BMB Reports - Manuscript Submission

Manuscript Draft

Manuscript Number: BMB-22-073

Title: Role of post-translational modifications on the alpha-synuclein aggregation-related pathogenesis of Parkinson's disease

Article Type: Mini Review

Keywords: Parkinson's disease; alpha-synuclein; post-translational modification; protein aggregation; PTMs

Corresponding Author: Yohan Oh

Authors: Hajung Yoo[1], Jeongmin Lee[1], Bokwang Kim[1], Heechang Moon[1], Huisu Jeong[1], Kyungmi Lee[1], Woo Jeung Song[1], Junho K. Hur[1], Yohan Oh[1,*]

Institution: [1]Department of Biomedical Science, Graduate School of Biomedical Science and Engineering, Hanyang University, Seoul 04763, Republic of Korea, [2]Department of Medicine and [3]Department of Medical Genetics, College of Medicine, Hanyang University, Seoul 04763, Republic of Korea, [4]Hanyang Institute of Bioscience and Biotechnology, Hanyang University, Seoul 04763, Republic of Korea, [5]Department of Biochemistry and Molecular Biology, College of Medicine, Hanyang University, Seoul 04763, Republic of Korea,

FOR REVIEW

Manuscript Type: Mini Review

Title: Role of post-translational modifications on the alpha-synuclein aggregation-related pathogenesis of Parkinson's disease

Author's name: Hajung Yoo[1,6], Jeongmin Lee[1,6], Bokwang Kim[1,6], Heechang Moon[1,6], Huisu Jeong[1], Kyungmi Lee[2], Woo Jeung Song[3], Junho K. Hur[1,3,4] and Yohan Oh[1,4,5,*]

Affiliation:

[1] Department of Biomedical Science, Graduate School of Biomedical Science and Engineering, Hanyang University, Seoul 04763, Republic of Korea

[2] Department of Medicine, College of Medicine, Hanyang University, Seoul 04763, Republic of Korea

[3] Department of Medical Genetics, College of Medicine, Hanyang University, Seoul 04763, Republic of Korea

[4] Hanyang Institute of Bioscience and Biotechnology, Hanyang University, Seoul 04763, Republic of Korea

[5] Department of Biochemistry and Molecular Biology, College of Medicine, Hanyang University, Seoul 04763, Republic of Korea

[6] These authors contributed equally to this work.

Running Title: Role of PTMs of α-syn in the pathogenesis of PD

Keywords: Parkinson's disease; alpha-synuclein; post-translational modification; protein aggregation

Corresponding Author's Information: Tel.: +82-2-2220-2423; E-mail: yoh@hanyang.ac.kr

FOR REVIEW

ABSTRACT

Together with neuronal loss, the existence of insoluble inclusions of alpha-synuclein (α-syn) in the brain is widely accepted as a hallmark of synucleinopathies including Parkinson's disease (PD), multiple system atrophy, and dementia with Lewy body. Because the α-syn aggregates are deeply involved in the pathogenesis, there have been many attempts to demonstrate the mechanism of the aggregation and its potential causative factors including post-translational modifications (PTMs). Although no concrete conclusions have been made based on the previous study results, growing evidence suggests that modifications such as phosphorylation and ubiquitination can alter α-syn characteristics to have certain effects on the aggregation process in PD; either facilitating or inhibiting fibrillization. In the present work, we reviewed studies showing the significant impacts of PTMs on α-syn aggregation. Furthermore, the PTMs modulating α-syn aggregation-induced cell death have been discussed.

FOR REVIEW

INTRODUCTION

Alpha-synuclein (α-syn) encoded by *SNCA* gene is very well known as a potential key protein for the onset and the progression of Parkinson's disease (PD), which is one of the most common neurodegenerative diseases (1, 2). Although it has been suggested that α-syn in neurons would have certain roles in synaptic trafficking (3), it is mostly studied in relation to multiple neurodegenerative diseases, in particular, synucleinopathies because it is a highly amyloidogenic protein, and consequently prone to form aggregation. Synucleinopathies including PD, multiple system atrophy (MSA), and dementia with Lewy body (DLB) are characterized by accumulated α-syn insoluble inclusions observed in the patient's brain (4, 5). Therefore, the formation of the abnormal α-syn aggregates and their physiological features' effects on the brain function are mainly studied for understanding the synucleinopathies pathogenesis.

α-Syn aggregates are detected in different cell types and intracellular locations, depending on the associated disease. For example, in PD and DLB, aggregates called Lewy body (LB) or Lewy neurite (LN) are mainly observed in the cytoplasm of neurons (6); in MSA, glial cytoplasmic inclusions in the oligodendrocyte are predominant, although cytoplasmic inclusions and nuclear inclusions are found in the neurons as well (7). Consequently, α-syn can develop into inclusions in various locations under specific pathological circumstances. In normal physiology, however, α-syn exists in a state of equilibrium between soluble tetramers, unstructured monomers, and membrane-bound multimers (8, 9). Pathological conformations arise from the arrangement of β-sheet structure recruited in the process of forming insoluble α-syn oligomers, also known as protofibrils, which may eventually develop into LB (10-12). Organelles such as mitochondria and endosomes are known to be deeply engaged in the LB formation as the LB compartments thereby failing the original functional role inside the cell

FOR REVIEW

(13). In addition, oligomers and fibrils are known to have toxic effects on cells and therefore the whole aggregation process might result in dysfunction and degeneration of the neural cells (14-16). Furthermore, pathological propagation of α-syn aggregates to other brain regions occurs through cell-to-cell transmission in a prion-like manner (17, 18), which has been elucidated in the previous research using preformed fibrils (PFFs) generated from recombinant α-syn (19). Together, evidence from various studies suggests that α-syn is responsible for neuronal cell death; in consequent, its accumulation might be the leading force of the synucleinopathy progression (20).

Due to its pathological significance, the risk factors for α-syn aggregation have been a subject of great research interest. One of possible risk factors for the aggregation is the *SNCA*-related genetic risk factor, i.e., the familial PD-linked missense mutations in *SNCA* gene and copy number variations in *SNCA* locus (21). Several PD associated physiological environments, such as mitochondrial dysfunction, oxidative stress, and impaired protein degradation systems, known to be toxic to the cells and therefore causes of neuronal cell death have been studied in relation to their effect on the protein aggregation; however, the exact mechanisms of the α-syn aggregation process to form insoluble inclusion like LB remains to be further explored (22-25). In this review, the role of various post-translational modifications (PTMs), which alter conformations and functions of their target proteins, on the α-syn aggregation-related PD pathogenesis was investigated.

FOR REVIEW

PATHOLOGICAL IMPLICATIONS OF ALPHA-SYNUCLEIN POST-TRANSLATIONAL MODIFICATIONS ON FIBRIL FORMATION AND TOXICITY

It has been reported that α-syn is a viable target for a variety of PTMs in various sites; for example, acetylation, glycosylation, glycation, nitration, phosphorylation, ubiquitination, SUMOylation, and truncation. In this review, the possible role of PTMs in the α-syn aggregation process, either in developing or restraining the LB formation is investigated (Table 1). Since PTMs modify the translated proteins chemically resulting in alteration of physical or chemical properties of their targets, it is likely that PTMs in α-syn would also change their characteristics, such as structure and the propensity to interact with other organic or inorganic substances (26). The changes in the physiological properties of α-syn might either cause or prevent the accumulation of insoluble α-syn aggregates; therefore, it is expected that examining the PTMs on α-syn will give a revealing insight into the mechanism of the protein aggregation process and better understanding on the pathophysiological features relevant to the synucleinopathies including the LB formation in PD.

