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Development of new inbred transgenic strains of rats with LacZ or GFP

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Abstract

The ideal goal of regeneration medicine is to restore form and function to damaged tissues. While stem cell transplantation is considered a promising therapeutic approach, knowing the fate of transplanted cells using appropriate markers is essential. We developed new inbred transgenic rat strains with lacZ and GFP based on the transgenic (Tg) animal technique in rats. These Tg animals expressed most of their marker genes ubiquitously, compared to previous Tg rats. Immunological antigenicity against marker proteins was evaluated using conventional skin grafting, and results suggested lacZ-Tg-derived skin was much less immunogenic than that of GFP-Tg. However, GFP-positive cells from parental transgenic rats were still potential candidates for the study of cellular fate in immune privilege sites, such as the brain. Taking advantage of less immunogenic lacZ, we also examined the role of bone marrow-derived cells (BMDCs) in skin wound healing using an in vivo biological imaging system. Although transplantation of BMDCs enhanced wound healing at the injection site, BMDCs were detected only for a short time, suggesting a transient contribution of autologous BMDC-transplantation in wound healing. Our Tg-rat system may provide great benefits for the elucidation of the cellular process of regenerative medicine, including cell and tissue transplantation.

Keywords: Transgenic rat; LacZ; GFP; Immunogenicity; Regenerative medicine; Cellular trafficking

To restore form and function to damaged tissues, a cell and/or tissue transplantation strategy has emerged as a potential therapeutic approach involving the use of autologous cells [1–3]. To examine the fate of the transplanted cells, however, appropriate and stable marking is required for visualization. While it is easy to use fluorescent dye, there is a drawback in that fluorescent intensity decreases during in vivo cellular proliferation. Based on the genetic engineering technique used in rodents [4–7], it is possible to express stable reporter proteins such as β-galactosidase (LacZ) and green fluorescent protein (GFP) in rats and mice. Cells from transgenic animals that express marker genes provide potential benefits for the stable visualization of cellular fate.

We previously developed lacZ- and GFP-Tg rats on a different genetic background, and demonstrated that these Tg rats were suitable for the monitoring of cellular fate in certain experiments [4,5]. However, these Tg animals have a few weak points: all of the tissues did not express enough marker genes in the established animal lines, although both reporter genes were driven under a ubiquitous CMV enhancer/chicken β-actin promoter (CAG promoter) [8]. Furthermore, transplanted cells

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or tissues were occasionally rejected by immune responses due to a mismatch of the minor histocompatibility complex (mHC) derived from an outbred strain of Wistar rats [4]. Herein we redeveloped both lacZ- and GFP-expressing Lewis rats harboring the same genetic background (MHC haplotype: $RT1^l$). These Tg animals express most of their marker genes ubiquitously, compared to previous Tg rats. We also evaluated immunological antigenicity against these marker proteins using conventional skin grafting, and discovered that the skin from lacZ-Tg rats was much less immunogenic than that of GFP-Tg. Nonetheless, GFP-positive cells from parental transgenic rats were available for the observation of cellular fate in an immune privilege site, such as the brain. We also examined the role of bone marrow-derived cells (BMDCs) in skin wound healing using the less immunogenic lacZ-Tg rat and autologous cell transplantation. Although transplantation of BMDCs enhanced wound healing at the injection site, BMDCs were not detected for a long times despite using a sensitive biological imaging system, suggesting the transient and limited contribution of autologous BMDC transplantation in wound healing. An animal model using our Tg-rat system may provide great benefits for the study of regenerative medicine, including cellular and tissue transplantation.

Materials and methods

Animals. An inbred rat strain, Lewis (LEW) (MHC haplotype: $RT1^l$), was purchased from Charles River Japan, (Yokohama, Japan). LacZ-expressing DA (CAG/LacZ-DA) transgenic rats (MHC haplotype: $RT1^a$) and GFP-expressing Wistar (CAG/GFP-Wistar) transgenic rats (MHC haplotype: $RT1^k$) have been described previously [4,5]. Both of the transgenes were driven under the CAG promoter [8]. All animals had free access to standard chow and drinking water, and were maintained on a 12-h light/dark cycle. All experiments in this study were performed in accordance with the Jichi Medical School Guide for Laboratory Animals.

