

The role of lysosomes in alpha-synucleinopathies: a focus on glial cells

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Lysosomes are the major degradative compartments within eukaryotic cells. Besides their role in the degradation and recycling of intra- and extracellular molecules, they further mediate important biological processes, such as immune signaling and perpetuation of nutrient- and energy homeostasis. Impairment of lysosomal function triggers the accumulation of catabolic products within the organelle resulting in lysosomal storage disorders (LSDs). Interestingly, clinical, molecular, and genetic studies further indicate a strong link between lysosomal dysfunction and neurodegenerative disorders, including Parkinson's disease (PD). Because of the association of lysosomal dysfunction and protein aggregation of α -synuclein (α -Syn) in PD or multiple system atrophy (MSA), the role of lysosomal pathways has been a matter of recent studies, mostly focusing on neuronal cells. Although it is known that glial cells play an important role in disease pathology of PD and MSA, only few studies on the lysosomal pathways, within glial cells have been carried out. Hence, a better understanding of lysosomal function in glia is needed to elucidate disease pathogenesis and to search for novel therapeutic approaches.

Lysosomal biogenesis: The prerequisite for unhampered lysosomal degradation is constituted by about 60 different acidic hydrolases and approximately 25 integral lysosomal membrane proteins. The latter preserves the acidic pH within the lysosomal lumen, maintain the ionic gradient and homeostasis, transport proteins into the lysosome, dispose catabolism products into the cytosol, and are important for membrane trafficking/fusion. The pathways in which lysosomes receive their biomaterial for the catabolic processing involve three main types of autophagy: micro- and macroautophagy, as well as chaperone-mediated autophagy (Trivedi et al., 2020). Lysosomal dysfunction, depending on the genetic defect and biochemical property of the accumulating substrate, can lead to severe pathology, accompanied with deficits in the central nervous system (CNS). There are several therapeutic strategies for LSDs which focus on either increasing the activity of the specific target enzyme, reducing substrate production, or modulating lysosomal exocytosis (Kreher et al., 2021).

Role of lysosomes in neurons during synucleinopathies: An efficient lysosomal function is pivotal for neuronal survival. As neurons reach a post-mitotic state after differentiation, the degradation of neurotoxic protein aggregates is essential to prevent an irreversible loss of neurons. Focusing on α -synucleinopathies, the aggregation of α -Syn represents a key factor for the development of neurodegenerative disorders like PD or MSA. There are several hypotheses for the occurrence of pathological α -Syn species and their toxicity, especially within dopaminergic neurons in the substantia nigra. It is suggested that in the early stages of PD, α -Syn pathology could have its origin in the olfactory bulb, or the dorsal motor nucleus of the vagus. Recent studies have identified the possibility of α -Syn aggregates to spread and seed pathology from cell-to-cell, reaching dopaminergic neurons by distribution through synaptic coupled networks. Further, it was observed that oxidative stress can trigger the conversion of the neurotransmitter dopamine into a reactive quinone species within dopaminergic neurons, which might contribute to their vulnerability. This reactive compound was shown to further cause lysosomal dysfunction, disturb lysosomal enzyme activity, and cause neurotoxicity (Surmeier, 2018).

Next to proteasomal processing, α -Syn can also get degraded via autophagy within neuronal cells. Recent studies implicate the lysosomal proteases cathepsin D (CTSD), cathepsin B (CTSB), and cathepsin L in the degradation of α -Syn. Additionally, impairments in the lysosomal degradation pathway or enzyme dysfunction can lead to the aggregation of α -Syn. Hence, disease-associated CTSD variants were shown to be impaired in their maturation and exhibited altered α -Syn degradation properties in human cell models (Bunk et al., 2021). Furthermore, it is known that mutations within the gene encoding for the lysosomal enzyme β -glucocerebrosidase (*GBA1*) (Figure 1) present a common genetic risk factor for the development of PD. *GBA1* gene mutations in PD patients lead to an insufficient degradation of its substrate glucosylceramide in lysosomes, which has been shown to interfere and accelerate α -Syn aggregation (Zunke et al., 2018). Interestingly, the aggregation of α -Syn also contributes to further lysosomal dysfunction, probably by interrupting lysosomal protein

trafficking. Genetic studies have associated further genes linked to lysosomal function, like the lysosomal hydrolases CTSD and CTSB, the cation-transporting ATPase 13A2 (*ATP13A2*) or vacuolar protein sorting-associated protein 35 (*VPS35*) to an increased risk of developing PD (Figure 1), indicating the importance of lysosomal function in neuronal homeostasis (Puska et al., 2018; Zunke et al., 2018).

