

REVIEW

Transcriptional mutagenesis by 8-oxodG in α -synuclein aggregation and the pathogenesis of Parkinson's disease

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Parkinson's disease (PD) is an age-related progressive neurodegenerative disease associated with selective loss of dopaminergic neurons. The characteristic hallmark of the disease is intracytoplasmic proteinaceous inclusion bodies called Lewy bodies, primarily consisting of a presynaptic protein α -synuclein. Oxidative stress-mediated damage to macromolecules have been shown to occur frequently in PD. Oxidative damage to DNA in the form of oxidized guanine (8-oxodG) accumulates in both the mitochondrial and nuclear DNA of dopaminergic neurons of the substantia nigra in PD. 8-oxodG-mediated transcriptional mutagenesis has been shown to have the potential to alter phenotype of cells through production of mutant pool of proteins. This review comprehensively summarizes the role of oxidative stress-mediated damage incurred during neurodegeneration, and highlights the scope of transcriptional mutagenesis event in leading to α -synuclein aggregation as seen in PD.

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INTRODUCTION

Parkinson's disease (PD) is an age-related progressive neurodegenerative disorder which is associated with selective loss of dopaminergic neurons from the substantia nigra pars compacta region of the midbrain.¹ PD is broadly classified into a familial form (resulting from genetic alterations like mutations or multiplication in the *SNCA* gene encoding alpha-synuclein (α -SYN), early-onset form) and the idiopathic form with unknown etiology (late-onset form).² The majority of idiopathic PD cases represent a late-onset sporadic form with cytoplasmic α -SYN aggregates which are the major component of Lewy bodies and Lewy neurites, the characteristic proteinaceous cytoplasmic deposits that are pathological hallmark of the disease.³ Increasing evidence suggest that oxidative stress is a key contributor to the pathogenesis of PD, which causes damage to nucleic acids (both DNA and RNA), proteins, lipids and other cellular macromolecules whose functions are indispensable for cell survival. The metabolism of dopamine (DA) itself contributes to oxidative stress that renders the nigral neurons particularly vulnerable in PD.^{4,5} The most frequent DNA lesion generated by oxidative stress is 8-oxo-7,8-dihydroguanine (8-oxodG), the oxidized form of guanine, often associated with neurodegenerative diseases

including PD and Alzheimer's disease (AD).^{1,5} 8-oxodG, being a nonbulky DNA lesion, has high mutagenic potential by misincorporation of an adenine instead of cytosine causing G:C \rightarrow T:A transversion mutation.⁶ 8-oxodG has also been implicated in an event called transcriptional mutagenesis (TM), whereby a misincorporated adenine in the transcribing mRNA leads to the generation of mutated species of protein^{7,8} (Figure 1). It is well documented that oxidative DNA damage accumulates in ageing brains and this accumulation is significantly increased in brains of patients with PD and AD compared to their age-matched controls.^{5,9} These increased levels of DNA damage are also corroborated by decrease in the DNA repair capacity of specific enzymes such as 8-oxodG DNA glycosylase1 (OGG1).¹⁰ In addition to its involvement in tumor development, TM may have a very important role in the neurodegenerative disorders, in which a nucleation-dependent protein aggregation process has a pivotal role in neuronal degeneration as seen in PD and AD.¹¹ As shown in α -SYN A53T mutant species that was reported in the familial form of PD,¹² the pathologically misfolded proteins drive the template-directed misfolding of the native monomeric proteins, which contributes to the nucleation-dependent fibrillation process.^{13,14} Moreover, compared with replicating

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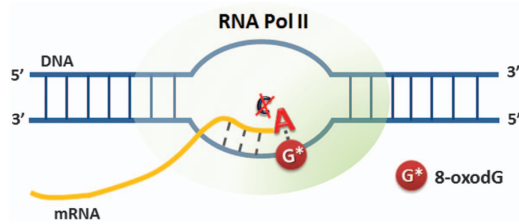


Figure 1 8-OxidG-mediated transcriptional mutagenesis event. TM event occurs when 8-oxodG present on the transcribing strand (3' → 5') of a gene can misinsert an adenine instead of a cytosine in the growing mRNA chain, thus introducing a mutation on the nascent mRNA strand.

cells, neurons that are post-mitotic cells might be even more vulnerable to 8-oxodG-mediated TM as pathogenic effects caused by mutant species generated through TM events could be accumulated over a lifetime.⁸ In the following review, we discuss the importance of oxidative damage in PD and its scope in the pathogenesis of the disease through 8-oxodG-mediated TM events.

