Author's response to reviews

Title: long non-coding RNA MEG3 inhibits NSCLC cells proliferation and induces apoptosis by affecting p53 expression

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Version: 4 Date: 26 July 2013

Author's response to reviews: see over
Dear Editors and Reviewers:

First of all, let me thank you and all the other reviewers for the critical feedback. We feel very fortunate that our manuscript went to these reviewers as the valuable comments from them not only helped us with the improvement of our manuscript, but also suggested some valuable ideas for future studies. Please forward our heartfelt thanks to these experts.

Based on the comments we received, careful modifications have been made to the manuscript. We hope the new manuscript will meet your journal’s standard. Below you will find our point-by-point responses to the reviewers’ comments/questions.
To Reviewer: Valentina Profumo

We are sorry that previous revised manuscript did not satisfy all your requests. Based on your comments, we have tried our best to revise and improve the manuscript and we hope the new manuscript will meet your standard. Below you will find our point-by-point responses to your comments/questions:

To satisfy my main request, in the revised version of the manuscript “Long non-coding RNA MEG3 inhibits NSCLC cells proliferation and induces apoptosis by affecting p53 expression” Kai-hua Lu and colleagues included a supplementary table showing MEG3 qRT-PCR data in all normal and NSCLC tissues. In addition, they also prepared exclusively for the reviewers two representative figures of the qRT-PCR data. Nevertheless, it not so clear how the authors obtained the first figure showing the qRT-PCR results in all normal and NSCLC tissues.

We thank you to raise this important issue, and qRT-PCR data of MEG3 expression represented in the first figure (Figure1A in manuscript) was normalized to GAPDH expression (as $\Delta \Delta CT$, the CT value of normal or tumor tissues' MEG3 expression minus that of GAPDH).

Rather, I suggest the authors to include the second figure, with the relative p-value, in the final version of the manuscript.

We thank you for this suggestion; however, another reviewer suggested us to include the first figure in the manuscript. Therefore, we have also carefully thought about both of your suggestions, and these two figures actually represent the same results. However, the first figure may be more intuitive representation (the expression of MEG3 in all normal and tumor tissues that normalized to GAPDH). We hope this modification can satisfy your request.

The two figures in previous rebuttal letter are shown in below.
To Reviewer: Paolo Gandellini

We are sorry that previous revised manuscript did not satisf all your requests. Based on your comments, we have tried our best to revise and improve the manuscript and we hope the new manuscript will meet your standard. Below you will find our point-by-point responses to your comments/questions:

In the revised version of the manuscript, the authors addressed my request to show MEG3 expression levels in individual samples. However, they did not prove that correlations with pathological and clinical parameters, as shown in figure 1B,C,D, hold true also when MEG3 expression levels in tumors are used instead of tumor/normal ratios.

We thank you to raise this important issue, according to your suggestion, the correlations of MEG3 expression levels with pathological and clinical parameters, was shown in figure 1B,C,D, using deltaCts of tumor samples only.

1) The authors provided individual Delta Cts in supplementary table 1 (note that expression levels are missing for patient #44). My suggestion is to include the figure showing deltaCts in normal and tumors tissues that they showed in the rebuttal letter as panel of figure 1. The evidence that expression of MEG3 is differential in tumor compared to normal tissues both in case the data are expressed as absolute values as well as when they are expressed as ratios strengthens the authors’ findings. It is necessary however to provide also p-value of the difference between deltaCts in tumor and normal tissues in the graph.

We are sorry for the mistake, and the expression of patuent #44 has been suuplied in new table. Moreover, according to your suggestion, we have include the figure showing deltaCts in normal and tumors tissues that shown in the peiviouse rebuttal letter as figure 1A in our new manuscript, and p-value of the difference between deltaCts in tumor and normal tissues was provided in the graph.

2) The first figure provided in the rebuttal letter showing relative MEG3 expression in normal tissues and tumors is not clear. How these relative expression levels have been calculated from deltaCts?
We thank you to raise this important issue, and qRT-PCR data of MEG3 expression represented in the first figure (Figure 1A in manuscript now) was normalized to GAPDH expression (as ΔΔCT, the CT value of normal or tumor tissues’ MEG3 expression minus that of GAPDH).

3) Please show, at least in the rebuttal letter (but preferably in the main text), the correlations of MEG3 expression levels with pathological and clinical parameters, as shown in figure 1B,C,D, using deltaCts of tumor samples only.

We thank you to raise this important issue, according to your suggestion, the correlations of MEG3 expression levels with pathological and clinical parameters, was shown in figure 1B,C,D, using deltaCts of tumor samples only.

4) Page 13: honestly I can’t understand the meaning of the sentence “The different expression level of MEG3 in NSCLC cell line may be due to the expression pattern of IncRNA is more cell sepecificall and those cells ability to proliferate or migrate/in evade”. Please clarify.

We thank you to raise this important issue, and wo have modified in the new manuscript.

The two figures in previous rebuttal letter are shown in below.