Effects of lentiviral shRNA-mediated targeting of selected genes on alpha-synuclein aggregation in cortical neurons

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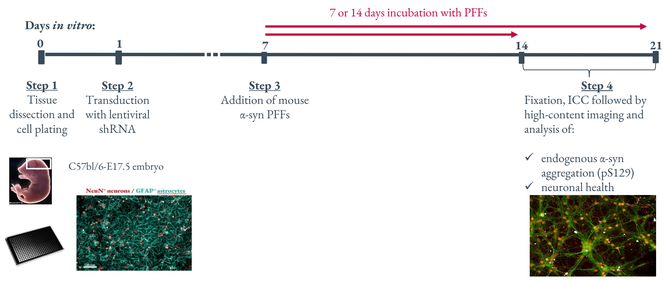
INTRODUCTION

Insoluble aggregates consisting of the misfolded protein alpha-synuclein (αSyn) progressively accumulate in the nervous system of most Parkinson's disease (PD) patients. In the current study, the aim was to identify targets involved in modulation of αSyn aggregation using an established in vitro model. Based on literature evidence, 20 targets were chosen for their potential involvement in αSyn aggregation pathways, such as regulation of αSyn expression, post-translational modifications, or autophagy/lysosomal degradation.

METHODS

Developed assay for assessment of modulation of α-syn aggregation



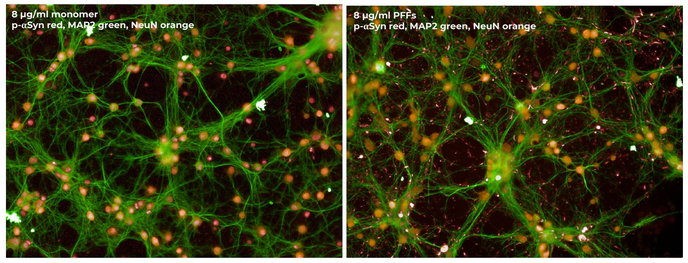
As previously described (Lardell et al., 2022), the assay is based on embryonic mouse cortical cultures cultured in the 384-well format.

Cells were transduced with lentiviral shRNA, aimed for down-regulation of target, at 1 day in vitro (DIV) and endogenous phosphorylated αSyn (p-αSyn) aggregation was induced at 7 DIV using mouse αSyn pre-formed fibrils (PFFs, StressMarq Biosciences).

At 14 or 21 DIV, the cultures were stained with an antibody binding to pS129 for assessment of phosphorylated endogenous p-αSyn aggregation as well as MAP2, NeuN and Hoechst for assessment of cell health. High content analysis (HCA) was performed using an Operetta automated high content imager (PerkinElmer).

The model has been validated using lentiviral shRNAs for knockdown of genes known for their involvement in PD where both genes causing an increase as well as a decrease in the p-αSyn aggregation were identified.

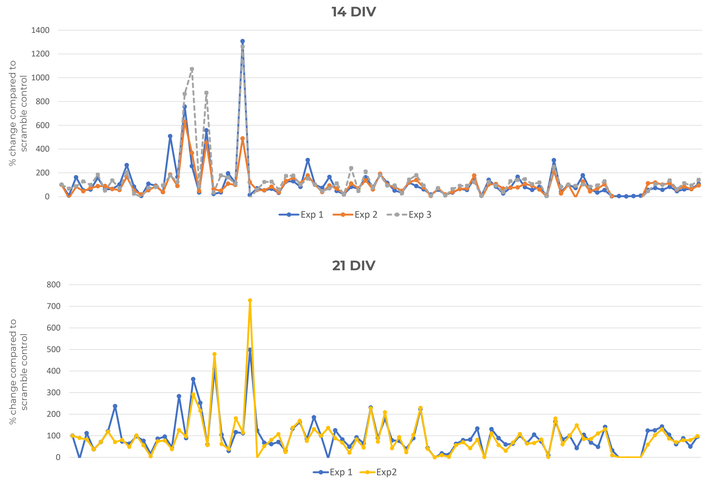
p-αSyn aggregate evaluation



In the established model, in cultures treated with αSyn PFFs, p-αSyn aggregates were identified, whereas in cultures treated with monomers at corresponding concentrations, p-αSyn aggregates were rarely found. However, p-αSyn immunostaining was observed in cell nuclei which is a well-known phenomenon. The neuronal network (visualized by MAP2) and number of NeuN positive nuclei appeared similar between cultures treated with αSyn PFFs and monomers (Lardell et al., 2022).

RESULTS

Excellent correlation between independent experiments



Knockdown of twenty targets was carried out using lentiviral shRNA (four oligos per target) in 2-3 independent experiments. Even though the level of αSyn aggregation varied between experiments, the correlation between the experiments was very high. Graphs represents αSyn aggregation in percent change compared to PFF + scrambled control (100%). Each point represents one oligo of shRNA.

