We are IntechOpen, the world's leading publisher of Open Access books
Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

154
Countries delivered to

Our authors are among the

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Alpha-Synuclein Aggregation, Cholesterol Transport, and the 18-kDa Translocator Protein

Jasmina Dimitrova-Shumkovska and Ljupcho Krstanoski

Abstract

The molecular responses to counteract diseases, including insulting conditions such as injury and pathogen infection, involve coordinated modulation of gene expression programs. The association of alpha synuclein (α-Syn) with several progressive disorders has focused the research on its induced conformational behavior as critical for uncovering the "secrets" for progression of α-synucleinopathies. Cholesterol is one of the lipid components crucial for regular proliferation of the nervous tissue. Its interaction with α-Syn may offer other insights to α-Syn normal expression. Discovering that the molecular regulatory mechanisms responsible for prevention of α-Syn aggregation may be manifested through microRNA (miRNA) regulated gene expression is also crucial for widening the perception of neuropathology. The 18-kDa translocator protein (TSPO) localized on the outer mitochondrial membrane is able to regulate various cellular and tissue functions, with key role as cholesterol transporter for neurosteroid synthesis. TSPO up-regulation, has been connected to several diseases, including cancer, neuronal damage, and inflammation. Connection may also be established between TSPO expression and fatty acid oxidation, thus unveiling new possibilities in the research of α-Syn overexpression. However, expression of TSPO in the neuroinflammatory environment is probably the best starting point for targeting TSPO as a suitable therapeutic target.

Keywords: alpha synuclein, lipid interaction, inflammation, oxidative stress, TSPO ligands

1. Synucleins family—new insights and prospects

Synucleins are a family of small and soluble proteins expressed mostly in neural tissue and cancer cells. Existing findings have identified three members of this family: α, β, and γ—synucleins (α-Syn, β-Syn, and γ-Syn, respectively). Rather than unveiling their physiological properties and functions in normal brain tissue, the synucleins are mostly exploited as biomarkers for neurodegenerative diseases since the discovery of their involvement in proteinaceous aggregation in patients with Alzheimer's disease (AD) [1]. In the following years, synucleins have been linked with other neuronal disorders increasing the interest of elucidating their connection with these diseases.

α-Synucleinopathies are severe neurodegenerative disorders caused by abnormal accumulation and subsequent aggregation of insoluble α-Syn, a small and

intrinsically unfolded cytosolic protein localized at synaptic terminals, in structures called Lewy bodies (LBs) in neuronal or glial cells [2, 3]. Establishing its involvement in synaptic maintenance, mitochondrial homeostasis, and neurotransmitter release regulation, α-Syn impaired function is considered as a direct cause for several progressive disorders such as Parkinson's disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA). Other rare diseases, mainly associated with neuroaxonal dystrophy have also shown α-Syn pathologies [4].

Despite early discovery of α-Syn as a product of SNCA gene whose dysfunction was considered as the primary cause for PD development [5], excessive research has been carried out in order to fully disclose the reasons for α-Syn aggregation. In the following years, nine other genes such as PARK, PINK, and LRRK involved in PD pathology were discovered [6–8], but so far missense mutations and multiplication of α-Syn-encoding gene are considered as the most often causation of familial form of PD [9]. Intensive research is mainly focused on discovering the effects of fibrillization, oligomerization, and misfolding of this protein as well as developing a suitable methods for its quantification in biological fluid enabling early diagnosis of PD [10–12]. Efforts are also being made to elucidate the participation of other molecules in the α-Syn altered dynamics. Namely, Abeyawardhane et al. reported the contribution of oxygen and redox active iron in conformational change and oligomerization of α-Syn, which can be useful in understanding the mechanisms of its physiological and/or pathological role [13]. The strong ability of α-Syn to form complexes with other biomolecules such as lipid moieties and cholesterol has also been reported. This capability is due to the presence of the repeats of lipo-protein-like hexamer sequence (KTKEGV) in synucleins, which may reveal other approaches for the diagnosis and therapy of neurodegenerative diseases [14, 15].

Furthermore, it has been shown that this protein was also expressed in erythroid precursors, megakaryocytes, and platelets [16, 17]. α-Syn is assumed to participate in negative regulation of calcium dependent α-granule release, thus implying that its presence is crucial for normal development and functioning of platelets [18, 19]. Relevant to this context, platelets have immense diagnostic value for neurocognitive diseases since several studies reported the significantly decreased levels of amyloid β protein precursor (AβPP) and mean platelet volume (MPV) in AD patients [20, 21]. Further supporting the concept, the presence of α-Syn in platelets impacts MPV level through the SCNA gene expression, whereas its concentration remains unaltered in patients with cognitive impairment [19]. However, α-Syn concentration in plasma supernatant is considered as a significant marker for the quality of single donor platelet samples during storage time [22].

β-synuclein (β-Syn) although somewhat smaller protein than α-Syn is also localized in presynaptic terminals, secreted and expressed in similar levels [23]. Early research concerning β-Syn properties and function suggests that this protein, even though 78% identical to α-Syn, is not present in LBs, and therefore, it is not directly involved in neurodegenerative and neurocognitive pathology. The main "advantage" of β-Syn against the induced changing of its conformation is the absence of nonamyloid β component (NAC) domain in its structure. Hence, β-Syn can significantly reduce the initiation of self-assembly and aggregation of α-Syn since it lacks this highly hydrophobic domain, which may prove beneficial against abnormal accumulation of α-Syn, thus preventing neurodegeneration [24, 25].

Several studies have shown the natural antagonism between the two molecules providing the mechanisms for inhibition of α-Syn aggregation both *in vivo* and *in vitro* [26, 27]. Janowska et al. reported that β-Syn-mediated inhibition of α-Syn aggregation occurs by direct interaction between the molecules at specific sites. This ultimately results in the formation of heterodimers further implying that balance between the specificity and affinity of α-Syn/β-Syn interactions is crucial

for maintaining favorable reduction rates of α-Syn aggregation [28]. A study by Brown et al. also suggested that β-Syn molecules can only inhibit the nucleation of lipid bound and fibril forms of α-Syn aggregates by competitive binding to the surface of vesicles prepared from the phospholipid 1,2-dimyristoyl-sn-glycero-3-phospho-L-serine (DMPS), and this inhibition is pH dependent. In addition, they confirmed that β-Syn has no effect on elongation of α-Syn aggregates [29]. It seems that further research is needed in order to highlight the exact mechanism and conditions in which β-Syn prevents the aggregation of α-Syn, therefore enabling its use as antiparkinsonian agent.

