Author's response to reviews

Title: Epigenetic regulation of caloric restriction in aging

Authors:

Yuanyuan Li (lyy@uab.edu)
Michael Daniel (michael5@uab.edu)
Trygve Tollefsbol (trygve@uab.edu)

Version: 3 Date: 16 June 2011

Author's response to reviews: see over
Dear Dr. Lee:

Thank you very much for the prompt reviews of our manuscript and for the constructive criticisms from you and the reviewers. We are pleased that the Editors could consider our manuscript after revision in response to editorial and the reviewers’ critiques. We appreciate the valuable comments from you and the reviewers and we believe that all these comments truly helped us to improve this manuscript. We have now revised the manuscript point by point based on the comments from the editor and the reviewers and the changes have been highlighted in the resubmitted manuscript. Our responses to the editorial and the reviewers’ comments follow:

Detailed reply to Dr. Lee’s editorial comments

1. Comment 1. Introduction: This introduction is clear and concise, and gives enough background and interest for the broad readership of BMC Medicine. In order to give it more of a clinically relevant flavour and to draw the audience in, I have moved one of the paragraphs relating to the background and discovery of CR into the introduction, and then deleted any subsequent duplicated text.

Authors’ Reply 1: We totally agree with the reviewer’s comments and have made corresponding changes as the editor suggested in our revised manuscript (see on Page 3, Lines 39-55).

2. Comment 2. Introduction: I also think that the addition of a table (see comment LL 5) to expand upon the sentence about the types of diseases that CR is known to have an effect upon would strengthen the manuscript. This would both summarise the discussion in the manuscript, and also refer interested parties to the initial studies.

Authors’ Reply 2: We have added a table addressing CR involving age-related diseases studied in rodents, non-human primates and humans according to the editor’s comment (shown in Table 1, Page 27)

3. Comment 3. DNA methylation affects aging during CR: I have stated the long form of CR for this important subheading to make it clearer. The way in which the mechanism of DNA methylation during CR is explained is very clear.

Authors’ Reply 3: We have changed the statement of “CR” to its original explanation as “caloric restriction” in all our sub-titles to ensure it is clear and consistent for the reader (shown on Page 4, Line 72; Page 7, Line 166; Page 13, Line 346).
4. Comment 4. DNA methylation affects aging during CR: I also thought that the section discussing a specific disease relating to CR and DNA methylation was very important. However, although epigenetic effects of CR in weight loss is interesting, this paragraph doesn’t fit too well in this section which is about aging. Therefore, I wonder if there is any data that exists linking weight loss to longevity that you could add to this paragraph to make it more relatable to aging? If not, I would suggest removal of this section, and replacement with another disease that is affected by DNA methylation changes due to CR. For example, you could expand upon your previous point about CR on cancer prevention.

Authors’ Reply 4: We fully agree with the editor. Actually, obesity is highly related to various human diseases and we have addressed this correlation in our revised manuscript (seen on Page 6, Lines 141-147). In addition, we have incorporated some DNA methylation therapies for cancer to further strength our points regarding clinical aspects (seen on Page 6, Lines 129-137).

5. Review comment 5. Effects of histone remodeling in control of aging during CR: Again, I have stated the long form of CR for this important subheading to make it clearer. Generally, this section is very detailed and gives a lot of useful information. However, I thought this could benefit from having subsections that would enable the reader to quickly focus in on their area of interest and also allow you to expand upon certain points. I have suggested three. These are: ?Sirtuin 1 and its substrates?, ?Histone methylation?, and ?Potential epigenetic treatments for age-related diseases?.

Authors’ Reply 5: The reviewer is absolutely right and we have added four sub-titles in this section. These are: Histone acetylation and HDACs; Sirtuin 1 and its substrates; Histone methylation and Potential epigenetic treatments for age-related diseases.

