J. Biol. Res. 2006 n. 1 - Vol. LXXXI - Rubbettino - Soveria Mannelli

Analysis with electronic and confocal microscope of GFP-stable transfected adult mesenchymal bone marrow stems cells

C. Penna, S. Raimondo

Department of Clinical and Biological Sciences, University of Turin

KEY WORDS: stem cell, green-fluorescent-protein, ultrastructural characterization

Abstract

Growing attention has been recently brought to the use of adult stem cells instead of embryonic stem cells for research purposes and for the development of transplantation treatments for various human diseases. Among the various types of adult stem cells, mesenchymal bone marrow stem cells have been studied extensively because of their ability to self-renew and to give rise to a variety of differentiated cell types as well as of the relative easiness for obtaining and culturing them by bone biopsy. In addition, the possibility of labelling stem cells with green fluorescent protein before transplantation has opened new and very promising perspectives for their employment in basic research. We have described the morphology, at light and electron microscope level, of cultured adult mesenchymal stem cells derived from the bone marrow of transgenic rats for the green fluorescent protein. This analysis provides a useful baseline for the employment of these cells in various experimental models designed to investigate their differentiation fate in normal and pathological tissues.

Introduction

Since stem cells are the precursors of many tissue in the body (Geuna et al., 2001; Lovell and Mathur, 2004) and thus have the potential to provide replacement tissue for damaged organs, they are good candidates for being transplanted for the treatment of various human diseases such as Parkinson's disease (Dunnett et al., 2001), diabetes (Henningson et al., 2003), and heart disease (Orlic et al., 2002; Herzog et al., 2003).

The first description of bone marrow mesenchymal stem cells (MSC) dates back to the 1970's (Friedenstein et al., 1974). These cells have multilineage differentiation capacity in vitro and maintain an undifferentiated and stable phenotype over many generations (Orlic et al., 2002). Although MSCs represent a very small fraction of the total population of nucleated cells in marrow (Pittenger et al., 1999), they can be isolated and expanded with high efficiency (Orlic et al., 2002). In spite of the interest that MSCs have raised over the last years, structural and ultrastructural characterization of the adult MSCs phenotype is lacking. Therefore, this study was aimed at providing a morphological description, at light and electron microscopy, of rat cultured MSCs. In addition, since the many experimental studies based on MSCs transplantation use green-fluorescent-protein (GFP)-positive cells to enable their easy localization in the receiving tissues, we focused our investigation on GFP-positive MSCs.

Materials and methods

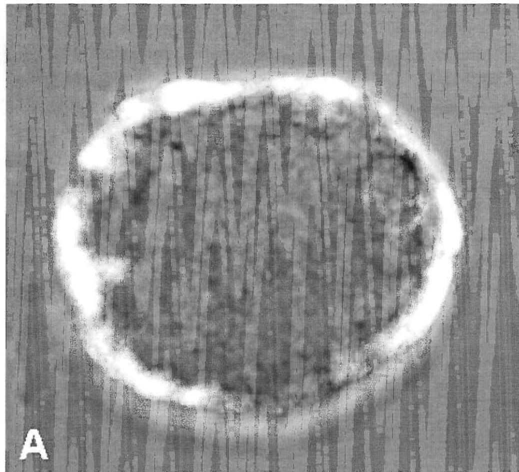
MSCs were harvested from the bone marrow of the femurs of GFP-stable transfected (GFP under β-actin promoter, Okabe et al., 1997) adult rats and treated in culture as described in Raimondo et al. (2006). To verify the mesenchymal origin of these cell, they were stimulated (as described in Raimondo et al., 2006) with dexamethasone, insulin and IBMX for 5 days. With this treatment they differentiate in adypocites.

For morphological analysis stem cells were stained with haematoxylin and eosin, CD90 (Thy-1.1 monoclonal, mouse, 1:50, BD Pharmingen) and propidium iodide (Sigma, St. Louis, MO, USA).

For ultrastructural analysis by electron microscopy sample were treated as described in Raimondo et al. (2006).

