Published in final edited form as:

Neuroreport. 2012 March 7; 23(4): 259–264. doi:10.1097/WNR.0b013e3283509842.

Neuronal to oligodendroglial alpha-synuclein redistribution in a double transgenic model of MSA

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Abstract

Multiple System Atrophy is a sporadic, progressive, neurodegenerative disease characterized by an oligodendroglial accumulation of α -syn. The mechanisms underlying the oligodendroglial accumulation of α -syn in the brains of patients with Multiple System Atrophy has attracted a great deal of interest given the primarily neuronal role reported for this protein.

We examined the interactions between neuronal and oligodendroglial α -syn in the progeny of crosses between parental transgenic (tg) mouse lines that express α -syn either under the oligodendroglial specific myelin-basic protein promoter (MBP1- α -syn tg) or under the neuronal platelet-derived growth factor promoter (PDGF- α -syn tg). Our results demonstrate that progeny from the cross (α -syn double (dbl) tg mice) displayed a robust redistribution of α -syn accumulation, with a re-localization from a neuronal or mixed neuronal/oligodendroglial α -syn expression to a more oligodendroglial pattern in both the neocortex and basal ganglia which closely resembled the parental MBP- α -syn tg line. The α -syn dbl tg mice also displayed motor deficits, concomitant with reduced levels of tyrosine hydroxylase and augmented neuropathological alterations in the basal ganglia.

These results suggest that the CNS milieu in the α -syn dbl tg mice favors an oligodendroglial accumulation of α -syn. This model represents an important tool with which to examine the interactions between neuronal and oligodendrocytic α -syn in disease such as Multiple System Atrophy.

Keywords

bigenic; alpha-synuclein; behavior; neurodegeneration; propagation

INTRODUCTION

The term alpha-synucleinopathy is used to encompass a varied group of neurodegenerative disorders characterized by the abnormal accumulation of the natively soluble neuronal protein alpha-synuclein (α -syn). Alpha-synucleinopathies include Parkinson' disease and

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Conflicts of interest: None

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Dementia with Lewy Bodies, which are characterized by a primarily neuronal accumulation of α -syn, and Multiple System Atrophy, which is characterized by an oligodendroglial accumulation of α -syn.

Multiple System Atrophy is a sporadic, progressive, neurodegenerative disease characterized clinically by motor and autonomic dysfunction. Neuropathologically, Multiple System Atrophy is characterized by glial cytoplasmic inclusions of α -syn in the oligodendrocytes of affected individuals [1]. Although glial cytoplasmic inclusions are the primary neuropathological hallmark of Multiple System Atrophy, neuronal inclusions (have also been reported as well as neuronal loss in the striatum, cerebellum, brainstem and cortex accompanied by astrogliosis, microgliosis and myelin loss [1,2].

Given the primarily neuronal roles reported for α -syn [3-5], its accumulation in oligodendroglial cells in Multiple System Atrophy brains has attracted a great deal of interest, however the mechanisms underlying this apparent redistribution of α -syn remain unclear. In this context we sought to examine the interactions between neuronal and oligodendroglial α -syn in the progeny of crosses between parental transgenic (tg) mouse lines that express either predominantly oligodendroglial or predominantly neuronal α -syn.

The results demonstrate that progeny from this cross, (hereafter referred to as the h α -syn double (dbl) tg mice), displayed a robust redistribution of α -syn, with a re-localization from neuronal α -syn to a more oligodendroglial pattern. This was accompanied by a worsening of motor behavior and neurodegenerative pathology.

MATERIALS AND METHODS

Breeding and maintenance of transgenic mouse lines

Mice expressing human α -syn under the control of the oligodendroglial-specific myelin basic protein promoter (MBP) were generated as previously described [6]. The MBP-h α syn line 1 mice (MBP1-h α syn tg mice) were chosen for this study as they express an intermediate level of α -syn expression. These mice have previously been shown to accumulate α -syn in oligodendrocytes from 3 months of age and to display neuropathological alterations including myelin loss and astrogliosis and behavioral deficits [6].