Role of lysosomes within glial cells: The interaction between neurons and glial cells is essential for balanced brain homeostasis. Glial cells can be differentiated into three subtypes: astrocytes, microglia, and oligodendrocytes. Astrocytes, as the most abundant glial cell type in the CNS, have diverse supporting functions on neurons. These include nutrition supply, modulation of the blood-brain barrier, immune signaling, and neurotransmitter recycling. Microglia are CNS-resident macrophages, important for the immune defense within the brain, whereas oligodendrocytes are specialized in the synthesis of myelin for axonal insulation, which is crucial for proper neuronal function (Kreher et al., 2021). Many processes which are vital for glial cell function involve lysosomal or autophagic pathways and are summarized below for each glial cell type.

Lysosomes within astrocytes play an important role in membrane recycling, cell signaling, and clearance of protein compounds. Astrocytes can release proteolytic enzymes and signal molecules via lysosomal exocytosis. One important signal molecule is the gliotransmitter ATP, which contributes to the crosstalk between astrocytes and other cells of the CNS, facilitating neuronal activity as well as synaptic plasticity. The interaction between neurons and astrocytes becomes further evident in endocytic events: extracellular protein aggregates (e.g., α -Syn), myelin debris or toxic lipid droplets secreted from neurons can be taken up by astrocytes and processed within their lysosome. This indicates a potential molecular mechanism of the CNS to deal with harmful neuronal products (Kam et al., 2020; Kreher et al., 2021). In microglia, lysosomes are crucial for modulating synaptic plasticity and immune responses. The exocytosis of the brain-derived neurotrophic factor plays an important role regarding the development of dendritic spines of neurons. Furthermore, exocytosis of the acidic hydrolase cathepsin S, as expressed in antigen-presenting cells like microglia, could also contribute to spine formation by degradation processes of the extracellular matrix. Endo- and phagocytosis events of microglia are responsible for the clearance of myelin debris. Additionally, microglial phagocytosis is critical for the degradation of extracellular aggregates and pathogens, which underlines their essential role in immune response (Kreher et al., 2021).

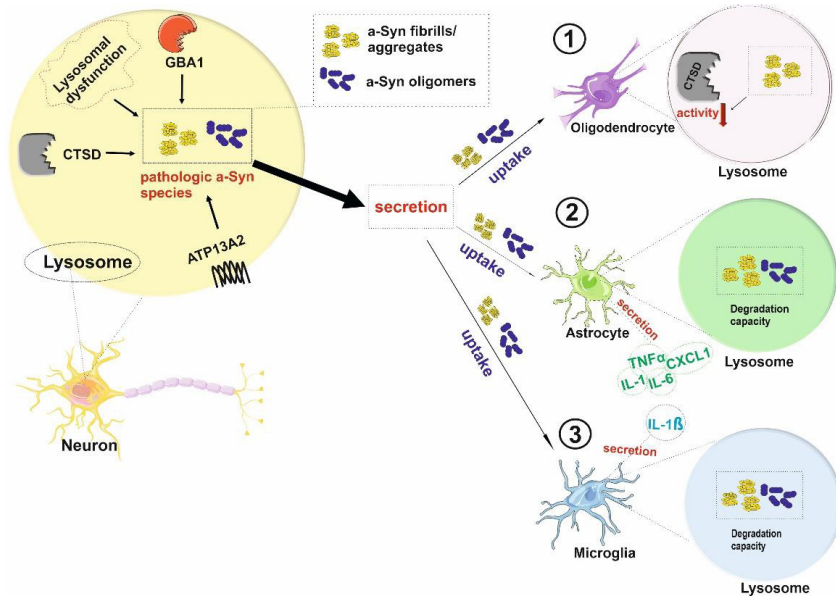


Figure 1 | Effects of secreted neuronal α -synuclein (α -Syn) species on different glial cells in the context of lysosomal pathways.

Lysosomal dysfunction as well as mutations within lysosomal enzymes, as for example *CTSD* and *GBA1*, or lysosomal membrane transport protein *ATP13A2* can trigger the formation of pathologic α -Syn species (fibrils/aggregates, oligomers) in neurons and potentially in lysosomes (Puska et al., 2018; Zunke et al., 2018). The resulting neuronal degeneration process can further lead to the secretion of α -Syn. Glial cells are able to degrade neurotoxic protein aggregates by their uptake and lysosomal clearance, thus contributing to neuroprotection. Secreted α -Syn protein aggregates can influence glial cells and their lysosomal function in certain ways: (1) The uptake of α -Syn aggregates in oligodendrocytes has been shown to decrease lysosomal CTSD activity (Kaji et al., 2018). (2) Astrocytes can clear endocytosed α -Syn aggregates in lysosomes, however, simultaneously stimulate the secretion of astrocytic pro-inflammatory factors like TNF α , IL-1, IL-6, or CXCL1. These factors induce neuroinflammation and further contribute to neurodegeneration processes (Kaji et al., 2020). (3) Microglia are critical participants in the immune response of the CNS by eliminating pathogens and protein aggregates. Microglial α -Syn uptake can also trigger neuroinflammation in neurodegenerative processes by the transmission of pro-inflammatory molecules, like IL-1 β (Kam et al., 2020; Kreher et al., 2021). Overall, the lysosomal function within glial cells could contribute towards protecting neurons from neurotoxic protein aggregates by degrading processes. Nevertheless, glial clearance capacity of protein aggregates is limited, and an overload of α -Syn accumulation can promote neuroinflammation. Moreover, it is unclear if and to what extent α -Syn aggregates within glial cells interfere with general lysosomal function and other intracellular pathways. Source of images (neurons and glial cells): <https://smart.servier.com/>.

