Insights into the pathogenesis of multiple system atrophy: focus on glial cytoplasmic inclusions

Seiji Kaji *, Takakuni Maki, Tomoyuki Ishimoto, Hodaka Yamakado and Ryosuke Takahashi *

Abstract

Multiple system atrophy (MSA) is a debilitating and fatal neurodegenerative disorder. The disease severity warrants urgent development of disease-modifying therapy, but the disease pathogenesis is still enigmatic. Neurodegeneration in MSA brains is preceded by the emergence of glial cytoplasmic inclusions (GCIs), which are insoluble α-synuclein accumulations within oligodendrocytes (OLGs). Thus, preventive strategies against GCI formation may suppress disease progression. However, although numerous studies have tried to elucidate the molecular pathogenesis of GCI formation, difficulty remains in understanding the pathological interaction between the two pivotal aspects of GCIs; α-synuclein and OLGs. The difficulty originates from several enigmas: 1) what triggers the initial generation and possible propagation of pathogenic α-synuclein species? 2) what contributes to OLG-specific accumulation of α-synuclein, which is abundantly expressed in neurons but not in OLGs? and 3) how are OLGs and other glial cells affected and contribute to neurodegeneration? The primary pathogenesis of GCIs may involve myelin dysfunction and dyshomeostasis of the oligodendroglial cellular environment such as autophagy and iron metabolism. We have previously reported that oligodendrocyte precursor cells are more prone to develop intracellular inclusions in the presence of extracellular fibrillary α-synuclein. This finding implies a possibility that the propagation of GCI pathology in MSA brains is mediated through the internalization of pathological α-synuclein into oligodendrocyte precursor cells. In this review, in order to discuss the pathogenesis of GCIs, we will focus on the composition of neuronal and oligodendroglial inclusions in synucleinopathies. Furthermore, we will introduce some hypotheses on how α-synuclein pathology spreads among OLGs in MSA brains, in the light of our data from the experiments with primary oligodendrocyte lineage cell culture. While various reports have focused on the mysterious source of α-synuclein in GCIs, insights into the mechanism which regulates the uptake of pathological α-synuclein into oligodendroglial cells may yield the development of the disease-modifying therapy for MSA. The interaction between glial cells and α-synuclein is also highlighted with previous studies of post-mortem human brains, cultured cells, and animal models, which provide comprehensive insight into GCIs and the MSA pathomechanisms.

Keywords: Multiple system atrophy, α-Synuclein, Glial cytoplasmic inclusion, Prion, Neurodegeneration, Oligodendrocyte, Microglia, Astrocyte, Oligodendrocyte precursor cell

* Correspondence: seijik@kuhp.kyoto-u.ac.jp; ryosuket@kuhp.kyoto-u.ac.jp
Department of Neurology, Graduate School of Medicine, Kyoto University, 54 Shogoin-Kawahara-cho, Sakyo-ku, Kyoto, Japan

© The Author(s). 2020 Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
Background

Multiple system atrophy (MSA) is a progressive neurodegenerative disorder involving multiple nervous systems. The median survival from onset is about 9 years [1]. MSA symptoms, which are mainly characterized by autonomic failure, cerebellar ataxia, and parkinsonism that poorly respond to treatment used for Parkinson’s disease (PD). Depending on the predominant clinical feature, MSA patients are classified as either MSA-P, a Parkinsonian feature-dominant type, or MSA-C, a cerebellar ataxia-dominant type [2, 3]. MSA-P is the more common phenotype in most countries except some Asian countries. This difference was also confirmed pathologically by comparisons between British and Japanese post-mortem MSA cases [4].

Glial cytoplasmic inclusions (GCIs), the diagnostic hallmark of MSA, are fibrillar structures composed of misfolded α-synuclein (α-syn) [5]. This important discovery of α-syn-immunoreactive inclusions in oligodendrocytes (OLGs) raised fundamental questions: 1) what is the primary event which triggers the generation of misfolded α-syn leading to the formation of GCIs? 2) how does α-syn accumulate in OLGs, which produce few α-syn mRNA transcripts? and 3) how much are other glial cells involved in the pathogenesis of GCIs and neurodegeneration? In terms of glial cells other than OLGs, not only microglia and astrocytes but also oligodendrocyte precursor cells (OPCs) may be a role player of deep significance, considering their potential to become OLGs even in adult CNS [6]. In this review, current insights into the pathogenesis of GCIs will be highlighted based on studies from post-mortem human cases and in vitro/in vivo experiments replicating MSA pathology. While important viewpoints have been stated in the previously published excellent reviews, we provide an insight into the possible involvement of OPCs, featuring their notable response to extracellularly applied misfolded α-syn [7–11].

Main text

Diagnosis and treatment of MSA

Current consensus criteria for the diagnosis of MSA are based on three categories: definite, probable, and possible MSA [2]. Diagnosis of definite MSA requires neuropathological findings of widespread and abundant α-syn-positive GCIs, which are concomitant with striatonigral or olivopontocerebellar neurodegeneration. Diagnosis of both probable and possible MSA requires autonomic dysfunction accompanied by parkinsonism and/or by cerebellar syndrome, which symptoms are progressive and adult (>30 years old)-onset lacking family history.

Treatment of MSA is supportive and depends on the target symptoms ranging from parkinsonism (Levodopa), orthostatic hypotension (midodrine and droxidopa), urinary tract dysfunction (anticholinergic agents), constipation (laxative therapy), breathing disorders (continuous positive air pressure and tracheotomy) and dystonia (botulinum toxin injection) [12]. Although several clinical trials have been conducted, significant improvement of MSA symptoms has not been documented except for a few medications such as rotigotine and intra-arterial administration of autologous mesenchymal stem cells [13]. Considering the efficacy of mesenchymal stem cells, intrathecal administration of these cells may be beneficial and its safety profile has just been examined through phase I/II study [14]. Nevertheless, currently, there is no potent disease-modifying therapies, which emphasizes the importance of various insights into MSA pathogenesis based on post-mortem and biochemical investigations.

GCIs and other inclusions in MSA brains

In order to understand the non-genetically triggered pathogenesis of MSA, capturing the earliest changes during MSA disease progression is a key to unravelling its pathomechanisms. The term “minimal change” MSA describes a pathological condition of MSA that is characterized by neuronal loss restricted to the substantia nigra and locus coeruleus with widespread GCIs [15]. These cases provide critical insights into the primary changes of MSA pathology because abundant GCIs throughout the brain are found even in the absence of any clinical signs or neuronal loss, possibly serving as a key regulator of MSA disease progression [16].

GCIs were initially reported as loosely packed tubular structures adjacent to oligodendroglial nuclei by electron microscopy [17]. The majority of α-syn in GCIs is phosphorylated at Ser 129. The frequency of GCIs and the severity of neuronal cell loss are significantly correlated [18]. Other MSA-associated inclusions are less frequently observed than GCIs, including neuronal cytoplasmic inclusions (NCIs), neuronal nuclear inclusions (NNIs), and glial nuclear inclusions (GNIs), all of which are labeled with an antibody against phosphorylated α-syn and silver impregnation techniques [19]. Ultrastructurally, both GCIs and NCIs consist of granule-associated filaments with diameters of approximately 25 nm, and the filaments in these inclusions are morphologically indistinguishable [20]. Although the distribution of GCIs is always more striking than that of neuronal inclusions, some regions such as the anterior cingulate cortex and agranular frontal cortex seem to develop neuronal inclusions very frequently [21]. In MSA brains, the frequency of GCIs correlates with the severity of neuronal loss, although the possibility still exists that NCIs play a significant role in neurodegeneration, especially in the pons [18].

Difference between GCIs and Lewy bodies (LBs)

Gai et al. described that GCI filaments are composed of central core fibrils coated with amorphous materials. Removal of this amorphous material allows visualization of
10-nm-sized central core fibrils that are strongly labeled with antibodies against α-syn, but not with antibodies against other proteins (αβ-crystallin, ubiquitin, and tubulins) [5]. Moreover, quantitative analysis of the protein composition of GCIs and LBs with immunomagnetic analysis showed that GCIs consist of 11.7% α-syn, 1.9% αβ-crystallin, and 2.3% 14–3–3 proteins (8.5, 2.0, and 1.5% in LBs, respectively) [22], with predominance of α-syn accumulation. These data suggest that fibrillary α-syn is the main component of GCIs and plays a pivotal role in GCI formation. Despite these differences, most GCI components such as cytoskeletal proteins, molecular chaperones, aggresomal proteins, and apoptosis mediators are similarly observed as components of LBs (Table 1). With all these observations in mind, some GCI components such as cytoskeletal proteins, molecular chaperones, aggresomal proteins, and apoptosis mediators are similarly observed as components of LBs (Table 1). Taken together, many morphological profiles seen in NCIs of MSA brains are different from LBs in PD brains, possibly reflecting different cellular milieus in which each α-syn aggregate species is originally generated.