Results

Figures 1 A and B show confocal imaging of MSCs. All cells were labelled with CD90 (Fig. 1A), a stem cell marker that is expressed by cultured MSCs (Young, 2004). In figure 1B the nucleus was stained with propidium iodide thus appearing eccentric and irregularly shaped. The cells



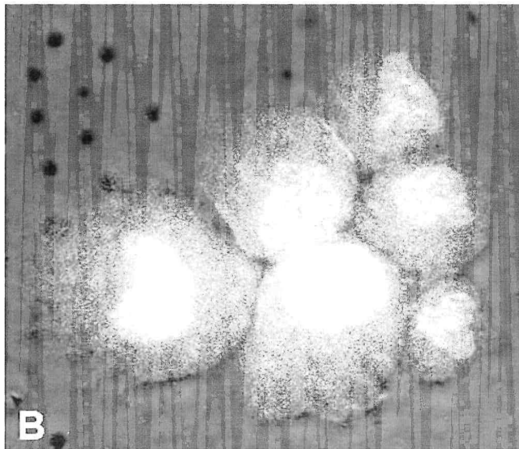


Fig. 1 - *Confocal laser imaging of GFP-positive mesenchymal stem cells in suspension labelled with CD90 (white), stem cell marker (A) and propidium iodide (white), nuclear marker (B).*

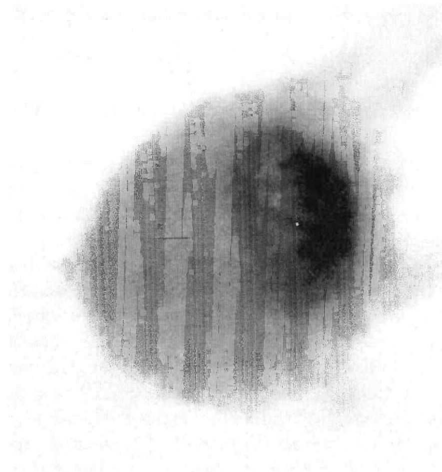


Fig. 2 - *Light micrographs of mesenchymal stem cells in suspension stained with haematoxylin and eosin. The cells have an eccentric nucleus and the cytoplasm divided into two differently stained areas: an inner zone more intensely stained and a thin peripheral zone with a pale appearance. The plasma membrane showed an irregular profile.*

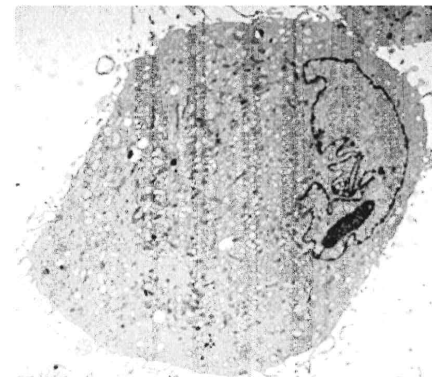


Fig. 3 - *Electron microscopy images of MSCs cut near equatorial level. The cell shows an eccentric nucleus with a nucleolo located near the perinuclear cisternae, a rich granular endoplasmic reticulum and many mitochondrial profiles.*

showed a rounded shape and a size ranging from 10 to 20 μm.
In haematoxylin and eosin stained medium drops (Fig. 2), MSC showed a basophilic and eccentric nucleus and an eosinophil cytoplasm in which two differently stained areas can be clearly detected: an inner zone more intensely stained and a thin peripheral zone with a pale appearance. The plasma membrane showed an irregular profile explained with electron microscope observation (Fig. 3) that showed the irregularities of the plasma membrane were due to small pseudopodia located all around the cells. Electron microscopy of these cells allowed describing their ultrastructure clarifying some of the pictures obtained at light and confocal laser microscopy.
At low magnification, all MSCs shared a very similar appearance with a pale, eccentric and irregular shaped nucleus with one nucleolo located near the perinuclear cisternae. Chromatin is spread throughout the nucleus except for a thin dense layer located immediately inside the perinuclear cisternae. Ultrastructural observations disclosed the cytological basis of the two differently stained cytoplasmic areas observed at light microscopy since the inner part of the cytoplasm was rich in organellae while the peripheral zone was not. In particular, the inner cytoplasmic area was rich in mitochondria profiles with both rounded and elongated shape, an electrondense matrix, and thick cristae. On the other hand, the endoplasmic reticulum was detectable both in the inner and peripheral cytoplasmic zones. The endoplasmic reticulum was mainly granular and organized into small elements. The Golgi apparatus was also well represented and showed the typical stacks of flatterned cisternae,

vescicles and vacuoli, some of which were very large, (data not show).

