

## Response to the Reviewers Letter

Dear Editors and Reviewers:

Thank you for the comments concerning our manuscript entitled “Long non-coding RNA: The Functional Regulator of Mesenchymal Stem Cells” (Manuscript Number: 45695). These comments were valuable and very helpful for improving our manuscript to better convey the significance of our research. We have carefully reviewed all the comments and completed point-by-point revisions. **The responses to the comments in this letter and the revised portions of the manuscript are marked in red.** We appreciate the work of the Editors and Reviewers, and we hope that the revisions will meet with their approval. We are happy to respond to any further comments that you may have.

Yours sincerely,

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## Responses to Reviewer 1

Reviewer's comments:

It is very nicely written review. The topic is actual and this review will serve as good guide for future research. I have only some minor comments (see below) Abstract I suggest substituting “Living in various tissues” by “residing in various tissues” Main Text Some abbreviations are not explained in the text, e.g. DANCR, BDNF-AS, VEGF etc The nature of some cell lines mentioned in the review must be explained (e.g. C2C12 cells)

Response: Thank you very much for your helpful comment. We have revised the abstract and added a list of abbreviations at the end of our manuscript. Besides, explanations of cell lines were added in the manuscript.

## Responses to Reviewer 2

Reviewer's comments:

The review by Zhong-Yu and coll. is an extensive analysis of the putative role of long non-coding RNA into MSC functional regulation. The title and the Abstract well reflect and summarize the paper's content. Despite the review is well organized, it has the fault to be really too long-winded and rambling, thus becoming difficult to be read. The revision of the text, an the effort to make it more concise should be made. In addition, it would be helpful the introduction of diagrams to summarize the arguments. In addition, there are some mistakes: the version I received contained the "revision", with the comments of the authors! and there was not any tracks of Figures 1 and 2 into the text, they were only present at the end of the paper.

Response: Thank you very much for your helpful comment. According to the comments, we revised our manuscript with the help of a native English speaker from AJE, which making our paper easier to read. Besides, the track of Figure 1 and 2 were also added in the text.

### **Responses to Reviewer 3**

Reviewer's comments:

Authors have written an extensive review on the potential differentiation functions and immunoregulatory abilities of mesenchymal stem cells (MSCs). They further highlight the critical role of LncRNA as a functional regulator of MSCs and how their modulation could guide the directional differentiation of MSCs into specific cells and thus can greatly improve the clinical use of MSCs in the diagnosis and treatment of diseases. This paper although provides a detailed description, nevertheless, limitations of the study can be further elaborated. Typological errors are to be taken care of at certain places.

**Response:** Thank you very much for your helpful comment. We have revised the part of conclusion and perspective. Besides, typological errors were carefully checked and revised.

## Responses to Reviewer 4

Reviewer's comments:

In this review article, the Authors aim at presenting an overview on the role of Long non-coding RNAs (LncRNAs) in the regulation of MSC dynamics. This is one of most promising area of inquiry recently emerging in biological studies, holding promise for unprecedented deployment within the context of stem cell patterning, regenerative medicine and precision medicine. The Authors have provided a nice and updated dissection of the current knowledge on LncRNAs in many interconnected fields of MSC biology. They have carefully highlighted the actual understanding of LncRNA-mediated patterning in the establishment of MSC multilineage potential. An accurate description of the current lack of knowledge in multiple hot fields is also provided, such as the needs for understanding the mechanisms of LncRNA in cardiogenesis, skeletal myogenesis, neurogenesis. The involvement of altered LncRNA dynamics in the onset and progression of pathological conditions (i.e. autoimmune diseases, neurodegenerative diseases, and cancer) is also carefully presented. On the whole, I have appreciated the overall consideration of LncRNAs within a context of "flexible" modulation of cellular functions, capable of generating "pockets of information", rather than considering these molecules as activators and inhibitors of MSC functions. Minor points: The Authors refers to LncRNA sometimes using the "singular", other times the "plural". It will be more consistent always considering them as LncRNAs (plural). The Authors initially consider MSCs as pluripotent elements, and then refer to them as multipotent stem cells. Referring to

MSCs as multipotent elements throughout the manuscript would be more appropriate.

