α-syn PFF Triggers Stress Responses in Human Astrocytes That Induce Senescence

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1 Introduction

The substantia nigra pars compacta (SNpc) is a structure of the midbrain that is crucial for modulating the initiation of motor movement, among other specific cognitive and emotion-processing functions. SNpc is composed of a group of neurons that fire rhythmically at a rate of 2–10 Hz. This characteristic makes SNpc vulnerable to metabolic stress.(Ni et al, 2022; Lin et al 2021). Moreover, the rhythmic activity of dopaminergic neurons is altered in Parkinson's Disease (PD) patients and the abnormalities seems to be related to α-synuclein (Bove et al, 2019). In normal conditions, astrocytes sustain neuronal function but what happens if they become toxic or unresponsive? Recently, it came to our attention that there is a wide spectrum of phenotypes that astrocytes may acquire depending on the signaling they encounter, (Escartin et al, 2021). However, the most striking observation is that astrocytes become progressively impaired under protein-misfolded pathological conditions (Zimmer et al, 2024). We hypothesize that astrocytes exposed to neurodegeneration neurons that leave behind a-syn aggregates become reactive and Maresin-1 revert this status. Mar-1 synthesis is linked to the changes observed in the phenotype of astrocytes (Fig1). Here we uncover a novel reactivity acquired by astrocytes exposed to a-syn-PFF characterized by the expression of senescence and stress markers along with the induction of the NFkB nuclear translocation.

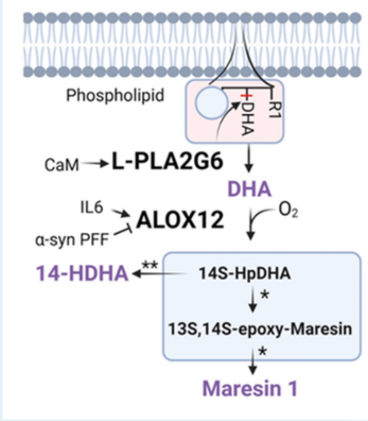

Figure 1: Proposed synthetic pathway of Maresin 1 (Mar1) in astrocytes. DHA is hydrolyzed by L-PLA2G6 from membrane phospholipid. L-PLAG6 activity is modulated by Calmodulin (CaM) (Zhou et al, 2016) DHA is converted to 14S-peroxyde-DHA (14S-HDHA) by ALOX12. ALOX12 activity is increased by IL6 and inhibited by a-syn PFF in astrocytes. 14S-HDHA is converted enzymatically to 13S, 14S-epoxy-Mar and subsequently to Mar1 (Serhan et al, 2015). 14-HDHA is highly unstable; what is not enzymatically transformed, will be converted to 14-HDHA spontaneously. Because Mar works at very low concentrations endogenously, 14-HDHA increase is an indication that the synthesis was activated. Thus, by LC-MS/MS, we measure free DHA (L-PLAG6 activity), 14-HDHA (ALOX12 activity) and Mar1 (purple)

2 Methods

- **Human and rat astrocyte culture:** primary cultures of astrocytes (Cell Applications Inc., San Diego, CA) were culture following media and directions provided by distributors. Briefly, rat and astrocyte cultures were thawed and plated at passage 2 and expanded up to passage 5. The cells were plated and treated as described in each figure.
- **Immunocytochemistry:** Immunostaining took place as follows: cells were fixed using 4% paraformaldehyde solution for 20 min, washed with PBS and permeabilized with 0.1% Triton-X. After blocking with 1% BSA and 10% Donkey normal serum, primary culture was added overnight in humid chambers at 4C. The cells were washed and incubated for one hour with secondary antibody conjugated with Alexa-fluor 555. DAPI (Thermo Fisher cat# D1306) was used for nuclear, and cell mask (Thermo Fisher cat# C10046) for cell membrane staining. Z-stacks were obtained for 5 random fields in a FluoView 3000 laser confocal microscope. Images were converted to and processed using IMARIS 9.9 using the Cell module.
- **Real-Time PCR**: Cells were scrapped using RLT buffer and processed for total RNA extraction using RNAeasy plus kit (Qiagen, Germantown, MD). Total RNA was measured using Nanodrop. First strand synthesis of cDNA was performed using iScript Mastermix cDNA synthesis kit (BioRAD, Hercules CA). Real-Time PCR was performed using Taq-Man pre-design primers from LifeTechnologies (ThermoFisher, Whaltham, MA).
- **Statistical analysis:** q-PCR CT were processed by the deltaCT method.. Plots and statistical treatment were performed using GraphPad 10.0. The data was first analyzed using one-way analysis of variance (ANOVA) and multiple comparisons via Tukey's honest significant differences test. The pairwise comparison was performed using a two-tailed Student's t-test.

3 Results

Figure 2

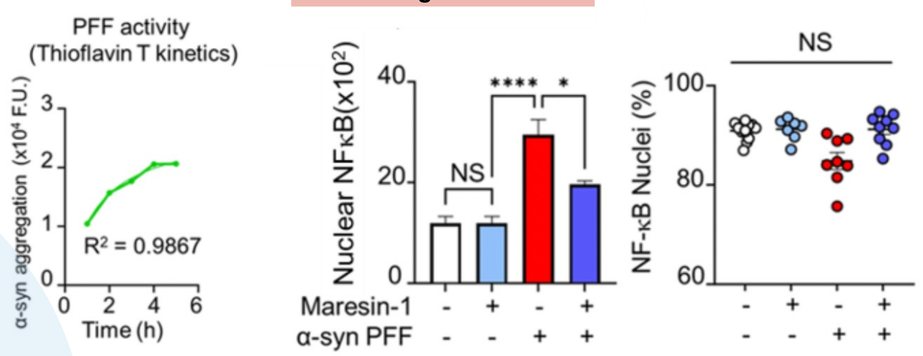

Figure 2: Marl restores p65 status in astrocytes undergoing phenotype changes induced by a-syn PFF in rat astrocytes passage 3 (p3). A) a-syn PFF aggregation activity test using Thioflavin T. (Polinski et al, 2018). Non-linear regression was performed using GraphPad. B-C) Quantification of nuclear NF-KB/p65 (intensity B&E; number of cells C) using Imaris 10.0 software of rat astrocytes treated with 100 ng/ml sonicated a-syn PFF for 24 hours in the presence or absence of 200M Mari and immunostained using anti-p65 (Genetax cat# GTX102090). *p<0.05; ****p<0.00001.. ANOVA plus Tukey's HSD.

