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Invited Mini Review

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

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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. [BMB Reports 2022; 55(7): 323-335]

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 (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

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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.

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 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 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

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

PTM (site/residue)	Enzyme	Experimental model	Aggregation	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, 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)

Table 1. Continued 1

PTM (site/residue)	Enzyme	Experimental model	Aggregation	Cell death	Note	Ref.
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)
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)

Table 1. Continued 2

PTM (site/residue)	Enzyme	Experimental model	Aggregation	Cell death	Note	Ref.
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)
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)

Table 1. Continued 3

PTM (site/residue)	Enzyme	Experimental model	Aggregation	Cell death	Note	Ref.
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.

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 pro-

tein 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, SUMOylation, and truncation, have been reported to have both enhancing and suppressing aspects on the pathological α-syn aggregation (Table 1). 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) 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, 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 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.

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

As described in the case of the phosphorylation at S129, it has been widely demonstrated that certain PTMs of α-syn is involved in the aggregation process and subsequent formation of insoluble inclusions, LBs. However, it is still not clear: *i)* whether a certain PTM of α-syn actually accelerates/inhibits the aggregation or occurs as an event accompanying the aggregation process, and *ii)* whether it is a leading factor inducing/

reducing fibrillization. Although it is too hasty to conclude that PTMs repress or accelerate the pathogenesis of LB formation, according to a lot of evidence, PTMs can regulate α-syn in different ways, and it might be critical for the progression of α-syn aggregation-related pathogenesis including synucleinopathies. However, it is not negligible that α-syn not only undergoes PTMs, but also interacts with proteins including chaperons and HDAC, and metal ions such as Ca^{2+}, which alter the properties of α-syn and consequently possibly impact the progression of synucleinopathies (32, 123, 124). Therefore, α-syn constantly interacts with the surroundings in the physiological condition, and its conversion to pathological inclusion might be the consequence of collaboration between PTMs, interacting proteins, and metal ions. Further 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.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

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