differentiated ReNcells at all three doses with complete transduction occurring at a minimum of 50K MOI.

TDP-43 overexpression causes cytotoxicity in ReNcells

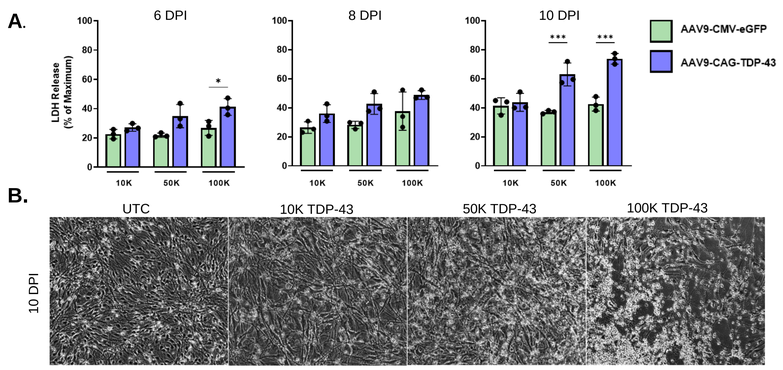

Figure 5. AAV9-mediated TDP-43 overexpression causes toxicity in differentiated ReNcell culture. (A) At DIV 9, ReNcells were transduced with either AAV9-CMV-eGFP or AAV9-CAG-TDP-43 at 10,000, 50,000, or 100,000 MOI. LDH activity was measured at 6, 8, and 10 DPI and normalized to the maximum cell death condition. **(B)** After 10 days of TDP-43 overexpression in ReNcells, brightfield images show dose-dependent changes in morphology.

Conclusions

- AAV9 leads to complete transduction of primary mouse cortical neurons without causing toxicity at 50,000 MOI.
- AAV9-mediated TDP-43 overexpression in primary mouse cortical neurons leads to reproducible dose-dependent cytotoxicity.
- Differentiated ReNcells express various neural markers such as GFAP and βIII-tubulin.
- AAV9-mediated overexpression of TDP-43 in differentiated ReNcells results in a dose dependent cytotoxic response.
- Together, our data demonstrate that our *in vitro* models are useful assays to screen neuroprotective candidates and develop novel gene therapies.

References

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2. Wang, D.B. et al. Expansive Gene Transfer in the Rat CAN Rapidly Produces Amyotrophic Lateral Sclerosis Relevant Sequelae When TDP-43 is Overexpressed. *Mol Ther.* **18(12)**, 2064-2074 (2010).
3. Boyer, L.F. et al. Dopaminergic Differentiation of Human Pluripotent Cells. *Curr Protoc Stem Cell Biol.* Chapter 1 (2012).