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The Extracellular α-Synuclein and its Biological Significance

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Abstract

α-synuclein plays an important role in the development of Parkinson's disease (PD). It was believed that α-synuclein elicits its effects in the neuronal cytosol. However, the finding of extracellular α-synuclein expands the orthodox spectrum. The α-synuclein can be secreted via the multi-vesicle bodies-mediated exosome and the recycling-endosome pathway. In the intercellular milieu, the secreted α-synuclein is degraded by enzymes or engulfed by neighboring cells. The remaining α-synuclein can induce the neurotoxicity, activate microglia, and promote the pathogenesis of Parkinson's disease. In the review, we focus on the recent findings of extracellular α-synuclein and its biological significance.

Keywords: α-synuclein; Parkinson's disease; Protein secretion; Exosome; Neuroinflammation

The Introduction of α-Synuclein

Parkinson's disease (PD) is pathologically characterized by α-synuclein immunopositive intracellular deposits termed as Lewy's bodies [1]. It is well known that α-synuclein consists of 140 amino acids and is divided into three distinct regions: (1) The N-terminal region (1-60 amino acid residues), containing KTKEGV repeats and forming amphipathic α-helices; (2) The central region (61-95 amino acids residues), serving as a hydrophobic NAC (non-Aβ component of Alzheimer's disease) peptide; (3) The C-terminal region (96-140 amino acid residues) [2].

In nature, α-synuclein is an unfolded protein which can spontaneously aggregate into oligomers and fibrils. The aggregation of α-synuclein is considered as the pivotal step for the development of PD. Locus duplication/triplication and mutations in α-synuclein gene make the α-synuclein prone to aggregate, and lead to the early-onset of PD [3-6].

The Finding of Extracellular α-Synulein

In the beginning of the 21st century, α-synuclein monomer was firstly detected in human cerebrospinal fluid (CSF) and blood plasma in both PD patients and the normal human subjects [7]. Following this initial finding, Millter, et al found that the blood level of α-synuclein monomers was doubled in familial PD patients with α-synuclein gene locus triplication [8].

In 2005, the aggregated extracellular α-synuclein (eSNCA) was found in cell culture medium. Both soluble oligomeric and monomeric species of α-synuclein were detected as early as 2 h followed the transient overexpression of human α-synuclein in the differentiated SH-SY5Y cells and accumulated over time [9]. In the next year, El-Agnaf et al. [10] confirmed the existence of aggregated eSNCA in the normal plasma and postmortem CSF from either PD patients or control subject. In 2012, Danzer et al. [11] further established that eSNCA oligomers either dispersed freely or lay in the exosomes.

The Production of eSNCA

Although eSNCA is detected the plasma and CSF, it doesn't mean that eSNCA is secreted *in vivo* by the neuronal cells since α-synuclein is also released from peripheral cells (such as red cells) or dead neurons [12,13]. In 2011, using a novel highly sensitive ELISA in conjugation with an *in vivo* microdialysis technique, Emmanouilidou et al. [14] provided the first solid evidence. They found that α-synuclein was readily detected in the interstitial fluid of both α-synuclein transgenic mice and human patients with traumatic brain injury.

The Compartment of α-Synuclein and the Formation of Multivesicular Bodies

In the physiological condition, α-synuclein often exists as monomer. Some disperses in the cytosol; some are loosely attached to the cytosolic surface of endoplasmic reticulum vesicles; and the others are compartmented into the lumen of vesicles [9].

The vesicles containing α-synuclein can be transformed to early endosomes. Part of early endosome is casted out of the cells through the Ras-related protein Rab11a-dependent recycling endosome pathway, especially in the physiological conditions [15]. For the remainders, the peripheral membrane around the endosome is invaginated into the endosome lumen, which forms luminal vesicles. Thus, the early endosomes are transformed into the late endosomes, and are also known as multivesicular bodies (MVBs). In this process, α-synuclein is also sorted into luminal vesicles.

