# Color-engineered rats and luminescent LacZ imaging: a new platform to visualize biological processes

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**Abstract.** The rat represents an excellent mammalian model for broadening medical knowledge, and a wealth of information on its physiology has been obtained from its use as an experimental organism. Furthermore, its ample body size allows various surgical manipulations that cannot be performed on a mouse. Many rat models mimic human diseases and have therefore been used in a variety of biomedical studies, including physiology, pharmacology, and transplantation. In an effort to create specifically designed rats for new biomedical research and the field of regenerative medicine, we develop an engineered rat system on the basis of transgenic technology and succeed in establishing unique rats that possess genetically encoded color probes: green fluorescent protein (GFP), DsRed2 (red liver), Cre/LoxP (red to green), and LacZ (blue and luminescence). In this work, we highlight their characteristics and describe recent applications for tissue engineering and regeneration. Coupled with recent progress in modern imaging systems, these transgenic rats are providing powerful tools for the elucidation of many cellular processes in biomedical science, and may lead to innovative medical treatments. © *2005 Society of Photo-Optical Instrumentation Engineers.* [DOI: 10.1117/1.2007947]

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## 1 Background

### 1.1 Rat as a Model Organism

Model organisms have been used for over a century to understand a variety of biological processes.[1] Rodents have been utilized to study mammalian physiology and pathology in an attempt to understand disease processes and pathogenesis.[2–4] Though the rat is often viewed as a pest, it was the first mammalian species to be domesticated for scientific research.[5] The first inbred rat strain (PA) was established in the same year that systematic inbreeding began for the mouse,[6] but the mouse became the dominant model for mammalian geneticists under the early support of genetic engineering. Recent advances in genetic manipulation have progressed to a lesser degree for the rat than the mouse,[7,8] but the Rat Genome Project is helping biomedical researchers with a powerful set of tools with which to develop much better models of human disease at the phenotypic and genomic level.[9,10] The role of the rat in medicine has changed from a carrier of infectious diseases to an indispensable tool for biomedical science and drug development.

Based on a wealth of physiological and pharmacological data, the current use of the rat in biomedical research includes transplantation,[11–13] cancer,[14,15] diabetes,[16] and psychiatric disorders,[17] including behavioral intervention[18] and addiction.[19] In drug development, the rat is routinely employed to demonstrate therapeutic efficacy[12,20] and assess toxicity of novel therapeutic compounds before use in human clinical trials.[21–23] The rat therefore represents an important animal model for modern human health and medicine.

### 1.2 Visualization of Biological Processes

In an effort to enhance our understanding of biological processes as they occur in living animals, imaging strategies have been developed and refined that reveal cellular and molecular biological events in real time. The development of molecular tags such as green fluorescent proteins (GFP) from the jellyfish (*Aequorea victoria*) and luciferase from the firefly (*Photinus pyralis*) has accelerated a revolution over the past decade, allowing complex biochemical processes to be associated with the functioning of proteins in living cells.[24,25] In particular, fluorescence imaging, which utilizes fluorescent proteins (e.g., GFP and DsRed2) as internal biological light sources, offers important opportunities for the investigation of a wide variety of biological processes in living cells (e.g., protein geography, movement, and chemistry).[24,26] Results from these applications are providing new, profound insights into cellular processes occurring in the complex environment of the cell.

Cell differentiation or fusion, for example, is the end result of a complex series of biologic events that potentially lead to functional recovery in damaged tissues.[27–30] Visualization of

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their fate needs an appropriate cellular marking procedure. Indeed, it is easy to use fluorescent dye, but there is a drawback in that fluorescent intensity decreases during *in vivo* cell proliferation. Thus, genetically encoded biological probes can act as high-performance tools to visualize cellular fate in living animals.

### 1.3 Transgenic Rat System

Laboratory mice represent a convenient experimental animal for research and monitoring the progression of genetic manipulation.[2] However, recent advances in rat genetic engineering are catching up to those based on mouse technology.[7,8,31] The transgenic rat system may not have been a popular model for biomedical research, but new translational research fields in medicine (such as regeneration medicine) demand an excellent animal model system, in which the larger body size allows us to test various techniques in medicine. A complete survey and analysis of strategies used in mouse models[25,26,32] is beyond the scope of this work, which focuses instead on the use of genetically colored rats to investigate cellular fate in living animals. We highlight our transgenic rat system and demonstrate the following potential applications to particular biologic events, including cellular trafficking, transdifferentiation, and tissue repair: 1. use of GFP-transgenic rats in cellular trafficking and fate, 2. the role of bone marrow-derived cells in liver regeneration using the liver-specific DsRed2 transgenic rat, 3. spatio-temporal gene control by the Cre/LoxP system and cellular fusion, and 4. the role of bone marrow-derived cells in skin wound healing using the LacZ-mediated luminescent technique.

