Validating genetic tools and molecules preventing pathological protein aggregation in mouse neurons in culture and in vivo

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Introduction

Parkinson's disease (PD) is an age-related and progressive neurodegenerative disorder. Accumulation of misfolded and aggregated alpha-synuclein (αSyn) into intraneuronal inclusions known as Lewy bodies is considered as a major pathological hallmark of PD. Currently, there is no cure for PD and the mechanisms regulating αSyn aggregation remain poorly understood.[1]

αSyn preformed fibrils (PFFs) have been widely utilized to model PD. PFFs can trigger intracellular misfolding and aggregation of endogenous αSyn and thus, they have considerably increased the understanding of αSyn aggregation.[2] In this study, Accell siRNAs and an antibody targeting αSyn, BIIB054 antibody, were tested and validated in mouse neurons and brain sections as robust research tools for studying αSyn aggregation.

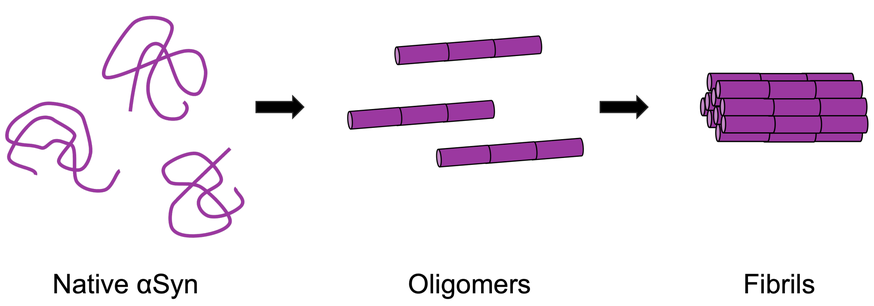

Figure 1. Natively unfolded αSyn monomers can misfold and aggregate into oligomers and fibrils which accumulate within Lewy bodies.[1,2]

Materials and methods

Accell small interfering RNAs (siRNA) were added to primary mouse cortical neurons to induce knockdown of two housekeeping genes (cyclophilin B and GAPDH), without viral delivery or transfection reagent, and knockdown efficiency was assessed with quantitative PCR (qPCR) after 72-hour and 10-day treatment.

BIIB054 antibody was studied in western blot analyses and immunofluorescent staining of mouse primary cortical cultures to evaluate specificity of the antibody for monomer and aggregated αSyn. Immunohistochemical staining of mouse brain sections was also performed to assess the specificity of BIIB054 on *in vivo* experiment samples.

Results

Accell siRNAs induced efficient knockdown of two selected housekeeping genes after 72-hour and 10-day treatments in primary neurons.

BIIB054 antibody selectively detected aggregated forms of αSyn, by immunohistochemical staining, in brain sections of mice injected with PFFs.

Efficient knockdown by Accell siRNAs in primary cortical neurons after 72-hour and 10-day treatment in dose-dependent manner

