

ORIGINAL ARTICLE

Tracking GFP-labeled Transplanted Mouse MSC in Nude Mice Using in Vivo Fluorescence Imaging

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Synopsis

The purpose of this study was to track the fate of GFP-labeled transgenic mouse mesenchymal stem cells (MSC) transplanted in nude mice. Mouse MSC had been collected from tibia of whole body GFP-labeled mice; were transplanted into five nude mice by tail vein injection; and were fed for 4 weeks. After comfort sacrifice, the existence and accumulation of GFP-labeled mouse MSC transplanted in nude mice was examined by in Vivo Florescence imaging. The searched cells were absent in bone marrow regions in dissected long bones (femurs). On the contrary, exposed abdominal cavities revealed that one out of five mice had a small fluorescent node on the liver surface. This means that at 20% probability, transplanted GFP-labeled mouse MSC could circulate in the vein, and settle and multiply in the terminal organ of a host mouse. This finding appears to contribute to future stem cell therapy by homing phenomenon of MSC.

Key words: *stem cell therapy, mesenchymal stem cells, cell transplantation, cell homing*

Introduction

Stem cell therapy is now considered as a new therapeutic method to restore damaged organ including injured liver [1]. One important source of stem cells is mesenchymal stem cells (MSC) [2]. MSC can be easily collected from bone marrows of patients [3]. MSC is chemotactic to damaged organs and tissues which often secrete cytokines and chemokines [4]. Subsequently, MSC can settle and multiply in the damaged zones (namely, by homing phenomenon), and often heal the damaged or inflamed organ and

tissues [5]. This phenomenon has, however, not well been understood, yet.

The fluorescent nature of the cells from green fluorescent protein (GFP)-transgenic mice facilitate the use in many kinds of cell transplantation experiments [6]. Immunity-compromised mice (nude mice) have been used as host animal so that the relation between transplanted GFP-transgenic mouse cells and host nude mouse body could be clarified [7].

The purpose of this study was, therefore, to monitor the fate of GFP-labeled transgenic

mouse mesenchymal stem cells (MSC) transplanted in nude mice by tail vein injection.

Materials and Methods

1. Compliance for animal welfare

All experimental procedures were conducted in accordance with the guidelines established by the Animal Studies Committee at Iwate Medical University (#25-015).

2. GFP-labeled MSC

GFP transgenic (under control of beta-actin promoter) (BCF1) mice [8] were obtained from Center for In Vivo Sciences, Institute for Biomedical Sciences and Library, Iwate Medical University, Iwate, Japan. Bone marrow-derived cells were flushed out from the tibia of three-week-old GFP transgenic mice with phosphate buffered saline (PBS) containing 0.5% fetal bovine serum (FBS) and 2 mM EDTA, and were seeded into plastic cell culture dishes in Dulbecco's modified Eagle medium (DMEM) containing 10% FBS, and cultured for one week in a hypoxia condition (5% O₂, 5% CO₂, and 90% N₂). When the culture reached 80% confluency, cells were re-plated. The cells after eight to ten passages were used in transplantation experiments as MSC [9].

3. Transplantation of GFP-labeled MSC into nude mice

GFP-labeled MSC (1.25×10^5) were transplanted into the tail veins of five 40-weeks old male nude (BALB/cAJcl) mice with 18 G needles. The mice were then fed with free water and diet. Four weeks after implantation, the mice were sacrificed with CO₂ gas.

4. In Vivo Florescence imaging

The existence and accumulation of GFP-labeled MSC in the body of nude mice was examined by in Vivo Florescence imaging device (IVIS Lumina Imaging System, Xenogen, Lincolnshire, U.K.). Fig. 1 indicates GFP reflection from GFP-labeled MSC (1×10^6) in collagen gel transplanted into the back of a nude mouse by 18 G needle, demonstrating the GFP sensitivity. This was a representative result of preliminary experiments.

5. Histological observation

The GFP-fluorescent tissue was fixed in 10 % formalin (Mildform 10N, Wako, Osaka, Japan) for 2 weeks at 4°C; dehydrated sequentially in ethyl-alcohol (70, 80, 90, 95, 100 %) and xylene; embedded in paraffin; and sectioned to a thickness of 5 μm with microtome (TU-213, Yamato Kouki, Tokyo, Japan). The tissue was then stained with hematoxylin and eosin (HE); and examined histologically with fluorescence microscope (All-in-One BZ-9000, Keyence, Osaka, Japan).