Transgenic mice over expressing wild type human (h) alpha-syn under the control of the neuronal platelet-derived growth factor (PDGF) promoter were also used. The PDGF promoter drives the expression of α -syn exclusively in neuronal cells and the PDGF- α -syn tg mice display accumulation of α -syn in the frontal cortex and limbic system accompanied by behavioral deficits, early motor alterations, loss of dopaminergic terminals and formation of inclusion bodies [7].

These mice were crossed to produce the h α -syn dbl tg mice, which were analyzed at 8 months of age and compared to age-matched mice from the parental lines with a total of 10 mice per group. Offspring were identified by PCR analysis of tail DNA, and were shown to contain both parental transgenes. Genomic DNA was extracted and analyzed as previously described [7]. The control mice were littermates of the same age and mixed gender.

Motor Behavioral analysis using the Pole Test

The pole test is a well-documented test used to assess basal ganglia-related motor function [8]. For the test mice were placed head upwards on top of a vertical wooden pole 50 cm long (1 cm in diameter). The base of the pole was placed in the home cage. When placed on the pole, animals orient themselves downward and descend the length of the pole back into their

home cage. Groups of mice received two days of training that consisted of five trials for each session. On the test day, animals received five trials and the total time to descend (T-total) was measured.

Tissue processing

Following NIH guidelines for the humane treatment of animals, under anesthesia mice were killed and brains removed. The right hemisphere was immersion-fixed in 4% paraformaldehyde in pH 7.4 PBS and serially sectioned at 40 μ m with a Vibratome (Leica, Deerfield, IL). The left hemisphere was kept at -80 °C for biochemical analysis.

Immunohistochemistry

40 μ m vibratome sections were immunolabeled overnight with antibodies against α -syn using monoclonal (1:500, BD Biosciences) or polyclonal (1:350, Chemicon) antibodies, phosphorylated α -syn (1:500, Millipore), the dendritic marker, microtubule-associated protein-2 (MAP2; 1:250, mouse monoclonal; Chemicon) and the neuronal marker NeuN (1:1000, Chemicon) followed by incubation with species-appropriate secondary antibodies (1:2000, Vector Laboratories). Sections were transferred to SuperFrost slides (Fisher Scientific, CA) and mounted with anti-fading media (Vector Laboratories). The immunolabeled blind-coded sections were analyzed with the laser scanning confocal microscope (MRC1024, BioRad). Stereological analysis was conducted as previously described [9] to examine the neuronal density as evidenced by NeuN immunoreactivity.

Western Blot Analysis

Protein levels of total α -syn, phosphorylated α -syn (S129) and nitrosylated α -syn were determined by immunoblot analysis. Briefly, tissue was processed to obtain the detergent soluble and insoluble fractions as previously described [6], 20 μ g of total protein from the soluble fraction per mouse were loaded onto 10% Bis-Tris (Invitrogen) SDS-PAGE gels, transferred onto Immobilon membranes, incubated with an anti- α -syn (1:1000, Chemicon, polyclonal), phosphorylated α -syn (S129) (1:1000, Millipore), nitrosylated α -syn (1:1000, Millipore). After overnight incubation with primary antibodies, membranes were incubated in appropriate secondary antibodies, reacted with ECL, and developed on a VersaDoc gel-imaging machine (Bio-Rad, Hercules, CA). Anti-beta-actin (1:1000, Sigma) was used to confirm equal loading.

Statistical methods

Differences between groups were tested using one and two factor ANOVA with Dunnett's post hoc tests. All the results are expressed as mean $\pm$ SEM.

RESULTS

Augmentation of motor deficit and neuropathology in a double transgenic model of α -synucleinopathy

The specific parental lines chosen for this study were the MBP1- α -syn tg and the PDGF- α -syn tg mice, which express α -syn under the control of an oligodendroglial or neuronal promoter, respectively.

