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Can Upregulation of Pluripotency Genes Enhance Stemness of Mesenchymal Stem Cells?

Kejia Li[1] · Yangzi Jiang[2,3] · Zheng Yang[4] · Boon Chin Heng[5] · Hua Tian[6] · Zigang Ge[1]

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Dear editor,

Stem cells stand out among the diverse array of seed cells available for cell therapy because of their stemness, which encompasses self-renewal capacity and multilineage differentiation potential. Different stem cells possess varying stemness, with embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) having significantly higher degrees of stemness than mesenchymal stem cells (MSCs) derived from postnatal tissues. It is broadly accepted that stemness is critical for achieving regeneration and other therapeutic goals, while pluripotency genes emerge as the key parameters, as well as the major molecular targets for enhancing stemness. However, it is still controversial whether pluripotency genes can be reliable stemness markers of MSCs, similar to that of ESCs and iPSCs. In this letter, we first review how the pluripotency genes regulate the stemness of pluripotent stem cells. Then, we systematically examine the roles that pluripotency genes play in the regulation of stemness in MSCs, in comparison with their corresponding roles in pluripotent stem cells.

Octamer-binding transcription factor (Oct4), Sex-determining region Y-box 2 (Sox2) and Nanog are considered the most important pluripotency genes, as these are thought to be the key regulators of other genes that balance self-renewal and differentiation in stem cells, which work together with many other diverse transcriptional co-factors and chromatin regulators, from the expanded transcriptional regulatory network to maintain the pluripotency of pluripotent stem cells [1]. Oct4 and Sox2 can directly regulate Nanog expression by binding to the Nanog promoter, and are essential for maintaining the self-renewing and undifferentiated state of the inner cell mass of the blastocyst, embryonic stem cell lines and iPSCs [2]. Stem cell fate is controlled by these key differentiation and reprogramming factors. By changing the culture conditions and supplementing with growth/differentiation factors or the Yamanaka cocktail (including Sox2 and Oct4), researchers can either induce differentiation of ESCs/MSCs, or reprogram somatic cells into iPSCs (Fig. 1).

Expression levels of pluripotency genes are closely associated with stemness of MSCs. MSCs at early passages exhibit high proliferative capacity and differentiation potential with small size and spindle-like morphology, but they gradually become larger and flattened in morphology during a prolonged period of *in vitro* expansion, along with losing their stemness, and meanwhile, surprisingly, showing downregulation in the expression of pluripotency genes [3]. Interestingly, restoring Oct4/Nanog/Sox2 expression can efficiently recover the stemness of MSCs [4]. In our previous study, we found that nanosecond pulsed electric fields can enhance the expression of pluripotency genes as well as the differentiation potentials of MSCs [5]. Additionally, prolong nanosecond pulsed electric fields successfully maintained upregulation of pluripotency genes of MSCs for weeks [6]. There are many biological, chemical and physical methods to regulate the expression of pluripotency genes, thereby improving the stemness of MSCs (Fig. 1). These researches suggested that pluripotency genes Oct4, Sox2 and Nanog directly or indirectly regulate stemness of MSCs.

Despite that the aforementioned experiments in vitro support the relevance between pluripotency genes and

✉ Zigang Ge
gez@pku.edu.cn

[1] Department of Biomedical Engineering, College of Engineering, Peking University, Beijing 100871, China

[2] Institute for Tissue Engineering and Regenerative Medicine, School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong 999077, SAR, China

[3] School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong 999077, SAR, China

[4] Department of Orthopedic Surgery, School of Medicine, National University of Singapore, Singapore 119260, Singapore

[5] Peking University School of Stomatology, Beijing 100871, China

[6] Department of Orthopaedics, Peking University Third Hospital, Beijing 100191, China