What happens within the OLGs of MSA brains prior to the emergence of GCIs?

The study also revealed that even in vitro generation of aggregates from monomeric α-syn in the presence of OLG lysate is sufficient to generate GCI-α-syn strain [97]. These observations provide critically important insight into the pathogenesis of GCIs, clarifying that OLGs are the likely sites of aggregate formation from primary seeds.

In addition, these findings emphasize the importance of post-mortem investigations about the difference between two types of neuronal inclusions: NCIs and LBs. Analogous to GCIs, NCIs, the ubiquitin-immunoreactive inclusions, are labeled with antibodies to α-syn and stained with Gallyas-Braak impregnation [94]. Investigations with immunoelectron microscopy confirmed that the ultrastructure of perinuclear NCIs resembles that of GCIs rather than that of LBs [94]. Most NCIs are immunonegative for antibodies against tau, neurofilaments, and αβ-crystallin, which are the components of LBs [94] (Table 1). Taken together, many morphological profiles seen in NCIs of MSA brains are different from LBs in PD brains, possibly reflecting different cellular milieus in which each α-syn aggregate species is originally generated.
Table 1 Comparison of molecular components within GCIs, NCIs, and LBs* through analysis with post-mortem human brains

| Protein                                      | GCIs | NCIs | LBs* | References |
|----------------------------------------------|------|------|------|------------|
| **Chaperons**                                |      |      |      |            |
| α-synuclein                                  | +    | +    | +    | [23]       |
| Heat shock protein 70 and 90                 | ND   | +    |  +   | [24–26]    |
| DJ-1                                         | ND   | +    | –    | [27]       |
| αB-Crystallin                                | –    | +/−  | +/−  | [20, 28]   |
| **Cytoskeletal proteins**                    |      |      |      |            |
| α/β-tubulin                                  | +    | –    | +    | [29–31]    |
| Tau (non-phosphorylated)                     | +/−  | −    | +/−  | [20, 30, 32, 33] |
| Tau (phosphorylated)                         | −    | ND   | +/−  | [33–35]    |
| Microtubule associated protein-1             | ND   | +    |  +   | [29, 31]   |
| Microtubule associated protein-2             | −    | −    | +    | [29, 31, 36] |
| p25α/TPPP (tubulin polymerization-promoting protein) | +    | +    |  +   | [37–39]    |
| **Ubiquitin and autophagy-related proteins** |      |      |      |            |
| Ubiquitin                                    | +    | +    | +    | [20, 40]   |
| SUMO-1 (small ubiquitin modifier 1)          | ND   | +/−  | +    | [41, 42]   |
| 20s proteasome subunits                      | ND   | +/−  | +    | [24, 43]   |
| HDAC6                                        | ND   | +    |  +   | [44]       |
| Parkin                                       | ND   | +/−  | +    | [45, 46]   |
| Parkin-R                                     | −    | ND   | +    | [45]       |
| Dorfin                                       | ND   | +/−  | +    | [47, 48]   |
| NEDD-8                                       | ND   | +/−  | +    | [49]       |
| NUB1 (Negative regulator of ubiquitin-like protein 1) | +    | +    |  +   | [50, 51]   |
| Synphilin-1                                  | +    | +/−  | +    | [52, 53]   |
| F-box only protein (FBXO7)                   | ND   | +    |  +   | [54]       |
| p62/SQSTM1                                   | ND   | +    |  +   | [35]       |
| LC3                                          | ND   | +/−  | +    | [55, 56]   |
| NBR1                                         | ND   | +/−  | +    | [57]       |
| AMBRA1                                       | ND   | +/−  | +    | [58, 59]   |
| **Apoptosis regulators**                     |      |      |      |            |
| Bcl-2                                        | ND   | +    | ND   | [60]       |
| HtrA2/Omi                                     | ND   | +    |  +   | [61]       |
| Parkin co-regulated gene (PACBG)             | ND   | +/−  | +    | [62]       |
| XIAP (X-linked inhibitor of apoptosis protein)| ND   | +/−  | +    | [63, 64]   |
| Apoptosome (cytochrome c, Apaf-1, caspase-9) | +    | +    |  +   | [65, 66]   |
| **Signal transduction**                      |      |      |      |            |
| 14–3–3 protein                               | ND   | +    |  +   | [67, 68]   |
| Mitogen-activated protein kinase (MAPK)       | ND   | +    | ND   | [69]       |
| LRRK2                                        | ND   | +/−  | +    | [46, 70, 71] |
| **Metal-related proteins**                   |      |      |      |            |
| Transferrin                                  | ND   | +    | ND   | [29]       |
| Ferritin                                     | ND   | +    | +/−  | [72, 73]   |
| Metallothionein-III                          | ND   | +/−  | ND   | [74]       |
| Copper/zinc superoxide dismutase             | ND   | +    | ND   | [72, 75]   |

**Oligodendroglial markers (proteins predominantly expressed in OLGs)**

* LBs with clinical signs of parkinsonism; ** OLGs; "ND" means not determined.
recent years. This trend may have been accelerated by the
notion of neuronal inclusion formation in autophagy-
deficient Atg5-knockout mice, as well as by the disclosure
of the genetic association between GBA variants and inci-
dence of MSA in the context of autophagic dysregulation
[3, 105]. Indeed, in vitro observations have revealed that
pharmacologic and genetic inhibition of autophagy causes
significant accumulation of both endogenous and exogen-
ously applied α-syn in oligodendroglial cells [11, 106]. Dys-
function of the autophagy-lysosome system in MSA is also
regulated by transcriptional and epigenetic mechanisms. A
recent report showed that the autophagy-suppressing micro-
RNAs, miR-101 and let-7b, are significantly increased in the
striatum of MSA brains [107]. The report also clarified that
lentiviral delivery of an anti-miR-101 construct to the stri-
atum of the MBP-α-syn transgenic mouse model of MSA re-
sults in reduced oligodendroglial α-syn accumulation and
improved autophagic clearance. Another post-mortem study
showed that GCIs contain an upstream protein of autophagy,
autophagy/beclin1 regulator 1 (AMBRA1), the overexpres-
sion of which leads to mild reduction of abnormal α-syn in
HEK293 cells co-transfected with S129E α-syn [58]. More-
over, the protein expression levels of AMBRA1 are increased
in MSA brains, whereas those of an upstream regulator,
TNF receptor associated factor 6, are significantly decreased,
suggesting that the upstream autophagy regulation pathways
are impaired [58]. Nevertheless, evidence is still lacking re-
garding whether autophagic dysregulation precedes α-syn
accumulation in OLGs. Interestingly, induced pluripotent
stem cell (iPSC)-derived dopaminergic neurons from
MSA patients show aberrant autophagic machinery with-
out obvious inclusion formation [108]. These findings sug-
gest the presence of a general autophagy defect in MSA
brains as a prodromal condition predisposing the individ-
ual to the emergence of GCIs.

Iron accumulation in affected areas of MSA brains is a
pathological hallmark of the disease [109]. In addition to
the increase in the total iron concentration, expression of
the iron storage protein, ferritin, is increased, and the iron
export protein, ferroportin, is decreased in the pons of pa-
tients with MSA, suggesting the presence of dysregulated
bioavailability of iron in MSA brains [110]. OLGs play
critical roles in iron homeostasis, as these cells contain
iron and express iron-binding proteins such as transferrin
in the normal CNS [111]. Iron levels in basal ganglia (pu-
tamen, globus pallidus, and caudate nucleus) are physiolo-
gically higher than those of the other brain areas [112].
These physiological profiles of iron expression may be
partially associated with the predisposition of iron accumu-
lation in OLGs and basal ganglia of MSA brains. The

Table 1 Comparison of molecular components within GCIs, NCIs, and LBs * through analysis with post-mortem human brains
(Continued)

| Protein                                    | GCIs | NCIs | LBs * | References |
|--------------------------------------------|------|------|-------|------------|
| Midkine                                    | +    | −    | ND    | [76]       |
| Leu-7                                      | +    | ND   | ND    | [77]       |
| **Others**                                 |      |      |       |            |
| Elk1                                       | +    | ND   | +     | [78, 79]   |
| cdk-5                                      | +    | ND   | +     | [69, 80]   |
| P39                                        | +    | ND   | ND    | [81]       |
| DARPP32                                    | +    | ND   | ND    | [82]       |
| Rab5, Rabaptin5                            | +    | ND   | +     | [83, 84]   |
| Sept4                                      | +    | ND   | +     | [85]       |
| Protein disulfide isomerase (PDI)          | +    | ND   | +     | [71, 86]   |
| Apolipoprotein E                            | +/-  | ND   | +***  | [72, 87]   |
| Clusterin/apolipoprotein J                 | +/-  | –    | +/-   | [88]       |
| matrix metalloproteinase-2                 | +    | ND   | ND    | [89]       |
| transactive response DNA-binding protein of 43 kDa (TDP-43) | +/- | +/- | +/- | [90, 91] |
| **Silver stain**                            |      |      |       |            |
| Campbell-Switzer                           | +    | +/-  | +     | [92, 93]   |
| Bodian                                     | +    | +/-  | +     | [29, 92, 93] |
| Bielshowsky                                | +    | +/-  | +     | [29, 92, 93] |
| Gallyas                                    | +    | +    | –     | [29, 92–94] |

The presence/absence of each protein’s expression within GCIs and NCIs in MSA brains, and within LBs in PD brains is displayed. The lists of proteins and their profiles described above are modified from [72, 95, 96].

