Production and optimization of brain-targeted viral tools for target validation in Parkinson's disease models

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MOLECULAR SYSTEMS BIOLOGY

Background

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the loss of dopamine-producing neurons in substantia nigra pars compacta (SNc) with several environmental and genetic factors interplay. PD manifests with motor and non-motor dysfunctions.

Among several genetic risk factors, mutation in the Leucine-rich repeat kinase 2 (LRRK2) gene is the most common mutation causing PD. This mutation increases LRRK2 kinase activity.

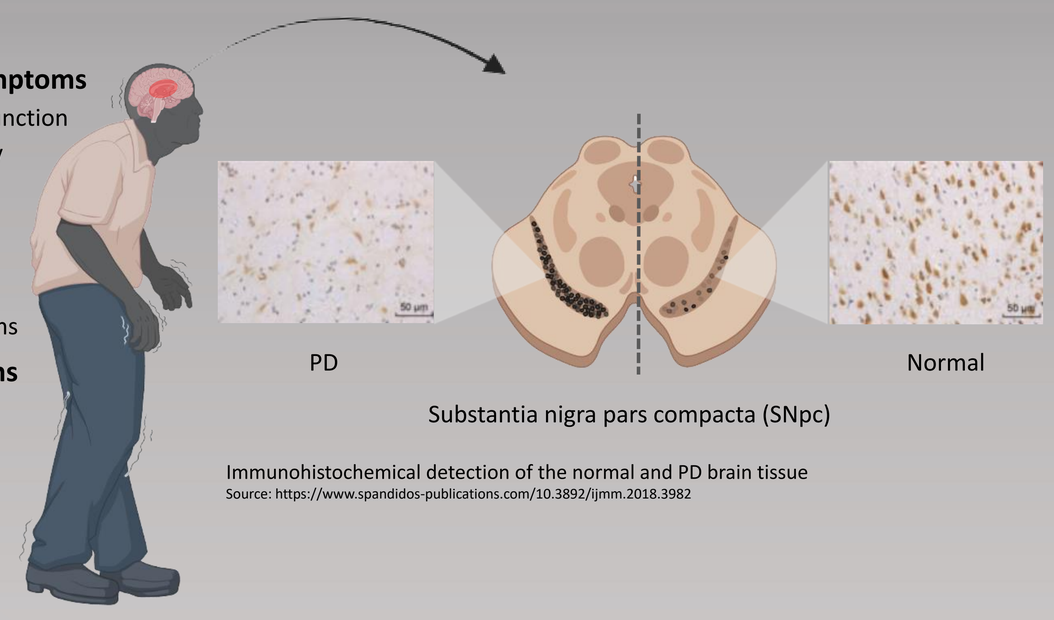
Our research group identified disturbance in phosphorylation of three proteins downstream of the LRRK2 kinase activity in rat models of PD. Those proteins play important roles in synaptic vesicle trafficking, the disruption of which may contribute to PD development. In this study, we mimic the phosphorylation changes using phospho-mutant transgenes in cultured neuronal cells to evaluate their involvement in cellular distruption.

Non- Motor Symptoms

- Autonomic dysfunction
- Pain and Sensory symptoms
- Constipation
- Anxiety and depression
- Sleeping problems

Motor Symptoms

- Bradykinesia
- Rigidity
- Tremor



Immunohistochemical detection of the normal and PD brain tissue
Source: https://www.spandidos-publications.com/10.3892/ijmm.2018.3982

Prospect

By investigating the impacts of phospho-variant target proteins on the function of vesicular trafficking in dopaminergic neurons, we may validate a novel drug target in the follow-up studies and improve our understanding of early mechanisms that occurs in PD

Aims

1. Generate viral vector tools to transfer the transgenes into primary cultured neurons
2. To functionally assess the involvement of target proteins in degeneration of dopaminergic neurons

Materials and Methods

DNA construction (Previous work)

To mimic the phosphorylation changes, site-directed mutagenesis PCR on **Serine** residue(s) of target proteins was performed previously in our lab.

