Enrichment of Adipose-derived Mesenchymal Stem Cells

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Introduction: Mesenchymal stem cells (MSC) are multipotent cells that can differentiate into a variety of lineages, including adipocytes, chondrocytes, and osteoblasts. Adipose tissue has been shown to contain a supply of MSCs as well as adipoprogenitor cells (APC), chondroprogenitor cells (CPC), and osteoprogenitor cells (OPC). Enrichment techniques are required before these cells can be used effectively. One method is to grow the adipose-derived cells in media containing an array of growth factors and chemicals that mimic a specific tissue environment, thereby increasing the ability of MSCs to differentiate down a particular pathway. This technique has been used to show that the population of cells derived from adipose tissue contains MSCs and/or progenitor cells. Another enrichment technique is to specifically inhibit non-desired lineages pharmacologically. The present study tested the hypothesis that greater enrichment of MSC subpopulations can be achieved in this manner. Resveratrol is known to inhibit cyclooxygenase and activate Sirt1, which inhibits adipogenesis and induces apoptosis in adipocytes. Activation of Sirt1 has been shown to decrease adipocyte development from preadipocytes through inhibition of PPARG2[1]. This suggests that resveratrol could reduce the number of adipocyte progenitor cells and enrich the MSC population with osteoprogenitor cells (OPC). Accordingly, we used treatment with resveratrol as a model to deplete the pre-adipocyte population in adipose derived MSCs.

Methods: Adherent cells were isolated from the inguinal fat pads of Sprague-Dawley rats and plated at 5,000 cells/cm^2. One group of cells was grown to confluence in MSC growth media (GM) and then passaged onto 6-well plates containing GM, adipogenic media (AM), chondrogenic media (CM), or osteogenic media (OM) (Lonza). At 7, 14, and 21 days post-confluence, RT-PCR was used to assess expression of mRNAs for marker proteins of adipogenesis (PPARγ2 and leptin), chondrogenesis (collagen type II and aggrecan), and osteogenesis (RUNX2, collagen I, and osteopontin)[6]. In another study the same cells were cultured in GM containing 0, 12.5, or 25μM resveratrol for 7 or 14 days. Flow cytometry was used to assess expression of MSC or osteoblast markers in the original population as well as following growth in GM. MSCs were defined as CD73+[2], CD271+[3], and CD45-[4]. Osteoprogenitors were defined as E11[5] and osteocalcin positive.

Results: mRNAs varied as a function of media in which the cells were cultured and the age of the cultures post-confluence (Fig 1). MSCs cultured in GM (C) for 21 days were positive for all genes examined. Expression of collagen I and II remained constant throughout the culture period; mRNAs for PPARg2, leptin, aggrecan and RUNX2 were low at day 7. RUNX2 was elevated at day 14 and 21, but the other differentiation markers did not increase appreciably until day 21 and still were lower than cells grown in differentiation media. Growth in AM caused increased expression of PPARγ2 and leptin; growth in CM increased expression of collagen II and aggrecan; and growth in OM increased RUNX2. Resveratrol caused a dose and time-dependent increase in cell number and of the percentage of both MSCs and OPCs (Figures 2 and 3). In GM containing 25μM resveratrol, there was a 577-fold increase in MSCs at 7 days (5% of population – Figure 2), and a 106-fold increase in OPCs (21% of population – Figure 3) at 14 days.

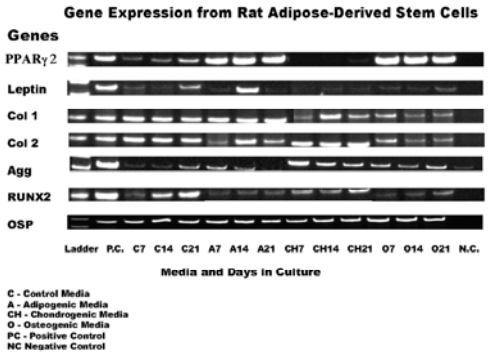

Figure 1: PCR Results

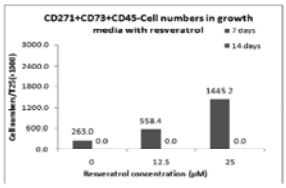

Figure 2: Flow Cytometry of MSC Markers

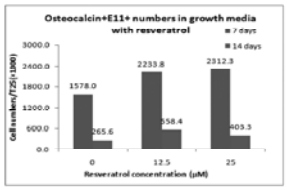

Figure 3: Flow Cytometry of Osteogenic Markers

Conclusion: RT-PCR demonstrated the multipotent potential of adipose-derived MSCs. The results also indicate that the adipose-derived cells contained a mixed population of MSCs, adipoprogenitor cells, chondroprogenitor cells, and osteoprogenitor cells. The MSCs and progenitors cells were pushed towards differentiation depending on the specific media provided during the 21-day experimental time course. The flow cytometry results also showed that MSCs and OPCs were present in the original adherent cell population. The resveratrol treatment was able to enrich both the MSC and OPC population in a dose and time-dependent manner.

References: (1) Backesjo CM. J Bone Min Res. 2006;21:993-1002. (2) Mitchell JB. Stem Cells. 2006;24:376-385. (3) Quirici, N. Exp. Hematol. 2002;30:783-791. (4) Schaffler A. Stem Cells. 2007;25:818-827. (5) Zhang, K. Mol Cell Bio. June 2006:4539-4552. (6) Zuk, PA. Mol Biol Cell. 2002;13:4279-4295.

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