21st Century Publisher

21 ST CENTURY PATHOLOGY

21st Century Pathology

Short Communication

Open Access

Unexpected Roles of Soluble α-Synuclein Post-translational Modifications in Pathological α-Synuclein Amplification

Yuhan Sun, Chao Peng*, Shujing Zhang*

Department of Neurology, David Geffen School of Medicine, University of California–Los Angeles, USA

***Corresponding Author:** Chao Peng, Department of Neurology, David Geffen School of Medicine, University of California–Los Angeles, Los Angeles, CA, USA; E-mail: HYPERLINK "mailto:CPeng@mednet.ucla.edu"CPeng@mednet.ucla.edu

Shujing Zhang, Department of Neurology, David Geffen School of Medicine, University of California–Los Angeles, Los Angeles, CA, USA; E-mail: ShujingZhang@mednet.ucla.edu

Received: 01 June 2023; **Revised:** 12 July 2023; **Accepted:** 13 July 2023; **Published:** 18 July 2023

Abstract

This short review summarizes the effect of soluble α-Synuclein (α-Syn) post-translational modifications (PTMs) on pathological α-Syn amplification. Most previous studies of pathological α-Syn transmission and amplification focused on pathological α-Syn seeds themselves. Our recent study showed, for the first time, how phosphorylation and acetylation of soluble and nonpathological α-Syn affect the amplification of pathological α-Syn in a modification site- and conformation-specific manner. Also, a series of novel modifications on soluble α-Syn have been discovered for future research. Moreover, we proposed the importance of the different roles of PTMs during the different stages of pathology development.

Keywords: *Acetylation; Pathological α-Synuclein; Neurodegenerative diseases; Phosphorylation; Post-translational modifications*

Introduction

Characterized by the intracellular aggregation of pathological α-syn, neurodegenerative diseases such as Parkinson's disease (PD), dementia with Lewy bodies (DLB), multiple system atrophy (MSA), and part of Alzheimer's disease (AD) are collectively known as α-synucleinopathies [1-3]. Mountains of research support that pathological α-Syn seeds can lead to the cell-to-cell transmission and amplification of α-Syn pathology and eventually promote the progression of the α-synucleinopathies [4-7]. Therefore, previous research mainly focused on exploring the function of pathological α-Syn seeds during the progression of α-synucleinopathies [8,9].

There are two stages for the amplification of pathological proteins, the formation of pathological α-Syn seeds and the transformation of soluble α-Syn to pathological α-Syn. The soluble α-Syn has not been investigated for their role in regulating the amplification of pathological α-Syn.

Previously, quite a few post-translational modifications (PTMs) have been identified on α-Syn [10,11]. Many extensively studied PTM sites exhibit diverse effects on pathological α-Syn, including amplification, toxicity, and fibrillization, etc., [12-15]. However, the understanding of the roles of PTMs on soluble α-Syn in pathological α-Syn amplification is quite limited. In our recent investigation, we systematically identified the PTM sites on soluble α-Syn from a large number of patient samples, and evaluated the effect of PTMs at a few known and novel sites of soluble α-Syn on the modulation of amplification and seeding properties of pathological α-Syn using different cell models.

Discussion

The potential regulatory role of soluble α-Syn in the transmission of pathological α-Syn has generally been overlooked. In our study, LC-MS/MS was employed to identify PTMs on soluble α-Syn from diseased and healthy brains. The conformations of pathological α-Syn are significantly different across various α-synucleinopathies. In Lewy body disease (LBD), the misfolded α-Syn aggregates in neurons as Lewy bodies (LB-α-Syn) which is considered a distinct strain of pathological α-Syn from the one observed in Multiple system atrophy (MSA). In MSA, the pathological α-Syn manifests as Glial cytoplasmic inclusion (GCI-α-Syn) [16-18]. To assess the impact of α-Syn PTMs on the transmission of pathological α-Syn with distinct conformations, we simultaneously utilized LB-α-Syn, GCI-α-Syn, α-Syn preformed fibrils (PFF, artificially generated misfolded α-Syn), and GCI-α-Syn passaged in mouse primary neurons (GCI-N, 99% composed of mouse α-Syn) as seeds for the test. For the first time, we have provided evidence demonstrating the influence of phosphorylated soluble α-Syn on the amplification of pathological α-Syn. Importantly, we observed that this effect is contingent upon the conformation of pathological α-Syn and sites of modification rather than the

amino acid sequence of α-Syn. Furthermore, these observations extend beyond phosphorylation and are also evident in our investigation of acetylation.