The endosomal sorting complex required for transport (ESCRT) complex play an important rolein the formation of MVBs, which can recognize cargo proteins, sort α-synulcein into subregionals of the endosomal membrane. It has been reported that the charged multivesicular body protein 2B (CHMP2B), a subunit of ESCRT-III complex, was found in the lewy's bodies in the brain of PD patients [16]. Moreover, the vacuolar protein sorting 4 (VPS4), a regulator of ESCRT-III, was also found to participate in the formation of MVBs. As an ATPase, VPS4 releases the ESCRT-III machinery from the endosomal membrane, promotes the membrane invagination and the formation of MVB vesicles. The dominant-negative mutant of VPS4 interfered with the lysosomal targeting of α-synuclein, and facilitated α-synuclein secretion [15].

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In the physiological condition, MVB fuses with the autophagosome for lysosomal degradation [15]. However, when α-synuclein is excessively produced or lysosomes fail to clear α-synuclein, the superfluous α-synuclein is secreted out of the cells.

MVB-mediated Exosome Secreting Pathway

Due to the lack of the signal recognition sequence in the structure of α-synuclein, its translocation is independent on the ER/Golgi exporting pathway. This is supported by the fact that disruption of the classical export with brefeldin A, a classical inhibitor for the protein transportation from endoplasmic reticulum to the Golgi complex, failed to block the release of α-synuclein.

It is known that at least four non-classical secreting (export) pathways may exist [17]: the endosomal pathways (for instance, interleukin-1b and thioredoxin), direct translocation across the plasma membrane (for instance, FGF-1 and FGF-2), the transporter-assisted flip-flop mechanism and formation of exosomes. For the secretion of α-synuclein, the most attractive one is the MVB-mediated exsosome pathway [13]. Some MVB specific containing proteins, such as alix and flottilin, have been found in the α-synulcein-positive vesicles. Also, α-synuclein oligomers exist in the exosomal fraction of primary neurons, presumably lying on the outer surface of exosomes [14]. Furthermore, since the exosome-mediated exocytosis is a calcium-dependent process, the decrease in intracellular calcium concentration was shown to inhibit the secretion of α-synuclein [13].

Alternatively, the recycling endosome pathway may also play an important role in the process [18]. It has been found that α-synuclein is also aggregated in the recycling endosome [15]. Although the recycling endosome pathway is mainly intended to clear up the extracellular materials via the endocytosis, accumulating evidences indicate that it may be also involved in the secretion of α-synuclein. Firstly, the engulfed endosomes can be re-secreted out of the cells. Secondly, by the unknown mechanism, the α-synuclein in early endosomes is transported into the recycling endosome, and then is casted into the extracellular milieu [15]. This pathway is found to be regulated by rab11a and heat shock protein 90 (HSP90) [18]. Rab11a is the marker for this kind of endosome. Overexpression of Rab11a can alleviate the α-synuclein accumulation and protect dopaminergic neuron from degeneration in animal models of PD [19]. HSP90 was shown to interact with Rab11a and co-localize with Rab11a in the substantia nigra pars compacta (SNPc) of PD patients. Moreover, inhibition of HSP90 attenuated the exocytosis of internalized eSNCA [18].

In addition, other secreting pathways also exist in the secreting process of α-synuclein. For instance, α-synuclein can be directly integrated into secretory vesicles and subsequently released by exocytosis [9]. The low temperature, a classical blocker of vesicular exocytosis, reduced the secretion of α-synuclein.

Although two pathways have been proposed, it is difficult to evaluate the role of each pathway in the labile cell microenvironment. Based on the fact that the level of the monomer is higher than that of the aggregated forms, it appears that that the recycling pathway may be predominant, especially in the physiological condition [15]. However, the controversial result was also reported [11].

The Fate of the eSNCA

The eSNCA is disposed via either enzymatic degradation or phagocytosis.

