INTRODUCTION: It is well known that bone morphogenetic protein-2 (BMP-2) is a member of the transforming growth factor-β superfamily, and is a critical regulator for bone differentiation of pluripotent human bone marrow-derived mesenchymal stem cells (hBM-MSCs) [1, 2].

Recently, it has been reported that growth and differentiation factor-5 (GDF-5), also known as cartilage-derived morphogenetic protein-1 (CDMP-1) and BMP-14, regulates and promotes osteoblastic and osteogenic differentiation [3]. For this reason, we hypothesized that GDF-5 can be regarded as a convincing candidate for stimulating osteogenic differentiation.

Although GDF-5 and BMP-2 have been investigated for the ability of osteogenic differentiation, those effects have not yet been compared in hBM-MSCs. Therefore, we here compared effects of GDF-5 and BMP-2 on the proliferation and osteogenic differentiation of hBM-MSCs.

MATERIALS AND METHODS:
Study design: The study protocol was approved by the IRB of our institute. The experimental groups were divided into 4 groups for this study: (1) control group (no differentiation), (2) osteogenic group without GDF-5 or BMP-2, (3) BMP-2 group (osteogenic differentiation with BMP-2), and (4) GDF-5 group (osteogenic differentiation with GDF-5).

Cell isolation and culture: hBM-MSCs were isolated from iliac crest of donors undergoing total hip arthroplasty using a modified procedure described by Pittenger et al. [2]. Most cells expressed CD73 and CD105. In addition, the majority of cells were negative for CD34 and CD45.

Cells of control group were cultured in Dulbecco’s Modified Eagle Medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum (FBS, Invitrogen) and 100 U/ml penicillin/streptomycin. Growth factors were added to osteogenic differentiation media at a concentration of 100 ng/ml.

DNA content assay: Cells were scraped into PBS containing 1% tritylphenoxypolyethoxyethanol (Triton X-100, Sigma-Aldrich) at 7 days after seeding. These cell lysates were homogenized by freeze and thaw followed by sonication. Deoxyribonucleic acid (DNA) content was determined using the Hoechst 33258 dye assay with calf thymus DNA as a standard. The fluorescence was read at 360 nm excitation and 460 nm emission on fluorescent plate reader.

Alkaline phosphatase (ALP) activity assay: Homogenized samples were prepared on day 14 after seeding. The ALP activity was analyzed using p-nitrophenyl phosphate as a substrate (Sigma-Aldrich). Aliquots (10 µl) of each sample were incubated with 50 µl alkaline phosphatase substrate buffer for 30 min at room temperature. The reaction was stopped with 50 µl 0.05N NaOH. The absorbance was read in duplicates at 405 nm. Enzyme activity was expressed as umol/min/µg protein of p-nitrophenol released, normalized to the protein content of the sample as measured by the Bradford protein assay method.

Calcium (Ca) contents: On day 14 after seeding, cells were incubated in 0.6N HCl for 4 hours and supernatant was used as sample. Calcium concentration was measured using a QuantChrom® calcium assay kit.

Histology: The calcium deposition was determined by von Kossa staining at 21 days after seeding. Cells were fixed 4% paraformaldehyde, were rinsed with distilled water, and then incubated in 5% silver nitrate solution. After washing with distilled water, the cells were exposed to UV light for 30 minutes to detect the calcium deposition.

Statistical analysis: Data were expressed as mean and SD. The significance of differences was determined using Student’s t-test. Differences with p values <0.05 were considered statistically significant.

RESULTS: Cell proliferation was significantly increased in GDF-5 treated group compared to those in BMP-2 treated group (P<0.05) (Figure 1). ALP activities of osteogenic differentiation group, BMP-2 treated group, and GDF-5 treated group were higher than in control group. The ALP activity of GDF-5 group was significantly higher than in BMP-2 group (P<0.05, Figure 2-A).

When the Ca activity was compared in all groups, the Ca level of GDF-5 group was highest and was significantly higher than BMP-2 group (P<0.05, Figure 2-B).

CONCLUSION: We have demonstrated that GDF-5 is a more effective stimulator of proliferation and osteogenic differentiation than BMP-2. To this regard, GDF-5 may be useful regulator for promoting the bone regeneration and formation by inducing hBM-MSCs proliferation and osteogenic differentiation.

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