

Review

Possible Alterations in β -Synuclein, the Non-Amyloidogenic Homologue of α -Synuclein, during Progression of Sporadic α -Synucleinopathies

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Abstract: α -Synucleinopathies are neurodegenerative disorders that are characterized by progressive decline of motor and non-motor dysfunctions. α -Synuclein (α S) has been shown to play a causative role in neurodegeneration, but the pathogenic mechanisms are still unclear. Thus, there are no radical therapies that can halt or reverse the disease's progression. β -Synuclein (β S), the non-amyloidogenic homologue of α S, ameliorates the neurodegeneration phenotype of α S in transgenic (tg) mouse models, as well as in cell free and cell culture systems, which suggests that β S might be a negative regulator of neurodegeneration caused by α S, and that “loss of function” of β S might be involved in progression of α -synucleinopathies. Alternatively, it is possible that “toxic gain of function” of wild type β S occurs during the pathogenesis of sporadic α -synucleinopathies, since tg mice expressing dementia with Lewy bodies-linked P123H β S develop progressive neurodegeneration phenotypes, such as axonal pathology and dementia. In this short review, we emphasize the aspects of “toxic gain of function” of wild type β S during the pathogenesis of sporadic α -synucleinopathies.

Keywords: α -synucleinopathies; α -synuclein; β -synuclein; dementia with Lewy bodies; toxic gain of function

1. Introduction

β -Synuclein (β S) is a presynaptic protein of unknown function that was originally isolated as a phosphoprotein from bovine brain by Nakajo and colleagues in 1993 [1–3]. During the same period, two structurally related proteins; α -synuclein (α S) and γ -synuclein (γ S), were also found independently by several groups. After extensive characterization of the synuclein family of peptides, it is now well established that α S plays a causative role in stimulation of α -synucleinopathies, including Parkinson's disease (PD), dementia with Lewy bodies (DLB), multiple system atrophy, neurodegeneration with brain iron accumulation, type 1 (formerly known as Hallervorden-Spatz disease), and the Lewy body variant of Alzheimer's disease [4,5], while γ S is involved in tumor progression and metastasis of cancers such as breast cancer, ovarian tumor and brain tumors [6].

In contrast to the other two members, the pathogenic role of β S has been elusive. In this regard, we previously showed that the neurodegeneration phenotype of the α S transgenic (tg) was ameliorated by cross-breeding with a tg mouse expressing wild type β S, and proposed that non-amyloidogenic β S might negatively regulate neurodegeneration caused by amyloidogenic α S [7]. More recently, we showed that tg mice expressing DLB-linked P123H β S develop progressive neurodegeneration, as characterized by axonal pathology and memory disorder, suggesting that the DLB-linked mutant β S is pathogenic [8]. This raises the question of how these apparently opposite effects of wild type β S and DLB-linked mutant β S in tg mice brain should be interpreted. Is it sufficient to conclude that wild type β S is simply neuroprotective and that this is distinct from the stimulatory effect of neurodegeneration by DLB-linked mutant β S?

In this review, we propose a new perspective that “toxic gain of function” of wild type β S might be a critical event during the pathogenesis of sporadic α -synucleinopathies. This scenario may permit development of a comprehensive model of β S actions in the brain in α -synucleinopathies, including findings from our studies using tg mice and reports by other laboratories.

2. β S with a DLB-Linked Mutation Shows “Gain of Function” in α -Synucleinopathies

A series of studies in our laboratory have shown that β S with a DLB-linked mutation is an aggregate-prone protein and that “toxic gain of function” of mutant β S occurs in cell-free, cellular and tg experimental models.

