Institute of Neurosurgery, The PLA Navy General Hospital, Beijing, China

Extracellular α-synuclein - a possible initiator of inflammation in Parkinson's disease

Wen-Qing Ren, Zeng-Min Tian, Feng Yin, Jun-Zhao Sun, Jian-Ning Zhang

Received April 18, 2015, accepted July 17, 2015

Jian-Ning Zhang, Institute of Neurosurgery, General Hospital of Navy, 6 Fucheng Rd., Beijing 100048, China
yanwenfen@126.com

Pharmazie 71: 51–55 (2016) *doi: 10.1691/ph.2016.5070*

Parkinson's disease (PD) is a progressive neurodegenerative disease involving the loss of dopamine-producing neurons of the substantia nigra and the presence of Lewy bodies which contain high levels of α-synuclein. Although the causative factors of PD remain unclear, the progression of PD is accompanied by a highly localized inflammatory response mediated by reactive microglia. Recently, attention has focused on the relationship between α-synuclein and microglial activation. This review examines the role of α-synuclein on microglia in PD pathogenesis and progression, we also discuss the way of α-synuclein induced microglial activation.

1. Introduction

Parkinson's disease (PD) is the second most prevalent age-related neurodegenerative disease, after Alzheimer's disease (AD). PD affects 3% of people over 60 years (Chauhan and Jeans 2015). The pathological hallmarks of PD are the loss of dopaminergic neurons in the substantia nigra of the brain and the presence of intraneuronal cytoplasmic inclusions, termed "Lewy Bodies" (LBs) (Schulz and Falkenburger 2004). Although the etiology of PD is incompletely understood, dysfunction of the ubiquitin proteasome pathway, protein aggregation, mitochondrial dysfunction, oxidative stress and inflammation have all been identified as important pathways leading to excitotoxic and apoptotic death of dopaminergic neurons (Barzilai and Melamed 2003; Moore et al. 2005; Ryan et al. 2015). Among these possible causes of PD, microglia and neuroinflammation have gained great attention during the last years. Further epidemiological studies have shown that NSAIDs (Non-Steroidal Anti-inflammatory Drugs) could attenuate the development of PD (Bornebroek et al. 2007; Gagne and Power 2010; Gao et al. 2011; Rees et al. 2011).

α-Synuclein, a main component of LBs, can be secreted from neuronal cells. It is confirmed that extracellular α-synuclein stimulated the abnormal microglial activation in many models of Parkinson's disease, accompanied by a large number of inflammatory factor secretion *in vitro* (Klegeris et al. 2008; Su et al. 2008; Codolo et al. 2013; Lei et al. 2014). Therefore, the interaction between microglial activation and extracellular α-synuclein seems to have become paramount in the pathogenesis of PD. A clear understanding about possible mechanisms of microglial activation by extracellular α-synuclein may be a valuable reference to potential strategy of PD treatment. Thus, we do a literature research and make a review about the structure and function of α-synuclein, the effect of microglial activation in PD and the possible pathway of microglial activation induced by extracellular α-synuclein.

2. Structure and function of α-synuclein in PD

α-Synuclein is an abundant and intrinsically disordered protein that is predominantly localized around synaptic vesicles in presynatic terminals. α-Synuclein is a small protein (140 amino acid) firstly described in *Torpedo californica* (Maroteaux et al. 1988). The protein is composed of three distinct regions (Fig. 1): (1) an amino terminus, residues1–60, contains four 11-amino acid imperfect repeats. It is coding for amphipathic helices, with a conserved motif (KTKEGV); (2) a central hydrophobic, residues 61–95, is called non-Aβ component (NAC). This domain was first discovered as the non-amyloid component of amyloid-β plaques in Alzheimer disease, and allows for fibrillization of α-synuclein to undergo a conformational change from random coil to β-sheet structure (el-Agnaf and Irvine 2002); (3) a carboxyl terminus, residues 96-140, a highly negatively charged, is thought to contain protein–protein and protein–small molecule interaction sites. The carboxy-terminus of α-synuclein may regulate aggregation of full-length α-synuclein and determine the diameter of α-synuclein filaments (Murray et al. 2003). In recent years, it has been hypothesized that the extent of α-synuclein may be a key factor in mediating transmission of disease pathology from one brain area to another. Braak and colleagues described their widely accepted staging system of PD progression. They identified six stages in PD. Starting first with the appearance of α-synuclein aggregates in the olfactory bulb and anterior olfactory nucleus. Thereafter, the pathology spreads rostral, indicated by LBs. During the second stage, the pathology manifests itself in the caudal raphe nucleus, in the gigantocellular reticular nucleus, and in the coeruleus–subcoeruleus complex. It is not until stage 3 where α-synuclein-positive deposits appear in the substantia nigra. At stage 4, lesions are detected in proencephalic regions and finally reach areas of neocortex and prefrontal cortex at the last stages.