6. Review comment 6. Sirtuin 1 and its substrates: I split this off into its own section due to the importance of this HDAC in aging. I have recommended that you define ?Sirt 1? for its first use within the manuscript. Reviewer 2 also has some recommendations regarding word order, nomenclature and references (see comments LL 16-19, 22) and suggests you re-write two sentences for scientific sense (comments LL 20-21) as the meaning is currently unclear.

Authors’ Reply 6: We have fully complied with the editorial and reviewer 2’s comments by making corresponding changes as suggested (seen on Page 9, Lines 222-223; Page 10, Lines 256-257, 269-270). The detailed reply to reviewer 2 will be addressed following.

7. Comment 7. Sirtuin 1 and its substrates: You briefly mention that there have been promising effects of Sirt 1 in mediating CR and longevity supported by various models. I feel that your manuscript would be greatly strengthened expansion of this sentence either by an additional few paragraphs or a table detailing these experiments and their results (see comment LL 17).

Authors’ Reply 7: We have expanded this section to two more paragraphs by providing more detailed information and current advances in this area as suggested by the editor (Page 9, Lines 228-242; Page 10, Lines 243-251)
8. Comment 8. Histone methylation: I added this in as another subsection title. Reviewer 2 recommended a re-wording a sentence in this section.

Authors’ Reply 8: We have fully complied with the editorial and reviewer 2’s comments (shown on Page 11, Lines 298, 303-304)

9. Comment 9. Potential epigenetic treatments for age-related diseases: Again, I have added this title as another subsection heading. I think this would be sometime our audience would find particularly interesting, so I wonder if you would be able to expand on this with a few extra paragraphs (see comment LL 26). Reviewer 2 also suggests you add an additional reference about resveratrol.

Authors’ Reply 9: We have discussed the related epigenetic treatments in different sections, for example, DNA methyltransferases (DNMTs) inhibitors and histone deacetylases (HDACs) inhibitors on cancer therapy and additional resveratrol studies on age-related diseases, in our revised manuscript (seen in Page 6, Lines 129-137; Page 8, Lines 204-211; Page 9, Lines 212-218; Page 12, Lines 322-329). We also included an additional table to further explain the clinical trials that have been performed with these epigenetic medications (Table 3, Page 29).

10. Comment 10. Extra references from Reviewer 1

Authors’ Reply 10: We have incorporated three references into our revised manuscript as suggested by reviewer 1.

11. Comment 11. References: As I have changed the order of a few paragraphs and suggested that you add additional references, please ensure that the number order of your references is still correct.

Authors’ Reply 11: We have re-numbered our references based on new organization of our revised manuscript.

12. Comment 12. Figures: I thought this manuscript would be greatly strengthened by having another figure showing diagrammatically the mechanisms of histone modification.

Authors’ Reply 12: We have included a figure to demonstrate the mechanisms of histone modification (see in Figure. 2)

Detailed reply to Reviewer 1

Review comments: Excellent revision about the current advances in epigenetic regulations in response to caloric restriction and how this impacts cellular senescence, aging and potential extension of a healthy lifespan for humans. The authors deep into SIRTUINS as specially implicated in these mechanisms. I can suggest as discretionary recommendations to include some of these cites:


Authors’ Reply: We are very pleased that the reviewer considers our manuscript to be “Excellent” and we have incorporated these three references into our revised manuscript as suggested. The reference numbers are 81, 82 and 116, respectively.

Detailed reply to Reviewer 2

Discretionary Revisions

1. Review comment 1: the review would benefit from including a paragraph on page 7 describing the known types of HDACs before in depth description of the sirtuins

Authors’ Reply 1: We agree with the reviewer and have addressed HDAC family members in our current manuscript as suggested by the reviewer (seen on Page 8, Lines 187-191).

2. Review comment 2. the distinction between genes and proteins encoded by genes should be made clearer throughout the manuscript

Authors’ Reply 2: We have clarified the statement by using italic font to represent gene names based on the reviewer’s suggestion.