Two missense mutations of β S were found in unrelated DLB patient pedigrees in 2004. A valine to methionine substitution at position 70 (V70M) was found in a sporadic DLB case in Japan, while a proline to histidine mutation at position 123 (P123H) was identified in a familial DLB pedigree in Seattle. Since P123H β S was inherited as an autosomal dominant trait, the β S gene mutations might have caused a toxic gain of function, similarly to cases with α S mutations. However, there was no pathological evidence of β S in autopsy brains of patients with the P123H β S mutation, including the absence of immunoreactivity of β S in Lewy bodies, and no aggregated form of β S in biochemical analysis. Thus, it was proposed that the mutated forms of β S might have lost the protective function of wild type β S.

To understand the role of the DLB-linked mutant form of β S in neurodegeneration more clearly, we analyzed P123H and V70M recombinant β S proteins in a cell-free system, cell culture, and transgenic (tg)

mice. Under cell-free conditions, both proteins were prone to aggregation and acted synergistically with α S to stimulate protein aggregation [9]. In B103 neuroblastoma cells expressing P123H or V70M β S, abnormal lysosomal inclusions were formed, in which mutant β S accumulated due to impairment of the autophagy-lysosome pathway [9]. Furthermore, the number of lysosomal inclusions was markedly increased by coexpression of α S and mutant β S [9]. Taken together, these results suggest that both mutant forms of β S could themselves be pathogenic and might act synergistically with α S.

This view was further supported by experiments in a tg mouse expressing P123H β S under the Thy-1 promoter. These mice displayed several neuropathological abnormalities, including formation of P123H β S-immunoreactive axonal swellings in basal ganglia and extensive astrogliosis in various brain regions, including the hippocampus, cerebral cortex and basal ganglia, indicating that P123H β S is pathogenic *in vivo* [8]. Strikingly, P123H β S tg mice also showed significant memory dysfunction at a relative early age (~6 months), while motor dysfunction was apparent in a later stage (over 12 months) [8]. These behavioral features were distinct from tg mice expressing Thy-1 promoter-driven α S, which showed significant motor dysfunction at an earlier age (~4 months). The discovery of β S mutations in DLB patients suggests that β S may be involved more specifically in memory functions, compared to α S. Consistent with this idea, β S was recently shown to be a strain-independent hippocampal protein that is upregulated during the Morris water maze test [8]. Thus, these results suggest that the pathological effects of β S might be distinct from those of α S.

Further cross-breeding experiments revealed that bigenic mice expressing α S and P123H β S exhibited greatly enhanced neurodegeneration phenotypes, including earlier appearance of axonal swelling, a decreased level of dopamine in the striatum, motor dysfunction, neuroinflammation, and neuronal cell death [8]. These results indicate that α S and P123H β S may synergistically enhance the neuropathological effect of each protein.

Taken together, the analyses of our P123H β S tg mice suggest that P123H β S is pathogenic through a “gain of function” mechanism and may cooperate with pathogenic α S to stimulate neurodegeneration in mouse brain, indicating a causative role of P123H β S in the pathogenesis of familial DLB.

3. Does “Loss of Function” Explain the Role of Wild Type β S in Sporadic α -Synucleinopathies?

We have shown that wild type β S is protective, whereas β S with a DLB-linked mutation is stimulatory for neurodegeneration. These results prompted us to consider if wild type β S might be involved in the pathogenesis of sporadic DLB and other α -synucleinopathies. To date, several studies have shown that “loss of function” of wild type β S could contribute to the pathogenesis of α -synucleinopathies.

Masliyah and colleagues used a RNA-protection assay that was previously used to show that the ratio of β S to α S at the mRNA level was significantly decreased in diseased brains, including PD, DLB and AD brains, compared to healthy brains [10]. These results are intriguing because decreased expression of β S may lead to relative loss of protective functions of β S against neurotoxicity caused by α S. Furthermore, it is possible that downregulation of β S could occur not only in α -synucleinopathies, but also in other types of neurodegenerative disease. More recently, Ariza and colleagues performed a real-time polymerase chain reaction analysis to show that β S mRNA expression was significantly decreased in cortical areas of pure DLB pathology and in the clinical phenotype of DLB, but not in

cases with diffuse Lewy body pathology and concomitant AD pathology or in the clinical phenotype of PD (PD with dementia) [11]. These results suggest the existence of a specific molecular subtype of DLB characterized by a strong decrease of β S.

