progress in neurobiology

Peer Review Overview

Manuscript Title: A multi-faceted genotoxic network of alpha-synuclein in the nucleus and mitochondria of dopaminergic neurons in Parkinson's disease: Emerging concepts and challenges

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1st Decision Letter

Ref: PRONEU_2019_77
Title: A multi-faceted neurotoxic network of alpha-synuclein in the nucleus and mitochondria of dopaminergic neurons in Parkinson's disease: Emerging concepts and challenges
Journal: Progress in Neurobiology

Dear Dr. Hegde,

Thank you for submitting your manuscript to Progress in Neurobiology. We have completed the review of your manuscript and a summary is appended below. The reviewers recommend reconsideration of your paper following major revision. We invite you to resubmit your manuscript after addressing all reviewer comments.

When resubmitting your manuscript, please carefully consider all issues mentioned in the reviewers' comments, outline every change made point by point, and provide suitable rebuttals for any comments not addressed.

We look forward to receiving your revised manuscript as soon as possible.

Kind regards,

Jeanne Paz, Associate Editor

Sabine Kastner, Editor-in-Chief
Progress in Neurobiology

Comments from the editors and reviewers:

Reviewer 1

The review article *„A multi-faceted neurotoxic network of alpha-synuclein in the nucleus and mitochondria of dopaminergic neurons in Parkinson's disease: Emerging concepts and challenges"* by Vasquez et al. outlines the role of alpha-synuclein for DNA damage and its interference with proteins of the DNA repair machinery, they predict DNA binding residues

within the alpha-synuclein protein in different conformational states, and discuss how alpha-synuclein could mediate mitochondrial dysfunction. Furthermore, they discuss the role of alpha-synuclein metal binding, especially the role of Fe^{2+} and ferroptosis. In the last paragraph they discuss challenges of alpha-synuclein cell models and introduce a new inducible Tet-ON cell line with neuronal differentiation.

The article is overall well and clearly written and gives a relevant insight into current research for alpha-synuclein and its potential mechanisms of interaction/function in DNA damage and repair. Only, some paragraphs stand alone and are not directly linked with each other, like chapter 4 on ferroptosis or chapter 11 on cell models. Also, for alpha-synuclein function in the nucleus and in/on mitochondria different mechanisms are discussed. While in the nucleus a direct interaction between alpha-synuclein and DNA is presented, but for mitochondria an indirect effect on mtDNA, via inhibition of protein import thereby affecting import of other essential proteins and the interference with the PINK1/parkin pathway are discussed.

Major Comments:

Chapter 5

- Maybe also mention binding to Ca^{2+}.

Chapter 6.2

- Paragraph 2 and 3 should be written clearer, its quite hard to follow. For example “Mutant mice lacking OGG1 and MutT homolog 1 (MTH1) demonstrated that hMUTYH up regulation and activity promotes neurodegeneration. More specifically, SSBs are generated in neuronal mtDNA during MUTYH-initiated BER of adenine that is inserted opposite to 8-oxoG. (Sheng et al., 2012).” is hard to understand without knowing the reference. Is it because mistakes are introduced?

Chapter 6.3

- Please add reference for linkage to aging.

- Should be neurodegenerative disorders, not syndromes.

- Also here, this chapter is a bit hard to read.

Chapter 7

- Please add reference for increase of asyn nuclear localization upon oxidative stress (in first paragraph).

- Maybe add and reference papers from Outeiro lab on phosphorylation of asyn and nuclear localization.

Chapter 8

- Please clarify the meaning “B to altered B-DNA”

- For the last sentence of the first paragraph: “Together this evidence indicates a possible function for α-synuclein binding because the association with distinct chromosomal regions may affect the expression of genes related to mitochondrial homeostasis or differentiation.“, where comes the rational from, that mitochondrial genes are affected?

- Please add reference for “In α-synuclein, pSer129 is associated with enhanced nuclear localization in vivo.“ – paper from Outeiro lab as suggested before?