Figure 3

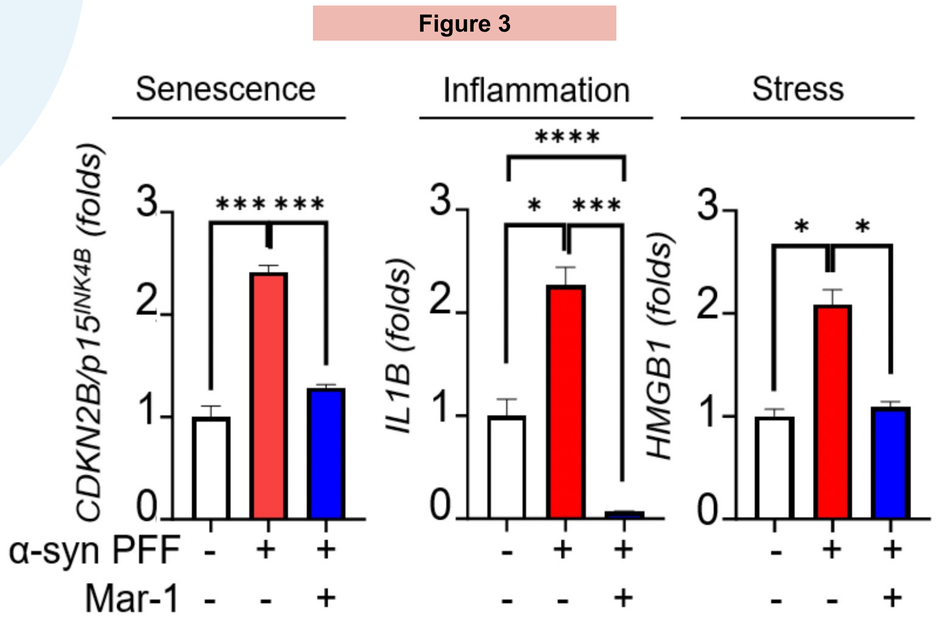

Figure 3: a-syn PFF triggers stress responses in human astrocytes that induce senescence and inflammation. Expression of senescence marker CDKN2B/p15/NK4B (left panel), was elevated by 100 ng/ml of a-syn PFF 24-hour incubation and reverse by 200M of Mar1. The expression of IL1-ß, an inflammation cytokine, and HMGB1, a stress alarmin that can also be a facilitator of senescence (2021), was increased by a-syn PFF and return to normal levels by Mar1. *p< 0.001; ***p<0.0001; **p<0.0001. ANOVA plus Tukey's HSD.

Figure 4

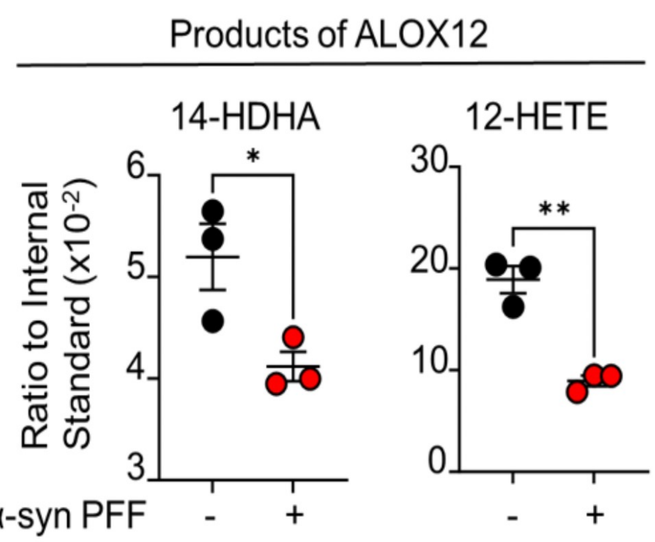

Figure 4: a-syn-PFF depressed the activity of ALOX12 in human astrocytes. Incubation of astrocytes for 24 hours with 100 ng/ml of a-syn-PFF induced a decrease in the activity of the ALOX12 noticed in the two measured products 12-HETE (right panel) and 14-HDHA (left panel, stabilization of 14-HpDHA;
Fig.1). *p<0.05; **p<0.01

4 Conclusion

1. Mar-1 decreased the activation of NFkB/p65 by α-syn PFF (Fig.2)

2. α-syn PFF induced the upregulation of the senescence marker *CDKN2B*/p15^{INKB}; (Fig. 3)

3. the increase transcription of *HMGB1* and *IL1B* (Fig.3)

4. Mar-1 counteracted the effect of Mar-1 on the transcription of stress, inflammation, and senescence markers.

5. The activity of ALOX12 was decreased by α-syn PFF (Fig.4)

Overall, α-syn PFF disabled astrocytes to respond to cues by induce senescence and Mar-1 prevent this change of phenotype.

5 Future Direction

In future directions we will focus on the mechanisms by which α-syn PFF induces senescence and the link between this cellular process and the decrease in the synthesis of Mar-1.

6 References

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