Non-Invasive, Targeted Overexpression of AAV2.SIRT3-myc using MR-guided Focused Ultrasound: A Disease-Modification Strategy in Parkinson's Disease

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Introduction

Upon diagnosis of Parkinson's disease (PD), individuals are relatively mobile, and able to lead independent lives. In the advanced stages, symptoms of PD become disabling, and often palliative care is required. If a therapeutic was available that could slow, halt or reverse the progression of PD, the prognosis of individuals with PD would be significantly improved. **The aim of the current study was to develop a disease-modifying therapy to slow the progression of PD pathology, that can be safely administered to individuals with PD.**

Post-mortem and genetic studies have shown that mitochondrial dysfunction is central to PD pathology[1]. Mitochondrial abnormalities, such as increased oxidative stress, mutations in mtDNA, decreased function of the electron transport chain, and reduced ATP output have all been linked with PD pathology. Sirtuin 3 (SIRT3), is the major protein deacetylase in the mitochondria, linked with cytoprotective and longevity-enhancing effects, with over 700 substrates[2] (**Fig. 9**). In non-neuronal cells, such as liver, muscle and heart, SIRT3 has been shown to reduce oxidative stress, increase ATP, and generally maintain mitochondrial health[3].

Hypothesis

1. **In a rat model of PD, viral-mediated overexpression of SIRT3 will prevent motor dysfunction by preventing PD-like pathology such as dopaminergic neuronal loss. This occurs through the stabilisation of mitochondrial bioenergetics.**
2. **MR-guided focused ultrasound (MR-g-FUS) induced blood brain barrier opening (BBBO) can be used to non-invasively and selectively target AAV2.SIRT3 to brain regions affected in PD.**

Materials and Methods

Mutant (A53T) α-synuclein rat model of PD: In rats, adeno-associated virus (AAV1/2) expressing A53T α-synuclein or control (EV) was intra-nigrally infused ipsilaterally to induce a hemi-parkinsonian PD model. Following AAV-α-synuclein infusion, this model shows motor impairment after 3 weeks, and dopaminergic neuron loss after 6 weeks.

Stereotaxic Infusion of AAV.SIRT3: Rats received intra-nigral or intra-striatal infusions of AAV.SIRT3-myc (serotypes: AAV1 & AAV2 for substantia nigra pars compacta (SNc) and striatum respectively), volume: 2 µL; [AAV1 = 2.59 x 10^{11} GC/mL, AAV2 = 9.86 x 10^{12} GC/mL] using stereotaxic surgery.

Behavioural Assessment: Three and 6 weeks following AAV-α-synuclein, forelimb asymmetry was assessed using the cylinder test. Following 20 forelimb placements, bias in forelimb use was calculated as % asymmetry (positive indicates parkinsonism).

Dopaminergic neuron loss: 6 weeks following AAV-α-synuclein, brains were removed and immunohistochemistry (IH) performed in the SNc, using antibodies against tyrosine hydroxylase (TH) and NeuN, followed by quantitative stereology.

rAAV2 MR-g-FUS Delivery: MR-imaging was performed to identify and select the target regions in the striatum (2) and SNc (1). Activated microbubbles were incubated with 4C10 antibody linker and Artenga activator, then AAV2.SIRT3-myc incubated with microbubbles to allow conjugation to cross-linked microbubbles. The AAV-conjugate (300-400 µL) was administered via a tail-vein catheter following 0.5 mL of saline. FUS was performed at a fixed pressure of 0.4 MPa, pulse length 10 ms, frequency 1 Hz, for a total duration of 120 seconds (RK100, FUS Instruments Inc.). BBBO was confirmed using MR-imaging with gadolinium contrast agent[4].

Immunological Analysis: Two weeks following MR-g-FUS-mediated delivery of AAV2.SIRT3-myc, IH was performed using antibodies against myc to label ectopic SIRT3-myc, SIRT3 to label ectopic and endogenous SIRT3, and DAPI to label the nucleus.