The degradation of eSNCA is highly associated with the enzyme of neurosin [20]. As a specific enzyme for the degradation of α-synuclein, neurosin cleaves α-synuclein between lysine 80 and threonine 81 in the NAC region and degrades eSNCA fibril and oligomers [20,21]. The enzyme is secreted from the cells and activated in the extracellular space. Insufficient or abnormal function of neurosin might lead to the aggregation of α-synuclein in the extracellular space.

However, α-synuclein monomers may not be good substrates for neurosin. Several matrix metalloproteinases (MMPs), especially MMP3, play a critical role for the degradation of eSNCA monomers. As zinc-dependent endopeptidases, MMPs are synthesized primarily by astrocytes, microglias, and neurons. It was shown that MMPs (such as MMP-3, MMP-14, MMP-2, and MMP-9) effectively cleaved α-synuclein monomers [22]. In the content, MMP3 shows the most potent enzymatic activity. Besides, overexpression of α-synuclein remarkably up-regulates the MMP3 expression.

Recently, plasmin is found to cleave α-synuclein, mainly in the N-terminal region. Both aggregates and monomers of α-synuclein are good substrates for plasmin. Furthermore, eSNCA is shown to promote the expression of plasminogen activator inhibitor-1 and increases the plasmin activity [23].

Phagocytosis is another pathway for removing eSNCA. As mentioned above, the α-synuclein is exported in the form of exosomes, suggesting that α-synuclein shuttles between the host neurons and the neighboring cells [24]. Exosome-associated α-synuclein oligomers are more likely to be taken up by recipient cells, which is more potent in toxicity as compared with free α-synuclein oligomers [11,25]. After being taken up, fibrillar α-synuclein is transported in the manner of anterograde axonal transport, and released into the intercellular space, which can be taken up by other neurons [26].

Endocytosis is involved in numerous cellular processes such as nutrients uptake, synaptic vesicle recycling, and regulation of cell-surface expression of signaling receptors [27,28]. The aggregated eSNCA was found to be internalized into neuronal cells via clathrin-dependent endocytosis, then move into the lysosome through the recycling endosomal pathway [29]. In contrast, the monomeric α-synuclein may be transported into the cytosol directly across the plasma membrane. Low temperature was shown to effectively inhibit the internalization of fibrillar α-synuclein and oligomers, but do not affect the internalization of monomers. The internalization of monomeric α-synuclein into microglia is, however, independent on the classical pathways, rather dependent on the lipid raft in the plasma membrane [30]. The disruptors of lipid raft, such as methyl-β-cyclodextrin and filipin, inhibited the internalization of α-synuclein into microglia in a dose-dependent manner [30]. The ganglioside GM1 in the lipid raft may serve as the receptor of α-synuclein [30]. It has been reported that α-synculein binds specifically to gangioside GM1-containing small unilamellar vesicles [31].

Moreover, α-synculein can also be taken up by COS-7 cells via the dynamin-dependent endocytosis. Upon internalization, oligomer, not monomer and fibril, alters the process of membrane trafficking. For instance, oligomer of α-synculein significantly promotes the internalization and recycling of transferrin receptor (TfR), and subsequently increases the surface levels of TfR [32].