Minor Essential Revisions

Review comments 1-26

1. P4 L21: change “aberrant gene expressions” to “aberrant gene expression”
2. p4 L22-23: change “of distribution of ….genome leading a decrease” to “in distribution of….genome leading to a decrease”
3. p5 L2: change “including yeast, worms, fish” to “yeast, worms, flies, fish” to better reflect the references cited.
4. p5 L5: a reference is needed
5. p5 L10: change “maintain the chromatin function” to “maintain chromatin function”
6. p5 L16: change “oncogenes” to “protooncogenes”
7. p5 L22: move “DNA” to read “hypermethylation of an E2F-1 binding site’s DNA”
8. p6 L4: change “involve” to “induce”
9. p6 L7: change “Due to the promising” to “Due to promising”
10. p6 L17: Indent if this is a new paragraph
11. p6 L21: change “prevention” to “preventive”
12. p7 L7: change “repressive transcription” to “transcriptional repression”
13. p7 L11: change “and the most notable are” to “including”
14. p7 L17: remove “and determines lifespan accordingly”
15. p7 L27: define H4K16 when first used to allow the reader to understand the nomenclature used
16. p8 L1-2: the first sentence of page 8 should precede the last sentence of page 7
17. p8 L3: change “regulatory genes” to “regulatory proteins”
18. p8 lines 9-10: the sentence “Another important gene that influences apoptosis such as Foxo was deacetylated and underwent reduced expression by SIRT1, therefore repressing Foxo-mediated apoptosis [67, 68].” does not make sense since Sirt1 is not a DNA deacetylase. This needs rewriting.
19. p8 line 11-13: the sentence “In addition, the DNA repair protein, Ku70, can become deacetylated by SIRT1 allowing it to inactivate the pro-apoptotic factor, Bax, resulting in apoptosis inhibition [69]. “ does not make sense either since Ku is not known to directly interact with Bax so this also needs rewriting.
21. p8 L26: “that activation of SIRT1 by CR can directly bind” should be changed to “that CR-activated SIRT1 can directly bind”
22. p9 L22-23: change “Histone methylations at lysine residues could be mono-,” to Lysine residues on histones can be mono-,”
23. p9 L31: change “regulating” to “regulate”
24. p9,10: although many studies have reported positive effects of resveratrol upon lifespan in model organisms, it would also be worth citing studies showing other results such as Bass TM, Weinkove D, Houthoofd K, Gems D, Partridge L Effects of resveratrol on lifespan in Drosophila melanogaster and Caenorhabditis elegans. Mech Ageing Dev. 2007 Oct;128(10):546-52.
25. p11 L14: change “and key gene expressions” to “the expression of key genes”
26. p22: delete the word “important” in the title since it implies that other genes are unimportant.

Authors’ Reply: We have summarized our replies to Reviewer 2’s minor comments in the following table.