Generation of transgenic rats. To generate ROSA/LacZ- and CAG/GFP-LEW Tg rats, the authentic microinjection technique was used as described previously [4]. Briefly, the *Nco*I and *Nhe*I fragment (LacZ cDNA) from a pMOD-LacZ plasmid (InvivoGen, San Diego, CA) was inserted into the *Nco*I and *Xba*I sites of a pBROAD2 expression plasmid (InvivoGen) containing ROSA26 promoter [9], and the *Pac*I fragment containing the promoter and coding sequence was injected into the fertilized Lewis rat egg. Transgene expression was examined by β-galactosidase staining (detailed below).

For the GFP-expression plasmid, GFP cDNA from a pEGFP vector (Clontech, Palo Alto, CA) was inserted into a pCAGGS expression plasmid [4,8], and the *Hin*dIII–*Sal*I fragment was injected into the fertilized Lewis rat egg. Transgene expression was confirmed under an excitation light (489 nm). In this study, hemizygous Tg rats (LEW-Tg(Rosa-LacZ)15Jmsk and LEW-Tg(CAG-GFP)ys) were used.

Detection of lacZ expression. Samples were embedded in OCT compound (Miles laboratory, IN), frozen in liquid nitrogen, and cut into thin (8–10 μm) sections under freezing conditions. Sections were fixed with a fixative solution (0.2% glutaraldehyde, 2 mM $MgCl_2$, and 5 mM EGTA) in phosphate-buffered saline (PBS) for 5 min at room temperature (RT), and washed three times in a washing solution (2 mM $MgCl_2$, 0.01% sodium deoxycholate, and 0.02% Nonidet-P40) in PBS. Specimens were treated with a β-gal staining solution (1 mg/ml of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside, 2 mM $MgCl_2$, and 5 mM potassium hexacyanoferrate [III], 5 mM potassium hexacyanoferrate [II] trihydrate) at 37 °C for 1–4 h [10]. Eosin was then used for counter-staining.

To visualize lacZ expression in vivo and in vitro, an in vivo bioimaging system (IVIS) (Xenogen, Alameda, CA) was used. Animals were anesthetized using isoflurane (Abbot, Chicago, IL), and Beta-glo (Promega, Madison, WI) was administered locally to the artificial dermis or skin graft (50 μl of the reagent/animal). LacZ expression photo-images were taken by IVIS and quantified using Living Image software (Xenogen), which measured photon/s/cm^2/steradian. Longitudinal changes in lacZ expression of artificial dermis in the same animal were followed using IVIS.

Skin grafting. Skin transplantation was performed using 6- to 8-week-old female rats. Full-thickness donor skin grafts were transplanted onto a dorsal area of recipients under diethyl ether anesthesia using our coupled skin grafting method [11]. Grafted skins were fixed physically using 5–0 nylon tie-over sutures and bandages. Grafts were monitored regularly by visual and tactile inspection after the removal of the bandage on day 6, as described by Billingham and Medawar [12]. Rejection was defined as the start of contraction of the skin graft according to previously defined criteria [11].

Neurosphere preparation and transplantation into the injured brain. Neurospheres were prepared by culturing cells from the fetus of CAG/GFP-LEW Tg rats as described by Reynolds et al. [13]. GFP-positive fetuses of 14.5 day gestation were obtained from pregnant rats. After decapitation, brains were mechanically excised and collected in cold PBS. Each sample was mined with a razor blade following centrifugation for 500*g*, 10 min. The precipitate was resuspended in PBS containing 0.1% trypsin and 0.04% DNase, and then incubated at 37 °C for 30 min to facilitate dissociation into single cells. The dissociated cells were cultured in serum-free Dulbecco's modified Eagle's medium/Ham's F12 (DEMEM/F12: Gibco, Grand, Island, NY) with basic fibroblast growth factor (bFGF, 10 mg/ml, Sigma) and epidermal growth factor (EGF, 20 mg/ml, Sigma). GFP expression in the cultured cells was analyzed using a flow cytometer.

The left-middle cerebral artery (MCA) in adult male LEW rats was occluded for 60 min using the intraluminal filament method [14]. Five days after MCA occlusion, the rats were anesthetized with ketamine (60 mg/kg, ip) and xylazine (6 mg/kg, ip) and then placed in a stereotactic frame (Type SR-50 Narishige, Tokyo, Japan). GFP^+ neural stem cells (1.1×10^4 cells) were transplanted into the left side of the lateral ventricle through a glass micropipette.

