Author’s response to reviews

Title: WIF1 enhanced dentinogenic differentiation in stem cells from apical papilla

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Author’s response to reviews:

Editor Comments:

1. Under the 'Funding' declaration please state the role of the funding body in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript. If the funding body played no such role please state this.

Response: Thank you for this suggestion. In the revised manuscript, we state the role of the funding body under the 'Funding declaration'.

2. For the 'Authors' contributions' declaration please write the authors as initials as opposed to full names (e.g. Haifeng Wang to HW)

Response: Thank you for this suggestion. In the revised manuscript, we write the authors as initials as opposed to full names under the Authors contributions declaration.

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Reviewer reports:

Oriana Trubiani (Reviewer 1): Authors should include the Approval number of Ethical committee in the materials and methods section.
Response: Thank you for this suggestion. In the manuscript, we revised Ethical committee agreement (EthicalCommittee Agreement, Beijing Stomatological Hospital Ethics Review No. 2011-02) in Materials and Methods section. All research involving human stem cells comply with the ISSCR “Guidelines for the Conduct of Human Embryonic Stem Cell Research”.

Shaomian Yao (Reviewer 2): This manuscript reports expression of Wnt inhibitory factor 1 (WIF1) in Stem cells from apical papilla (SCAPs) and its role in dentinogenesis of SCAPs by overexpression of the gene. In vitro and in vivo experiments were performed. The results are of interest for the field. The manuscript needs major revision for publication. Specific comments are as follows:

Abstract:

Lines 17-19: Revise the sentence. "---to investigate the function of WIF1 for ---"

Response: Thank you for this suggestion. In the manuscript, we revised the sentence, "In present study, we used SCAPs to investigate the function of WIF1 for dentinogenic differentiation."

Lines 22-32: Methods of the abstract is incomplete.

Methods:

Lines 39-42: This paragraph is redundant as the information are provided in the next paragraph regarding human subjects.

Under "Alkaline Phosphatase and Alizarin Red Detection". The authors described "The final calcium level in each group was normalized to the total protein concentration -------". There is no description about protein collection and quantification in the Methods. Please provide the description.

Response: Thank you for this suggestion. In the manuscript, we revised description about protein collection and quantification in the Methods.

Under "Transplantation in nude mice". Was any treatment to induce dentinogenesis applied after transplantation? If not, how could the SCAPs spontaneously undergo dentinogenesis after transplantation? Did the cells undergo other types of differentiation after transplantation?

Response: Thank you for this suggestion. There was no treatment to induce dentinogenesis applied after transplantation.
Our nude mice transplantation experiments demonstrated that newly formed bone/dentin-like tissues were derived from transplanted SCAPs-Vector and SCAPs-WIF1 cells and revealed that WIF1 also promoted osteo/dentinogenesis in vivo. These results indicated WIF1 enhanced osteo/dentinogenic differentiation in SCAPs. In order to clarify the role of WIF1 in dentinogenic differentiation, we further investigated the dentinogenic differentiation indicators. DSPP and DMP1 are classic odontogenic markers, DSPP is a key gene in the process of dentin formation, and DMP1 can regulate the DSPP. We found the expressions of DSPP and DMP1 were also enhanced by WIF1 in SCAPs by in vitro study. And in transplanted tissues, more DSPP protein was found in SCAPs-WIF1 group.

The reason of SCAPs spontaneously undergo dentinogenesis after transplantation was not clear.

Results and Discussion:

Fig 1B: What means 0W (0 week)? Does it mean the control without subjecting to differentiation induction? The method for the in vitro experiment of dentinogenic differentiation should be provided in the Methods Section.

Response: Thank you for this suggestion. 0W (0 week) means the control without subjecting to differentiation induction.

Cell differentiation assay in vitro

Osteo/dentinogenesis differentiation assays were performed as previously reported [4]. The gene expression of DSPP、DMP1、OSX and Runx2 was assayed by real-time RT PCR.

Under "WIF1 enhanced dentinogenic differentiation potential of SCAPs in vitro". The Authors state "---the expression of RUNX2 was not significantly different (data not shown)". It would be interested to present the RUNX2 data as it is as important gene in stem cell differentiation. This results of no significant change (SCAP-vector vs SCAP-WIF1) should be discussed.

Response: Thank you for this suggestion. In the revised manuscript, we have show the data.

Over-expression of WIF1 the expressions of RUNX2 was not significantly different. Real-time RT-PCR results showed that the expressions of RUNX2 was not significantly different in WIF1 over-expressed SCAPs compared to control group. GAPDH was used as an internal control. Student’s t test was performed to determine statistical significance.
Response: Thank you for this suggestion. In the revised manuscript, we discuss the results of the expression of RUNX2 no significant change in the discussion section.

Otherwise, we found that the transcription factor RUNX2, the mRNA expression levels of RUNX2 was not significantly different in SCAP-WIF1 cells than inSCAP-Vector cells (data not shown), Han’s in vitro study findings indicated that Wnt/β—catenin could enhance DPSCs cell dentinogenic differentiation by activating RUNX2.

Fig 4. More specific bone/dentin staining other than H&E would strengthen the result.

Response: Thank you for this suggestion. In the study we have done the immunohistochemistry staining to detect bone/dentin marker- DSPP protein.

Fig 4C. The difference in immunohistochemistry staining of DSPP is not very obvious. A negative control staining without primary antibody should be included to make sure no non-specific staining present.

Response: Thank you for this suggestion. In the revised manuscript, we have show the negative control staining without primary antibody.