POST-TRANSLATIONAL MODIFICATIONS REDUCING THE AGGREGATION-RELATED PATHOGENESIS OF ALPHA-SYNUCLEIN

Some of the PTMs of α-syn have been reported to show protective effects by interrupting the aggregation of α-syn or decreasing cellular toxicity (Table 1). A comparative analysis with wild-type α-syn and modified α-syn is a popular method for determining the effect of PTMs on α-syn. Acetylation, one of the major PTMs that happens inside the cell, occurs at the very end of the amino-terminal region of α-syn, being a common event that is not limited to pathological conditions (9, 27, 28). *In vitro* studies using purified α-syn, however, revealed that acetylation can alter α-syn protein to aggregation resistant type by augmenting its α-helical

FOR REVIEW

structure (9, 28-31). Recent research showed that metal ions and 3,4-dihydroxyphenylacetaldehyde are involved in the reduction of the aggregation propensity of acetylated α-syn (32, 33). *O*-linked β-*N*-acetylglucosaminylation (*O*-GlcNAcylation) is a type of glycosylation that transfers *N*-acetylglucosamine (GlcNAc) to threonine or serine residues of target proteins by *O*-GlcNAc transferase, and *O*-GlcNAcylated α-syn proteins were detected in the human brain tissues (34). Multiple sites in α-syn, including threonine 72 (T72), T75, T81, and serine 87 (S87) residues located in the critical region for the aggregation (NAC, non-amyloid component, amino acid residues 65-90) (35, 36), are redundantly reported to be targeted by *O*-GlcNAcylation (34, 37-44). In studies using synthetic α-syn peptides, each of the four *O*-GlcNAcylation sites exhibited inhibitory effects on the α-syn fibril formation, which are synergistically amplified by the triple *O*-GlcNAcylation at T72, T75, and T81 residues (40-44). Interestingly, a cell-based study reported that α-syn PFF-treated mouse hippocampal primary neurons showed diminished seeding efficiency in α-syn aggregation in the presence of *O*-GlcNAcylated α-syn (40). A number of reports have suggested that glycation, the covalent binding of sugar to a protein without enzymatic regulation, may induce aggregation and cytotoxicity (45-47). However, other studies claimed that glycation of α-syn can suppress the formation of aggregates by reducing the conformational flexibility of α-syn (48, 49). All four tyrosine residues, tyrosine 39 (Y39), Y125, Y133, and Y136, in α-syn are known to be capable of being nitrated (50-55). It is not clearly revealed whether the nitration of α-syn enhances or inhibits the progression of α-syn aggregation-related PD pathogenesis. While the nitration of α-syn tends to strengthen oligomer formation (53, 55), some studies suggested that the nitration down-regulates the α-syn aggregation (51, 54, 56). The results imply that the nitration-induced soluble oligomers might inhibit the pathological α-syn inclusion formation by blocking fibril formation (56); suggesting a neuroprotective function in

FOR REVIEW

α-syn nitration. In both healthy and pathological physiology, a portion of α-syn is phosphorylated (57, 58). Numerous kinases are able to phosphorylate α-syn at various sites; for example, α-syn is phosphorylated by G protein-coupled receptor kinases (GRKs) at S129 (59-62), by casein kinases (CKs) at S87 and S129 (57, 63-67), by polo-like kinases (PLKs) at S129 (68-70), by death-associated protein kinase 1 (DAPK1) at S129 (71), and by tyrosine kinases c-Fgr, Syk, Lyn, Fyn, and Src at Y125 (72-74). The evidence supporting the neuroprotective feature of phosphorylation-induced modification of α-syn is presented by the works demonstrating the implication of kinase treated α-syn and the studies using mutant α-syn in which the phosphorylation site is replaced with another amino acid to mimic or block the phosphorylation (61, 64, 67, 70, 74-76).

The ubiquitin-proteasomal system (UPS) is one of the main mechanisms mediating the clearance of impaired α-syn (77). As might be expected, ubiquitination of α-syn is prevalently linked to the α-syn aggregation process, therefore, inhibiting aggregates formation. The ubiquitination of α-syn and its regulatory effects on the aggregation process have been studied with various ubiquitin E3 ligases. Co-chaperone carboxyl-terminus of Hsp70-interacting protein (CHIP) not only encourages degradation of α-syn by UPS and autophagy-lysosomal pathways (78), but cell-based research showed that ubiquitination mediated by CHIP eliminates oligomers (79). Similarly, neural precursor cells expressed developmentally down-regulated protein 4 (NEDD4)-mediated ubiquitination of α-syn exerted neuroprotective roles both *in vitro* and *in vivo* studies (80, 81). Besides the type of ubiquitin E3 ligases, the number and location of the ubiquitinated sites on α-syn are relevant factors for the anti-aggregation effect (82, 83). Interestingly, recent studies on the relationship between the ubiquitinated α-syn and its aggregation propensity supported the protective role of α-syn ubiquitination against synucleinopathies (78-87). SUMOylation is a process in which a small ubiquitin-like modifier

(SUMO) is attached to the target proteins, and it has been revealed that the SUMO protein co-localizes with pathological α-syn inclusion (88-90). The physiological roles of α-syn SUMOylation have been investigated in various model systems; converging data from *in vitro* protein fibrillization assay, cell-based model, animal model, and yeast model imply that SUMOylation enhances α-syn degradation and prevents fibrillization (91-93). Although 15% of the α-syn found in LB is truncated, the truncation of α-syn is a common modification that exists under healthy physiological conditions (94-96). Among the studies that have investigated the effect of truncation on α-syn, some data support the idea that truncation of α-syn plays a protective role in neurodegenerative diseases (75, 97-102). Interestingly, a previous study showed that soluble α-syn protein fibril formation is inhibited by calpain 1 (CAPN1)-mediated truncation while further fibrillization of already formed α-syn fibrils is enhanced by truncation (99, 100). Also, neurosin (KLK6, kallikrein-related peptidase 6)-mediated truncation can impede aggregation of α-syn site-specifically; α-syn truncation between K80-T81 may show an anti-aggregation effect, whereas the truncation between K97-D98 may enhance the aggregation (101).

Taken together, the above findings suggest that α-syn can be post-translationally modified by various enzymes or stimuli to reduce its propensity to form pathological aggregates. This implies that a lack of these anti-aggregation related PTMs might enhance the progression of synucleinopathy; this provides a new therapeutic target.

POST-TRANSLATIONAL MODIFICATIONS INCREASING THE AGGREGATION-RELATED PATHOGENESIS OF ALPHA-SYNUCLEIN

Various studies have shown that α-syn PTMs can enhance the aggregation-related PD pathogenesis; PTMs such as glycation, nitration, phosphorylation, ubiquitination,

FOR REVIEW

SUMOylation, and truncation, have been reported to have both enhancing and suppressing aspects on the pathological α-syn aggregation (Table1). Glycation of α-syn has an inhibitory effect against α-syn aggregation (48, 49), but the general consensus suggested by numerous reports is that glycation fosters α-syn aggregation and induces toxicity in the brain; these findings are further supported by the existence of advanced glycation end products (AGEs) identified in PD patients' brains (45-47, 103). There are a number of studies indicating that the aggregation of α-syn is promoted by nitration in a site-specific manner (53, 55). Nitrated monomeric or dimeric α-syn induces fibrillization by recruiting unmodified α-syn, while nitration-induced oligomeric α-syn blocks the development of pathological inclusion; this suggests that the oligomeric status of nitrated α-syn might be an important feature determining the propensity for accelerating the aggregate-related PD pathogenesis (53). Through comparative studies either expressing or inhibiting the relative kinases or using mutated α-syn in which S129 is replaced with alanine (S129A) to impede phosphorylation, it has been speculated that the phosphorylation of α-syn is a factor that possibly induces or enhances the aggregation of α-syn and neurotoxicity (57, 61, 62, 65, 66, 69, 71). *In vitro* study with recombinant human α-syn also showed that α-syn in the presence of its kinase, CK2, is prone to aggregation (57). Converging results from recent works demonstrate that kinases increase the level of phosphorylation at S129 of α-syn, and the phosphorylation exacerbates the aggregation and toxicity of α-syn (61, 65, 69, 71). Another known kinase of α-syn, c-Abl, can phosphorylate α-syn at Y39, and this Y39-phosphorylation increases the α-syn aggregation and induces neurodegeneration (104).

While numerous researchers have reported that ubiquitination inhibits α-syn aggregation and reduces its toxicity by promoting its clearance (78-87), a couple of cell-based studies suggest that α-syn mono- or di-ubiquitinated by seven in absentia homolog (SIAH)

FOR REVIEW

ubiquitin E3 ligase is more likely to form inclusion and induce cell death in SH-SY5Y and PC12 cells (105, 106). This inconsistency may result from differences in enzymes that mediate α-syn ubiquitination. It has been demonstrated that SUMOylation can enhance α-syn aggregation-related PD pathogenesis; cell-based studies proposed a strong correlation between SUMOylation and aggregation of α-syn (89, 90, 107, 108). Recent research has reported that in the presence of ginkgolic acid which inhibits SUMOylation, α-syn aggregation is inhibited and pre-formed aggregates are eliminated; this implies that SUMOylation might play an important role in the formation of α-syn inclusion (108). The impact of α-syn truncation is still controversial. Although some studies have demonstrated the inhibitory effect of truncation on α-syn aggregation (75, 97-102), there is evidence from *in vivo* and *in vitro* studies indicating that α-syn truncation tends to enhance α-syn aggregation (99-102, 109-118). *In vitro* studies using recombinant short α-syn variants and full-length α-syn to examine the aggregation propensity of truncated α-syn demonstrated that truncation can effectively promote the fibrillization of α-syn, with comparative analysis (99-102, 114, 116-118).