γ-Synuclein (γ-Syn) has been identified in various human tissues, and its expression is significantly upregulated in ovary, liver, and cervical cancer, with specific overexpression in breast cancer linked with tumor development and promoting of cancer metastasis through demethylation of CpG islands and activation of insulin-like growth factor pathway [30–32]. Similar to β-Syn, γ-Syn is also naturally found in peripheral neurons, and it has not been directly correlated with pathology of neurocognitive diseases, although differences have been reported in its expression [33]. Beside the existence of γ-Syn in nervous and malignant tissue, studies also reported its presence in the skin particularly in *stratum granulosum* where it could be included in modulation of keratin [34]. This synuclein member is also found in retinal ganglion cells (RGCs) where its decreased expression was first correlated with the development of glaucoma [35]. Later, research indicates that γ-Syn downregulates kinases involved in activation of pro-apoptotic signaling pathways in RGCs, therefore playing a key regulatory role in progression of this disease [36]. The protective mechanism of γ-Syn antibodies in neuroretinal cells against oxidative stress by increasing the viability and altering their apoptosis rate has also been reported [37].

2. Regulation of α-synuclein expression

Because of the genetic background of α-synucleinopathies, research must also be focused toward discovering the exact molecules and mechanisms for posttranscriptional and epigenetic regulation of SNCA gene. Up to this point, it is established that not only changes in the gene sequence (multiplications, missense mutations, and single nucleotide polymorphisms) but also activation of certain transcriptional factors and RNAs may affect α-Syn regular expression [38]. MicroRNAs (MiRNAs) are small non-coding RNA molecules encoded as independent genomic transcription units predominantly engaged as regulators of protein expression mostly through inhibition of mRNA translation or cleavage [39, 40]. Ever since their discovery, miRNA dysregulation is correlated with the pathogenesis of numerous diseases and disorders such as cancer, diabetes, nonalcoholic fatty liver disease (NAFLD), neurological and cardiovascular diseases (CVD) [41, 42]. As mentioned earlier, the main causes for PD development are mutations in genes resulting in a α-Syn overexpression and modification, so it is highly possible that PD progression and/or inhibition can be managed by alteration of certain miRNAs. So far, it has been confirmed that they can affect several signaling pathways involved in PD development, therefore enabling their use as biomarkers or alternative therapy for PD, as well as other types of dementia.

Because the oligomerization and fibrillation of α-Syn is primarily associated with increased production of reactive oxygen species (ROS) and subsequent mitochondrial dysfunction in neuronal cells, research has been conducted in order to identify the key miRNAs involved in regulation of brain mitochondrial function [43]. Namely, several studies have reported the down regulatory effects

of miR-34b and miR-34c on the expression of protein deglycase DJ-1, involved in α-Syn degradation via chaperone-mediated autophagy (CMA), thus preventing the ROS outburst from complex I or other constituents from the electron transport chain (ETC) [44–46]. Recent publication by De Miranda et al. also marked the DJ-1 as an essential for maintaining the integrity of dopaminergic neurons which is accomplished by reduction of nitrosative stress and suppression of rotenone-induced inflammatory response, thus highlighting its value as potential therapeutic target [47]. Furthermore, it was also elucidated that decrease of miR-34 b/c levels in neuronal cells leads to the loss of mitochondrial potential and reduction of ATP production. Accordingly, the depletion of these miRNAs directly contributes for decreased levels of DJ-1 in brains from PD, with a direct binding to the 3′-untranslated region (3′-UTR) of their mRNAs which proves their neuroprotective role [46]. Additionally, miR-4639 and miR-494 were identified in the list of potential inhibitors of DJ-1 expression, suggesting the measurement of their levels in human plasma as prognostic biomarkers of PD [48, 49] (**Figure 1**).
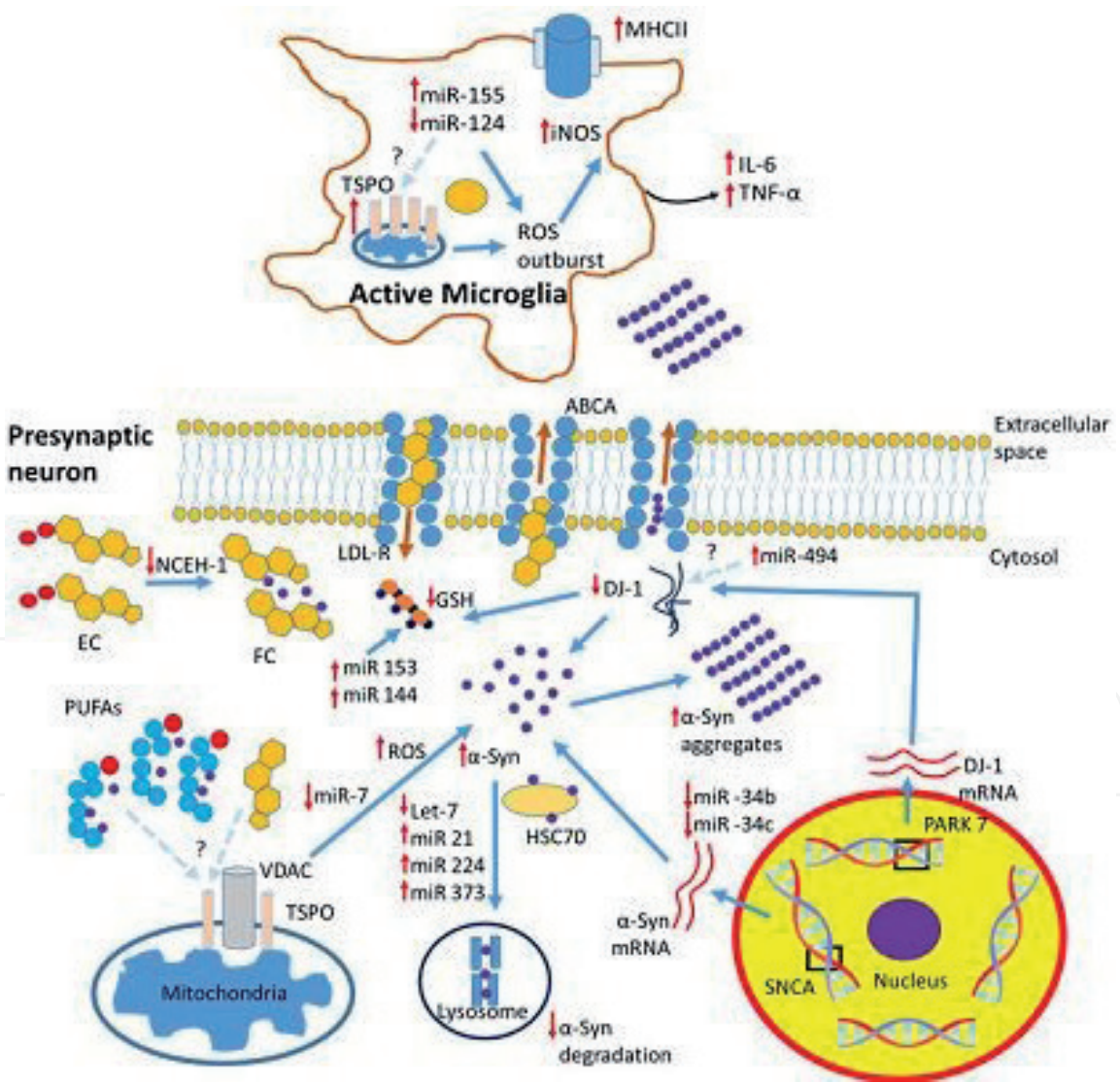