In its native monomeric state, α-synuclein behaves as an intrinsically unstructured protein, characterized by the lack of a stable

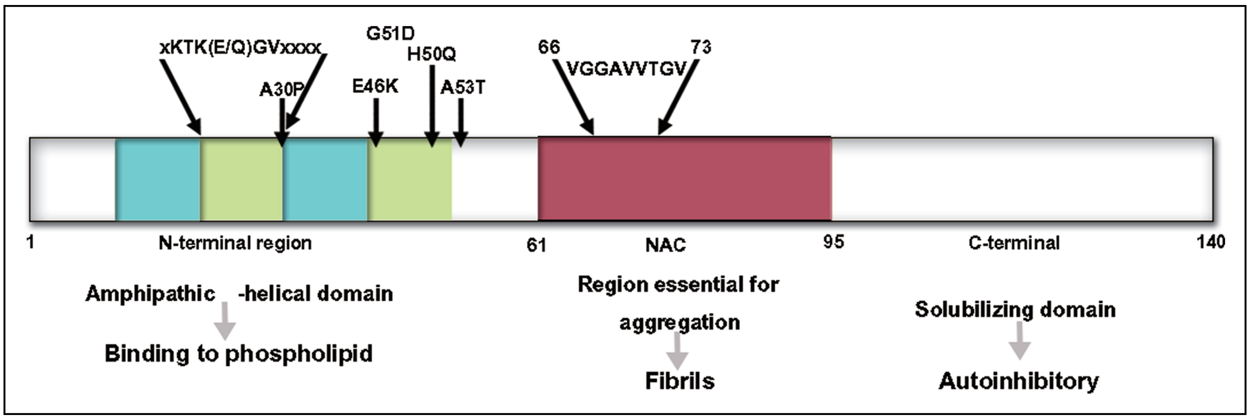

Fig. 1: Schematic structure of α-synuclein.

tertiary structure. However, it may adopt a secondary structure upon binding to protein interactors or lipid membranes. The assembly of α-synuclein is accompanied by transition from random coil to a-pleated sheet conformation (Roberts and Brown 2015). X-ray fiber and electron diffraction studies show α-synuclein filaments to exhibit a conformational conversion and fibril formation (Bousset et al. 2013). The pathological form of α-synuclein consists of oligomers and fibrils being rich in β-sheets. The conversion of its α-helical structure to the β-sheet rich fibril is a defining pathologic feature of α-synuclein.

α-Synuclein is a member of the family of synuclein proteins, which also include β- and γ-synuclein. Although the physiological function of α-synuclein has not been completely defined, studies have shown that it may be closely related to synaptic vesicle (Nakamura et al. 2008) transport function and synaptic plasticity, signal transmission, and the dopamine synthesis and so on. Several models have been made to investigate the pathological features of α-synuclein in PD. Early studies sing the zebra finch indicated that the expression of α-synuclein in neuron terminals is related to synaptic plasticity. Abeliovich and collaborators first developed feasible α-synuclein-knockout mice models, having normal synaptic architecture and brain morphology, showing that α-synuclein deletion only led to slight changes in synaptic transmission (Abeliovich et al. 2000). The second type of model are rodent and primate models where cell death is induced using neurotoxins such as MPTP and rotenone, 6-OHDA. These models have studied how oxidative stress and metabolic challenges alter the structure, expression and aggregation of α-synuclein. Otherwise, there are genetic models, where mutations (A53T, A30P, and E46K as well as G51D and H50Q) in proteins are associated with the development of PD (Appel-Cresswell et al. 2013; Proukakis et al. 2013). They have been expressed in transgenic mice or delivered using viral vectors in *Drosophila*. In all cases, α-synuclein accumulates in neurons, leading to the formation of protofibrils, fibrils and LBs.

α-Synuclein is known as an intraneuronal protein. However, a series of studies demonstrated the presence of α-synuclein in extracellular fluids. A nanomolar concentration of extracellular α-synuclein enhanced dopaminergic neuronal survival; this effect disappeared when the level of α-synuclein reached a micromolar concentration (Kim et al. 2013b). Published reports demonstrated a statistically significant quantification of α-synuclein in PD. The concentrations of α-synuclein in biological fluids were determined by sandwich ELISA, as measured in cell-free CSF (1.57 + 0.6 ng/ml), serum (12.69 + 10.3 ng/ml), plasma (59.54 + 35.1 ng/ml) and whole blood lysates (5002.03 + 3827.6 ng/ml) (Scherzer et al. 2008; Golpich et al. 2015). Recently, studies have put forward the presence of extracellular α-synuclein, suggesting that the pathogenic action of this protein may also be a crucial factor in the pathogenesis of PD (Pacheco et al. 2012; Gassowska et al. 2014; Lee et al. 2014).