Taken together, these results suggest that α-syn can be post-translationally modified by various enzymes or stimuli to increase its propensity to form pathological aggregates. This suggests that an excess of the aggregation-related PTMs might contribute to the progression of synucleinopathy, thus providing another therapeutic target.

PROPERTY OF THE PHOSPHORYLATION AT SERINE 129 OF ALPHA-SYNUCLEIN

Among the PTMs associated with the formation of pathological α-syn inclusions, the phosphorylation at S129 is widely used as a biomarker for PD because 90% of α-syn incorporated in PD patients' LBs is S129-phosphorylated-α-syn (pS129-α-syn)-positive, while only 4% of α-syn is phosphorylated at S129 under physiological condition (57, 119). It is,

FOR REVIEW

however, premature to conclude that S129-phosphorylation triggers initiation or elongation of the α-syn aggregation. According to a study using PFFs, treating cells with the PFFs of C-terminally truncated α-syn without S129 residue induced the development of pS129 with newly recruited endogenous full-length α-syn; this implied that the S129-phosphorylation might not be the essential inducer to start the aggregation (110, 120). In addition, the PFFs of phosphorylation-incompetent α-syn^{S129A} could induce the aggregation even in cells stably expressing α-syn^{S129A}; indicating that α-syn aggregate seeding and the subsequent recruitment of endogenous α-syn occur even in the S129-phosphorylation-incapable environment (120). On the other hand, however, various *in vivo* and *in vitro* research models using specific kinase or mutant α-syn showed that the S129-phosphorylation plays a role in the aggregation process (57, 60, 61, 65-67, 69-71, 75). One possible speculation is that the aggregation starts before the α-syn S129-phosphorylation occurs, which may be triggered by a certain factor(s) or just by chance. Thereafter, the S129-phosphorylation shows up and may foster the aggregation process by stabilizing its structure; it supports the evidence that not only monomeric α-syn but also the aggregated forms of α-syn are subjected to phosphorylation (68, 121). Since pS129 is not necessarily needed for the seed fibril elongation in α-syn PFF seeded cells (120), the aggregation promoting effect of pS129 might be limited to further events after the formation of α-syn fibrils in the pathophysiological condition. It is also worth noting that the S129-phosphorylation of α-syn accelerates the clearance of abnormal α-syn (70, 122). Its neuroprotective feature further suggests that the S129-phosphorylation of α-syn happens after the initiation of aggregation to promote degradation of abnormal protein accumulation. According to this hypothesis, the S129-phosphorylation of α-syn inhibits aggregation under healthy conditions but is a double-edged sword and promotes α-syn aggregation under pathophysiological conditions. However, it should clearly be demonstrated: *i)* whether the

FOR REVIEW

S129-phosphorylation of α-syn occurs later after the initial aggregation step at the molecular level in pathophysiological conditions, and *ii)* whether the pS129-fibrillar α-syn results in solidifying and enlarging the aggregates in pathophysiological conditions.

FOR REVIEW

DISCUSSION

The findings of experimental research on the effects of some PTMs on α-syn aggregation do not come to an accord as shown in Table 1 in which most of the PTMs are reported to act in both directions, either up-regulating or down-regulating α-syn aggregation. The contradiction may arise from the different features between site-specifically modified α-syn proteins. As it is well described in the glycosylation, glycation, nitration, phosphorylation, ubiquitination, SUMOylation, and truncation, the tendency of α-syn toward aggregation and its extent differ depending on the modification sites in α-syn (40, 44-46, 51, 54, 61, 67, 74, 75, 81, 82, 84, 86, 91-93, 98-102, 105, 110, 112, 114, 116-118). While a majority of research on phosphorylation at S129 residue suggests that it encourages α-syn aggregation (57, 61, 65, 66, 69, 71), phosphorylation at other sites such as S87 and Y125 have been reported might to inhibit aggregation (61, 64, 67, 74, 76). In the case of ubiquitination, the anti-aggregation effects may vary depending on the modification sites of α-syn and the length of the ubiquitin chain (80-87, 105). Truncation is generally linked to the acceleration of α-syn fibrillization especially when it happens at the carboxy terminus of α-syn (99-102, 109-118); however, a study showed that the α-syn aggregation is inhibited when the NAC region is truncated (99, 101). Besides modification sites, the difference in α-syn aggregation is also attributed to the enzymes related to PTMs. Generally, more than one enzyme is associated with a PTM, and the effect of PTM on α-syn may vary depending on the enzymes involved. For instance, ubiquitination mediated by SIAH is reported to enhance the propensity of α-syn fibrillization (105, 106), whereas other ubiquitin E3 ligases-mediated ubiquitination, such as CHIP and NEDD4, are linked to aggregation inhibitory effect (78-81). One interesting point is that not only α-syn monomers but also fibrils of α-syn are subject to modification. Notably, the consequences of a PTM on different forms of α-syn may be inconsistent; a couple of studies suggested that truncated α-

FOR REVIEW

278 syn monomers are resistant to aggregation while the truncation of α-syn fibril induces further
279 fibrillization (99, 100). Some contradictory research results make it hard to determine the
280 pathological impact of the PTMs on synucleinopathies; for example, one research suggested
281 that the glycation reduces conformational flexibility of α-syn and thereby inhibits further
282 fibrillization (49), however, these findings were inconsistent with results of similar studies (45,
283 47). In addition, a study showed that when the phosphorylation by GRK2 (Gprk2 in *Drosophila*
284 *melanogaster*) is blocked, the aggregation of α-syn is increased, whereas the cytotoxicity is
285 alleviated (60); this implied that there might be a neuroprotective effect in α-syn aggregation,
286 contrary to the conventional belief (40, 41, 45-47, 55, 61, 65, 69-71, 75, 76, 79-81, 92, 93, 105,
287 106, 109, 111, 115).

288 As described in the case of the phosphorylation at S129, it has been widely
289 demonstrated that certain PTMs of α-syn is involved in the aggregation process and subsequent
290 formation of insoluble inclusions, LBs. However, it is still not clear: *i)* whether a certain PTM
291 of α-syn actually accelerates/inhibits the aggregation or occurs as an event accompanying the
292 aggregation process, and *ii)* whether it is a leading factor inducing/reducing fibrillization.
293 Although it is too hasty to conclude that PTMs repress or accelerate the pathogenesis of LB
294 formation, according to a lot of evidence, PTMs can regulate α-syn in different ways, and it
295 might be critical for the progression of α-syn aggregation-related pathogenesis including
296 synucleinopathies. However, it is not negligible that α-syn not only undergoes PTMs, but also
297 interacts with proteins including chaperons and HDAC, and metal ions such as Ca^{2+}, which
298 alter the properties of α-syn and consequently possibly impact the progression of
299 synucleinopathies (32, 123, 124). Therefore, α-syn constantly interacts with the surroundings
300 in the physiological condition, and its conversion to pathological inclusion might be the
301 consequence of collaboration between PTMs, interacting proteins, and metal ions. Further

FOR REVIEW

study to precisely reveal the α-syn modifications and their associated roles in pathophysiological conditions to fully uncover the mechanism of aggregation, and taking this further, to develop a better understanding of the synucleinopathies is warranted.

FOR REVIEW

Table 1. Pathological implications of alpha-synuclein post-translational modifications for the aggregation and toxicity.