Figure 1.
Summary of signaling pathways involved in α-Syn aggregation and potential connection of 18-kDa translocator protein (TSPO) with neurodegeneration and neuroinflammatory response. Abbreviations: alpha synuclein (α-Syn), low density lipoprotein receptor (LDL-R), esterified cholesterol (EC), free cholesterol (FC), polyunsaturated fatty acids (PUFAs), heat shock cognate 71-kDa protein (hsc70), interleukin 6 (IL-6), tumor necrosis factor alpha (TNFα), neutral cholesterol ester hydrolase (NCEH-1), 18-kDa translocator protein (TSPO), major histocompatibility complex class II protein (MHC II), and ATP-binding cassette subfamily A (ABCA).

Similarly to the effects of miR-34 b/c, it was confirmed that miR-7 also plays a key role in α-Syn repression by directly binding to the 3′-UTR sequence of its mRNA in different experimental models such as SH-SY5Y cells, HEK293T cells, primary neurons, and pancreatic islets [50–52]. Moreover, Junn et al. discovered the protective role of miR-7 against hydrogen peroxide-mediated cell injury in cells expressing mutant A53T form of α-Syn [51]. The effects of MiR-7 on cell death reduction in experimentally induced PD symptomatology by various neurotoxins such as MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) and its active metabolite MPP$^+$ (1-methyl-4-phenylpyridinium) was reported as well [53]. A recent study indicated that miR-7 can also achieve its protective role by regulating the expression of the voltage dependent anionic channel (VDAC) in the outer mitochondrial membrane (OMM), thereby preventing MPP$^+$-induced cellular damage [54]. Since VDAC is crucial part of mitochondrial permeability transition pore, its function is primarily associated with maintaining the polarization of OMM and balancing ROS production. Research has confirmed that VDAC overexpression can increase free radical generation and cause the release of pro-apoptotic proteins ultimately promoting mitochondrial swelling which inevitably triggers α-Syn aggregation [55]. The discovery that cells producing A53T had swollen mitochondria puts VDAC in the list of key molecules involved in progression of α-synucleinopathies [56]. Overall, it can be concluded that miR-7 optimal expression is directly "responsible" for regular neural development, and future research should be focused on finding suitable vectors in order to include this molecule in gene therapy for neurodegenerative diseases.

As indicated earlier, cellular mechanisms for α-Syn degradation such as CMA can also be affected by miRNAs. Studies revealed that the increase of miR-21, miR-224, and miR-373 levels leads to suppression of heat shock cognate 71-kDa protein (hsc70) which impairs α-Syn degradation via CMA [57]. Moreover, Shamsuzzama et al. reported that Let-7 miRNA knockdown might influence CMA by modulating gene expression and enhancing ROS production in *C. elegans* [58].

MiRNAs have also been connected with pathways related to synthesis and expression of enzymatic and nonenzymatic radical scavengers within brain cells effectively "delaying" any genetic aberrations and expression α-Syn mutant forms. These are included in regulation of nuclear factor erythroid 2-related factor 2-antioxidant response element (Nrf2-ARE), which is also one of the reasons for PD progression [59]. Narasimhan et al. observed that the overexpression of miR-153, miR-27a, miR-142-5p, and miR-144 weakened the antioxidant response in SH-SY5Y cells throw decreasing the activity glutathione reductase (GSR) with impact on GSH/GSSG homeostasis [60].

Progression of PD due to α-Syn aggregation results in brain inflammatory response as primary defensive mechanism against neurodegeneration achieved through microglial cells. This process is mainly associated with activation of several components in the inflammatory cascade such as interleukins, members of the complement system, and receptors or enzymes whose expression is essential for proper immune defense [61, 62]. Neuroinflammation can also be aggravated by dietary components such as artificial sweeteners who additionally enhance dopamine degeneration and gravity of the immune response [63]. Discovery of miRNAs highlighted the possibilities of regulating the intensity and severity of the immune response against α-Syn-mediated inflammation. Specifically, Thome et al. reported the pro-inflammatory effects of miR-155 in brain microglia against α-Syn fibrils manifested through elevation of inducible nitric oxide synthase (iNOS) and major histocompatibility complex class II proteins (MHCII) expression, thus keeping the integrity of dopamine neurons [64]. A recent study confirmed that miR-124 could suppress microglial activation by regulating the expression of inflammatory cytokines [65] (**Figure 1**).

Because of the complexity of brain inflammatory response, it is necessary to extend the research for other molecules that might be included in its mediation. The number of studies has suggested the 18 kDa mitochondrial translocator protein (TSPO) as potential biomarker for neurodegenerative disorders [66, 67]. This protein is included in cholesterol transport into the mitochondria where it serves as a substrate for neurosteroid biosynthesis [68]. As previously reported that brain injury increases TSPO binding affinity for its ligand PK11195 [69, 70], the connection between TSPO expression and α-synucleinopathies has not been sufficiently explained [71]. Namely, it was reported that TSPO exhibited increased striatal PK11195 binding potential in patients with PD and DLB, but its expression remained unaltered compared to healthy controls [72, 73]. Regarding neuroinflammation, TSPO overexpression is also associated with activation of microglia/macrophages, revealing yet another unexplored role of this receptor [74]. There is also overwhelming evidence that TSPO ligands and agonists possess neuroprotective properties, but so far little is known about the precise functions of TSPO itself in brain cells [75, 76]. Overall, it seems that further research is needed in order to elucidate the regulatory mechanism of miRNAs in neuroinflammation and the possible correlation with TSPO.

