

Format for ANSWERING REVIEWERS

Feb 10, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: FoxO3a-revised).



Title: FoxO3a and disease progression

Author: Richard Seonghun Nho, Polla Hergert

Name of Journal: *World Journal of Biological Chemistry*

ESPS Manuscript NO: 7763

The manuscript has been improved according to the suggestions of reviewers:

1. We responded all comments raised by reviewers.
2. Revision has been made according to the suggestions of the reviewer

1) Reviewer 1: This review is written well and requires little refinement for its publishing. Firstly, a) it would be desirable to supplement the review by data on interplay of FoxO3a with NF-kB. Also, b) authors can improve MS by adding a simple diagram describing cross-talks of FoxO3a with other signaling pathways. Finally c) the manuscript requires minor polishing as for example: p.4 Furthermore, d) the phosphorylation of FoxO3a by Akt is subsequently polyubiquitinated p. 10 Sirti (lab slang?)

Response 1: a-b) We included additional information on the relationship between FoxO3a and NF-KB, and new figures (Figures 2&3) and table (Table 1) were added to this revised manuscript. c) We also edited this manuscript suitable for the publication quality in WJBC. d) We corrected abbreviation.

2) Reviewer 2: This review article describes the current knowledge of FoxO3a in moderate detail. It is informative and relatively well-written. a) There are a few sentences however that are not grammatically correct and are difficult to understand such as this sentence on page 4. b) Furthermore, “the phosphorylation of FoxO3a...” The full manuscript should be carefully edited before publication... One more issue is that within the manuscript there are several instances of under-citing references and c) I would recommend the authors check back over the manuscript to ensure that references are being cited when required. d) A final suggestion is that figures be added if possible to illustrate and summarize the main points of the review article (regulatory mechanisms, function, etc). Overall, the article was easy to follow and informative.

Response 2: a) We corrected and rewrote sentences that were not grammatically correct and difficult to understand. b) We also edited and included detailed mechanisms on how FoxO3a becomes degraded by proteasome. c) We also edited and updated references. d) We included several figures for the illustration of the function of FoxO3a in our revised manuscript.

3) Reviewer 3: Authors present a review on FoxO3a biology and cancer.

a). The topic is interesting but the manuscript has some aspects that should be modified. Title is too

much ambiguous. I believe that the topic of the manuscript is FoxO3 biology and cancer. Introduction, line 10-14. There are sentences redundant. Genes regulated by FoxO3a is an important aspect of this review.

b) A Table or Figure could be necessary to develop this point. This Table may contain oxidative-stress response genes mentioned in section 3.2 Introduction, line 15. "...other functions of FoxO3a in various cell types" It is too much ambiguous for a review.

c). Authors should mention these functions, and cells with the corresponding references. Section 1, line 4. This sentence about Fox regulation should be presented in the next section.

d). The role of kinases/phosphatases on FoxO3a phosphorylation and cell distribution could be presented in a figure that illustrates section 2.

e). The role of oxidative stress on FoxO3a phosphorylation should be also presented in this part of the review. Section 2.3 says "...oxidative stress triggers FoxO acetylation and affects localization..." How? This aspect should be more deeply explained. "Acetylation plays an important role in regulating FoxO3a" How? Protein acetylase, p300, Sirt1...modulate FoxO3a function, acetylation or both? This section is too much ambiguous.

f). The role of FoxO3a on cell proliferation/apoptosis and cancer should be illustrated with a Table which contains information about FoxO3a in cancer and/or cancer cell lines.

g). The section title FoxO3a in clinical applications is too much simple and ambiguous. Thus, this section says "...FoxO3a has been targeted as a way to treat several types of cancers" What cancers? How were these treated? Please, include appropriate references respecting these ideas. Future application of FoxO3a section proposes that FoxO3a may be deregulated by environmental factors. What? How? These aspects should be mentioned in the revised manuscript. "...management of optimum FoxO3a activity" What means? How is performed?