In order to evaluate the extent of motor disturbance in the α -syn dbl tg mice their performance was assessed using the pole test. The non tg mice were able to descend the pole in an average of 11.41 ($\pm$ 1.49) secs, consistent with previous reports both the PDGF- α -syn tg and MBP1- α -syn tg mice [6,10,11] took longer than non tg mice to descend the pole (32.08 $\pm$ 2.26, and 23.28 $\pm$ 1.66 secs, respectively), indicative of motor deficits (Figure 1A).

The α -syn dbl tg mice took 56.2 ± 6.75 secs to complete the test suggesting that these mice also display motor dysfunction (Figure 1A). The α -syn dbl tg took longer to complete the test than each of the parental lines, indicative of a worsening of the motor deficit in the progeny in comparison to the parents.

Increased motor deficit in the α -syn dbl tg mice was accompanied by a marked reduction in basal ganglia tyrosine hydroxylase (TH) immunoreactivity in comparison to non tg mice and the PDGF- α -syn tg and MBP1- α -syn tg parental lines (Figure 1B). Here again, although the PDGF- α -syn tg mice and MBP1- α -syn tg parental lines displayed reduced TH immunoreactivity in comparison to non tg mice (Figure 1B), the reduction observed in the α -syn dbl tg was greater than that observed in either parental line.

Neuronal to oligodendroglial α -syn redistribution in a double transgenic model of α -synucleinopathy

Immunohistochemical examination of total α -syn levels demonstrated a robust increase in the PDGF- α -syn tg (Figure 2A, B, E, F (n= neurons, arrows = oligodendrocytes)) and MBP1- α -syn tg mice (Figure 2A, C, E, G, 2D, H (n= neurons, arrows = oligodendrocytes)) in the neocortex in comparison to non tg mice. A similar increase was also in levels of phospho(p129)- α -syn immunoreactivity in the neocortex of the parental lines in comparison to non tg mice (Figure 2I-L)

Analyses of the neuronal/oligodendroglial distribution pattern of α -syn in both the frontal cortex and basal ganglia showed that the PDGF- α -syn tg mice displayed a predominantly neuronal α -syn accumulation pattern in both regions with little or no detectable oligodendroglial accumulation (Figure 2B, F, M-P), this is consistent with previous reports in these mice. In contrast, the MBP1- α -syn tg displayed little or no detectable neuronal α -syn accumulation (Figure 2C, G, M, N) in the frontal cortex and basal ganglia, they did however display robust levels of oligodendroglial α -syn (Figure 2C, G, O, P) in both regions, these results are also in line with previous studies in this model [6].

The α -syn dbl tg mice displayed low levels of neuronal α -syn in the frontal cortex and basal ganglia (Figure 2D, H, M, N), but much more robust levels of oligodendroglial α -syn accumulation in both regions examined. Levels of oligodendroglial α -syn in the α -syn dbl tg mice were significantly higher than those observed in the MBP1- α -syn tg (Figure 2D, H, O, P).

In order to examine the cell type of α -syn immunoreactive cells in more detail, a series of double-labeling studies were performed. Colocalization of α -syn and NeuN in the PDGF- α -syn tg mice confirmed the neuronal identity of the α -syn positive cells in these mice (Figure 3D-F). In contrast, the α -syn signal in the MBP1- α -syn tg or the α -syn dbl tg mice did not localize with the NeuN in these mice (Figure 3G-I, J-L).

Immunohistochemical analysis of NeuN immunoreactivity showed a marked decrease in both the MBP1- α -syn tg and the α -syn dbl tg mice in comparison to both non tg control mice and PDGF- α -syn tg mice (Figure 3M). NeuN immunoreactivity in the PDGF- α -syn tg mice did not differ from that observed in non tg control mice (Figure 1M). Analysis of MAP2 immunoreactivity in the neuropil of the basal ganglia demonstrated a decrease in the MBP1- α -syn tg parental line and the α -syn dbl tg in comparison to non tg mice (Figure 3N). The α -syn dbl tg also displayed lower levels of MAP2 immunoreactivity in comparison to the PDGF- α -syn tg mice (Figure 3N).