2.1 α-Synuclein, lipid homeostasis, and TSPO

As mentioned earlier, α-Syn possesses intriguing and still not fully characterized affinity of interacting with fatty acids, cholesterol and phospholipids, and other cell lipid molecules. This implies that high levels of polyunsaturated fatty acids (PUFAs) normally present in healthy brain tissue, which not only increase its membrane fluidity and permeability but also serve as energy sources and second messengers, could be one of the reasons for α-Syn increased expression in the nervous system [77]. Further *in vivo* investigation, revealed that α-Syn overexpression in patients with α-synucleinopathies caused an increase of PUFA levels without alteration on saturated and monounsaturated fatty acid composition [78]. The decline in n-6/n-3 ratio during aging, increased lipid peroxidation and decreased brain volume, are between main factors promoting neurodegenerative disorder. Following this assumption, many studies proved that the enhanced multimerization and interaction of this protein with PUFAs, particularly with arachidonic and docosahexaenoic acid, result in the formation and aggregation of insoluble high-molecular complexes in LBs, which might unveil new insights into PD diagnostics [79, 80]. Based on the aforementioned findings, research should be focused on discovering whether α-Syn/PUFA interactions could be a sufficient proof of the alleged scavenging activity of α-Syn in experimental models of PD. So far, results based on spectrometric analysis confirm that PUFA interacts solely with α-helical secondary structure of α-Syn in optimal protein/PUFA ratio, which strongly suggests that α-Syn may prevent the initiation of lipid peroxidation given the high autoxidation rates of PUFAs [81]. Researchers have also reported the regulatory role of α-Syn of other lipid molecules such as triacylglycerols and cholesteryl esters, considering their increased conversion to lipid droplets in α-Syn expressing cells due to the modulating activity of lipid metabolizing enzymes, such as acyl-CoA synthase [82, 83].

Other important biomarkers for neurodegenerative disorders are the phospholipase D (PLD) isoforms which are crucial enzymes mostly involved in cytoskeleton structure and cellular signaling processes in the brain. More recent studies reported that inflammation caused by oxidative stress triggers PLD signaling as part of the synaptic response in neurodegeneration indirectly insinuating a connection

between PLD and α-Syn overexpression [84, 85]. Conde et al. has confirmed this connection by proving that this protein acts as an inhibitor of PLD1 in WT α-Syn neurons [86].

Cholesterol is also one of the lipid components which homeostasis is crucial for regular proliferation of the nervous tissue, if properly regulated. It acts as an integral membrane component, improving its structure and function. As mentioned earlier, studies have already established the interaction between α-Syn and cholesterol, indirectly making a correlation between cholesterol levels and α-Syn normal expression [15]. In a study by Hsiao et al., α-Syn was described as mediator of cholesterol efflux from SK-N-SH neuronal cells enabled by an ATP-binding cassette subfamily A (ABCA1) [87]. In accordance with these discoveries, the possible neuroprotective role of enzymes included in "cellular capturing and release" of cholesterol such as *neutral cholesterol ester hydrolase* (*NCEH*) or *Acyl Co-A:cholesterol acyltransferase* (*ACAT*) was also investigated. Namely, Zhang et al. discovered that *NCEH-1* knockdown increases the aggregation of α-Syn and dopamine neural damage in *C. elegans*, while inhibition of ACAT gave the opposite effect. Moreover, they confirmed the previous hypothesis concerning normal cholesterol levels and α-Syn expression, further implying that this relation is also a highly important factor for triggering the neuroprotective role of *NCEH-1*. Finally, these authors suggested that exogenous cholesterol does not have beneficial effects against neural degeneration [88].

Taking into account that TSPO is also involved in alterations of cytosolic cholesterol levels, there is also a possibility for its involvement in modulation of α-Syn aggregation rates. Connection has also been established between TSPO binding capacity and ROS levels, which are as mentioned earlier one of the reasons for PD development [89, 90]. In accordance with these findings, Gatliff et al. reported that SH-SY5H cells exhibited enhanced ROS production after TSPO overexpression establishing connection between TSPO, VDAC, and Ca^{2+} homeostasis [91]. On the other hand, it is also suggested that TSPO expression is inversely correlated with fatty oxidation rates in steroidogenic cells [92], which may be a plausible starting point in discovering whether TSPO has the same effect in neurons and if so, could altered expression of TSPO prove beneficial against neurodegenerative disorders considering the α-Syn interactions with PUFAs and cholesterol (**Figure 1**).

3. Conclusions

A systematic research in the last two decades highlights the precise mechanisms and pathways for regulation of α-Syn expression and aggregation, involved in neuropathologies. Success has also been made in demonstrating the possible therapeutic values of miRNAs, receptors, and other bioactive molecules with specific intentions for their inclusion in modern therapy for dementias. Future research should be focused on discovering the proposed beneficial actions of the interactions between lipids and α-Syn with particular interest in the potential involvement of TSPO in cholesterol homeostasis of the neural cells (**Figure 1**).

Acknowledgements

Abbreviations and symbols

α-Syn	alpha synuclein
β-Syn	beta synuclein
γ-Syn	gamma synuclein
NAC	non-amyloid-β component
SNCA	alpha synuclein gene
PINK	PTEN-induced kinase
PD	Parkinson's disease
AD	Alzheimer's disease
DLB	dementia with Lewy Bodies
LBs	Lewy Bodies
AβPP	amyloid β protein precursor
MPV	mean platelet volume
RGCs	retinal ganglion cells
DMPS	1,2-dimyristoyl-sn-glycero-3-phospho-L-serine
NAFLD	nonalcoholic fatty liver disease
CVD	cardiovascular disease
UTR	untranslated region
A53T	mutant form of alpha synuclein
VDAC	voltage dependent anionic channel
OMM	outer mitochondrial membrane
ROS	reactive oxygen species
CMA	chaperone-mediated autophagy
hsc70	heat shock cognate 71-kDa protein
Nrf2-ARE	erythroid 2-related factor 2-antioxidant response element
DJ-1	protein deglycase DJ-1
iNOS	inducible nitric oxide synthase
MHCII	major histocompatibility complex class II proteins
TSPO	18-kDa translocator protein
PK11195	1-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-3-isoquinoline-carboxamide
PUFAs	polyunsaturated fatty acids
PLD	phospholipase D
NCEH-1	neutral cholesterol ester hydrolase
ACAT	acyl Co-A:cholesterol acyltransferase
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MPP$^+$	1-methyl-4-phenylpyridinium

Author details

Jasmina Dimitrova-Shumkovska* and Ljupcho Krstanoski
Department of Experimental Biochemistry and Physiology, Faculty of Natural
Sciences and Mathematics, Institute of Biology, Ss. Cyril and Methodius University,
Skopje, Republic of Macedonia