3. Microglial activation in PD

Microglia, the brain-resident macrophages, are the major immune cells in PD (Chung et al. 2010; Fellner and Stefanova 2013). Under physiological conditions, microglia continually monitor brain microenvironment in a resting state characterized by ramified morphology. In response to certain cues such as brain injury or immunological stimuli, microglia are readily activated (Fetler and Amigorena 2005). Key features of microglial activation are morphological changes, increased expression of cell surface receptors, the production and release of proinflammatory cytokines as well as neurotoxic factors (e.g: tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), nitric oxide (NO) and reactive oxygen species (Block et al. 2007). Furthermore microglia-mediated neuroinflammation may contribute significantly to neurodegeneration in parkinsonian animals or even human Parkinson's disease (Kohutnicka et al. 1998; Ouchi et al. 2005; Qian and Flood 2008). Subsequently, microglia evoke a cascade of cell signaling, resulting in the activation of classical pathways (Block et al. 2007). The generation of reactive oxygen species proinflammatory cytokines are believed to be largely responsible for dopaminergic cell death in PD (Tansey and Goldberg 2010; Li et al. 2013; Frakes et al. 2014).

4. Mechanisms of microglial activation by extracellular α-synuclein

In recent years, extracellular α-synuclein has received a lot of scientific attention due to its potential role in the initiation and progression of PD. Extracellular α-synuclein exerts extracellular pathogenic actions as well. Recent studies suggest that neuronal cells release small amounts of α-synuclein by unconventional exocytosis (Lee et al. 2005; Jang et al. 2010; Recasens and Dehay 2014). Released α-synuclein can be transferred to neighbouring neurons and glia cells, inducing cell death and contributing to the major pathological features of PD (Lee et al. 2010b; Wilkaniec et al. 2013; Gallegos et al. 2015). Thus, extracellular α-synuclein may be a possible initiator of the neuroinflammation in Parkinson's disease.

Several papers have reported that extracellular α-synuclein immunoreactive surrounded by activated microglia or inflammatory mediators (McGeer et al. 1988; Chao et al. 2014; Gassowska et al. 2014; Heneka et al. 2014). Overexpression of α-synuclein leads to increased numbers of activated microglia

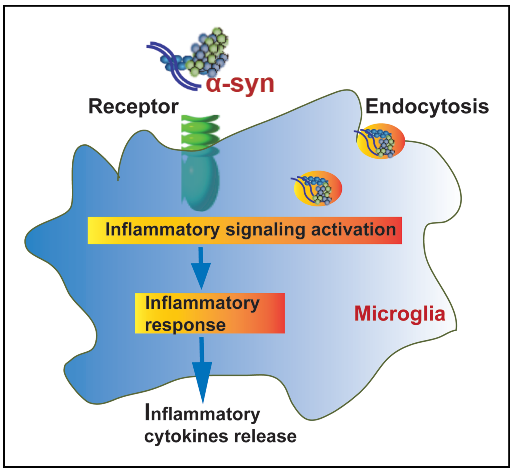

Fig. 2: Interaction between α-synuclein and microglia.

and the absence of dopamine neuron death in the substantia nigra pars compacta (SNpc) (Su et al. 2008). Altered α-synuclein expression in microglial cells, such as α-synuclein knockout mice, have been shown to exhibit a remarkably different phenotype compared to Wt cells with elevated levels of pro-inflammatory cytokines, for example tumour necrosis factor -α and interleukin -6 (Austin et al. 2006). This shows that α-synuclein plays a key role in modulating the activation of microglia. However, the mechanism involved in microglia activation by α-synuclein is controversial (Sung et al. 2001; Ahn et al. 2006; Golpich et al. 2015). Several modes have been proposed that mediate interaction of α-synuclein with microglial activation.

4.1. Receptor–independent endocytosis of microglia

Exocytosis/endocytosis and passive membrane translocation are classical mechanisms for the transfer of proteins between cells. Extracellular α-synuclein can enter recipient cells by endocytosis through the plasma membrane (Fig. 2). α-Synuclein is known to associate with membranous compartments in cultured cells and in brain tissue (George et al. 1995; McLean et al. 2000). He-Jin Lee et al.'s experimental results showed that the activation way of extracellular α-synuclein is dependent on the assembly state of the protein; while monomeric α-synuclein passively diffuses across the plasma membrane (Lee et al. 2008). The *in vitro* studies also showed that α-synuclein can interact with lipid layers, through the conformational changes to the α-helical structure (Recasens and Dehay 2014). It was also reported that both the N- and C-terminal regions of α-synuclein play a critical role in this translocation, particularly the 11-amino acid imperfect repeats at the N-terminal region of the protein (Ahn et al. 2006).

