Reviewer's report

Title: MEG3 promotes proliferation and inhibits apoptosis in osteoarthritis chondrocytes by miR-361-5p/FOXO1 axis

Version: 2 Date: 21 Sep 2019

Reviewer: Shantibhusan Senapati

Reviewer's report:

General comment: In the current manuscript entitled "MEG3 inhibits proliferation and promote apoptosis in osteoarthritis chondrocytes by miR-361-5p/wnt/β-catenin axis" the authors have shown the possible role of MEG3 in osteoarthritis (OA). The authors tried to establish a miRNA mediated pathway of MEG3 in OA disease progression using in vitro and in vivo model. The article is quite interesting but clarification to the below mentioned points is indispensable to accept the hypothesis.

Minor comments: 1. The manuscript need to be thoroughly read by the authors to correct the typo errors and sentences. e.g. "proved to participated", "closed associated", "MEG3 3'UTR containing miR-361-5p was synthesized", "followed by Kit (Promega….) detection", "100ul/50mL", "was competitive binding", "rats was". What is NA3.1-NC? 2. The materials and methods section needed to be clearly written. Which platform was used for qPCR assays? Why DAB is used as a detection method for western blots. What kind of secondary antibody is used? In the OA rat model, how the authors have ensured the delivery of si-NC or si-MEG3 into the cells/chondrocytes.

3. The quantification graphs of Figure 1F and 1G were interchanged and need to be rectified.

4. The authors have mentioned that the proliferation is reduced upon MEG3 over-expression. The authors have checked for the Ki67 and PCNA level as proliferation marker and found increased upon siMEG3 treatment in Fig 1E; however the statement in text is inconsistent with the Fig1E.

5. The authors have checked different molecules in rat OA model, but it was unclear that how the siMEG3 and siNC were delivered into the chondrocytes and the MEG3 level. Please clarify the source of protein isolated in rat OA model that is used for western blots (whole tissue/chondrocytes isolated from the tissue).

6. Please correct the figure legends of Fig 4A, 5C and 5D.

Major comments: 1. In comparison to OA patients how the control group were selected for the whole experiment, please explain. Were the samples age and sex matched?

2. The authors started with the fact that the lower expression level of MEG3 is associated with OA (Fig1A), and contrastingly, further mechanistic study revealed that if we reduce the level of MEG3 in chondrocytes, the condrocytes survive better, proliferate and helps in maintaining the bone marrow matrix proteins and prevents from degeneration. Please explain these two contrasting evidences that was presented in this manuscript.

3. The authors have claimed MEG3 to be an apoptosis inducing molecule, however upon MEG3 overexpression the anti-apoptotic protein BCL2 went up instead apoptotic protein (BAX). The authors should have checked more apoptosis related molecule to draw any conclusion regarding the role of MEG3 in inducing apoptosis. Please check the blots of Fig1G and its corresponding quantification.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.
Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

No

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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