Discussion

We report the morphology of cultured MSCs harvested from bone marrow of GFP-stable transfected adult rats. This study provides the first comprehensive morphological analysis of these cells using light, confocal and electron microscopy imaging.

MSCs are a relatively uniform cell population especially regarding ultrastructural morphology, an occurrence that might be particularly important to facilitate their detection after their injection in other tissues and to describe their post-transplantation modifications over time. Among the various morphological features detected, the presence of many small pseudopodia all around MSCs is interesting since it is suitable to be at the basis of their high capacity of migrating inside the receiving tissue (Wu et al., 2003; Lee et al., 2004). In addition, electron microscope observation allowed describing two peculiar ultrastructural features of MSCs that differentiate them from fibroblasts. First, the presence of an eccentric nucleus with an irregularly shaped appearance. Second, the relative richness in cytoplasmic organellae (especially mitochondria and Golgi apparatus) in the inner cytoplasmic zone.

In conclusion, the comprehensive structural and ultrastructural description of GFP-positive rat mesenchymal stem cells reported here can represent a useful baseline for researcher that wish to employ these cells in various experimental models; in particular these results may be helpful in order to investigate MSC differentiation fate in normal and pathological tissues.

Acknowledgments

This work was supported by Compagnia di S. Paolo, University of Turin, Regione Piemonte, MIUR (FIRB and PRIN), and INRC.

References

Dunnett SB, Bjorklund A, Lindvall O. 2001. Cell therapy in Parkinson's disease – stop or go? Nat Rev Neurosci 2: 365–369.

Friedenstein AJ, Deriglasova UF, Kulagina NN, Panasuk AF, Rudakowa SF, Luria EA, Ruadkow IA. 1974. Precursors for fibroblasts in different populations of hematopoietic cells as detected by the in vitro colony assay method. Exp Hematol 2: 83-92.

Geuna S, Borrione P, Fornaro M, Giacobini-Robecchi MG. 2001. Adult stem cells and neurogenesis: historical roots and state of the art. Anat Rec 265: 132-141.

Henningson CT, Jr, Stanislaus MA, Gewirtz MA. 2003. Embryonic and adult stem cell therapy. J Allergy Clin Immunol 111: S745-S753.

Herzog EL, Chai L, Krause DS. 2003. Plasticity of marrow-derived stem cells. Blood 102: 3483-3493.

Lee MS, Lill M, Makkar RR. 2004. Stem Cell Transplantation in Myocardial Infarction. Rev Cardiovasc Med 5: 82-98.

Lovell MJ, Mathur A. 2004. The role of stem cells for treatment of cardiovascular disease. Cell Prolif 37: 67–87.

Okabe M, Ikawa M, Kominami K, Nakanishi T, Nishimune Y. 1997. 'Green mice' as a source of ubiquitous green cells. FEBS Lett 407: 313-319.

Orlic D, Hill JM, Arai AE. 2002. Stem Cells for Myocardial Regeneration. Circ Res 91: 1092-1102.

Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. 1999. Multilineage potential of adult human mesenchymal stem cells. Science 284: 143-147.

Raimondo S, Penna C, Pagliaro P, Geuna S. 2006. Morphological characterization of GFP stably transfected adult mesenchymal bone marrow stem cells. J Anatomy 208, 3-12.

Wu GD, Nolta JA, Jin YS, Barr ML, Yu H, Starnes VA, Cramer DV. 2003. Migration of mesenchymal stem cells to heart allografts during chronic rejection. Transplantation. 75: 679-685.

Young HE, Black AC Jr. 2004. Adult stem cells. Anat Rec Part A 276A: 75-102.