Response: Thank you very much for your helpful comment. We have revised the MSCs as the multipotent elements throughout the manuscript according to this comment.

## **Responses to Reviewer 5**

Reviewer's comments:

The article from Zhong-Yu Xie et al constitutes a detailed summary of the role of different Long non-coding RNAs (lncRNAs) in osteogenic, adipogenic and chondrogenic tissue differentiation from mesenchymal stem cells (MSCs), as well as of the role of these molecules in disease, diagnosis and treatment. This work will be a useful reference for the scientific community working in lncRNAs and MSCs. The sections of the manuscript are well organized and the figures presented are relevant. However, there are a number of issues that do not allow me to recommend the publication of the manuscript in its current form. Major points. 1.- Currently, the main point precluding the publication of this manuscript is the use of the English language. Although no spelling mistakes have been detected by this reviewer, numerous expressions/phrases should be checked for meaning and syntax since some sentences, are not readable and highly difficult to understand (i.e. "These studies confirm that the differentially expressed LncRNA in the culture materials to mediate the osteogenic differentiation of MSCs" or "On day 14 of induction as the chondrogenic phase....."). The English language in the text would undoubtedly

benefit from improvement, for clarity and readability. I recommend that the text is revised a colleague (a scientist) whose native language is English.

**Response:** According to the comments, we revised our manuscript with the help of a native English speaker from AJE, which making our paper easier to read.

2.- A figure depicting the different mechanism of gene expression modulation by nuclear and cytoplasmic lncRNAs would certainly improve the manuscript.

**Response:** We surely agree with this comment. However, figure about the regulatory mechanism about nuclear and cytoplasmic lncRNAs have been showed in many other reviews. Besides, this manuscript mainly focuses on the lncRNA in regulating the MSC function. Therefore, we showed a figure about this topic rather than the mechanism of nuclear and cytoplasmic lncRNAs.

3.- In the 'Long non-coding RNA section'; the way of action of nuclear lncRNAs on the enhancer and/or promoter regions of nearby genes should be explained further.

**Response:** Thank you for this comment. The sentence have been revised into "Moreover, the location and transcriptional status of other nuclear lncRNAs will directly affect their nearby or partial overlapping genes expression mainly through bringing transcriptional enhancers closer to the promoters of the specific gene."

4.- When talking about immortalized MSCs vs non-immortalized MSCs in the 'Osteogenic differentiation' section, a brief explanation of the immortalization method used, would help to underscore the importance of analysing non-immortalized MSCs

**Response:** Thank you for this comment. An explanation of immortalized MSCs has

been added in the paper.

5.- The authors use in the text the words non-coding gene. This is drastically wrong and goes against the definition of gene. Please, avoid the use of this expression.

**Response: Thank you for this comment. We replace it as non-coding DNA.**

6.- In the 'Osteogenic differentiation' section the authors refer to ceRNA without having stated the meaning of this word previously. When a new word or gene, or lncRNA is cited for the first time, the complete name should follow the abbreviation in brackets.

**Response: Thank you very much. CeRNA means competitive endogenous RNA, and we have mentioned in the manuscript. Besides, an abbreviation has been added.**

7.- Throughout the text, the authors give or cite some examples of lncRNA regulation of cell differentiation processes through the modulation of epigenetic mechanisms, mainly histone acetylation. A brief explanation of the mechanism by which lncRNAs could regulate histone acetylation should be given. This would certainly improve the manuscript.