+, positive; +/-, partially or weakly positive; −, negative; ND, not described. *, described as +, or +/- when the positivity was recognized in either brainstem-type or cortical LBs; **, proteins other than iron-related proteins; ‘***,’ amino-terminal 17 kDa fragment of Apolipoprotein E.
presence of an iron responsive element in the 5′-untranslated region of the α-syn transcript implies the potential for induction of excessive α-syn production triggered by iron accumulation [113]. In vitro experiments have clarified that various metals including iron cause significant acceleration in the rate of α-syn fibril formation, and both ferritin and transferrin are contained within GCIs [29, 114]. Although these findings suggest that iron dysregulation may be a potential predisposing event of MSA pathogenesis, the alteration in the iron concentration has not been documented in the locus coeruleus of MSA brains where severe neuronal loss is frequently observed [115]. Moreover, a MRI study shows that putaminal iron accumulation occurs under volume atrophy or change in microstructural integrity, implying that putaminal iron deposition in MSA brains is a secondary byproduct of neurodegeneration [116]. At present, whether iron dysregulation is a primary event underlying the pathogenesis of GCIs or a secondary event as the result of neuronal degeneration and subsequent microglial activation is unclear [117].

How GCI pathology spreads?

The concept of α-syn as a prion-like protein originates from the description of LBs in grafted neurons within the post-mortem brains of PD patients who were transplanted with human fetal mesencephalic dopaminergic neurons [118, 119]. Further in vitro studies have proven that extracellularly applied α-syn fibrils induce endogenous soluble α-syn in primary neurons to form insoluble fibrillary α-syn aggregates [120]. The prion-like property of GC1-α-syn persists even after serial propagation in α-syn transgenic mice [121]. These findings led to the hypothesis that prion-like propagation of GC1-α-syn pathology contributes to the disease progression of MSA. Interestingly, GCIs are immunoreactive to Rab5, a Rab protein that is involved in the transport of cell surface molecules to early endosomes [83]. Rab5 proteins are expressed not only in neurons but...
also in OLGs, and their expression increases with OLG differentiation [122, 123]. The colocalization of fibrillary α-syn and early endosomes may be proof of cell-to-cell transfer by which α-syn pathology spreads in MSA brains. Nevertheless, trans-synaptic propagation of α-syn pathology, which is suggested in PD pathology, cannot be simply applied to the OLG-specific distribution of α-syn pathology in MSA [124, 125]. In fact, GCIs seem to be distributed randomly or present in clusters, and their spatial patterns are different from those of neuronal inclusions in other neurodegenerative diseases [126]. Considering these observations, cell-to-cell propagation of GCI-α-syn pathology may occur in a distinctive pattern.

Identifying the route and regulator of OLG-specific disease propagation in MSA pathology is challenging. Although some ELISA-based studies did not detect a significant change in total α-syn concentrations in the cerebrospinal fluid (CSF) of MSA patients, an increase in phosphorylated oligomeric α-syn may be present [127, 128]. However, α-syn-immunoreactive inclusions in mouse brains injected with GCI-α-syn are observed predominantly within neurons, rather than within OLGs [97, 99]. These observations may indicate the existence of exclusive OLG-to-OLG communications specific to MSA brains.

Overall, some unanswered questions remain regarding the pathological contribution of the prion-like property of GCI-α-syn for MSA disease progression. Even if the prion-like property of GCI-α-syn explains how pathological conformation spreads from cell to cell in MSA brains, the explanation of what triggers the emergence of the primary seeds is still missing.

Future hot topics: the possible involvement of OPCs in the spreading of GCI pathology

To further our understanding of the mechanism underlying the propagation of GCI pathology, two aspects of the GCI development require scrutiny: 1) How does misfolded α-syn induce prion-like propagation between OLGs? and 2) Which cell produces the excessive amount of α-syn, serving as the main source of aggregated α-syn in GCIs?

Unlike neurons, OLGs possess a unique repair mechanism, which is enabled by the presence of OPCs. OPCs are abundant in adult brains, making up 5–8% of the glial cell population [6]. In response to various types of CNS damage, OPCs show extensive proliferation, migration, and differentiation in an attempt to compensate for demyelination [129, 130]. A few investigations suggest that the numbers of OPCs are increased in MSA brains [131]. However, the number of mature OLGs is reported to be not decreased, even in the presence of abundant GCIs, neuronal loss, and myelin loss [101, 132, 133]. The pathological finding of myelin loss accompanied by preserved numbers of OLGs in MSA brains may imply that α-syn-induced impairment of remyelination involves reduced myelin turnover and defective replacement of damaged OLGs [101].

An ongoing debate remains regarding whether OPCs are involved in MSA pathology. The difficulty in the immunohistochemical detection of OPCs in human tissues is probably due to the excessive vulnerability to fixation and the low specificity of antigens which are enriched in OPCs [134]. However, it seems very likely that some immature oligodendrocytes in MSA brains contain α-syn-immunoreactive inclusions [11, 131]. In vitro experiments using primary culture indicate that extracellularly applied α-syn is taken up by OPCs and disrupts their maturation [11]. Importantly, although recombinant human α-syn pre-formed fibrils (PFFs), which induce inclusions in neurons, are incorporated into OPCs leading to intracellular inclusion formation, α-syn PFFs do not induce inclusions when applied to OLGs (Fig. 2a and b) [11, 120]. Furthermore, once α-syn PFFs are incorporated into OPCs, intracellular inclusions can be detected even after their maturation, causing insufficient neurosupportive function (Fig. 2c) [11]. The presence of unique resistance against inclusion formation in mature OLGs is also speculated from in vivo observations using mice injected with brain extracts from MSA patients [99]. Given the fact that normal OLGs cannot take up extracellular seeds, three hypothetical explanations have been proposed for seed propagation between OLGs in MSA brains: 1) the uptake of extracellular seeds occurs before complete OPC maturation (Fig. 3a), 2) OLGs in MSA acquire an abnormal uptake mechanism that allows the invasion of extracellular seeds (Fig. 3b), and 3) seeds are transferred to OLGs via tunneling nanotubes or extracellular vehicles such as exosomes and other glial cells (microglia and astrocytes) [135–137] (Fig. 3c).

At present, the uptake mechanism of α-syn PFFs into OPCs is not clarified. Considering the possibility that normal OPCs and OLGs in MSA brains share the same mechanism of α-syn PFFs uptake (Fig. 3c), it is critically important to elucidate the regulators of their uptake in OPCs. Inhibitors of clathrin-mediated endocytosis did not affect α-syn PFFs uptake into OPCs, although RNA-seq analysis of α-syn PFF-treated OPCs showed increased gene expressions of Rab proteins which mediate endocytosis [11]. The quantification of gene and protein expression levels of lymphocyte activation gene-3 (LAG3), which is a membranous protein known to bind specifically with α-syn PFFs, also failed to specify how α-syn PFFs uptake into OPCs is controlled [11, 138]. Thorough investigations of oligodendroglial transmembrane proteins and their association with pathological α-syn may reveal the mechanism of cell-to-cell propagation of the pathological seeds.

It is also of note that disclosure of OPC pathology in MSA brains may contribute to the development of cell therapies. In fact, OPC transplantation successfully promoted remyelination and functional recovery in a chronic demyelinated
Transplantation of OPCs, which are genetically modified to resist \( \alpha \)-syn pathology, may not only encourage the replacement of the impaired OLGs but also arrest disease progression in MSA brains.

