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α-Synuclein impairs oligodendrocyte progenitor maturation in multiple system atrophy

Verena E.L. May [a], Benjamin Ettle [a], Anne-Maria Poehler [a], Silke Nuber [b], Kiren Ubhi [b], Edward Rockenstein [b], Beate Winner [c], Michael Wegner [d], Eliezer Masliah [b], Jürgen Winkler [a,b,*]

[a] Department of Molecular Neurology, Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen, Germany
[b] Department of Neurosciences and Pathology, School of Medicine, University of California San Diego, La Jolla, CA, USA
[c] Junior Research Group III, Interdisciplinary Centre of Clinical Research, Nikolaus Fiebiger Centre for Molecular Medicine, University Hospital Erlangen, Erlangen, Germany
[d] Institute of Biochemistry, Emil-Fischer-Zentrum, Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen, Germany

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ABSTRACT

Multiple system atrophy (MSA), an atypical parkinsonian disorder, is characterized by α-synuclein (α-syn$^+$) cytoplasmatic inclusions in mature oligodendrocytes. Oligodendrocyte progenitor cells (OPCs) represent a distinct cell population with the potential to replace dysfunctional oligodendrocytes. However, the role of OPCs in MSA and their potential to replace mature oligodendrocytes is still unclear. A postmortem analysis in MSA patients revealed α-syn within OPCs and an increased number of striatal OPCs. In an MSA mouse model, an age-dependent increase of dividing OPCs within the striatum and the cortex was detected. Despite of myelin loss, there was no reduction of mature oligodendrocytes in the corpus callosum or the striatum. Dissecting the underlying molecular mechanisms an oligodendroglial cell line expressing human α-syn revealed that α-syn delays OPC maturation by severely downregulating myelin-gene regulatory factor and myelin basic protein. Brain-derived neurotrophic factor was reduced in MSA models and its in vitro supplementation partially restored the phenotype. Taken together, efficacious induction of OPC maturation may open the window to restore glial and neuronal function in MSA.

1. Introduction

Multiple system atrophy (MSA) is an age-related neurodegenerative synucleinopathy characterized by fast progression and severe disability (Wenning and Colosimo, 2010). Distinct MSA subtypes predominantly present besides parkinsonism (MSA-P) or cerebellar ataxia (MSA-C) with autonomic failure and poor levodopa responsiveness. The MSA related neuropathological hallmark is glial cytoplasmic inclusions (GCI) in oligodendrocytes frequently observed in the cortex, the corpus callosum, and the striatum (Papp et al., 1989) correlating with severe neurodegenerative changes such as axonal degeneration, severe myelin loss, and gliosis (Ahmed et al., 2012b; Ozawa et al., 2004). The most prominent constituent of GCIs is alpha-synuclein (α-syn), a presynaptic protein involved in vesicular and synaptic transport of neurons but its origin and function in oligodendrocytes still remains elusive (Nemani et al., 2010; Tofaris and Spillantini, 2007). More recently, studies indicate that extracellular α-syn may be taken up by neurons, astrocytes, microglia, and oligodendroglia (Hansen et al., 2011; Kisos et al., 2012; Kordower et al., 2011; Lee et al., 2008, 2010).

Importantly, numerous studies suggest that synucleinopathies interfere with neuronal plasticity in the human adult central nervous system (CNS) such as neurogenic regions, like the hippocampus and the subventricular zone and/or olfactory bulb (Marxreiter et al., 2012; Winner et al., 2011). However, much less is known about the influence of α-syn on glial plasticity.

Oligodendrogenesis occurs during development and is maintained throughout adulthood (Dawson et al., 2000; Miller, 2002). An OPC population is present throughout the gray and white matter (Richardson et al., 2011) and compromise approximately 5% of all

* Corresponding author at: Department of Molecular Neurology, Friedrich-Alexander-University Erlangen-Nürnberg, Schwabachanlage 6, D-91054 Erlangen, Germany. Tel.: +49 9131 85 39324; fax: +49 9131 85 36597.
E-mail address: juergen.winkler@uk-erlangen.de (J. Winkler).

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cells within the adult CNS. These cells are characterized by expression of the chondroitin sulfate proteoglycan NG2 (Trotter et al., 2010) and coexpress platelet derived growth factor receptor α (PDGFRα; Rivers et al., 2008). Upon further differentiation to mature oligodendrocytes, PDGFRα expression is continuously downregulated paralleled by increased expression of mature oligodendrocytic proteins, for example, proteolipid protein (PLP) and myelin basic protein (MBP; Fancy et al., 2011), recapitulating developmental signaling pathways during adult oligodendrogenesis and remyelination. However, very little is known on the effect of α-syn on oligodendrogenesis and remyelination under pathologic conditions. As MSA is characterized by widespread myelin loss, we hypothesized that α-syn interferes with adult oligodendrogenesis preventing oligodendrocyte progenitor cells (OPCs) from remyelination and therefore contributing to MSA pathology. Indeed, in postmortem analyses we detected an increase in the number of PDGFRα$^+$ OPCs in the striatum of MSA patients suggesting a maturation deficit of these cells. Supporting our hypothesis, mice overexpressing human wild-type α-syn under the control of the MBP promoter exhibit increased striatal OPC numbers while numbers of mature oligodendrocytes remain unaffected. A delayed expression pattern of stage-specific transcription factors accompanied by downregulation of MBP expression in differentiated central glia 4 cells (CG4, a permanent oligodendroglial cell line) stably expressing human wild-type α-syn also point towards interference of intracellular α-syn with maturation of OPCs. Taken together, our data suggest that α-syn impairs maturation of OPCs thereby contributing to MSA pathology.