Chapter 8.1

- Please define PDB structures already in the first paragraph (which one is broken a-helix, beta-sheet), then the rest of the text will be easier to read. Just like it is done later on: “Notably, the two α-helices of the micelle-bound α-synuclein structure (PDB:1XQ8) exhibits more predicted binding sites than the SLAS-micelle bound α-synuclein structure (PDB:2KKW) (Figure 4). Whereas, the α-synuclein fibril structure (PDB: 2N0A) had more predicted binding sites at the N-terminal distributed across the different protein chains.“ Is the difference between 1XQ8 and 2KKW the broken/non-broken a-helix?

- Are NPDock and HDOCK for the COAHC-D method?

- Is there an explanation why predictions are so different between the methods for the same structure, especially for 2N0A as seen in Figure 4, one predicts N-terminal residues, one C-terminal residues?

Chapter 10

- Please add reference for reduced complex I activity in sporadic PD.

Chapter 12

- “mitochondrial membrane abnormalities” – is this mentioned in the text?

- Last paragraph should be rewritten, formulation “We thus believe that our insightful, but broad survey of the multi-faceted, neurotoxic role of α-synuclein in the nucleus and mitochondria, with a particular emphasis on DNA binding and the repair of genomic DNA, will help drive future research efforts in this area.“ More appropriate for a cover letter but not a conclusion.

Figure 2

- Please be consistent how to describe the different PDB structures, throughout the text and the figure legends as well.

Figure 4

- Very good overview, but can it be explained in more detail why the predictions give so different results. A clear tendency cannot be draw, especially for 2N0A where completely different regions are presicted. Why is the prediction from NucBind not included in the overview (data from Table 1)?

Figure 5

- The table in B) need revision since several typos occur, spaces and “DNA strand”.

Minor Comments - Typos:

- Missing spaces in chapter 4 before (Kruman et al. 2004…

- Define Substantia nigra from the beginning with just SN (in Introduction) as used later in the text.

- Please add definition of APE1 in chapter 6.2.

- Instead of using the word “similar”, “like” would be better (Chapter 8 last paragraph).

- Chapter 10.2 – PD, AD, and ALS

- It is SH-SY5Y cells – page 12 and 14

Reviewer 2

In this article, Vasquez et al briefly introduced the structure, physiological properties, neurotoxicity of alpha-synuclein as well as the emerging mechanisms underlying its neurotoxicity, then discussed the links between alpha-synuclein overexpression and nuclear/mitochondrial DNA damage/repair in PD or related cellular/animal models. In addition, they also predicted the potential DNA binding residues of alpha-synuclein and introduced a newly established cell model in their lab. Overall, this review provided an informative update on the recent progress of studies on DNA damage in PD and related disorders, also hightlighted the neurotoxic role of alpha-synuclein in this aspect. However, major revision for quality improvement is definitely required before it can be considered for publication. Below are main issues raised by the reviewer:

1) The title of this article is about neurotoxic network of alpha-synuclein in the nucleus and mitochondria of dopaminergic neurons in Parkinson’s disease, however, the scope is mainly limited to DNA damage, which is only one aspect of synuclein-induced damages in nucleus and mitochondria. For examples, histone modification and membrane disruption could also be induced by synuclein overexpression. Therefore, either the title or the scope of content need to be adjusted to fit each other.

2) The outline and structure of this article are not reasonable. There were too many subtitles in parallel, which could make the readers unable to get the main points.

3) The aim of section 4 is confusing. If the authors just wanted to introduce newly emerging mechanisms of cell death-associated alpha-synuclein toxicity, two mechanisms are obviously not enough to cover this topic. For examples, alpha-synuclein-induced membrane poration, ER stress, lysosome dysfunction, cell-to-cell transmission were not mentioned.

4) The authors specifically used one and half sections (4.1 and 5) to talk about the effect of metal ions on alpha-synuclein aggregation. Since there are many other factors able to facilitate aggregation of alpha-synuclein, why the authors only focused on such effect from metal ions. Moreover, this article didn't clearly explain the direct link between metal ions and neurotoxic network of alpha-synuclein in the nucleus and mitochondria, is such detailed discussion really necessary?

