Substantial Mouse Calvarial Bone Defect Healing by Human Embryonic Stem Cells

INTRODUCTION

Human embryonic stem cells (hESCs) may one day provide an unlimited source of progenitor cells for orthopaedic repair applications. Early attempts at using osteogenic progenitor cells derived from hESCs for bone repair demonstrated many difficulties; such as, a lack of homogeneity of the cells within the cultures, the need for flow cytometry cell sorting to provide a more homogeneous source of cells [1], sparse new bone formation [1,2], and the formation of teratomas after in vivo implantation [3]. Our objective was to identify a simple, highly efficient method for deriving a large population of osteogenic precursor cells from hESCs and demonstrate successful in vitro and in vivo osteogenesis by these cells. Based on the successful use of adult MSCs to achieve in vivo bone repair [4], we selected a recently published efficient technique for transforming hESCs to mesenchymal-like cells [5] and evaluated their capability for in vitro and in vivo osteogenesis.

METHODS

Derivation of MSC-like cells from hESCs. The MSC-like cells were derived as described in [5] except that the undifferentiated H9 cells in this study were cultured on and transferred directly from matrigel to laminin-coated wells of a 6-well plate. The cells were cultured for 21 days on the laminin in microvascular endothelial growth media (EGM2-MV) (Lonza) with medium changes every other day without passaging. The cells were then passaged onto standard tissue culture plastic treated flasks three times after reaching near confluency, during which time the morphology of the cells underwent an epithelial to mesenchymal transition [5]. The cells were analyzed by flow cytometry for MSC markers: CD73, CD90, CD105, CD146, CD166, Stro-1, CD45, CD34.

In vitro mineralization experiments. 15 x 10^6/cm^2 MSC-like cells were plated in α-MEM with 10% fetal bovine serum (FBS), 100 U penicillin-100 μg/ml streptomycin (1% Pen/Strep), 50 μg/ml ascorbic acid 2-phosphate, magnesium salt (AA-P). Upon confluence, medium was changed to α-MEM, 10% FBS, 1% Pen/Strep, 100 nM dexamethasone (Dex), 50 μg/ml AA-P, 2 mM beta-glycerophosphate (β-GP). In some cultures, serum- and dexam-f replete medium with 10 nM 1,25-dihydroxyvitamin D3 (Vit D3) was added 24 hrs before the final time point at 21 days. Mineral deposition was detected by 20 μg/ml xylenol orange (XO) staining and analysis of calcium content. Gene expression was analyzed by real time-quantitative PCR using primers for RUNX2, COL1a1, ALP, BSP and OC with GAPDH as an internal control.

In vivo mouse calvarial defect study. The study protocol was approved by the Animal Care Committee at the University of Connecticut Health Center. One million of the MSC-like cells were loaded onto a 3.5 mm x 1 mm collagen/hydroxyapatite sponge (Healov®, DePuy Spine Inc, Raynham, MA) and placed into a 3.5 mm defect in the parietal bone of the NOD SCID gamma mouse calvaria. Each mouse received two identical implants. The mice were euthanized at 6 wks. VIVACT, x-ray, non-decalcified frozen and paraffin embedded decalcified histology including ALU in situ hybridizations were used to assess and analyze the cellular origin of new bone formation.

RESULTS

The FACS analysis of the cells prior to use in the osteogenesis assays indicated negligible expression of CD34, CD45 and Stro-1, and greater than 90% expression of CD73, CD105, CD166 and CD146 similar to adult MSCs. After 14 days in osteogenic supplements, the cells expressed RUNX2, ALP and COL1a1, but were negative for BSP and OC, except in the Vit D3 treated cultures which did have OC expression and mineral deposition (Fig. 1).

DISCUSSION

This is the first demonstration of substantial new bone formation from hESC-derived cells with a high proportion of cell engraftment with no evidence of teratoma formation. The presence of ALU positive human cells within the new bone demonstrates that MSC-like cells derived from hESCs are capable of becoming osteoblasts after implantation into a bone defect on a collagen/hydroxyapatite scaffold. The derivation method adopted in the present study is simple, highly efficient, reproducible and does not involve embryoid body (EB) formation, osteoblast differentiation protocols, FACS sorting or other special handling processes. The high efficiency and simplicity of the process make the method a suitable approach for deriving a large population of osteogenic precursor cells for bone tissue engineering.

In vitro mineralization studies were not predictive of the successful in vivo bone forming capability of these MSC-like hESCs. In vitro, despite culturing with osteogenic supplements, the cells appeared to remain in an immature osteoblast state based on lack of OC and BSP gene expression. Special care was taken to avoid dystrophic mineralization not associated with mature osteoblasts that we found occurred at β-GP concentrations above 2 mM in hESC-derived cell cultures. Thus mineral deposition and osteocalcin expression was essentially non-detectable without Vit D3 spiking. This emphasizes the need for investigators to continue to use caution when interpreting in vitro mineralization studies used as a screen for assessing functional capabilities of hESC-derived cells. The use of the 3-D collagen/hydroxyapatite scaffold in the in vivo studies may have provided important osteogenic cues.

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REFERENCES