OXIDATIVE STRESS-MEDIATED DAMAGE IN NEURODEGENERATION

Oxygen is an essential component to the survival of all living beings. But the greatest paradox remains in the fact that production of reactive oxygen species (ROS) as a by-product of oxygen metabolism is highly toxic to cells. ROS are molecules that can react with cellular macromolecules and impair their functions. It can include both free radicals like superoxide, hydroxyl radical and nitric oxide (containing highly reactive unpaired electrons) and molecules like hydrogen peroxide and peroxynitrite. Post-mortem brain tissues from patients of PD, AD and amyotrophic lateral sclerosis (ALS) have clearly demonstrated higher amount of ROS in the selective areas that undergoes neurodegeneration.

Oxidative stress originates when the rate of ROS production is significantly higher compared with its elimination from the system. Several markers of the oxidized cellular macromolecules have been identified under conditions of neurodegeneration. For example, elevated levels of malondialdehyde and 4-hydroxynonenal, which are markers of oxidized lipids, have been observed in the cortex and hippocampus of patients with AD, in the substantia nigra of patients with PD and spinal fluid of patients with ALS.^{13–16} Oxidative modification of unsaturated fatty acids can result in the generation of lipid peroxides which can further cause oxidation of the unsaturated fatty acids in a chain-like event, finally leading to the disruption of plasma membranes and membranes of other cellular organelles like mitochondria.¹⁷ The levels of protein carbonyls, a marker of protein oxidation, have been also reported to be consistently elevated in the hippocampus and neocortex of individuals with AD, in Lewy bodies in case of PD and motor neurons of ALS patients.^{18–21} Oxidation of proteins can disrupt the active site of enzymes, lead to conformational change, disrupt protein–protein interactions, and alter their binding capacity to DNA, eventually threatening cell survival. In

addition, increased levels of oxidative damage to DNA and RNA bases have been consistent with the neurodegenerative conditions like PD, AD and ALS. Although all DNA bases could be potentially oxidized, guanine is the most susceptible base to oxidative damage. It gets readily oxidized to form 8-oxodG and serves as a marker for oxidative damage.²² In AD, the level of nuclear DNA damage in the brain regions including frontal, parietal and temporal lobes is significantly higher compared with age-matched controls.²³ Overall, it can be concluded that oxidative stress is commonly associated with neurodegenerative conditions and has a critical role in mediating the disease processes.

The origin of oxidative stress and subsequent accumulation of damage can not only be attributed to the overproduction of ROS but also to the inefficient cellular defense and repair machinery against oxidative stress.^{17,24} The defense machinery refers to the antioxidant enzymes like superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase among many others whose primary function is to scavenge ROS generated in the cells. For example, superoxide dismutase converts superoxide to hydrogen peroxide, which is subsequently converted to water by either catalase or glutathione peroxidase. Glutathione peroxidase detoxifies hydrogen peroxide using reduced glutathione. During this process, glutathione is oxidized and it can be subsequently reduced by glutathione reductase.¹⁷ A number of reports have shown reduced activity of the antioxidant machinery in AD.^{25,26} In familial ALS, mutations in copper- and zinc-containing superoxide dismutase lead to a toxic gain of function, rendering superoxide dismutase itself to a pro-oxidant protein involved in ROS generation.^{27,28} PD is also characterized by significant loss of the reducing agent glutathione in the substantia nigra, which is one of the earliest known indicators of nigral neuronal degeneration.²⁹ Together, these evidences comprehensively indicate that reduced antioxidant potential might be a critical factor toward increased oxidative stress that is associated with neurodegenerative disorders.