5) Based on the subtitle, section 9 should focus on the synergistic role of alpha-synuclein in the pathogensis of neurodegerative diseases in the case that other amyloidogenic proteins coexist. However, there were too much content talking about other proteins, while the limited discussion on the role of alpha-synuclein in DNA damage was mainly based on assumption. This is not acceptable.

6)Section 11 discussed the limitation and challenges of in vitro alpha-synuclein cell line models. It would be nice to also include animal models if such discussion is initiated. Besides, the reviewer suggest that the introduction of the newly developed Tet-on SNCA cell line should be deleted due to its lack of novelty, or such discussion should include the similar cell lines established in other labs back to 2007(see PMID: 19476547, PMID: 17714183 and PMID: 18957893).

1st Author Response Letter

Reviewer 1

The review article A multi-faceted neurotoxic network of alpha-synuclein in the nucleus and mitochondria of dopaminergic neurons in Parkinson's disease: Emerging concepts and challenges" by Vasquez et al. outlines the role of alpha-synuclein for DNA damage and its interference with proteins of the DNA repair machinery, they predict DNA binding residues within the alpha-synuclein protein in different conformational states, and discuss how alpha-synuclein could mediate mitochondrial dysfunction.

The article is overall well and clearly written and gives a relevant insight into current research for alpha-synuclein and its potential mechanisms of interaction/function in DNA damage and repair. Only, some paragraphs stand alone and are not directly linked with each other, like chapter 4 on ferroptosis or chapter 11 on cell models. Also, for alpha-synuclein function in the nucleus and in/on mitochondria different mechanisms are discussed. While in the nucleus a direct interaction between alpha-synuclein and DNA is presented, but for mitochondria an indirect effect on mtDNA, via inhibition of protein import thereby affecting import of other essential proteins and the interference with the PINK1/parkin pathway are discussed.

Response: We thank the Reviewer for appreciating our review for clear and insightful writing. We also appreciate the Reviewer's important suggestions making ferroptosis and cell line model section more coherent with the rest of the text. We have carefully considered these suggestions and revised these sections with appropriate connecting statements for a better reading. While several recent studies suggest the possible involvement of non-apoptotic cell death pathways like ferroptosis in PD, which may be dependent on iron, these still need to be established in patients as well as appropriate in vivo models to firmly link them to alpha-synuclein-iron toxicity dynamics. We have now revised the text to clearly represent this notion. Regarding the chapter 11 on cell line models, as suggested by both Reviewers, we agree that this stand-alone section is not within the scope of this review and have thus remove it and instead have briefly mentioned this a challenge in the conclusion section.

Furthermore, regarding the different pathways of alpha-synuclein toxicity in nucleus and mitochondria, it is important to note that alpha-synuclein's involvement in inducing genomic DNA breaks has been demonstrated in nucleus. As alpha-synuclein localizes in mitochondria, it is likely that a similar DNA damage pattern may occur in mitochondrial genome as a direct effect of alpha- synuclein, however, this has not been experimentally tested. We have now clarified this in the

revised text and advocated the need to future studies to examine alpha-synuclein mediated DNA instability in mitochondria.

Chapter 5

- *Maybe also mention binding to Ca2+.*
  Response: We have now included recent findings of α-synuclein interaction with Ca2+ effects and potential binding sites to Ca2+. However, based on the Reviewer-2's suggestion, we have moved revised Chapter 5 as new Chapter 6.1.

Chapter 6.2

- *Paragraph 2 and 3 should be written clearer, its quite hard to follow. For example "Mutant mice lacking OGG1 and MutT homolog 1 (MTH1) demonstrated that hMUTYH up regulation and activity promotes neurodegeneration. More specifically, SSBs are generated in neuronal mtDNA during MUTYH-initiated BER of adenine that is inserted opposite to 8-oxoG. (Sheng et al., 2012)." Is hard to understand without knowing the reference. Is it because mistakes are introduced?*
  Response: We apologize for the cryptic statement. We have revised this section to clearly explain how modulation of activity or expression of early BER factors such as OGG1 and hMUTYH can cause imbalance in the homeostasis of repair complex(es), often leading to incomplete repair and accumulation of unrepaired intermediates like SSBs. These intermediate SSBs are more toxic than the initial base damage and thus eventually promote neurodegeneration.