A study using computer modeling and membrane simulations investigated the course of penetration of WT and A53T mutant α-synuclein in the membranes and pore formation activity. Their data showed that the N-terminal initially makes contact with the surface of the membrane, undergoing changes in secondary structure, and that the penetration of the A53T mutant α-synuclein across the membrane was faster than WT α-synuclein (Tsigelny et al. 2012). A similar study with the endocytosis inhibitors monodansylcadaverine or dynasore validated this entry of α-synuclein, these inhibitors decreased the number of cells interacting with α-synuclein inclusions, indicating that endocytosis plays an important role in the extracellular α-synuclein activating microglia (Hansen et al. 2011).

4.2. Receptor-mediated endocytosis of microglia

Previous studies showed that extracellular α-synuclein can activate microglia cells *via* receptor-independent endocytosis. However, somewhat conflicting results, were presented showing that aggregated forms of α-synuclein, both fibrils and oligomers, penetrate into microglia *via* unidentified receptors mediating endocytosis. Under treatment with endocytosis blockers, the membrane translocation of α-synuclein was not significantly affected (Ahn et al. 2006). Here, we present evidence that TLR2 (toll-like receptors 2), TLR4 (toll-like receptors 4) and the NLRP3 (nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin domain containing 3) inflammasome are involved in the activation mechanism of microglia.

Toll-like receptors (TLRs) are expressed on cells of the innate immune system, including microglia. TLRs are activated by PAMPs. There is a large body of evidence for the role of TLR-mediated neuroinflammation in the initiation and progression of PD (Lehnardt 2010; Arroyo et al. 2011). Dawn Béraud's team showed that extracellular α-synuclein acts as a DAMP which can directly activate microglia and modulate expression of toll-like receptors (Beraud et al. 2011). Oligomeric α-synuclein may induce inflammatory responses in the microglia through activating TLR2 *in vitro* and *in vivo*. The extracellular oligomeric α-synuclein are endogenous agonist of TLR2 (Kim et al. 2013a). It also said that only specific types of α-synuclein oligomers can bind and activate TLR2, however, monomeric α-synuclein binds to TLR4. Interestingly, there is a different point of view that monomeric and fibrillar species can also bind to TLR2 (Codolo et al. 2013). Although, there are controversies about the different effects of monomeric and oligomeric α-synuclein interaction with TLRs, TLRs are type-specific receptors for α-synuclein activating microglia.

The NLR family comprises pattern-recognition receptors that regulate innate immunity and inflammatory processes (Davis et al. 2011; Mason et al. 2012). There are three distinct subfamilies of the NLR family, the NODs, the NLRPs and the IPAF subfamily. NLRP3 has occupied particular attention due to its role in the NLRP3 inflammasome, a multi-protein complex consisting of the NLRP3 scaffold, the adaptor molecule apoptosis-associated speck-like protein containing a CARD (ASC) and the cysteine protease caspase-1 (Schroder and Tschopp 2010). Codolo and colleagues have recently demonstrated that insoluble α-synuclein fibrils induced monocytes to release IL-1β following the activation of NLRP3 inflammasome. This is the first strong evidence for the involvement of NLRP3 inflammasome in prion-associated inflammation. It was concluded that the NALP3 inflammasome is involved in neurotoxic prion peptide-induced microglial activation (Shi et al. 2012). Subsequently, a wealth of information linking the microglia-specific activation of NLRP3 inflammasome by Aβ have a critical role in AD pathogenesis by mediating a harmful chronic inflammatory response (Halle et al. 2008; Goldmann et al. 2013; Heneka et al. 2013; Wu et al. 2013). Some researchers suggest that α-synuclein has prion-like behavior and mechanisms (Lema Tome et al. 2013; Masuda-Suzukake et al. 2013; Narkiewicz et al. 2014). Moreover, a fragment of α-synuclein has also been identified as non-Aβ component of AD amyloids, α-synuclein has the ability to form β-sheets to form amyloid-like fibrils. Thus, we believe that α-synuclein existing in extracellular fluids may activate microglia inflammation through the NLRP3 inflammasome.