PTM (site/residue)	Enzyme	Experimental model	Aggre-gation	Cell death	Note	Ref.
Acetylation						
N-terminal	NatB	*in vitro*	Reduce	n.d.	Decreased aggregation rate of N-terminally acetylated α-syn than non-acetylated α-syn.	(28)
N-terminal	NatB	*in vitro*	Reduce	n.d.	Decreased aggregation of N-terminally acetylated α-syn due to the increased helical folding propensity.	(29)
N-terminal	NatB	*in vitro*	Reduce	n.d.	Decreased α-syn aggregation rate with N-terminal acetylation.	(30)
N-terminal	NatB	*in vitro*	Reduce	n.d.	Decreased N-terminally acetylated α-syn aggregation rate than non-acetylated α-syn; the aggregation rate more slows down by Fe^{3+}, but no effect by Cu^{2+}.	(32)
N-terminal	NatB	*in vitro*	Reduce	n.d.	N-terminally acetylated α-syn is less prone to oligomerize than the non-acetylated α-syn in the presence of DOPAL due to increased binding to vesicles.	(33)
N-terminal	NatB	*in vitro*	No effect	n.d.	No significant differences in the fibrillization kinetics between N-terminally acetylated α-syn and non-acetylated α-syn.	(27)
O-GlcNAcylation						
Thr72	n.d.	*in vitro*	Reduce	n.d.	*O*-GlcNAcylated synthetic α-syn peptide(68-77) reduces the aggregation.	(42)
Thr72	n.d.	Rat cortical neuron, SH-SY5Y, *in vitro*	Reduce	Decrease	Decreased α-syn aggregation and PFF-induced toxicity by *O*-GlcNAcylation at T72.	(41)
Thr72, 75, 81, Ser87	n.d.	Mouse hippocampal neuron, *in vitro*	Reduce	Decrease	Triply *O*-GlcNAcylated α-syn(gT72,75,81) inhibits the aggregation of unmodified α-syn.	(40)
Thr72, Ser87	n.d.	*in vitro*	Reduce	n.d.	*O*-GlcNAcylated α-syn at T87 also inhibits the aggregation, but to a lesser extent than at T72.	(44)
n.d.	OGT	*in vitro*	Reduce	n.d.	Enzymatic *O*-GlcNAcylation of α-syn inhibits aggregation	(39)
Glycation						
N-terminal, all Lys	n.d.	*in vitro*	Reduce	n.d.	Glycation inhibits α-syn fibril formation *in vitro*, but it cannot disassemble pre-existing fibrils.	(48)
n.d.	n.d.	SH-SY5Y, HeLa, *in vitro*	Reduce	No effect	Glycated α-syn inhibits fibrillation of itself or of unmodified α-syn *in vitro*.	(49)
Lys6, 10, 12, 21, 23, 32, 34, 43, 45	n.d.	hiPSC, mouse, fly, yeast, H4, LUHMES	Enhance	Increase	Glycation promotes the accumulation of toxic α-syn oligomers and enhances α-syn toxicity in cells and *in vivo*; glycation inhibitors reduce α-syn aggregation and alleviate motor phenotypes in fly.	(45)
Lys58, 60, 80, 96, 97, 102	n.d.	SH-SY5Y, *in vitro*	Enhance	Increase	Ribosylation, glycation with *D*-ribose, induces α-syn aggregation and cell death.	(46)
n.d.	n.d.	Mouse, N2a, *in vitro*	Enhance	Increase	DJ-1 activity controls to the accumulation of glycated α-syn.	(47)
Nitration						
Tyr39, 125	n.d.	*in vitro*	Reduce	n.d.	Semi-synthetic nitrated α-syn(nY39 or nY125) has slower aggregation kinetics than wild-type *in vitro*.	(51)
Tyr39, 125, 133, 136	n.d.	*in vitro*	Reduce	n.d.	Tyrosine-nitration blocks α-syn fibril formation *in vitro*.	(54)
n.d.	n.d.	*in vitro*	Reduce	n.d.	Nitrated α-syn inhibits fibrillation of itself or of unmodified α-syn *in vitro*.	(56)
Tyr39	n.d.	*in vitro*	Enhance	n.d.	Nitrated α-syn monomer or dimer accelerates the rate of fibrillation of unmodified α-syn *in vitro*.	(53)
Tyr39	n.d.	Mouse, SH-SY5Y	Enhance	Increase	Y39-nitration of α-syn may increase neuronal α-syn aggregation and apoptosis induced by METH.	(55)
Phosphorylation						
Ser87	n.d.	Rat	Reduce	Decrease	Intranigral injection of rAAV2/6-α-syn(WT or S87A) induces α-syn aggregation and loss of DA neurons in rat, but S87E does not.	(76)
Ser87	CK1	Human brain, rat, mouse, *in vitro*	Reduce	n.d.	Phosphorylation at S87 increases conformational flexibility of α-syn.	(64)
Ser87, 129	CK1, CK2	Human brain, mouse, SH-SY5Y, *in vitro*	Reduce	n.d.	Phosphorylation at S87 inhibits α-syn fibril formation *in vitro*, but pS87-α-syn is not abundant in LB; proteasomal dysfunction increases CK2 activity, which results in elevated pS129-α-syn level.	(67)
Tyr125	Shark	Human brain, fly	Reduce	Decrease	Y125-phosphorylation of α-syn is reduced in aged human and fly brains.	(61)

FOR REVIEW

Tyr125, 133, 136	SYK	Mouse, SH-N-BE, CHO	Reduce	n.d.	Syk-mediated phosphorylation prevents α-syn multimerization; Y125-α-syn is the major phosphorylation site by Syk.	(74)
Ser129	n.d.	Mouse, HEK293T	Reduce	Decrease	Prion-like progression and time to disease onset in S129E-α-syn PFFs-injected mouse are elongated.	(75)
Ser129	PLK2	Rat, HEK293T	Reduce	Decrease	S129-phosphorylation of α-syn is mediated by PLK2, and it enhances α-syn autophagic degradation.	(70)
Ser129	GRK2	Fly	Reduce	Increase	S129A-α-syn suppresses DA neuronal cell death induced by α-syn completely and increases inclusion formation; S129D-α-syn or Gprk2-mediated pS129-α-syn enhances α-syn toxicity.	(60)
Ser129	GRK6	Rat	No effect	Increase	Increased levels of pS129-α-syn enhances A53T α-syn toxicity in the rAAV-based rat model.	(62)
Tyr39	c-Abl	Mouse, SH-SY5Y, HEK293T, *in vitro*	Enhance	Increase	Deletion of *c-Abl* reduces α-syn aggregation and neurodegeneration in the hA53T α-syn mice; overexpression of constitutively active c-Abl accelerates α-syn aggregation and neurodegeneration in the hA53T α-syn mice.	(104)
Ser129	CK2	Human brain, *in vitro*	Enhance	n.d.	Phosphorylation of α-syn at S129 promotes fibril formation *in vitro*.	(57)
Ser129	DAPK1	SH-SY5Y, MEF	Enhance	Increase	DAPK1 plays an important role in stimulating toxic α-syn aggregation and neuronal cell death.	(71)
Ser129	CK2	SH-SY5Y	Enhance	n.d.	H_2O_2 induces S129-phosphorylation of α-syn and the inclusion formation.	(66)
Ser129	GRK2	Fly	Enhance	Increase	Co-expression of Gprk2 with α-syn increases α-syn aggregation; S129A-α-syn reduces α-syn toxicity; S129D-α-syn enhances α-syn toxicity.	(61)
Ser129	CK1	Fly	Enhance	Increase	CK1-mediated S129-phosphorylation of α-syn increases the aggregation.	(65)
Ser129	PLKs	Mouse, SH-SY5Y	Enhance	Increase	METH treatment increases PLK2 and pS129-α-syn levels, the aggregation, and apoptosis; BI2536, pan-PLK inhibitor, treatment reduces S129-phosphorylation of α-syn, the aggregation, and apoptosis, induced by METH.	(69)
Ubiquitination						
N-terminal	UBE2W	*in vitro*	Reduce	n.d.	N-terminal ubiquitination and the proteasome may together disturb α-syn aggregate formation.	(85)
Lys6	n.d.	*in vitro*	Reduce	n.d.	Ubiquitination at K6 results in prominent inhibition of α-syn fibril formation.	(83)
Lys6, 12, 21, 32, 34, 43, 96	n.d.	*in vitro*	Reduce	n.d.	Disulfide-directed ubiquitination at K32C, K34C, K43C or K96C strongly inhibits α-syn aggregation; disulfide-directed ubiquitination at K6C, K12C, or K21C inhibits α-syn aggregation; disulfide-directed ubiquitination at K10C or K23C may not inhibit α-syn aggregation.	(82)
Lys6, 23, 96	n.d.	*in vitro*	Reduce	n.d.	Disulfide-directed ubiquitination at K6C, K23C, or K96C inhibits α-syn aggregation; disulfide-directed ubiquitination at K96C may cause an alteration in the structure of α-syn aggregates.	(86)
Lys12	n.d.	*in vitro*	Reduce	n.d.	K12 tetra-ubiquitinated α-syn forms nonfibrillar aggregates but does not form amyloid fibrils; α-syn K12 di/tetra-ubiquitination abolishes PLK3-mediated phosphorylation at S129, but SYK-mediated phosphorylation at Y125 destabilizes K12 tetra-ubiquitinated α-syn.	(87)
Lys12, 21, 45, 58, 96	NEDD4	Human brain, SH-SY5Y, HEK293, yeast, *in vitro*	Reduce	Decrease	Nedd4-mediated ubiquitination promotes the destruction of α-syn by the endosomal–lysosomal pathway.	(81)
Lys45, 58, 60	SCF	Mouse, SH-SY5Y, HeLa, BV-2, COS7	Reduce	n.d.	SCF containing FBXL5 prevents LB-like pathology by extracellular α-syn fibrils, from the initiation and spreading in mice.	(84)
n.d.	CHIP	Human brain, H4	Reduce	n.d.	Overexpression of CHIP, a component of LBs, inhibits α-syn aggregation and reduces α-syn protein levels.	(78)
n.d.	CHIP	H4	Reduce	Decrease	Co-expression of CHIP selectively degrades toxic α-syn oligomers, thereby it selectively reduces α-syn oligomerization and toxicity.	(79)
n.d.	NEDD4	Rat, fly	Reduce	Decrease	Overexpressed-Nedd4-mediated degradation reduces the accumulation and aggregation of α-syn in rat SN; overexpression of Nedd4 decreases the α-syn-induced dopaminergic cell loss in a rat model.	(80)
Lys10, 12, 21, 23, 34, 43, 96	SIAH1/2	SH-SY5Y, *in vitro*	Enhance	Increase	Monoubiquitylation may trigger α-syn aggregation and LB formation.	(105)
n.d.	SIAH1	HeLa, PC12, *in vitro*	Enhance	Increase	Siah1-mediated ubiquitination promotes α-syn aggregation and enhances its toxicity.	(106)
SUMOylation						
Lys96,102	n.d.	*in vitro*	Reduce	n.d.	SUMOylation at K102 more inhibits the aggregation of α-syn than K96 SUMOylation; SUMO1 modification more inhibits the aggregation of α-syn than SUMO3.	(91)
Lys96,102	n.d.	Yeast	Reduce	Decrease	Impaired SUMOylation of α-syn aggravates cytotoxicity and increase the formation of inclusions.	(92)
Lys96,102	n.d.	Rat, HEK293T, *in vitro*	Reduce	Decrease	SUMOylation of α-syn impaired by K96/102R mutation increases propensity for both aggregation and cytotoxicity in rat SN DA neurons.	(93)