Chapter 6.3

- *Please add reference for linkage to aging.*
- *Should be neurodegenerative disorders, not syndromes.*
- *Also here, this chapter is a bit hard to read.*
  Response: Reference (Lu et al., 2004) was included to provide support for defective DNA repair linkage to aging. Paragraphs one to three were modified to ease readability as suggested.

Chapter 7

- *Please add reference for increase of asyn nuclear localization upon oxidative stress (in first paragraph).*
- *Add and reference papers from Outeiro lab on phosphorylation of asyn and nuclear localization.*

Response: Reference (Lu et al., 2004) was included to provide support for defective DNA repair linkage to aging. Paragraphs one to three were modified to ease readability. We have included references from Outeiro Lab on phosphorylation contribution to nuclear α-synuclein localization (Gonçalves and Outeiro, 2013; Pinho et al., 2019).

Chapter 8

- *Please clarify the meaning "B to altered B-DNA"*
  Response: "B to altered B-DNA" statement was removed as it refers to CD spectra changes for B-DNA (Gray et al., 1995; Hegde et al., 2006).

- *For the last sentence of the first paragraph: "Together this evidence indicates a possible function for α-synuclein binding because the association with distinct chromosomal regions may affect the expression of genes related to mitochondrial homeostasis or differentiation.", where comes the rational from, that mitochondrial genes are affected?*
  Response: The rationale comes from studies (Siddiqui et al., 2012) demonstrating that α-synuclein binding to the promoter region of PGC1 alpha gene, a mitochondrial transcription activator, reduces its expression and downstream transcriptional effects. This is clarified in the revised manuscript.

- *Please add reference for "In α-synuclein, pSer129 is associated with enhanced nuclear localization in vivo." – paper from Outeiro lab as suggested before?*
  Response: *Reference from Huang et al .2011 and Outeiro lab has been added.*

Chapter 8.1

- *Please define PDB structures already in the first paragraph (which one is broken a-helix, beta- sheet), then the rest of the text will be easier to read. Just like it is done later on: "Notably, the two α-helices of the micelle-bound α-synuclein structure (PDB:1XQ8) exhibits more predicted binding sites than the SLAS-micelle bound α-synuclein structure (PDB:2KKW) (Figure 4). Whereas, the α-synuclein fibril structure (PDB: 2N0A) had more predicted binding sites at the N-terminal distributed across the different protein chains".*
  Response: *The PDB structure names were assigned as mentioned in (Rao et al., 2010; Tuttle et al., 2016; Ulmer et al., 2005).*

- *Is the difference between 1XQ8 and 2KKW the broken/non-broken a-helix?*
  Response: Both structural models are comprised of two anti-parallel helices. However, the main difference between these two structures is the helix motif that connects the antiparallel helices. For instance, 1XQ8 shows a helix-loop-helix motif whereas 2KKW shows a helix-turn-helix.

- *Are NPDock and HDOCK for the COAHC-D method?*
  Response: The NucBind is for the COACH-method, which is a template-based method for protein- ligand binding residues prediction. This method calculates interactions with information from homologous ligand-binding templates in the BioLiP database. On the other hand NPDock and HDOCK (template-free based method) are algorithms designed to perform rigid docking without information from homologous ligand-binding templates. Instead, rigid docking considers nuclei acid three-dimensional structures as a single

immobile entity that only changes its overall coordinates by rotational and translational transformations when bound on the receptor protein (Krüger et al., 2018).