**Response: In the lncRNA section, we have mentioned about the nuclear lncRNA and their mechanism, including histone modification mechanism. The nuclear lncRNA could regulate gene expression through histone acetylation mechanism. Besides, our study about lncRNA-OG to affect histone acetylation also showed in our manuscript.**

8.- When citing works carrying out analysis of lncRNA expression it is necessary to clarify the organism in which those works have been performed (human/mouse) and the technique used (microarray/RNA sequencing) for clarity.

Response: In this manuscript, most studies were performed using human cell and RNA sequencing. Those researches using mouse cells or detected by microarray have been stated in our paper.

9.- Please, give a brief explanation of the regulation of gene expression mediated by the formation of RNA-DNA-DNA triplets (Reference 48).

Response: Thank you for this help comment. Considering that the RNA-DNA-DNA triplet has less relationship with our manuscript, we choose to delete this sentence to avoiding confusing the readers.

10.- The distinct role of a given lncRNA (i.e. lncRNA-MEG3) in MSCs of different origin (adipose or bone marrow tissue) should be explained further, since this is a crucial point to understand the nature of lncRNA regulation of gene expression.

Response: Actually, although many studies have reported that a lncRNA exhibited different functions in MSCs from different origin, the reason of this phenomenon and their detailed mechanism are still unclear. It is of great importance to study this topic in the future, and we have mention this point in the perspective part.

Minor points

1.- Check punctuation, spacing and spelling throughout the text.

Response: The punctuation, spacing and spelling have been carefully checked in our manuscript.

2.- Avoid the use of contractions (lncRNA's wide involvement in all aspects of cells "

Response: We revise this sentence into wide involvement of lncRNAs

3.- Use lncRNAs as plural of lncRNA.

Response: Revision have been made about this comment.

## Responses to Reviewer 6

Reviewer's comments:

Comments to authors: Nice and easy to follow manuscript and relevant to the field of utilizing Long non-coding RNA for the functional regulator of Mesenchymal stem cells. The review showed powerful capacities allow of MSCs and extensive participation of LncRNA in cell growth and development progressing fate. Then, it also elaborated the role of LncRNA in regulating the function of MSCs, and discussed their participation in the pathogenesis of diseases and clinical use in diagnosis and treatment. Overall, the topic of this review is comprehensive and important and some viewpoints are also interesting. However, there are some points need to be addressed.

1. English writing of this paper need to be much more precisely and some expressions should be checked. e.g. for later phase of adipogenic differentiation should be late phase in the part of Adipogenic Differentiation; deeper understand in depth the occurrence and development of diseases should be canceled with deeper or in derth in the last sentence of second paragraph of LncRNA of MSCs in Diseases Diagnosis and Treatment .

Response: According to the comments, we revised our manuscript with the help of a

native English speaker to improve the expression and avoid grammatical mistakes.

2. In the Abstract, on the basis of summarizing the full text, the author should describe the abstract more accurately and strengthen the logic line of the whole part, please. You need to describe what kind of ideas you want to express and how to support them, instead of only generalizing the two concepts of MSC and LncRNA and combine them directly in this part. And in this part, the author showed illuminating the functional mechanism of MSCs will help to improve their curative effect and promoting their clinical use; in my opinion, the author should clarify what aspect of the functional mechanism of MSC.

Response: Thank you very much for your helpful comment. Due to the word limitation (200 words), in the abstract we could only emphasize on two key centres, MSCs and LncRNA, of our manuscript. We greatly agree with the reviewer's comment, but we can only detailedly discussed this points in our manuscript.

3. In the Mesenchymal Stem Cells, the author showed benefiting from the self-renewal and low immunogenicity characteristics, MSCs with these powerful capacities have been widely used in clinical practice, but the above mentioned in this section is immunoregulation and multilineage differentiation. Also, in this part, the author showed illuminating the mechanism of transcriptional regulation of MSCs could not only improve their curative effect but also elucidate the pathogenesis of the diseases; but, the general order should be illuminating the mechanism of transcriptional regulation of MSCs could not only elucidate the pathogenesis of the diseases but also improve their curative effect.