4.3. α-Synuclein induced signaling in microglia

Microglial activation induced by α-synuclein is likely to evoke multiple intracellular signaling pathways, resulting in the induction of innate immune responses, including the activation of transcription factors that regulate the induction of genes encoding proinflammatory cytokines. However, the underlying signaling mechanisms are different.

Ample evidence suggests that nuclear factor-κB (NF-κB) signal cascade plays a key role in microglial activation induced by monomeric, oligomeric, aggregated or nitrated α-synuclein, owing to its function in the regulation of pro-inflammatory mediators (Austin et al. 2006; Klegeris et al. 2008; Su et al. 2014). In addition, NF-kB regulates the expression and activation of matrix metalloproteinases (MMPs), involved in the cellular activation process initiated by α-synuclein (Lee et al. 2010a). P38 and ERK1/2 MAP kinases have been consistently implicated with exposing to α-synuclein *in vitro* studies and in rodent cell lines and human microglia (Wilms et al. 2009; Prabhakaran et al. 2011).

CD14 has also been associated with α-synuclein induced microglial activation. Victoria Ingham and his teams found the cytokine production from CD14 knockout mice reduced in microglia, when microglial cells responsed to α-synuclein. Beyond that, there are other signaling pathways, such as PI(3)K/AKT (Wang et al. 2012), TLR4-MyD88 pathway (Fellner et al. 2013), CD36 (Su et al. 2008) and so on.

5. Conclusion

The activation of microglia and the damage of dopaminergic neurons may play an important role in the development of PD. α-Synuclein can be released and spread into the extracellular environment. Extracellular α-Synuclein may directly react with the adjacent microglia by endocytosis or bind to the receptor of cell surfaces resulting in the activation of microglia. Extracellular α-Synuclein mediated microglial activation aggravates the damage of dopaminergic neurons, contributing to the pathological process of PD. Therefore, to interfere with the extracellular α-synuclein induced microglia activation may because a strategy for the treatment of Parkinson's disease.

References

Abeliovich A, Schmitz Y, Farinas I, Choi-Lundberg D, Ho WH, Castillo PE, Shinsky N, Verdugo JM, Armanini M, Ryan A, Hynes M, Phillips H, Sulzer D, Rosenthal A (2000) Mice lacking alpha-synuclein display functional deficits in the nigrostriatal dopamine system. Neuron 25: 239–252.

Ahn KJ, Paik SR, Chung KC, Kim J (2006) Amino acid sequence motifs and mechanistic features of the membrane translocation of alpha-synuclein. J Neurochem 97: 265–279.

Appel-Cresswell S, Vilarino-Guell C, Encarnacion M, Sherman H, Yu I, Shah B, Weir D, Thompson C, Szu-Tu C, Trinh J, Aasly JO, Rajput A, Rajput AH, Jon Stoessl A, Farrer MJ (2013) Alpha-synuclein p.H50Q, a novel pathogenic mutation for Parkinson's disease. Mov Disord 28: 811–813.

Arroyo DS, Soria JA, Gaviglio EA, Rodriguez-Galan MC, Iribarren P (2011) Toll-like receptors are key players in neurodegeneration. Int Immunopharmacol 11: 1415–1421.

Austin SA, Floden AM, Murphy EJ, Combs CK (2006) Alpha-synuclein expression modulates microglial activation phenotype. J Neurosci 26: 10558–10563.

Barzilai A, Melamed E (2003) Molecular mechanisms of selective dopaminergic neuronal death in Parkinson's disease. Trends Mol Med 9: 126–132.

Beraud D, Twomey M, Bloom B, Mittereder A, Ton V, Neitzke K, Chasovskikh S, Mhyre TR, Maguire-Zeiss KA (2011) alpha-Synuclein Alters Toll-Like Receptor Expression. Front Neurosci 5: 80.

Block ML, Zecca L, Hong JS (2007) Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. Nature Rev Neurosci 8: 57–69.

Bornebroek M, de Lau LM, Haag MD, Koudstaal PJ, Hofman A, Stricker BH, Breteler MM (2007) Nonsteroidal anti-inflammatory drugs and the risk of Parkinson disease. Neuroepidemiology 28: 193–196.

Bousset L, Pieri L, Ruiz-Arlandis G, Gath J, Jensen PH, Habenstein B, Madiona K, Olieric V, Böckmann A, Meier BH, Melki R (2013) Structural and functional characterization of two alpha-synuclein strains. Nature Commun 4: 2575.

Chao Y, Wong SC, Tan EK (2014) Evidence of inflammatory system involvement in Parkinson's disease. Biomed Res Int 2014: 308654.

Chauhan A, Jeans AF (2015) Is Parkinson's disease truly a prion-like disorder? An appraisal of current evidence. Neurol Res Int 2015: 345285.