FOR REVIEW

n.d.	PIAS2	Human brain, SH-SY5Y, HEK293	Enhance	n.d.	PIAS2-mediated SUMOylation leads to α-syn accumulation by reducing its degradation *via* UPS; PIAS2 expression along with SUMOylated α-syn in PD brains.	(89)
n.d.	CBX4	HEK293, COS7	Enhance	Decrease	Increased α-syn aggregation and decreased staurosporine-induced cell death by CBX4-mediated SUMOylation.	(107)
n.d.	n.d.	Rat cortical neuron, SH-SY5Y	Enhance	n.d.	SUMOylation inhibitor, ginkgolic acid, promotes autophagy-dependent clearance of α-syn aggregates.	(108)
Truncation						
1-57, 1-73, 1-75, 1-83	CAPN1	*in vitro*	Reduce	n.d.	CAPN1-cleaved soluble α-syn fragments prevent fibrillization of full-length α-syn.	(99)
1-108, 1-124	n.d.	*in vitro*	Reduce	n.d.	Truncation of the C-terminal 16 amino acid residues of α-syn results in an approximately 8-fold reduction of $t_{1/2}$ in aggregation kinetics.	(98)
1-115, 1-119, 1-122, 1-125, 1-129	n.d.	Mouse, HEK29T, *in vitro*	Reduce	Decrease	Prion-like progression and time to disease onset in C-terminally truncated α-syn PFFs-injected mouse are elongated.	(75)
1-120	n.d.	Mouse, SH-SY5Y	Reduce	n.d.	C-terminally truncated α-syn fibrils induce sparse α-syn pathologies in mouse.	(102)
11-140, 31-140	n.d.	*in vitro*	Reduce	n.d.	N-terminally truncated α-syn slows down the aggregation *in vitro*.	(102)
1-57	CAPN1	Mouse, *in vitro*	*Reduce	n.d.	The major cleavage site of soluble α-syn by CAPN1 is between E57-K58.	(100)
1-80	KLK6	*in vitro*	*Reduce	n.d.	The cleavage of α-syn between K80-T81 (within the NAC region) may impede α-syn aggregation.	(101)
1-108	n.d.	*in vitro*	*Reduce	n.d.	Strongly twisted β-sheets in α-syn(1-108) fibrils resist incorporation of full-length α-syn monomers.	(97)
1-87, 1-120	n.d.	*in vitro*	Enhance	n.d.	C-terminally truncated α-syn is most rapidly assembled.	(114)
1-102, 1-110	n.d.	*in vitro*	Enhance	n.d.	C-terminally truncated α-syn aggregates more rapidly than full-length protein.	(116)
1-103	AEP	Rat ventral midbrain neuron, mouse	Enhance	Increase	AEP-cleaved α-syn(1-103) enhances the aggregation and the neurotoxicity.	(111)
1-103, 1-122	n.d.	*in vitro*	Enhance	n.d.	Increased fibril helix twists upon removal of C-terminal residues.	(117)
1-114, 1-122	CAPN1	*in vitro*	Enhance	n.d.	CAPN1-cleaved fibrillar α-syn promotes further co-assembly with full-length α-syn.	(99)
1-120	n.d.	*in vitro*	Enhance	n.d.	C-terminally truncated α-syn quickens up the aggregation *in vitro*.	(102)
1-120	n.d.	Fly	Enhance	Increase	α-Syn(1-120) increases the aggregation and enhances the neurotoxicity *in vivo*.	(109)
1-120	n.d.	Mouse	Enhance	*Increase	Rat *TH*-specific expression of α-syn(1-120) leads to the formation of pathological inclusions in SN and OB and to a reduction in striatal dopamine levels.	(115)
1-120, 1-123	n.d.	Mouse, SH-SY5Y, HEK29T, N2a, Ltk-, COS-1	Enhance	n.d.	C-terminally truncated α-syn enhances the aggregation of full-length α-syn.	(110)
1-120, 1-123	AEP	Mouse brain, N27	Enhance	n.d.	C-terminal cleavage of α-syn is directly induced by lysosomal activity.	(112)
1-122	CAPN1	Human brain lysate, SH-SY5Y	Enhance	n.d.	Cleavage of α-syn by CAPN1 enhances self-aggregation and induces β-sheet structure.	(113)
11-140, 31-140	n.d.	Mouse, SH-SY5Y	Enhance	n.d.	N-terminally truncated α-syn fibrils induce abundant α-syn pathologies in mouse.	(102)
1-97	KLK6	*in vitro*	*Enhance	n.d.	The cleavage of α-syn between K97-D98 may enhance the aggregation.	(101)
1-103, 1-119	n.d.	in vitro	*Enhance	n.d.	C-terminally truncated α-syn promotes the aggregation at neutral pH.	(118)
1-122	CAPN1	Mouse, *in vitro*	*Enhance	n.d.	Fibrillized α-syn is cleaved predominantly after E114 and N122 by CAPN1.	(100)

*, speculation without experimental evidence. AGEs, advanced glycation end-products. DA neuron, dopaminergic neuron. DOPAL, 3,4-dihydroxyphenylacetaldehyde. hiPSC, human induced pluripotent stem cell. LB, Lewy body. MATH, methamphetamine. n.d., not determined. NAC, non-amyloid component. *O*-GlcNAcylation, *O*-linked β-N-acetylglucosaminylation. OB, olfactory bulb. PD, Parkinson's disease. PFF, pre-formed fibril. SN, substantia nigra. SUMO, small ubiquitin-like modifier. UPS, ubiquitin proteasome system.

307

FOR REVIEW

ACKNOWLEDGMENTS

This work was supported by Korea Drug Development Fund funded by Ministry of Science and ICT (MSIT), Ministry of Trade, Industry, and Energy, and Ministry of Health and Welfare (MOHW) (HN21C1258, Republic of Korea), by the National Research Foundation of Korea grants funded by MSIT (NRF-2019M3A9H1103783, NRF-2020R1A2C1009172; Republic of Korea), and by Korean Fund for Regenerative Medicine funded by MSIT and MOHW (2021M3E5E5096744, Republic of Korea). I sincerely apologize to colleagues whose work has not been cited in this review due to space limitations.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