Taken together, downregulation of β S mRNA in autopsy brains of α -synucleinopathies suggest that “loss of function” of β S occurs in pathogenesis of sporadic α -synucleinopathies. In this regard, future studies are warranted to examine this possibility at the protein level, since expression levels of proteins are not directly reflected by the mRNA level and may be affected by the clearance of protein. This is critical in the aging process and under disease conditions in which protein degradation systems, such as the proteasome and autophagy-lysosome pathways, are compromised.

4. Rebutting Genetic Reports Showing a Negative Association of Wild Type β S with Sporadic α -Synucleinopathies

Several studies have indicated that synuclein gene polymorphisms could be involved in the onset of α -synucleinopathies. Among the synuclein genes, α S and γ S gene polymorphisms and SNPs have been related to disease. In contrast, few relationships have been proposed between β S gene polymorphisms and the onset of α -synucleinopathies, although one SNCB (β S) SNP (rs1352303) has been associated with delayed age at onset of PD in women, despite SNCB not being a susceptibility gene for PD [12]. Another study indicated that α S and γ S genes have particular effects on the risk of developing diffuse Lewy body disease (DLBD), whereas variants of the β S gene showed the least evidence of an association with DLBD [13].

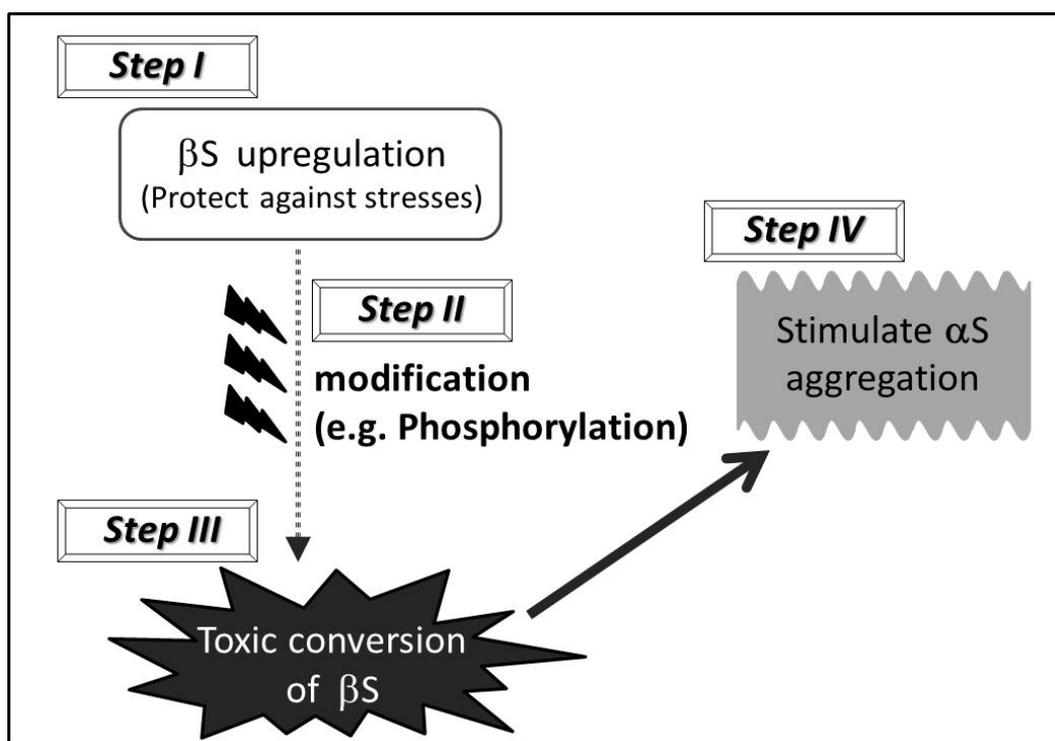
Based on these findings, it may be argued that β S is not related to the pathogenesis of α -synucleinopathies, or at least that β S does not obtain a “toxic gain of function”, distinct from α S and γ S. However, it is important to remember that diagnosis of DLBD is based on the presence of Lewy bodies. It has been well characterized that formation of Lewy bodies is a protective reaction in surviving neurons, especially during an advanced stage of neurodegeneration. Since β S is involved in axonal pathology but not in Lewy body pathology, it is reasonable that SNPs of the β S gene are not associated with DLBD. In this context, it is intriguing to speculate that SNCB SNPs may correlate with the extent of axonopathy in DLBD.