The Biological Significance of eSNCA

Neurotoxicity

eSNCA has shown the potent neurotoxicity. The A30P, E46K

and A53T α-synuclein mutations are more potent than the wild type of α-synuclein. It seems that α-synuclein aggregates are more toxic than the monomers, for the oligomer-interfering compounds can rescue the recipient neuronal cells from the observed toxicity [13]. The fibrillar aggregates are found to be toxic, while the profibrillar and oligomeric aggregates are also regarded as the toxic culprit [33]. Several mechanisms have been proposed for α-synuclein-induced neurotoxicity: (1) α-synuclein is taken up into recipient cells and interferes with the intracellular homeostasis. It has been shown that α-synuclein enhances the reactive oxygen and nitrogen species level, thus leads to macromolecule damage and neurodegeneration reviewed by Wilkeniec et al. [34]. It has been found that exosome-associated α-synuclein oligomers are more likely to be taken up by recipient cells than free α-oligmers [11]. Fibrillar α-synuclein was also internalized by primary neurons and transported to axons [26]. (2) The aggregated α-synuclein forms annular structures with a central pore. When they are inserted into the plasma membrane, the aggregated α-synuclein compromises the membrane integrity, alters the equilibrium of ions and small metabolites between the cytoplasm and the extracellular space, ultimately leads to neuronal degeneration [35]; (3) Receptor-dependent cytotoxicity. It has been reported that ciliary neurotrophic factor receptor-α (CNTF-α) mediates eSNCA-induced neurotoxicity, at least in part, via the JAK1/STAT3 pathway [36].

Neuroinflammation

Neuroinflammation is a dispensable part in the pathophysiological process of PD. eSNCA , particularly the aggregated form, can trigger the neuroinflammatory response. In a primary mesencephalic neuron-glia co-culture system, α-synuclein activated microglia and led to dopaminergic neurodegeneration, which was found to depend on the activation of NADPH oxidase and the production of reactive oxygen species [37].

Different signal pathways have been found in the microglia activation. The mitogen-activated protein (MAP) kinase pathways appeared to mediate the process [38]. Moreover, the activation of MAP kinases occurred within minutes following exposure to α-synuclein, suggesting that the activation of these pathways may be a receptor-mediated event. Further study showed that CD36 may be one of possible receptors with which α-synuclein interacts, since knockout of CD36 reduced the microglial activation and dampened the proinflammatory response to α-synuclein [39].

Some pattern recognition receptors are also involved in the activation of microglias. It has been established that toll-like receptor 4 (TLR4) mediated α-synuclein-induced microglial phagocytic activity, pro-inflammatory cytokine release, and ROS production. Knockout of TLR4 suppressed the proinflammatory response and decreased ROS production triggered by α-synuclein [40]. Moreover, the latest study revealed that eSNCA oligomer can also interact with TLR2 and thus activate microglias [41].

The amino acid sequence motifs, which are responsible for macrophage activation, have been identified. Lee et al. [42] found that N-terminal KTKEGV repeat of α-synuclein is essential for its penetration into cells, the 1–60 region of α-synuclein serves as a carrier that transports α-synuclein into cells, whereas the acidic C-terminal is the region responsible for activating primary human macrophage and RAW264.7 cell (a mouse macrophage cell line). Other studies also supported the observation. When the α-synuclein 71-82 fragment is deleted, the α-synuclein is unable to aggregate due to the lack of a corresponding middle hydrophobic region, but still effectively stimulates THP-1 cells (human acute monocytic leukemia cell line) [38,43]. Fellner et al. [40] reported that the C-terminus of α-synuclein appears to be the strongest activator of microglia as compared with the full length soluble or fibrillized α-synuclein.

Microglia migration is also a hallmark for the neuroinflammation. It is established that α-synuclein-induced microglia migrates into the SNpc in the 6-hydroxydopamine mouse model of PD. α-synuclein activates ERK1/2 and subsequently up-regulates the expression of the cell adhesion molecule CD44. The soluble CD44 can liberate microglia from the surrounding extracellular matrix for migration. The identical effects were also displayed in the murine microglia BV-2 cells [44].

Except for microglia, astrocytes are also involved in the inflammatory responses triggered by extracellular α-synuclein. It has been shown that α-synuclein strongly stimulates the human primary astrocytes as well as the human U-373 MG astrocytoma cells, and increases the expression of intercellular adhesion molecule-1 and interleukin-6. The mutated forms of α-synuclein showed more potency than the wild-type [45]. Moreover, the most recent research demonstrated that extracellular α-synuclein can be taken up by astrocytes through endocytosis and form inclusion bodies. Besides, the engulfing astrocytes can highly express various pro-inflammatory cytokines and chemokines, such as IL-1α, TNF-α, CX3CL-1 [25].