As discussed previously, accumulation of ROS-induced DNA damage, like oxidation of bases and single strand breaks have been implicated in the etiology of AD, PD, ALS and other neurological disorders. This accumulation of the DNA damage may imply the defect in the DNA repair machinery of the cells. It has been shown that ROS-induced DNA damages are primarily repaired via highly conserved base excision repair pathway.^{30,31} Neuronal dysfunctions have been linked to mutations or differential expression of base excision repair enzymes like OGG1, XRCC1,^{32–34} single-strand break repair enzymes like TDP1, aprataxin^{35,36} and double-strand break repair enzymes like ATM and NBS1.³⁷ Furthermore, it has been shown that *Ogg1* knockout mice exhibit age-associated loss of nigrostriatal pathways and increased sensitivity to the dopaminergic neuronal toxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, than their wild-type littermates.³⁸ Together, these evidences strongly suggest that oxidative stress-mediated damage to cellular macromolecules including

DNA coupled with inefficient repair leads to progressive neurodegeneration as seen in AD, PD, ALS and others.

OXIDATIVE DNA DAMAGE IN THE FORM OF 8-OXODG IN PARKINSON'S DISEASE

Oxidative stress has been classically linked to the etiology of PD. The high metabolic activity of neurons along with their long life span makes them highly susceptible to oxidative damage.^{39,40} Moreover, dopaminergic neurons in the substantia nigra, the most affected brain region by PD, are particularly vulnerable to oxidative stress. Although the exact causes for this selective vulnerability is yet to be elucidated, DA metabolism itself has been considered as a major culprit for selective degeneration. DA has the capacity to auto-oxidize at normal pH into toxic quinone species, producing superoxide and hydrogen peroxide.⁴¹ Monoamine oxidase can also enzymatically deaminate DA into nontoxic metabolite 3,4-dihydroxyphenylacetic acid and hydrogen peroxide.⁴² Hydrogen peroxide can in turn be broken down into hydroxyl radical in a reaction catalyzed by iron. The level of iron is reportedly higher in the nigral dopaminergic neurons as compared with the other regions of the brain, owing to its binding affinity to neuromelanin.^{14,43,44} Therefore, when it is synthesized or transported into cells from extracellular space, DA is rapidly stored into synaptic vesicles which provides a stable environment for DA without monoamine oxidase and low pH. Under conditions of PD, nigral neurons appear to be in an exaggerated oxidative stress, causing severe damage to cellular macromolecules.

Damage to nucleic acids is particularly very hazardous amongst all the cellular macromolecules, because it can change genetic information present in both nuclear and mitochondrial genome.⁴⁵ DNA damage by oxidative stress can result in the production of either nonbulky DNA lesions like 8-oxodG that can be repaired by the base excision repair pathway or bulky DNA lesions which are generally repaired by the nucleotide excision repair pathway.⁴⁶ 8-oxodG, the most frequent DNA lesion caused by oxidative stress is often associated with neurodegenerative diseases including PD.⁵ Immunocytochemical analysis of 8-oxodG revealed a significant increase of this DNA oxidation marker in the substantia nigra of patients with PD although the extent of nuclear 8-oxodG accumulation is not as high as mitochondrial 8-oxodG.^{4,5,47} Despite the presence of 8-oxodG-specific DNA repair enzyme, OGG1, a significant percentage of this DNA lesion remains unrepaired and accumulated under disease conditions. Moreover, it is reported that the overall activity of OGG1 in brain decreases over ageing in a mouse model.⁴⁸ The 8-oxodG generated by direct oxidation of DNA, can be base paired with adenine as well as cytosine during replication, and consequently lead to spontaneous G:C to T:A transversion mutation.⁴⁹ Thus, 8-oxodG remains the extensively studied route for mutagenesis in proliferating cells. However, majority of the cells in our body, including neurons, exist in non-proliferating quiescent state. Neurons being post-mitotic cells, face a major challenge of DNA repair during transcription.⁵⁰