Chung YC, Ko HW, Bok E, Park ES, Huh SH, Nam JH, Jin BK (2010) The role of neuroinflammation on the pathogenesis of Parkinson's disease. BMB Rep 43: 225–232.

Codolo G, Plotegher N, Pozzobon T, Brucale M, Tessari I, Bubacco L, de Bernard M (2013) Triggering of inflammasome by aggregated alpha-synuclein, an inflammatory response in synucleinopathies. PloS one 8: e55375.

Davis BK, Wen H, Ting JP (2011) The inflammasome NLRs in immunity, inflammation, and associated diseases. Ann Rev Immunol 29: 707–735.

el-Agnaf OM, Irvine GB (2002) Aggregation and neurotoxicity of alpha-synuclein and related peptides. Biochem Soc Transact 30: 559–565.

Fellner L, Irschick R, Schanda K, Reindl M, Klimaschewski L, Poewe W, Wenning GK, Stefanova N (2013) Toll-like receptor 4 is required for alpha-synuclein dependent activation of microglia and astroglia. Glia 61: 349–360.

Fellner L, Stefanova N (2013) The role of glia in alpha-synucleinopathies. Molr Neurobiol 47: 575–586.

Fetler L, Amigorena S (2005) Neuroscience. Brain under surveillance: the microglia patrol. Science 309: 392–393.

Frakes AE, Ferraiuolo L, Haidet-Phillips AM, Schmelzer L, Braun L, Miranda CJ, Ladner KJ, Bevan AK, Foust KD, Godbout JP, Popovich PG, Guttridge DC, Kaspar BK (2014) Microglia induce motor neuron death *via* the classical NF-kappaB pathway in amyotrophic lateral sclerosis. Neuron 81: 1009–1023.

Gagne JJ, Power MC (2010) Anti-inflammatory drugs and risk of Parkinson disease: a meta-analysis. Neurology 74: 995–1002.

Gallegos S, Pacheco C, Peters C, Opazo CM, Aguayo LG (2015) Features of alpha-synuclein that could explain the progression and irreversibility of Parkinson's disease. Front Neurosci 9: 59.

Gao X, Chen H, Schwarzschild MA, Ascherio A (2011) Use of ibuprofen and risk of Parkinson disease. Neurology 76: 863–869.

Gassowska M, Czapski GA, Pajak B, Cieślik M, Lenkiewicz AM, Adamczyk A (2014) Extracellular alpha-synuclein leads to microtubule destabilization via GSK-3beta-dependent Tau phosphorylation in PC12 cells. PloS one 9: e94259.

George JM, Jin H, Woods WS, Clayton DF (1995) Characterization of a novel protein regulated during the critical period for song learning in the zebra finch. Neuron 15: 361–372.

Goldmann T, Tay TL, Prinz M (2013) Love and death: microglia, NLRP3 and the Alzheimer's brain. Cell Res 23: 595–596.

Golpich M, Amini E, Hemmati F, Ibrahim NM, Rahmani B, Mohamed Z, Raymond AA, Dargahi L, Ghasemi R, Ahmadiani A (2015) Glycogen synthase kinase-3 beta (GSK-3beta) signaling: Implications for Parkinson's disease. Pharmacol Res 97: 16–26.

Halle A, Hornung V, Petzold GC, Stewart CR, Monks BG, Reinheckel T, Fitzgerald KA, Latz E, Moore KJ, Golenbock DT (2008) The NALP3 inflammasome is involved in the innate immune response to amyloid-beta. Nature Immunol 9: 857–865.

Hansen C, Angot E, Bergstrom AL, Steiner JA, Pieri L, Paul G, Outeiro TF, Melki R, Kallunki P, Fog K, Li JY, Brundin P (2011) alpha-Synuclein propagates from mouse brain to grafted dopaminergic neurons and seeds aggregation in cultured human cells. J Clin Invest 121: 715–725.

Heneka MT, Kummer MP, Latz E (2014) Innate immune activation in neurodegenerative disease. Nature Rev Immunol 14: 463–477.

Heneka MT, Kummer MP, Stutz A, Delekate A, Schwartz S, Vieira-Saecker A, Griep A, Axt D, Remus A, Tzeng TC, Gelpi E, Halle A, Korte M, Latz E, Golenbock DT (2013) NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. Nature 493: 674–678.

Jang A, Lee HJ, Suk JE, Jung JW, Kim KP, Lee SJ (2010) Non-classical exocytosis of alpha-synuclein is sensitive to folding states and promoted under stress conditions. J Neurochem 113: 1263–1274.