- *Is there an explanation why predictions are so different between the methods for the same structure, especially for 2N0A as seen in Figure 4, one predicts N-terminal residues, one C-terminal residues?*
  Response: We thank the reviewer for pointing out this important issue, which has been now rectified in the revised manuscript. Although both NPDock and HDOCK utilize Fast Fourier Transformation (FFT) algorithm to perform rigid base docking, they differ on the type of statistical scoring function used to asses and rank the binding modes. NPDock utilizes a combination of all- atom statistical functions specific to protein-DNA interactions (Robertson and Varani, 2007; Tuszynska et al., 2015; Zhang et al., 2005). Alternatively, HDOCK uses a distance-dependent knowledge-based scoring function for protein-RNA interactions based on DNA similarity to RNA in terms of residues and atom types (Huang and Zou, 2014; Yan et al., 2017).
  Based on the reviewer's comments, we carefully re-evaluated our submission process to the servers. Initially, we submitted the structure to the servers in different ways. We first provided the PDB ID to the HDOCK server, which is designed to obtain the structure directly from the Protein Data Bank website. Once the prediction for all the structures was gathered, we then decided to compare the HDOCK prediction to the NPDock. For the NPDock server, we submitted PDB structures downloaded from the Protein Data Bank website as the server is not enabled to obtain the structures directly from this website. Moreover, NPDock only accepts structures within 1000 amino acids range. Therefore, only chains A-D of the 2N0A structure were selected for further prediction. Based on these differences, we decided to resubmit all the downloaded structures to the HDOCK server following the procedure we did for the NPDock. In addition, we generated a new 2N0A PDB structure displaying chain A-D using PyMOL to maintain an equal amount of atoms.
  Importantly, we did not observe any difference for the resubmitted alpha-helical structures to HDOCK. However, there were major differences for the new 2N0A structure, as the results for the HDOCK server now shows that only the N-terminal of chain C interacts with DNA. We have discussed and clarified this in the text.

Chapter 10

- *Please add reference for reduced complex I activity in sporadic PD.*
  Response: Reference from Flones et al. 2018 and Keeney et al., 2006 were added to support the statement that sporadic PD patients present reduced complex I activity.

Chapter 12

- "mitochondrial membrane abnormalities" – is this mentioned in the text?
  Response: *This phrase was replaced with "outer mitochondrial membrane protein degradation".*

- *Last paragraph should be rewritten, formulation "We thus believe that our insightful, but broad survey of the multi-faceted, neurotoxic role of α-synuclein in the nucleus and mitochondria, with a particular emphasis on DNA binding and the repair of genomic DNA, will help drive future research efforts in this area." More appropriate for a cover letter but not a conclusion.*

Response: Thank you for the suggestion. We have revised the conclusion section accordingly.

Figure 2

- *Please be consistent how to describe the different PDB structures, throughout the text and the figure legends as well.*
  Response: PDB structures are consistently described as SLAS-bound α-synuclein (PDB: 2KKW), SDS-bound α-synuclein (PDB: 1X8Q), and fibril α-synuclein (PDB: 2N0A).

Figure 4

- *Very good overview, but can it be explained in more detail why the predictions give so different results. A clear tendency cannot be draw, especially for 2N0A where completely different regions are predicted. Why is the prediction from NucBind not included in the overview (data from Table 1)?*
  Response: The Nucbind prediction has been included in the overview. A clear tendency cannot be drawn due to the different scoring function for protein-DNA interaction utilized by each method. However, a tendency can be observed for α-helical structures 1XQ8 and 2KKW residues in the NAC domain. In addition, we performed an additional prediction model for fibril α-synuclein structure (PDB: 2N0A) chain A-D, and observed that both algorithms predict the N-terminal as the most likely DNA binding domain for this structure.

Figure 5

- *The table in B) need revision since several typos occur, spaces and "DNA strand".*
  Response: We apologize for the typos which are now fixed.

- *Missing spaces in chapter 4 before (Kruman et al. 2004...*
- *Define Substantia nigra from the beginning with just SN (in Introduction) as used later in the text.*
- *Please add definition of APE1 in chapter 6.2.*
- *of using the word "similar", "like" would be better (Chapter 8 last paragraph). - Chapter 10.2 – PD, AD, and ALS*
- *It is SH-SY5Y cells – page 12 and 14*
  Response: Thank you for the detailed corrections. They have been incorporated.