How \( \alpha \)-syn accumulates in OLGs: the source of \( \alpha \)-syn in GCIs

The internalized misfolded \( \alpha \)-syn (Fig. 3 left, pre-GCI) presumably self-assembles through the interaction with a large amount of \( \alpha \)-syn to eventually form the perinuclear fibrillary structure (Fig. 3 left, mature GCI). There are two possibilities regarding which cell produces the majority of \( \alpha \)-syn composing GCIs in MSA brains: OLGs and neurons (Fig. 3d, e).

OLGs express less \( \alpha \)-syn protein than neurons. Previous assays with in situ hybridization were not sufficiently sensitive to detect \( \alpha \)-syn mRNA within OLGs of either control or MSA brains [140, 141]. In contrast, analysis of oligodendroglial mRNA expression using laser-capture microdissection...
showed a 1.6-fold increase in SNCA mRNA expression in MSA OLGs compared with control OLGs; however, this increase was not statistically significant for inducing pathological aggregate formation [142]. In rat primary culture, α-syn protein expression in OLGs is approximately 30% of that in neurons [11]; iPSC-derived OLGs from healthy individuals and MSA patients showed the presence of α-syn protein expression in OLG lineage cells, although the expression is reduced by up to 70% when cells are fully mature [143]. Curiously, our recent in vivo observation of wild-type mice brains injected with aggregated α-syn revealed that the emergence of oligodendroglial α-syn pathology occurs over several months after that of neuronal α-syn pathology [144]. This notion is extremely important considering that modest amount of endogenous α-syn expression in wild-type OLGs can contribute to GCI formation over a long period.

Currently, the leading hypothesis for α-syn accumulation within OLGs is that α-syn is transferred from neurons to OLGs (Fig. 3e). Given that GCI-specific α-syn fibrils can be generated only within OLGs, the soluble form of α-syn may be transferred from neurons to OLGs [97]. A few in vitro and in vivo studies have tried to observe neuronal release and oligodendroglial uptake of α-syn [145, 146]. The neuron-to-OLG communication may be mediated not only by the delivery of proteins but also by the delivery of mRNA and microRNA [147]. In spite of these possibilities, however, the elevation of neuronal α-syn mRNA expression has not been confirmed in MSA brains [142, 148]. These facts may imply that the presence of α-syn overproduction in OLGs or neurons is less relevant to the pathomechanism of inclusion formation compared with the conformational change of α-syn [149, 150]. This notion is also supported by recent studies which revealed even minute amount of endogenous α-syn in oligodendroglial cells can contribute to inclusion formation [11, 151].

The involvement of microglia and astrocytes in MSA pathology

Microglia and astrocytes are involved in the disease progression of MSA, which notion is supported by observation of increased numbers of these cells in MSA brains [133, 152, 153]. Activation of microglia seems evident in white matter regions where α-syn inclusions are abundantly observed [154]. Evidence is still lacking with regard to whether microglial activation precedes the emergence of GCIs or prodromal symptoms such as rapid eye-movement sleep behavior disorder [155]. Microglial activation due to fibrillar α-syn and subsequent production of pro-inflammatory cytokines such as interleukin (IL)-1β through NACHT, LRP and PYD domain-containing protein 3 (NLRP3) and Apoptosis-associated speck-like protein containing a CARD (ASC) inflammasome activation is closely linked to dopaminergic neurodegeneration in MSA brains [152, 156]. In addition, some in vitro studies have demonstrated that misfolded α-syn is responsible for microglial activation via signaling through Toll-like receptors [157, 158].

Reactive microglia in α-syn pathology may also serve as a potential vehicle for cell-to-cell α-syn spread. This notion is supported by the presence of microglial cells bearing α-syn inclusions distal from GCIs in MSA brains [137]. Microglia take up exosomes released by α-syn-containing OLGs via macropinocytosis, but have a low capability of degrading fibrillar α-syn in vitro [159]. Given the crucial roles of the NLRP3-ASC inflammasome in amyloid β (Aβ)- and tau-induced microglia on accumulation and propagation of Aβ and tau [160, 161], reactive microglia with a high migratory capacity in α-syn pathology may also accelerate development of α-syn aggregates and spread via uptake and re-release of α-syn. Further studies are needed to examine if blocking the microglial inflammasome, α-syn uptake, or migration could indeed mitigate disease progression in MSA animal models.

Astrocytes also appear to be activated by α-syn in MSA brains [162]. Accumulation of α-syn inclusions in astrocytes can be observed among subpial and periventricular regions of MSA patients, especially those with a long disease duration [163]. Emergence of α-syn inclusions is also reported with primary astrocyte culture, which is exposed to synthetic and patient-derived aggregated α-syn [164, 165]. It is, however, still unclear whether these astrocytic responses enhance neurodegeneration or neuroprotection in MSA brains. On one hand, reactive astrocytes, which take up α-syn, secrete increased levels of cytokines (IL-1α, IL-1β, IL-6, etc.), colony-stimulating factors, and chemokines, triggering inflammatory neurodegenerative processes [166]. On the other hand, the accumulation of α-syn within astrocytes may reflect the process of astrocytic degradation, which is neuroprotective against cytotoxic α-syn [164]. Another possible mechanism of astrocyte-mediated neuroinflammation may be mediated through the conversion of normal astrocytes into the neurotoxic A1 phenotype through α-syn-induced microglial activation. Notably, a recent study reported that the glucagon-like peptide-1 agonist, NYL01, which blocks microglial activation and the generation of A1 astrocytes, may prolong the survival and reduce the neuropathology in a model of α-synucleinopathy [167]. In terms of non-cell autonomous α-syn spread, PD patient-specific iPSC-derived dysfunctional astrocytes accumulate and transfer pathological α-syn species to healthy dopaminergic neurons, resulting in neurodegeneration [168].

From GCI to neurodegeneration: how can this process be prevented?

Although various players are suspected to contribute to neurodegeneration in MSA, the main contributing factor remains elusive. One important finding from post-mortem analysis is the unique cell populations in MSA brains:
neuronal loss with preserved numbers of OLGs and increased numbers of microglia [101, 131–133]. Based on these observations, α-syn-induced neuronal loss in MSA can be attributed to three possible components: 1) cytotoxicity of abnormal α-syn against neurons, 2) insufficient neuronal support from OLGs, and 3) cytotoxicity mediated through microglial and astrocytic activation.

NCIs are commonly observed in MSA brains, and this observation suggests the presence of direct interactions between α-syn and neurons [21]. Some oligomeric α-syn species exert their neurotoxicity through induction of calcium ion flux [169]. CSF from MSA patients induces cytotoxicity via activation of endoplasmic reticulum stress and autophagy in cultured neuroblastoma cells and the substantia nigra of CSF-injected mice [170]. This cytotoxicity may be mediated through the uptake of soluble oligomeric α-syn species.

Reduced expression of myelin-associated proteins and neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) and glial-derived neurotrophic factor (GDNF), was reported in an MSA mouse model [101, 131, 171]. In experiments with primary rat OLG culture, OLGs show specifically high mRNA expression of GDNF, and conditioned medium from OLGs has a strikingly positive effect on the survivability of primary neurons [11]. Yet the impaired neurotrophic support from OLGs has not been fully evaluated on a cellular level in MSA brains.

**MSA animal models: how useful?**

The main approaches to replicating MSA pathology in animals include administration of neurotoxins and generation of transgenic animals. Intracerebral injection of 6-hydroxydopamine and quinolinic acid and systemic administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, rotenone, and 3-nitropropionic acid are commonly used [172]. With the objective of replicating GCI formation, the pathological hallmark of MSA, transgenic overexpression of α-syn under control of the promoter of OLG markers is often used. Each transgenic model using the promoter of 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNP), myelin basic protein (MBP), and proteolipid protein (PLP) has slightly different characteristics, although all models develop insoluble aggregates containing phosphorylated α-syn within OLGs (Table 2). Moreover, in all of these models, neuronal degeneration and predominant oligodendrogial α-syn aggregate formation were demonstrated, consistent with the non-cell autonomous mode of neurodegeneration in MSA [173, 175, 177]. The limitations applicable to all types of models are as follows: 1) high expression levels of oligodendroglial α-syn mRNA/protein are not consistent with MSA brain pathology [148], and 2) none of these animal models replicates olivopontocerebellar pathology of MSA brains (Table 2).