FOR REVIEW

REFERENCES

1. Klein C and Westenberger A (2012) Genetics of Parkinson's disease. Cold Spring Harb Perspect Med 2, a008888
2. Polymeropoulos MH, Lavedan C, Leroy E et al (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 276, 2045-2047
3. Emamzadeh FN (2016) Alpha-synuclein structure, functions, and interactions. J Res Med Sci 21, 29
4. Spillantini MG, Crowther RA, Jakes R, Hasegawa M and Goedert M (1998) alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies. Proc Natl Acad Sci U S A 95, 6469-6473
5. Trojanowski JQ, Revesz T and Neuropathology Working Group on MSA (2007) Proposed neuropathological criteria for the post mortem diagnosis of multiple system atrophy. Neuropathol Appl Neurobiol 33, 615-620
6. McCann H, Stevens CH, Cartwright H and Halliday GM (2014) alpha-Synucleinopathy phenotypes. Parkinsonism Relat Disord 20 Suppl 1, S62-67
7. Nishie M, Mori F, Yoshimoto M, Takahashi H and Wakabayashi K (2004) A quantitative investigation of neuronal cytoplasmic and intranuclear inclusions in the pontine and inferior olivary nuclei in multiple system atrophy. Neuropathol Appl Neurobiol 30, 546-554
8. Fauvet B, Mbefo MK, Fares MB et al (2012) alpha-Synuclein in central nervous system and from erythrocytes, mammalian cells, and Escherichia coli exists predominantly as disordered monomer. J Biol Chem 287, 15345-15364
9. Bartels T, Choi JG and Selkoe DJ (2011) alpha-Synuclein occurs physiologically as a helically folded tetramer that resists aggregation. Nature 477, 107-110
10. Lashuel HA, Petre BM, Wall J et al (2002) Alpha-synuclein, especially the Parkinson's disease-associated mutants, forms pore-like annular and tubular protofibrils. J Mol Biol 322, 1089-1102
11. Cremades N, Cohen SI, Deas E et al (2012) Direct observation of the interconversion of normal and toxic forms of alpha-synuclein. Cell 149, 1048-1059
12. Chen SW, Drakulic S, Deas E et al (2015) Structural characterization of toxic oligomers that are kinetically trapped during alpha-synuclein fibril formation. Proc Natl Acad Sci U S A 112, E1994-2003
13. Mahul-Mellier AL, Burtscher J, Maharjan N et al (2020) The process of Lewy body formation, rather than simply alpha-synuclein fibrillization, is one of the major drivers of neurodegeneration. Proc Natl Acad Sci U S A 117, 4971-4982
14. Danzer KM, Haasen D, Karow AR et al (2007) Different species of alpha-synuclein oligomers induce calcium influx and seeding. J Neurosci 27, 9220-9232
15. Fusco G, Chen SW, Williamson PTF et al (2017) Structural basis of membrane disruption and cellular toxicity by alpha-synuclein oligomers. Science 358, 1440-1443
16. Peelaerts W, Bousset L, Van der Perren A et al (2015) alpha-Synuclein strains cause distinct synucleinopathies after local and systemic administration. Nature 522, 340-344
17. Li JY, Englund E, Holton JL et al (2008) Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. Nat Med 14, 501-503
18. Braak H, Ghebremedhin E, Rub U, Bratzke H and Del Tredici K (2004) Stages in the development of Parkinson's disease-related pathology. Cell Tissue Res 318, 121-134
19. Volpicelli-Daley LA, Luk KC, Patel TP et al (2011) Exogenous alpha-synuclein fibrils induce Lewy body pathology leading to synaptic dysfunction and neuron death. Neuron 72, 57-71
20. Yasuda T, Nakata Y and Mochizuki H (2013) alpha-Synuclein and neuronal cell death. Mol Neurobiol 47, 466-483
21. Benskey MJ, Perez RG and Manfredsson FP (2016) The contribution of alpha synuclein to neuronal survival and function - Implications for Parkinson's disease. J Neurochem 137, 331-359
22. Sherer TB, Betarbet R, Stout AK et al (2002) An in vitro model of Parkinson's disease: linking mitochondrial impairment to altered alpha-synuclein metabolism and oxidative damage. J Neurosci 22, 7006-7015
23. Scudamore O and Ciossek T (2018) Increased oxidative stress exacerbates alpha-synuclein aggregation in vivo. J Neuropathol Exp Neurol 77, 443-453
24. Musgrove RE, Helwig M, Bae EJ et al (2019) Oxidative stress in vagal neurons promotes parkinsonian pathology and intercellular alpha-synuclein transfer. J Clin Invest 129, 3738-3753
25. Xilouri M, Brekk OR and Stefanis L (2013) alpha-Synuclein and protein degradation systems: a reciprocal relationship. Mol Neurobiol 47, 537-551
26. Seo J and Lee KJ (2004) Post-translational modifications and their biological functions: proteomic analysis and systematic approaches. J Biochem Mol Biol 37, 35-44
27. Maltsev AS, Ying J and Bax A (2012) Impact of N-terminal acetylation of alpha-synuclein on its random coil and lipid binding properties. Biochemistry 51, 5004-5013

PREVIEW

28. Kang L, Moriarty GM, Woods LA, Ashcroft AE, Radford SE and Baum J (2012) N-terminal acetylation of alpha-synuclein induces increased transient helical propensity and decreased aggregation rates in the intrinsically disordered monomer. Protein Sci 21, 911-917
29. Bartels T, Kim NC, Luth ES and Selkoe DJ (2014) N-alpha-acetylation of alpha-synuclein increases its helical folding propensity, GM1 binding specificity and resistance to aggregation. PLoS One 9, e103727
30. Watson MD and Lee JC (2019) N-terminal acetylation affects alpha-synuclein fibril polymorphism. Biochemistry 58, 3630-3633
31. Dikiy I and Eliezer D (2014) N-terminal acetylation stabilizes N-terminal helicity in lipid- and micelle-bound alpha-synuclein and increases its affinity for physiological membranes. J Biol Chem 289, 3652-3665
32. Lorentzon E, Kumar R, Horvath I and Wittung-Stafshede P (2020) Differential effects of Cu(2+) and Fe(3+) ions on in vitro amyloid formation of biologically-relevant alpha-synuclein variants. Biometals 33, 97-106
33. Lima VA, do Nascimento LA, Eliezer D and Follmer C (2019) Role of Parkinson's disease-linked mutations and N-terminal acetylation on the oligomerization of alpha-synuclein Induced by 3,4-dihydroxyphenylacetaldehyde. ACS Chem Neurosci 10, 690-703
34. Wang S, Yang F, Petyuk VA et al (2017) Quantitative proteomics identifies altered O-GlcNAcylation of structural, synaptic and memory-associated proteins in Alzheimer's disease. J Pathol 243, 78-88
35. Li Y, Zhao C, Luo F et al (2018) Amyloid fibril structure of alpha-synuclein determined by cryo-electron microscopy. Cell Res 28, 897-903
36. Tuttle MD, Comellas G, Nieuwkoop AJ et al (2016) Solid-state NMR structure of a pathogenic fibril of full-length human alpha-synuclein. Nat Struct Mol Biol 23, 409-415
37. Alfaro JF, Gong CX, Monroe ME et al (2012) Tandem mass spectrometry identifies many mouse brain O-GlcNAcylated proteins including EGF domain-specific O-GlcNAc transferase targets. Proc Natl Acad Sci U S A 109, 7280-7285
38. Morris M, Knudsen GM, Maeda S et al (2015) Tau post-translational modifications in wild-type and human amyloid precursor protein transgenic mice. Nat Neurosci 18, 1183-1189
39. Zhang J, Lei H, Chen Y et al (2017) Enzymatic O-GlcNAcylation of alpha-synuclein reduces aggregation and increases SDS-resistant soluble oligomers. Neurosci Lett 655, 90-94
40. Levine PM, Galesic A, Balana AT et al (2019) alpha-Synuclein O-GlcNAcylation alters aggregation and toxicity, revealing certain residues as potential inhibitors of Parkinson's disease. Proc Natl Acad Sci U S A 116, 1511-1519
41. Marotta NP, Lin YH, Lewis YE et al (2015) O-GlcNAc modification blocks the aggregation and toxicity of the protein alpha-synuclein associated with Parkinson's disease. Nat Chem 7, 913-920
42. Marotta NP, Cherwien CA, Abeywardana T and Pratt MR (2012) O-GlcNAc modification prevents peptide-dependent acceleration of alpha-synuclein aggregation. ChemBioChem 13, 2665-2670
43. Ryan P, Xu M, Davey AK et al (2019) O-GlcNAc modification protects against protein misfolding and aggregation in neurodegenerative disease. ACS Chem Neurosci 10, 2209-2221
44. Lewis YE, Galesic A, Levine PM et al (2017) O-GlcNAcylation of alpha-synuclein at serine 87 reduces aggregation without affecting membrane binding. ACS Chem Biol 12, 1020-1027
45. Vicente Miranda H, Szego EM, Oliveira LMA et al (2017) Glycation potentiates alpha-synuclein-associated neurodegeneration in synucleinopathies. Brain 140, 1399-1419
46. Chen L, Wei Y, Wang X and He R (2010) Ribosylation rapidly induces alpha-synuclein to form highly cytotoxic molten globules of advanced glycation end products. PLoS One 5, e9052
47. Sharma N, Rao SP and Kalivendi SV (2019) The deglycase activity of DJ-1 mitigates alpha-synuclein glycation and aggregation in dopaminergic cells: Role of oxidative stress mediated downregulation of DJ-1 in Parkinson's disease. Free Radic Biol Med 135, 28-37
48. Marino L, Ramis R, Casasnovas R et al (2020) Unravelling the effect of N(epsilon)-(carboxyethyl)lysine on the conformation, dynamics and aggregation propensity of alpha-synuclein. Chem Sci 11, 3332-3344
49. Lee D, Park CW, Paik SR and Choi KY (2009) The modification of alpha-synuclein by dicarbonyl compounds inhibits its fibril-forming process. Biochim Biophys Acta 1794, 421-430
50. Sevcsik E, Trexler AJ, Dunn JM and Rhoades E (2011) Allostery in a disordered protein: oxidative modifications to alpha-synuclein act distally to regulate membrane binding. J Am Chem Soc 133, 7152-7158
51. Burai R, Ait-Bouziad N, Chiki A and Lashuel HA (2015) Elucidating the role of site-specific nitration of alpha-synuclein in the pathogenesis of Parkinson's disease via protein semisynthesis and mutagenesis. J Am Chem Soc 137, 5041-5052
52. Giasson BI, Duda JE, Murray IV et al (2000) Oxidative damage linked to neurodegeneration by selective alpha-synuclein nitration in synucleinopathy lesions. Science 290, 985-989
53. Hodara R, Norris EH, Giasson BI et al (2004) Functional consequences of alpha-synuclein tyrosine nitration: diminished binding to lipid vesicles and increased fibril formation. J Biol Chem 279, 47746-47753
54. Norris EH, Giasson BI, Ischiropoulos H and Lee VM (2003) Effects of oxidative and nitrative challenges on alpha-synuclein fibrillogenesis involve distinct mechanisms of protein modifications. J Biol Chem 278, 27230-27240