Ser to Ala → phospho-deficient

Ser to Asp→ phosphomimetic

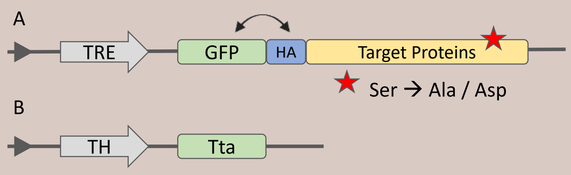


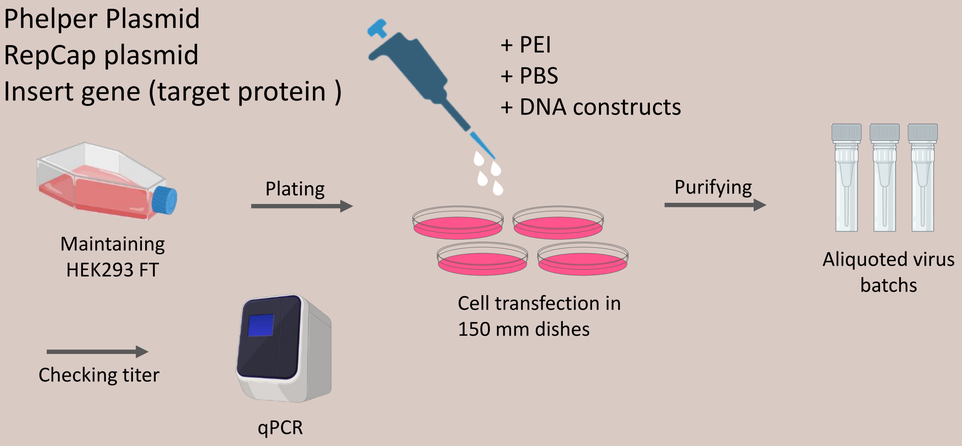
Fig.1. DNA constructs required for AAV generation. **A.** All DNA constructs were subcloned from the pEGFP-c3 vector into the pAAV-TRE-mTurquoise2 plasmid. According to AAV packaging capacity (~4.5 kb), either GFP or HA tag was used for labeling (Previous work). **B.** DNA required for tetracycline-inducible expression.

Table 1. number of different phospho-mutated genes used for viral vector preparation

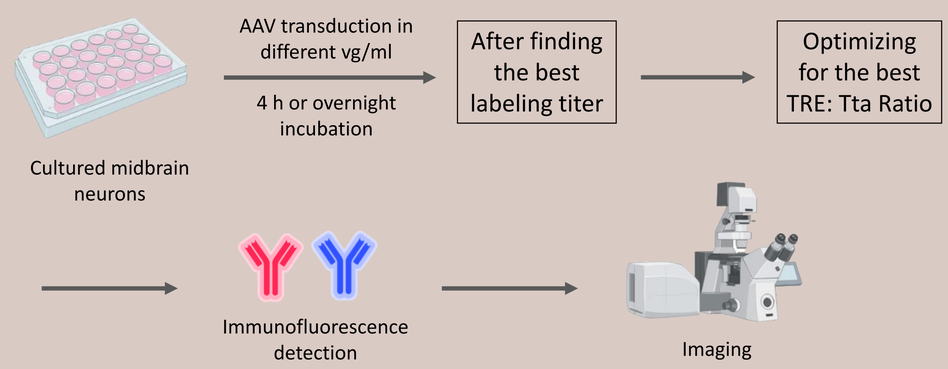
Target One	Target Two	Target Three
2 Phospho-varient	2 Phospho-varient	6 Phospho-varient
WT	WT and 1 KD	WT

High titer AAV generation

AAV requires:
1- Phelper Plasmid
2- RepCap plasmid
3- Insert gene (target protein)



Functional titer, and AAV transduction to cultured neurons



Results

AAV generation

The AAV generating protocol was developed by the Gradinaru lab (source:https://www.nature.com/articles/s41596-018-0097-3). This protocol is optimized for high titer AAVs and typically yields ≥1 × 10^{13} vector genomes (VG) per ml with high transduction efficiency in vivo. The titer for generated constructs was measured by quantitative PCR. The resulted range of titration was - between 0.46 × 10^{12} to 6.6 × 10^{13} vg/ml.