It is also of note that injection of synthetic α-syn PFF into the brains of non-α-syn-overexpressing wild-type mice enables replication of oligodendrogial α-syn pathology after long post-injection intervals of several months [144]. Further approaches for the development of non-α-syn-overexpression MSA models may contribute to the interpretation of disease pathogenesis, which precedes the accumulation of pathological α-syn in OLGs.

**Conclusions**

As recently described, the GCI-α-syn species can maintain its conformational and prion-like property even in a different cellular environment [97]. Although direct evidence is still lacking with regard to whether prion-like propagation contributes to the disease progression of MSA, further understanding of α-syn conformation and the components of pathological inclusions may provide critical information about the pathogenesis.

| Promotor | CNP | MBP | PLP |
|----------|-----|-----|-----|
| Phosphorylated α-syn aggregates in OLGs | ++ | ++ | ++ |
| Neuronal loss | | | |
| Striatonigral system | – | ++ | ++ |
| Olivopontocerebellar system | ND | ND | – |
| Spinal cord | + | – | ++ |
| Demyelination | + | ++ | – |
| Microglial activation | ND | ++ | ++ |
| Phenotype | | | |
| Motor | ++ | ++ | + |
| Non-motor | – | ND | ++ |
| Reference | [173, 174] | [175, 176] | [177, 178] |

The results of immunohistochemical and phenotypic analysis of each MSA mouse model are highlighted. ++, present within 12 months of age; +, present after 12 months of age; –, not clearly observed; ND, not described; *, moderate expresser line; **, not significant in substantia nigra; ***, mainly parasympathetic outflow.
Given that GCIs precede neuronal loss in MSA brains and misfolded α-syn exerts neurotoxicity, prevention of the generation and propagation of GCIs seems to modify the disease progression of MSA. As for the primary generation of GCIs, even though age-dependent decline of OLG function may precede triggering the misfolding of α-syn in MSA, GCIs have not been replicated in vivo without overexpressing oligodendroglial α-syn [7]. While a few recent studies have described the role of endogenous α-syn in GCI formation, the mechanism of misfolded α-syn uptake into oligodendroglial cells needs to be elucidated in order to block the OLG-to-OLG propagation of GCIs [11, 151].

Importantly, our recent observations have shown that OPCs incorporate extracellularly applied fibrillar α-syn, whereas their maturation results in decreased uptake activity [11]. Although not only OPCs but also other glial cells can take up misfolded α-syn, the emergence of α-syn-immunoreactive inclusions in OPCs is of great pathological significance, considering their capability to become OLGs [10]. Disclosure of the uptake mechanism, which regulates the entry of misfolded α-syn into oligodendroglial cells probably contributes to the development of disease-modifying therapy against MSA.

Abbreviations
AMBR1: Autophagy/beclin1 regulator 1; BDNF: Brain-derived neurotrophic factor; CNP: 2',3'-cyclic nucleotide 3'-phosphodiesterase; CNS: Central nervous system; COQ2: Coenzyme Q2; CSF: Cerebrospinal fluid; DARP32: Dopamine- and cAMP-regulated neuronal phosphoprotein; FBXO7: F-box only protein 7; GBA: Glucocerebrosidase; GCC: Glial cytoplasmic inclusion; GDNS: Glial-derived neurotrophic factor; GNI: Glial nuclear inclusion; HDAC6: Histone deacetylase 6; IL: Interleukin; IPSC: Induced pluripotent stem cell; LE: Lewy body; LRRK2: Leucine-rich repeat kinase 2; MAPK: Mitogen-activated protein kinase; MBP: Myelin basic protein; MSA: Multiple system atrophy; NBR1: Next to BRCA1 gene 1 protein; NCI: Neuronal cytoplasmic inclusion; NEDD8: Neural cell death modifier 8; NLPR3: NLR family, pyrin domain containing 3; NNI: Neuronal nuclear inclusion; NUB1: Negative regulator of ubiquitin-like protein 1; OLG: Oligodendrocyte; OPC: Oligodendrocyte precursor cell; PACBG: Parkin co-regulated gene; PARP-1: Poly(adenosine 5'-diphosphate-ribose) polymerase-1; PD: Parkinson’s disease; PDI: Protein disulfide isomerase; PFF: Pre-formed fibril; PNP: Polynucleotide phosphorylase; Ser: Serine; SNCA: α-synuclein; SUMO-1: Small ubiquitin modifier 1; TDP-43: Transactive response DNA-binding protein of 43 kDa; TPPP: Tubulin polymerization promoting protein; TREM2: Triggering receptor expressed on myeloid cells 2; XAP: X-linked inhibitor of apoptosis protein; αBARK: αB adrenergic receptor; α-syn: α-synuclein.

Acknowledgements
We thank all of our colleagues and staffs at the department of Neurology, Graduate School of Medicine, Kyoto University, including M. Ikuno, E. Nakanishi, M. Sawamura, S. Okuda, T. Taguchi, J. Ueda, S. Matsuzawa, I. Amano and R. Hikawa for their expert advice.

Authors’ contributions
SK performed the background studies, collected data, made all the figures, and wrote the manuscript. TM, TI, HY and RT reviewed the manuscript and provided scientific advice. All authors read and approved the final manuscript.

Funding
This work was funded by the Japan Agency for Medical Research and Development (AMED, 18ek0109384h0001, 19ek0109384h0002; T.M., H.Y., R.T.) and Kyoto University MSA Research Fund (RT). SK is supported by Grant-in-Aid for Research Activity start-up (18H06088) and Grant-in-Aid for Young Scientists (B) (19K16915) from Japan Society for the Promotion of Science in Japan.

Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate
Not applicable.

Consent for publication
All authors agreed to publish this article.

Competing interests
The authors declare that they have no competing interests.

Received: 24 October 2019 Accepted: 31 January 2020
Published online: 17 February 2020

References
1. Watanabe H, Saito Y, Terao S, Ando T, Kachi T, Muka E, et al. Progression and prognosis in multiple system atrophy: an analysis of 230 Japanese patients. Brain. 2002;125(Pt 5):1070–83.
2. Gilman S, Wenning GK, Low PA, Brooks DJ, Mathias CJ, Trojanowski JQ, et al. Second consensus statement on the diagnosis of multiple system atrophy. Neurology. 2008;71(9):670–6.
3. Mitsui J, Matsukawa T, Sasaki H, Yabe I, Matsushima M, Durr A, et al. Variants associated with Gaucher disease in multiple system atrophy. Ann Clin Trans Neurol. 2015;2(4):17–26.
4. Ozawa T, Tada M, Kakita A, Onodera O, Tada M, Ishihara T, et al. The phenotype spectrum of Japanese multiple system atrophy. J Neurol Neurosurg Psychiatry. 2010;81(11):1232–3.
5. Gai WP, Pountney DL, Power JH, Li QX, Culveron JG, McLean CA, et al. Alpha-Synuclein fibrils constitute the central core of oligodendroglial inclusion filaments in multiple system atrophy. Exp Neurol. 2003;181(1):68–78.
6. Levine JM, Reynolds R, Fawcett JW. The oligodendrocyte precursor cell in health and disease. Trends Neurosci. 2001;24(1):39–47.
7. Lee HJ, Ricarte D, Ortiz D, Lee SJ. Models of multiple system atrophy. Exp Mol Med. 2019;51(11):13139.
8. Vieira BD, Radford RA, Chung RS, Guillemin GJ, Pountney DL. Neuroinflammation in multiple system atrophy: response to and cause of alpha-Synuclein aggregation. Front Cell Neurosci. 2015;9:437.
9. Valera E, Masliah E. The neuropathology of multiple system atrophy and its therapeutic implications. Auton Neurosci. 2018;111:1–6.
10. Valdiniacci D, Radford RAW, Goulding M, Hayashi J, Chung RS, Pountney DL. Extracellular Interactions of Alpha-Synuclein in Multiple System Atrophy. Int J Mol Sci. 2018;19(12):4129.
11. Kaji S, Maki T, Kinosita H, Uemura N, Ayaki T, Kawamoto Y, et al. Pathological endogenous alpha-Synuclein accumulation in oligodendroglial precursor cells potentially induces inclusions in multiple system atrophy. Stem Cell Reports. 2018;10(2):356–65.
12. Perez-Lloret S, Flabeau O, Fernagut PO, Pavy-Le Traon A, Rey MV, Foubert-Samier A, et al. Current concepts in the treatment of multiple system atrophy. Mov Disord Clin Pract. 2015;2(1):16–16.
13. Moretti DV. Available and future treatments for atypical parkinsonism: a systematic review. CNS Neurol Ther. 2019;25(2):119–74.
14. Singer W, Dietz AB, Zeller AD, Gehring TL, Schmechel JD, Hauser SS, et al. Intrathelial administration of autologous mesenchymal stem cells in multiple system atrophy. Neurology. 2019;93(1):77–87.
15. Wenning GK, Quinn N, Magalhaes M, Mathias C, Daniel SE. “Minimal change” multiple system atrophy. Mov Disord. 1994;9(2):161–6.
16. Fukushima H, Atsumi T, Frigeno R, DeRicci J, Josephs KA, Parisi JE, et al. Glial cytoplasmic inclusions in neurologically normal elderly: prodomal multiple system atrophy? Acta Neuropathol. 2008;116(3):269–78.
17. Papp MI, Kahn JE, Lantos PL. Glial cytoplasmic inclusions in the CNS of patients with multiple system atrophy (striotrigonal degeneration, olivopontocerebellar atrophy and shy-Drager syndrome). J Neurol Sci. 1989;94(1–3):79–100.
Arima K, Murayama S, Mukoyama M, Inose T. Immunocytochemical and ultrastructural studies of neuronal and oligodendroglial cyttoplasmic inclusions in multiple system atrophy. 2. Oligodendroglial cyttoplasmic inclusions. Acta Neuropathol. 1992;84(1):32–8.

