



Tumor-homing effect of human mesenchymal stem cells in a TH-MYCN mouse model of neuroblastoma



Koseki Kimura^a, Tsunao Kishida^b, Junko Wakao^a, Tomoko Tanaka^a, Mayumi Higashi^a, Shigehisa Fumino^a, Shigeyoshi Aoi^a, Taizo Furukawa^a, Osam Mazda^b, Tatsuro Tajiri^{a,*}

^a Department of Pediatric Surgery, Kyoto Prefectural University of Medicine, Kyoto, Japan

^b Department of Immunology, Kyoto Prefectural University of Medicine, Kyoto, Japan

ARTICLE INFO

Article history:

Received 18 August 2016

Accepted 12 September 2016

Key words:

Neuroblastoma

Mesenchymal stem cell

Homing

TH-MYCN transgenic mouse

IVIS

ABSTRACT

Background: Human mesenchymal stem cells (hMSCs) are multipotent stem-like cells that are reported to have tumor-suppression effects and migration ability toward damaged tissues or tumors. The aim of this study was to analyze the tumor-homing ability of hMSCs and antitumor potency in a transgenic TH-MYCN mouse model of neuroblastoma (NB).

Methods: hMSCs (3×10^6) labeled with DiR, a lipophilic near-infrared dye, were intraperitoneally (i.p.) or intravenously (i.v.) administered to the TH-MYCN mice. hMSC *in vivo* kinetics were assayed using the IVIS® imaging system for 24 h after injection. Immunohistochemistry using human CD90 antibody was also performed to confirm the location of hMSCs in various organs and tumors. Furthermore, the survival curve of TH-MYCN mice treated with hMSCs was compared to a control group administered PBS.

Results: i.p. hMSCs were recognized in the tumors of TH-MYCN mice by IVIS. hMSCs were also located inside the tumor tissue. Conversely, most of the i.v. hMSCs were captured by the lungs, and migration into the tumors was not noted. There was no significant difference in the survival between the hMSC and control groups.

Conclusion: The present study suggested that hMSCs may be potential tumor-specific therapeutic delivery vehicles in NB according to their homing potential to tumors.

© 2016 Elsevier Inc. All rights reserved.

Neuroblastoma (NB) is the most common extracranial solid tumor in children. NB is noteworthy for its broad spectrum of clinical behavior. Although a great improvement in the outcome of low-risk patients has been achieved during the past few decades, the prognosis of high-risk patients remains poor. The 5-year survival ratio of progressed NB remains at around 30%–40% with the present multidisciplinary therapy consisting of surgery, chemotherapy, radiotherapy, and autologous bone marrow transplantation [1]. New approaches to high-risk NB including 13-*cis*-retinoic acid, GD2-targeted immunotherapy, and new molecular-targeted therapy has been developed recently; however, these remain a clinical challenge. Therefore, it is an urgent issue to develop a novel therapy for NB to improve its prognosis in this setting.

Mesenchymal stem cells (MSCs) have the ability to differentiate into multiple lineages of cells, *e.g.*, osteoblasts, adipocytes or chondroblasts *in vitro* [2,3]. Recent studies suggested that MSCs may migrate into damaged neurogenic tissues (peripheral nerves or spinal cord) [4,5] or tumors [6–12]. The potential involvement of MSCs in tumor suppression or progression remains controversial [12]. Regarding the antitumor

effect on NB, the secretion of growth factors from MSCs was reported to promote NB differentiation *in vitro* [13], and an *in vivo* experiment also showed that the direct injection of MSCs into NB resulted in tumor suppression [14]. Therefore, we considered that MSCs might be a potentially novel tool for NB treatment.

The TH-MYCN transgenic mouse is a widely used mouse model of NB, in which the human *MYCN* gene is engineered to be activated in neural crest derived cells of developing mice under the influence of the rat tyrosine hydroxylase promoter [15]. TH-MYCN transgenic mouse was shown to closely replicate the clinical form of the disease both in its behavior as well as in its genetic characteristics [16]. In this study, we analyzed the tumor-homing ability and antitumor potency of MSCs in TH-MYCN mice using an *in vivo* cell-tracking system to investigate the potential of MSCs as a novel tool for NB treatment.