Reviewer 2

In this article, Vasquez et al briefly introduced the structure, physiological properties, neurotoxicity of alpha-synuclein as well as the emerging mechanisms underlying its neurotoxicity, then discussed the links between alpha- synuclein overexpression and nuclear/mitochondrial DNA damage/repair in PD or related cellular/animal models. In addition, they also predicted the potential DNA binding residues of alpha-synuclein and introduced a newly established cell model in their lab. Overall, this review provided an informative update on the recent progress of studies on DNA damage in PD and related disorders, also hightlighted the neurotoxic role of alpha-synuclein in this aspect. However, major revision for quality improvement is definitely required before it can be considered for publication.

Response: We thank the reviewer for appreciating the concept and the topics covered in this review. We also appreciate the reviewer's incisive comments and suggestions for improvements and reorganization, which we have now carefully considered in revising it, as pointed out in our response to specific comments.

1) *The title of this article is about neurotoxic network ofalpha-synuclein in the nucleus and mitochondria of dopaminergic neurons in Parkinson's disease, however, the scope is mainly limited to DNA damage, which is only one aspect of synuclein-induced damages in nucleus and mitochondria. For examples, histone modification and membrane disruption could also be induced by synuclein overexpression. Therefore, either the title or the scope of content need to be adjusted to fit each other.*
   Response: We agree with the Reviewer and have modified the title to reflect the scope of the contents more appropriately, as "A multi-faceted genotoxic network of alpha-synuclein in the nucleus and mitochondria of dopaminergic neurons in Parkinson's disease: Emerging concepts and challenges"

2) *The outline and structure of this article are not reasonable. There were too many subtitles in parallel, which could make the readers unable to get the main points.*
   Response: We have removed the subtitles/sub-sub-titles from the contents page, which now only highlights the major topics discussed.

3) *The aim of section 4 is confusing. If the authors just wanted to introduce newly emerging mechanisms of cell death-associated alpha-synuclein toxicity, two mechanisms are obviously not enough to cover this topic. For examples, alpha-synuclein-induced membrane poration, ER stress, lysosome dysfunction, cell-to-cell transmission were not mentioned.*
   Response: We thank the reviewer for this suggestion. We have now included a subsection on other emerging mechanisms of cell death associated with alpha-synuclein toxicity in section 4.3, describing the topics of membrane poration, ER stress, lysosome dysfunction, cell-to-cell transmission.

4) *The authors specifically used one and half sections (4.1 and 5) to talk about the effect of metal ions on alpha-synuclein aggregation. Since there are many other factors able to facilitate aggregation of alpha-synuclein, why the authors only focused on such effect from metal ions. Moreover, this article didn't clearly explain the direct link between metal ions and neurotoxic network of alpha-synuclein in the nucleus and mitochondria, is such detailed discussion really necessary?*
   Response: We agree with the reviewer that this elaborative section on metal binding may come across as a bit of out scope of this review. We have thus moved the section 5 as part of section 6 (6.1.1), only highlighting the impact of metals on alpha-synuclein's DNA binding activity.

5) *Based on the subtitle, section 9 should focus on the synergistic role of alpha- synuclein in the pathogenesis of neurodegerative diseases in the case that other amyloidogenic proteins coexist. However, there were too much content talking about other proteins, while the limited discussion on the role of alpha-synuclein in DNA damage was mainly based on assumption. This is not acceptable.*

Response: We strongly believe that this is an important topic of how alpha-synuclein toxicity impacts other pathologies when they co-exist, which should not only affect the disease progression and severity, but may also influence the patients' response to therapies. However, as the reviewer mentioned, there are not many studies on such cross-talk of pathologies and our aim here is to highlight the importance of topic and emphasize this as an important topic for future studies. We have now revised the section to reflect this limited goal of this section, without sounding overly speculative. We thank the reviewer for the suggestions.

6) *Section 11 discussed the limitation and challenges of in vitro alpha-synuclein cell line models. It would be nice to also include animal models if such discussion is initiated. Besides, the reviewer suggest that the introduction of the newly developed Tet-on SNCA cell line should be deleted due to its lack of novelty, or such discussion should include the similar cell lines established in other labs back to 2007(see PMID: 19476547, PMID: 17714183 and PMID: 18957893).*
Response: We have deleted this section on the cell line as suggested.