Cairns NJ, Atkinson PF, Hanger DP, Anderton BH, Daniel SE, Lantos PL. Tau protein in the glial cytoplasmic inclusions of multiple system atrophy can be distinguished from abnormal tau in Alzheimer's disease. Acta Neuropathol. 2003;105(4):409–41.

Chiba Y, Takei S, Kawamura N, Kawaguchi Y, Sasaki K, Hasegawa-Ishii S, et al. Accumulation of NEDD8 in neuronal and glial inclusions in multiple system atrophy. Brain Pathol. 2019;29(6):803–809.

Chiba Y, Takei S, Kawamura N, Kawaguchi Y, Sasaki K, Hasegawa-Ishii S, et al. Immunohistochemical localization of aggresomal proteins in glial cytoplasmic inclusions in multiple system atrophy. Neuropathol Appl Neurobiol. 2012;38(4):659–71.

Kawamoto Y, Aiguchi J, Shirakashi Y, Hanjo Y, Tomimoto H, Takahashi R, et al. Accumulation of Hsc70 and Hsp70 in glial cyttoplasmic inclusions in patients with multiple system atrophy. Brain Res. 2007;1136(1):219–27.

Uyta K, Richter-Landsberg C, Welch W, Sun A, Siddiqua O, Norris EH, et al. Converge of heat shock protein 90 with ubiquitin in filamentous alpha-synuclein inclusions of alpha-synucleinopathies. Am J Pathol. 2006;168(3):947–61.

Neumann M, Muller V, Gorner K, Kretzschmar HA, Haass C, Kahle PJ. Pathological properties of the Parkinson's disease-associated protein DJ-1 in alpha-synucleinopathies and tauopathies: relevance for multiple system atrophy and Pick's disease. Acta Neuropathol. 2004;107(6):489–96.

Lowe J, McDermott H, Pike I, Spendlove I, Landon M, Mayer RJ. Alpha B crystallin expression in non-lesional tissues and selective presence in ubiquitinated inclusion bodies in human disease. J Pathol. 1992;166(1):151–8.

Abe H, Yagishita S, Amano N, Iwabuchi K, Hasegawa K, Kowa K. Argyrophilic glial inclusions in multiple system atrophy - synuclein and neuronal degeneration. Rinsho shinkeigaku. 2011;51(11):838–42.

Lowe J, Blanchard A, Monrell K, Lennox G, Reynolds L, Billett M, et al. Ubiquitin is a common factor in intermediate filament inclusion bodies of diverse type in man, including those of Parkinson's disease, Pick's disease, and Alzheimer's disease, as well as Rosenthal fibres in cerebellar astrocytomas, cyttoplasmic bodies in muscle, and malory bodies in alcoholic liver disease. J Pathol. 1988;155(1):9–15.

Pountney DL, Chegini F, Shen X, Blumbergs PC, Gai WP. SUMO-1 masks subdomains within glial cyttoplasmic inclusions of multiple system atrophy. Neurosci Lett. 2005;381(1):74–9.

Kim YM, Jiang WH, Quezado MM, Oh Y, Chung KC, Junn E, et al. Proteasome inhibition induces alpha-synuclein SUMOylation and aggregate formation. J Neurosci. 2011;31(71):257–61.

Olanow CW, Perl DP, DeMartino GN, Naughts KS. Lewy-body formation is an aggresome-related process: a hypothesis. Lancet Neurol. 2004;3(8):496–503.

Miki Y, Mori F, Tanji K, Kakita A, Takahashi H, Wakabayashi K. Accumulation of histone deacetylase 6, an aggresome-related protein, is specific to Lewy bodies and glial cyttoplasmic inclusions. Neuropathology. 2011;31(6):561–8.

Murakami T, Shoji M, Imai Y, Inoue H, Kawarabayashi T, Matsubara E, et al. Pael-R is accumulated in Lewy bodies of Parkinson's disease. Ann Neurol. 2004;55(3):439–42.

Huang Y, Song YJ, Murphy K, Holton JL, Lashley T, Revesz T, et al. LRRK2 and parkin immunoreactivity in multiple system atrophy inclusions. Acta Neuropathol. 2008;116(6):639–46.

Hishikawa N, Niwa J, Doyu M, Ito T, Ishigaki S, Hishizume Y, et al. Dorfin localizes to the ubiquitinated inclusions in Parkinson's disease, dementia with Lewy bodies, multiple system atrophy, and amyotrophic lateral sclerosis. Am J Pathol. 2003;162(2):609–19.

Ito T, Niwa J, Hishikawa N, Ishigaki S, Doyu M, Sobe G. Dorfin localizes to Lewy bodies and ubiquitates synphilin-1. J Biol Chem. 2003;278(31):29106–14.

Mori F, Nishie M, Piao YS, Kito K, Kamitani T, Takahashi H, et al. Accumulation of NEDD8 in neuronal and glial inclusions of neurodegenerative disorders. Neuropathol Appl Neuropathol. 2005;31(1):53–61.

Tanji K, Mori F, Kakita A, Zhang H, Kito K, Kamitani T, et al. Immunohistochemical localization of NUB1, a synphilin-1-binding protein, in neurodegenerative disorders. Acta Neuropathol. 2007;114(4):365–71.

Tanji K, Miki Y, Mori F, Kon T, Kakita A, Takahashi H, et al. Phosphorylated NUB1 distinguishes alpha-synuclein in Lewy bodies from that in glial cyttoplasmic inclusions in multiple system atrophy. Brain Pathol. 2019;29(6):803–12.

Wakabayashi K, Engelender S, Tanaka Y, Yoshimoto M, Mori F, Tsujii S, et al. Immunohistochemical localization of synphilin-1, an alpha-synuclein-associated protein, in neurodegenerative disorders. Acta Neuropathol. 2002;103(3):209–14.

Wakabayashi K, Engelender S, Yoshimoto M, Tsujii S, Ross CA, Takahashi H. Synphilin-1 is present in Lewy bodies in Parkinson's disease. Ann Neurol. 2000;47(4):521–3.

Zhao T, Severijnen LA, van der Weiden M, Zheng PP, Oostra BA, Hukema RK, et al. FBXO7 immunoreactivity in alpha-synuclein-containing inclusions in Parkinson disease and multiple system atrophy. J Neuropathol Exp. 2013;72(6):482–8.

Tanji K, Odaigiri S, Maruyama A, Mori F, Kakita A, Takahashi H, et al. Alteration of autophagosomal proteins in the brain of multiple system atrophy. Neurobiol Dis. 2013;49:190–8.

Tanji K, Mori F, Kakita A, Takahashi H, Wakabayashi K. Alteration of autophagosomal proteins 0C3, GABARAP and GATE-16) in Lewy body disease. Neurobiol Dis. 2011;43(3):690–7.

Odaigiri S, Tanji K, Mori F, Kakita A, Takahashi H, Wakabayashi K. Autophagic adapter protein NBR1 is localized in Lewy bodies and glial cyttoplasmic inclusions and is involved in aggregate formation in alpha-synucleinopathy. Acta Neuropathol. 2012;124(2):173–86.

Miki Y, Tanji K, Mori F, Tada Y, Utsunomiya S, Sasaki H, et al. AMBRA1, a novel alpha-synuclein-binding protein, is implicated in the pathogenesis of multiple system atrophy. Brain Pathol (Zurich, Switzerland). 2016;26(3):359–67.