*Address all correspondence to: jasminad@pmf.ukim.mk

IntechOpen

References

[1] Ueda K, Fukushima H, Masliah E, Xia Y, Iwai A, Yoshimoto M, et al. Molecular cloning of cDNA encoding an unrecognized component of amyloid in Alzheimer disease. Proceedings of the National Academy of Sciences of the United States of America. 1993;**90**(23):11282-11286

[2] McCann H, Stevens CH, Cartwright H, Halliday GM. α-Synucleinopathy phenotypes. Parkinsonism & Related Disorders. 2014;**20**:S62-S67. DOI: 10.1016/s1353-8020(13)70017-8

[3] Wakabayashi K, Tanji K, Odagiri S, Miki Y, Mori F, Takahashi H. The Lewy body in Parkinson's disease and related neurodegenerative disorders. Molecular Neurobiology. 2013;**47**(2):495-508. DOI: 10.1007/s12035-012-8280-y

[4] Uversky VN. A protein-chameleon: Conformational plasticity of alpha-synuclein, a disordered protein involved in neurodegenerative disorders. Journal of Biomolecular Structure & Dynamics. 2003;**21**(2):211-234. DOI: 10.1080/07391102.2003.10506918

[5] Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, et al. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science. 1997;**276**(5321):2045-2047

[6] Bonifati V, Rizzu P, van Baren MJ, Schaap O, Breedveld GJ, Krieger E, et al. Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. Science. 2003;**299**(5604):256-259. DOI: 10.1126/science.1077209

[7] Valente EM, Abou-Sleiman PM, Caputo V, Muqit MM, Harvey K, Gispert S, et al. Hereditary early-onset Parkinson's disease caused by mutations in PINK1. Science. 2004;**304**(5674):1158-1160. DOI: 10.1126/science.1096284

[8] Zimprich A, Biskup S, Leitner P, Lichtner P, Farrer M, Lincoln S, et al. Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. Neuron. 2004;**44**(4):601-607. DOI: 10.1016/j.neuron.2004.11.005

[9] Hardy J. Genetic analysis of pathways to Parkinson disease. Neuron. 2010;**68**(2):201-206. DOI: 10.1016/j.neuron.2010.10.014

[10] Emamzadeh FN, Surguchov A. Parkinson's disease: Biomarkers, treatment, and risk factors. Frontiers in Neuroscience. 2018;**12**:612. DOI: 10.3389/fnins.2018.00612

[11] Mollenhauer B, Batrla R, El-Agnaf O, Galasko DR, Lashuel HA, Merchant KM, et al. A user's guide for alpha-synuclein biomarker studies in biological fluids: Perianalytical considerations. Movement Disorders. 2017;**32**(8):1117-1130. DOI: 10.1002/mds.27090

[12] Tran HT, Chung CH, Iba M, Zhang B, Trojanowski JQ, Luk KC, et al. Alpha-synuclein immunotherapy blocks uptake and templated propagation of misfolded alpha-synuclein and neurodegeneration. Cell Reports. 2014;**7**(6):2054-2065. DOI: 10.1016/j.celrep.2014.05.033

[13] Abeyawardhane DL, Fernandez RD, Murgas CJ, Heitger DR, Forney AK, Crozier MK, et al. Iron redox chemistry promotes antiparallel oligomerization of alpha-synuclein. Journal of the American Chemical Society. 2018;**140**(15):5028-5032. DOI: 10.1021/jacs.8b02013

[14] Clayton DF, George JM. The synucleins: A family of proteins

involved in synaptic function, plasticity, neurodegeneration and disease. Trends in Neurosciences. 1998;**21**(6):249-254

[15] Fantini J, Carlus D, Yahi N. The fusogenic tilted peptide (67-78) of alpha-synuclein is a cholesterol binding domain. Biochimica et Biophysica Acta. 2011;**1808**(10):2343-2351. DOI: 10.1016/j.bbamem.2011.06.017

[16] Hashimoto M, Yoshimoto M, Sisk A, Hsu LJ, Sundsmo M, Kittel A, et al. NACP, a synaptic protein involved in Alzheimer's disease, is differentially regulated during megakaryocyte differentiation. Biochemical and Biophysical Research Communications. 1997;**237**(3):611-616. DOI: 10.1006/bbrc.1997.6978

[17] Nakai M, Fujita M, Waragai M, Sugama S, Wei J, Akatsu H, et al. Expression of alpha-synuclein, a presynaptic protein implicated in Parkinson's disease, in erythropoietic lineage. Biochemical and Biophysical Research Communications. 2007;**358**(1):104-110. DOI: 10.1016/j.bbrc.2007.04.108

[18] Kim KS, Park JY, Jou I, Park SM. Regulation of Weibel-Palade body exocytosis by alpha-synuclein in endothelial cells. The Journal of Biological Chemistry. 2010;**285**(28):21416-21425. DOI: 10.1074/jbc.M110.103499

[19] Xiao W, Shameli A, Harding CV, Meyerson HJ, Maitta RW. Late stages of hematopoiesis and B cell lymphopoiesis are regulated by alpha-synuclein, a key player in Parkinson's disease. Immunobiology. 2014;**219**(11):836-844. DOI: 10.1016/j.imbio.2014.07.014

[20] Srisawat C, Junnu S, Peerapittayamongkol C, Futrakul A, Soi-ampornkul R, Senanarong V, et al. The platelet amyloid precursor protein ratio as a diagnostic marker for Alzheimer's disease in Thai patients.

Journal of Clinical Neuroscience. 2013;**20**(5):644-648. DOI: 10.1016/j.jocn.2012.06.008

[21] Wang RT, Jin D, Li Y, Liang QC. Decreased mean platelet volume and platelet distribution width are associated with mild cognitive impairment and Alzheimer's disease. Journal of Psychiatric Research. 2013;**47**(5):644-649. DOI: 10.1016/j.jpsychires.2013.01.014

[22] Stefaniuk CM, Hong H, Harding CV, Maitta RW. Alpha-synuclein concentration increases over time in plasma supernatant of single donor platelets. European Journal of Haematology. 2018;**101**:630-634. DOI: 10.1111/ejh.13152

[23] Jakes R, Spillantini MG, Goedert M. Identification of two distinct synucleins from human brain. FEBS Letters. 1994;**345**(1):27-32

[24] Park JY, Lansbury PT Jr. Beta-synuclein inhibits formation of alpha-synuclein protofibrils: A possible therapeutic strategy against Parkinson's disease. Biochemistry. 2003;**42**(13):3696-3700. DOI: 10.1021/bi020604a