After transplantation of the neurosphere, the host rats were sacrificed at 28 days. Each rat was perfused transcardially with heparinized saline followed by a phosphate-buffered solution containing 4% paraformaldehyde and 7.5% sucrose. The brains were removed and serially sectioned in the coronal plane at a thickness of 2-mm. GFP expression in each section was observed using a fluorescence stereoscopic microscope.

BMDC preparation and artificial dermis grafting. BMDCs were harvested by flushing femurs, tibiae and humeri of rats with ice cold PBS. Red blood cells were lysed with ACK buffer (150 mM NH_4Cl, 10 mM $KHCO_3$, and 0.1 mM EDTA, pH 7.2) at 4 °C for 30 min. Cells were then washed with PBS three times and re-suspended in PBS just before injection.

The head hair of rats was clipped under anesthetic conditions. Full-thickness skin defects (2-cm × 2-cm) were made on the head. The artificial dermis (Terudermis, Terumo, Tokyo, Japan) containing 10^7 BMDC cells (100 μl) was grafted onto the skin defects. As a control group, artificial dermis containing PBS (100 μl) was grafted. For histological evaluation, animals were killed 1, 2, 3, 4, and 8 weeks after transplantation, and specimens were stained using a β-gal solution.

Results

LacZ and GFP expression in inbred (Lewis) transgenic rats

In an effort to examine the expression pattern of newly developed transgenic rats, various organs were removed from transgenic animals and their expression pattern and intensity were determined. Regarding LacZ-LEW transgenic rats, we compared lacZ expression of the previously established LacZ-DA Tg (CAG/LacZ-DA) line [5] with that of the LacZ-LEW (ROSA/LacZ-LEW) line. While skeletal muscle and myocardium revealed strong lacZ expression in CAG/LacZ-DA rats, ROSA/LacZ-LEW rats showed weak and heterogeneous expression in these tissues (Fig. 1). In contrast, ROSA/LacZ-LEW rats showed superior expression in the liver (hepatocytes), skin (epidermis and hair follicles), small intestine, and cartilage, compared to CAG/LacZ-DA rats. Expression patterns of lacZ and intensity are summarized in Table 1. It is notable that bone marrow cells were not stained by this histological staining procedure, but their expression was visualized by the Beta-glo bioluminescent system (described later).

On the other hand, we also evaluated the GFP expression pattern between CAG/GFP-LEW and CAG/GFP-Wistar Tg rats. The new CAG/GFP-LEW line expressed GFP strongly and ubiquitously in most of the organs, compared with the former GFP-Tg line of Wistar [4] (Table 2). As shown in Fig. 2, representative organs such as the brain, liver, and intestine demonstrated higher levels of GFP-expression in the new GFP-LEW Tg line.

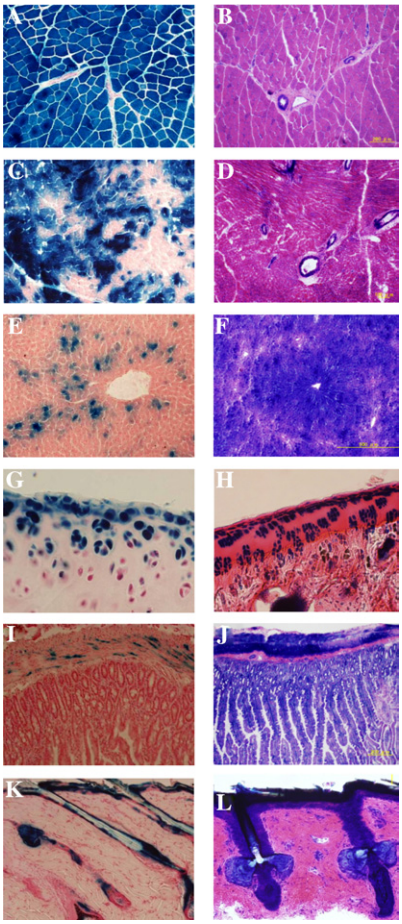

Fig. 1. Differential LacZ-expression pattern between CAG/LacZ-DA and ROSA/LacZ-LEW Tg rats. Tissues skeletal muscle (A,B), myocardium (C,D), liver (E,F), cartilage (G,H), small intestine (I,J), and skin (K,L) were removed from CAG/LacZ-DA (A,C,E,G,I, and K) [5] and ROSA/LacZ-LEW Tg rats (B,D,F,H,J, and L). Specimens were fixed with 0.2%-glutaraldehyde and stained with a β-gal staining solution (original magnification 100×). Experiments were performed two to three times, each with similar results.

