**BONE MARROW DERIVED MESENCHYMAL STEM CELL RECYCLING IN THE BONES OF THE NEONATAL MICE DIRECTS THE CELLS TOWARDS BONE AND CARTILAGE CELL LINEAGES**

*Wang, S; *Mi, Z; *Robbins, PD; **Niyibizi, C
*Penn State College of Medicine, Hershey, PA
*University of Pittsburgh School of Medicine, Pittsburgh, PA

**Introduction**

Treatment of the skeletal genetic diseases using gene therapy approaches via stem cells will require clear understanding of engraftment characteristics of the cells in different tissues and organs. As an attempt to understand mesenchymal stem cell commitment and engraftment in bone, we tested the hypothesis that mesenchymal stem cell recycling in bone will induce commitment of the cells to osteoblastic lineage.

**Methods**

**Cell isolation:** Murine bone marrow mesenchymal stem cells were established from the marrow harvested from femurs and tibia of 8 week-old mice. The murine adherent cells were maintained in culture in DMEM supplemented with 50 µg/ml of ascorbic acid with media changes every 3 days.

**Cell surface marker determination:** The cells were evaluated for stem cell characteristics by examining cell surface marker expression. The cell surface markers evaluated were CD13, CD34, CD45, CD90, CD105 and CD117 using specific antibodies.

**Transduction of the MSCs with a retrovirus carrying e-GFP-cDNA:** For cell tracking in vivo or in vitro, the murine MSCs were transduced with a retrovirus carrying the GFP and zeocin resistant genes. Cells were selected in a medium supplemented with zeocin to select for the GFP positive (GFP+) cells. The selected cells were maintained in culture in a medium supplemented with zeocin.

**Transplantation of the GFP positive cells in neonatal mice:** The GFP positive cells (5x10^4) were infused into 2-day old immunocompetent mice via the temporal vein using the methods described previously. Twenty five days after cell transplantation, lung, liver and femurs were harvested from the recipient mice; cells were isolated from these tissues and selected in a medium supplemented with zeocin.

**Recycling of MSCs in neonatal mice:** The GFP+ cells recovered from bone, 25 days after cell transplantation, were re-injected into the two-day old neonatal mice as above. The injected mice were sacrificed at day 35 after cell infusion, lung, liver and femurs were harvested and GFP positive cells were isolated from the tissues. The GFP+ cells recovered from bone were re-injected into the 2-day old mice and the mice were sacrificed after 14 days of cell infusion. Lung, liver, femurs and cartilage from knee joints and ribs were harvested, subjected to cell isolation and selection for the GFP positive cells. The recovered cells were characterized in terms of cell surface markers, osteogenic potential and collagen analysis.

**Characterization of the GFP+ cells recovered from the Cartilage:** The GFP+ cells recovered from the cartilaginous tissues of the recipient mice after 14 days, were analyzed for collagen synthesis by immunofluorescence and western blotting.

**Results:**

**Cell surface marker expression:** The adherent murine MSCs used in the present study were CD13, CD34, CD45 and CD105 positive. The Cells were CD34 and CD117 negative suggesting that the isolated cells exhibited mesenchymal stem cell characteristics. The cells were used as such for subsequent experiments without further purification.

**RetroGFP-transduction:** The initial transduction efficiency of the cells with GFP was about 70%. This was achieved by repeated treatment of the cells with the retrovirus, after selection in a medium supplemented with zeocin, 100% of the MSCs were GFP+.

**Recycling of the GFP positive cells into neonatal mice:** Twenty five days after cell infusion into neonatal mice, GFP positive cells were recovered from lung, liver and femurs of the recipient mice. When the cells recovered from bone at 25 days were re-injected into neonatal mice, tissue analysis for the GFP+ cells after 35 days, demonstrated presence of the donor cells in lung, liver and femurs of the recipient mice (fig 1). Cell surface marker analysis demonstrated that the cells recovered from bone at 35 days after cell infusion were CD13, CD90 and CD105 positive indicating that the cells still possessed stem cell characteristics. Re-injection of the cells recovered from bone at 35 days and subsequent analysis of the tissues harvested from the recipient mice at 14 days after cell infusion, demonstrated presence of the GFP+ cells in femurs and cartilage. The cells recovered from cartilages exhibited chondrocyte morphological appearance (fig 2a,b). At this time point however, there were no GFP+ cells recovered from liver or lung. These data suggest that, after three rounds of cell recycling in bone, only cells with propensity to migrate to bone and cartilage were present.

**Collagen synthesis by the cells recovered from cartilages of the recipient mice:** Immunofluorescence localization using a mouse type II specific monoclonal antibody demonstrated specific staining for type II collagen in the GFP+ cells recovered from cartilage (fig 3a). Western blotting of the collagens synthesized by the GFP+ cells from cartilage demonstrated presence of type II collagen thus confirming the immunofluorescence results (fig 3b). These data demonstrate clearly that the cells transplanted into neonatal mice differentiated into chondrocytes in vivo and became part of the cartilage of the growing mice.

**Discussion:**

The present data clearly demonstrate that cell recycling in bone selected out or induced commitment of these cells to osteoblastic and chondrocytic cell lineages. The cells recovered from bone after 35 days of cell infusion still exhibited stem cell characteristics as demonstrated by the expression of the cell surface markers characteristic of stem cells. The cells recovered from cartilage however did not express any of the cell surface markers analyzed indicating that these cells were fully differentiated. There are two possible explanations for the absence of the cells recycled in bone in other tissues other than bone and cartilage; 1) Cell recycling in bone may have induced differentiation of the cells toward pre-osteoblastic and pre-chondrogenic cell lineages as a result of microenvironmental cues provided by the bone, 2) Cell recycling, fractionated out other cell types and only cells with commitments to bone and cartilage lineages were selected out. All these possibilities remain to be evaluated. The present findings offer opportunities to explore mechanisms of cell commitment to specific tissues.

**Acknowledgement:** This work was supported by NIH AR049688-01 and Children’s Brittle Bone Foundation grants.

*Paper No: 0189*