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Genetic expression and functional characterization of the *RUNX2* gene in human adult bone marrow mesenchymal stem cells

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ABSTRACT. Past studies have revealed the critical role of runt-related transcription factor 2 (RUNX2) in the proliferation and differentiation of mesenchymal stem cells (MSCs). This study therefore aimed to investigate the expression profile of the *RUNX2* gene in human bone marrow MSCs and its biological characteristics. Bone marrow MSCs were separated from 12 patients who had received hip joint replacement surgery. After purification and culture, the MSCs were subjected to the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, flow cytometry, the alkaline phosphatase assay, reverse transcription polymerase chain reaction, and RUNX2 protein quantification. The cell growth curve, staining images, and information on the membrane antigens and the levels of *RUNX2* mRNA and protein were obtained based on the results. The growth curve showed, after a 2-day lag period, cultured MSCs entered into the log phase between d3 (Day 3) and d6, when they reached a plateau. Flow cytometry data suggested 94.38% of MSCs were CD90-positive, while only 3.99 and 1.71% of total cells were positive for CD35 and CD45, respectively. With

the elongated induction period, cultured MSCs were polygonal in shape. After a 14-day induction, cell fusion occurred in the center of the cell nodule accompanied by the disappearance of cellular structure to form the calcium nodule, which was stained red. There was also a statistically significant increase in the level of RUNX2 protein at d7 and d14. An osteogenic medium is required for the differentiation of adult MSCs, which is also under *RUNX2* regulation. These findings are potentially valuable for clinical practice.

Key words: Mesenchymal stem cells; Runt-related transcription factor 2; RUNX2; Stem cell differentiation