55. Qiao HH, Zhu LN, Wang Y et al (2019) Implications of alpha-synuclein nitration at tyrosine 39 in methamphetamine-induced neurotoxicity in vitro and in vivo. Neural Regen Res 14, 319-327
56. Yamin G, Uversky VN and Fink AL (2003) Nitration inhibits fibrillation of human alpha-synuclein in vitro by formation of soluble oligomers. FEBS Lett 542, 147-152
57. Fujiwara H, Hasegawa M, Dohmae N et al (2002) alpha-Synuclein is phosphorylated in synucleinopathy lesions. Nat Cell Biol 4, 160-164
58. Nakajo S, Tsukada K, Omata K, Nakamura Y and Nakaya K (1993) A new brain-specific 14-kDa protein is a phosphoprotein. Its complete amino acid sequence and evidence for phosphorylation. Eur J Biochem 217, 1057-1063
59. Pronin AN, Morris AJ, Surguchov A and Benovic JL (2000) Synucleins are a novel class of substrates for G protein-coupled receptor kinases. J Biol Chem 275, 26515-26522
60. Chen L and Feany MB (2005) Alpha-synuclein phosphorylation controls neurotoxicity and inclusion formation in a Drosophila model of Parkinson disease. Nat Neurosci 8, 657-663
61. Chen L, Periquet M, Wang X et al (2009) Tyrosine and serine phosphorylation of alpha-synuclein have opposing effects on neurotoxicity and soluble oligomer formation. J Clin Invest 119, 3257-3265
62. Sato H, Arawaka S, Hara S et al (2011) Authentically phosphorylated alpha-synuclein at Ser129 accelerates neurodegeneration in a rat model of familial Parkinson's disease. J Neurosci 31, 16884-16894
63. Okochi M, Walter J, Koyama A et al (2000) Constitutive phosphorylation of the Parkinson's disease associated alpha-synuclein. J Biol Chem 275, 390-397
64. Paleologou KE, Oueslati A, Shakked G et al (2010) Phosphorylation at S87 is enhanced in synucleinopathies, inhibits alpha-synuclein oligomerization, and influences synuclein-membrane interactions. J Neurosci 30, 3184-3198
65. Prasad V, Wasser Y, Hans F et al (2019) Monitoring alpha-synuclein multimerization in vivo. FASEB J 33, 2116-2131
66. Smith WW, Margolis RL, Li X et al (2005) Alpha-synuclein phosphorylation enhances eosinophilic cytoplasmic inclusion formation in SH-SY5Y cells. J Neurosci 25, 5544-5552
67. Waxman EA and Giasson BI (2008) Specificity and regulation of casein kinase-mediated phosphorylation of alpha-synuclein. J Neuropathol Exp Neurol 67, 402-416
68. Mbefo MK, Paleologou KE, Boucharaba A et al (2010) Phosphorylation of synucleins by members of the Polo-like kinase family. J Biol Chem 285, 2807-2822
69. Ding J, Wang Y, Huang J et al (2020) Role of alpha-synuclein phosphorylation at Serine 129 in methamphetamine-induced neurotoxicity in vitro and in vivo. Neuroreport 31, 787-797
70. Oueslati A, Schneider BL, Aebischer P and Lashuel HA (2013) Polo-like kinase 2 regulates selective autophagic alpha-synuclein clearance and suppresses its toxicity in vivo. Proc Natl Acad Sci U S A 110, E3945-3954
71. Shin WH and Chung KC (2020) Death-associated protein kinase 1 phosphorylates alpha-synuclein at Ser129 and exacerbates rotenone-induced toxic aggregation of alpha-synuclein in dopaminergic SH-SY5Y cells. Exp Neurobiol 29, 207-218
72. Nakamura T, Yamashita H, Takahashi T and Nakamura S (2001) Activated Fyn phosphorylates alpha-synuclein at tyrosine residue 125. Biochem Biophys Res Commun 280, 1085-1092
73. Ellis CE, Schwartzberg PL, Grider TL, Fink DW and Nussbaum RL (2001) alpha-synuclein is phosphorylated by members of the Src family of protein-tyrosine kinases. J Biol Chem 276, 3879-3884
74. Negro A, Brunati AM, Donella-Deana A, Massimino ML and Pinna LA (2002) Multiple phosphorylation of alpha-synuclein by protein tyrosine kinase Syk prevents eosin-induced aggregation. FASEB J 16, 210-212
75. Sorrentino ZA, Hass E, Vijayaraghavan N et al (2020) Carboxy-terminal truncation and phosphorylation of alpha-synuclein elongates survival in a prion-like seeding mouse model of synucleinopathy. Neurosci Lett 732, 135017
76. Oueslati A, Paleologou KE, Schneider BL, Aebischer P and Lashuel HA (2012) Mimicking phosphorylation at serine 87 inhibits the aggregation of human alpha-synuclein and protects against its toxicity in a rat model of Parkinson's disease. J Neurosci 32, 1536-1544
77. Stefanis L, Emmanouilidou E, Pantazopoulou M, Kirik D, Vekrellis K and Tofaris GK (2019) How is alpha-synuclein cleared from the cell? J Neurochem 150, 577-590
78. Shin Y, Klucken J, Patterson C, Hyman BT and McLean PJ (2005) The co-chaperone carboxyl terminus of Hsp70-interacting protein (CHIP) mediates alpha-synuclein degradation decisions between proteasomal and lysosomal pathways. J Biol Chem 280, 23727-23734
79. Tetzlaff JE, Putcha P, Outeiro TF et al (2008) CHIP targets toxic alpha-Synuclein oligomers for degradation. J Biol Chem 283, 17962-17968
80. Davies SE, Hallett PJ, Moens T et al (2014) Enhanced ubiquitin-dependent degradation by Nedd4 protects against alpha-synuclein accumulation and toxicity in animal models of Parkinson's disease. Neurobiol Dis 64, 79-87
81. Tofaris GK, Kim HT, Hourez R, Jung JW, Kim KP and Goldberg AL (2011) Ubiquitin ligase Nedd4 promotes alpha-synuclein degradation by the endosomal-lysosomal pathway. Proc Natl Acad Sci U S A 108, 17004-17009