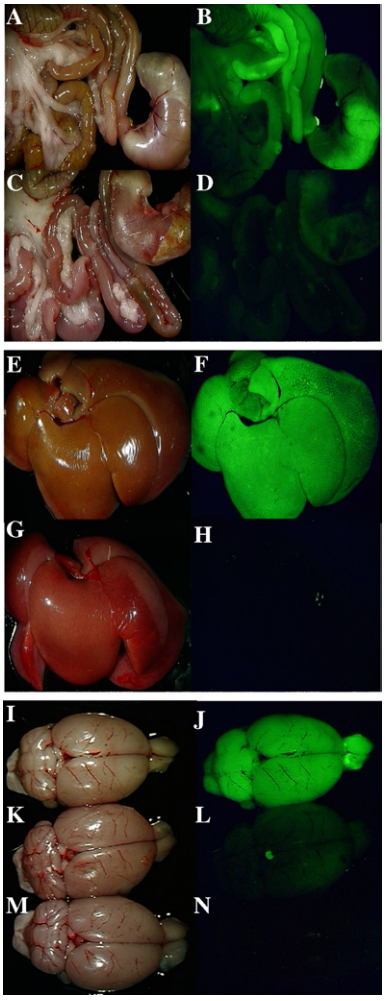

Fig. 2. Differential GFP-expression pattern between CAG/GFP-Wistar and CAG/GFP-LEW Tg rats. Representative organs (small intestine (A–D), liver (E–H), and brain (I–N)) were removed from CAG/GFP-LEW (A,B,E,F,I, and J) and CAG/GFP-Wistar Tg rats [4] (C,D,G,H,K, and L), and examined under a visible (A,C,E,G,I,K, and M) or 489 nm excitation light (B,D,F,H,J,L, and N). (M) and (N) represent wild-type LEW rats (as a negative control). Results derived from one of two independent experiments showing similar results.

Table 1
Differential expression profile between the adult CAG/LacZ-DA and ROSA/LacZ-LEW Tg rats

Organ	CAG/LacZ-DA	ROSA/LacZ-LEW
Brain	–	+
Heart	+++	+
Skeletal muscle	+++	+
Vessels	–	++
Liver	+	+++
Pancreas	++	++
Small intestine	+	+++
Kidney	+	+
Cartilage	++	+++
Nerve	–	–
Skin	++	+++
Bone[a]	–	–
Bone marrow	–	–

[a] Born matrix was LacZ-negative, but osteocytes were LacZ-positive.

Table 2
Differential expression profile of GFP between the adult CAG/GFP-Wistar and CAG/GFP-LEW Tg rats

Organ	CAG/GFP-Wister	CAG/GFP-LEW
Brain	+/–	+++
Eye	++	+++
Lung	+/–	+++
Heart	++	+++
Thymus	+	+++
Liver	+	+++
Pancreas	++	+++
Small intestine	+	+++
Colon	+	+++
Kidney	++	+++
Muscle	++	+++
Skin	+	+++

LacZ is less immunogenic than GFP

The transplantation of cells and tissues is a well-established strategy used in an effort to evaluate the nature of cellular processes. Transplanted cells expressing a marker protein, however, have occasionally disappeared during the observation period, even with syngeneic transplantation, and this has sometimes been explained in terms of immunogenicity [15–17]. Therefore, we evaluated how tissues from newly developed inbred Tg animals possess immunogenic potential. The skin grafting model was used to clarify the antigenic relationship between Tg and wild-type LEW rats because the skin is the most immunogenic organ and the grafting technique provides an easy experimental procedure. The skin grafts of either CAG/GFP-LEW or ROSA/LacZ-LEW rats were transplanted onto a dorsal area of parental LEW rats. The skin of CAG/GFP-LEW Tg rats resulted in graft rejection (Figs. 3A–C); this rejection reaction occurred within 6–9 days after skin transplantation (Table 3), suggesting acute graft rejection. By contrast, Rosa/LacZ-LEW skin graft

