*Cytotherapy*, 2013; 15: 951–960

International Society for Cellular Therapy
ISCT®

# Evidence for crossing the blood barrier of adult rat brain by human adipose-derived mesenchymal stromal cells during a 6-month period of post-transplantation

ALIAKBAR HADDAD-MASHADRIZEH[1,2], AHMAD REZA BAHRAMI[1,2], MARYAM M. MATIN[1,2], MOHAMMAD AMIN EDALATMANESH[3], ALIREZA ZOMORODIPOUR[4], ALI FALLAH[5], MOSSA GARDANEH[4], NAGHMEH AHMADIAN KIA[6] & NASER SANJARMOOSAVI[7]

[1]*Cell and Molecular Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran,* [2]*Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran,* [3]*Department of Physiology, Science & Research Branch, Islamic Azad University, Fars, Iran,* [4]*National Institute for Genetic Engineering and Biotechnology, Tehran, Iran,* [5]*Molecular Medicine Group, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran,* [6]*School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran, and* [7]*Faculty of Medicine, Azad University of Medical Sciences, Mashhad, Iran*

**Abstract**

*Background aims.* Therapeutic promises of adult stem cells have been overshadowed by an elicited immune response, low maintenance of implanted cells or concerns regarding their migration to non-target sites. These problems might be lessened by the use of immune privilege cells and tissues for implantation. *Methods.* In this study, human adipose-derived mesenchymal stromal cells (hADMSCs) were stably transfected with a vector containing Turbo green fluorescent protein (GFP) and JRed, which allows tracing the cells after transplantation. Labeled hADMSCs were transplanted into the adult rat brain followed by assessment of their survival and migration during 6 months after transplantation. *Results.* Results indicate that there were no postsurgical complications, and the animals thrived after transplantation. The lesions of the surgical process were remarkable at the first weeks, and a high number of transplanted cells were accumulated around them. Cell populations declined over time as they partly migrated away from the injection sites; nonetheless, they were detectable at each examination time point. Although the cells could survive and remain at the injection site for up to 6 months, some of them drifted to spleen, which is an indication of their ability to cross the blood-brain barrier. *Conclusions.* Despite the high survival rate of hADMSCs in the xenogenic condition, which is an ideal criterion in cell therapy, irregular migration tendency must be handled with caution.

**Key Words:** *cell migration, cell survival, immune privilege, mesenchymal stromal cells, stem cell therapy, xenotransplantation*

## Introduction

The limited regenerative capacity of the central nervous system necessitates the development of cell replacement strategies for neurodegenerative diseases (1). Cell-based therapeutic strategies, especially with the use of cells derived from fetal neural tissues, have been developed over the past two decades (2,3). However, ethical and logistical hurdles surrounding tissue procurement highlight the need to identify an alternative cell source.

Considerable efforts for mining suitable cell sources have introduced stem cells as the best source. Stem cells have generated great interest in the past decade as potential tools for cell-based treatment of human high-grade gliomas and neurodegenerative diseases (4,5). Thus far, three types of stem cells have been tested as vehicles for various therapeutic agents: embryonic, neural and mesenchymal (4). Each strategy has specific advantages and disadvantages. Irrespective of the source and/or type of stem cells, there are several areas of concern for their translation to the clinical setting, such as migration in the adult human brain, potential teratogenesis, immune rejection and regulatory and ethical issues (4). The desired properties of stem cells to make them well suited for regenerative medicine include high abundance, easy to harvest without invasive protocols,

Correspondence: Dr **Ahmad Reza Bahrami**, Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran. E-mail: AR-Bahrami@um.ac.ir

*(Received 4 November 2012; accepted 11 March 2013)*

ISSN 1465-3249 
http://dx.doi.org/10.1016/j.jcyt.2013.03.003

easy *ex vivo* expansion, multi-lineage differentiation capacity and relatively safe and effective rate of transplantation with regard to current good manufacturing practice guidelines (6,7).