Kim C, Ho DH, Suk JE, You S, Michael S, Kang J, Joong Lee S, Masliah E, Hwang D, Lee HJ, Lee SJ (2013a) Neuron-released oligomeric alpha-synuclein is an endogenous agonist of TLR2 for paracrine activation of microglia. Nature Comm 4: 1562.

Kim JY, Jeon BS, Kim HJ, Ahn TB (2013b) Nanomolar concentration of alpha-synuclein enhances dopaminergic neuronal survival via Akt pathway. Neural Regen Res 8: 3269–3274.

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

Kohutnicka M, Lewandowska E, Kurkowska-Jastrzebska I, Członkowski A, Członkowska A (1998) Microglial and astrocytic involvement in a murine model of Parkinson's disease induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Immunopharmacology 39: 167–180.

Lee EJ, Woo MS, Moon PG, Baek MC, Choi IY, Kim WK, Junn E, Kim HS (2010a) Alpha-synuclein activates microglia by inducing the expressions of matrix metalloproteinases and the subsequent activation of protease-activated receptor-1. J Immunol 185: 615–623.

Lee HJ, Bae EJ, Lee SJ (2014) Extracellular alpha–synuclein-a novel and crucial factor in Lewy body diseases. Nature Rev Neurol 10: 92–98.

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

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

Lee HJ, Suk JE, Patrick C, Bae EJ, Cho JH, Rho S, Hwang D, Masliah E, Lee SJ (2010b) Direct transfer of alpha-synuclein from neuron to astroglia causes inflammatory responses in synucleinopathies. J Biol Chem 285: 9262–9272.

Lehnardt S (2010) Innate immunity and neuroinflammation in the CNS: the role of microglia in Toll-like receptor-mediated neuronal injury. Glia 58:253–263.

Lei B, Mace B, Dawson HN, Warner DS, Laskowitz DT, James ML (2014) Anti-inflammatory effects of progesterone in lipopolysaccharide-stimulated BV-2 microglia. PloS one 9: e103969.

Lema Tome CM, Tyson T, Rey NL, Grathwohl S, Britschgi M, Brundin P (2013) Inflammation and alpha-synuclein's prion-like behavior in Parkinson's disease–is there a link? Mol Neurobiol 47: 561–574.

Li J, Yang JY, Yao XC, Xue X, Zhang QC, Wang XX, Ding LL, Wu CF (2013) Oligomeric Abeta-induced microglial activation is possibly mediated by NADPH oxidase. Neurochem Res 38:443–452.

Maroteaux L, Campanelli JT, Scheller RH (1988) Synuclein: a neuron-specific protein localized to the nucleus and presynaptic nerve terminal. J Neurosci 8: 2804–2815.

Mason DR, Beck PL, Muruve DA (2012) Nucleotide-binding oligomerization domain-like receptors and inflammasomes in the pathogenesis of non-microbial inflammation and diseases. J Innate Immun 4:16–30.

Masuda-Suzukake M, Nonaka T, Hosokawa M, Oikawa T, Arai T, Akiyama H, Mann DM, Hasegawa M (2013) Prion-like spreading of pathological alpha-synuclein in brain. Brain 136:1128–1138.

McGeer PL, Itagaki S, Boyes BE, McGeer EG (1988) Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. Neurology 38:1285–1291.

McLean PJ, Kawamata H, Ribich S, Hyman BT (2000) Membrane association and protein conformation of alpha-synuclein in intact neurons. Effect of Parkinson's disease-linked mutations. J Biol Chem 275: 8812–8816.

Moore DJ, West AB, Dawson VL, Dawson TM (2005) Molecular pathophysiology of Parkinson's disease. Ann Rev Neurosci 28: 57–87.

Murray IV, Giasson BI, Quinn SM, Koppaka V, Axelsen PH, Ischiropoulos H, Trojanowski JQ, Lee VM (2003) Role of alpha-synuclein carboxy-terminus on fibril formation in vitro. Biochemistry 42: 8530–8540.

Nakamura K, Nemani VM, Wallender EK, Kaehlcke K, Ott M, Edwards RH (2008) Optical reporters for the conformation of alpha-synuclein reveal a specific interaction with mitochondria. J Neurosci 28: 12305–12317.

Narkiewicz J, Giachin G, Legname G (2014) In vitro aggregation assays for the characterization of α-synuclein prion-like properties. Prion 8:19–32.

Ouchi Y, Yoshikawa E, Sekine Y, Futatsubashi M, Kanno T, Ogusu T, Torizuka T (2005) Microglial activation and dopamine terminal loss in early Parkinson's disease. Ann Neurol 57: 168–175.