Failure to maintain both transcriptional and translational fidelity is expected to result in functional impairment of the cells. Studies have shown that many of these nonbulky lesions present on the sense strand of DNA could be bypassed by RNA polymerase during transcription, leading to misinsertion of ribonucleotides to the growing mRNA strands, producing mutant transcripts.^{51–53} This phenomenon is referred as TM event in the cells. Since a significant increase of 8-oxodG was observed in the substantia nigra of patients with PD,^{4,5,47} it is highly possible that TM event might significantly contribute to the pathogenesis of PD. In the next few sections of this review, the perspective of TM in PD will be closely explored with the emphasis on α -SYN that is reckoned a major pathogenic molecule.

8-OXODG-MEDIATED TRANSCRIPTIONAL MUTAGENESIS

DNA damage-mediated mutagenesis in a replication-based model has provided a wide range of information for better understanding of the origin of mutation and subsequently its contribution to the pathogenesis of human diseases such as cancers. However, as discussed briefly in the last section, majority of cells under normal physiological conditions are not frequently engaged in division and do not undergo continuous cycles of replication.⁵⁴ Most of the multicellular organs of eukaryotes including brain or heart are mainly comprised of nondividing or terminally differentiated cells. Maintenance of complex physiological functions of these organs primarily depends on securing the high fidelity of both transcription and translation.⁷ Accumulation of TM-derived mutant transcripts and subsequently generated erroneous proteins has the potential to produce functional impairment of nonproliferating cells and organs.¹¹ As the aging process may be accompanied by progressive deterioration of normal cellular functions such as DNA repair machinery and antioxidation processes, TM-mediated adverse effects on cells could be exacerbated over aging. In fact, under *in vitro* conditions allowing TM event, it has been shown that each round of transcription keeps producing a mutant transcript as long as 8-oxodG lesion is not repaired. This event is expected to generate a fairly large population of mutant transcripts which will be translated multiple times, leading to a relatively large amount of the mutant protein.⁷

A number of studies have shown that a plethora of DNA damage can lead to a TM event.¹¹ The structural analysis of yeast RNA polymerase II at an 8-oxodG lesion revealed the possible mechanism of TM. In this study, it has been shown that 8-oxodG can mispair with adenine instead of cytosine through a Hoogsteen base-pairing with the 8-oxodG lesion at the polymerase active center, thereby escaping the proofreading of the polymerase and maintained in the nascent RNA stand.⁵⁵

The potential of 8-oxodG to cause TM event had been demonstrated in a bacterial system using *Escherichia coli* and its role in developing antibiotic resistance.⁷ Later, using a luciferase-based reporter system, TM-derived mutation event has been also proved in mammalian cells, demonstrating that

TM event is strongly affected by factors, such as promoter strength of the gene, flanking sequence around the 8-oxodG lesion and position of the lesion with reference to the promoter.⁸ In the same study, *OGG1* knockout cells showed more frequent TM event compared with the cells lacking a transcription-coupled repair machinery, suggesting that a 8-oxodG lesion is not efficiently recognized by transcriptional machinery for initiating repair and rather *OGG1* has a critical role in preventing TM.⁸ Interestingly, TM event has been also linked to activation of oncogenic pathway.⁵⁶ Replacement of a guanine with 8-oxodG at codon 61 of *HRAS*, a proto-oncogene, led to generation of a constitutively active mutant form of *HRAS* (Q61K) through TM event. Moreover, under condition of *Ogg1* null background in mouse embryonic fibroblast, sufficient amount of the mutant protein was generated to activate the downstream MAPK pathway, leading to ERK phosphorylation.⁵⁶

Together it suggests that 8-oxodG lesion is often bypassed by RNA polymerase II without efficient detection by the transcription-coupled repair or the base excision repair machinery, generating transcription mutant species and contributing to the pathogenesis of various diseases including cancer, neurodegeneration or cardiovascular disease.