<table>
<thead>
<tr>
<th>Comments</th>
<th>Changed according to reviewer’s comment</th>
<th>Location in revised paper</th>
<th>Further explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yes</td>
<td>Page 5, line 89</td>
<td>N/A</td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
<td>Page 5, lines 91-92</td>
<td>N/A</td>
</tr>
<tr>
<td>3</td>
<td>Yes</td>
<td>Page 3, line 43</td>
<td>N/A</td>
</tr>
<tr>
<td>4</td>
<td>Yes</td>
<td>Reference no. 13, 22-24</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>5</td>
<td>Yes</td>
<td>Page 5, line 102</td>
<td>N/A</td>
</tr>
<tr>
<td>6</td>
<td>Yes</td>
<td>Page 5, line 108</td>
<td>N/A</td>
</tr>
<tr>
<td>7</td>
<td>Yes</td>
<td>Page 5, line 116</td>
<td>N/A</td>
</tr>
<tr>
<td>8</td>
<td>Yes</td>
<td>Page 6, line 138</td>
<td>N/A</td>
</tr>
<tr>
<td>9</td>
<td>Yes</td>
<td>Page 6, line 146</td>
<td>N/A</td>
</tr>
<tr>
<td>10</td>
<td>Yes</td>
<td>Page 7, line 159</td>
<td>N/A</td>
</tr>
<tr>
<td>11</td>
<td>Yes</td>
<td>Page 7, line 163</td>
<td>N/A</td>
</tr>
<tr>
<td>12</td>
<td>Yes</td>
<td>Page 7, line 180</td>
<td>N/A</td>
</tr>
<tr>
<td>13</td>
<td>Yes</td>
<td>Page 9, line 221</td>
<td>N/A</td>
</tr>
<tr>
<td>14</td>
<td>Yes</td>
<td>N/A</td>
<td>The sentence is removed.</td>
</tr>
<tr>
<td>15</td>
<td>Yes</td>
<td>Page 10, line 256-257</td>
<td>N/A</td>
</tr>
<tr>
<td>16</td>
<td>N/A</td>
<td>N/A</td>
<td>We feel our statement is more appropriate.</td>
</tr>
<tr>
<td>17</td>
<td>Yes</td>
<td>Page 10, line 263</td>
<td>N/A</td>
</tr>
<tr>
<td>18</td>
<td>Yes</td>
<td>Page 10, lines 269-270</td>
<td>The reviewer is correct and SIRT1 deacetylates protein residues other than DNA. We have corrected this ambiguous statement.</td>
</tr>
<tr>
<td>19</td>
<td>N/A</td>
<td>N/A</td>
<td>Actually, the Ku70 protein can directly bind to Bax based on the reference: Cohen et al. Acetylation of the C terminus of Ku70 by CBP and PCAF controls Bax-mediated apoptosis. Mol Cell. 2004 13(5): 627-38.</td>
</tr>
<tr>
<td>20</td>
<td>Yes</td>
<td>Reference no. 101</td>
<td>N/A</td>
</tr>
<tr>
<td>21</td>
<td>Yes</td>
<td>Page 11, line 287</td>
<td>N/A</td>
</tr>
<tr>
<td>22</td>
<td>Yes</td>
<td>Page 11, lines 303-304</td>
<td>N/A</td>
</tr>
<tr>
<td>23</td>
<td>Yes</td>
<td>Page 12, line 313</td>
<td>N/A</td>
</tr>
<tr>
<td>24</td>
<td>Yes</td>
<td>Reference no. 113</td>
<td>N/A</td>
</tr>
<tr>
<td>25</td>
<td>Yes</td>
<td>Page 14, line 374</td>
<td>N/A</td>
</tr>
<tr>
<td>26</td>
<td>Yes</td>
<td>Table 2</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Finally, we would like to draw to your attention that we have revised our manuscript thoroughly according to the editorial and the reviewers’ comments. We have also extended our manuscript by including more detailed information, current advances in related topics and more tables and figures in our revised paper. We appreciate the significant efforts of the editor and the reviewers for all the insightful suggestions, which have helped to greatly improve the quality and significance of this manuscript.

We are grateful for the points raised by the review which has helped us to improve our work and to present our paper more clearly to the readers. We have revised our manuscript by carefully
addressing every single point raised by the reviewer and we believe that these changes will better strengthen our findings and to illustrate our paper more clearly to the readers. All of the references have been checked to conform to the standard style of *BMC Medicine*, and each reference has been cited and numbered in the order of its first mention within the text of article. We feel that our manuscript has been extensively revised in response to the critique of the reviewers. We hope that you will now find this manuscript suitable for publication in *BMC Medicine*.

Thank you very much for giving us this opportunity to update and improve our manuscript, and we will look forward to hearing favorably from you soon.

Sincerely

Yuanyuan Li  
Research Assistant Professor  
Department of Biology  
175 Campbell Hall  
1300 University Boulevard  
University of Alabama at Birmingham  
Birmingham, AL 35294-1170

205-934-4587 (tel)  
205-975-6097 (fax)  
lyy@uab.edu (e-mail)