Levels of α -syn species in a double transgenic model of α -synucleinopathy

Modification such as phosphorylation and nitrosylation have been linked to α -syn aggregation [12,13] and recent studies have reported differential expression of α -syn species between Parkinson's disease and Multiple System Atrophy [14]. In light of these studies we sought to further characterize the levels and species of α -syn in the $h\alpha$ -syn dbl tg mice by immunoblot. Consistent with the immunohistochemistry, both the parental lines displayed a robust increase in α -syn and phospho(p129)- α -syn immunoreactivity in comparison to non tg mice (Figure 4A, B) though these levels were more robust in the MBP1- $h\alpha$ -syn tg mice. The $h\alpha$ -syn dbl tg mice displayed levels of total and phospho(p129)- α -syn comparable to those observed in the MBP1- $h\alpha$ -syn tg mice (Figure 4A, B). The MBP1- $h\alpha$ -syn tg mice demonstrated more robust levels of nitrosylated α -syn immunoreactivity in comparison to both PDGF- $h\alpha$ -syn tg and non tg mice (Figure 4A, E), the $h\alpha$ -syn dbl tg mice displayed levels of nitrosylated α -syn comparable to the MBP1- $h\alpha$ -syn tg mice (Figure 4D).

Collectively the immunohistochemical and immunoblot data demonstrate that, rather than displaying the mixed neuronal-oligodendroglial α -syn phenotype that may be expected, the $h\alpha$ -syn dbl tg mice appear to display a profile of α -syn that has re-localized to more closely resemble that observed in the MBP1- $h\alpha$ -syn tg parental line. These results suggest that the CNS milieu in the $h\alpha$ -syn dbl tg may favor the oligodendrocytic accumulation of α -syn over neuronal accumulation.

DISCUSSION

Multiple System Atrophy is characterized by the oligodendrocytic accumulation of α -syn, a neuronal protein. The mechanisms leading to α -syn aggregation in Multiple System Atrophy remain unclear, especially in light of reports suggesting that oligodendrocytes themselves do not produce α -syn [15,16]. Recent studies have suggested that α -syn may be capable of cell-to-cell transmission, and this has been posited as a potential mechanism underlying α -syn aggregation in Multiple System Atrophy [17].

This study examined the interactions between neuronal and oligodendroglial α -syn in the $h\alpha$ -syn dbl tg mice, progeny of parents that express either predominantly oligodendroglial (MBP1- $h\alpha$ -syn tg) or predominantly neuronal (PDGF- $h\alpha$ -syn tg) α -syn. The expression pattern of α -syn in each of the parental lines is controlled by the transgene and given that the progeny of this cross express both the neuronal and oligodendroglial α -syn transgenes (confirmed by PCR), they would reasonably be expected to express a mixed neuronal-oligodendroglial α -syn expression pattern. Remarkably, our results indicate that $h\alpha$ -syn dbl tg mice display a re-localization of α -syn expression to more closely resemble the oligodendrocytic expression pattern observed in the MBP1- $h\alpha$ -syn tg parental line. Moreover, the α -syn in these mice displays a phosphorylation and nitrosylation profile similar to the MBP1- $h\alpha$ -syn tg mice. These results indicate a re-localization of α -syn from neurons to oligodendrocytes in the $h\alpha$ -syn dbl tg mice and a species shift towards more phosphorylated and nitrosylated form of α -syn in these mice.

The precise mechanisms underlying this apparent redistribution and species shift remain unclear, however possible mechanisms could include; 1) the translocation of neuronal α -syn in the $h\alpha$ -syn dbl tg mice into the oligodendrocytes, 2) down-regulation of the production of neuronal α -syn by as yet unidentified signals from the oligodendrocytes or 3) the clearance of neuronal α -syn, leaving the oligodendroglial α -syn unaffected. Of these proposed mechanisms, the translocation of α -syn from the neurons to the oligodendrocytes has the most support in the literature and is consistent with studies suggesting that α -syn, a cytosolic protein, is able to enter the extracellular space and undergo cell-to-cell transmission [18-21] and with studies demonstrating the neuroprotective effects of immunotherapy approaches in

α -syn tg mice [22,23]. Further, more detailed studies are needed in order to thoroughly examine this process and to elucidate the precise mechanism governing the cell-to-cell movement of α -syn.

