Free Radical Biology and Medicine 53 (2012) 993–1003

Contents lists available at SciVerse ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original Contribution

# Selective binding of nuclear alpha-synuclein to the PGC1alpha promoter under conditions of oxidative stress may contribute to losses in mitochondrial function: Implications for Parkinson's disease

Almas Siddiqui, Shankar J. Chinta, Jyothi K. Mallajosyula, Subramanian Rajagopolan, Ingrid Hanson, Anand Rane, Simon Melov, Julie K. Andersen *

*Buck Institute for Age Research, 8001 Redwood Boulevard, Novato, CA 94945, USA*

ARTICLE INFO

*Article history:*
Received 17 February 2012
Received in revised form
10 May 2012
Accepted 14 May 2012
Available online 15 June 2012

*Keywords:*
Parkinson's disease (PD)
α-Synuclein
PGC1-α
Histone
Chromatin
Mitochondria
Oxidative stress

ABSTRACT

Alpha-synuclein has been reported to be present in the nucleus and levels enhanced by oxidative stress. Herein, we sought to investigate the mechanistic role of nuclear alpha-synuclein. We found that alpha-synuclein nuclear localization coincided with enhanced chromatin binding both in an in vitro and a corresponding in vivo brain oxidative stress model previously characterized by our laboratory as well as in PD brain tissues. Genome-wide chromatin immunoprecipitation (ChIP)-on-chip analysis of alpha-synuclein:promoter binding in response to oxidative stress in vitro revealed that binding occurs at several promoters belonging to a range of functional categories including transcriptional regulation. Interestingly, given the important role of mitochondrial dysfunction in PD, this included binding to the promoter for the master mitochondrial transcription activator, PGC1alpha in vitro, in vivo, and in human brain tissue with age and PD. To test the possible mechanistic impact of alpha-synuclein PGC1alpha promotor binding, we assessed PGC1alpha promoter activity, mRNA, and protein levels and expression of candidate PGC1alpha target genes in our in vitro model. All were found to be reduced in conjunction with increased levels of aberrant mitochondrial morphology and impaired mitochondrial function. Exogenous PGC1alpha expression was found to attenuate alpha-synuclein-mediated mitochondrial dysfunction and subsequent neurotoxicity in vitro. Our data suggest that nuclear alpha-synuclein localization under conditions of oxidative stress may impact on mitochondrial function in part via the protein's capacity to act as a transcriptional modulator of PGC1alpha. This represents a novel role for alpha-synuclein as it relates to mitochondrial dysfunction in PD.



## Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder that impacts approximately 1:100 people in the United States over the age of 65 [1]. PD is accompanied by hallmark formation of intraneuronal inclusions termed "Lewy bodies" which contain alpha-synuclein as their major protein component. Interest in alpha-synuclein was initially sparked when rare autosomal dominant familial disease forms were linked first to point mutations in the gene and then to wildtype gene duplication and triplication events which increase wildtype protein levels; interest was further heightened when the protein was revealed to be a major component of Lewy bodies in sporadic PD [2–5]. Recent gene-wide association studies have identified several polymorphisms of the alpha-synuclein gene as susceptibility factors for the idiopathic form of the disorder [6,7]. Postmortem studies suggest that temporal patterns of alpha-synuclein-containing Lewy body accumulation within various brain regions track with disease progression [8–11]. It has become increasingly clear that obtaining a fuller mechanistic "picture" of how this protein contributes to PD-associated neuropathology would not only improve our understanding of the disease itself but also potentially uncover novel therapeutic targets for its treatment.

Nuclear α-synuclein localization has recently been demonstrated in a variety of experimental systems and nuclear levels have been reported to increase under conditions of oxidative stress both in vitro and in vivo [1]. The function of nuclear alpha-synuclein is unknown [12], but it has been reported to colocalize with histones in conjunction with reduced levels of histone acetylation [13,14]. Alpha-synuclein expression has also been

*Abbreviations:* A53T, mutant alpha-synuclein; ChIP, chromatin immunoprecipitation; dox, doxycycline; –dox, absence of doxycycline; +dox, doxycycline addition; IP, immunoprecipitation; I, 100% input; Nuc, nuclear; syn, synuclein; TSS, transcription start site; WT, wildtype alpha-synuclein.

* Corresponding author. Fax: +1 415 899 1810.
*E-mail address:* jandersen@buckinstitute.org (J.K. Andersen).

0891-5849/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.freeradbiomed.2012.05.024

shown to affect the expression of genes involved in various cellular or neuronal functions including transcription [15,16]. Given known affects of alterations in histone acetylation on transcription, this suggests that nuclear alpha-synuclein may contribute to neurotoxicity in part via its ability to impact on this epigenetic event [17–21]. In this report, we demonstrate select binding of alpha-synuclein to promoters including PGC1 alpha which may contribute to mitochondrial affects associated with alpha-synuclein via its transcriptional modulation of this master regulator of mitochondrial gene expression.

## Materials and methods

All chemicals were obtained from Sigma unless otherwise noted.

### *Cell culture and transfection*

Stable doxycycline (dox)-inducible MAO-B PC12 cell lines used for these studies were previously described [22]. Cells were maintained in DMEM containing 10% FBS, 5% horse serum, 1% streptomycin–penicillin, and 200 mg/ml of G418. Cells differentiated via 50 ng/ml nerve growth factor (NGF, 2 days) were transfected with either wildtype (WT) or mutant (A53T) alpha-synuclein cDNAs (plasmids, gift of Dr. C. Ross; John Hopkins) using Lipofectamine 2000 reagent (Invitrogen). To assure equivalent transfection efficiency, cells were collected 4 h after transfection, mixed, and replated for subsequent experiments. At 32 h following transfection, oxidative stress was induced via dox addition (40 μg/ml, 16 h). Untransfected noninduced cells were used as negative controls.