1. Materials and methods

1.1. Human mesenchymal stem cells (hMSCs)

hMSCs derived from the adipose tissue of a Caucasian female were purchased from KURABO, Japan. The cells were cultured using a StemLife™ MSC Comp Kit (KURABO, Japan) with 1% (v/v) penicillin and streptomycin (Nacalai Tesque, Japan). When 70%–80% adherent

* Corresponding author at: Department of Pediatric Surgery, Kyoto Prefectural University of Medicine, 465 Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto, 602-8566, Japan. Tel.: +81 75 251 5809; fax: +81 75 251 5828.

E-mail address: taji@koto.kpu-m.ac.jp (T. Tajiri).

cells were confluent, the cells were trypsinized, harvested, and expanded. The cells were maintained in a humidified atmosphere at 37 °C with 5% CO₂. The cells were then trypsinized and harvested at about 70%–80% confluency to inject into mice. All the experiments were performed using hMSCs at 10–20 passages.

1.2. TH-MYCN transgenic mouse

TH-MYCN transgenic mice were first established by Weiss et al. [15]. TH-MYCN tumors predominantly arise in a paraspinous location. The tumors are highly malignant and grow very rapidly. This model shows almost 100% tumor development and dies shortly after birth in homozygous mice. Therefore, TH-MYCN transgenic mice are used as a model of aggressive NB [16]. The strain of such transgenic mouse was kindly provided by Professor Kenji Kadomatsu (Department of Biochemistry, Nagoya University Graduate School of Medicine). These mice were maintained by continuous inbreeding with 129^{+Ter}/SvJ strain mouse (CLEA, Japan). Mice were housed under a 12-h light/dark cycle in a specific pathogen-free facility with controlled temperature and humidity and allowed access to food and water *ad libitum*. Genotyping was performed according to the modified version of the protocol previously published to identify homozygous or heterozygous mice [17]. All experimental procedures and protocols for the animals conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Committee for Animal Research of Kyoto Prefectural University of Medicine.

1.3. hMSCs labeling and IVIS[®] *in vivo* imaging

Near-infrared (NIR) imaging has become a popular modality that can penetrate biological tissues such as the skin and blood more efficiently than visible light. DiR dye is a lipophilic, NIR fluorescent cyanine dye ideal for staining the cytoplasmic membrane. DiR combined with NIR light has been found to enable real-time observation of stem cell tracking [18]. In this study, hMSCs were labeled with NIR DiR dyes and monitored using an *in vivo* imaging system.

Briefly, 25 mg of XenoLight DiR (PerkinElmer, USA) was dissolved in 3 ml of ethanol, and this solution was diluted in PBS to be 320 µg/ml of working solution. Approximately 3×10^6 hMSCs were incubated in DiR solution for 30 min. After washing twice in PBS, the cells were dissolved in 100 µl of PBS and injected into TH-MYCN transgenic mice. The mice were homozygous, 5 weeks of age, with the hair removed in order to detect the internal signals clearly. The cells were injected into the mice intraperitoneally at the left lower abdominal quadrant (i.p.), or intravenously through a tail vein (i.v.). The *in vivo* distribution of hMSCs in mice was monitored and visualized by the IVIS[®] imaging system (PerkinElmer, USA) with 710 nm excitation and 760 nm emission at 0 min, 10 min, 30 min, 4 h and 24 h after injection. Mice images were taken in the decubitus and lateral positions. The tumors and normal organs (heart, lungs, liver, spleen, and kidneys) were resected from the mice to take *ex vivo* images with IVIS[®] 24 h after injection.