[25] Uversky VN, Li J, Souillac P, Millett IS, Doniach S, Jakes R, et al. Biophysical properties of the synucleins and their propensities to fibrillate: Inhibition of alpha-synuclein assembly by beta- and gamma-synucleins. The Journal of Biological Chemistry. 2002;**277**(14):11970-11978. DOI: 10.1074/jbc.M109541200

[26] Hashimoto M, Bar-On P, Ho G, Takenouchi T, Rockenstein E, Crews L, et al. Beta-synuclein regulates Akt activity in neuronal cells. A possible mechanism for neuroprotection in Parkinson's disease. The Journal of Biological Chemistry. 2004;**279**(22):23622-23629. DOI: 10.1074/jbc.M313784200

[27] Windisch M, Hutter-Paier B, Rockenstein E, Hashimoto M, Mallory M, Masliah E. Development of a new treatment for Alzheimer's disease and Parkinson's disease using anti-aggregatory beta-synuclein-derived peptides. Journal of Molecular Neuroscience. 2002;**19**(1-2):63-69. DOI: 10.1007/s12031-002-0012-8

[28] Janowska MK, Wu KP, Baum J. Unveiling transient protein-protein interactions that modulate inhibition of alpha-synuclein aggregation by beta-synuclein, a pre-synaptic protein that co-localizes with alpha-synuclein. Scientific Reports. 2015;**5**:15164. DOI: 10.1038/srep15164

[29] Brown JW, Buell AK, Michaels TC, Meisl G, Carozza J, Flagmeier P, et al. Beta-Synuclein suppresses both the initiation and amplification steps of alpha-synuclein aggregation via competitive binding to surfaces. Scientific Reports. 2016;**6**:36010. DOI: 10.1038/srep36010

[30] Hibi T, Mori T, Fukuma M, Yamazaki K, Hashiguchi A, Yamada T, et al. Synuclein-gamma is closely involved in perineural invasion and distant metastasis in mouse models and is a novel prognostic factor in pancreatic cancer. Clinical Cancer Research. 2009;**15**(8):2864-2871. DOI: 10.1158/1078-0432.ccr-08-2946

[31] Ji H, Liu YE, Jia T, Wang M, Liu J, Xiao G, et al. Identification of a breast cancer-specific gene, BCSG1, by direct differential cDNA sequencing. Cancer Research. 1997;**57**(4):759-764

[32] Surguchov A. Molecular and cellular biology of synucleins. International Review of Cell and Molecular Biology. 2008;**270**:225-317. DOI: 10.1016/s1937-6448(08)01406-8

[33] Rockenstein E, Hansen LA, Mallory M, Trojanowski JQ, Galasko D, Masliah E. Altered expression of the synuclein family mRNA in Lewy body and Alzheimer's disease. Brain Research. 2001;**914**(1-2):48-56

[34] Ninkina NN, Privalova EM, Pinon LG, Davies AM, Buchman VL. Developmentally regulated expression of persyn, a member of the synuclein family, in skin. Experimental Cell Research. 1999;**246**(2):308-311. DOI: 10.1006/excr.1998.4292

[35] Surgucheva I, McMahan B, Ahmed F, Tomarev S, Wax MB, Surguchov A. Synucleins in glaucoma: Implication of gamma-synuclein in glaucomatous alterations in the optic nerve. Journal of Neuroscience Research. 2002;**68**(1): 97-106. DOI: 10.1002/jnr.10198

[36] Surgucheva I, Shestopalov VI, Surguchov A. Effect of gamma-synuclein silencing on apoptotic pathways in retinal ganglion cells. The Journal of Biological Chemistry. 2008;**283**(52):36377-36385. DOI: 10.1074/jbc.M806660200

[37] Wilding C, Bell K, Beck S, Funke S, Pfeiffer N, Grus FH. Gamma-synuclein antibodies have neuroprotective potential on neuroretinal cells via proteins of the mitochondrial apoptosis pathway. PLoS One. 2014;**9**(3):e90737. DOI: 10.1371/journal.pone.0090737

[38] Piper DA, Sastre D, Schule B. Advancing stem cell models of alpha-synuclein gene regulation in neurodegenerative disease. Frontiers in Neuroscience. 2018;**12**:199. DOI: 10.3389/fnins.2018.00199

[39] Macfarlane LA, Murphy PR. MicroRNA: Biogenesis, function and role in cancer. Current Genomics. 2010;**11**(7):537-561. DOI: 10.2174/138920210793175895

[40] Perron MP, Provost P. Protein interactions and complexes in human microRNA biogenesis and function. Frontiers in Bioscience. 2008;**13**:2537-2547

[41] Tufekci KU, Oner MG, Meuwissen RL, Genc S. The role of microRNAs in human diseases. Methods in Molecular Biology. 2014;**1107**:33-50. DOI: 10.1007/978-1-62703-748-8_3

[42] Xiao J, Bei Y, Liu J, Dimitrova-Shumkovska J, Kuang D, Zhou Q, et al. miR-212 downregulation contributes to the protective effect of exercise against non-alcoholic fatty liver via targeting FGF-21. Journal of Cellular and Molecular Medicine. 2016;**20**(2):204-216. DOI: 10.1111/jcmm.12733

[43] Recasens A, Perier C, Sue CM. Role of microRNAs in the regulation of alpha-synuclein expression: A systematic review. Frontiers in Molecular Neuroscience. 2016;**9**:128. DOI: 10.3389/fnmol.2016.00128

[44] Hayashi T, Ishimori C, Takahashi-Niki K, Taira T, Y-c K, Maita H, et al. DJ-1 binds to mitochondrial complex I and maintains its activity. Biochemical and Biophysical Research Communications. 2009;**390**(3):667-672. DOI: 10.1016/j.bbrc.2009.10.025

[45] Lopert P, Patel M. Brain mitochondria from DJ-1 knockout mice show increased respiration-dependent hydrogen peroxide consumption. Redox Biology. 2014;**2**:667-672. DOI: 10.1016/j.redox.2014.04.010

[46] Minones-Moyano E, Porta S, Escaramis G, Rabionet R, Iraola S, Kagerbauer B, et al. MicroRNA profiling of Parkinson's disease brains identifies early downregulation of miR-34b/c which modulate mitochondrial function. Human Molecular Genetics. 2011;**20**(15):3067-3078. DOI: 10.1093/hmg/ddr210

[47] De Miranda BR, Rocha EM, Bai Q, El Ayadi A, Hinkle D, Burton EA, et al. Astrocyte-specific DJ-1 overexpression protects against rotenone-induced neurotoxicity in a rat model of Parkinson's disease. Neurobiology of Disease. 2018;**115**:101-114. DOI: 10.1016/j.nbd.2018.04.008

