Biochemical and Biophysical Research Communications 454 (2014) 341–346

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Transplantation of mature adipocyte-derived dedifferentiated fat cells promotes locomotor functional recovery by remyelination and glial scar reduction after spinal cord injury in mice

Hiromi Yamada [a], Daisuke Ito [a], Yoshinao Oki [b], Masato Kitagawa [a], Taro Matsumoto [c], Tosihiro Watari [d], Koichiro Kano [b,*]

[a] *Laboratory of Comprehensive Veterinary Medicine, College of Bioresource Sciences, Nihon University, 1866 Kameino, Fujisawa, Kanagawa 252-0880, Japan*
[b] *Laboratory of Cell and Tissue Biology, College of Bioresource Sciences, Nihon University, 1866 Kameino, Fujisawa, Kanagawa 252-0880, Japan*
[c] *Division of Cell Regeneration and Transplantation, Department of Functional Morphology, Nihon University School of Medicine, 30-1, Ohyaguchi-Kamimachi, Itabashi-ku, Tokyo 173-8610, Japan*
[d] *Laboratory of Veterinary Internal Medicine, College of Bioresource Sciences, Nihon University, 1866 Kameino, Fujisawa, Kanagawa 252-0880, Japan*

ARTICLE INFO

Article history:
Received 7 October 2014
Available online 22 October 2014

Keywords:
Adipocyte-derived dedifferentiated fat cell
Spinal cord injury
Remyelination
Scar reduction
Cell transplantation

ABSTRACT

Mature adipocyte-derived dedifferentiated fat cells (DFAT) have a potential to be useful as new cell-source for cell-based therapy for spinal cord injury (SCI), but the mechanisms remain unclear. The objective of this study was to examine whether DFAT-induced functional recovery is achieved through remyelination and/or glial scar reduction in a mice model of SCI. To accomplish this we subjected adult female mice ($n = 22$) to SCI. On the 8th day post-injury locomotor tests were performed, and the mice were randomly divided into two groups (control and DFAT). The DFAT group received stereotaxic injection of DFAT, while the controls received DMEM medium. Functional tests were conducted at repeated intervals, until the 36th day, and immunohistochemistry or staining was performed on the spinal cord sections. DFAT transplantation significantly improved locomotor function of their hindlimbs, and promoted remyelination and glial scar reduction, when compared to the controls. There were significant and positive correlations between promotion of remyelination or/and reduction of glial scar, and recovery of locomotor function. Furthermore, transplanted DFAT expressed markers for neuron, astrocyte, and oligodendrocyte, along with neurotrophic factors, within the injured spinal cord. In conclusion, DFAT-induced functional recovery in mice after SCI is probably mediated by both cell-autonomous and cell-non-autonomous effects on remyelination of the injured spinal cord.

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1. Introduction

Traumatic spinal cord injury (SCI) results in severe neurological deficits that can include paraplegia, but there is no effective clinical therapy to improve the locomotor function. Recently, the therapeutic efficacy from locomotor functional recovery following SCI have been reported in animal models by transplantation of several types of cells, such as embryonic stem cells, induced pluripotent stem cells, neural stem/progenitor cells (NPCs), and mesenchymal stem cells (MSCs) [1–6]. The mechanisms proposed for promoting locomotor function after SCI include: replacement of lost neurons to reconstruct local circuitry, remyelination of demyelinated axons by transplantation-derived oligodendrocytes, and the provision of trophic support that reduces the damage and glial scar to create a permissive substrate for axonal growth, etc. [1,2,5,6].