The Extracellular α-Synuclein and Parkinson's Disease

The finding of extracellular α-synuclein may aid in elucidating some pathological traits of Parkinson's disease (PD). It has been reported that dopamine accelerates the α-synuclein oligomerization in intracellular vesicles and promotes the production of extracellular α-synuclein [46], which may contribute to the progressive loss of the dopaminergic neuronal population in the SNpc in the PD patients.

Moreover, the finding of extracellular α-synuclein may pave the new way for the treatment of PD. As reviewed by Park and Kim [47], the potentiation in the proteolytic clearance of eSNCA can inhibit the spreading of eSCNA and serve as a new therapeutic approach against PD. In addition, it has been found that antibodies against α-synuclein reduced α-synuclein accumulation and synaptic loss in the PD mouse model. And, this kind of antibodies specifically promotes microglia to clear eSNCA proteins through the Fcγ receptor, thereby preventing their toxic actions on neighboring cells [48].

Conclusion

In this review, we outlined the production and degradation of eSNCA and its biological significance. When excessive α-synuclein is accumulated in cytosol, it is secreted into the extracellular space. In the extracellular space, it invades into the neighboring cells and triggers the neuroinflammation. The finding of eSNCA not only helps us to understand the pathology of PD and other neurodegenerative diseases, and supplies new ways for the therapy of PD.

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References

1. Goedert M (2001) Alpha-synuclein and neurodegenerative diseases. Nat Rev Neurosci 2: 492-501.
2. Beyer K (2006) Alpha-synuclein structure, posttranslational modification and alternative splicing as aggregation enhancers. Acta Neuropathol 112: 237-251.

3. Chartier-Harlin MC, Kachergus J, Roumier C, Mouroux V, Douay X, et al. (2004) Alpha-synuclein locus duplication as a cause of familial Parkinson's disease. Lancet 364: 1167-1169.

4. Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, et al. (2003) alpha-Synuclein locus triplication causes Parkinson's disease. Science 302: 841.

5. Krüger R, Kuhn W, Müller T, Woitalla D, Graeber M, et al. (1998) Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. Nat Genet 18: 106-108.

6. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, et al. (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 276: 2045-2047.

7. Borghi R, Marchese R, Negro A, Marinelli L, Forloni G, et al. (2000) Full length alpha-synuclein is present in cerebrospinal fluid from Parkinson's disease and normal subjects. Neurosci Lett 287: 65-67.

8. Miller DW, Hague SM, Clarimon J, Baptista M, Gwinn-Hardy K, et al. (2004) Alpha-synuclein in blood and brain from familial Parkinson disease with SNCA locus triplication. Neurology 62: 1835-1838.

9. Lee HJ, Patel S, Lee SJ (2005) Intravesicular localization and exocytosis of alpha-synuclein and its aggregates. J Neurosci 25: 6016-6024.

10. El-Agnaf OM, Salem SA, Paleologou KE, Curran MD, Gibson MJ, et al. (2006) Detection of oligomeric forms of alpha-synuclein protein in human plasma as a potential biomarker for Parkinson's disease. FASEB J 20: 419-425.

11. Danzer KM, Kranich LR, Ruf WP, Cagsal-Getkin O, Winslow AR, et al. (2012) Exosomal cell-to-cell transmission of alpha synuclein oligomers. Mol Neurodegener 7: 42.

12. Barbour R, Kling K, Anderson JP, Banducci K, Cole T, et al. (2008) Red blood cells are the major source of alpha-synuclein in blood. Neurodegener Dis 5: 55-59.

13. Emmanouilidou E, Melachroinou K, Roumeliotis T, Garbis SD, Ntzouni M, et al. (2010) Cell-produced alpha-synuclein is secreted in a calcium-dependent manner by exosomes and impacts neuronal survival. J Neurosci 30: 6838-6851.