POTENTIAL OF TM EVENT TO CAUSE A-SYN AGGREGATION IN PARKINSON'S DISEASE

α -SYN is a 140 amino acid protein and natively unfolded. It can exist as random-coil state as well as β -sheet conformation upon aggregation or a α -helical conformation upon binding to membranes. Sequence of α -SYN can be divided into three regions with distinct characteristics (Figure 2): (1) the amphipathic lysine-rich N-terminus (residues 1–60), which is mainly involved in membrane interactions; (2) the middle hydrophobic region (non-A β component of amyloid plaques, residues 61–95), which is prone to β -sheet formation and fibrillization;

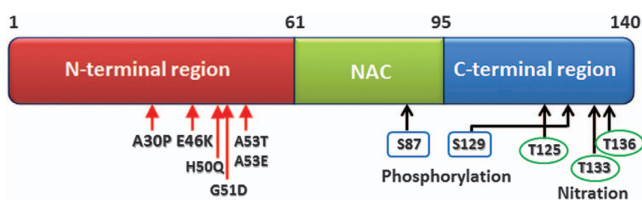


Figure 2 Structure of full length α -SYN protein and its functional components. N-terminal (1–60), non-A β component of amyloid plaques domain (61–95) and C-terminal (96–140); point mutations reported in familial PD (autosomal dominant form) are present in the N-terminal (red arrows). N-terminal contributes to the α -helical structure of α -SYN upon binding to lipid membranes. Non-A β component of amyloid plaques domain contains most hydrophobic residues and one phosphorylation site (blue rectangle, serine 87). Non-A β component of amyloid plaques domain promotes aggregation of the molecule by its propensity to form β -sheet structure. C-terminal has three nitration sites (green circle, tyrosine 125, 133, 136) and one phosphorylation site (blue rectangle, serine S129) and is mainly responsible for maintaining the protein solubility.

and (3) the C-terminus (residues 96–140), which is a highly acidic and proline-rich region and primarily controls the nuclear localization and interaction with other proteins.⁵⁷

Multiplication as well as various single-nucleotide polymorphisms of *SNCA* have been reported in dominantly inherited early-onset PD.^{12,58–61} α -SYN mutant species, A30P, A53T and E46K have been shown to alter the aggregation process and interfere with oligomerization, fibril formation and the distribution in cellular compartments.^{12,62–68} More recently, three additional mutations of α -SYN, H50Q, G51D and A53E, have been identified in PD patients.^{63–65} Increasing body of evidence from various experimental models has shown that elevated levels of wild-type and various mutant species are prone to accelerate the aggregation process. In misfolded state, α -SYN is characterized by twisted, nonbranched filaments of β -sheets.⁶⁹ The mutant α -SYN proteins are transformed into amyloid fibrillar species consisting of β -sheets, which have properties to serve as templates to drive soluble proteins to adopt similar structural changes, leading to the formation of highly ordered aggregated structure.^{69–71} Increased oxidative stress is a key contributor to the pathogenesis of PD and several *in vitro* and *in vivo* experiments have linked oxidative stress to α -SYN aggregation. Exposure of neuronal cells to various oxidative stressors including ferrous ions, hydrogen peroxide, MPP⁺ (1-methyl-4-phenylpyridinium), rotenone, nitric oxide and superoxide all promoted the aggregation process.^{72–75} *In vivo* studies also corroborated the same idea that chronic and systemic exposure of rodents to rotenone, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine or paraquat leads to selective nigrostriatal dopaminergic lesions accompanying degeneration with α -SYN aggregation.^{76–79}

Thus, how the 8-oxodG-mediated TM event might have a critical role in the process of α -SYN fibrillogenesis depends on whether the mutant species generated by this process can acquire a misfolded state by themselves, which will eventually act as a seed in the nucleation-dependent aggregation process as seen for reported mutant α -SYN proteins.⁸⁰ If the 8-oxodG-mediated TM mutant species are more stable in the β -sheet form, then a limited amount of TM species could be enough to promote prion-like nucleation of α -SYN.^{80,81} Over the past few years, increasing number of studies have provided evidence that α -SYN aggregates can propagate from one brain region to another in a prion-like manner.^{82,83} This self-perpetuating cycle of α -SYN fibrillation and propagation could be initiated by the addition of a minute amount of pathogenic proteins that can potentially serve as aggregate seeding. 8-oxodG-derived TM species may trigger this process, leading to the pathogenesis of PD.

