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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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 truly grateful to your critical comments and thoughtful suggestions. Based on these comments and suggestions, we have tried our best to revise and improve the manuscript and made great changes in the manuscript. All changes made to the text are in red color. We hope the new manuscript will meet your standard. Below you will find our point-by-point responses to your comments/questions:

To better understand the functional role of MEG3 in affecting cell proliferation and apoptosis, the authors should perform loss-of-function experiments in normal human bronchial epithelial cells (16BHE) or tumor cells in which MEG3 RNA is expressed at high levels.

We thank you to raise this important issue, according to your valuable suggestion, we performed loss-of-function experiments in H1299 cells in which MEG3 is expressed at high levels. The results were shown in figure S1.

The authors are also encouraged to discuss why MEG3 is not down-regulated in some NSCLC adenocarcinoma cell lines (e.g., NCI-H358 and NCI-H1299) and tissues. For example, do the authors know whether the different NSCLC cell lines, in which they evaluated the expression levels of MEG3, differ for their ability to proliferate or migrate/intrude? Furthermore, for a better understanding of MEG3 expression pattern, it should be also helpful to represent MEG3 qRT-PCR data in all normal and NSCLC tissues (by dividing them into adenocarcinomas and squamous cell carcinomas).

The expression pattern of IncRNA is more tissue and cell sepecific, which may be due to their ability to proliferate or migrate/intrude. According to your valuable suggestion, we have discussed in the new manuscript. The MEG3 expression data was represented in each tumor as normalized to matched normal tissues, because we attempted to avoid the impact of individual difference on data represent. However, we divided them into adenocarcinomas and squamous cell carcinomas, and there was no significant difference between the two groups(figure 1 D).
The authors claim that MEG3 may regulate NSCLC cell proliferation and apoptosis by activating p53. However, they also found that a p53 target, p21, is not modulated upon MEG3-dependent p53 activation. So, I encourage the authors to evaluate the expression levels of other p53 target genes upon MEG3 over-expression. In addition, do the authors know whether NSCLC cells (SPC-A1), that they used for their experiments, express wild-type p53 protein? This may help understand why p21 is not activated after MEG3-dependent p53 activation in SPC-A1 cells.

We thank you to raise this issue, and we have checked that SPC-A1 cells can expression wild-type p53 protein. P53 is an important tumor suppressor, and we will evaluate the expression levels of other p53 target genes upon MEG3 over-expression in our further studies to clarify the underlying pathways involved in MEG3 mediated NSCLC cells growth arrest.

With reference to the text and legends, the authors should invert figure 3C and 3D.

We are sorry for the mistake, and thank you for pointing out this error, and we have inverted figure 3C and 3D.

The manuscript is nicely written, but some typesetting errors occur in the text (e.g., spaces between words just before references and references often miss).

We have carefully edited the mispelling and typing errors and tried our best to improve the quality of english. We hope the new manuscript will meet your standard.

Page 5 (line 6), the authors write “Maternally expressed gene 3 (MEG3), encoding IncRNA…”. I am not sure that “encode” can be used with reference to a long non-coding RNA. The authors are encouraged to check.

We are sorry for the misunderstanding due to unclear descriptions in the previous manuscript. We have checked and modified it.

With reference to pages 6 and 7 (“Cell lines and culture conditions” paragraph), replace “Four NSCLC adenocarcinoma…” with “Six NSCLC adenocarcinoma…”; replace “NCI-358” with “NCI-H358”; mention NCI-H1975
cell line; describe how 16HBE cell line is cultured.

We are sorry for the mistake, and we have checked and modified it.

Pages 8 (two lines before the end) and 9 (last line), please check “Sigma-Aldrich (country???)”.
Legend to table 1, rectify “correlation of the expression of HOTAIR”; add superscript “a” to the title in column 3.
Page 14, the authors write “MEG3 was overexpressed in SPC-A1 and A549 cells by transfecting them with pCDNA-MEG3 or empty vector”. That is conceptually incorrect, since I am sure that they did not achieve an over-expression of MEG3 by transfecting cells with empty vector.

We are sorry for the mistake, and we have checked and modified it.

Page 15, the authors write “Eighteen days after injection, the tumors formed in empty vector group were substantially bigger than those in the pCDNA-MEG3 group”. To emphasize the effect of MEG3 up-regulation, I suggest the authors write “Eighteen days after injection, the tumors formed in pCDNA-MEG3 group were substantially smaller than those in the empty vector group”.

We thank you to raise this issue, and according to your valuable suggestion, we have checked and modified it.
To Reviewer: Paolo Gandellini

We are truly grateful to your critical comments and thoughtful suggestions. Based on these comments and suggestions, we have tried our best to revise and improve the manuscript and made great changes in the manuscript. All changes made to the text are in red color. We hope the new manuscript will meet your standard. Below you will find our point-by-point responses to your comments/questions:

Figure 1: MEG3 expression data have been reported for each tumor as normalized to the matched normal sample. This is suitable to present the data as in panel A, where the authors want to highlight the general down-modulation of MEG3 in tumors. However, it would be informative to report the expression of MEG3 also for normal samples. To this purpose the authors may choose one sample (i.e., one among the normal samples) as a calibrator to assess the expression of MEG3 for all individual normal and tumor specimens. This would actually add valuable information about the distribution of MEG3 expression across samples. In addition, it is my opinion that tumor/normal ratios are not suitable to be used for the analyses shown in panels B, C and D, where expression in tumor samples (not ratios) could be more appropriate.

We thank you to raise this important issue, we have also carefully thought about this suggestion; however, to avoid the impact of individual difference on data represent, the MEG3 expression data was represented in each tumor as normalized to matched normal tissues may be better.

2) Figure 2: concentrations of 5-aza are 5 and 10 uM (as in the graph) or 2 and 5 uM (as in the text)?

We are sorry for the mistake, and we have checked and modified it.

Figure 3: panels C and D are inverted in the legend, please correct. Please add standard deviations and p-values to percentages of cells in the different cell cycle phases.

We are sorry for the misunderstanding due to unclear descriptions in the previous manuscript. We have checked and modified it.

legend to fig.3: specify that the graph in panel A is the result of RT-PCR for MEG3 upon transfection.
correct for typing mistakes
methods: insert the country for Sigma products
We are sorry for the misunderstanding due to unclear descriptions in the previous manuscript. We have checked and modified it. And we have carefully edited the mispelling and typing errors and tried our best to improve the quality of English. We hope the new manuscript will meet your standard.