Response: Thank you very much for your helpful comment. We have revised the manuscript about the order of this sentence. The points about disease pathogenesis or treatment have been discussed in the part of LncRNA of MSCs in Diseases Diagnosis and Treatment.

4. In the Figure 1, the author should add the information of other Aspects of MSCs Functions at least containing Neurogenesis, Myogenesis and Endothelial Differentiation rather than just tri-lineage differentiation because they also were important compositions for your article story.

Response: Considering that tri-lineage differentiation was the mainly function of MSCs and the controversy still exist about neurogenesis, myogenesis and endothelial differentiation, we decided to show the figure only containing tri-lineage differentiation ability. If the reviewer think that it is necessary, we will add these function into the figure 1.

5. We could not find the indication of Figure 1 and Figure 2 in the text, please add them to better understand the article for readers.

Response: The indication have been added in the manuscript.

## **Responses to Reviewer 7**

Reviewer's comments:

In this review, Zhong-Yu Xie and colleagues describe the role of lncRNAs in regulating different functions of MSCs and discuss their involvement in the

pathogenesis as well as clinical approach of diseases. The manuscript is well written and simple to understand but, sometimes, the English language should be improved. For example, the Authors write LncRNA both in the singular and in the plural and always they use capital letter. Maybe, they should write without capital letter and distinguish singular and plural form. Fifth line of par. Long non-coding RNA: the Authors state “Non-coding RNA is defined into two classes, the one less than 200nt including microRNA, small nuclear RNA and small interfering RNA and the other one larger than 200nt called LncRNA”. It would be better: “Based on their length, non-coding RNAs are discerned into two classes, the one includes molecules less longer than 200nt as microRNA, small nuclear RNA and small interfering RNA and the other one RNA longer than 200nt called lncRNA”. Throughout the manuscript, when the Authors introduce a molecule for the first time (ceRNA, lncRNA-HULC, lncRNA-HOTAIR and so on), they should specify the acronym and briefly describe it (where it is expressed, its most common role). In various paragraphs as “Adipogenic differentiation” and “Neurogenesis, Myogenesis and Endothelial differentiation”, the authors describe MEG3 and, in the first par. previously mentioned, they assert that: ”Therefore, we suggest that on one hand the difference in differentiation potential of MSCs from various sources may result from the expression and function of LncRNA, and on the other hand a same LncRNA could exhibit different functions in MSCs from various origin”. It is possible generalize the role of MEG3 and sustain that it functions as negative regulator in differentiation processes? In particular, in bone marrow mesenchymal stem cells that are stem cells but yet addressed to

osteogenic differentiation, MEG3 inhibits the osteogenesis and in human adipose-derived stem cells, yet addressed to adipose differentiation, MEG3 inhibits the adipogenesis. We can observe a similar situation in the endothelial differentiation of MSCs (ref 67), where the differentiation process is stimulated by VEGF and MEG3 is down-regulated. In sum, both when the tissue of belonging or the stimuli in the microenvironment address the cells to a precise differentiation, MEG3 function as suppressor. The authors should extensively discuss this point. The Title of paragraph: "Other aspects of MSCs function" is not appropriate because the review is focused on lncRNA. The Authors might change the title as follows "Other mechanisms regulated by lncRNAs in MSCs. Paragraph "LncRNA of MSCs in Diseases Diagnosis and Treatment". In the first part, the Authors should introduce the role or the putative role of lncRNAs in these pathologies, because the review is focused on lncRNAs and this paragraph too. In the last part of the same paragraph, from "So far, clinical research....." until "However, the clinical efficacy.....enhancing the capacities of MSCs." In contrast with the title of paragraph, the Authors discuss about MSCs and not lncRNA expressed in these cells.

**Response: Thank you very much for your helpful comment. Our manuscript has been revised again by a native English speaker. Besides, a list of abbreviation has been added at the end of our paper. The expression and description have been carefully checked, and several related revision were made.**