In a paraquat-based animal model of PD, it has been shown that nuclear 8-oxodG accumulation in the substantia nigra is clearly correlated with increase in proteinase-K resistant species of α -SYN aggregates.⁷⁸ A recent study has pointed that genomic distribution of 8-oxodG is not a random event, instead it is localized preferentially to specific areas of the chromosome and is negatively correlated with transcriptional activity of a gene.⁸⁴ This fact implies that TM could affect various genes in the

WT : MDVFMKGLSK AKEGVVAAAE KTKQGVAAEA GKTKEGVLYV GSKTKEGVVH GVATVAEIKT
 TM : MDVFMKGLSK **D**KEGVV**D**DE **K**N**K**KG**V**EE**E** **G**K**K**KEGV**L**YV **G**Y**K**K**K**EGV**V**N **G**V**E**K**V**DE**K**K
 WT : EQVTNVGGAV VTGVTAVAQK TVEGAGSIAA ATGFVKKDQL GKNEEGAPQE GILEDMFPDP
 TM : **E**K**V**K**N**VGG**E**V **V**K**G**V**K**E**V**D**K**K **K**V**E**G**E**G**S**I**E**E **A**T**G**F**V**K**D**K**L** **G**K**N**E**E**G**D**T**K**E **G**I**L**E**D**M**H**V**D**H
 WT : DNEAYEMPSE EGYQDYEEPA
 TM : DNE**D**YEM**T**Y**E** EGY**D**Y**E**T**E**D

Figure 3 All the possible mutant amino acid sequences of α -SYN generated by 8-oxodG-driven TM. Upper line (wild type) represents the reference amino acid sequence of the wild-type protein; lower line (TM) shows amino acid changes can be caused by 8-oxodG-mediated transcriptional mutagenesis arising from misincorporated adenine instead of cytosine in the mRNA sequence during transcription (red); green letters designate reported α -SYN mutants (A30P, E46K, H50Q, G51D, A53E & A53T) in familial PD; and serine 87 and 129 (blue) is the site of phosphorylation as seen during aggregation of the molecule.

α -SYN mutants	β -aggregation score
WT	896.07
L38I	962.23
S42Y	1053.17
H50N	943.49
Q62K	917.93

Figure 4 Predicted score for wild-type and some of the possible mutant α -SYN species generated by TM event to form β -aggregate by TANGO. β -aggregation score for wild-type and some of the possible mutant α -SYN species which can be generated through TM event as predicted by the structure analyzing algorithm TANGO (see text for reference). Abbreviations: L38I, leucine to isoleucine; S42Y, serine to tyrosine; H50N, histidine to asparagine; Q62K, glutamine to lysine; WT, wild type.

context of chromatin structure, and mutant species originating from TM event should be outnumbered by the normal physiological form. Therefore, for most proteins, generation of small portion of nonfunctional TM species would not be likely to cause entire functional impairment. However, α -SYN could become cytotoxic in the nucleation-dependent oligomerization process, in which small addition of mutant α -SYN species to wild-type population may initiate the seeding process and fibrillogenesis^{80,85}. This unique biochemical feature of α -SYN would strongly support the feasibility of the proposed model.

