Reduced Surface Expression of CD44, CD49b, CD49e, and CD105 Associated with Growth of Human Bone Marrow Stem Cells Is Preserved During Growth in Osteogenic Differentiation Medium

INTRODUCTION: Multipotent mesenchymal stem cells (MSCs) have been isolated from human bone marrow (BMSCs), and shown to have osteogenic and chondrogenic potential when cultured in defined differentiation media. Surface markers have been extensively characterized for the undifferentiated cells [1], but how expression of these markers changes during osteogenic and chondrogenic induction has not been described. It is clear that proliferation and acquisition of the bone or cartilage phenotype depend on the cell’s ability to interact with its surrounding extracellular matrix [2,3]. For example, the alpha-2 subunit of the α2β1 integrin pair (CD49b) and the α5 subunit of the α5β1 (CD49e) have been associated with osteoblast differentiation in a number of studies [4,5,11]. The objective of this study was to quantify changes in surface phenotypic expression during osteogenic and chondrogenic culture of human bone marrow-derived MSCs, focusing on those cell surface proteins that are specifically associated with cell/matrix interactions.

METHODS: MSCs were isolated from bone marrow aspirates from the iliac crest of normal adult donors [3] and expanded for 4 passages in growth media consisting of low glucose DMEM with 10% FBS. A portion of undifferentiated MSCs was resuspended in PBS to assess expression of surface markers by flow cytometry at day 0. Remaining cells were re-plated at 5000 cells/cm² with either growth media (GM) or osteogenic media (OM) consisting of GM supplemented with 1nM dexamethasone, 3mM beta-glycerophosphate, and 50 µg/mL ascorbic acid 2-phosphate. To assess effects of chondrogenic culture conditions, cells seeded in 1.2% Keltone alginate at a density of 12-15x10^5 cells/ml were loaded on 24-well transwell insert membranes [6]. Once hydrogel discs were cross-linked in 100mM CaCl₂, samples were cultured in either incomplete chondrogenic medium (ICM) consisting of high glucose DMEM with 40µg/ml L-proline, 50 µg/mL ascorbate-2-phosphate, 0.1 µM dexamethasone, and 1% ITS+ or complete chondrogenic medium (CCM) consistent of ICM with 10ng/ml TGF-β3 and 50ng/ml BMP-6. After 7 days, cells in osteogenic culture were trypsinized and cells in chondrogenic culture were recovered by uncrosslinking the alginate with sodium citrate [7] and then incubated in 0.125% trypsin-EDTA solution with 2mg/ml collagenase at 37°C for 2 hours. Isolated cells were divided into aliquots of 1.25x10^5 cells, washed in PermWash buffer, and incubated with PE-labeled anti-human mouse monoclonal antibodies specific for CD34, CD44, CD73, CD49b, CD49e, CD105 and CD146. Percent change in surface expression was calculated by normalizing the difference between day 7 and day 0 expression percentage to the day 0 expression percentage. Data were analyzed by ANOVA; * p< 0.01.

RESULTS: At T=0, BMSCs were positive for CD105, CD73, and negative for CD34. CD49b and CD146 expression was low whereas negative for CD44. CD49e was high (Fig 1). The pattern of surface marker expression changed with time. Both control and osteogenic cultures exhibited down-regulation of CD146 (data not shown). However, cells cultured in OM exhibited distinct differences in surface marker expression compared to GM and control cultures (Fig 2). CD49e and CD44 were upregulated in OM cultures but down-regulated in GM cultures. CD49b was down-regulated in both media conditions; however this effect was reduced in OM cultures, although this was not a consistent observation. CD105 was regulated in a similar manner to CD49b but the change in surface expression, while statistically significant, was less pronounced. BMSCs undergoing chondrogenic differentiation exhibited comparable changes in surface markers to cells undergoing osteogenic differentiation.

DISCUSSION: Surface marker expression varied as a function of both time and the medium in which the cells were cultured. CD146, which is a cell adhesion molecule generally used as a marker of endothelial cell lineage [8], decreased with time in culture but was independent of the medium used, suggesting that endothelial progenitor cells were present in low numbers and the GM media did not support endothelial lineage progression. BMSCs treated with OM in monolayer culture had significantly higher expression of the hyaluronan receptor (CD44), the α5 integrin subunit of the fibronectin receptor (CD49e), and the regulatory component of the TGF-β receptor (CD105) compared to GM cultures. The reduction in these surface markers observed in the cultures with time was mitigated by growth in OM or under chondrogenic culture. These results indicate that growth under appropriate conditions can help maintain or up-regulate cell adhesion molecules and surface proteins associated with tissue organization and cell differentiation [3,7,9,10]. Immunohistochemistry should be performed to confirm that the cell isolation process from tissue culture plates and hydrogels does not alter the surface phenotype. Gene expression of surface markers and proteins associated with osteoblasts and chondrocytes should also be investigated to determine if changes in the surface phenotype reflect post-translational regulation and to determine potential markers of differentiation. This study shows that exploring the temporal changes in surface phenotypic expression during osteogenesis and chondrogenesis can provide a better understanding of how cells interact with their surrounding environment throughout tissue development.

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