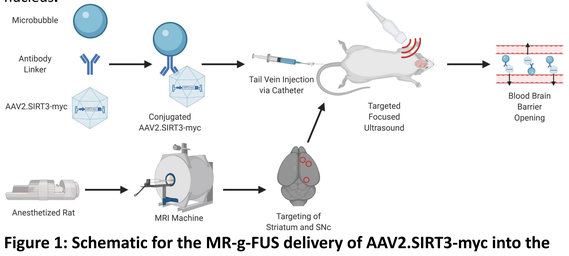


Figure 1: Schematic for the MR-g-FUS delivery of AAV2.SIRT3-myc into the rat brain. Created with BioRender.com

Results

In a rat model of PD, AAV.SIRT3 reverses motor dysfunction

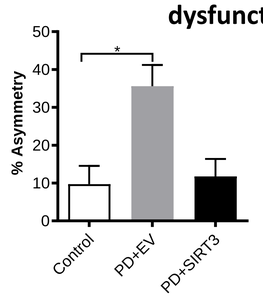


Figure 2: In the virally-mediated mutant α-synuclein overexpressing rat model of PD, SIRT3 overexpression in the SNc reversed motor asymmetry. Data are expressed as mean % asymmetry in number of forelimb placements ± SEM. Abbreviations: Control = Empty Vector + Empty Vector, PD = A53T α-synuclein. ANOVA with Tukey *post-hoc*. n = 10-12 per group. * p<0.05

In a rat model of PD, AAV.SIRT3 prevents loss of dopaminergic neurons

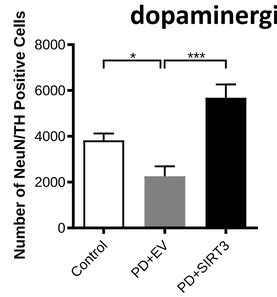
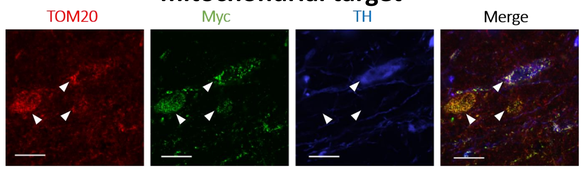


Figure 3: In the virally-mediated mutant synuclein overexpressing rat model of PD, nigral SIRT3 overexpression prevented dopamine (DA) cell loss. Data are expressed as mean number of TH-positive cells ± SEM. (Abbreviations: Control = Empty Vector + Empty Vector, PD = A53T α-synuclein). ANOVA with Tukey *post-hoc*. n = 10-12 per group. * p<0.05, *** p<0.001

Ectopically expressed SIRT3 engages the mitochondrial target



Scale bar = 20 µm

Figure 4: Following infusion of AAV.SIRT3-myc into the SNc, SIRT3 overexpression is observed in the mitochondria of neurons, shown using immunofluorescence labelling. This shows that ectopically expressed SIRT3 can translocate from the nucleus to the mitochondria (white arrows). Antibodies: TOM20 (mitochondria), myc (ectopic SIRT3-myc), and TH (dopaminergic neurons).

Ectopically expressed SIRT3 deacetylates mitochondrial proteins

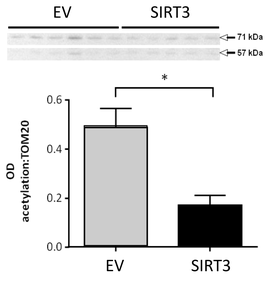


Figure 5: Following infusion of AAV.SIRT3-myc into the SNc, brains were removed, and the mitochondrial fraction isolated. SIRT3-myc overexpression resulted in deacetylation of mitochondrial proteins as shown by SDS-PAGE and Weston blot followed by quantification of optical density (OD) . Data are expressed as mean OD ratio acetylation: TOM20 ± SEM. (Abbreviations: EV = Empty Vector).. Mann-Whitney U test. n = 4-5 per group. * p<0.05

SIRT3 overexpression decreases oxygen consumption rate in the mitochondria

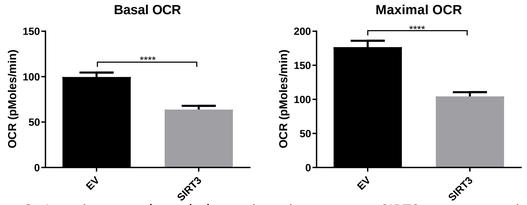


Figure 6: In primary cultured dopaminergic neurons, SIRT3 overexpression decreases oxygen consumption rate (OCR) as measured using Seahorse XFe24. Data are expressed as mean OCR ± SEM. . (Abbreviations: EV = Empty Vector). Student two-tailed t-test. n = 10-30 wells per group. **** p<0.0001

MR-g-FUS temporarily opens the BBB to target brain regions affected in PD

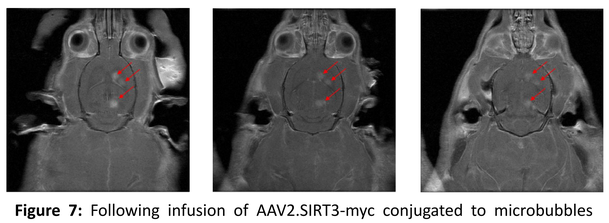


Figure 7: Following infusion of AAV2.SIRT3-myc conjugated to microbubbles then MR-g-FUS, MR imaging with gadolinium contrast agent shows the FUS targeted areas as bright spots (red arrows), indicating the BBB was opened and contrast agent was able to concentrate in the SNc and striatum.