Mature adipocyte-derived dedifferentiated fat cells (DFAT) are one of the cell types studied for cell-based therapy for SCI. DFAT are fibroblast-like cells that sustain high proliferative activity and multilineage differentiation capacity similar to MSCs [7–9]. DFAT have several properties that are well suited to cell-based therapy as follows [9,10]: First, DFAT can be obtained from donors regardless of age. In our previous study using human DFAT from a total of 18 donors, ranging in age from 4 to 81 years old, we successfully prepared DFAT from every donor. Moreover, subcutaneous fat tissues can be easily collected from large numbers of healthy donors who undergo surgery or liposuction, because these tissues are typically discarded after surgery. From these perspectives, it is

* Corresponding author. Fax: +81 466 84 3790.
E-mail addresses: brhi10509@g.nihon-u.ac.jp (H. Yamada), dice-ito@brs.nihon-u.ac.jp (D. Ito), oki.yoshinao@nihon-u.ac.jp (Y. Oki), kitagawa.masato@nihon-u.ac.jp (M. Kitagawa), matsumoto.taro@nihon-u.ac.jp (T. Matsumoto), watari@brs.nihon-u.ac.jp (T. Watari), kkano@brs.nihon-u.ac.jp (K. Kano).

http://dx.doi.org/10.1016/j.bbrc.2014.10.082
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possible that DFAT provide a useful cell source for allogenic transplantation. Second, DFAT can be obtained from small amounts of tissue, because DFAT originate from a homogeneous fraction of mature adipocytes, the density of which is high in the tissue. Third, DFAT are highly homogenous. DFAT contain almost no other cell type even during first passage, because the cells are prepared from an isolated mature adipocyte fraction. This property of DFAT may not only lead to higher safety and efficacy for clinical cell therapies, but also be useful for mechanistic studies in cell-based therapy because of the small risk of contamination from other cell types.

Previously, we reported that DFAT transplantation had the efficacy to promote locomotor functional recovery in SCI rats [11]. However, it remains unclear whether the transplanted DFAT contributed to remyelination, glial scar reduction, and differentiated into a neural lineage after SCI. The aim of this study was to ascertain whether remyelination and reduction of glial scar by DFAT transplantation directly promotes functional recovery after SCI.

2. Materials and methods

2.1. Isolation and ceiling culture of DFAT

For analysis of DFAT survival after transplantation into the injured spinal cord, DFAT-Green Fluorescent Protein (DFAT-GFP) were prepared using a previously described method [7]. Briefly, mature adipocytes were obtained from the adipose tissue of GFP transgenic mice, from the Mitsubishi Kagaku Institute of Life Sciences. The tissues were minced and digested in collagenase solution (Collagenase Type II; Sigma) at 37 °C for 1 h under gentle agitation. After filtration and centrifugation at 135×g for 3 min, the floating top layer containing adipocytes was collected. After washing with phosphate-buffered saline, the cells were placed in culture flasks (BD Falcon 3107, Bedford) that were completely filled with Dulbecco's modified Eagle's medium (DMEM, Nissui Pharmaceutical), supplemented with 10% fetal bovine serum (FBS; Moregate BioTech), and incubated in a humidified 5% CO_2 atmosphere. The cells floated and adhered to the top inner ceiling of the flask. Approximately 1 week later, the medium was removed and the flasks were turned upside-down so that the cells were at the bottom surface.

2.2. Spinal cord injury model and transplantation

All experiments were performed with the approval of the animal experiment committee of Nihon University (approval number: AP13B057). Spinal cord injury was induced at the Th10 level of adult female mice ($n = 22$, C57BL/6N, Charles River Laboratories Japan, 8 weeks old), using an Infinite Horizon Impactor (Precision Systems and Instrumentation; 60-kilodyne) under general anesthesia (isoflurane). The spinal cord was contused at a point on the midline, where the central canal was estimated to exist. The midline was confirmed by the anterior spinal vein, which was matched to a line connecting the spinous processes of vertebra at T9 and T11 levels. On the 8th day post-injury (dpi), SCI mice were randomly divided into DFAT and Control groups. In the DFAT group, 1×10^5 -DFAT-GFP in 2 μl were injected into the spinal cord at the Th10 level (lesion area) using a 26-gauge needle and a 10-μl Hamilton syringe (Sigma), which was vertically inserted using a stereotaxic injector (Muromachikikai) (0.5 μl/min). Controls received DMEM (2 μl) injections. Locomotor function score of hindlimbs, the Basso mouse scale (BMS) score [12], was assessed by two researchers at 1, 8, 9, 11 14, 17, 22, 29, and 36 dpi. When differences in BMS score between the right and left hind limbs were observed, an average of the two score was used. Any movements of mice during the sustained adduction of hind limbs were not scored.