Probst-Cousin S, Rickert CH, Schmid KW, Gullotta F. Cell death mechanisms in multiple system atrophy. J Neuropath Exp. 1998;57(9):814–23.

Kawamoto Y, Kobayashi Y, Suzuki Y, Inoue H, Tomimoto H, Aiguchi I, et al. Accumulation of HtrA2/Omi in neuronal and glial inclusions in brains with alpha-synucleinopathies. J Neuropathol Exp. 2008;67(10):984–93.

Taylor JM, Song YJ, Huang Y, Farrer M, Delatycki MB, Halliday GM, et al. Parkin co-regulated gene (PACRG) is regulated by the ubiquitin-proteasomal system.
system and is present in the pathological features of parkinsonian diseases. Neurobiol Dis. 2007;27(2):238–47.

63. Kawamoto Y, Ito H, Iwara M, Takahashi R. XIAP immunoreactivity in glial and neuronal cytoplasmic inclusions in multiple system atrophy. Clin Neuropathol. 2014;33(1):76–83.

64. Kawamoto Y, Ito H, Iwara M, Takahashi R. Immunohistochemical localization of X-linked inhibitor of apoptosis protein in brainstem-type and cortical Lewy bodies. Neuroreport. 2012;23(3):162–7.

65. Kawamoto Y, Ayaki T, Urashtani M, Ito H, Takahashi R. Activated caspase-9 immunoreactivity in glial and neuronal cytoplasmic inclusions in multiple system atrophy. Neurosci Lett. 2016;628:207–12.

66. Kawamoto Y, Ito H, Ayaki T, Takahashi R. Immunohistochemical localization of apoptosome-related proteins in Lewy bodies in Parkinson’s disease and dementia with Lewy bodies. Brain Res. 2014;1571:39–48.

67. Kawamoto Y, Akiguchi I, Nakamura S, Budka H. Accumulation of 14-3-3 proteins in glial cytoplasmic inclusions in multiple system atrophy. Ann Neurol. 2002;52(6):722–31.

68. Kawamoto Y, Akiguchi I, Nakamura S, Honjyo Y, Shibasaki H, Budka H. 14-3-3 proteins in Lewy bodies in Parkinson disease and diffuse Lewy body disease brains. J Neuropathol Exp Neurol. 2002;61(3):245–53.

69. Nakamura S, Kawamoto Y, Nakano S, Akiguchi I, Kimura J. Cyclin-dependent kinase 5 and mitogen-activated protein kinase in glial cytoplasmic inclusions in multiple system atrophy. J Neuropathol Exp Neurol. 1998;57(7):690–9.

70. Alegre-Abarrategui J, Aronso O, Estiri M, Wade-Martin R, LRKRR is a component of granular alpha-synuclein pathology in the brainstem of Parkinson’s disease. Neurobiol Appl Neurobiol. 2006;34(3):372–83.

71. Honjo Y, Ito H, Horibe T, Takahashi R, Kawakami K. Protein disulfide isomerase immunopositive glial cytoplasmic inclusions in patients with multiple system atrophy. Int J Neuroscience. 2011;121(10):543–50.

72. Wakabayashi K, Takahashi H. Cellular pathology in multiple system atrophy. Neuropathology. 2006;26(4):338–45.

73. Jellinger K, Paulus W, Grundke-Iqbal I, Riederer P, Youdim MBH. Brain iron and ferritin in Parkinson’s and Alzheimer’s diseases. J Neural Transmission – Parkinson’s Disease Dementia Section. 1990;24:327–40.

74. Pountney DL, Dickson TC, Power JH, Vickers JC, West AJ, Gai WP. Midkine, a new neurotrophic factor, is present in glial cytoplasmic inclusions of brains with multiple system atrophy. Acta Neuropathol. 1995;89(6):471–8.

75. Pountney DL, Dickson TC, Power JH, Vickers JC, West AJ, Gai WP. Immunocytochemical study. Acta Neuropathol. 1995;89(6):471–83.

76. Kawamoto Y, Ito H, Ihara M, Takahashi R. XIAP immunoreactivity in glial and neuronal cytoplasmic inclusions in multiple system atrophy. Neuropathol Appl Neurobiol. 2010;36(5):789–801.

77. Uchihara T. Silver diagnosis in neuropathology: principles, practice and revised interpretation. Acta Neuropathol. 2007;113(5):483–99.

78. Saito M, Hara M, Ebashi M, Morita A, Okada K, Homma T, et al. Perinuclear accumulation of neuronal alpha-synuclein in a multiple system atrophy patient with dementia. Neurology. 2017;5(7):431–40.

79. Takeda A, Arai N, Komori T, Katayama K, Oda M. Neuronal inclusions in the dentato-rubral-pallidal fasciculus in patients with multiple system atrophy. Neurosci Lett. 1997;227(3):157–60.

80. Jellinger KA. Multiple system atrophy: an Oligodendrogialneural Synucleinopathy1. J Alzheimers Dis. 2018;62(3):1141–70.

81. Wakabayashi K, Tanji K, Mori F, Takahashi H. The Lewy body in Parkinson’s disease: molecules implicated in the formation and degradation of alpha-synuclein aggregates. Neuropathology. 2007;27(5):494–506.

82. Peng X, Gathagan RJ, Covell DJ, Medellin C, Stieber A, Robinson JL, et al. Cellular milieu imparts distinct pathological alpha-synuclein strains in alpha-synucleinopathies. Nature. 2018;557(7706):558–63.

83. Guo JL, Covell DJ, Daniels JP, Iba M, Stieber A, Zhang B, et al. Evidence for alpha-synuclein prions causing multiple system atrophy in humans with parkinsonism. Proc Natl Acad Sci U S A. 2015;112(28):E3088–3097.

84. Wilnening GK, Stefanova N, Jellinger KA, Poewe W, Schlossmacher MG. Multiple system atrophy: a primary oligodendrogliopathy. Ann Neurol. 2008;63(4):239–46.

85. Ettle B, Kerman BE, Valera E, Gillmann C, Schlachetzki JC, Reiprich S, et al. Alpha-Synuclein-induced myelination deficit defines a novel interventional target for multiple system atrophy. Acta Neuropathol. 2016;132(1):155–79.

86. Rohn TT, Mack JM. Apolipoprotein E Fragmentation within Lewy Bodies of Parkinson’s Disease Brain. Int J Neurodegenerative Disorders. 2018;11(5):2013–45.
109. Dexter DT, Carayan A, Javoy-Agid F, Agid Y, Wells FR, Daniel SE, et al. Alterations in the levels of iron, ferritin and other trace metals in Parkinson’s disease and other neurodegenerative diseases affecting the basal ganglia. Brain. 1991;114(Pt Pt 4):1953–75.

110. Visanji NP, Collinge JD, Finnegan ME, Tandon A, House E, Hazati LN. Iron deficiency in parkinsonism: region-specific iron dysregulation in Parkinson’s disease and multiple system atrophy. J Parkinson Dis. 2013;3(4):233–37.

111. Connor JR, Menzies SL, St Martin SM, Mufson EJ. Cellular distribution of transferrin, ferritin, and iron in normal and aged human brains. J Neurosci Res. 1990;27(4):595–611.

112. Ward RJ, Zucca FA, Duyn JH, Crichton RR, Zecca L. The role of iron in brain aging and neurodegenerative disorders. Lancet Neurol. 2014;13(10):1045–60.

113. Zhou ZD, Tan EK. Iron regulatory protein (IRP)-iron responsive element (IRE) signaling pathway in human neurodegenerative diseases. Mol Neurodegener. 2017;12(1):75.

114. Uversky VN, Li J, Fink AL. Metal-triggered structured transformational processes, aggregation, and fibrillation of human alpha-synuclein. Mol Neurodegener. 2003;18(Suppl):52–12.

115. Jellinger KA. Neuropathological spectrum of synucleinopathies. Mov Disord. 2005;20(4):504–6.

116. Lee MJ, Kim TH, Kim SJ, Mun CW, Shin JH, Lee GH, et al. Speculating the timing of iron deposition in the putamen in multiple system atrophy. Parkinsonism Relat Disord. 2019;63:106–10.

117. Kaindlstorfer C, Jellinger KA, Eschibock S, Stefanova N, Weiss G, Wenning GK. The relevance of iron in the pathogenesis of multiple system atrophy: a viewpoint. J Alzheimers Dis. 2018;64(1):1253–73.

118. Kordower JH, Chu Y, Hauser RA, Freeman TB, Olano CW. Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson’s disease. Nat Med. 2008;14(5):504–6.

