Author's response to reviews

Title: Multiple proteins and biological processes up-regulated during maxillary expansion in rats

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Author's response to reviews:

Dear Dr da-Silva

Thank you very much for your letter dated 2007-12-14, regarding the review of our aforementioned manuscript. We are grateful for your editorial efforts and the reviewers' comments and thoughtful suggestions. We have now made the modifications suggested by the reviewers. Changes made to the text are marked in red so that they can be easily identified.

Thank you for your consideration. We are looking forward to your review and to publication of this manuscript.

Sincerely,
Dr Junqing Ma

Replies to Reviewer comments:

Response to Reviewer Dr. Antonella Forlino:

1. To accept the presented proteomic data obtained by 2-DE and mass spectrometry in Table 1 the authors should also report the fold difference of the differentially expressed proteins, the accession number, the Entrez Gene ID, the number of mass values matched, the Sequence coverage (%), the MASCOT Score. Table 1 will also be easier to read if the identified proteins could be organized based on their role/function.

[Response]:
More information has been added in Table 1. Since some proteins have more than one function, such as vimentin, it is hard to list these proteins to certain category based on their role/function.

2. Also the CV (%) for the number of detected spots in the replicates as well as the CV of their intensity should be reported to demonstrate the validity and reproducibility of the data.

[Response]:

Based on the reviewer's comments, further information on this issue has now been added.

3. To prove the presence of new bone it will be nice to have some immunostaining of bone specific protein together with type I collagen like osteocalcon or ALP.

[Response]:

The review is essentially correct. Although HE and Masson's trichrome can provide basic evaluation of new bones, more immunostaining of bone specific proteins, like osteocalcon or ALP, would strengthen the data. However, to generate additional data more time and experiments is need. The time for preparation of the revised manuscript is somewhat short and would not allow us to perform additional experiments. We apologize for this. Nevertheless, our current data supports our conclusions.

4. In the growth curve it is necessary to include the SD and to evaluate if the decrease in body weight even at the end of the treatment is statistically significant. If this is the case the authors should discuss possible effects of this on their data.

[Response]:

The corrections have been done according to the reviewer's suggestions.

5. Fig.2 should include at least other two panels showing the sections of control samples at the beginning and at the end of the treatment (d=0 and d=11) to allow the effective comparison of control and treated animals.

[Response]:

The corrections have been done according to the reviewer's suggestions.

6. Nothing is mention about the diet of the rats. Do they receive normal pellet food or wet food and was the food the same for control and treated animals?

[Response]:
The related information has now been added into the revised manuscript, according to the reviewer’s suggestions.

7. In the discussion the paragraph on UPR need to be changed, UPR is not simply activated by GRP78, it is a more complex system that includes GRP78.

[Response]:
The corrections have been done according to the reviewer’s suggestions.

8. The discussion in general is too long, it should be shortened and more focused on the message that the authors want to give.

[Response]:
The corrections have been done according to the reviewer’s suggestions.

9. In Fig. 8 SD bars are missing.

[Response]:
The corrections have been done according to the reviewer’s suggestions.

10. The paper need a carefully spelling check.

[Response]:
The corrections have been done according to the reviewer’s suggestions.

Response to Reviewer Dr. Tara L Aghaloo:

In the abstract, background section, the authors state that maxillary expansion (ME) is an accepted practice, but its treatment effect needs improvement. This statement should be clarified, as to what type of improvement is needed, and how is a rat study on the molecular mechanisms going to answer these questions or issues.

[Response]:
The related information has now been added into the revised manuscript, according to the reviewer’s suggestions.

In the background section, much more information is needed. More should be written about proteomics and disease understanding using proteomics. More about tissue remodeling should be written, and how this pertains to the current study. The authors state that the biological mechanism of ME is so complicated. They must elaborate more on the complexity, and how proteomics will help make this less complicated. Also, how does proteomic understanding relate to further interpretation of the procedure, because the authors state that a
global proteomic understanding is needed to interpret further the procedure.

[Response]:

Based on the reviewer’s comments, further discussion on this issue has now been added.

The results in general, need much more explanation and detail. In the histological alterations subsection, the authors say significant tissue remodeling. Much more information is needed here, with detailed histological description. In the PCNA and collagen I expression subsection, to identify the tissue in the suture shown in Fig 2F of the ME group, immunohistochemical analysis of collagen I is not nearly descriptive enough for a result in this paper. Please clarify this significantly. In the western blot subsection, figures 7 and 8 should be combined into one figure and error bars should be used in the quantitation.

[Response]:

The corrections have been done according to the reviewer’s suggestions.

The discussion first sentence rats were used in the present study for practical reasons does not fit here in this paper. This should be placed in the body of the discussion. Also, much more expansion on the rat models of ME should be discussed here. Why does proteomics apply here? Why is it necessary in this model? Please describe more in detail about this point. Much of the discussion focuses on processes such as UPR and stress reaction, angiogenesis, cell proliferation, and reconstruction of cytoskeleton. However, the authors did not focus on any of these processes with the experiments in this study. They only focused on bone tissue remodeling proteins, so why is all of this discussion here? The osteogenesis subsection should be expanded, including why these proteins are differentially expressed. Elaborate more on OPG, PTH, and vimentin.

[Response]:

Done accordingly.

Are the weights significantly different in figure 1? Figure 2, 3, and 4 need to be labeled, and as stated previously, figures 7 and 8 should be combined.

[Response]:

The body weights were significantly different between the two groups. However, at the end of the study period, the mean weight difference was only about 5%. The figures were modified as suggested.

The methods section should elaborate on the expansion procedure including rate, frequency, amount of expansion, etc. In the 2-DE section, it is stated, based on the daily body weight monitoring and histological observations, rats at
3d were chosen for proteomic analysis of the midpalatal sutures. How was this time point chosen as an important point for evaluation? This is not clear.

[Response]:

Based on the reviewer's comments, we have added some information in these parts. Appropriate references also have been given for precise details of the expansion procedure used.