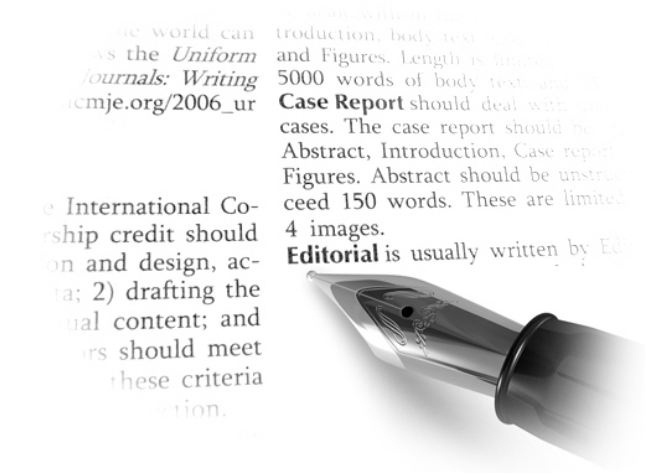

BLOOD RESEARCH
Volume 52 · Number 1 · March 2017
https://doi.org/10.5045/br.2017.52.1.1

Editorial

Alternative approaches to preserve MSC progenitor potency

Nayoun Kim, Ph.D.,[1,2], Seok-Goo Cho, M.D., Ph.D.[1,2,3]

[1]Institute for Translational Research and Molecular Imaging, [2]Laboratory of Immune Regulation, Convergent Research Consortium for Immunologic Disease, [3]Catholic Blood and Marrow Transplantation Center, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea

Over the years, mesenchymal stem cells (MSCs) have gained increasing interest in various clinical fields due to its unique properties including immune modulation and tissue regeneration. The mechanisms by which MSCs exert its therapeutic potential can be broadly categorized into two groups: (1) stimulation of neighboring cells to facilitate repair by secretion of immunoregulatory, cell-mobilization, and growth factors and (2) direct replacement of damaged cells through multilineage differentiation. Despite high expectations on its success, the clinical outcomes of MSC therapy have been controversial with unclear efficacy [1]. Many studies now suggest that MSCs, itself, have inherent limitations due to its high immunoregulatory plasticity and significant degeneration in progenitor potency during culture expansion.

As a transition, researchers are now reversely translating from clinical trials to pre-clinical research in order to overcome these limitations and to enhance the therapeutic functions of MSCs. Strategic approaches to overcome immunoregulatory plasticity of MSCs have been previously discussed, which includes gene modification to intentionally over-express anti-inflammatory genes, pre-conditioning of MSCs with various cytokines or growth factors to allow MSCs to acquire immunoregulatory functions, and combined cell-based immune modulation to promote empowering and synergistic interactions between MSCs and immunoregulatory cells [2-5]. Similarly, approaches to preserve and enhance progenitor potency of MSCs are being addressed. Proliferation, multilineage potential, and colony-forming efficiency are fundamental MSC progenitor properties that can improve the therapeutic potential for tissue repair and regeneration [6].

In this issue of **Blood Research**, Bae *et al.* [6] suggest that three dimensional (3D) culture system could promote the osteogenic and adipogenic differentiation potential of bone-marrow derived mouse MSCs, and additional treatment of azacitidine (AZA), a DNA methyltransferase inhibitor, could further enhance the osteogenic differentiation ability. However, other progenitor properties, including proliferation and colony-forming efficiency were not significantly affected by either treatment compared to conventionally cultured MSCs.

Bone-marrow derived MSCs reside within a complex environment in which MSCs are subjected to various mechanical stimuli of the bone and also environmental stimuli from interacting cell types. However, classic two dimensional (2D) cultures lack these mechanical stimuli and vital cues by hematopoietic cells and nonadherent population of mesenchymal progenitors. Therefore, to preserve MSC progenitor potency, studies have focused on expansion techniques to recreate an elaborate bone marrow niche through 3D expansion, hypoxia, and growth factors [7]. 3D expansion cultures of MSCs can be performed through scaffolds [8] and bioreactors [9]. Scaffold-based approaches may reflect tissues targeted for therapies by substrates and pore sizes; however, scaffold-less suspension approaches

using bioreactor devices may be more feasible for clinical application. Furthermore, dynamic perfusions can enhance mechanical stimuli during 3D expansion inducing increased differentiation toward the desired lineages [10]. In majority of 3D culture systems, osteogenic and adipogenic potential were enhanced compared to control, as shown by Bae *et al*. It has also been consistently reported in other studies that 3D culture systems may increase proliferation and colony forming efficiencies of MSCs.