Results

Before sacrifice, no florescent images were obtained from the entire surface of hairless nude mice. Immediately following comfort sacrifice, abdominal cavity of the nude mice was exposed so that major still-living-organs and tissues could be examined by in Vivo Florescence imaging. From one mouse, a florescent zone, a node on the liver, was found (Fig. 2) while four mice did not display any florescent region. This means that at 20% probability, the GFP-labeled MSC settled and multiplied at the terminal organ (i.e. a node on one liver) of nude mice four weeks after implantation. The lumen was lined by mono-layer cells. (Fig. 3). The still-living long bones (femur and tibia) of five nude mice were extracted and dissected in the long direction. No florescent image was, however, obtained from bone marrow regions as well as surrounding bones.

Discussion

It became evident from experimental results obtained that injected GFP-labeled MSC were difficult to settle in the body of host healthy nude mice. The homing probability of MSC in nude mice was only 20% that occurred on the terminal end of the liver of one nude mouse. The considerations concerning MSC transplantation into nude mice follow:

It was reported that human MSC were attracted to and settle on the injured liver of nude mice, followed by cure of the injured liver of host nude mouse by transplanted human MSC [10] while most human MSC were lost in healthy nude mouse [11]. It was retrospectively postulated that damaged or inflamed region had been artificially pre-created on nude mice prior to transplantation of GFP-labeled MSC in our study.

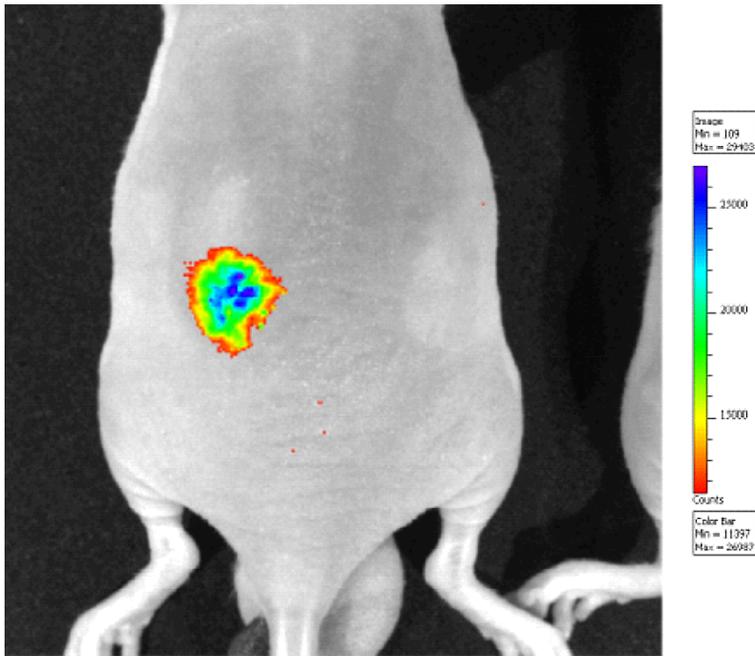


Fig. 1 GFP reflection from GFP-labeled MSC (1×10^6) in collagen gel transplanted in the back of a nude mouse by 18 G needle. Note: Four days after injection, GFP reflection disappeared from the back, indicating that transplanted MSC disappeared; either died or flew away from the back into the blood circulation.



Fig. 3 HE image of a GFP-reflected node on the liver of one nude mouse, indicated in Fig. 2. Note: The lumen was lined by mono-layer cells. Bar = 100 μ m.