In a preliminary study, to determine the timing of DFAT transplantation, we excised the contused spinal cord of mice, that were not used in this study, at 1, 3, 6, 9, and 14 dpi (Sup. 1A, $n = 36$), and performed histological analysis by hematoxylin and eosin (HE) staining (data not showed). In this preliminary study, the pathological condition until 6 dpi was mainly hemorrhage or cellular infiltration (may be inflammatory cells), and scar-like constitutions were observed after 9 dpi. Therefore, we decided to transplant DFAT on the 8th dpi, when the infiltration cells are low, and just before the appearance of a scar-like constitution.

2.3. Histological analysis

To reveal the potential mechanism of functional recovery by DFAT transplantation, we conducted histological analyses of the spinal cords at 36 dpi. Under deep isoflurane anesthesia, mice were intracardially perfused with 4% paraformaldehyde and the spinal cords harvested. Dissected spinal cords were post-fixed overnight in 4% paraformaldehyde, embedded in paraffin, and sectioned at 7 μm. After drying and deparaffinization processing, sections were subjected to Luxol fast blue (LFB) staining, HE staining, or immunohistochemistry (IHC). In IHC, the sections were incubated with the primary antibodies overnight at 4 °C, followed by antigen activation and blocking processes. The primary antibodies used were as follows: GFP (1:100, Santa Cruz), nestin (1:250, Santa Cruz), β III tubulin (1:250, Covance), neurofilament 200 kDa (NF-H, 1:5000, Aves Labs), glial fibrillary acidic protein (GFAP, 1:5000, Aves Labs),

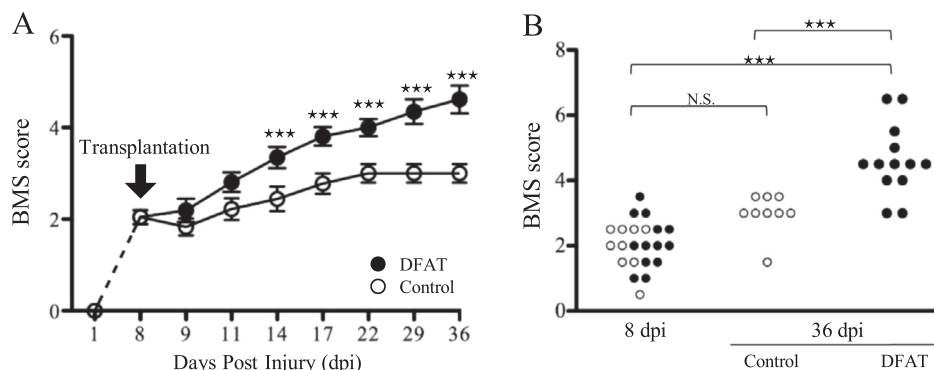

Fig. 1. Recovery of locomotor function by DFAT transplantation. (A) Comparison between pre-transplantation (8 dpi) and post-transplantation (9, 11, 14, 17, 22, 29, and 36 dpi). Mice in the DFAT group significantly recovered after 14 dpi compared with 8 dpi pre-transplantation. No significant difference in functional recovery was observed in the Control group. (B) Differences in the distribution of BMS score at 8 dpi and 36 dpi in the Control and DFAT groups. The locomotor function of mice in the DFAT group was significantly improved compared with the Control group. dpi, days post-injury. (DFAT group, $n = 13$; Control group, $n = 9$.) ***$P < 0.001$.

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