Author’s response to reviews

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

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MGNM-D-19-00237R2MEG3 inhibits proliferation and promote apoptosis in osteoarthritis chondrocytes by miR-361-5p/wnt/β-catenin axis.Anying Wang; Naixia Hu; Yefeng Zhang; Yuanzhen Chen; Changhui Su; Yao Lv; Yong ShenBMC Medical GenomicsDear Editor,On behalf of my co-authors, we thank you very much for giving us an opportunity to revise our manuscript, we appreciate editor and reviewers very much for their positive and constructive comments and suggestions on our manuscript.We have studied reviewer’s comments carefully and have made revision which marked in red in the paper. We have tried our best to revise our manuscript according to the comments. Due to there is a controversy in Wnt/β-catenin pathway and MEG3, we changed the direction and explored the MEG3/miR-361-5p/FOXO1 axis to replace the Wnt/β-catenin pathway. Attached please find the revised version, which we would like to submit for your kind consideration.We would like to express our great appreciation to you and reviewers for comments on our paper. Looking forward to hearing from you. Thank you and best regards.Yours sincerely, Yong Shen.Reviewer reports:Shantibhusan Senapati (Reviewer 1): 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",
"100µl/50mL", "was competitive binding", "rats was". What is NA3.1-NC? Answer: We are very sorry for incorrect expressions in this article. Based on your suggestion, we checked it and corrected the corresponding mistakes. The pcDNA3.1-NC means an empty vector, which was regarded as the control; the experimental group was transfected with pcDNA3.1-MEG3.2. 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. Answer: The section of Materials and methods has been corrected in according to your comments. RT-PCR was performed on the ABI7500 platform. Similar to ECL, DAB is also a commonly used protein coloring reagent. Due to its high specificity, we selected DAB in this present study. The secondary antibody used in this study was HRP-conjugated secondary antibody. To ensure the delivery of indicated agents into the cells/chondrocytes, we performed qRT-PCR analysis, such as Figure.1B and Figure.6A.3. The quantification graphs of Figure 1F and 1G were interchanged and need to be rectified. Answer: Thank you for your review. We have changed the Figure 1F and 1G in the revised version. 4. The authors have mentioned that the proliferation is reduced upon MEG3 overexpression. 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. Answer: We are very sorry for our negligence of results you mentioned. In fact, our figures were mislabeled, so that the description in the text was also incorrect. According to our original results, MEG3 promoted cell proliferation and the expression levels of Ki67 and PCNA were significantly increased due to the overexpression of MEG3. Corresponding descriptions have been corrected in the revision. We are very grateful if you can forgive us for our mistakes. 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). Answer: The “OA rat model construction” section have showed that how the siMEG3 and siNC were delivered into the chondrocytes: “One week after the operation, si-NC and si-MEG3 (1×109 PFU, 20 µl) were injected into the knee joint of the recipient rat (n = 6 for each group, 20 µL per joint) twice a week for 4 weeks. Eight weeks after the operation, the rats were sacrificed with cervical dislocation method (external force dislocated the cervical vertebra of rat and disconnects the spinal cord from the cerebrospinal cord), and then the knee joints were harvested.” The MEG3 levels were also showed in Figure.1B and Figure.6A. The chondrocytes isolated from the tissue were used to provide the underlying proteins and corrections have been made in the “Western blotting” section. 6. Please correct the figure legends of Fig 4A, 5C and 5D. Answer: We have made correction according to your comments. 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? Answer: The control group was collected from 20 patients who had not undergone OA or RA (rheumatoid arthritis). There was no statistical difference in age and sex, and the age and sex of all samples were matched. Corrections have been made in the revised version. 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. Answer: It is really true as you suggested that the previous version of this article showed the contradictory results. We are