Introduction: Cell therapy of genetic or non genetic diseases of bone will require delivery of a sufficient number of cells to the bones. Systemic delivery of the cells is attractive because it would provide the distribution of the cells to all the bones of the recipients. There is however a lot of controversy as to whether cells delivered systemically in recipient animals migrate and engraft in bone. Most of the studies on cell engraftment of the cells delivered systemically into mice have used cells harvested from bone marrow. In the present study we investigated the potential of murine adipose derived mesenchymal stem cells (MAMSCs) to migrate and engraft in the bones of adult and developing mice so as to understand their potential for skeletal regeneration.

Methods

Cell Isolation: MAMSCs were isolated from the adipose tissue harvested from neonatal (F/B/CFe a/a) using the methods described by Zuk et al. (1). Briefly, adipose tissue was extensively washed with PBS, minced and enzymatically digested with 0.075%/5% Type IA collagenase. The digested adipose tissue was centrifuged to obtain a cell pellet. The pellet was resuspended and passed through a 100 um filter to remove debris. Following plating MAMSCs were maintained at 37 °C 5% CO2 in DMEM medium, and expanded until passage 4 for further experiments.

Transduction of the Cells with a Retrovirus Carrying eGFP-Zeocin cDNA: The cells were plated in six-well plates in DMEM until 60% confluency. The cells were then treated with 1 ml of a high-titer DFG-eGFP retrovirus in serum free medium for 24 h. After 24 h, the medium was replaced with new medium containing the viral vector 2 more times. The GFP positive cells were selected in a medium supplemented with zeocin and the cells were expanded in culture.

ALP activity: MAMSCs were treated with BMP-2 (100ng/ml) for 7 days. Alkaline phosphatase (ALP) activity was determined in cell lysates using an ALP assay kit and normalized for total protein concentration.

Osteogenic Differentiation: The cells were maintained in osteogenic medium with medium changes every 3 days. After 28 days of culture, the cells were fixed and stained for calcium deposition with Alizarin Red S solution.

Adipogenic differentiation: The cells were cultured in the adipogenic medium for 14 days followed by Oil Red O staining. The cells were observed under microscope and photographed.

Transplantation of MAMSCs into neonatal and adult mice: 0.5–1×10⁶ cells of GFP positive cells were injected into 2-day-old mice via the superficial temporal vein (2). In other sets of experiments, 3×10⁶ GFP+ cells were injected intramedullary into the femurs of the adult mice. The cells were maintained in osteogenic medium for 14 days followed by Oil Red O staining. The cells were observed under microscope and photographed.

Results

Cell Isolation: The MAMSCs consisted of a heterogeneous population of fibroblast-like cells. After three rounds of transduction with a retrovirus carrying eGFP, about 70% of the cells were GFP positive. After selection in a medium supplemented with Zeocin, 100% of the cells were GFP+ (Fig. 1a)

Osteogenesis: The MAMSCs GFP+ expressed high levels of ALP when treated with BMP-2. The cells also demonstrated calcium deposits when they were stained with Alizarin Red S (Fig. 1b, c).

Adipogenesis: The MAMSCs cultures in adipogenic medium, for 14 days demonstrated positive oil red staining for adipocytes (Fig 1d).

Discussion: In the present study we have shown that MAMSCs exhibit osteogenic and adipogenic potential suggesting that the cells have stem cell potential. Injection of the MAMSCs into neonatal mice demonstrated that the cells colonized multiple tissues of the recipient mice including bone and bone marrow. Intramedullarily injection of the GFP+ cells at 14 days also demonstrated a large number of GFP+ cells in bone and bone surfaces. Re-injection of the GFP+ cells recovered from the adult mice at 14 days into neonatal mice indicated that the cells also colonized multiple tissues of the recipient mice. These recovered cells however appeared to persist in bones of the recipient mice when they were re-injected into neonatal mice.

Conclusion: The data suggest that Adipose derived MSCs (AMSCs) will migrate and engraft in the bones of the developing mice and that their engraftment may be enhanced by prior exposure to bone microenvironment. The data also suggest that MAMSCs may be good targets for skeletal repair and regeneration.

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2. Niyibizi C et al., Mol Ther. 2004

Fig. 1. Morphology of MAMSCs (a) and their Multilineage differentiation potential (b, ALP activity; lane c, Alizarin Red S staining; d, Oil Red O staining)

Fig. 2. Tracking of GFP positive MAMSCs in neonatal mice. a, whole bone (femur) showing cells on surface; b, tissue section showing GFP + cells lined on bone surface in the endostemum.

Fig. 3. Intramedullary injection and the distribution of eGFP+ MAMSCs in femur (a) and the recovered cells from bone and BM (b, c).

Fig. 4. Tracking of the bone retrieved cells into neonatal mice. GFP+ cells. a, whole bone; b, tissue section