82. Meier F, Abeywardana T, Dhall A et al (2012) Semisynthetic, site-specific ubiquitin modification of alpha-synuclein reveals differential effects on aggregation. J Am Chem Soc 134, 5468-5471
83. Hejjaoui M, Haj-Yahya M, Kumar KS, Brik A and Lashuel HA (2011) Towards elucidation of the role of ubiquitination in the pathogenesis of Parkinson's disease with semisynthetic ubiquitinated alpha-synuclein. Angew Chem Int Ed Engl 50, 405-409
84. Gerez JA, Prymaczok NC, Rockenstein E et al (2019) A cullin-RING ubiquitin ligase targets exogenous alpha-synuclein and inhibits Lewy body-like pathology. Sci Transl Med 11
85. Ye Y, Klenerman D and Finley D (2020) N-terminal ubiquitination of amyloidogenic proteins triggers removal of their oligomers by the proteasome holoenzyme. J Mol Biol 432, 585-596
86. Moon SP, Balana AT, Galesic A, Rakshit A and Pratt MR (2020) Ubiquitination can change the structure of the alpha-synuclein amyloid fiber in a site selective fashion. J Org Chem 85, 1548-1555
87. Haj-Yahya M, Fauvet B, Herman-Bachinsky Y et al (2013) Synthetic polyubiquitinated alpha-Synuclein reveals important insights into the roles of the ubiquitin chain in regulating its pathophysiology. Proc Natl Acad Sci U S A 110, 17726-17731
88. Pountney DL, Huang Y, Burns RJ et al (2003) SUMO-1 marks the nuclear inclusions in familial neuronal intranuclear inclusion disease. Exp Neurol 184, 436-446
89. Rott R, Szargel R, Shani V et al (2017) SUMOylation and ubiquitination reciprocally regulate alpha-synuclein degradation and pathological aggregation. Proc Natl Acad Sci U S A 114, 13176-13181
90. Kim YM, Jang WH, Quezado MM et al (2011) Proteasome inhibition induces alpha-synuclein SUMOylation and aggregate formation. J Neurol Sci 307, 157-161
91. Abeywardana T and Pratt MR (2015) Extent of inhibition of alpha-synuclein aggregation in vitro by SUMOylation is conjugation site- and SUMO isoform-selective. Biochemistry 54, 959-961
92. Shahpasandzadeh H, Popova B, Kleinknecht A, Fraser PE, Outeiro TF and Braus GH (2014) Interplay between sumoylation and phosphorylation for protection against alpha-synuclein inclusions. J Biol Chem 289, 31224-31240
93. Krumova P, Meulmeester E, Garrido M et al (2011) Sumoylation inhibits alpha-synuclein aggregation and toxicity. J Cell Biol 194, 49-60
94. Baba M, Nakajo S, Tu PH et al (1998) Aggregation of alpha-synuclein in Lewy bodies of sporadic Parkinson's disease and dementia with Lewy bodies. Am J Pathol 152, 879-884
95. Muntane G, Ferrer I and Martinez-Vicente M (2012) alpha-synuclein phosphorylation and truncation are normal events in the adult human brain. Neuroscience 200, 106-119
96. Campbell BC, McLean CA, Culvenor JG et al (2001) The solubility of alpha-synuclein in multiple system atrophy differs from that of dementia with Lewy bodies and Parkinson's disease. J Neurochem 76, 87-96
97. Iyer A, Roeters SJ, Kogan V, Woutersen S, Claessens M and Subramaniam V (2017) C-terminal truncated alpha-synuclein fibrils contain strongly twisted beta-sheets. J Am Chem Soc 139, 15392-15400
98. Hoyer W, Cherny D, Subramaniam V and Jovin TM (2004) Impact of the acidic C-terminal region comprising amino acids 109-140 on alpha-synuclein aggregation in vitro. Biochemistry 43, 16233-16242
99. Mishizen-Eberz AJ, Norris EH, Giasson BI et al (2005) Cleavage of alpha-synuclein by calpain: potential role in degradation of fibrillized and nitrated species of alpha-synuclein. Biochemistry 44, 7818-7829
100. Mishizen-Eberz AJ, Guttmann RP, Giasson BI et al (2003) Distinct cleavage patterns of normal and pathologic forms of alpha-synuclein by calpain I in vitro. J Neurochem 86, 836-847
101. Kasai T, Tokuda T, Yamaguchi N et al (2008) Cleavage of normal and pathological forms of alpha-synuclein by neurosin in vitro. Neurosci Lett 436, 52-56
102. Terada M, Suzuki G, Nonaka T, Kametani F, Tamaoka A and Hasegawa M (2018) The effect of truncation on prion-like properties of alpha-synuclein. J Biol Chem 293, 13910-13920
103. Munch G, Luth HJ, Wong A et al (2000) Crosslinking of alpha-synuclein by advanced glycation endproducts--an early pathophysiological step in Lewy body formation? J Chem Neuroanat 20, 253-257
104. Brahmachari S, Ge P, Lee SH et al (2016) Activation of tyrosine kinase c-Abl contributes to alpha-synuclein-induced neurodegeneration. J Clin Invest 126, 2970-2988
105. Rott R, Szargel R, Haskin J et al (2008) Monoubiquitylation of alpha-synuclein by seven in absentia homolog (SIAH) promotes its aggregation in dopaminergic cells. J Biol Chem 283, 3316-3328
106. Lee JT, Wheeler TC, Li L and Chin LS (2008) Ubiquitination of alpha-synuclein by Siah-1 promotes alpha-synuclein aggregation and apoptotic cell death. Hum Mol Genet 17, 906-917
107. Oh Y, Kim YM, Mouradian MM and Chung KC (2011) Human Polycomb protein 2 promotes alpha-synuclein aggregate formation through covalent SUMOylation. Brain Res 1381, 78-89
108. Vijayakumaran S, Nakamura Y, Henley JM and Pountney DL (2019) Ginkgolic acid promotes autophagy-dependent clearance of intracellular alpha-synuclein aggregates. Mol Cell Neurosci 101, 103416

109. Periquet M, Fulga T, Myllykangas L, Schlossmacher MG and Feany MB (2007) Aggregated alpha-synuclein mediates dopaminergic neurotoxicity in vivo. J Neurosci 27, 3338-3346
110. Li W, West N, Colla E et al (2005) Aggregation promoting C-terminal truncation of alpha-synuclein is a normal cellular process and is enhanced by the familial Parkinson's disease-linked mutations. Proc Natl Acad Sci U S A 102, 2162-2167
111. Zhang Z, Kang SS, Liu X et al (2017) Asparagine endopeptidase cleaves alpha-synuclein and mediates pathologic activities in Parkinson's disease. Nat Struct Mol Biol 24, 632-642
112. McGlinchey RP, Lacy SM, Huffer KE, Tayebi N, Sidransky E and Lee JC (2019) C-terminal alpha-synuclein truncations are linked to cysteine cathepsin activity in Parkinson's disease. J Biol Chem 294, 9973-9984
113. Dufty BM, Warner LR, Hou ST et al (2007) Calpain-cleavage of alpha-synuclein: connecting proteolytic processing to disease-linked aggregation. Am J Pathol 170, 1725-1738
114. Serpell LC, Berriman J, Jakes R, Goedert M and Crowther RA (2000) Fiber diffraction of synthetic alpha-synuclein filaments shows amyloid-like cross-beta conformation. Proc Natl Acad Sci U S A 97, 4897-4902
115. Tofaris GK, Garcia Reitbock P, Humby T et al (2006) Pathological changes in dopaminergic nerve cells of the substantia nigra and olfactory bulb in mice transgenic for truncated human alpha-synuclein(1-120): implications for Lewy body disorders. J Neurosci 26, 3942-3950
116. Murray IV, Giasson BI, Quinn SM et al (2003) Role of alpha-synuclein carboxy-terminus on fibril formation in vitro. Biochemistry 42, 8530-8540
117. Ni X, McGlinchey RP, Jiang J and Lee JC (2019) Structural insights into alpha-synuclein fibril polymorphism: Effects of Parkinson's disease-related C-terminal truncations. J Mol Biol 431, 3913-3919
118. van der Wateren IM, Knowles TPJ, Buell AK, Dobson CM and Galvagnion C (2018) C-terminal truncation of alpha-synuclein promotes amyloid fibril amplification at physiological pH. Chem Sci 9, 5506-5516
119. Anderson JP, Walker DE, Goldstein JM et al (2006) Phosphorylation of Ser-129 is the dominant pathological modification of alpha-synuclein in familial and sporadic Lewy body disease. J Biol Chem 281, 29739-29752
120. Luk KC, Song C, O'Brien P et al (2009) Exogenous alpha-synuclein fibrils seed the formation of Lewy body-like intracellular inclusions in cultured cells. Proc Natl Acad Sci U S A 106, 20051-20056
121. Waxman EA and Giasson BI (2011) Characterization of kinases involved in the phosphorylation of aggregated alpha-synuclein. J Neurosci Res 89, 231-247
122. Machiya Y, Hara S, Arawaka S et al (2010) Phosphorylated alpha-synuclein at Ser-129 is targeted to the proteasome pathway in a ubiquitin-independent manner. J Biol Chem 285, 40732-40744
123. Moons R, Konijnenberg A, Mensch C et al (2020) Metal ions shape alpha-synuclein. Sci Rep 10, 16293
124. Burmann BM, Gerez JA, Matecko-Burmann I et al (2020) Regulation of alpha-synuclein by chaperones in mammalian cells. Nature 577, 127-132

FOR REVIEW