5. “Toxic Gain of Function” Scenario of Wild Type β S

At present, it is unknown whether “toxic gain of function” of wild type β S is indeed the case for the pathogenesis of sporadic α -synucleinopathies. In this regard, we propose a hypothetical multiple-step mechanism (steps I–IV) through which wild type β S might stimulate the axonal pathology of α -synucleinopathies in human brains (Scheme 1). In the initial phase, β S might be accumulated in the presynapse (or distal axon) due to genetic factors, environmental factors, and aging. In particular, given the neuroprotective effect of wild type β S, β S might be upregulated to protect against increasing stresses, such as oxidative stress and chronic inflammation, during the course of aging (step I). Accumulated β S might then gradually undergo posttranslational modifications, such as phosphorylation and glycosylation, particularly in the C-terminal region (step II). As a consequence, a small amount of wild type β S with extensive modifications might adopt altered structures (e.g., toxic oligomers, protofibrils) due to aberrant regulation of the C-terminal region. This step may be regarded

as “transformation” of β S from a neuroprotective to a neurodegenerative molecule (step III). Then, wild type β S with an altered structure may sequester α S, further stimulating the process of α S aggregation. Once aggregation of α S starts, amyloidogenesis of α S might proceed even in the absence of β S (step IV). Overall, alteration of β S might be involved in the early stage of the amyloidogenesis of α S, whereby altered β S may interact with α S, resulting in initial seeding of aggregated α S and leading to promotion of α S pathology.

Scheme 1. Schematic hypothesis of a multiple-step mechanism through which β S may stimulate the pathogenesis of sporadic α -synucleinopathies.



6. Evidence that Wild Type β S Exhibits “Gain of Function” Properties in α -Synucleinopathies

According to the “gain of function” scenario, wild type β S might be accumulated in the initial stage of sporadic α -synucleinopathies (step I). Indeed, accumulation of wild type β S has been observed in various aspects of neurodegeneration. For instance, β S was found to be abnormally concentrated in dystrophic neurites in the hippocampal region in brains from PD and DLB patients [14]. Similarly, β S was detected in spheroids, but not in Lewy body-like or glial inclusions, in neurodegeneration with brain iron accumulation, type 1 [15]. These results suggest that accumulation of wild type β S in the axonal pathology could be an early step in sporadic α -synucleinopathies.

Accumulated wild type β S might be subjected to posttranslational modifications (step II). Indeed, several serine/threonine residues of β S are constitutively phosphorylated [1]. In particular, it is of interest to determine if phosphorylation of S118 confers enhanced aggregation properties on β S, as is the case for phosphorylation of S129 of α S [16]. In a similar context, tyrosine residues Y119, Y127 and Y130 of β S, which are potential targets of non-receptor tyrosine kinases (e.g., src family kinases), might affect aggregation of β S, since aggregation of α S is negatively regulated by phosphorylation of

the corresponding Y125, Y133 and Y136 [17,18]. Furthermore, it is of note that β S is a major *O*-glycosylated protein in the presynapse of rat brain [19]. Assuming that the serine residues compete for glycosylation and phosphorylation, glycosylation may also be involved in aberrant posttranslational modifications of β S during aging or due to environmental factors. Finally, it is also of note that a polyproline II (PPII) helix is clustered in the *C*-terminal region (EPLXEPLXEPE motif: 105–115) of β S and that posttranslational modifications and P123H mutation could affect this important structure [20]. Given the role of PPII helices in protein-protein interactions through binding with SH3 domains [21], it is possible that the PPII domain might be a locally important regulatory domain for the aggregation properties of β S.