[48] Chen Y, Gao C, Sun Q, Pan H, Huang P, Ding J, et al. MicroRNA-4639 is a regulator of DJ-1 expression and a potential early diagnostic marker for Parkinson's disease. Frontiers in Aging Neuroscience. 2017;**9**:232. DOI: 10.3389/fnagi.2017.00232

[49] Xiong R, Wang Z, Zhao Z, Li H, Chen W, Zhang B, et al. MicroRNA-494 reduces DJ-1 expression and exacerbates neurodegeneration. Neurobiology of Aging. 2014;**35**(3):705-714. DOI: 10.1016/j.neurobiolaging.2013.09.027

[50] Doxakis E. Post-transcriptional regulation of alpha-synuclein expression by mir-7 and mir-153. The Journal of Biological Chemistry. 2010;**285**(17):12726-12734. DOI: 10.1074/jbc.M109.086827

[51] Junn E, Lee KW, Jeong BS, Chan TW, Im JY, Mouradian MM. Repression of alpha-synuclein expression and toxicity by microRNA-7. Proceedings of the National Academy of Sciences of the United States of America. 2009;**106**(31):13052-13057. DOI: 10.1073/pnas.0906277106

[52] Latreille M, Hausser J, Stutzer I, Zhang Q, Hastoy B, Gargani S, et al. MicroRNA-7a regulates pancreatic beta cell function. The Journal of Clinical Investigation. 2014;**124**(6):2722-2735. DOI: 10.1172/jci73066

[53] Fragkouli A, Doxakis E. miR-7 and miR-153 protect neurons against MPP(+)-induced cell death via upregulation of mTOR pathway. Frontiers in Cellular Neuroscience. 2014;**8**:182. DOI: 10.3389/fncel.2014.00182

[54] Chaudhuri AD, Choi DC, Kabaria S, Tran A, Junn E. MicroRNA-7 regulates the function of mitochondrial permeability transition pore by

targeting VDAC1 expression. The Journal of Biological Chemistry. 2016;**291**(12):6483-6493. DOI: 10.1074/jbc.M115.691352

[55] Abou-Sleiman PM, Muqit MM, Wood NW. Expanding insights of mitochondrial dysfunction in Parkinson's disease. Nature Reviews. Neuroscience. 2006;**7**(3):207-219. DOI: 10.1038/nrn1868

[56] Martin LJ, Semenkow S, Hanaford A, Wong M. Mitochondrial permeability transition pore regulates Parkinson's disease development in mutant α-synuclein transgenic mice. Neurobiology of Aging. 2014;**35**(5):1132-1152. DOI: 10.1016/j.neurobiolaging.2013.11.008

[57] Alvarez-Erviti L, Seow Y, Schapira AH, Rodriguez-Oroz MC, Obeso JA, Cooper JM. Influence of microRNA deregulation on chaperone-mediated autophagy and alpha-synuclein pathology in Parkinson's disease. Cell Death & Disease. 2013;**4**:e545. DOI: 10.1038/cddis.2013.73

[58] Shamsuzzama KL, Nazir A. Modulation of alpha-synuclein expression and associated effects by MicroRNA Let-7 in Transgenic *C. elegans*. Frontiers in Molecular Neuroscience. 2017;**10**:328. DOI: 10.3389/fnmol.2017.00328

[59] Ramsey CP, Glass CA, Montgomery MB, Lindl KA, Ritson GP, Chia LA, et al. Expression of Nrf2 in neurodegenerative diseases. Journal of Neuropathology and Experimental Neurology. 2007;**66**(1):75-85. DOI: 10.1097/nen.0b013e31802d6da9

[60] Narasimhan M, Patel D, Vedpathak D, Rathinam M, Henderson G, Mahimainathan L. Identification of novel microRNAs in post-transcriptional control of Nrf2 expression and redox homeostasis in neuronal, SH-SY5Y cells. PLoS One.

2012;**7**(12):e51111. DOI: 10.1371/journal.pone.0051111

[61] McGeer PL, Itagaki S, Tago H, McGeer EG. Occurrence of HLA-DR reactive microglia in Alzheimer's disease. Annals of the New York Academy of Sciences. 1988;**540**(1):319-323. DOI: 10.1111/j.1749-6632.1988.tb27086.x

[62] Mogi T, Saiki K, Anraku Y. Biosynthesis and functional role of haem O and haem A. Molecular Microbiology. 1994;**14**(3):391-398. DOI: 10.1111/j.1365-2958.1994.tb02174.x

[63] Amin SN, Hassan SS, Rashed LA. Effects of chronic aspartame consumption on MPTP-induced parkinsonism in male and female mice. Archives of Physiology and Biochemistry. 2018;**124**(4):292-299. DOI: 10.1080/13813455.2017.1396348

[64] Thome AD, Harms AS, Volpicelli-Daley LA, Standaert DG. microRNA-155 regulates alpha-synuclein-induced inflammatory responses in models of Parkinson disease. The Journal of Neuroscience. 2016;**36**(8):2383-2390. DOI: 10.1523/JNEUROSCI.3900-15.2016

[65] Yao L, Ye Y, Mao H, Lu F, He X, Lu G, et al. MicroRNA-124 regulates the expression of MEKK3 in the inflammatory pathogenesis of Parkinson's disease. Journal of Neuroinflammation. 2018;**15**(1):13-13. DOI: 10.1186/s12974-018-1053-4

[66] Gavish M, Weizman R. Role of peripheral-type benzodiazepine receptors in steroidogenesis. Clinical Neuropharmacology. 1997;**20**(6):473-481

[67] Venneti S, Lopresti BJ, Wiley CA. The peripheral benzodiazepine receptor (translocator protein 18 kDa) in microglia: From pathology to imaging. Progress in Neurobiology. 2006;**80**(6):308-322. DOI: 10.1016/j.pneurobio.2006.10.002

[68] Papadopoulos V, Liu J, Culty M. Is there a mitochondrial signaling complex facilitating cholesterol import? Molecular and Cellular Endocrinology. 2007;**265-266**:59-64. DOI: 10.1016/j.mce.2006.12.004

[69] Bae EJ, Lee HJ, Jang YH, Michael S, Masliah E, Min DS, et al. Phospholipase D1 regulates autophagic flux and clearance of alpha-synuclein aggregates. Cell Death and Differentiation. 2014;**21**(7):1132-1141. DOI: 10.1038/cdd.2014.30

[70] Gerhard A, Pavese N, Hotton G, Turkheimer F, Es M, Hammers A, et al. In vivo imaging of microglial activation with [11C](R)-PK11195 PET in idiopathic Parkinson's disease. Neurobiology of Disease. 2006;**21**(2):404-412. DOI: 10.1016/j.nbd.2005.08.002

