The Role of the Growth Factor-Coated Nano-Beads in Chondrocytic Differentiation of Bone Marrow Stromal Cells

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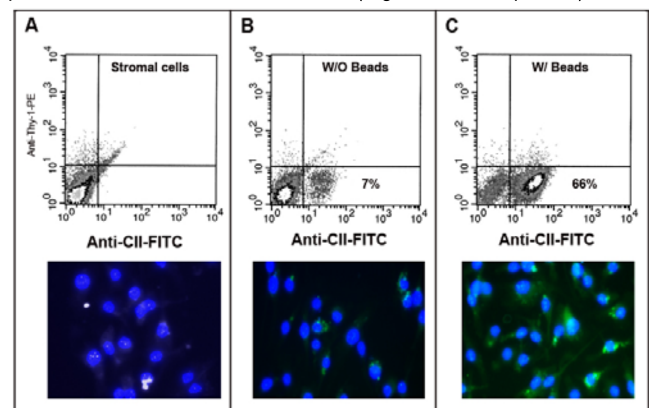
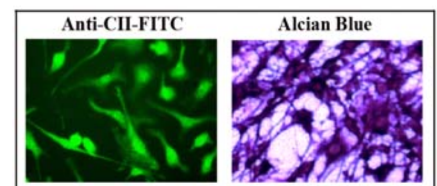
ABSTRACT: Mesenchymal precursor cells (MPC) are pluripotent precursors, that give rise to osteocytes, chondrocytes, endothelial, adipose, and muscle cells. The reported results indicate that MPC chondrogenesis depends on genetic regulation of growth factors such as PDGF, TGF-β3, FGF, IGF and BMP2. Our previous *in vitro* data demonstrated that chondrocytic-like cells can be directly derived from fibroblast-like MPC after TGF-β3 and BMP2 treatment. During the MPC chondrocytic differentiation, these growth factors regulate morphological and genetic changes of MPC from initiation to final differentiation. We have also noticed that the RhoA/RAK genetic signals are critical for MPC differentiation. However, the differentiation rate of MPC chondrogenesis remains at very low levels (<10%) although numerous cocktails of growth factors have been tried by others. To enhance the differentiation rate of chondrogenesis from bone marrow MPC, our newly designed growth factor-coated beads (GF-beads) were used in culture with MPC after stromal cells were isolated and defined ($SH2/SH3^{+}CD29^{+}CD140^{+}/CD34^{-}CD117^{-}$). The gene expression profiles of MPC treated with uncoated beads or GF-beads were analyzed for markers of chondrogenesis using cDNA microarray representing 1000 mRNAs from the mouse isolated with stromal protein markers $SH2/SH3^{+}CD29^{+}CD140^{+}/CD34^{-}CD117^{-}$. The significant alterations in RhoA and RhoA-Rho kinase (RAK) gene expression levels were confirmed. Both collagen II proteoglycan gene and protein expressions were predominant in the cultures with GF-beads but not in the cultures incubated with control beads. Comparing of the differentiation rate from the MPC cultures treated with either GF-beads or control beads, the collage II positive cells make up about 60% of the population in the GF-beads cultures. With the control beads, the differentiation rate was similar to cultures without beads. The data suggest that the GF-beads have a practical potential role in MPC chondrocytic differentiation, and warrant further studies.

METHODS: Stromal Cell Purification: Bone marrow from mice(12-14 weeks) used in this study is part of an ongoing research protocol that is approved by the Wayne State University Animal Investigations Committee. For SH2/3 positive cell preparation, single cells were isolated from bone marrow using modified density gradient centrifugation method and then positively selected with a commercial MASC magnetic cell purification kit (Hao et al, 2003). The cell viability was monitored by trypan blue exclusion at each purification step. The cell phenotypes of purified cells were estimated by flow cytometric assays. Differentiation Studies: The isolated MPC (2 x10^5 cells/well) cultures were incubated with either GF-beads or control beads (0.3 mg wet weight) at a final concentration of 0.3 mg/ml. After gently mixing, cultures were incubated in the 5% oxygenated incubator for 18 hours allowing cells attachment. After changing the media, 0.5 ml mineral oil was added to each culture, and the same culture environment was maintained for 5 days. Cultures without beads served as an additional control. The collagen positive cells were isolated and identified after trypsin (1.0 %) incubation. Molecular and Biochemical Analyses: 1). RT-PCR was carried out to examine the mRNA expression of cell type specific protein markers of both MPC and chondrocytes. 2). Protein expression from induction cultures were also determined using immunological assays.

RESULTS: 1). The purity of MPC isolated from mouse bone marrow was monitored and defined with both flow cytometric and immunocytochemical analyses. 2). The inductive effects of GF-beads on chondrogenesis were estimated using cDNA microarray and protein analyses. Gene profiles revealed that both regulatory and maturation genes of chondrocytic specific proteins are expressed predominately in the GF-beads treated cultures. 3). The differentiation rate of chondrogenesis was enhanced 5 folds in the GF-beads cultures compared to the controls (Figure 1, upper panels). The micrographs from morphological studies also reveal a high rate of collagen II production in GF-bead treated cells (Figure 1, lower panels).



4). Following the induction procedures, the further maturation of chondrogenesis was examined using typical cytochemical and immunocytochemical methods. With GF-beads, the cells were stained strongly with both anti-collagen II antibody and Alcian blue (Figure 2).



SUMMARY: The *in vitro* results suggest that our newly designed GF-beads significantly enhance chondrogenesis. Alcian blue stains chondrocytes and strongly indicate characteristics of chondrocytic maturation. Although the mechanism of GF-beads on chondrocytic differentiation remains to be determined, the methodology may provide a practical strategy to increase the population of collagen II positive cells from MPC that may prove to be useful clinically.

REFERENCES:

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