Reviewer 03671529

1. **The authors of the article discuss the role of methylation and demethylation in adipo-, chondro- and osteogenic differentiation of MSCs. Is there evidence of the role of methylation and demethylation in the differentiation of MSCs towards other cell types?**

Response: We appreciate the reviewer's question. We added a new paragraph named "**Myogenic differentiation associated with DNA demethylation**" in the manuscript. Cardiogenic differentiation is another important property of MSCs as stem cell therapy for cardiovascular diseases is now in clinical-trial[24]. Bhuvanalakshmi et al.[25] found that in differentiated cardiomyocytes from MSCs, 6 out of the 10 CpG islands of the promoter regions of *Nkx2.5*, the early cardiac gene, underwent demethylation. What's more, the CpG promoter demethylation of *sFRP4*, a Wnt antagonist, was also observed. This result is consistent with the previous findings that 5-Azacytidine treatment of BMMSCs inhibited the ventricular scar from thinning and expanding, and minimized left ventricular chamber dilatation thus improved myocardial function[26]. Antonitsis et al.[27] treated hBMMSCs with 5-Aza *in vitro* to induce them to differentiate towards a cardiomyogenic lineage. Nakatsuka et al.[28] also used 5-Aza to investigate the myogenic differentiation potential of mouse dental pulp stem cells (mDPSCs). DNA demethylation induced by 5-Aza and forced expression of Myod1 upregulated the muscle-specific transcriptional factors such as Myogenin and Pax7.

2. **One of the possible mechanisms of the immunomodulating activity of MSCs may be their effect on the population of organ macrophages. Is there evidence of the role of methylation and demethylation in the effect of MSCs on macrophages?**

Response: We appreciate the reviewer's concern. So far we haven't found paper about the role of methylation and demethylation in the effect of MSCs on macrophages. Most of the researches about DNA methylation and demethylation of MSCs focused on their effect on T cells, as we wrote in the manuscript. We agree with the reviewer's opinion and added the following sentence in the manuscript： "Nevertheless, further investigations are required to reveal whether the methylation of MSCs involved in

other immune cells regulation such as macrophages and NK cells and the underlying mechanisms."

Reviewer 00609434

The manuscript from Xin et al. is a review describing the importance of epigenetic regulation, in terms of DNA methylation/demethylation, in the differentiation capacity and immunomodulatory properties of mesenchymal stem cells (MSC) from various sources (i.e. adipose tissue, bone marrow). The manuscript is well written, although it could be better updated, anyway I find it worthy of publication although I would suggest to enhance the impact of the section describing adipogenic differentiation epigenetic changes in MSC which is really too short and poor of information. Here are some suggestions of papers that could be mentioned in the section, but of course there would be much more to be considered: Fujiki et al, BMC Biol, 7 (1), 2009: on the methylation and function of PPARgamma promoter during adipogenesis Melzner at al, JBC, 277 (47), 2002: on the role of Leptin demethylation and expression in adipogenic differentiation Barrand et al, BBRC, 391 (1), 2010: on the role of OCT4 regulation in adipogenesis Also the chondrogenic differentiation section could give a better description of the current literature.

Response: We appreciate the reviewer's suggestion. We found more literature and enhanced the impact of the description of adipogenic and chondrogenic differentiation according to the reviewer's suggestion. We added the following paragraph in the "Adipogenic differentiation of MSCs is related to DNA methylation and demethylation" section: Barrand *et al*[22] showed that in adipose mesenchymal stem cells, the promoter of *OCT4* was hypermethylated consistent with its repression. Melzner *et al*[23] found that the promoter of leptin underwent extremely demethylation(9.4%±4.4%) during the maturation of human preadipocytes toward terminally differentiated adipocytes. What's more, methyl-CpG binding proteins could bind to specific sites in the promoter and repressed leptin expression. Fujiki *et al*[24] reported that during the differentiation of 3T3-L1 preadipocytes to adipocytes, the hypermethylated PPARγ2 promoter was progressively demethylated, while

5-azacytidine could increase the expression of PPARγ2, indicating the methylation of its promoter inhibited the gene expression. We added the following paragraph in the "Chondrogenic differentiation is regulated by DNA methylation and demethylation" section: Lin *et al*[27] found that stepwise preconditioning–manipulated BMMSCs showed improved cell proliferation and chondrogenic differentiation potential *in vitro* and enhanced therapeutic effect on the progression of osteoarthritis *in vivo*, and one mechanism of that is the reduction in CpG methylation at the promoter of *Nanog* and *Oct4*. Pollock *et al*[28] demonstrated an experimental DMSO-free formulation which could improve post-thaw function of MSCs including chondrogenesis, as DMSO is a strong inducer of demethylation which may affect the potential of MSCs for therapeutic use in treatment of human diseases. These researches reminded us that epigenetic modification of MSCs could be a promising approach to improve their therapeutic effects.