Hit selection at 14 DIV

	p-αSyn				NeuN			
	Oligo A % change	Oligo B % change	Oligo C % change	Oligo D % change	Oligo A % change	Oligo B % change	Oligo C % change	Oligo D % change
Trib3	53	72	158	60	64	91	93	81
Zscan21	64	70	205	50	77	99	90	75
Ppp1r15a	6	73	87	38	71	86	93	53
Vps35	185	124	757	367	63	103	66	40
Atp13a2	46	557	34	48	81	84	57	78
Fyn	161	107	1262	14	114	109	118	1
Tmem163	59	53	84	37	88	100	83	62
Tpcn2	131	159	99	177	95	98	97	104
Rhot1	68	21	110	67	86	95	82	116
Aimp2	163	74	186	97	78	81	89	100
Rhot2	82	34	121	140	87	97	95	77
Usp8	77	8	70	17	76	10	69	25
USP14	39	65	69	152	81	94	97	96
Trim11	6	123	92	37	15	120	103	36
Dclk1	75	140	107	92	80	102	104	93
Ttbk1	81	4	250	41	72	76	98	73
Bach1	61	68	104	3	81	56	73	72
Hspa8	-15	-15	-14	-14	7	12	15	5
Tbk1	56	106	99	112	79	80	83	88
Usp30	59	85	64	107	96	87	47	95

Hit selection at 21 DIV

	p-αSyn				NeuN			
	Oligo A % change	Oligo B % change	Oligo C % change	Oligo D % change	Oligo A % change	Oligo B % change	Oligo C % change	Oligo D % change
Trib3	37	71	120	154	52	94	87	76
Zscan21	77	56	100	66	78	102	97	74
Ppp1r15a	11	80	87	42	63	96	92	58
Vps35	205	93	327	234	54	96	52	26
Atp13a2	58	444	83	34	82	73	71	61
Fyn	149	113	613	62	112	112	131	58
Tmem163	60	71	89	27	104	107	92	59
Tpcn2	135	166	81	158	107	101	109	126
Rhot1	75	35	88	55	86	82	70	92
Aimp2	229	81	194	60	74	85	82	100
Rhot2	85	32	96	226	90	99	98	84
Usp8	41	0	14	7	53	8	31	18
USP14	59	75	62	107	79	97	114	98
Trim11	-1	121	73	45	13	107	104	37
Dclk1	65	105	65	86	69	87	110	102
Ttbk1	78	6	173	71	56	75	105	60
Bach1	77	80	135	21	70	63	85	88
Hspa8	-2	0	-1	-2	14	20	10	9
Tbk1	91	114	136	96	72	99	92	100
Usp30	65	84	65	97	101	100	70	106

PFF + scrambled = 100 %, Green = increase in aggregation ≥ 200%, red = decrease in aggregation ≤ 50% . For neuronal health, red = decrease of number of NeuN-positive cells with more than 40%. N=3 experiments for 14 DIV and n=2 experiments for 21 DIV.

Endogenous p-αSyn and cell health (number of NeuN-positive cells) after lentiviral shRNA treatment were evaluated at 14 and 21 DIV in two or three independent experiments. Tables show percent change in aggregation after lentiviral shRNA treatment in a median of the two or three experiments.

Lentiviral shRNA-mediated downregulation of some targets caused effects on neuronal survival. However, one week after PFF addition, targeting Zscan21, Vps35 and Fyn resulted in an increase, whereas targeting Ppp1r15a, USP14, Rhot1, Rhot2 and Bach1 resulted in a decrease in p-αSyn aggregation without effects on neuronal health. The results correlate well with literature, with anticipated effects for e.g. Vps35, Ppp1r15a and Bach1.

Two weeks after PFF addition, targeting Atp13a2, Fyn and Aimp2 resulted in an increase, and targeting Ppp1r15a, Rhot1, Rhot2, Ttbk1 and Bach1 resulted in a decrease in p-αSyn aggregation without effects on neuronal survival. For some of the targets, the effect on p-αSyn aggregation was thus time-dependent.

CONCLUSIONS

- Our in vitro model is useful for identification of potential targets involved in modulation of αSyn aggregation and for understanding the αSyn aggregation process over time.
- Next step is to confirm that the results seen are due to downregulation of the target of interest by gene and/or protein expression analyses.
- We provide a list of potential targets for modulation of αSyn aggregation, suitable for further evaluation in αSyn aggregation models in human neurons and in vivo for the identification and development of disease-modifying therapies for PD.
- The developed model, besides being relevant for target validation, is also due to the high degree of reproducibility and the capacity, amenable for target discovery applications.

REFERENCES

Lardell et al., 2022: Development of an in vitro α-synuclein aggregation assay in primary cortical cultures for target discovery and validation. SfN meeting 2022.