References Cited in the author response:

Gonçalves, S., Outeiro, T.F., 2013. Assessing the subcellular dynamics of alpha-synuclein using photoactivation microscopy. *Mol Neurobiol* 47**,** 1081-1092.

Gray, D.M., Hung, S.H., Johnson, K.H., 1995. Absorption and circular dichroism spectroscopy of nucleic acid duplexes and triplexes. *Methods Enzymol* 246**,** 19-34.

Hegde, M.L., Gupta, V.B., Anitha, M., Harikrishna, T., Shankar, S.K., Muthane, U., Subba Rao, K., Jagannatha Rao, K.S., 2006. Studies on genomic DNA topology and stability in brain regions of Parkinson's disease. *Arch Biochem Biophys* 449**,** 143-156.

Huang, S.Y., Zou, X., 2014. A knowledge-based scoring function for protein-RNA interactions derived from a statistical mechanics-based iterative method. *Nucleic Acids Res* 42**,** e55.

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Lu, T., Pan, Y., Kao, S.Y., Li, C., Kohane, I., Chan, J., Yankner, B.A., 2004. Gene regulation and DNA damage in the ageing human brain. *Nature* 429**,** 883-891.

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Rao, J.N., Jao, C.C., Hegde, B.G., Langen, R., Ulmer, T.S., 2010. A combinatorial NMR and EPR approach for evaluating the structural ensemble of partially folded proteins. *J Am Chem Soc* 132**,** 8657-8668. Robertson, T.A., Varani, G., 2007. An all-atom, distance-dependent scoring function for the prediction of

protein-DNA interactions from structure. *Proteins* 66**,** 359-374.
Siddiqui, A., Chinta, S.J., Mallajosyula, J.K., Rajagopolan, S., Hanson, I., Rane, A., Andersen, J.K., 2012.

Selective binding of nuclear alpha-synuclein to the PGC1alpha promoter under conditions of oxidative stress may contribute to losses in mitochondrial function: implications for Parkinson's disease. *Free radical biology & medicine* 53**,** 993-1003.

Tuszynska, I., Magnus, M., Jonak, K., Dawson, W., Bujnicki, J.M., 2015. NPDock: a web server for protein-nucleic acid docking. *Nucleic Acids Res* 43**,** W425-430.

Tuttle, M.D., Comellas, G., Nieuwkoop, A.J., Covell, D.J., Berthold, D.A., Kloepper, K.D., Courtney, J.M., Kim, J.K., Barclay, A.M., Kendall, A., Wan, W., Stubbs, G., Schwieters, C.D., Lee, V.M., George, J.M., Rienstra, C.M., 2016. Solid-state NMR structure of a pathogenic fibril of full-length human alpha-synuclein. *Nat Struct Mol Biol* 23**,** 409-415.

Ulmer, T.S., Bax, A., Cole, N.B., Nussbaum, R.L., 2005. Structure and Dynamics of Micelle-bound Human α-Synuclein. *J Biol Chem* 280**,** 9595-9603.

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Zhang, C., Liu, S., Zhu, Q., Zhou, Y., 2005. A knowledge-based energy function for protein-ligand, protein- protein, and protein-DNA complexes. *J Med Chem* 48**,** 2325-2335.

2nd Decision Letter

Ref: PRONEU_2019_77_R1
Title: A multi-faceted genotoxic network of alpha-synuclein in the nucleus and mitochondria of dopaminergic neurons in Parkinson's disease: Emerging concepts and challenges
Journal: Progress in Neurobiology

Dear Dr. Hegde,

Thank you for submitting your manuscript to Progress in Neurobiology. We have received comments from reviewers on your manuscript. Your paper should become acceptable for publication pending suitable minor revision and modification of the article in light of the appended reviewer comments.

When resubmitting your manuscript, please carefully consider all issues mentioned in the reviewers' comments, outline every change made point by point, and provide suitable rebuttals for any comments not addressed.

We look forward to receiving your revised manuscript as soon as possible.