We analyzed 11 different soluble α-Syn PTMs using mimetic mutants, including Y39E, S87E, Y125E, Y133E, K21Q, K34Q, K43Q, K45Q, K60Q, K96Q, and K102Q (as seen in Table1). There are eight PTMs that can modulate the amplification of at least one of the pathological α-Syn strains, including Y39E, S87E, Y125E, Y133E, K21Q, K43Q, K45Q, and K60Q. This finding supports the notion that the impact of α-Syn post-translational modifications (PTMs) on the amplification of pathological α-Syn is widespread and not limited to specific types of modifications or particular sites of modification. While only K21Q and K43Q have the same effect on three distinct strains, demonstrating that the effects of soluble α-Syn PTMs on transmission are highly selective to specific conformations. We indicated that the same α-Syn PTMs may change pathological α-Syn amplification differently across various α-Syn strains. This will eventually contribute to the clinical diversity of different α-synucleinopathies.

Table 1: Effects of α-syn PTMs on different pathological a-syn strains.

α-syn PTMs	LB-α-syn amplification	GCI-α-syn amplification	PFF amplification	GCI-N amplification	GCI seeding property	PFF seeding property
Y39 phosphorylation	↓	↓	-	↓	P1: seeding ↓ P2: seeding ↓	P1: seeding - P2: seeding -
S87 phosphorylation	↑	↓	-	↓	P1: seeding ↓ P2: seeding ↓	P1: seeding - P2: seeding ↓
Y125 phosphorylation	↓	-	-	-	P1: seeding - P2: seeding -	P1: seeding - P2: seeding -
Y133 phosphorylation	↓	↓	-	↓	P1: seeding - P2: seeding -	P1: seeding - P2: seeding -
K21 acetylation	↓	↓	↓	↓	N/A	N/A
K34 acetylation	-	-	-	-	N/A	N/A
K43 acetylation	↓	↓	↓	↓	N/A	N/A
K45 acetylation	↓	↓	-	↓	N/A	N/A
K60 acetylation	↓	↑	-	-	N/A	N/A
K96 acetylation	-	-	-	-	N/A	N/A
K102 acetylation	-	-	-	-	N/A	N/A

Notes: ↑ represents increased amplification; ↓ represents reduced amplification; - represents no significant effect on amplification; seeding ↑ represents increased seeding ability; seeding ↓ represents reduced seeding ability; seeding - represents no significant effect on seeding ability; N/A represents not tested.

Based on previous studies, the acetylation on the N-terminal domain of α-Syn can block α-Syn aggregation[12]. In our investigation, we examined acetylation at seven distinct sites within α-Syn and discovered that acetylation diminishes the amplification of various α-Syn strains. These findings suggest that enhancing acetylation levels in α-Syn could hold therapeutic potential for individuals affected by diverse α-Synucleinopathies. Additionally, Glycation has been reported to promote the aggregation of α-Syn [19] and we have identified multiple glycation sites on soluble α-Syn. Investigating the role of glycation PTMs in regulating α-Syn aggregation could yield significant insights and therapeutic avenues. Moreover, O-GlcNAc, another modification, has been reported to impede α-Syn aggregation [16,20]. We have identified two O-GlcNAc sites on soluble α-Syn. Although α-Syn arginlation has been demonstrated to block aggregation [21-23], and we also identified one arginlation site (E57) in our study, the specific effects of arginlation on soluble α-Syn PTMs remain unexplored. Furthermore, we have discovered multiple

methylations and dimethylation sites in soluble α-Syn, presenting another promising research direction to pursue.

In addition to the influence of different post-translational modifications on soluble α-Syn across various sites, the distinct roles of PTMs during the different stages of pathology development could also be a potential area to explore. Notably, phosphorylation of α-Syn monomers at S87 has been demonstrated to inhibit α-Syn oligomerization in vitro and attenuate α-Syn aggregation in rat models of α-synucleionpathies [14,24]. However, our recent study revealed an increased trend of LB-α-Syn amplification associated with phosphorylation at S87, whereas phosphorylation blocked GCI-α-Syn amplification and diminished its seeding capacity. These findings highlight the diverse roles of PTMs throughout different stages of pathology development. Similarily, phosphorylation at Y39 has been shown to impede α-Syn fibrilization in vitro [25] yet it appears to enhance α-Syn aggregation in vivo [26]. However, our study demonstrated that Y39 phosphorylation on soluble α-Syn can attenuate LB-α-Syn and GCI-α-Syn amplification, while also reducing the seeding potency of amplified GCI-α-Syn. This underscores the notion that the same PTM can have distinct implications at different stages of pathology development.

