Dear Prof. Ying Dou and Reviewers:

Thank you for your letter and the four reviewers' comments concerning our manuscript entitled "An overview of noncoding RNAs involved in the osteogenic differentiation of periodontal ligament stem cells" (No.: **53645**). Those comments are valuable and very helpful for revising and improving our paper. We have studied comments carefully and have made correction in modified version. Revised portion are highlighted in yellow in the manuscript. The main corrections in the paper and the responds to the reviewer's comments are as following:

Reviewer ID: 02446223

Conclusion: Accept (General priority)

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Specific Comments To Authors: The present review gives a comprehensive overview of noncoding RNA, in particular those involved in some way with osteogenic differentiation of a particular class of stem cells, the periodontal ligaments derived stem cells. The review is very well written and exhaustive, with tables and figures which help the reader to understand and outline the argument. Both title and abstract fully reflect the manuscript subject.

Response:

Thank you for your positive review.

Reviewer ID: 02446120

Conclusion: Accept (General priority)

Scientific Quality: Grade B (Very good)

Language Quality: Grade A (Priority publishing)

Specific Comments To Authors: Comments to the authors The review by Qiu W et al. focusses on the role of non-coding RNA (ncRNA) on regeneration of periodontium during periodontal diseases. So far, little is known about the functions of ncRNA and even less about their roles during regenerative processes of periodontium tissues. The finding (by Seo et al.) that there are multipotent stem cells in human PDL (PDLSCs) opens interesting possibilities for repairing these dental tissues. However, up to now, it is unclear how to guide stem cells toward

and specific differentiation pathway. The work by Qiu et al, covers these issues gathering information, which is useful, not only for osteogenic differentiation in dental tissues but also for many other regenerative processes during diseases. The authors revise the general classification of ncRNA including long ncRNA and small RNA, circular RNA, which is very useful for the general readers, and then they focus on miRNAs, lncRNAs, and circRNAs which have a critical role in osteogenic differentiation. The authors added information gathered from their own laboratory: they highlight the changes in many miRNAs, some of which were increased while others were downregulated in PDLSCs during osteogenic induction. The authors concluded that miRNA may play an important regulatory role in osteogenic differentiation of PDLSCs. Also, the review highlights lncRNAs involved in osteogenic differentiation of pdlscs and transcriptional and Posttranscriptional regulation of lncRNAs in PDLSCs and Epigenetic regulation of lncRNAs in PDLSCs. The information is clearly exposed, the bibliography is helpful and extensive. In general, the review is excellent and provides useful and updated information. Minor recommendations. In my opinion the paragraph referring to the history of PDLSCS could be significantly shortened.

Response:

Thanks for your suggestion. According to your recommendation, we have shortened the content in the section of “HISTORY OF PDLSCS” in the revised manuscript. Please check the revised manuscript. Thanks.

Revised content is as follows:

HISTORY OF PDLSCS

The progenitor cells residing within the PDL (periodontal ligament progenitor, PDLPs) were first described in seminal studies by Melcher in 1994[26]. Seo et al described the identification and characterization of multipotent stem cells in human PDL in 2004, although these cells had been suspected to be present in the PDL for a long time[11]. Nevertheless, there is no uniform standard for defining the features of PDLSCs. Often, reports suggest that the isolation of particular subsets of cells from bulk explant cultures is far less rigorous and was too liberal for the use of the term PDLSC[27]. Prateeptongkum et al[28] reported that the isolation methods of PDLPs and PDLSCs from PDL tissues are different and demonstrated that PDLPs could be isolated using outgrowth methods, while PDLSCs need single-cell isolation methods for isolation. PDLSCs can be further characterized by their cell surface

expression of CD29, CD44, STRO-1, STRO-4, CD146, CD73, CD90, CD105 and CD166 and the lack of expression of endothelial (CD31), haematopoietic (CD14, CD34, CD45, and CD79a), and helper immune antigens (HLA-DR, CD40, CD54, CD80, and CD86)[10,29]. Functionally, PDLSCs have been determined to fulfil all of the criteria of identifiable MSC-like properties, including self-renewal capacity, multipotency in vitro, tissue regenerative capacity in vivo, and immunomodulation[30,31]. These processes are illustrated in Figure 1.

Reviewer ID: 03811054

Conclusion: Minor revision

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Specific Comments To Authors: There are minor grammatical errors

Response:

Thanks for your suggestion. According to your instruction, we have modified those grammatical errors carefully in the revised manuscript. In addition, we have asked American Journal Experts to check the English again (please see the certification below). We hope that the language is now acceptable for the next review process. Please check the revised manuscript. Thanks.

Reviewer ID: 02537336

Conclusion: Minor revision

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Specific Comments To Authors: This is a comprehensive review on the role of ncRNAs in the osteogenic differentiation of periodontal ligament stem cells. The manuscript contains useful information that may be helpful to the researchers in the field of periodontal diseases, particularly for researchers that are interested in periodontium regeneration.

