Molecular and Cellular Neuroscience 62 (2014) 60–67

Contents lists available at ScienceDirect

Molecular and Cellular Neuroscience

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

CD31$^+$ cell transplantation promotes recovery from peripheral neuropathy

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ARTICLE INFO

Article history:
Received 5 May 2014
Revised 20 July 2014
Accepted 12 August 2014
Available online 13 August 2014

Keywords:
Angiogenesis
CD31
Cell therapy
Engraftment
Peripheral nerve

ABSTRACT

Recently, we reported that human peripheral blood (PB)-derived CD31$^+$ cells are highly angiogenic. In this study, we investigated the beneficial effects of CD31$^+$ cells on peripheral neuropathy in mice. CD31$^+$ cells were collected from the peripheral blood using magnetic activated cell sorting. CD31$^+$ cells exhibited higher levels of expression of angiogenic genes on real-time reverse transcriptase polymerase chain reaction. Peripheral neuropathy was induced by crushing the sciatic nerve with a hemostat, and CD31$^+$ cells were then injected intramuscularly along the sciatic nerve. CD31$^+$ cell transplantation restored motor nerve conduction velocity and voltage amplitude and improved motor coordination. In addition, CD31$^+$ cell transplantation significantly improved blood perfusion and increased intraneural vascularity in the sciatic nerve. Whole-mount fluorescent imaging and dot blot analysis showed that CD31$^+$ cells in the nerve possessed high engraftment and anti-apoptotic properties. Additionally, injected CD31$^+$ cells displayed neurovascular tropism and are highly incorporated with vasculature. Angiogenic cytokines were augmented in CD31$^+$-injected nerve tissue, suggesting increased neovascularization. Taken together, these results indicate that CD31$^+$ cells might be a novel therapeutic strategy in the treatment of peripheral neuropathy.

1. Introduction

Over the last few decades, there have been significant advances in approaches that involve physical repairing of injured peripheral nerves. However, restoring functional activity in these nerves continues to be a major challenge (Lundborg, 2000). Many therapeutic approaches have attempted to enhance the regenerative process or reconstruct nerve defects using nerve conduits, nerve grafts, and alternative materials (Archibald et al., 1991; Hu et al., 2007; Rodriguez et al., 2000).

Recently, cell transplantation has been emerged as a novel therapeutic strategy for nerve regeneration. In particular, Schwann cell transplantation has been shown to regenerate injured nerves (Bryan et al., 1996). In addition, stem or progenitor cells derived from several sources, such as bone marrow, adipose and brain tissues, and amniotic fluid, have demonstrated favorable effects in injured peripheral and sciatic nerves (Chen et al., 2007; di Summa et al., 2010; Murakami et al., 2003; Pan et al., 2006; Sekiguchi et al., 2013). The possible mechanisms underlying therapeutic effects of cell implantation that have been postulated include transdifferentiation of transplanted cells, secretion of neurotrophic factors, immune modulation, and microenvironmental stabilization (Fawcett and Keynes, 1990; Hall, 2001; Kim and Ahn, 2012).

Platelet endothelial cell adhesion molecule-1 (PECAM/CD31) is a 130-kD cell surface marker belonging to the immunoglobulin (Ig) superfamily (Newman et al., 1990). CD31, which is expressed in vascular endothelial cells, plays a pivotal role in the processes of leukocyte transmigration, anti-apoptotic signaling, and cell adhesion (Muller et al., 1993; Newman and Newman, 2003). Recently, we documented that CD31$^+$ cells comprise the circulating angio-vasculogenic cell population (S.W. Kim et al., 2010). However, the therapeutic potential of CD31$^+$ cells in peripheral nerve injury is currently unknown.

In this study, we determined whether CD31+ cell implantation could ameliorate neuropathy, thereby inducing revascularization, and ultimately treat peripheral nerve injury.

2. Results

2.1. Angiogenic Characteristics of CD31$^+$ Cell

Human PB-derived CD31$^+$ and CD31$^-$ cells were isolated using a magnetic column and their shapes were shown in Fig. 1A. To examine the characteristics of isolated cells, fluorescent-activated cell sorter

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http://dx.doi.org/10.1016/j.mcn.2014.08.005
1044-7431/