### *MAO-B transgenic primary cultures and brain tissue*

Primary cortical cultures were prepared as previously described [25]. Briefly primary mixed cultures were prepared from the midbrain of 14-day-old mice embryos ($n=5$ per condition) from MAO-B transgenics and WT controls. Tissue was digested in Neurobasal medium containing 30 U/ml papain and 20 μg/ml DNase at 37 °C for 30 min and mechanically triturated. Dissociated cells were centrifuged at 500*g*, resuspended in growth medium (Neurobasal medium supplemented with 10% FBS, 2 mM glutamate, B25 supplement without antioxidants, 50 U/ml penicillin, 50 U/ml streptomycin, and 50 ng/ml GDNF [24]), and plated on poly-D-lysine-coated 8-well chamber slides (BD-Biocoat) at a density of $10^5$ cells/ml. Mixed cultures were grown at 37 °C for 3–5 days before induction with 40 μg/ml doxycycline for 12 h as per [22]. Cells isolated from MAO-B transgenic mice [25] were either treated with dox or left untreated (24 h). Cortical tissues were isolated from inducible transgenic MAO-B lines fed dox versus vehicle for 3 weeks for further analyses as previously described [25].

### *Human brain sample collection*

Postmortem tissues isolated from late-onset sporadic PD patients with mild-to-moderate neuronal loss versus age-matched controls ($n=3$; average postmortem period, $7.25 \pm 5$ h; average age, $69.7 \pm 9$ years) were provided by Dr. Carole Miller, the University of Southern California brain bank. All PD cases were diagnosed clinically and neuropathologically confirmed whereas controls had no clinical or neuropathological signs of PD or dementia.

### *Cellular fractionation*

Cellular fractionation was performed as previously described [23]. Briefly, cells were homogenized in L1 buffer (10 mM Hepes/0.1 mM EGTA/10 mM KCl/1.5 mM $mg_2Cl$) and centrifuged for 5 min at 5000*g*, and the pellet (P1) and supernatant (S) retained. Following 10% glycerol addition, S1 was retained as the cytoplasmic fraction. The P1 pellet was resuspended in L1 buffer, passed through a 16 gauge needle, and centrifuged at 5000*g*, 5 min. The pellet (P2) was resuspended in L2 buffer (10 mM Hepes/0.1 mM EGTA/400 mM NaCl/1.5 mM mg2Cl) at 4 °C for 60 min and centrifuged at 14,000*g*, 30 min, and the supernatant retained as the nuclear fraction.

### *Immunoblotting*

For Western blots, 25 μg protein samples were separated on 4–12% bisacrylamide gels prior to transfer to polyvinylidine difluoride membranes. Membranes were blocked with 5% powdered milk solution in 0.3% Triton/phosphate-buffered saline solution before incubation with either 1:500 alpha-synuclein (BD Transduction, San Jose CA), 1:250 PGC1alpha (Abcam, Cambridge, UK), or 1:1000 histone H3 or acetylated H3 (Abcam, Cambridge, UK) antibodies. Beta-actin (1:5000, Sigma) antibody was used as loading control and Parp (1:1000, Cell Signaling, Danvers, MA) and beta-tubulin (1:1000, Sigma) antibodies were used to assess cytoplasmic and nuclear subfraction purity. Protein bands were detected via chemiluminescence substrate for horseradish peroxidase (Amersham Biosciences), the resulting bands were scanned, and densitometry was measured by NIH image J.

### *Chromatin immunoprecipitation*

Chromatin was prepared from cells or tissues using the MAGnify chromatin immunoprecipitation system (Invitrogen, Carlsbad, CA). The amount of 200 μg of chromatin was immunoprecipitated using alpha-synuclein, H3, acetyl H3, or PGC1alpha Abs and de-crosslinked by heating at 65 °C, 3 h prior to Western blot analysis. For DNA isolation for ChIP-chip promoter tile arrays, the MAGnify DNA isolation protocol was followed per the manufacturer's instructions.

### *Nuclear colocalization of alpha-synuclein via confocal immunocytochemistry*

Cells or tissues were fixed with 4% paraformaldehyde (PFA) for 15 min, blocked with 10% donkey serum for 30 min, and then subjected to primary antibodies (1:500H3, Cell Signaling; 1:1700 alpha-synuclein, BD Transduction) overnight, 4 °C. Samples were incubated with Alexa-conjugated secondary antibodies (1:1000) for 60 min in the dark, rt. DAPI staining was performed as a nuclear localization marker. Images were captured on a Zeiss LSM 510 confocal microscope.

### *Biochemical histone acetylation assay*

Histone acetylation was assayed biochemically in vitro via the histone acetyltransferase assay (Active Motif kit, Carlsbad, CA). The assay was carried out using 0.1 μg of highly purified nuclear protein fractions obtained as described above and incubated in 17 μl HAT assay buffer containing 50 mm Tris–HCl, pH 8.0, 10% (v/v) glycerol, 1 mm dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 0.1 mm EDTA, pH 8.0, and 10 mm sodium butyrate. A 0.5 mM acetyl-co-A was added followed by 50 μM H3 peptide, and the reaction incubated for 30 min, rt. The reaction was terminated by 50 μl stop solution followed by addition of 100 μl