Repopulation of Rapidly Proliferative and Multi-potent Cells from Senescent MSCs

1,2Yoon, Dong Suk; 2Kim, Yun Hee; 1,2Jung, Ho Sun; 1Paik, Seungil; 1,2Lee, Jin Woo

INTRODUCTION
Major factor causing mesenchymal stem cell (MSC) senescence can be largely divided into donor age and culture condition. Previous studies have shown that MSCs established from aged donors showed larger, flattened morphology compared to that of the younger donors. In addition to the age of a bone marrow (BM) donor, the ex vivo expansion of MSC principally depends on the culture condition and require medium containing 10–20% serum as well as basic fibroblast growth factor. Although these findings provide valid alternative to delay MSC senescence and maintain its stemness, there are no valid experiments identifying methods to re-activate already senescent MSCs up to date. In absence of such knowledge, patients must endure repeated bone marrow extraction for clinical or research purposes in order to retrieve the pluripotent early passage MSC. This study was aimed to develop a method to reactivate the stemness potential of late passage (senescent) MSC to that of early passage MSCs. In order to eliminate any room for side-effect, treatment of growth factors, other than those included in serum, were completely removed.

METHODS

Cell culture and three lineage differentiation: Passage 7 (P7) MSCs were re-plated using standard (STD, 5000 cells/cm²) method or low density (LD, 17 cells/cm²) method in 10-cm² dishes. After re-plating cells using each method, the cells were grown in DMEM-LG containing 10 or 20% FBS and 1% antibiotic-antimycotic solution. Fresh medium was replaced every 3 days for about 6–12 days. For three lineage differentiation, the cells were incubated in osteogenic, adipogenic, and chondrogenic medium, respectively.

Colonies-forming unit fibroblast (CFU-F) assay and calcium contents assay: P7-STD and P7-LD cells subcultured from passage 7 MSCs were plated into 10-cm² culture dishes at the density of 1000 cells per dish in DMEM-LG containing 20% FBS. After 10–12 days, the cultures were stained with 20% crystal violet solution (Merck) for 30 minutes in dark. After washing with distilled water, colony-forming ability of stained cells was observed. To detect colony contents, 300 uL of fresh reagent (O-Cresolphthalein Complexon, ethanolamine/boric acid, hydroxyquinol, Sigma) was added to 50 uL of sample supernatants, and the absorbance was measured at 560 nm. 0.3% oil red O solution was used to measure adipogenic potentials, and the absorbance was detected at 500nm by destaining with isopropanol. For chondrogenic differentiation, 0.1% safranin O solution was used, and he absorbance was detected at 490nm following destaining with 100% ethanol.

RESULTS
We were interested in changing the cell fate of senescent MSCs by altering cell culture method. We observed that most of the early passage MSCs showed spindle-shaped morphology and small-sized cells. In contrast to primary MSC, however, P7 MSCs appeared to become flattened in morphology and enlarged in size. We, therefore, cultured P7 MSC using STD and LD method to compare the changes of cell morphology and colony-forming ability. As predicted, morphology of MSCs subcultured from P7 MSC by STD method (P7-STD cells) was not recovered. On the other hand, morphology of MSCs subcultured from P7 MSC using LD method (P7-LD cells) was recovered at similar level as that of primary MSC (Fig. 1).

DISCUSSION
This study showed that the reduced colony-forming ability of senescent MSCs was recovered by LD method at similar level as that of primary MSC. In addition to the enhanced colony-forming ability, LD method reduced both cell size and ALP expression of P7 MSCs compared to cells cultured by STD method. It has been well-known that differentiation potentials of BM-derived MSCs become exclusively restricted in osteoblastic cell lineage during repeated serial subculture. LD method seems to not only enhance the colony-forming ability and multi-lineage differentiation potential of senescent MSCs but also to prevent senescent MSCs from being committed into osteoblastic cell lineage by reducing the enhanced ALP expression.

Therefore, the application of LD method may not only eliminate the need of repeated bone-marrow extraction from the patients, but also provide an efficient alternative to recover the reduced stemness of senescent MSC while maintaining its pluripotency as well.

REFERENCES

ACKNOWLEDGEMENT
This study was supported by a grant of the Korea Healthcare technology R&D Project, Ministry for Health & Welfare Affairs, Republic of Korea (Code : A085136).