Kind regards,

Jeanne Paz, Associate Editor

Sabine Kastner, Editor-in-Chief
Progress in Neurobiology

Comments from the editors and reviewers:

Reviewer 2

The reviewer is happy to see that major changes have been made and the quality of this revised version is significantly improved. However, due to the big problem in the structure of this manuscript, a further revision is still required. It seems that the authors just simply put different sections together without proper arrangement although the content of each section is acceptable. For example, based on the title, this review should focus on genotoxic network of alpha-synuclein, but the whole section 5 almost has nothing to do with alpha-synuclein. Also, the second paragraph starting from "While the origins of mtDNA deletions are unclear……" in section 9.1, the reviewer couldn't find any link to alpha-synuclein. For the same reason, the subtitle 8, if the authors insist on keeping it, "Crosstalk between alpha-synuclein and other amyloidogenic proteins in genotoxicity" may be better in order to fit the title. As for the 10 subtitles, it is still very confusing. There is no a clear order to connect each section, in another word, each section seems to be isolated without a reasonable transition/connection. The readers can easily get lost by jumping from one section to another completely different section.

Therefore, the reviewer strongly suggests that this manuscript should be well-organized to improve its readability

2nd Author Response Letter

Reviewer 2

The reviewer is happy to see that major changes have been made and the quality of this revised version is significantly improved. However, due to the big problem in the structure of this manuscript, a further revision is still required. It seems that the authors just simply put different sections together without proper arrangement although the content of each section is acceptable. For example, based on the title, this review should focus on genotoxic network of alpha-synuclein, but the whole section 5 almost has nothing to do with alpha-synuclein. Also, the second paragraph starting from "While the origins of mtDNA deletions are unclear....." in section 9.1, the reviewer couldn't find any link to alpha-synuclein. For the same reason, the subtitle 8, if the authors insist on keeping it, "Crosstalk between alpha-synuclein and other amyloidogenic proteins in genotoxicity" may be better in order to fit the title. As for the 10 subtitles, it is still very confusing. There is no a clear order to connect each section, in another word, each section seems to be isolated without a reasonable transition/connection. The readers can easily get lost by jumping from one section to another completely different section.

Therefore, the reviewer strongly suggests that this manuscript should be well-organized to improve its readability.

Response: We thank the Reviewer for appreciating our significantly improved revised article in general and the contents and topics covered in this review.
We also appreciate the Reviewer's important suggestions for reorganization, and condensing the review by removing sections that are not directly relevant to the focus of alpha-synuclein mediated genotoxicity. We completely agree with the suggestions, which infact has significantly improved the readability of review. We have now carefully considered these suggestions and re-revised the manuscript, as pointed out below.

1) *Based on the title, this review should focus on genotoxic network of alpha-synuclein, but the whole section 5 almost has nothing to do with alpha-synuclein.*
Response: We have now removed section 5 and extracted only the alpaha-synuclein relevant text from this section with section 6 (old section 8).

2) *The second paragraph starting from "While the origins of mtDNA deletions are unclear....." in section 9.1, the reviewer couldn't find any link to alpha-synuclein.*
Response: We have again deleted this paragraph and included a Segway statement to connect the topics.

3) *The subtitle 8, if the authors insist on keeping it, "Crosstalk between alpha-synuclein and other amyloidogenic proteins in genotoxicity" may be better in order to fit the title.*
Response: After carefully considering the reviewer's concern, we have decided to delete this subtitle. Wehave now mentioned in the under the 'Concluding Remarks' section, 2-3 sentences about the importance of possible cross-talk between alpha-synuclein and other pathologies in disorderes with overlapping pathology such as mixed etiology dementia, as a topic for future direction.

4) *As for the 10 subtitles, it is still very confusing. There is no a clear order to connect each section, in another word, each section seems to be isolated without a reasonable transition/connection.*
Response: Agreeing with the Reviewer, we have now removed two subtitles and carefully reorganized the re-revised article, with appropriate Segway statements connecting the subtopics. We sincerely thank the Reviewer for his/her time in going over our article incisively, and helping us in presenting this significantly improved, re-revised version.