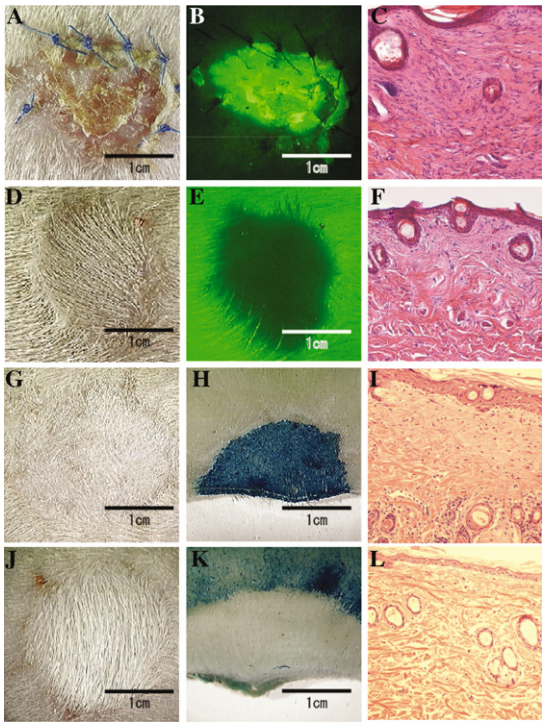

Fig. 3. Skin transplantation between transgenic and wild-type rats. (A–F) represent typical skin grafts between CAG/GFP-LEW and wild-type LEW rats at 14 days after transplantation. The skin graft from CAG/GFP-LEW on wild-type LEW rats under a visible (A) or 489 nm excitation light (B). Note hyperkeratosis and erythema with many cellular infiltrates (C) in the skin graft. The skin graft of wild-type LEW on CAG/GFP-LEW rats under a visible (D) or 489 nm excitation light (E). (F) Microscopic appearance indicates hair follicles without cell infiltrates at 24 days post-transplantation (hematoxylin and eosin (H and E), 200× magnification). (G) The skin graft of ROSA/LacZ-LEW vs. wild-type rats at 28 days post-transplantation. (H) Blue area represents the skin border resulting from β-gal staining. Note the almost histologically intact skin, except for premature hair growth with fewer cell infiltrations (H and E, 200× magnification). The skin graft from wild-type LEW rats placed onto ROSA/LacZ-LEW Tg rats has an appearance equivalent to the skin graft from the wild-type placed onto CAG/GFP-LEW rats at 28 days post-transplantation (J,K, β-gal stain; L,H, and E, 200× magnification).

remained intact for more than 90 days (Figs. 3G–I). Microscopic studies showed that CAG/GFP-LEW Tg skin grafting introduced more intense cellular infiltrate than ROSA/LacZ skin grafting (Figs. 3C and I). These cellular infiltrates represented $CD8^+$ T cells, while $CD4^+$ T cells were scarcely observed using immunostaining (data not shown). Rejection patterns after grafting are summarized in Table 3. Skin grafting of wild-type rats to both Tg animals did not show any rejection reaction more than 30 days after transplantation (Figs. 3D–F, and J–L). These results therefore demonstrate that LacZ is less immunogenic than GFP, and suggest that cells from ROSA/LacZ-LEW Tg rats may be more useful for the evaluation of cellular process through a transplantation technique than those of GFP-LEW Tg rats.

Table 3
Skin graft survival in Lewis rats

Donor	Recipient	*n*	Graft survival (days)	MST	Median (days)
GFP-Tg	WT	6	6, 6, 6, 6, 6, 9	6.5	6
WT	GFP-Tg	5	>60, >60, >60, >60, >60	>60	>60
LacZ-Tg	WT	2	>90, >90	>90	>90
WT	LacZ-Tg	2	>90, >90	>90	>90

WT, wild-type; MST, mean survival time.

Immune privilege site accepts GFP-Tg-derived neural progenitor cells

Notwithstanding the immunogenic response of GFP, the use of cells from GFP-Tg is still attractive for studies of cellular monitoring due to the stable marker expression and easy visualization under excitation light. Since the brain is well known as an immunologically privileged site, we further examined the cellular fate of neural progenitor cells from CAG/GFP-LEW Tg rats without any immunosuppressive drugs in a rat cerebral infarction model. Neural progenitor cells were established from E14.5 of CAG/GFP-LEW Tg rats and maintained in vitro for 20 days under appropriate culture conditions (see Materials and methods). Neurosphere cells strongly expressed GFP (Fig. 4A) and nestin (Fig. 4B), and kept the phenotype as the neural progenitor. The sphere cells were then transplanted stereotactically into the cerebral ventricle space of wild-type LEW rats at 5 days post-infarction. As shown in Fig. 4D, GFP-positive cells accumulated in the cerebral infarction area and were able to survive. Thus, even if GFP is highly immunogenic, GFP-positive cells are still useful and attractive materials for the study of cellular fate in immune privilege sites.