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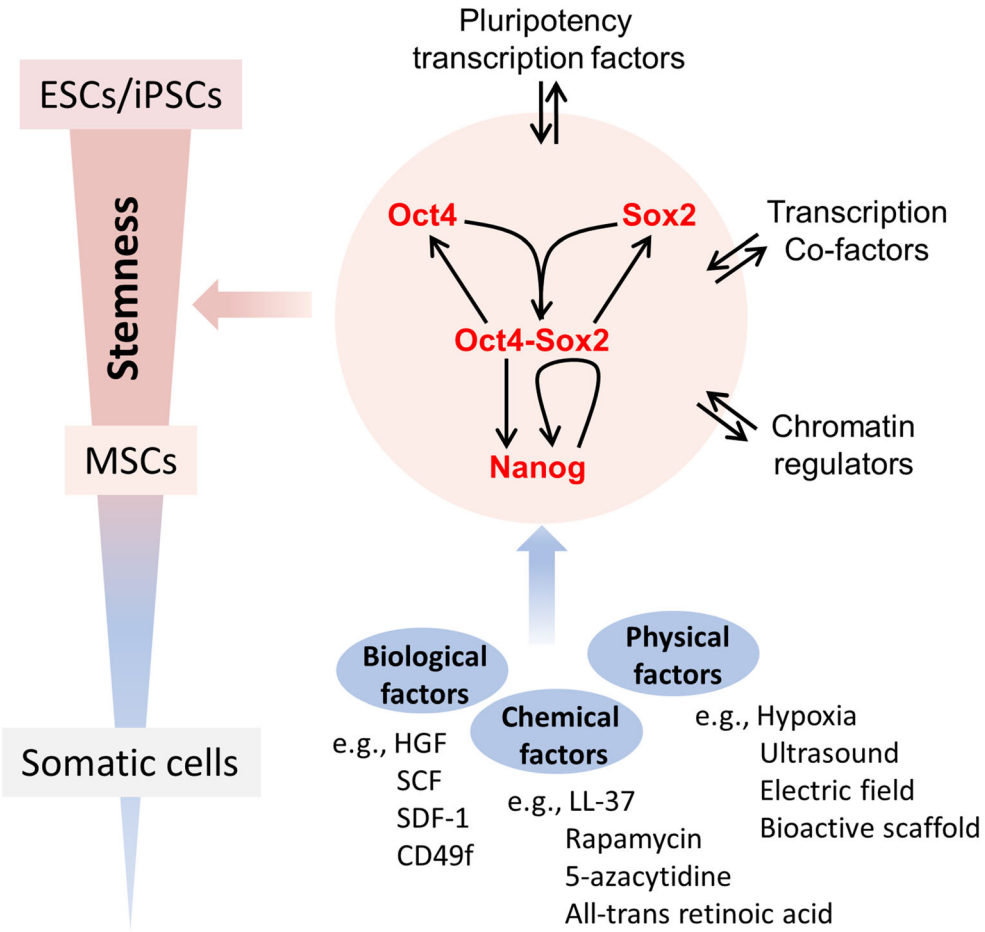

Fig. 1 Schematic illustration of the nexus of stemness maintenance by transcriptional regulation, epigenetic regulation, and signaling pathways. The expression of pluripotency genes can be regulated by many biological, chemical and physical factors. **ESCs**, embryonic stem cells; **iPSCs**, induced pluripotent stem cells; **MSCs**, mesenchymal stem cells; **Oct4**, octamer-binding transcription factor; **Sox2**, sex-determining region Y-box 2; **HGF**, hepatocyte growth factor; **SCF**, stem cell factor; **SDF-1**, stromal-derived factor-1

the stemness of MSCs, there exist some controversies. In addition to the works by Jauković et al. [4], Li et al. [5], and Chen et al. [6], Oct4 was found to interact with the Nanog and Sox2 genes and regulate a similar profile of target genes in MSCs as observed in ESCs, and Oct4 knockdown resulted in a marked decrease of Sox2 and Nanog expression at the mRNA and protein levels with loss of stemness [3]. However, another study by Lengner et al. found that the expression of Oct4 genes was not essential for MSCs multi-potency [7]. It was reported that the MSCs derived from mouse with bone marrow-specific ablation of Oct4 exhibited comparable proliferative capacity, osteogenic and chondrogenic differentiation potentials *in vitro*, to the MSCs derived from wild-type mouse [7]. Although Lengner et al. has opposed the role of Oct4 in MSCs, there is not enough experimental evidence to support this conclusion, as only colony-formation assay and staining for osteogenic and chondrogenic differentiation were performed. Besides, it is possible that Oct4 has other functions in MSCs that cannot be tested in the experiments. Because of the complicated interaction between Oct4, Nanog and Sox2, some other related pathways may compensate for the shortfall of Oct4. For example, the Yamanaka cocktail without Oct4 but remaining the three other factors, i.e., Sox2, Klf4, and cMyc (SKM), could generate mouse iPSCs with dramatically enhanced developmental potential [8], which means that Oct4 can actually be replaced by other factors under certain conditions.

Based on literature review and our previous studies, we concluded that Sox2/Oct4/Nanog can serve as markers of the stemness of MSCs, similar to ESCs, and that the expression of pluripotency genes can be manipulated for the enhancement of MSCs stemness. However, the definitive roles of pluripotency genes in MSCs need to be further elucidated with more studies of cellular and animal models of pluripotency gene knockout and knock-in.

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Boon Chin Heng: Manuscript writing.
Hua Tian: Manuscript writing.
Zigang Ge: Conception and design, manuscript writing, final approval of manuscript.

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Data Availability The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate All authors have approved the content of the submitted manuscript.

Consent to Publish All authors have agreed to the submission policies.

Conflict of Interest The authors declare that they have no competing interests.

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