119. Li JY, Englund E, Holton JL, Soulet D, Hagell P, Lees AJ, et al. Lewy bodies in Parkinson’s disease and multiple system atrophy: implications for the prion-like transmission hypothesis. Lab Invest. 2005;112(12):1613–24.

120. Visanji NP, Collingwood JF, Finnegan ME, Tandon A, House E, Hazrati LN. Iron system atrophy. Neurobiol Aging. 2014;35(10):2357–62.

121. Dexter DT, Carayon A, Javoy-Agid F, Agid Y, Wells FR, Daniel SE, et al. Alterations in the levels of iron, ferritin and other trace metals in Parkinson’s disease and other neurodegenerative diseases affecting the basal ganglia. Brain. 1991;114(Pt Pt 4):1953–75.

122. Visanji NP, Collinge JD, Finnegan ME, Tandon A, House E, Hazati LN. Iron deficiency in parkinsonism: region-specific iron dysregulation in Parkinson’s disease and multiple system atrophy. J Parkinson Dis. 2013;3(4):233–37.

123. Connor JR, Menzies SL, St Martin SM, Mufson EJ. Cellular distribution of transferrin, ferritin, and iron in normal and aged human brains. J Neurosci Res. 1990;27(4):595–611.

124. Ward RJ, Zucca FA, Duyn JH, Crichton RR, Zecca L. The role of iron in brain aging and neurodegenerative disorders. Lancet Neurol. 2014;13(10):1045–60.

125. Foulds PG, Yokota O, Thurston A, Davidson Y, Ahmed Z, Holton J, et al. Post neuronal alpha-synuclein fibrils induce Lewy body pathology leading to synaptic dysfunction and neuron death. Neurokin. 2011;72(1):57–71.

126. Woerman AL, Oehler A, Kazmi SA, Lee J, Halliday GM, Middleton LT, et al. Multiple system atrophy prions retain strain specificity after serial propagation in two different TgSNCA(AS3T) mouse lines. Acta Neuropathol. 2019;137(3):437–54.

127. Simonos M, Trajkovic K. Neuro-glia communication in the control of oligodendrocyte function and myelin biogenesis. J Cell Sci. 2006;119(Pt 21):4381–95.

128. Rodriguez-Gabin AG, Almazan G, Larocca JN. Vesicle transport in oligodendrocyte function and myelin biogenesis. J Cell Sci. 2001;17(4):4284–96.

129. Maki T, Lie N, Arai K. alpha-synuclein and TPPP/p25alpha signaling pathway in human neurodegenerative diseases. Mol Neurodegener. 2017;12(1):75.

130. Visanji NP, Collingwood JF, Finnegan ME, Tandon A, House E, Hazrati LN. Iron system atrophy. Neurobiol Aging. 2014;35(10):2357–62.

131. Kubler D, Wachter T, Cabanel N, Su Z, Turkheimer FE, Dodel R, et al. Widespread microglial activation in multiple system atrophy. Mov Disord. 2019;34(4):756–8.
154. Hoffmann A, Ettle B, Battis K, Reiprich S, Schlachetzki JC, Masliah E, et al. Oligodendroglial alpha-synucleinopathy-driven neuroinflammation in multiple system atrophy. Brain Pathol. 2019;29(3):380–96.

155. Stokholm MG, Iranzo A, Ostergaard K, Serrellid M, Otto M, Svendsen KB, et al. Assessment of neuroinflammation in patients with idiopathic rapid-eye-movement sleep behaviour disorder: a case-control study. Lancet Neurol. 2017;16(10):789–96.

156. Guo H, Callaway JB, Ting JP. Inflammasomes: mechanism of action, role in disease, and therapeutics. Nat Med. 2015;21(7):677–87.

157. Kim C, Ho DH, Suk JE, You S, Michael S, Kang J, et al. Neuron-released oligomeric alpha-synuclein is an endogenous agonist of TLR2 for paracrine activation of microglia. Nat Commun. 2012;4:1562.

158. Fellner L, Schmatz R, Schanda K, Reindl M, Klimaschewski L, Poewe W, et al. Toll-like receptor 4 is required for alpha-synuclein dependent activation of microglia and astroglia. Glia. 2013;61(3):349–60.

159. Fitzner D, Schnaars M, van Rossum D, Krishnamoorthy G, Dibaj P, Bakhti M, et al. Selective transfer of exosomes from oligodendrocytes to microglia by macropinocytosis. J Cell Sci. 2011;124(Pt 3):447–58.

160. Venegas C, Kumar S, Franklin BS, Derks T, Blinkschute R, Tejera D, et al. Microglia-derived ASC specks cross-seed amyloid-beta in Alzheimer’s disease. Nature. 2017;552(7685):355–61.

161. Stancu IC, Cremers N, Vanrussel H, Couturier J, Vanooijwuyse A, Kessels S, et al. Aggregated Tau activates NLRP3-ASC inflammasome exacerbating exogenously seeded and non-exogenously seeded Tau pathology in vivo. Acta Neuropathol. 2019;137(4):599–617.

162. Radford R, Rcom-H’cheo-Gauthier A, Wong MB, Eaton ED, Quilty M, Blizzard L, et al. The degree of astrocyte activation in multiple system atrophy is inversely proportional to the distance to alpha-synuclein inclusions. Mol Cell Neurosci. 2015;65:68–81.

163. Nakamura K, Mori F, Kon T, Tanji K, Miki Y, Tomiyama M, et al. Accumulation of phosphorylated alpha-synuclein in subpial and periventricular astrocytes in multiple system atrophy of long duration. Neurology. 2016;36(2):157–67.

164. Loria F, Vargas J, Boussert L, Yen S, Sales A, Melki R, et al. Alpha-Synuclein transfer between neurons and astrocytes indicates that astrocytes play a role in degradation rather than in spreading. Acta Neuropathol. 2017;134(5):89–100.

165. Krejciova Z, Carlson GA, Giles K, Prusiner SB. Replication of multiple system atrophy prions in primary astrocyte cultures from transgenic mice expressing human alpha-synuclein. Acta Neuropathol Communications. 2019;7(1):81.

166. Lee HJ, Suk JE, Patrick C, Bae EJ, Cho JH, Rho S, et al. Direct transfer of alpha-synuclein from neuron to astroglia causes inflammatory responses in synucleinopathies. J Biol Chem. 2010;285(12):9262–72.

167. Yun SP, Kim TJ, Panicker N, Kim S, Park JS, et al. Block of A1 astrocyte conversion by microglia is neuroprotective in models of Parkinson’s disease. Nat Med. 2018;24(7):931–8.

168. di Domenico A, Carola G, Calatayud C, Pons-Espinal M, Munoz JP, Richaud-Patin Y, et al. Patient-specific IPC-derived astrocytes contribute to non-cell-autonomous neurodegeneration in Parkinson’s disease. Stem Cell Reports. 2019;12(2):213–21.

169. D’Alessandro DM, Haasen D, Karow AR, Moussaud S, Habeck M, Giese A, et al. Different species of alpha-synuclein oligomers induce calcium influx and seeding. J Neurosci. 2017;27(23):9220–32.

170. Wang X, Ma M, Teng J, Zhang J, Zhou S, Zhang Y, et al. Chronic exposure to cerebrospinal fluid of multiple system atrophy in neuroblastoma and glioblastoma cells induces cytotoxicity via ER stress and autophagy activation. Oncotarget. 2015;6(15):13278–94.

171. Ufib K, Rockenstein E, Mante M, Inglis C, Adame A, Patrick C, et al. Neurodegeneration in a transgenic mouse model of multiple system atrophy is associated with altered expression of oligodendroglial-derived neurotrophic factors. J Neurosci. 2010;30(18):6236–46.

172. Stefanova N, Werning GK. Animal models of multiple system atrophy. Clin Auton Res. 2015;25(1):9–17.

173. Yazawa I, Glasson BI, Sasaki R, Zhang B, Joyce S, Uray K, et al. Mouse model of multiple system atrophy alpha-synuclein expression in oligodendrocytes causes glial and neuronal degeneration. Neuron. 2005;45(6):847–59.

174. Suzuki Y, Jin C, Isobe T, Yazawa I. Beta-III tubulin fragments inhibit alpha-synuclein accumulation in models of multiple system atrophy. J Biol Chem. 2014;289(35):24374–82.

175. Shults CW, Rockenstein E, Crews L, Adame A, Mante M, Larrea G, et al. Neurological and neurodegenerative alterations in a transgenic mouse model expressing human alpha-synuclein under oligodendrocyte promoter: implications for multiple system atrophy. J Neurosci. 2005;25(46):10689–99.