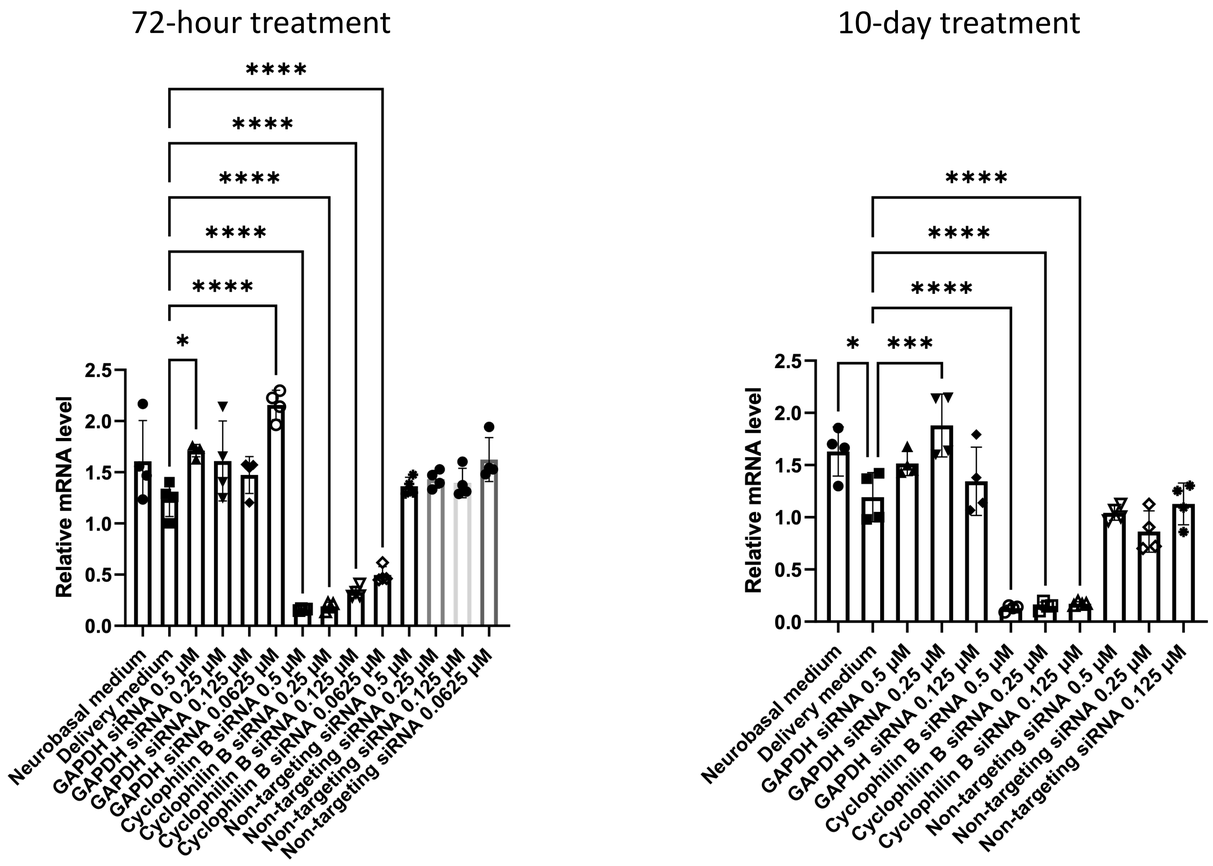

Figure 2. Relative cyclophilin B mRNA levels after 72-hour and 10-day Accell siRNA treatments. ****p<0.0001, ***p<0.001, *p<0.05 (one-way ANOVA followed by Šídák's multiple comparisons test), n=4 biological replicates. Data are represented as mean ± SD.

BIIB054 detects αSyn aggregates in cellular extracts

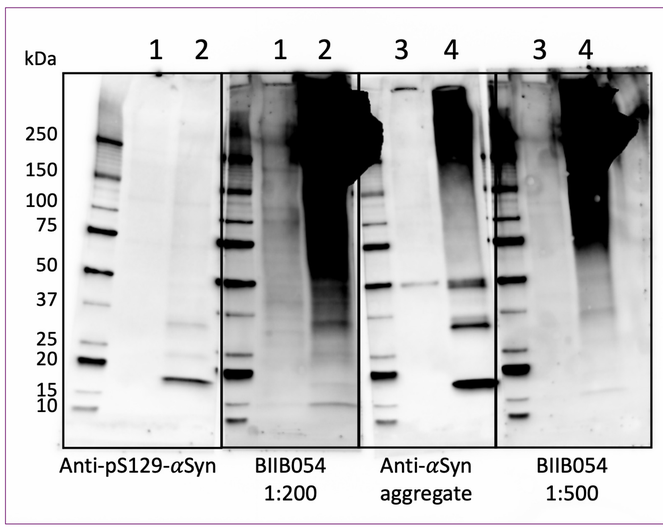

Figure 3. Western blot analysis for aggregated αSyn. Samples 1 and 3 were lysates from monomer-treated αSyn overexpressing primary cortical cultures. Samples 2 and 4 were lysates from PFF-treated αSyn overexpressing primary cortical cultures. BIIB054 was tested at two different dilutions (1:200 and 1:500).

BIIB054 specifically detects αSyn aggregates in mouse brain sections

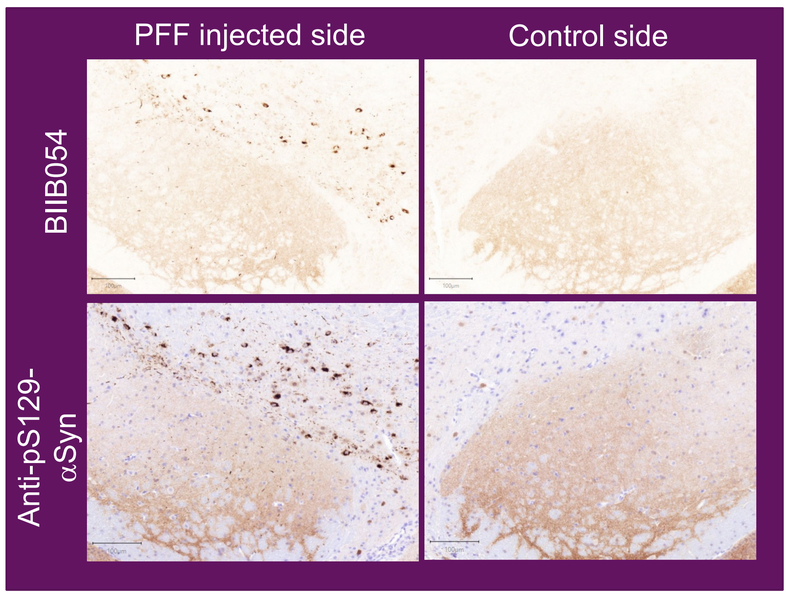

Figure 4. Immunohistochemical staining with BIIB054 and anti-pS129-αSyn antibodies. Brain sections from mice injected with 5 µg of PFFs were stained with BIIB054 to assess its ability to detect aggregated αSyn *in vivo*. Images of both injected side and control side of the brain are shown. Scale bar: 100 µm.

Conclusions

- Accell siRNAs are a valuable tool for efficient long-term knockdown of target genes in primary neurons.
- BIIB054 antibody detects aggregated αSyn in mouse brain sections and thus could be used to assess αSyn aggregation in preclinical PD models.
- This study validated research tools that will benefit future preclinical studies and aid in the identification of potential disease-modifying treatments in PD.

References

1. Fares, M. B., Jagannath, S. & Lashuel, H. A. (2021) Reverse engineering Lewy bodies: how far have we come and how far can we go? *Nat Rev Neurosci* **22**: 111-131.
2. Chmielarz, P. & Domanskyi, A. (2021) Alpha-synuclein preformed fibrils: a tool to understand Parkinson's disease and develop disease modifying therapy. *Neural Regen Res* **16**: 2219-2221.