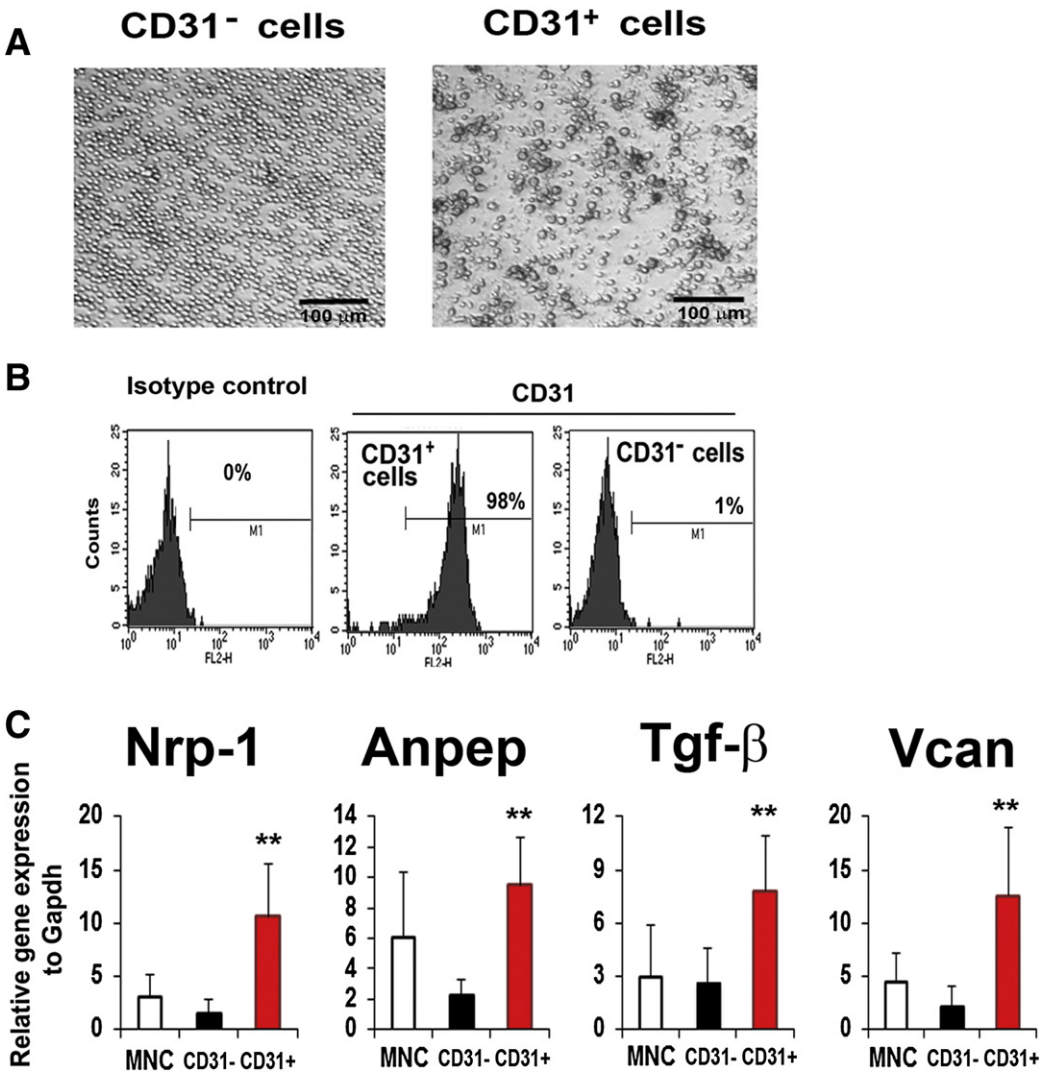

Fig. 1. Characteristics of CD31$^+$ cell. (A) Morphology of CD31$^+$ and CD31$^-$ cells. (B) Magnetic activated cell sorted CD31$^+$ and CD31$^-$ cells were analyzed by flow cytometry. (C) Multiple angiogenic factors were examined by qRT-PCR. **$p < 0.01$, CD31$^+$ vs. CD31$^-$. $n = 4$ per group. Abbreviation: MNC, mononuclear cells.

(FACS) analysis was performed and results revealed that >98% of isolated CD31$^+$ cells express CD31 (Fig. 1B).

To compare angiogenic gene expression, we conducted qRT-PCR analysis. Several angiogenic factors such as neuropilin (Nrp)-1, aminopeptidase (Anpep), transforming growth factor (TGF)-β and versican (VCAN) were significantly expressed in CD31$^+$ cells than CD31$^-$ cells (Fig. 1C) (Guzman-Rojas et al., 2012; Phillips et al., 1993; Zheng et al., 2004). Nrp-1 is a third specific vascular endothelial growth factor (Vegf) receptor and neuronal surface receptor for neuritic guidance molecules (Bagnard et al., 1998). This receptor plays an important role in angiogenesis and vasculogenesis in the brain (Fantin et al., 2013). Therefore, we hypothesize that CD31$^+$ cells expressing Nrp-1 may enhance angiogenesis in injured nerve tissue.

2.2. CD31$^+$ Cell Transplantation Restores Nerve Function

The mice were anesthetized and the sciatic nerve was exposed and crushed at the mid-thigh level for 15 s using a hemostat as previously described (De Koning et al., 1986). The mice were injected with 1×10^6 (DiI) dye-labeled CD31$^+$ and CD31$^-$ in 100 μl PBS or the same volume of PBS intramuscularly in the muscles along the sciatic nerve at six sites.

To investigate the functional change of injured nerves after implantation of CD31$^+$ cells, we measured motor nerve conduction velocity (MCV) for 4 weeks. MCV was undetectable on day 0 and there were no significant differences on day 5 between the treated groups. However, the mice treated with CD31$^+$ cells showed a significant recovery in MCV on day 28 compared to mice treated with CD31$^-$ cells or PBS (1.15 ± 0.31 vs. 0.75 ± 0.12, $p = 0.027$ and 0.36 ± 0.14, $p < 0.01$, respectively) (Fig. 2A).

We also examined nerve functional recovery using nerve voltage amplitude. After 4 weeks, the mice that were transplanted with CD31$^+$ cells showed significantly higher amplitudes compared to CD31$^-$ and PBS control groups (7.15 ± 2.01 vs. 4.27 ± 0.93, $p = 0.018$ and 2.55 ± 0.88, $p < 0.01$, respectively) (Fig. 2B).

Next, to measure the recovery of motor coordination, rotarod performance behavioral testing was conducted using a rotarod treadmill. The mice treated with CD31$^+$ cells performed better on rotarod task compared to the CD31$^-$ and PBS groups (419 ± 46 vs. 366 ± 39, $p = 0.045$ and 327 ± 32, $p < 0.01$, respectively) (Fig. 2C).

2.3. CD31$^+$ Cells Augment Blood Perfusion and Vascularization in Nerves

To determine whether CD31$^+$ cells promoted blood circulation to the injured nerve, we measured blood perfusion of the sciatic nerve using Laser Doppler Perfusion Imaging (LDPI) 4 weeks after the operation. Nerve blood flow was 1.35- and 1.87-fold higher in the