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Cytokine interactions in mesenchymal stem cells from cord blood

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Abstract

We used cytokine protein array to analyze the expression of cytokines from human cord blood-derived mesenchymal stem cells (CB-MSCs). Several cytokines, interleukins (IL), and growth factors, including ENA-78, GM-CSF, GRO, IL-1β, IL-6, IL-8, MCP-1, OSM, VEGF, FGF-4, FGF-7, FGF-9, GCP-2, IGFBP-1, IGFBP-2, IGFBP-3, IGFBP-4, IP-10, LIF, MIF, MIP-3α, osteoprotegerin, PARC, PIGF, TGF-β2, TGF-β3, TIMP-1, as well as TIMP-2, were secreted by CB-MSCs, while IL-4, IL-5, IL-7, IL-13, TGF-β1, TNF-α, and TNF-β were not expressed under normal growth conditions. IL-6, IL-8, TIMP-1, and TIMP-2 were the most abundant interleukins expressed by CB-MSCs. A set of growth factors were selected to evaluate their stimulatory effects on the IL6 secretion for CB-MSCs. IL-1β was the most important factor inducing CB-MSC to secret IL-6. The mechanism by which IL-1β promoted IL-6 expression in CB-MSCs was studied. By using various inhibitors of signal transduction, we found that activation of p38 mitogen-activated protein kinases (MAPK) and MAPK kinase (MEK) is essential in the IL-1β stimulated signaling cascade which leads to the increase in IL-6 synthesis. Additionally, continuous supplement of IL-1β in the CB-MSCs culture will facilitate adipogenic maturation of CB-MSCs as evidenced by the presence of oil drops in the CB-MSCs and secretion of leptin, a molecule marker of adipocytes. These results strongly suggest that cytokine induction and signal transduction are important for the differentiation of CB-MSCs.

Keywords: Mesenchymal stem cell; Cord blood; Cytokine; Interleukin-1β; Interleukin-6

1. Introduction

Mesenchymal stem cells (MSCs) are capable of self-renewal and differentiation into lineages of mesenchymal tissues, including bone, cartilage, fat, tendon, muscle, and hematopoietic-supporting stroma [1]. Although the existence of hematopoietic stem cells in cord blood (CB) is well documented, research on MSCs in cord blood is still in progress. Several researchers successfully isolated, expanded, and characterized the MSCs from umbilical cord blood and evaluated their potential for differentiation into osteogenic, chondrogenic or adipogenic lineage [2–6]. The circulation of pluripotent MSCs in the blood of preterm fetus between liver and bone marrow explains why MSCs can be isolated from cord blood [6]. While the most abundant source of MSCs is bone marrow [7], MSCs from umbilical cord blood can serve as an alternative for cell-based therapies for the following reasons including the significant reduction in number of MSCs with age and the high risk of viral contamination in bone marrow. Although MSCs from placental and umbilical cord blood can be successfully isolated and expanded [8,9], the fact that CB-MSCs are present at a very low frequency [10] may influence their autologous transplantation and other clinical applications. This limitation needs to be further solved by well-designed isolation and expansion procedures.

Abbreviations: MCP, monocyte chemotactic protein; IGFBP, insulin-like growth factor binding protein; ENA-78, epithelial neutrophil-activating protein 78; GRO, growth-related oncogene; OSM, oncostatin M; FGF, fibroblast growth factor; GCP, granulocyte chemotactic protein; IP, interferon-inducible protein; MIF, macrophage migration inhibitory factor; MIP, macrophage inflammatory protein; PARC, pulmonary and activation-regulated chemokine; PIGF, phosphatidylinositol glycan complementation class F; TGF, transforming growth factor; TIMP, tissue inhibitors of matrix metalloproteinases.

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MSCs in the bone marrow (BM) are involved in hematopoiesis, bone formation, bone resorption, and the development of cartilage and fat. From some of these events, BM-MSCs exert their influence through secreting soluble proteins. These macromolecules provide molecular signals to neighboring cells and modulate their mitotic, metabolic, and/or development states. Moreover, MSCs respond to components of the environmental milieu, including autocrine and paracrine factors synthesized by different cells, resulting in modulation of their own mitotic, metabolic, and/or developmental activity [11]. However, the cytokine expression profile of CB-MSCs and the influence of cytokine on the differentiation of CB-MSCs remains poorly documented. Utilization of this serum-free medium will facilitate analysis of interactions between growth factors and cytokines on the proliferation and differentiation of CB-MSCs without the complexity of exogenous serum. The serum-free medium for MSCs was reported and the differentiation potential of adipogenic, osteogenic, and chondrogenic lineages of MSCs was maintained under the serum-free medium [12]. In addition, serum-free medium was used to evaluate the effect of cytokines and growth factors on macrophage colony-stimulating factor secretion by human bone marrow stromal cells [13]. In this study, we used commercial human cytokine protein array to identify protein expression profiles of CB-MSCs under serum-free culture conditions. The study aimed at characterizing the secreted cytokine profile of CB-MSCs, evaluating the effect of cytokines on IL-6 release of CB-MSCs, and determining how their differentiation process was altered when CB-MSCs were exposed to exogenous IL-1β.