In addition to 3D expansion, progenitor potency of MSCs can be further enhanced by combining hypoxic culture conditions and specific growth factors. Hypoxic culture of MSCs enhanced proliferation and improved multilineage differentiation *in vitro* and *in vivo* [11]. Also growth factors that are found in the bone marrow niche can be used to supplement the media. While numerous growth factors are found in the bone marrow niche, fibroblast growth factor-2 was the most potent in preserving MSC progenitor potency with greater proliferation and multilineage potential [12].

Additionally, in Bae *et al*.'s study [6], AZA treatment was combined with 3D culture of MSCs. While the underlying mechanism of action of AZA on MSCS is unclear, it is hypothesized that AZA, can reduce DNA methylation and potentially activate transcriptionally silenced genes associated with pluripotency. However, depending on the dosage, duration of pre-treatment, number of exposures of AZA, the effects on MSCs may differ with varying differentiation potential [13]. In the present study, AZA only contributed to osteogenic differentiation and did not affect other progenitor properties. However, there have been reports that multiple exposures of AZA could increase MSCs' ability to generate chondrocytes [13]. While the effects of AZA on MSCs is still under investigation, these studies provide evidence that epigenetic modulations by demethylation agents or other modulators such as histone deacetylase inhibitors could be beneficial to preserve progenitor potency of MSCs.

To summarize, alternative approaches to preserve MSC progenitor potency include optimization of expansion techniques by 3D culture, hypoxia, growth factors and/or epigenetic modulators. With the increasing interest in the application of MSCs as a cell replacement therapy in regenerative medicine, further studies will be necessary to accurately define the optimal conditions of MSC culture for desired use in clinical settings.

REFERENCES

1. Kim N, Im KI, Lim JY, et al. Mesenchymal stem cells for the treatment and prevention of graft-versus-host disease: experiments and practice. Ann Hematol 2013;92:1295-308.
2. Kim N, Cho SG. Overcoming immunoregulatory plasticity of mesenchymal stem cells for accelerated clinical applications. Int J Hematol 2016;103:129-37.
3. Lim JY, Im KI, Lee ES, et al. Enhanced immunoregulation of mesenchymal stem cells by IL-10-producing type 1 regulatory T cells in collagen-induced arthritis. Sci Rep 2016;6:26851.
4. Lim JY, Park MJ, Im KI, et al. Combination cell therapy using mesenchymal stem cells and regulatory T-cells provides a synergistic immunomodulatory effect associated with reciprocal regulation of TH1/TH2 and th17/treg cells in a murine acute graft-versus-host disease model. Cell Transplant 2014;23:703-14.
5. Park MJ, Park HS, Cho ML, et al. Transforming growth factor β-transduced mesenchymal stem cells ameliorate experimental autoimmune arthritis through reciprocal regulation of Treg/Th17 cells and osteoclastogenesis. Arthritis Rheum 2011;63:1668-80.
6. Bae YJ, Kwon YR, Kim HJ, Lee S, Kim YJ. Enhanced differentiation of mesenchymal stromal cells by three-dimensional culture and azacitidine. Blood Res 2017;52:18-24.
7. Hoch AI, Leach JK. Concise review: optimizing expansion of bone marrow mesenchymal stem/stromal cells for clinical applications. Stem Cells Transl Med 2014;3:643-52.
8. Di Maggio N, Piccinini E, Jaworski M, Trumpp A, Wendt DJ, Martin I. Toward modeling the bone marrow niche using scaffold-based 3D culture systems. Biomaterials 2011;32:321-9.
9. Chen X, Xu H, Wan C, McCaigue M, Li G. Bioreactor expansion of human adult bone marrow-derived mesenchymal stem cells. Stem Cells 2006;24:2052-9.
10. Zhao F, Grayson WL, Ma T, Irsigler A. Perfusion affects the tissue developmental patterns of human mesenchymal stem cells in 3D scaffolds. J Cell Physiol 2009;219:421-9.
11. Tsai CC, Chen YJ, Yew TL, et al. Hypoxia inhibits senescence and maintains mesenchymal stem cell properties through down-regulation of E2A-p21 by HIF-TWIST. Blood 2011;117:459-69.
12. Martin I, Muraglia A, Campanile G, Cancedda R, Quarto R. Fibroblast growth factor-2 supports ex vivo expansion and maintenance of osteogenic precursors from human bone marrow. Endocrinology 1997;138:4456-62.
13. Rosca AM, Burlacu A. Effect of 5-azacytidine: evidence for alteration of the multipotent ability of mesenchymal stem cells. Stem Cells Dev 2011;20:1213-21.