However, the manuscript would improved if the following revisions can be made:

1. The language should be thoroughly revised by a native English speaker or a professional language service;

Response:

Thanks for your suggestion. We have asked American Journal Experts to check the English again (please see the certification below). We hope that the language is now acceptable for the next review process. Please check the revised manuscript. Thanks.

AJE Editing Certificate

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An overview of noncoding RNAs involved in the osteogenic differentiation of periodontal ligament stem cells

prepared by the authors

WEIQIU, BULING WU, FUCHUN FANG

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2. Tables 1-2 are too busy. The authors should use landscape; the contents are too much, should be made more concise;

Response:

Thanks for your suggestion. According to your instruction, we have deleted the information that is not the most important in Table 1 and 2 to make them concise in the revised manuscript. Please check the revised manuscript. Thanks.

3. Similarly, Figures 1, 2 are too busy. The authors may consider using a particular ncRNA to illustrate the regulatory mechanisms of ncRNAs.

Response:

Thanks for your suggestion. According to your instruction, we have deleted section B and relabeled figure 1 to make it concise in the revised manuscript. About figure 2, we enumerated all ncRNAs and their direct target molecules mentioned in this paper in this figure, so that

readers can clearly understand the regulatory mechanism of different ncRNAs during the osteogenic differentiation of PDLSCs. And according to your suggestion, we summarize that the action of ncRNAs is complementary base pairing in cytoplasm, while direct interacting with subunits of target proteins in nucleus in figure 2. Meanwhile, we have complemented the detailed explanation about the regulatory mechanism of ncRNAs in the legend of figure 2. Please check the revised manuscript. Thanks.

4. Fig. 3 should pinpoint a few biological consequences of the network, rather than just listing the possible pathways;

Response:

Thanks for your suggestion. Compared with the specific regulatory mechanism of miRNA, the action of lncRNA and circRNA is complex. Therefore, in figure 3, we focus on the signaling pathway of lncRNAs and circRNAs involved in osteogenesis of PDLSCs. We orderly list the core target miRNAs, genes, biomarkers and signaling pathways associated with lncRNAs and circRNAs during the process of osteogenic differentiation in PDLSCs referring to the format of Kyoto Encyclopedia of Genes and Genomes (KEGG) and the publication in **MOLECULAR CANCER** to make the figure concise and readable. And according to your suggestion, we have complemented the detailed explanation about the regulatory molecules involved in the pathway in the legend of figure 3. Please check the revised manuscript. Thanks.

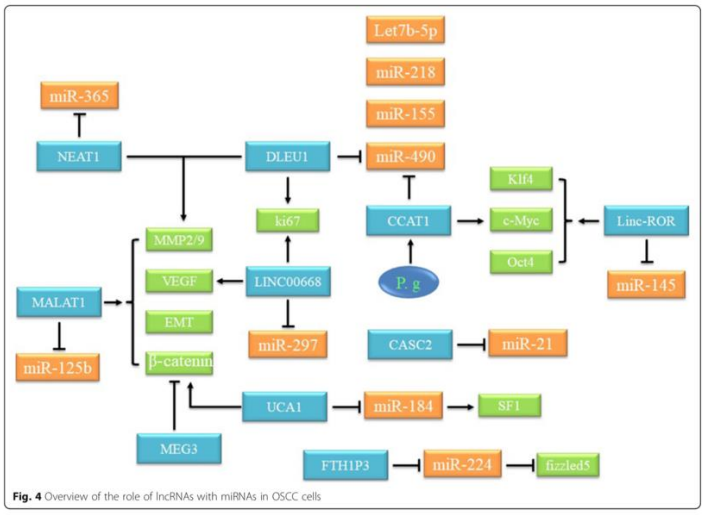

Fig. 4 Overview of the role of lncRNAs with miRNAs in OSCC cells

Reference Figure

(Zhang L, et al. Long non-coding RNAs in oral squamous cell carcinoma: biologic function, mechanisms and clinical implications. **Mol Cancer**. **2019** May 27;18(1):102.)

5. A summary digram illustrating how some of the key ncRNAs can be used to regulate the PDLSCs and then be used in the regeneration of periodontium should be provided.

Response:

Thanks for your suggestion. According to your instruction, we have drawn a schematic diagram to illustrate ncRNAs genetic modification-based stem cell transplantation therapy for the tissue regeneration of periodontal disease. However, most current studies of ncRNAs involved in PDLSCs focused on the cell level in vitro, in vivo experiments associated with stem cell transplantation used in the regeneration of periodontium need further in-depth exploration. So, there is no identified ncRNAs mentioned in the schematic diagram. Please check the revised manuscript and figure 4. Thanks.

Many thanks for your efforts to let us improve our manuscript. If you have any further questions about our manuscript, please contact us. We are waiting for your further messages.

Best Regards.

Sincerely,

A/Prof. Fuchun Fang

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