GENETICALLY ENGINEERED BONE MARROW STROMAL CELLS OVEREXPRESSING RUNX2/CBFA1 ENHANCE IN VITRO MINERALIZATION OF 3-D POLYMER SCAFFOLDS

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Introduction: Bone tissue engineering strategies are limited by inadequate supply of committed osteoprogenitor cells and loss of osteoblastic phenotype expression in vitro [1]. In an effort to address these cell sourcing limitations, our work focuses on overexpression of the osteoblast-specific transcriptional activator Runx2/Cbfa1 using retroviral gene delivery in potential target cells for bone tissue engineering applications. We have previously demonstrated that overexpression of Runx2 in the MC3T3-E1 immature osteoblast-like cell line enhanced osteoblastic gene and protein expression and upregulated in vitro matrix mineralization [2]. In the present study, we examined Runx2 overexpression in primary bone marrow stromal cells as a more clinically relevant autologous cell model. We hypothesized that the osteogenic cell population in bone marrow would be highly responsive to exogenous Runx2 expression. We demonstrate that sustained overexpression of Runx2 in primary bone marrow stromal cells enhances expression of multiple osteoblast-specific genes and upregulates in vitro matrix mineralization in 2-D culture and when grown in 3-D biodegradable polymeric scaffolds.

Materials and Methods: Primary bone marrow stromal cells were harvested from femora of young adult male Wistar rats in accordance with an IACUC-approved protocol. Passage 1 cells were transduced with Runx2 or left unmodified for controls and were cultured in α-MEM supplemented with 10% FBS, 1% pen-strep, 3 mM β-glycerophosphate, 50 μg/ml ascorbic acid, and 10 mM dexamethasone. Gene expression was investigated by real-time RT-PCR. ALP activity was examined by a biochemical assay, and matrix mineralization was quantified by von Kossa staining. Results reported are from three separate donors with two replicates each (n=6). Additionally, Runx2-expressing or unmodified stromal cells were trypsinized, counted with a hemacytometer, and seeded 1 day post infection onto fibronectin-coated Innopol 75/25 PLGA scaffolds (100-200 micron pore size, 85% porosity) at 4x10⁴ cells/cm² (n=5). Total DNA was quantified using PicoGreen reagent following Proteinase K digestion of the scaffolds, histological analysis was performed to determine cellular distribution throughout the scaffolds, and micro-CT was used to quantify mineralized matrix deposition following 3, 4, and 6 weeks in culture.

Results: Infection efficiencies (>50%) were observed in stromal cell transductions by flow cytometric detection of an eGFP co-selectable marker. Quantitative PCR (Fig. 1A) of 2-D cultures revealed significant upregulation in Runx2 (10-fold) and OCN (5 to 10-fold at various time points) gene expression in Runx2-infected cultures compared to controls (p<0.001). ALP activity was upregulated two-fold in Runx2-infected cultures compared to controls at 7 days (p<0.005).

Discussion: Bone marrow stromal cells engineered to overexpress the osteoblastic transcription factor Runx2 demonstrate enhanced in vitro osteoblastic differentiation and matrix mineralization capacity when compared to unmodified stromal cells in 2-D and 3-D culture. We expect that the use of dynamic culture conditions or a more macroporous scaffold will maintain greater differences between treatments at later time points by supporting cellular growth and differentiation throughout the interstitial regions of 3-D constructs. Current work focuses on evaluating the ability of these genetically enhanced cells to support ectopic bone formation in a subcutaneous implantation model. Primary stromal cells overexpressing Runx2 represent a potential alternative to address the clinical need for an osteogenic cell source for use in the development of tissue engineered constructs for treatment of damaged or diseased bone.

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