To date, fibril formation of wild type β S has not been reported *in vivo* or in cell cultures. However, in a cell-free system wild type β S was induced to form amyloid fibrils in the presence of specific metal ions and glycosaminoglycan macromolecular crowding agents [22]. Thus, aggregation and fibril formation of wild type β S may be possible *in vivo* (step III). Such aggregation may also involve effects of a minor transcript of β S. In the SNCA gene, SNCA112 and SNCA98 are aggregation-prone isoforms and SNCA126 is a potentially protective isoform [23,24]. Similarly to SNCA, Ariza and colleagues have shown the presence of novel minor splicing variants of SNCB, including variants lacking exon 4 (a homolog of SNCA126) and exon 6 (a homolog of SNCA112) [25]. These minor isoforms have not been detected at the protein level due to their extremely low expression compared to major isoforms, but it is an intriguing possibility that dysregulation of the products of minor transcripts, especially the variant lacking exon 6, might permit formation of aggregates that act as seeds that trigger α S aggregation.

7. Towards Novel Therapeutic Strategies

Since several studies have indicated decreased β S expression in some brain regions in α -synucleinopathies, compensation for decreased expression of β S may be an effective strategy. For instance, it was previously shown that viral delivery of β S to the brains of α S tg mice decreased α S aggregation in the brains of the treated mice [26]. In this study, expression of full length of β S was induced in the brain of α S tg mice using a lentivirus vector. In addition, short peptides derived from β S were effective for prevention of α S aggregation in the brain of α S tg mice. More recently, it was shown that locomotor activity and accumulation of A53T α S in the brain of a drosophila model of PD expressing A53T α S was significantly ameliorated when the flies were fed with retro-inverso β S peptides [27]. Thus, if “loss of function” occurs, then replenishment of β S is a potential therapeutic strategy.

On the other hand, β S might become pathological by gene mutation (e.g., P123H) or through other unknown modifications, as described in this review. In addition, considering the potential role of some β S isoforms in several brain regions in α -synucleinopathies, the involvement of β S in these diseases might be explained by “gain of function”. If some β S molecules are pathogenic, then it is reasonable to target the pathogenic β S. Vaccination with α S is effective for decreasing α S at the protein level [28], and recent studies indicate that pathological α S may transmit to other cells and result in progression of α -synucleinopathies [29]. Thus, vaccination of α S may contribute to reduction of extracellular α S and prevent progression of α -synucleinopathies. In a similar context, pathogenic β S could also be removed

by vaccination. Whether cytoplasmic β S is released into the extracellular space is currently unknown; however, it may be possible to decrease the intracellular β S level using vaccination, similarly to α S. Thus, if “gain of function” occurs, mixed vaccination with α S and β S could be a possible therapeutic strategy.

Finally, both “loss of function” and “gain of function” of wild type β S might occur in the same brain. In such a situation, the therapeutic strategy will become more complicated. Although vaccination with β S may be effective for removal of pathogenic β S, it may be difficult to control the expression level of β S throughout the brain. To establish novel therapies that are satisfactory for both compensation and vaccination, technological innovations are required to achieve region-specific regulation of β S expression in α -synucleinopathies.

8. Conclusion

In conclusion, we assume that not only “loss of function” of β S but also “toxic gain of function” of β S may be involved in progression of sporadic α -synucleinopathies. In this context, a better understanding of the role of wild type β S in the pathogenesis of sporadic α -synucleinopathies is critical for development of new avenues of therapy for these devastating diseases.

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