Lysosomes within oligodendrocytes play a crucial role in the recycling process of certain myelin proteins. Myelin serves as an insulating layer by enveloping the axons of neurons. It consists of different proteins such as myelin basic protein, proteolipid protein or myelin-associated glycoprotein. Lysosomal exocytosis in oligodendrocytes has been shown to modulate myelin plasticity by secreting myelin proteins. The protein lethal giant larvae 1 (*Lgl1*) is known to mediate vesicular acidification as well as lysosomal maturation, since knockout (KO) oligodendrocyte precursor cells (OPCs) indicate abnormal alterations of lysosomal shape. In cell culture and transgenic mouse models, the deficiency of proteins involved in vesicle transport such as VAMP3/VAMP7, Rab27 or CTSD, can disturb the exocytotic process and thus, lead to an impairment of the myelination process, often linked to LSDs (Kreher et al., 2021).

Lysosomal pathways in glia associated with synucleinopathies: Recent studies document the important role of glial cells in the disease progression of PD and MSA.

Importantly, a cell-to-cell transmission of α -Syn released from degenerated neurons to neighboring glial cells has been proposed. It has been shown that primary glial cells of mouse models overexpressing wild-type α -Syn, are able to process aggregated α -Syn, contributing to α -Syn homeostasis and preventing neurodegeneration (Choi et al., 2020).

Although astrocytes express low levels of α -Syn, a deficiency of α -Syn leads to impaired uptake and trafficking of fatty acids in these glial cells (Kam et al., 2020). The uptake of neuronal α -Syn in astrocytes occurs via phagocytosis, which has been shown in a primary astroglial culture. There are several suggestions for possible transfer mechanisms of neuronal α -Syn between neurons and astrocytes. During oxidative stress, astrocytes are able to form tunneling nanotubes, which can serve as connection to other non-stressed cells. Concurrently, the formation of these nanotubes can also enhance α -Syn spreading. Additionally, the transmission of α -Syn via exosomes comprises another intracellular mechanism (Mavroudi and Xilouri, 2021).

The uptake of secreted, neuronal α -Syn species like oligomers or fibrils was demonstrated in induced pluripotent stem cells-derived astrocytes from a healthy control, suggesting a protective function towards pathologic α -Syn clearance (Figure 1) (Tsunemi et al., 2020). Moreover, investigations from co-cultures of primary astrocytes with human neuroblastoma cells show, that exogenous α -Syn drives the formation of inclusion bodies in astrocytes (Kam et al., 2020). The astrocytic uptake of neuron-derived α -Syn aggregates can also promote the production of astroglial proinflammatory cytokines (IL-1, IL-6, TNF α) and chemokines (CXCL1) (Figure 1), leading to neuroinflammation contributing to neurodegeneration in PD and MSA (Kaji et al., 2020; Kam et al., 2020). Therefore, α -Syn can be considered as an exogenous stimulator of astrocytes (Kam et al., 2020). Furthermore, induced pluripotent stem cell-derived astroglia carrying *GBA1* mutations, showed disturbed lysosomal enzyme activity and consequently aggregation of α -Syn (Kam et al., 2020). In line with this data, also *GBA1* KO mice demonstrated astroglial activation and abnormal α -Syn accumulation. Another mouse model deficient for *GBA1* within neural and glial progenitor cells exhibited increased expression of lysosomal cathepsins within astrocytes as well as neurons. These cathepsins were further distributed to degenerating neurons of affected brain areas, potentially driving disease pathology (Kam et al., 2020). It is still a matter of debate if astrocytes drive disease progression or have a neuroprotective effect within α -synucleinopathies. On the one hand, α -Syn accumulation in astrocytes mediates inflammatory events due to the secretion of cytokines or chemokines. On the other hand, aggregates as found within glial cells could be an indication of a neuroprotective degradation mechanism protecting neuronal cells from toxic protein accumulations (Kaji et al., 2020).