Conclusion

Our study represents a pioneering effort in elucidating the regulatory roles of soluble α-Syn in the transmission of pathological α-Syn. Comprehensive understanding of the intricate post-translational modifications (PTMs) affecting soluble α-Syn, as well as the complexed interaction mechanisms during different stages, is crucial for making significant contributions to the clinical and pathological landscape of diverse α-synucleinopathies. Furthermore, investigating the regulatory role of soluble α-Syn PTMs on pathological α-Syn in vivo holds great promise for future research endeavours. This concept of regulatory roles for soluble proteins can readily be applied to other neurodegenerative diseases, such as tauopathies, where numerous PTMs on soluble tau have been reported [27] in association with various tau strains [28]. This breakthrough idea has far-reaching implications within the field of neurodegenerative diseases.

References

1. Lippa CF, Fujiwara H, Mann DMA, Giasson B, Baba M, Schmidt ML, et al. Lewy Bodies Contain Altered α-Synuclein in Brains of Many Familial Alzheimer's Disease Patients with Mutations in Presenilin and Amyloid Precursor Protein Genes. The American Journal of Pathology. 1998 Nov;153(5):1365-70. https://doi.org/10.1016/S0002-9440(10)65722-7

2. Spillantini MG, Schmidt ML, Lee VMY, Trojanowski JQ, Jakes R, Goedert M. α-Synuclein in Lewy bodies. Nature. 1997 Aug;388(6645):839-40. https://doi.org/10.1038/42166

3. Tu P hsien, Galvin JE, Baba M, Giasson B, Tomita T, Leight S, et al. Glial cytoplasmic inclusions in white matter oligodendrocytes of multiple system atrophy brains contain insoluble α-synuclein. Ann Neurol. 1998 Sep;44(3):415-22. https://doi.org/10.1002/ana.410440324

4. Luk KC, Kehm V, Carroll J, Zhang B, O'Brien P, Trojanowski JQ, et al. Pathological α-Synuclein Transmission Initiates Parkinson-like Neurodegeneration in Nontransgenic Mice. Science. 2012 Nov 16;338(6109):949-53. https://doi.org/10.1126/science.1227157

5. Kordower JH, Chu Y, Hauser RA, Freeman TB, Olanow CW. Lewy body–like pathology in long-term embryonic nigral transplants in Parkinson's disease. Nat Med. 2008 May;14(5):504-6. https://doi.org/10.1038/nm1747

6. Li JY, Englund E, Holton JL, Soulet D, Hagell P, Lees AJ, et al. Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. Nat Med. 2008 May;14(5):501-3. https://doi.org/10.1038/nm1746

7. Braak H, Del Tredici K. Neuroanatomy and pathology of sporadic Parkinson's disease. Adv Anat Embryol Cell Biol. 2009;201:1-119.

8. Jucker M, Walker LC. Propagation and spread of pathogenic protein assemblies in neurodegenerative diseases. Nat Neurosci. 2018 Oct;21(10):1341-9. https://doi.org/10.1038/s41593-018-0238-6

9. Guo JL, Lee VMY. Cell-to-cell transmission of pathogenic proteins in neurodegenerative diseases. Nat Med. 2014 Feb;20(2):130-8. https://doi.org/10.1038/nm.3457

10. Barrett PJ, Timothy Greenamyre J. Post-translational modification of α-synuclein in Parkinson's disease. Brain Research. 2015 Dec;1628:247-53. https://doi.org/10.1016/j.brainres.2015.06.002

11. Schmid AW, Fauvet B, Moniatte M, Lashuel HA. Alpha-synuclein Post-translational Modifications as Potential Biomarkers for Parkinson Disease and Other Synucleinopathies. Molecular & Cellular Proteomics. 2013 Dec;12(12):3543-58. https://doi.org/10.1074/mcp.R113.032730

12. De Oliveira RM, Vicente Miranda H, Francelle L, Pinho R, Szegö ÉM, Martinho R, et al. The mechanism of sirtuin 2–mediated exacerbation of alpha-synuclein toxicity in models of Parkinson disease. Bates G, editor. PLoS Biol. 2017 Mar 3;15(3):e2000374. https://doi.org/10.1371/journal.pbio.2000374

13. Yoo H, Lee J, Kim B, Moon H, Jeong H, Lee K, et al. Role of post-translational modifications on the alpha-synuclein aggregation-related pathogenesis of Parkinson's disease. BMB Rep. 2022 Jul 31;55(7):323-35. https://doi.org/10.5483/BMBRep.2022.55.7.073

14. Paleologou KE, Oueslati A, Shakked G, Rospigliosi CC, Kim HY, Lamberto GR, et al. Phosphorylation at S87 Is Enhanced in Synucleinopathies, Inhibits α-Synuclein Oligomerization, and Influences Synuclein-Membrane Interactions. J Neurosci. 2010 Mar 3;30(9):3184-98. https://doi.org/10.1523/JNEUROSCI.5922-09.2010