All possible mutant α -SYN proteins which could be generated through TM-mediated replacement of cytosine with adenine in the mRNA strand are listed in Figure 3. Among TM-generated α -SYN mutants, the following mutants, A30E, H50N and A53E, have mutations at the same amino acid position as the familial forms of mutants, A30P, H50Q and both A53E and A53T, respectively. It would strongly suggest that those TM mutants could similarly cause a nucleation-dependent α -SYN fibrillation. Apart from these mutant forms, there are several other residues that might disrupt the native structure of the protein when they get mutated and make them more prone to aggregation. To predict the aggregation propensity of expected TM species, we used TANGO, a statistical mechanics algorithm, which enables identifying β -aggregating regions within a protein based on the sequence information.⁸⁶ TANGO algorithm indicated that a couple of expected TM proteins has significantly higher β -aggregation scores than wild-type α -SYN, which include L38I, S42Y, H50N and Q62K in the N-terminal or the non-A β component of

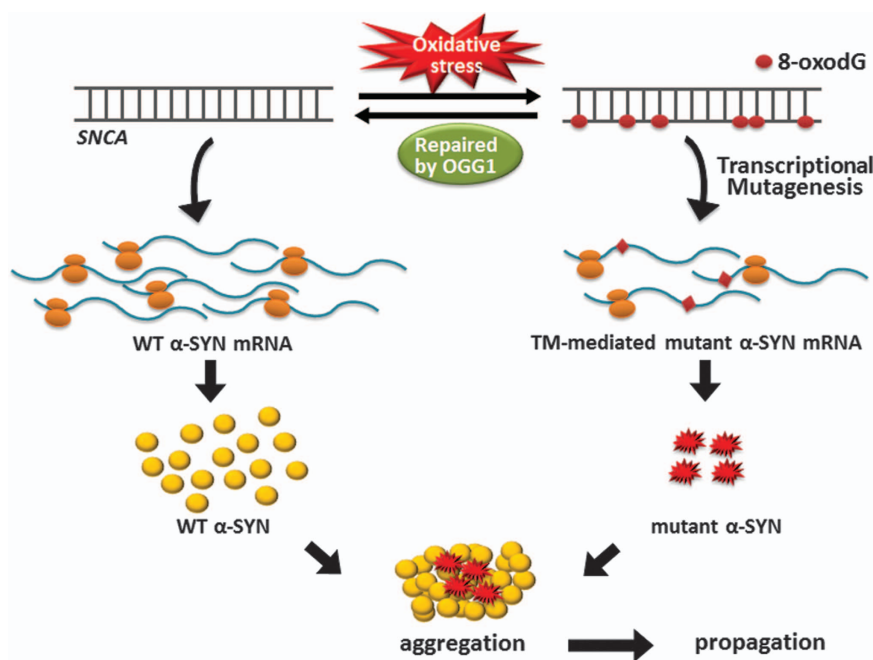


Figure 5 Proposed model for aggregation of wild-type α -SYN protein by TM-generated mutant α -SYN species and propagation. Mutant α -SYN generated through TM event may trigger a nucleation-dependent aggregation of the predominantly higher amount of wild-type species and lead to propagation of α -SYN aggregation.

amyloid plaques domain, predicting increases in aggregation property (Figure 4). Post-translational modifications such as phosphorylation on serine 87 and 129 may also affect the α -SYN aggregation process.^{87,88} TM event can replace the serine at 129 position with a tyrosine. Together we propose a hypothetical model by which 8-oxodG lesions in the protein coding region of *SNCA* could yield a small amount of TM protein species with higher aggregation propensity that potentially serve as a seeding for accelerating the aggregation of wild-type α -SYN, leading to the self-propagation of aggregates and causing degeneration of the nigrostriatal pathway in PD (Figure 5).

CONCLUSION

The majority of idiopathic PD cases are a late-onset sporadic form with cytoplasmic α -SYN aggregates, which indicates that increasing degree of aggregation does not depend only on genetic mutations in *SNCA*. However, till date, the approaches to understand the molecular mechanism of α -SYN aggregation have focused primarily on the biochemical properties of mutant protein species that were identified in rare familial form of PD and their behavior within the cells. The proposed model will give an insight into a novel mechanism called 'transcriptional mutagenesis' caused by the accumulation of oxidatively damaged DNA lesions, 8-oxodG, in the *SNCA* gene. Comprehensive investigation on age-dependent changes in α -SYN mRNA profiles as well as identification of TM species supported by functional studies on mutant proteins will definitely add a new dimension to the understanding of α -SYN pathology in conjunction with oxidative stress.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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