Contribution of ROSA/LacZ-LEW Tg-derived BMDCs to skin wound healing

BMDCs are also utilized in a transplantation strategy to restore and form damaged tissues. Considering the less immunogenic lacZ and sensitive luminescence assay for the expression of lacZ, we evaluated the contribution of BMDCs to skin wound healing. LacZ-expression of BMDCs was visualized in the presence of a luminescent substrate (Beta-glo, Promega), and at least 2000 cells were able to give rise to successful imaging in vitro (5×10^6 photons) (Fig. 5A). Full-thickness skin defects (2-cm × 2-cm) were made on the head of wild-type LEW rats, and BMDCs (10^7 cells/100 μl) from ROSA/LacZ-LEW rats with the artificial dermis (Terudermis) were transplanted into the skin defects of these rats (artificial dermis plus PBS 100 μl was grafted as the experimental control). Substantial luminescent images were only obtained for a few days (Figs. 5B and C), but BMDCs contributed to skin wound healing, as also

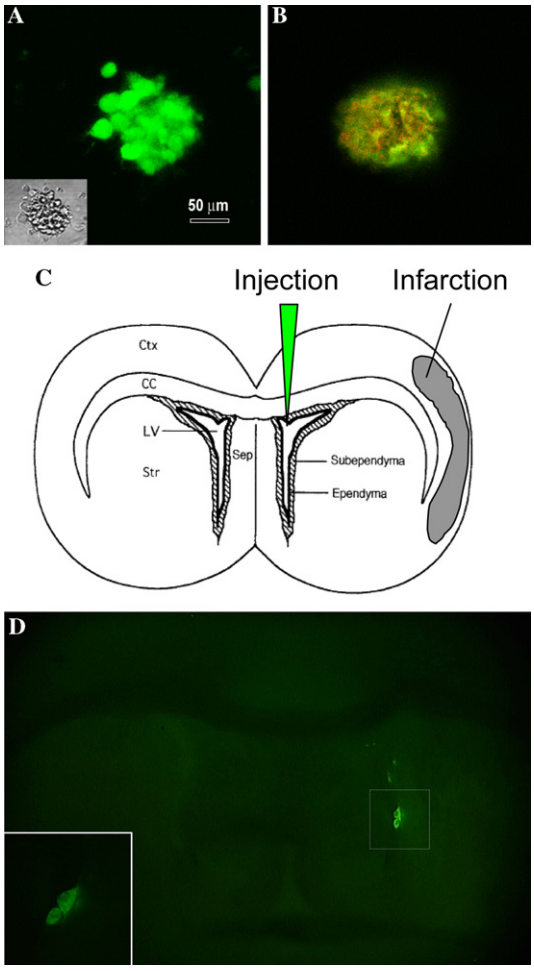

Fig. 4. Migration of neural progenitor-derived cells to the cerebral infarction area. (A) Neural progenitor cells from CAG/GFP-LEW Tg rats express substantial levels of GFP under a 489 nm excitation light 40× magnification). (B) Nestin (red color) was expressed in neural progenitor cells from CAG/GFP-LEW Tg rats (40× magnification). Cells were stained with anti-Nestin mAb (clone# Rat401) (Chemicon International, Temecula, CA) and followed by anti-mouse IgG-Cy3 (Chemicon International). Green color represents GFP. (C) Representative scheme of stereotactic microinjection to the cerebral ventricle. A glass micropipette was placed at an anterior position of 0.0-mm and lateral position of +1.5-mm from the center of the bregma, and cannula depth was 3.3-mm below the surface of the dura matter. The cell suspension (10 μl) was infused over 10 min. Gray area represents the infarction. (D) Accumulation of GFP^+ neural progenitor-derived cells to the cerebral infarction area. Neural progenitor cells (1.1×10^4 cells) from CAG/GFP-LEW Tg rats were injected into the cerebral ventricle, and transverse sections were made at 28 days post-cell transplantation (under a 489 nm excitation light, 10× magnification). One of two independent experiments with similar results.