14. Emmanouilidou E, Elenis D, Papasilekas T, Stranjalis G, Gerozissis K, et al. (2011) Assessment of α-synuclein secretion in mouse and human brain parenchyma. PLoS One 6: e22225.

15. Hasegawa T, Konno M, Baba T, Sugeno N, Kikuchi A, et al. (2011) The AAA-ATPase VPS4 regulates extracellular secretion and lysosomal targeting of α-synuclein. PLoS One 6: e29460.

16. Kurashige T, Takahashi T, Yamazaki Y, Hiji M, Izumi Y, et al. (2012) Localization of CHMP2B-immunoreactivity in the brainstem of Lewy body disease. Neuropathology.

17. Nickel W (2003) The mystery of nonclassical protein secretion. A current view on cargo proteins and potential export routes. Eur J Biochem 270: 2109-2119.

18. Liu J, Zhang JP, Shi M, Quinn T, Bradner J, et al. (2009) Rab11a and HSP90 regulate recycling of extracellular alpha-synuclein. J Neurosci 29: 1480-1485.

19. Cooper AA, Gitler AD, Cashikar A, Haynes CM, Hill KJ, et al. (2006) Alpha-synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models. Science 313: 324-328.

20. Tatebe H, Watanabe Y, Kasai T, Mizuno T, Nakagawa M, et al. (2010) Extracellular neurosin degrades Î±-synuclein in cultured cells. Neurosci Res 67: 341-346.

21. Little SP, Dixon EP, Norris F, Buckley W, Becker GW, et al. (1997) Zyme, a novel and potentially amyloidogenic enzyme cDNA isolated from Alzheimer's disease brain. J Biol Chem 272: 25135-25142.

22. Sung JY, Park SM, Lee CH, Um JW, Lee HJ, et al. (2005) Proteolytic cleavage of extracellular secreted {alpha}-synuclein via matrix metalloproteinases. J Biol Chem 280: 25216-25224.

23. Kim KS, Choi YR, Park JY, Lee JH, Kim DK, et al. (2012) Proteolytic cleavage of extracellular Î±-synuclein by plasmin: implications for Parkinson disease. J Biol Chem 287: 24862-24872.

24. Chivet M, Javalet C, Hemming F, Pernet-Gallay K, Laulagnier K, et al. (2013) Exosomes as a novel way of interneuronal communication. Biochem Soc Trans 41: 241-244.

25. Lee HJ, Suk JE, Patrick C, Bae EJ, Cho JH, et al. (2010) Direct transfer of alpha-synuclein from neuron to astroglia causes inflammatory responses in synucleinopathies. J Biol Chem 285: 9262-9272.

26. Freundt EC, Maynard N, Clancy EK, Roy S, Bousset L, et al. (2012) Neuron-to-neuron transmission of Î±-synuclein fibrils through axonal transport. Ann Neurol 72: 517-524.

27. Boettner DR, Chi RJ, Lemmon SK (2011) Lessons from yeast for clathrin-mediated endocytosis. Nat Cell Biol 14: 2-10.

28. Kiss AL, Botos E (2009) Endocytosis via caveolae: alternative pathway with distinct cellular compartments to avoid lysosomal degradation? J Cell Mol Med 13: 1228-1237.

29. Lee HJ, Suk JE, Bae EJ, Lee JH, Paik SR, et al. (2008) Assembly-dependent endocytosis and clearance of extracellular alpha-synuclein. Int J Biochem Cell Biol 40: 1835-1849.

30. Park JY, Kim KS, Lee SB, Ryu JS, Chung KC, et al. (2009) On the mechanism of internalization of alpha-synuclein into microglia: roles of ganglioside GM1 and lipid raft. J Neurochem 110: 400-411.

31. 31. Martinez Z, Zhu M, Han S, Fink AL (2007) GM1 specifically interacts with alpha-synuclein and inhibits fibrillation. Biochemistry 46: 1868-1877.