AAV transduction optimization

The transduction efficiency determines the average number of AAV integrations into the target cell. The functional titers were optimized in different dilutions and incubation times to have the best integration of AAVs. Optimization was performed in WT samples. A 2.5x10^{11} vg/ml titer and overnight incubation demonstrated optimal labeling efficiency. Tyrosine hydroxylase immunostaining indicates the dopaminergic neurons.

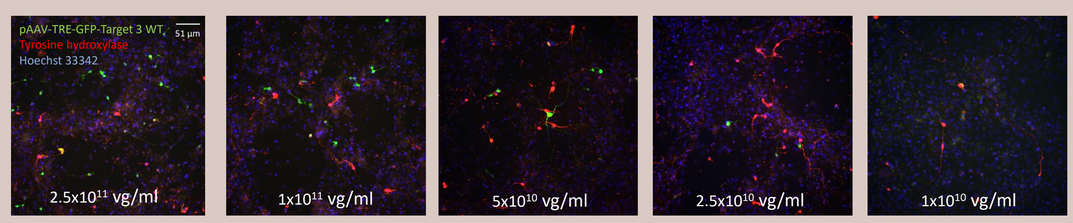


Fig.2. Optimizing the labeling titer.

In addition, the optimization of the TRE: Tta ratio was required to obtain better expression efficiency.

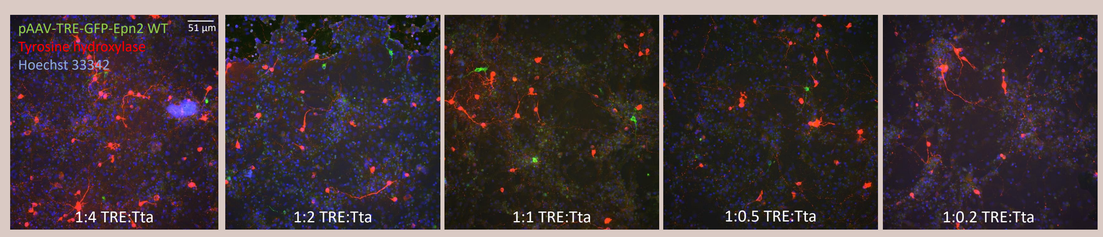


Fig.3. Optimizing the TRE: Tta ratio.

In the last step, all three WT construct transduced to cultured cells according to optimized results. HA stating shows reliable expression of the other two constructs.

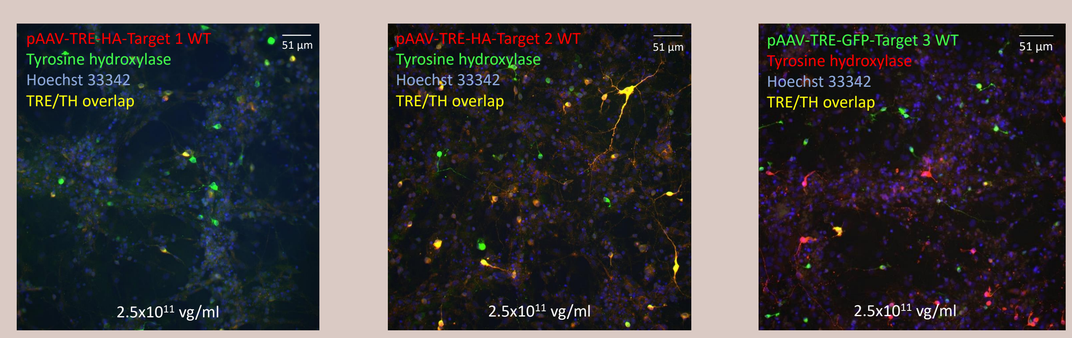


Fig.4. Immunofluorescence detection of transgene expression. Tyrosine hydroxylase staining shows the presence of dopaminergic neurons. Target 1 and 2 stained for HA tag.

Conclusion

Our results show reliable expression of phosphovarients in cultured midbrain neurons. Ongoing experiments will evaluate the effects of phosphorylation disturbance on synaptic vesicle endocytosis in neuronal cells using the pesticide (rotenone) model of PD.