Pacheco C, Aguayo LG, Opazo C (2012) An extracellular mechanism that can explain the neurotoxic effects of alpha-synuclein aggregates in the brain. Front Physiol 3: 297.

Prabhakaran K, Chapman GD, Gunasekar PG (2011) alpha-Synuclein overexpression enhances manganese-induced neurotoxicity through the NF-kappaB-mediated pathway. Toxicol Mech Methods 21: 435–443.

Proukakis C, Dudzik CG, Brier T, MacKay DS, Cooper JM, Millhauser GL, Houlden H, Schapira AH (2013) A novel alpha-synuclein missense mutation in Parkinson disease. Neurology 80: 1062–1064.

Qian L, Flood PM (2008) Microglial cells and Parkinson's disease. Immunol Res 41: 155–164.

Recasens A, Dehay B (2014) Alpha-synuclein spreading in Parkinson's disease. Front Neuroanat 8:159.

Rees K, Stowe R, Patel S, Ives N, Breen K, Clarke CE, Ben-Shlomo Y (2011) Non-steroidal anti-inflammatory drugs as disease-modifying agents for Parkinson's disease: evidence from observational studies. The Cochrane database of systematic reviews CD008454.

Roberts HL, Brown DR (2015) Seeking a mechanism for the toxicity of oligomeric alpha-synuclein. Biomolecules 5: 282–305.

Ryan BJ, Hoek S, Fon EA, Wade-Martins R (2015) Mitochondrial dysfunction and mitophagy in Parkinson's: from familial to sporadic disease. Trends Biochem Sci 40: 200–210.

Scherzer CR, Grass JA, Liao Z, Pepivani I, Zheng B, Eklund AC, Ney PA, Ng J, McGoldrick M, Mollenhauer B, Bresnick EH, Schlossmacher MG (2008) GATA transcription factors directly regulate the Parkinson's disease-linked gene alpha-synuclein. Proc Natl Acad Sci USA 105: 10907–10912.

Schroder K, Tschopp J (2010) The inflammasomes. Cell 140: 821–832.

Schulz JB, Falkenburger BH (2004) Neuronal pathology in Parkinson's disease. Cell Tissue Res 318: 135–147.

Shi F, Yang L, Kouadir M, Yang Y, Wang J, Zhou X, Yin X, Zhao D (2012) The NALP3 inflammasome is involved in neurotoxic prion peptide-induced microglial activation. J Neuroinflammation 9: 73.

Su X, Chen Q, Chen W, Chen T, Li W, Li Y, Dou X, Zhang Y, Shen Y, Wu H, Yu C (2014) Mycoepoxydiene inhibits activation of BV2 microglia stimulated by lipopolysaccharide through suppressing NF-kappaB, ERK 1/2 and toll-like receptor pathways. Int Immunopharmacol 19: 88–93.

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

Sung JY, Kim J, Paik SR, Park JH, Ahn YS, Chung KC (2001) Induction of neuronal cell death by Rab5A-dependent endocytosis of alpha-synuclein. J Biological Chem 276: 27441–27448.

Tansey MG, Goldberg MS (2010) Neuroinflammation in Parkinson's disease: its role in neuronal death and implications for therapeutic intervention. Neurobiol Dis 37: 510–518.

Tsigelny IF, Sharikov Y, Wrasidlo W, Gonzalez T, Desplats PA, Crews L, Spencer B, Masliah E (2012) Role of alpha-synuclein penetration into the membrane in the mechanisms of oligomer pore formation. FEBS J 279: 1000–1013.

Wang G, Pan J, Chen SD (2012) Kinases and kinase signaling pathways: potential therapeutic targets in Parkinson's disease. Progress Neurobiol 98: 207–221.

Wilkaniec A, Strosznajder JB, Adamczyk A (2013) Toxicity of extracellular secreted alpha-synuclein: Its role in nitrosative stress and neurodegeneration. Neurochem Int 62: 776–783.

Wilms H, Rosenstiel P, Romero-Ramos M, Arlt A, Schäfer H, Seegert D, Kahle PJ, Odoy S, Claasen JH, Holzknecht C, Brandenburg LO, Deuschl G, Schreiber S, Kirik D, Lucius R (2009) Suppression of MAP kinases inhibits microglial activation and attenuates neuronal cell death induced by alpha-synuclein protofibrils. Int J Immunopathol Pharmacol 22: 897–909.

Wu Z, Sun L, Hashioka S, Schwab C, Okada R, Hayashi Y, McGeer PL, Nakanishi H. (2013) Differential pathways for interleukin-1beta production activated by chromogranin A and amyloid beta in microglia. Neurobiol Aging 34: 2715–2725.