Interestingly, investigations of brain samples from MSA patients showed, that α -Syn aggregates, originated from oligodendroglia residues, were engulfed by astrocytes via phagocytosis and could be detected within astrocytic lysosomes (Puska et al., 2018). In fact, astrocytes show a higher endocytosis activity and lysosomal proteolysis compared to neurons, indicating a better capacity to degrade certain substrates, including α -Syn aggregates (Tsunemi et al., 2020). Therefore, astrocytic degradation processes regarding α -Syn clearance would comprise an essential therapeutic strategy, especially in α -synucleinopathies.

So far, there is no clear evidence for endogenous α -Syn expression in microglia. Nevertheless, a recent study verified the clearance of neuronal α -Syn by the autophagy-lysosomal pathway (Figure 1), which indicates the important role of

microglial neuroprotection (Choi et al., 2020). Moreover, microglia were able to take up α -Syn from exosomes released from oligodendrocytes (Kaji et al., 2020). However, pathologic α -Syn species, like oligomers or fibrils, can activate several microglial receptors and function as damage-associated molecular patterns (DAMPs). For instance, α -Syn can reduce microglial phagocytosis by binding on the surface receptor Fc γ R1IB and consequently, disturbs the clearance mechanisms of aggregated species or cell debris. Furthermore, fibrillar α -Syn can induce a series of pro-inflammatory events by activating the nuclear factor-kappa B (NF- κ B) pathway, which is crucial for microglial inflammatory response. The subsequent release of microglial cytokines (e.g., IL-1 β) contributes to neuroinflammation in PD (Kam et al., 2020). It is suggested that neuronal α -Syn can be phagocytosed by microglia via the lymphocyte-activation gene 3 (LAG3), and could further transmit α -Syn aggregates due to disturbed lysosomal clearance and exocytosis, which in turn induces pro-inflammatory microglial response (Figure 1; Kreher et al., 2021). Plasma levels of PD patients, carrying mutations in the coding gene for the lysosomal enzyme *GBA1*, were shown to have increased cytokine and inflammatory markers and promote microglia-mediated neuronal dysfunction (Kam et al., 2020). Surprisingly, the microglial β -glucocerebrosidase (*GBA1*) function has not been investigated on a mechanistic level to date.

Observations in MSA brains show a higher microglia cell density with increasing degeneration of neurons, possibly indicating a higher proliferation of microglia during neuroinflammation. With a high migration capacity, microglia possibly accelerate the formation and distribution of α -Syn aggregation and could promote α -Syn transmission by its uptake and release (Kaji et al., 2020).

Oligodendrocytes have been described to express low levels of endogenous α -Syn compared to neurons (Kaji et al., 2020). Accumulations of fibrillar α -Syn in oligodendrocytes are prevalent constituents of glial cytoplasmic inclusions in brain regions of MSA patients. Interestingly, an *in vitro* experiment in primary oligodendrocyte lineage cell cultures revealed, that external α -Syn fibrils did not affect the expression level of lysosomal enzymes, e.g., CTSD in OPCs and mature oligodendrocytes. However, α -Syn aggregates were able to diminish CTSD enzyme activity, especially in OPCs (Figure 1; Kaji et al., 2018). Importantly, CTSD deficiency in a transgenic mouse model has been shown to delay myelin maturation and oligodendrocyte development, underlining the importance of CTSD in oligodendrocyte function (Guo et al., 2018). Interestingly, in the terminal pathological phase of MSA, oligodendrocytes

rarely harbor lysosomal α -Syn in comparison to PD, where lysosomal α -Syn can be found in neurons (Puska et al., 2018). Surprisingly, the function and homeostasis of oligodendrocytes during disease progression in PD still remains elusive. In the future, the impact of OPCs and oligodendrocytes in α -synucleinopathy has to be investigated in more detail, allowing a better understanding of oligodendroglial alteration in α -Syn-related pathology.

Overall, α -Syn formations can trigger specific responses in the individual glial cells, which might contribute to either neuroprotection by lysosomal α -Syn degradation or further drive disease progression in neurodegeneration. This clearly underlines the importance of an unimpaired glial functionality with a special focus on lysosomal pathways.

Perspective: As depicted in this work, there is still a large lack of knowledge about the role of lysosomal pathways within glial cells under pathological, but also physiological conditions. In order to better understand the whole picture of disease pathways resulting in α -synucleinopathies, glial cell biology has to be studied in more detail. For instance, it is unknown if intracellular disease mechanisms, as found in neurons, recapitulate within glial cells. It would be interesting to know if α -Syn aggregates that have been shown to impact lysosomal function within neurons, for example by interfering with intracellular protein transport, exert the same effects within glia. Moreover, further studies will need to address the consequences of PD-associated genetic variants within lysosome-associated proteins in glial cells and how this compares to neurons. These future investigations are needed for a more detailed knowledge about the exact pathways of α -Syn uptake and clearance in glial cells to enable new therapeutic strategies in neurodegenerative diseases.

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