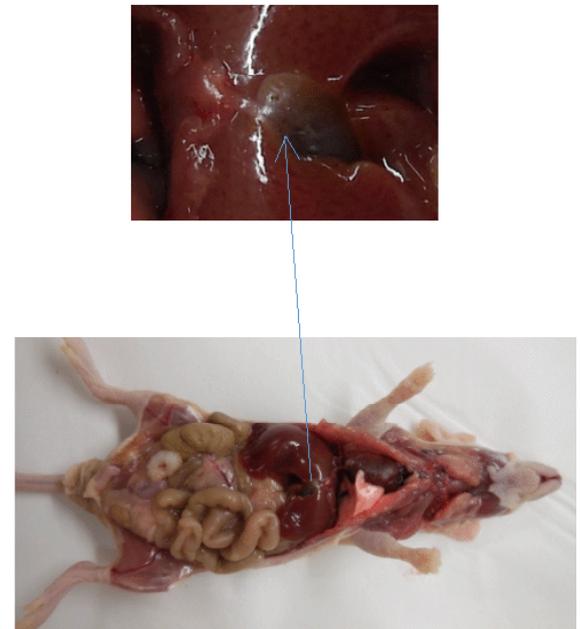
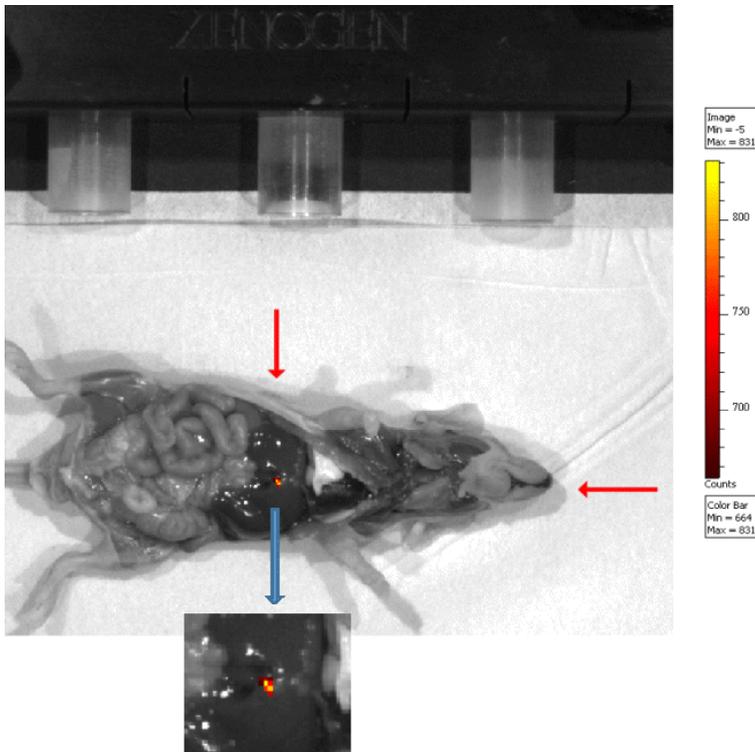


Fig. 2 Left: GFP reflection from a node on the liver of a nude mouse. Note: Intersection of two red arrows indicates the location of GFP illumination from homed GFP-labeled MSC. Blue arrow leads to the enlarged illuminated node. Right: Digital camera image of the exposed abdominal cavity of the same nude mouse. The exposed area corresponds to the GFP-traced image in left side. Blue arrow leads to the enlarged node on the liver.

MSC might be chemotactic to injured organ and tissues that abundantly secrete cytokines and chemokines, leading to settlement and homing of transplanted MSC into the injured region of host nude mouse. Once homing of MSC was established, re-generation of damaged organ and tissues of host nude mouse might have been obtained by transplanted MSC. It has also been reported that MSC can avoid immune attack from the host body [2,12]. Thus, trans-species transplantation and trans-individual transplantation of MSC could be feasible to human patients in future clinics.

In Vivo Fluorescence imaging was effective only on the surface of living tissues and organs. Without large accumulation GFP-labeled cells, GFP reflection from GFP-labeled cells could not be obtained from the surface of host animals. In this study, tiny amounts of GFP-labeled MSC necessitated open-up of the abdominal cavity of nude mice in order to identify homing of transplanted GFP-labeled MSC in one host nude mouse. Because the liver was the terminal end of blood circulation, we found one GFP-florescent node on the liver of one nude mouse out of five mice examined.

The culturing period of MSC in nude mice was four weeks. This duration was set because GFP-labeled MSC could be much multiplied on certain location, beyond the traceable level of in Vivo Florescence imaging device used. In another study [13], transplanted MSC in subcutaneous tissues of nude mice survived over three weeks in the host mouse.

It had been pre-anticipated that transplanted GFP-labeled MSC settled in bone marrows of nude mouse long bones, because stromal-cells derived factor (SDF)-1, a cytokine chemotactic to MSC, was abundantly secreted in bone marrows [14]. The results, however, denied this assumption. It was judged that transplanted GFP-labeled MSC was difficult to home on healthy bone marrows of nude mice. If damage or inflammation was superimposed on bone marrows, the situation might have varied [15].

In the future study, we plan to conduct animal experiments associated with stem cell therapy, which employs GFP-labeled MSC, scaffold/cytokine construct and cell homing phenomenon [16] in nude mouse hosts that are subject to certain body damage.

Disclosure

The authors report no conflicts of interest in this work.

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