 MCB, vol.3, no.4, pp.167-168, 2006

Isolation of Mesenchymal Stem Cells from Bone Marrow with Distinct Differentiation and Engraftment in Developing Mice

F. Li [1], X. Wang [1], X. Liao [1] and C. Niyibizi [1]

1 Introduction

Mesenchymal stem cells (MSCs) have been suggested as attractive cells for the musculoskeletal tissue repair and regeneration. Controversies however remain regarding engraftment of MSCs in the bones of the animal models after systemic delivery [2]. Since bone marrow contains a heterogeneous population of cells, it is not clear whether contradictory reports regarding MSCs engraftment in bone after systemic delivery may relate to the transplantation of undefined populations of MSCs. To help resolve some of the current controversies, we have isolated subpopulations of single cell expanded MSCs and assessed their differentiation and engraftment in developing mice bones after systemic transplantation.

2 Materials and Methods

Marrow derived MSCs (BMSCs) were established from the marrow harvested from femurs and tibia of 8 week-old mice as described [1]. The adherent cells were transduced with a retrovirus carrying EGFP-Zeocin^r genes. The GFP+ cells at passage 4 were used to generate single cell expanded MSCs (SMSCs) by limited serial dilution. Seven single cell expanded MSCs were established. Total RNA was extracted from 1 x 10^6 cells. Triplicate PCR reactions were amplified using specific primers and β-actin as a control for assessing PCR reaction efficiency. SMSCs at 30 population doublings were cultured in osteogenic medium for 21 days or in adipogenic medium for 14 days and stained with Alizarin Red or Red Oil O respectively. ALP activity was assessed after BMP-2 treatment. For cell surface antigen phenotyping, quantitative FACS was performed on a FACStar flow cytometer. The GFP+ SMSCs ($1x10^5$) were infused into 2-day old B6C3Fe mice via the superficial temporal vein using the methods described previously [(1)]. The neonatal mice that received the cells were sacrificed at 14 days after cell transplantation. GFP+ cells were tracked by isolating GFP+ cells from tissues.

[1]Pennsylvania State University College of Medicine Hershey, PA 17033

3 Results

In vitro differentiation.The seven established SMSCs differed in the level of ALP activity after BMP-2 treatment, matrix mineralization and adipogenic differentiation. **Fig. 1A**, shows the ALP activity levels in different SMSCs. All the SMSCs showed marked increase in ALP after treatment with BMP-2, clones 2 showing a slight higher response than the other SMSCs. Different SMSCs mineralized the matrix with varying degrees as indicated by the Alizarin staining (**Fig. 1B**). The SMSCs also exhibited differences in the level of surface marker expression (**Table 1**).Clone 3 differentiated toward osteogenic lineage but not adipogenic lineage. Clone 4 however was more efficient in differentiating toward adipogenic lineage but less efficient in differentiation toward osteogenic lineage (**Figs. 1B,C**).

In vivo engraftment and differentiation. The SMSCs differed in their level of engraftment in developing mice after systemic injection. **Fig. 2** shows GPF+ cells in different tissues harvested at 2 weeks from a mouse that received the cells at 2-days of age. The SMSCs derived from clone 2 colonized most of the tissues of the recipient mice including bone. SMSCs derived from clone 5 were more efficient in migrating to bone after systemic injection in developing mice (**Table 2**). Genotyping and gene expression analysis of the

cells retrieved from various tissues at 4 weeks after cell transplantation showed that the SMSCs underwent tissue specific differentiation in vivo with no evidence of cell fusion. These data suggest that subpopulations of MSCs exist in bone marrow and that each subpopulation exhibits distinct characteristics in terms of differentiation and engraftment in developing mice.