Much of the work conducted on adult stem cells has focused on mesenchymal stromal cells (MSCs), found within the bone marrow stroma (8,9). However, because of the invasive and painful extraction procedures, clinical application of these cells has been a subject of great controversy (10,11). This has led many researchers to investigate for alternative sources for MSCs. Adipose-derived mesenchymal stromal cells (ADMSCs), found within the adipose tissue, were propounded as a novel and ideal source in regenerative medicine (6,12–15). Adipose tissue as a reservoir of adult stem cells, similar to bone marrow, is derived from the embryonic mesenchyme and contains a stroma that can easily be isolated in abundant quantities (6,12–14,16). ADMSCs offer several advantages over other multipotent cells (such as bone marrow mesenchymal cells) including easy extraction procedures by commonly used surgical methods, high rate of proliferation, high abundance and robust mineralization within 1 week of *in vitro* differentiation (17).

Information relating to the biology, culture expansion and mechanisms relating to adipose-derived cells have advanced significantly in the past decade (18). The phenotypic and gene expression profiles of ADMSCs are similar to those of bone marrow MSCs (6), and these cells can be expanded in culture for extended periods (12). Both ADMSCs and bone marrow MSCs are derived from embryonic mesoderm, with similar phenotype and gene expression profiles (19). They have the ability to differentiate toward adipogenic, osteogenic, chondrogenic, myogenic, endothelial, hematopoietic, hepatic, islet and neurogenic cell lineages (12,20).

It has been shown that transplantation of ADMSCs results in functional recovery in animal models with peripheral nerve injury, suggesting that they may promote the peripheral nerve regeneration partly through paracrine secretion of trophic factors (15). However, the mechanism of enhanced nerve regeneration remains to be elucidated (15).

For treatment of neurodegenerated tissues, cell replacement or neurotrophic therapies must persist in the brain for a prolonged period (21). Thus, higher survival/maintenance rate and lower chance of cell migration to unwanted organs are ideal criteria in cell transplantation. On the basis of solid evidence of low immunogenic properties of MSCs during allograft implantation (22–24), this study sought to determine the utility of human ADMSCs (hADMSCs), equipped with JRed and Turbo green fluorescent protein (GFP) genes, as an experimental tool to track the survival of hADMSCs after direct transplantation to the intact adult rat brain. In doing so, the risk of migration from the brain was also assessed.

## Methods

### *hADMSCs isolation and expansion*

hADMSCs were isolated from liposuction samples of waste tissue by collagenase digestion and differential centrifugation (25). Briefly, samples were washed extensively with equal volumes of phosphate-buffered saline (PBS), containing antibiotics (100 units/mL of penicillin and 100 g/mL of streptomycin), and then digested with 0.1% collagenase type I (Invitrogen, Paisley, UK) in Hanks' balanced salt solution at 37°C under agitation for 60 min. Next, collagenase was inactivated with Dulbecco's modified Eagle's medium with high glucose (DMEM-HG) (Gibco, Roskilde, Denmark), supplemented with 10% (vol/vol) fetal bovine serum (FBS) (Gibco) and diluted by PBS. The stromal vascular fraction was then separated by centrifugation at 800 *g* for 5 min. This pellet was resuspended and seeded onto the tissue culture plates (Nunc) at 1000–3500 cells/cm$^2$ in DMEM-HG containing 10% FBS. Cultures were washed with PBS, 24–48 h after plating, to remove the unattached cells and fed with fresh medium. The cultures were maintained at 37°C and 5% $CO_2$. When they reached approximately 70% cell confluency, the cells were trypsinized (0.025%; Invitrogen) and plated at a density of 5000 cells/cm$^2$. Cultures were passaged repeatedly after achieving a density of 70–80% until passage 3.

### *In vitro differentiation assays*

To verify the identity of the isolated hADMSCs, before and after lentiviral transduction, adipogenic and osteogenic differentiation *in vitro* assays were performed *in vitro*. Cells from passage 3 before transduction and passage 12 after transduction were plated in six well plates at a concentration of 100,000 cells/well and maintained in standard growth medium to induce the phenotype of mesoderm-derived cells. For osteogenic induction, cultures were treated with 50 µg/mL ascorbate-2 phosphate, 100 nmol/L dexamethasone (Sigma, Munich, Germany) and 10 mmol/L $\beta$-glycerophosphate (Sigma) for a period of 4 weeks. Cells were then fixed with 70% (vol/vol) ethanol for 10 min at room temperature, washed three times with PBS and incubated with 0.1% (wt/vol) Alizarin red solution to stain for calcium deposition. For induction of an adipogenesis phenotype, cells were treated with 50 µg/mL ascorbate-2-phosphate, 100 nmol/L dexamethasone and 50 µg/mL indomethacin (Sigma) for 3 weeks and were