In conclusion, we present a double tg model of α -synucleinopathy in which $h\alpha$ -syn expression is being driven by both a neuronal and oligodendrocytic promoter and demonstrate that this model displays a predominantly oligodendroglial expression and Multiple System Atrophy-like species profile of α -syn. This model may represent an important tool with which to examine the cell-to-cell transmission of α -syn and the interactions between neuronal and oligodendrocytic α -syn in α -synucleinopathies such as Multiple System Atrophy.

(A) Characterization of motor abnormalities in non transgenic (non tg) mice, PDGF- $h\alpha$ -syn tg mice and MBP1- $h\alpha$ -syn tg parental lines and the $h\alpha$ -syn double tg mice. (B) Quantitative analysis of the dopaminergic marker tyrosine hydroxylase (TH) immunoreactivity in the basal ganglia. Analysis results are presented as mean $\pm$ SEM (mice were 8 months old, n=10 per group) and * indicates a statistically significant difference between groups indicated by bar (p<0.05) by one-way ANOVA and post-hoc Tukey.

(A-D) Representative images of α -syn immunoreactivity in the frontal cortex of non tg, PDGF- $h\alpha$ -syn tg, MBP1- $h\alpha$ -syn tg and $h\alpha$ -syn dbl tg mice. (E-H) Representative images of $h\alpha$ -syn immunoreactivity in the frontal cortex. (I-L) Representative images of phosphorylated $h\alpha$ -syn immunoreactivity (at the serine 129 phospho-epitope) in the frontal cortex. (M) Neuronal α -syn immunoreactivity in the frontal-temporal cortex. (N) Neuronal α -syn immunoreactivity in the basal ganglia. (O) Oligodendroglial α -syn immunoreactivity in the frontal-temporal cortex. (P) Oligodendroglial α -syn immunoreactivity in the basal ganglia. Scale bar (A-L) = 25 μ M, n=neuron, arrow=oligodendrocyte. Analysis results are presented as mean $\pm$ SEM (mice were 8 months old, n=10 per group) and * indicates a statistically significant difference between groups indicated by bar (p<0.05) by one-way ANOVA and post-hoc Tukey.

(A-C) Colocalization of α -syn and NeuN signal in frontal cortex of non tg mice. (D-F) Colocalization of α -syn and NeuN signal in PDGF- $h\alpha$ -syn tg mice. (G-I) Colocalization of α -syn and NeuN signal in MBP1- $h\alpha$ -syn tg mice. (J-L) Colocalization of α -syn and NeuN signal in $h\alpha$ -syn dbl tg mice. (M) Quantitative analysis of NeuN immunoreactivity in the basal ganglia of non tg, PDGF- $h\alpha$ -syn tg, MBP1- $h\alpha$ -syn tg and $h\alpha$ -syn double tg mice. (N) MAP2 immunoreactivity in the basal ganglia. Scale bar (A-L) = 25 μ M, n=neuron, arrow=oligodendrocyte. Analysis results are presented as mean $\pm$ SEM (mice were 8 months old, n=10 per group) and * indicates a statistically significant difference between groups indicated by bar (p<0.05) by one-way ANOVA and post-hoc Tukey

Acknowledgments

This work was supported by NIH grants AG 18440, NS 044233, AG 10435 and AG 022074 and the Donner Canadian Foundation.

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Figure 1. Behavioral and Neuropathological Characterization of the ha-syn double transgenic mice

Figure 2. Immunohistochemical Characterization of α -syn and phospho- α -syn

Figure 3. Double labeling of α -syn in the h α -syn double transgenic mice