2. Materials and methods

2.1. Isolation and culture of CB-MSCs

CB-MSCs were isolated and cultured according to modifications of a previous reported protocol [14]. Briefly, the term cord blood of newborns was layered onto Ficoll–Paque solution (1.077 g/ml, Amersham, Uppsala, Sweden) and centrifuged to deplete the residues of red blood cells, platelets and plasma (700 g for 40 min). Mononuclear cells in the interface were isolated, and seeded at the concentration of 10^6 cells/cm^2 in the serum-containing medium (SCM, IMDM + 20% FBS (Hyclone, Logan, UT) + 10 ng/ml basic fibroblast growth factor (bFGF)) at 37 °C for 2–3 weeks. The well-developed colonies of fibroblast-like cells were collected and preserved in liquid nitrogen for further experiments. Our laboratory routinely isolated MSCs from cord blood. MSCs can be successfully isolated in 108 units of cord blood out of a total of 144 donated samples. The efficacy of CB-MSCs isolation is 75% ($n = 144$). Because these isolated MSCs have similar morphology, differentiation potentials, growth characteristics, and the same immunophenotypic markers (CD26$^-$, CD31$^-$, CD34$^-$, CD45$^-$, HLA-DR$^-$, CD29$^+$, CD44$^+$, HLA-ABC$^+$, SH2$^+$, SH3$^+$, SH4$^+$, Fig. 1), one representative stain of CB-MSCs, CB-MSC no. 354, was used as a typical example in our experiments. CB-MSCs were routinely maintained in serum-containing medium (IMDM + 20% FBS + 10 ng/ml basic fibroblast growth factor (bFGF). Before performed the experiment under serum-free conditions, confluent CB-MSCs were digested with 0.25% trypsin-EDTA (JRH Biosciences, KS) from 20% FBS supplemented medium and seeded at the density of 10^5 cells/ml to serum-free medium. After CB-MSCs were confluent in T75 flasks under the serum-free condition, CB-MSCs were detached with 1 ml of NO-ZYME (JRH Biosciences) and seeded in the six-well plate at the density of 10^5 cells/well for the following experiments, including effects of cytokines on IL-6 expression and multi-lineage differentiation.

2.2. Effects of cytokines on IL-6 expression

For screen of different cytokines on IL-6 expression in CB-MSCs, the concentration of 10 ng/ml of different cytokines was added in the serum-free culture medium. The initial seeding density was 10^5 cells per well in 6-well culture dishes, and the cell-free conditioned medium was analyzed by ELISA method after a 3-day culture. In order to assay the kinase inhibitor effects, 10^5 cells were plated in 35-mm 6-well culture dishes under serum-free condition after 3 days of growth. Medium was then changed to fresh IMDM medium and cells were treated with freshly prepared kinase inhibitors (PD98059 and SB202190) at various doses for 1 h. The range of concentration tested in the evaluation procedure was 0–50 μM of PD98059, 0–50 μM of SB202190 and 10 ng/ml of IL-1β according to the preliminary experiments. After removal of medium, fresh serum-free medium and/or IL-1β were added and cells were cultured for 24 h according the protocol presented by Huang and Zhang [15]. The conditioned medium was collected and stored at −80 °C for further cytokine analysis.

2.3. Evaluation of differentiation

To test chronic supplement of IL-1β on the differentiation of CB-MSCs, serum-free medium supplemented with 10 ng/ml of IL-1β was replaced every 3–4 days for a total of 45 days. The conditioned serum-free medium was collected and preserved at a −80 °C refrigerator for further IL-6 and leptin assay. At term, the cultured cells were washed twice with PBS, fixed in 10% formaldehyde for histochemical staining. Differentiation of adipocytes, chondrocytes and osteocytes were evaluated by the appearance of Oil-red-O stained lipid vacuole [16], the Safranin-O stained red accumulation of sulfated proteoglycans [16] and von Kossa method stained mineralization of calcium nodules [17], respectively.

2.4. Immunophenotyping of CB-MSCs

After CB-MSCs expanded in SFM for two passages, the surface markers were analyzed using a FACSCalibur flow cytometry system (BD Biosciences, Canada). CB-MSCs (10^6 cells) were fixed with 70% ethanol (10 min at 4 °C). Antibodies against human antigens CD26, CD31, CD34, CD45,