2. Methods

2.1. Human specimen

A total of 12 human brains (n = 6 MSA-P cases; n = 6 controls) were analyzed for the present study (Table 1). Autopsies were performed within 24 hours of postmortem. Brains were fixed with 4% paraformaldehyde (PFA) and dissected according to standard procedure as described previously (Winner et al., 2012). Basal ganglia were processed in 40 μm sections on a vibratome for subsequent immunohistochemical analysis.

2.2. Animals and experimental design

Transgenic (tg) mice overexpressing human wild type α-syn under the control of the MBP promoter were compared with age-matched littermate controls. The generation of mice was previously described (Shults et al., 2005); MBP expresser line 1 was used for the present study. Animals were housed in a 12 hours light/12 hours dark cycle and had free access to nutrients. Body weight was monitored daily. Experiments for this study were performed for 2 animal groups with mixed gender, aged either 5 or 9 months at the time of perfusion (n = 5 − 8). To detect dividing cells, 5′-bromo-2′-deoxyuridine (BrdU; 50 mg/kg) was intraperitoneally injected twice a day for 3 consecutive days. Three weeks later, animals were perfused transcardially with 4% PFA (Sigma, USA) in 100 mM phosphate buffered saline (PBS), pH 7.4, brains were dissected and postfixed in 4% PFA (in 100 mM PBS) for 48 hours. Following NIH (National Institutes of Health) guidelines for the humane treatment of animals, mice were sacrificed under anesthesia. For longer storage, brains were transferred into a 30% sucrose (in PBS) solution. Brains were cut into 40 μm sections on a sliding microtome (Leica, Germany), sections were stored in cryoprotectant (ethylene glycol, glycerol, and PBS, pH 7.4, 1:1:2 by volume) at −20 °C until further processing.

2.3. Staining procedures

2.3.1. Primary antibodies

Stainings on tissue were performed with the following primary antibodies: rat monoclonal anti-BrdU (1:500; AbD Serotec, UK), mouse monoclonal anti-neuron-specific nuclear protein (NeuN; 1:100; Chemicon, USA), rat monoclonal anti-human α-synuclein 15G7 (1:10; Axxora GmbH, Switzerland), rabbit anti-PDGFRα (c-20; 1:100; Santa Cruz Biotechnology, USA), mouse monoclonal anti-glutathione-S-transfgerase π (GSTπ; 1:500 for fluorescent stainings, 1:1000 for DAB-stainings; BD Transduction Laboratories, Belgium), rabbit polyclonal anti-galactocerebroside (GalC; 1:100; AB 142; Chemicon, USA), and mouse monoclonal anti α-synuclein 211 for human samples (1:250; MA1-12874, Thermo Scientific, USA). For immunocytochemistry, monoclonal mouse anti-MBP (1:250; MCA184S; AbD Serotec, UK), rabbit anti-PDGFRα (c-20; 1:100; Santa Cruz Biotechnology, USA), rat monoclonal anti-human α-synuclein 15G7 (1:250; Axxora GmbH, Switzerland), and rabbit monoclonal anti-GFP (E385; 1:500; Abcam, UK) were used and counterstained with 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI; 1:10.000; Sigma, USA).

2.3.2. Secondary antibodies

For immunohistochemistry, donkey anti-mouse and anti-rat biotinylated secondary antibodies were used 1:1000, as well as the avidin-biotin-peroxidase complex, 1:100 (Vectastain Elite, Vector Laboratories, USA).

For immunofluorescence, donkey-anti-mouse antibodies were used coupled to Alexa 568 or Cy5 (both 1:1000), donkey-anti-rat coupled to Alexa 488 (1:1000) or Rhodamine Red-X (1:500), and donkey-anti-rabbit coupled to Alexa 488, Alexa 568, Cy5 or biotin (all 1:1000). Alexa-coupled antibodies were purchased from Invitrogen, Life Technologies, USA, Cy5-, Rhodamine Red-X- and biotin-coupled antibodies from Dianova, Germany, respectively.

2.3.3. Immunohistochemistry

Free-floating sections were incubated in 0.6% H_2O_2 in Tris-buffered saline (TBS: 0.15 M NaCl, 0.1 M Tris-HCl, pH 7.5) for 30 minutes. Afterwards, blocking was performed in TBS containing 0.25% Triton-X100 and 3% normal donkey serum for 30 minutes followed by incubation with primary antibodies in blocking solution overnight at 4 °C. Biotinylated secondary antibody incubation was performed for 1 hour in blocking solution. After rinsing in TBS,

Table 1
Summary of clinicopathological characteristics of patients

Diagnosis	n	Age (y)	PMT (h)	Gender (M/F)	Duration (y)	MMSE	Brain weight (g)
MSA-P	4	66.2 ± 3.2[a]	13.3 ± 2.3	3/1	7.8 ± 4.9	23.6 ± 0.9[b]	1425 ± 75.7
Control	4	80.8 ± 4.9	12.0 ± 1.5	1/3	NA	28.5 ± 0.7	1262 ± 83.9

Statistical analysis was performed using Student *t* test.
Key: F, female; M, male; MMSE, Mini-Mental State Examination; PMT, postmortem time.
[a] $p = 0.0313$.
[b] $p = 0.0018$.