32. 32. Chai YJ, Kim D, Park J, Zhao H, Lee SJ, et al. (2013) The secreted oligomeric form of Î±-synuclein affects multiple steps of membrane trafficking. FEBS Lett 587: 452-459.

33. Lee SJ (2008) Origins and effects of extracellular alpha-synuclein: implications in Parkinson's disease. J Mol Neurosci 34: 17-22.

34. Wilkaniec A, Strosznajder JB, Adamczyk A (2013) Toxicity of extracellular secreted alpha-synuclein: Its role in nitrosative stress and neurodegeneration. Neurochem Int .

35. Volles MJ, Lansbury PT Jr (2003) Zeroing in on the pathogenic form of alpha-synuclein and its mechanism of neurotoxicity in Parkinson's disease. Biochemistry 42: 7871-7878.

36. Liu J, Shi M, Hong Z, Zhang J, Bradner J, et al. (2010) Identification of ciliary neurotrophic factor receptor alpha as a mediator of neurotoxicity induced by alpha-synuclein. Proteomics 10: 2138-2150.

37. Zhang W, Wang T, Pei Z, Miller DS, Wu X, et al. (2005) Aggregated alpha-synuclein activates microglia: a process leading to disease progression in Parkinson's disease. FASEB J 19: 533-542.

38. Klegeris A, Pelech S, Giasson BI, Maguire J, Zhang H, et al. (2008) Alpha-synuclein activates stress signaling protein kinases in THP-1 cells and microglia. Neurobiol Aging 29: 739-752.

39. Su X, Maguire-Zeiss KA, Giuliano R, Prifti L, Venkatesh K, et al. (2008) Synuclein activates microglia in a model of Parkinson's disease. Neurobiol Aging 29: 1690-1701.

40. Fellner L, Irschick R, Schanda K, Reindl M, Klimaschewski L, et al. (2013) Toll-like receptor 4 is required for α-synuclein dependent activation of microglia and astroglia. Glia 61: 349-360.

41. Kim C, Ho DH, Suk JE, You S, Michael S, et al. (2013) Neuron-released oligomeric α-synuclein is an endogenous agonist of TLR2 for paracrine activation of microglia. Nat Commun 4: 1562.

42. Lee SB, Park SM, Ahn KJ, Chung KC, Paik SR, et al. (2009) Identification of the amino acid sequence motif of alpha-synuclein responsible for macrophage activation. Biochem Biophys Res Commun 381: 39-43.

43. Giasson BI, Murray IV, Trojanowski JQ, Lee VM (2001) A hydrophobic stretch of 12 amino acid residues in the middle of alpha-synuclein is essential for filament assembly. J Biol Chem 276: 2380-2386.

44. Kim S, Cho SH, Kim KY, Shin KY, Kim HS, et al. (2009) Alpha-synuclein induces migration of BV-2 microglial cells by up-regulation of CD44 and MT1-MMP. J Neurochem 109: 1483-1496.

45. Klegeris A, Giasson BI, Zhang H, Maguire J, Pelech S, et al. (2006) Alpha-synuclein and its disease-causing mutants induce ICAM-1 and IL-6 in human astrocytes and astrocytoma cells. FASEB J 20: 2000-2008.

46. Lee HJ, Baek SM, Ho DH, Suk JE, Cho ED, et al. (2011) Dopamine promotes formation and secretion of non-fibrillar alpha-synuclein oligomers. Exp Mol Med 43: 216-222.

47. Park SM, Kim KS (2013) Proteolytic clearance of extracellular α-synuclein as a new therapeutic approach against Parkinson disease. Prion 7: 121-126.

48. Bae EJ, Lee HJ, Rockenstein E, Ho DH, Park EB, et al. (2012) Antibody-aided clearance of extracellular Î±-synuclein prevents cell-to-cell aggregate transmission. J Neurosci 32: 13454-13469.