1.4. Immunohistochemical staining

To detect the migration of hMSCs into these tissues histologically, immunohistochemical staining using human CD90 antibody, one of the MSC surface markers, was performed. Briefly, the tumor and organs (heart, lungs, liver, spleen, and kidneys) were fixed in 4% paraformaldehyde for 8 h and embedded in paraffin. The samples were sliced into 3 µm thick sections from paraffin-embedded blocks, deparaffinized with xylene and rehydrated with graded alcohol, and washed in PBS. The sections were incubated in 1:200 of Anti-CD90/Thy1 antibody (Abcam, UK) as a primary antibody for 30 min at room temperature. The ImmPRESS Excel Anti-Rabbit IgG Staining Kit (Vector Laboratories, USA) was used according to the manufacturer's instructions. The

immune complex was visualized using liquid 3,3-diaminobenzidine (DAB) as a chromogen, and the slides were counterstained with hematoxylin. Images were obtained with a microscope BZ-X700 (Keyence, Japan).

1.5. Survival curves of TH-MYCN mouse

To evaluate the *in vivo* effect of hMSCs, survival curves of TH-MYCN mice treated with hMSCs and controls were compared. Approximately 3×10^6 hMSCs in 100 µl of PBS were injected intraperitoneally ($n = 17$) in the hMSC group, while 100 µl of PBS without hMSCs was injected intraperitoneally in the control group ($n = 17$) into homozygous mice of the same age. Injection was scheduled every 7 days from 3 weeks after birth. Survival curves were calculated from the survival days of mice since birth, and the survival rates were compared between the hMSC and control groups.

1.6. Statistical analysis

Statistical analyses were performed using the StatMate V software program (ATMS, Japan). Survival curves were generated using the method described by Kaplan and Meier and statistically compared using the generalized Wilcoxon test. All statistical tests were two-sided, and p values of less than 0.05 were considered to be statistically significant.

2. Results

2.1. IVIS analysis of TH-MYCN mice treated with hMSCs

DiR-labeled hMSCs injected into TH-MYCN mice were clearly identified by IVIS[®] through 24 h after injection, and the fluorescent hMSCs showed different distributions between i.p. and i.v. mice.

As shown in Fig. 1A, fluorescent signals of i.p. hMSCs migrating into retroperitoneal tumor tissues were clearly differentiated from the surrounding tissue at 30 min postinjection. The signals in tumor tissues gradually increased, and remained until 24 h after i.p. We also performed *ex vivo* imaging of dissected organs from i.p. hMSC mice. As shown in Fig. 1B, DiR-labeled hMSCs mainly existed in the tumor tissue, and weak fluorescent signals were found in the liver, spleen, and kidney. No obvious signal was detected in the heart or lung tissues.

On the other hand, the majority of fluorescent signals of i.v. hMSCs was noted in the whole lung 24 h after injection. *Ex vivo* imaging of organs from i.v. mice showed a strong accumulation in the lung and weak signals in the liver and spleen. There was no obvious homing of hMSCs to the tumors on *in vivo* imaging during the 24-h follow-up survey or *ex vivo* imaging (Fig. 2).

2.2. Immunohistochemical staining of hMSCs in the tumor tissues

To verify that the result of IVIS[®] coincided with the histological findings, immunohistochemical staining with an antibody for human CD90 was performed in the tumor section from a mouse 24 h after the administration of i.p. hMSCs. The distribution of CD90 staining was localized to the tumor periphery, not detected inside of the tumor. This result showed the existence of hMSCs migrating inside the tumor (Fig. 3). On the contrary, no positive hMSCs were detected by CD90 staining in the tumor section from a mouse 24 h after the administration of i.v. hMSCs. Furthermore, hMSCs were not detected by CD90 staining in other organs including the liver and spleen after 24 h, or in tumors or other organs 7 days after the day of injection of i.p. hMSCs (data not shown).

Download English Version:

<https://daneshyari.com/en/article/5718343>

Download Persian Version:

<https://daneshyari.com/article/5718343>

[Daneshyari.com](https://daneshyari.com)