Jan 20, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 7308-minireview-edited.docx).

Title: Extracellular O-GlcNAc: Its biology and relationship to human disease

Author: Mitsutaka Ogawa, Koichi Furukawa, Tetsuya Okajima

Name of Journal: World Journal of Biological Chemistry

ESPS Manuscript NO: 7308

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

1 Format has been updated

2 References and typesetting were corrected

3 Revision has been made according to the suggestions of the reviewer

Reviewed by 02446647
1) If there is information and discussion on the blood levels of N-acetylglucosamine, the sugar moiety and its metabolites in the mutant model animals and patients may be explained.

Response
-There is no information regarding the blood levels of N-acetylglucosamine, the sugar moiety and its metabolites in the mutant model animals and patients. We have noted following sentences in the main text.

P6. Line 11: Currently, the blood levels of extracellular O-GlcNAc, the sugar moiety and its metabolites in the patients have not yet been investigated.

Reviewed by 00484021
1) The manuscript needs language revision and would also benefit from some reorganization; for example, sections 4 and 5 could easily be organized into one, since they both mainly talk of O-GlcNAc in the context of AOS.

Response
-Language revision was reorganization was made. Sections 4 and 5 were organized into one as suggested.

2) The manuscript currently reads as a laundry list of extracellular proteins that have thus far been identified as being modified with O-GlcNAc. Little effort is given to providing context and significance for these early findings. For this reason the review fails to yield clear insight into the implications of current findings, the holes in our knowledge and the potential future directions for the field.

Response
-Following the comment from the reviewer, we have provided the context in which the O-GlcNAcylated protein was identified in the previous studies. We also emphasized that significance of the proteins was only tested in the context of Dumpy function, and the roles of O-GlcNAc in mammals have not been investigated.

3) Given the complex roles of intracellular O-GlcNAc modification and the associated signal transduction pathways and crosstalk with phosphorylation, the authors fail to address how O-GlcNAc of external proteins could interplay with other posttranslational modifications or modify interactions with the extracellular matrix.

Response
-Following the reviewer's comment, we have elaborated the discussion in terms of 1) relation to hexosamine pathway [p4 line 25] 2) other posttranslational modification including O-fucose and O-glucose [p7 line 12] 3) potential roles of O-GlcNAc to modify interaction with the extracellular matirix [p5 line 15].

4) It is therefore recommended that the review be revised both structurally and data wise to achieve the objective of the title before consideration of acceptance.

Response
-The reviewer has requested to add new experimental data to achieve the objective of the title of this paper "Extracellular O-GlcNAc: Biology and relevance to human disease." However, we cannot perform extra experiments within a short period of time requested by the editor. Due to the lack of data proving the functional relevance of extracellular O-GlcNAc in AOS, we have modified the title to "Extracellular O-GlcNAc: Its biology and relationship to human disease."

Reviewed by 00289703
1) First, the authors don't really add to our understanding of the phenomena. The review is essentially composed of a list of O-GlcNAc subtitles with a brief description. It would have been much more interesting if the authors had presented more details and some critical analysis of the findings they were describing or if they had provided some insight into a bigger picture of how O-GlcNAc is integrated into the framework of human disease.

Response
-We have revised the Figure 1A to include the insight into a bigger picture of how O-GlcNAc is integrated into the framework of human disease.

2) Second, I did not find the figure 1 particularly illuminating. Instead, the authors might consider including a figure outlining O-GlcNAc, emphasizing the extracellular O-GlcNAc.

Response
-I agree with the reviewer that figure1 is not particularly illuminating. As suggested by the reviewer the figure was replaced with the new figure focusing on the extracellular O-GlcNAc and relation to AOS.

3) Part B of that figure might include a panel to address any disease-relevance.

Response
-The function of the protein in the list was provided in the table. Potential relevance of each protein to AOS was illustrated in figure 1A.

4) Third, the authors need to expand more contents to address the upstream signal transduction or pathophysiological setting (s) that triggers extracellular O-GlcNAcylation. That is relevant to their functional consequences.

Response
-We have elaborated the description of the hexosamine pathway that acts upstream of EOGT and UDP-GlcNAc transporter that might trigger extracellular O-GlcNAcylation.(p4 line25).

5) Finally, the authors need to check their English usage carefully throughout the text. For example, "It is also possible that loss of Eogt directs the increased UDP-GlcNAc pool in the cytoplasm, leading to elevated pyrimidine synthesis, such as uracil, that is likely to promote wing blistering". It is too lengthy that could be easily reworded into two sentences.

Response
-We have revised the text as suggested and English is carefully corrected.

6) Additionally, many references should be cited. For example, they should add reference(s) to the statement of "The intracellular O-GlcNAcylation is reversible, and the cycling is dynamically regulated by O-GlcNAc transferase (OGT) and O-GlcNAcase".

Response
Following the reviewer's comments, new references were added.

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

Thank you very much for your kind consideration.

Best wishes,

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