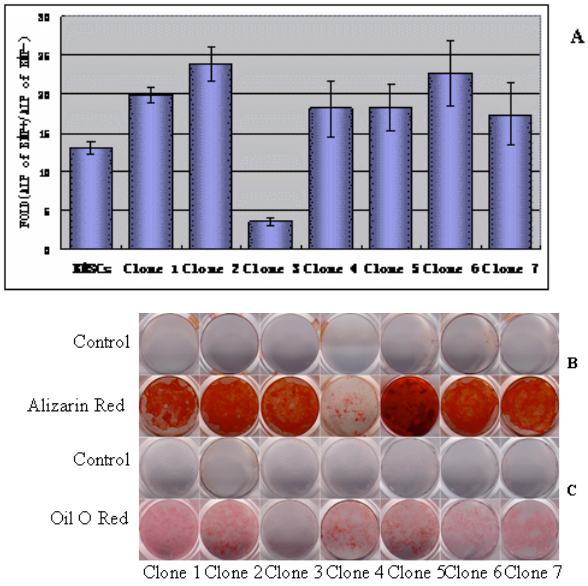

Figure 1 : The differentiation of SMSCs.

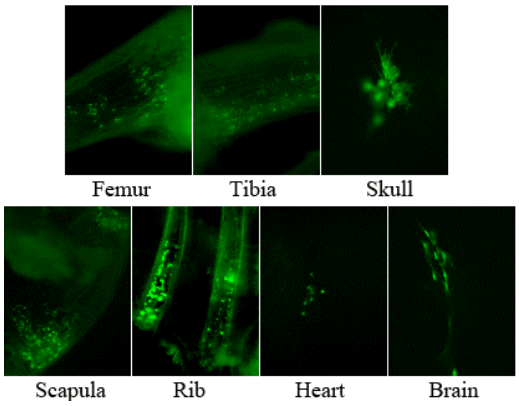

Figure 2 : Fluorescent images of eGFP+ SMSCs in whole tissues of a recipient mouse at 14 days after systemic injection of SMSCs derived from clone 2. Magnification: Femur, Tibia, Scapula, Rib, 40X; Heart,100X;Skull, Brain, 200X.

Table 1 FACS Analysis of Cell Surface Markers

	CD13	CD34	CD44	CD45	CD73	CD90	CD105
BMMSC	+++	-	+++	-	+	-	-
Clone 1	+++	±	++	-	-	-	-
Clone 2	+++	-	++	-	+	-	-
Clone 3	+	-	-	-	+	-	-
Clone 4	++	-	+	-	+	-	+
Clone 5	+++	-	++	-	+	-	-
Clone 6	±	-	-	-	++	-	-
Clone 7	+++	-	++	-	++	-	-

Table 2 Percentage of Mice that Were Positive for eGFP in Different Tissue Detected by Recovered GFP+ Cells after Infused EGFP+ Cells for 14 days

	Bone	BM	Lung	Heart	Rib	Liver	Kidney	Spleen	Brain
BMSC	2/4	3/4	4/4	2/4	1/4	0/4	0/4	0/4	0/4
Clone 1	1/5	1/5	5/5	0/5	0/5	0/5	0/5	0/5	0/5
Clone 2	4/4	4/4	4/4	3/4	3/4	2/4	2/4	0/4	2/4
Clone 5	5/5	5/5	5/5	3/5	1/5	0/5	0/5	0/5	0/5
Clone 6	2/5	2/5	5/5	0/5	1/5	0/5	0/5	0/5	0/5
Clone 7	0/5	0/5	5/5	0/5	0/5	0/5	0/5	0/5	0/5

4 Discussion

We have shown that, subpopulations of MSCs exist in bone marrow that possess differing capabilities of differentiation and engraftment in vivo. Some subpopulations exhibited high level of engraft into the multi-tissues of the recipient mice and other SMSCs exhibited higher affinity for migration to the bones of the developing mice, for example SMSCs derived from clone 5.

In summary, these data suggest that a well-defined population of murine SMSCs is capable of engrafting into various tissues and organs of developing mice when administered systemically. These data suggest that clear understanding of the characteristics of different subpopulations of MSCs will aid in isolation and use of MSCs for tissue repair and regeneration.

Acknowledgement: This work was supported by NIH/NIAMS AR49688.

References

1. Niyibizi, C., Wang, S., Mi, Z., and Robbins, P.D. (2004) *Mol. Ther*. **9**, 955-963.
2. Wang, L., Liu, Y., Kalajzic, Z., Jiang, X., and Rowe, D.W. (2005) *Blood*. **106**, 3650-3657.