MR-g-FUS induced BBBO successfully delivers AAV.SIRT3 to brain regions affected in PD

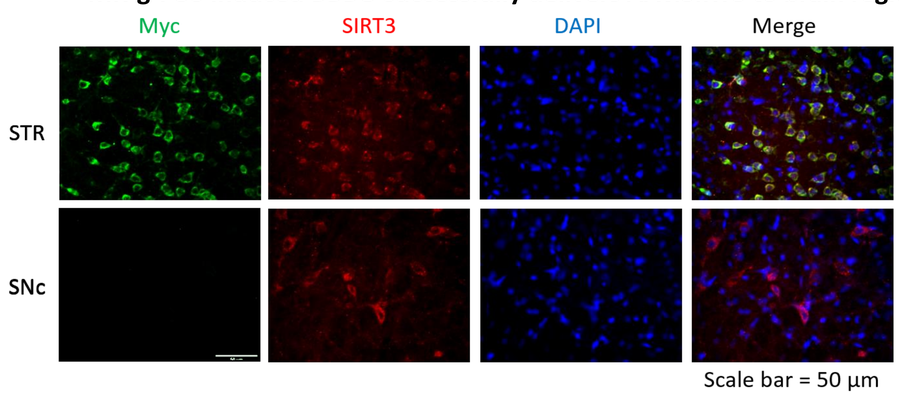


Figure 8: Following MR-g-FUS induced BBBO, SIRT3-myc is expressed in the rat striatum. Following tail vein injections of AAV2.SIRT3-myc plus microbubbles, MR-g-FUS was able to deliver AAV2.SIRT3-myc to the ipsilateral striatum (STR) but not the substantia nigra pars compacta (SNc). No SIRT3-myc expression was observed in the contralateral hemisphere. Antibodies: Myc (SIRT3-myc), SIRT3 (endogenous and ectopic SIRT3), and DAPI (nucleus). n = 5

Discussion

- In the AAV mutant α-synuclein rat model of PD, intra-nigral overexpression of SIRT3-myc using AAV has disease-modifying effects. Over-expression of SIRT3 reversed motor deficits, and prevented dopamine cell loss, showing proof of concept that SIRT3 may be a useful therapeutic in the clinic[5] (**Fig. 2, 3**).
- Transduction of SIRT3-myc using AAV results in mitochondrial targeting of SIRT3 and deacetylation of mitochondrial proteins, demonstrating target engagement *in vivo* (**Fig. 4, 5**).
- Ectopic SIRT3-myc improves mitochondrial health by decreasing the mitochondrial respiration rate of dopaminergic neurons, which decreases oxidative stress. These may underlie the disease modifying effects of SIRT3-myc (**Fig. 6**).
- MR-g-FUS induced BBBO to deliver AAVs is safe in humans, combined with the lower tropism and liver toxicity that can be achieved with AAV2 compared to other AAVs[6,7].
- Using MR-g-FUS induced BBBO, we have demonstrated the selective, non-invasive targeting of AAV2.SIRT3-myc into brain regions affected in PD (**Fig. 7**).
- Further pre-clinical studies are required to conclusively determine the efficacy of MR-g-FUS induced BBBO to deliver AAV2.SIRT3-myc as a disease-modification strategy for individuals with PD.

Conclusion

MR-g-FUS induced BBBO provides a non-invasive method for the delivery of AAV2.SIRT3-myc, a disease-modifying agent which has neurorestorative properties in parkinsonian rats.

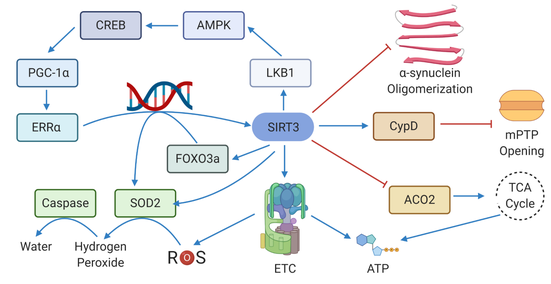


Figure 9: Notable protein targets of SIRT3. Created with BioRender.com

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Acknowledgements

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