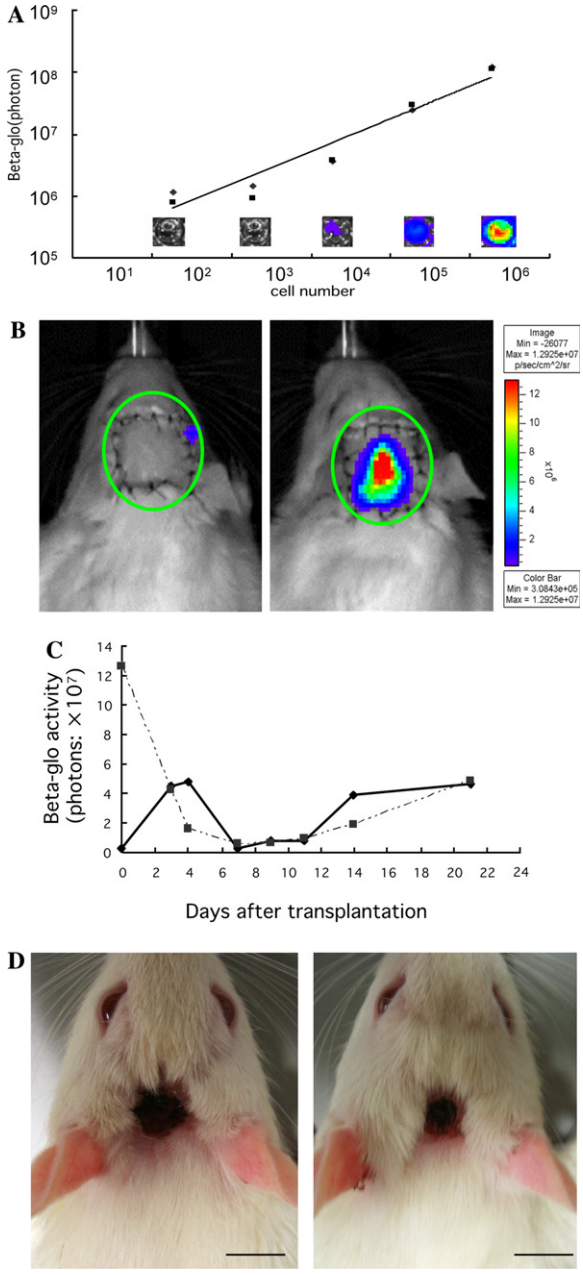

Fig. 5. Effect of BMDCs on skin wound healing. (A) Visualization of LacZ-expressing BMDCs. The number of BMDC cells from the ROSA/LacZ-LEW Tg rat was analyzed by an in vivo bio-imaging system using Beta-glo (Promega). At least 2000 cells contributed to the substantial imaging obtained from the in vitro investigation ($\sim 5 \times 10^6$ photons). Photons were correlated with cell number ($y = 1.3 \times 10^5\ x^r$, $r = 0.53$). (B) In vivo imaging of BMDCs from the ROSA/LacZ-LEW Tg rat at 2 days post-transplantation. Full-thickness skin defects (20-mm × 20-mm) were made on the head of rats, and BMDCs (10^7 cells) from ROSA/LacZ-LEW Tg rats with the artificial dermis (Terudermis) were transplanted onto the skin defects (right). The left panel represents a mock treatment. (C) Time-course quantification of LacZ-expressing BMDCs. The BMDC-treated animals were analyzed by the in vivo bio-imaging system every other day. (D) Representative image of wound healing using BMDCs at 21 days after post-operation. It is notable that the wound area decreased following administration of BMDCs (right panel), compared with that of the mock treatment (left panel). One of two independent experiments with similar results.

recently demonstrated by Yamaguchi et al. [18] (Fig. 5D). The wound area reduction rate was 9.6 ± 2.7% in the BMDC-administered group and 12.9 ± 6.9% in the control group. Nonetheless, their cellular signals were equivalent to the background level after 4 days post-grafting, and cell fate was not monitored throughout the healing period (Fig. 5C). These results therefore suggest that BMDCs can indeed enhance skin wound healing, but their contribution may be low and perhaps transitory.

Discussion

In this study, we established new inbred transgenic LEW rats with LacZ and GFP markers. Three remarkable features were demonstrated in our examinations: (1) ROSA/LacZ-Tg was strongly expressed in the liver, small intestine, cartilage and skin, and expressed to a lesser degree in the heart and skeletal muscle, in comparison with the former established CAG/LacZ-DA Tg line; (2) CAG/GFP-LEW Tg expressed GFP ubiquitously and strongly in all of the tissues we examined; and (3) cells from ROSA/LacZ-Tg were less immunogenic than those of CAG/GFP-Tg. We also demonstrated potential applications for cells from these transgenic animals by means of the cell transplantation technique.