Response 3: a) We included additional supporting findings on the defined role of FoxO3a in disease progression and the redundant sentence was corrected in our revised manuscript. b-c) New figure (Figure 1) showing the role of FoxO3a in response to oxidative stress and edited the sentence to elucidate the additional function of FoxO3a, and supporting references were also included in our revised manuscript. d) The phosphorylated/dephosphorylated residues by kinases and phosphatases were added and presented in Figures 2&3. e) The current underlying mechanisms of oxidative stress are described in more detail at page 8 to 9. f) New table (Table 1) was inserted in this manuscript to show the FoxO3a's role in cancer and other cell lines. g) More detailed clinical application of FoxO3a and their references are now included in this manuscript.

4) Reviewer 4: The structure of Nho's review is clear and the language is satisfied... There are some points to be addressed and also some advice for this review. a). At the end of paragraph "FOXO3a structure", the last two sentences are not necessary. They do not relate to FOXO3a structure. Move it to the later parts and add suitable reference. b). The paper mentions that FOXO3a can be phosphorylated/dephosphorylated at multiple sites by different kinases, such as AMPK, SGK etc. I find the author didn't introduce phosphorylation sites of some kinases (CK1, DYRK1a, MAPK, IKK β , MST1). It is recommended that add a table including the following information if possible: kinase name, FOXO3a phospho-sites, FOXO3a locates in nucleus or cytoplasm, stimuli which can activate/inactivate kinase and so on. It will be more readable and give more information to readers. c). More references are needed for the last sentence of paragraph "Acetylation, transcriptional regulation, microRNA and others" d). For histone deacetylase gene Sirt, there is a typo (Sirt3, Sirt3). e). The title "The unique features of FoxO3a in disease progression" seems too big. In disease part, author only

mentioned FOXO3a participates in sympathetic neuron cell death, TRAIL transcription in BCR-ABL-transformed human cells and chronic myeloid leukemia.

Response 4: a) We edited last two sentences as suggested. b) The additional phosphorylation sites by several kinases on FoxO3a and FoxO3a's location by these kinases are now included (Figures 2&3). c) More supporting references are now included in our revised manuscript. d) Typo was corrected. e). New title "FoxO3a and disease progression" is now included in our revised manuscript. We also included multiple FoxO3a target genes in various cell types (Table 1) as a reviewer suggested.

5) Reviewer 5: The authors reviewed recent advances about the functions of FoxO3a, a human homologue of the *C. elegans* cancer repressor DAF-16. The manuscript is publishable but requires extensive revision before it reaches the publication standard. a) Specifically, 1. Page 3, 2nd paragraph: The first 4 lines has nothing to do with the FoxO3a structure, but a general review about Forkhead family. It should be intergrated into "Introduction". The rest of the paragraph did not specifically talk about FoxO3a structure, but the structure of FoxO in general. This needs to be revised to focus more on the structure of FoxO3a, and compare with other FoxO3 families (Why FoxO3a is unique)! b). Page 4: "dephosphorylated" and "non-phosphorylated" are two different things. I did not find any specific discussion about dephosphorylation of FoxO3a, as the subtitle suggested. c). Page 7-9: The authors first describe FoxO3a promotes apoptosis (page 7). They then described that under ROS stress, the FoxO family were up-regulated and "blocks Caspase 3, 8 and 9", implying that the FoxO family prevents apoptosis? It is NOT clear then, under what condition FoxO3a promotes apoptosis? d) Minor revisions 1. The should add page numbers (and line numbers?), so it would be easier for future reviewers to make comments. 2. There are too many typos: a): Page 3, Should "FDNA-binding" be "DNA-binding"? b): Many places, such as page 3 line 2, "it's" should be "its" c): FoxO3a and FOXO3a are sometimes mis-used (see page 6). d): Page 6: "FoxO3a including FoxO1, FoxO4" should be "FoxO3a as well as FoxO1 and FoxO4". Same thing occurred in page 7. e): Page 7: What does "suppression of cell cycle inhibition" mean? f): Page 10: "sirti3", "SIRT3", "sirt3" - Are they supposed to be the same thing?

Response 5: a) We revised our manuscript as a reviewer suggested. b) Dephosphorylation and its detailed function is now included (Figure 3 and page 4 to 6). c) We clarified the role of FoxO3a in more detail and described how FoxO3a has a protective role in response to ROS (page 8, stress resistance effect to page 9, longevity). d) Page numbers were added, and we corrected all as a reviewer suggested.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Biological Chemistry*.

Sincerely yours,

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