15. Zhang J, Li X, Li JD. The Roles of Post-translational Modifications on α-Synuclein in the Pathogenesis of Parkinson's Diseases. Front Neurosci. 2019 Apr 18;13:381. https://doi.org/10.3389/fnins.2019.00381

16. Marotta NP, Lin YH, Lewis YE, Ambroso MR, Zaro BW, Roth MT, et al. O-GlcNAc modification blocks the aggregation and toxicity of the protein α-synuclein associated with Parkinson's disease. Nature Chem. 2015 Nov;7(11):913-20. https://doi.org/10.1038/nchem.2361

17. Schweighauser M, Shi Y, Tarutani A, Kametani F, Murzin AG, Ghetti B, et al. Structures of α-synuclein filaments from multiple system atrophy. Nature. 2020 Sep 17];585(7825):464-9. https://doi.org/10.1038/s41586-020-2317-6

18. Hejjaoui M, Butterfield S, Fauvet B, Vercruysse F, Cui J, Dikiy I, et al. Elucidating the Role of C-Terminal Post-Translational Modifications Using Protein Semisynthesis Strategies: α-Synuclein Phosphorylation at Tyrosine 125. J Am Chem Soc. 2012 Mar 21;134(11):5196–210. https://doi.org/10.1021/ja210866j

19. Vicente Miranda H, Szego ÉM, Oliveira LMA, Breda C, Darendelioglu E, De Oliveira RM, et al. Glycation potentiates α-synuclein-associated neurodegeneration in synucleinopathies. Brain. 2017 May 1;140(5):1399-419. https://doi.org/10.1093/brain/awx056

20. Levine PM, Galesic A, Balana AT, Mahul-Mellier AL, Navarro MX, De Leon CA, et al. α-Synuclein O-GlcNAcylation alters aggregation and toxicity, revealing certain residues as potential inhibitors of Parkinson's disease. Proc Natl Acad Sci USA. 2019 Jan 29;116(5):1511-9. https://doi.org/10.1073/pnas.1808845116

21. Pan B, Kamo N, Shimogawa M, Huang Y, Kashina A, Rhoades E, et al. Effects of Glutamate Arginylation on α-Synuclein: Studying an Unusual Post-Translational Modification through Semisynthesis. J Am Chem Soc. 2020 Dec 30;142(52):21786-98. https://doi.org/10.1021/jacs.0c10054

22. Wang J, Han X, Leu NA, Sterling S, Kurosaka S, Fina M, et al. Protein arginylation targets alpha synuclein, facilitates normal brain health, and prevents neurodegeneration. Sci Rep. 2017 Sep 12;7(1):11323. https://doi.org/10.1038/s41598-017-11713-z

23. Zhao J, Pan B, Fina M, Huang Y, Shimogawa M, Luk KC, et al. α-Synuclein arginylation in the human brain. Transl Neurodegener. 2022 Dec;11(1):20. https://doi.org/10.1186/s40035-022-00295-0

24. Oueslati A, Paleologou KE, Schneider BL, Aebischer P, Lashuel HA. Mimicking Phosphorylation at Serine 87 Inhibits the Aggregation of Human α-Synuclein and Protects against Its Toxicity in a Rat Model of Parkinson's Disease. J Neurosci. 2012 Feb 1;32(5):1536-44. https://doi.org/10.1523/JNEUROSCI.3784-11.2012

25. Dikiy I, Fauvet B, Jovicic A, Mahul-Mellier AL, Desobry C, El-Turk F, et al. Semisynthetic and in Vitro Phosphorylation of Alpha-Synuclein at Y39 Promotes Functional Partly Helical Membrane-Bound States Resembling Those Induced by PD Mutations. ACS Chem Biol. 2016 Sep 16;11(9):2428-37. https://doi.org/10.1021/acschembio.6b00539

26. Brahmachari S, Ge P, Lee SH, Kim D, Karuppagounder SS, Kumar M, et al. Activation of tyrosine kinase c-Abl contributes to α-synuclein–induced neurodegeneration. Journal of Clinical Investigation. 2016 Jun 27;126(8):2970-88. https://doi.org/10.1172/JCI85456

27. Morris M, Knudsen GM, Maeda S, Trinidad JC, Ioanoviciu A, Burlingame AL, et al. Tau post-translational modifications in wild-type and human amyloid precursor protein transgenic mice. Nat Neurosci. 2015 Aug;18(8):1183-9. https://doi.org/10.1038/nn.4067

28. Narasimhan S, Guo JL, Changolkar L, Stieber A, McBride JD, Silva LV, et al. Pathological Tau Strains from Human Brains Recapitulate the Diversity of Tauopathies in Nontransgenic Mouse Brain. J Neurosci. 2017 Nov 22;37(47):11406-23. https://doi.org/10.1523/JNEUROSCI.1230-17.2017