[71] Gerhard A. TSPO imaging in parkinsonian disorders. Clinical and Translational Imaging. 2016;**4**:183-190. DOI: 10.1007/s40336-016-0171-1

[72] Iannaccone S, Cerami C, Alessio M, Garibotto V, Panzacchi A, Olivieri S, et al. In vivo microglia activation in very early dementia with Lewy bodies, comparison with Parkinson's disease. Parkinsonism & Related Disorders. 2013;**19**(1):47-52. DOI: 10.1016/j.parkreldis.2012.07.002

[73] Koshimori Y, Ko JH, Mizrahi R, Rusjan P, Mabrouk R, Jacobs MF, et al. Imaging striatal microglial activation in patients with Parkinson's disease. PLoS One. 2015;**10**(9):e0138721. DOI: 10.1371/journal.pone.0138721

[74] Ft B, Alleyne CH Jr, Sukumari-Ramesh S. Augmented expression of TSPO after intracerebral hemorrhage: A role in inflammation? Journal of Neuroinflammation. 2016;**13**(1):151. DOI: 10.1186/s12974-016-0619-2

[75] Barron AM, Garcia-Segura LM, Caruso D, Jayaraman A, Lee JW, Melcangi RC, et al. Ligand for translocator protein reverses pathology in a mouse model of Alzheimer's disease. The Journal of Neuroscience. 2013;**33**(20):8891-8897. DOI: 10.1523/JNEUROSCI.1350-13.2013

[76] Girard C, Liu S, Cadepond F, Adams D, Lacroix C, Verleye M, et al. Etifoxine improves peripheral nerve regeneration and functional recovery. Proceedings of the National Academy of Sciences of the United States of America. 2008;**105**(51):20505-20510. DOI: 10.1073/pnas.0811201106

[77] Ruiperez V, Darios F, Davletov B. Alpha-synuclein, lipids and Parkinson's disease. Progress in Lipid Research. 2010;**49**(4):420-428. DOI: 10.1016/j.plipres.2010.05.004

[78] Sharon R, Bar-Joseph I, Frosch MP, Walsh DM, Hamilton JA, Selkoe DJ. The formation of highly soluble oligomers of alpha-synuclein is regulated by fatty acids and enhanced in Parkinson's disease. Neuron. 2003;**37**(4):583-595

[79] Perrin RJ, Woods WS, Clayton DF, George JM. Exposure to long chain polyunsaturated fatty acids triggers rapid multimerization of synucleins. The Journal of Biological Chemistry. 2001;**276**(45):41958-41962. DOI: 10.1074/jbc.M105022200

[80] Welch K, Yuan J. Alpha-synuclein oligomerization: A role for lipids? Trends in Neurosciences. 2003;**26**(10):517-519

[81] De Franceschi G, Fecchio C, Sharon R, Schapira AHV, Proukakis C, Bellotti V, et al. Alpha-synuclein structural features inhibit harmful polyunsaturated fatty acid oxidation, suggesting roles in neuroprotection. The Journal of Biological Chemistry. 2017;**292**(17):6927-6937. DOI: 10.1074/jbc.M116.765149

[82] Cole NB, Murphy DD, Grider T, Rueter S, Brasaemle D, Nussbaum

RL. Lipid droplet binding and oligomerization properties of the Parkinson's disease protein alpha-synuclein. The Journal of Biological Chemistry. 2002;**277**(8):6344-6352. DOI: 10.1074/jbc.M108414200

[83] Golovko MY, Rosenberger TA, Feddersen S, Faergeman NJ, EJ M. Alpha-synuclein gene ablation increases docosahexaenoic acid incorporation and turnover in brain phospholipids. Journal of Neurochemistry. 2007;**101**(1):201-211. DOI: 10.1111/j.1471-4159.2006.04357.x

[84] Mateos MV, Giusto NM, Salvador GA. Distinctive roles of PLD signaling elicited by oxidative stress in synaptic endings from adult and aged rats. Biochimica et Biophysica Acta. 2012;**1823**(12):2136-2148. DOI: 10.1016/j.bbamcr.2012.09.005

[85] Mateos MV, Kamerbeek CB, Giusto NM, Salvador GA. The phospholipase D pathway mediates the inflammatory response of the retinal pigment epithelium. The International Journal of Biochemistry & Cell Biology. 2014;**55**:119-128. DOI: 10.1016/j.biocel.2014.08.016

[86] Conde MA, Alza NP, Iglesias Gonzalez PA, Scodelaro Bilbao PG, Sanchez Campos S, Uranga RM, et al. Phospholipase D1 downregulation by alpha-synuclein: Implications for neurodegeneration in Parkinson's disease. Biochimica et Biophysica Acta. 2018;**1863**(6):639-650. DOI: 10.1016/j.bbalip.2018.03.006

[87] Hsiao JT, Halliday GM, Kim WS. Alpha-synuclein regulates neuronal cholesterol efflux. Molecules. 2017;**22**(10). DOI: 10.3390/molecules22101769

[88] Zhang S, Glukhova SA, Caldwell KA, Caldwell GA. NCEH-1 modulates cholesterol metabolism and protects against alpha-synuclein toxicity in a *C. elegans* model of Parkinson's disease. Human Molecular Genetics. 2017;**26**(19):3823-3836. DOI: 10.1093/hmg/ddx269

[89] Dimitrova-Shumkovska J, Veenman L, Ristoski T, Leschiner S, Gavish M. Chronic high fat, high cholesterol supplementation decreases 18 kDa translocator protein binding capacity in association with increased oxidative stress in rat liver and aorta. Food and Chemical Toxicology. 2010;**48**(3): 910-921. DOI: 10.1016/j.fct.2009.12.032

[90] Sun X, Guo S, Wang W, Cao Z, Dan J, Cheng J, et al. Potential involvement of the 18 kDa translocator protein and reactive oxygen species in apoptosis of THP-1 macrophages induced by sonodynamic therapy. PLoS One. 2018;**13**(5):e0196541. DOI: 10.1371/journal.pone.0196541

[91] Gatliff J, East DA, Singh A, Alvarez MS, Frison M, Matic I, et al. A role for TSPO in mitochondrial Ca(2+) homeostasis and redox stress signaling. Cell Death & Disease. 2017;**8**(6):e2896. DOI: 10.1038/cddis.2017.186

[92] Tu LN, Zhao AH, Hussein M, Stocco DM, Selvaraj V. Translocator protein (TSPO) affects mitochondrial fatty acid oxidation in steroidogenic cells. Endocrinology. 2016;**157**(3):1110-1121. DOI: 10.1210/en.2015-1795