It has been established that a generation of transgenic animals using an appropriate marker gene provides a useful strategy to monitor cellular fate, including migration, proliferation, and differentiation [9,19,20]. These cellular events play significant roles in organ development and tissue regeneration. To this purpose, our research groups and those of others have tried to generate LacZ and GFP transgenic rats. Previous established lines of LacZ-expressing Tg rats did not achieve ubiquitous lacZ expression, and only limited tissues such as the heart and skeletal muscle expressed enough of the marker gene, even though it had been driven under the ubiquitous promoter (CAG promoter) [5]. In this study, we hired the ROSA26 promoter [9] to obtain ubiquitous gene expression. In contrast to our previous line using the CAG promoter [5], lacZ expression was broadened to other tissues, whereas heart and skeletal muscle were less expressive, and it may be argued that such differential gene expression depended on the genomic integration sites of the expression plasmid. Furthermore, the new CAG/GFP-LEW Tg line also gave rise to much stronger GFP expression in more tissues. Indeed, the mouse versions of these marker genes have been used elsewhere [19,20], but their further use for biomedical research such as tissue or organ transplantation may be restricted due to limitations imposed by body size. Rats have provided fabulous animal models for neurological research investigations [21,22]. Thus, the use of these

Tg-rats allows for the potential elucidation of the uncharacterized regeneration process.

GFP is a fluorescent product of the jellyfish (*Aequorea victria*) and is used for a variety of biological experiments as a reporter molecule [19]. While GFP possesses advantages for the non-invasive imaging of viable cells, GFP-positive cells are still considered potential xeno-antigens [15,16]. Our authentic skin grafting experiments showed that GFP induced striking immune responses, but lacZ was less immunogenic than GFP (Fig. 3 and Table 3). Thus, long-term studies of GFP^+ cell transplantation should be limited to immune privilege sites (e.g., brain and testis), and it is much safer to use LacZ-positive cells in other sites.

Furthermore, although it was pointed out that constitutive expression of GFP might affect cellular differentiation and proliferation in human cells [23], our preliminary experiments using neurosphere cells from CAG/GFP-LEW Tg rats did not show that fluorescent intensity (amount of GFP) induced any change in their differentiation status, compared with that of wild-type rats (data not shown). We therefore consider that GFP-positive cells of rats are still a useful tool for the study of neural development and regeneration.

We presented a few experimental applications using newly developed Tg rats. Stereotactic injection of GFP-positive neural progenitors showed migration and accumulation from the safe cerebral ventricle to the infarction area. It has recently been reported that this biological event is strongly associated with the chemokine receptor CXCR4 and the ligand CXCL12/SDF-1 [24,25]. Rat neural progenitors from our transgenic line expressed significant levels of CXCR4 and the cerebral infarction area revealed enhanced mRNA expression of CXCL12 (data not shown). We therefore speculated that those cells were activated and targeted by the interaction of CXCR4 with CXCL12. Furthermore, since there are emerging data suggesting that the axis of CXCR4 and CXCL12 enhances survival of various cells [26,27], it is likely that cell survival, as well as chemotactic migration, may contribute to the accumulation of GFP-positive neural progenitor-derived cells in the infarction area.

As with the case shown by Yamaguchi et al. [18], BMDCs administered to a wound area shortened the healing period. Indeed, a limited number of BMDCs eventually differentiated into myofibroblasts, but their proliferation at wound sites was less frequently observed. Thus, the contribution of administered BMDCs to wound healing may involve an appropriate supply of certain kinds of beneficial growth factors. Although similar results have been reported by others [28–30], our in vivo bio-imaging studies of live animals substantially support the above speculations by providing precise data on the fate of BMDCs at wound healing sites.

Since the rat is larger than the mouse, studies using the former animal make available various physiological techniques that may prove to have biological significance. A variety of rat experimental systems, including disease models, have been developed for over a century. Coupled with recent advances in genetic engineering of the rat, the transgenic rats presented in this report should provide innovative animal tools and help to broaden understanding in new biomedical research fields, such as regeneration medicine.

Acknowledgments

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