## 2 GFP-Transgenic Rats

### 2.1 GFP Expression Profile

Our first-generation of GFP-Tg rat was derived from a Wistar background. Use of Wistar rats was indeed useful and effective in monitoring cellular fate in certain experiments, including organ transplantation[33–36] and cutaneous biology.[37] For example, it is known that the liver is a tolerogenic organ from an immunological aspect, and that it contains abundant hepatic lymphocytes.[38–40] Long-term tracking of rat donor passenger leukocytes (DPLs) by Inoue et al.[34] showed that a small number of major histocompatibility complex (MHC) class II[+]GFP[+] DPLs (from GFP-Wistar rats) were present at the graft site and in the spleen of recipient rats, but not in the bone marrow. They also demonstrated the substantial correlation of "leukocyte parking" with spontaneous tolerance in rat liver transplantation. Miyashita et al.[37] showed the usefulness of GFP-Wistar rats by characterizing the role of hair follicles as a source of stem cells by implantation of cultured keratinocyte sheets, and they obtained substantial data suggesting that stem cells are present in the induced follicle and that the induced follicle consists of polyclonally derived cells.

However, Tg animals derived from a Wistar strain 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 cytomegalovirus (CMV) enhancer/chicken beta-actin promoter (CAG promoter).[33,41] Furthermore, transplanted cells 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.[33] Thus, Inoue et al.[42] redeveloped GFP-expressing Lewis rats harboring the same genetic background (MHC haplotype: RT1[l]). The new CAG/GFP-LEW line expressed GFP strongly and ubiquitously in most of the organs, in contrast with the former GFP-Tg line of Wistar rats.[33,42] As shown in Fig. 1, representative organs such as the heart, brain, and kidney demonstrated higher levels of GFP expression in the new GFP-LEW Tg line.

### 2.2 Visualization of Cellular Fate Using a New GFP-Tg Strain

#### 2.2.1 Cell trafficking in the cerebral infarction

The use of cells from GFP-Tg is more appealing for studies of cellular monitoring due to stable marker expression and easy visualization under excitation light. Inoue et al.[42] focused on the central nervous system (brain) as an immunologically privileged site, and they examined the cellular fate of neural progenitor cells from CAG/GFP-LEW Tg rats using a rat cerebral infarction model in the absence of immunosuppressive drugs [Fig. 2(a)].[42] Of note is our later suggestion that some marker proteins might occasionally act as immunogens.

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.[42,43] Neurosphere cells strongly expressed GFP [Fig. 2(b)] and Nestin (data not shown), and maintained the phenotype as a neural progenitor. The sphere cells were then transplanted stereotactically into the cerebral ventricle space of wild-type LEW rats at 5 days postcerebral infarction. They showed that GFP-positive cells accumulated in the cerebral infarction area and were able to survive. We also observed a similar phenomenon in cases involving spinal cord injury (in preparation). The therapeutic potential of these neural progenitor cells is of great interest. Investigations of this migration have recently revealed that this biological event is strongly associated with the chemokine receptor CXCR4 and the ligand CXCL12/SDF-1$\alpha$.[44,45] Rat neural progenitors from the prior transgenic line expressed significant levels of CXCR4 [Fig. 2(b)], and the cerebral infarction area showed enhanced mRNA expression of CXCL12 [Fig. 2(c)], suggesting that the cellular migration results from the interaction of CXCR4 with CXCL12. Furthermore, since there is emerging data suggesting that the axis of CXCR4 and CXCL12 enhances survival of various cells,[46,47] it is likely that cell survival, as well as chemotactic migration, is highly implicated in the accumulation of GFP[+] neural progenitor-derived cells in the infarction area.

#### 2.2.2 Regeneration potential of the neonatal intestine

Based on advances in tissue engineering technology and stem cell research, newborn tissue possesses promising potential as a donor source in the field of organ transplantation, even when undergoing cryopreservation. Small intestinal transplantation is still an optional treatment for patients with severe intestinal failure as a means of sustaining life;[48] however, intestinal transplantation remains limited due to chronic donor shortages, and clinical results of intestinal transplantation do not always compare well with other organ transplantations. Interestingly, Tahara et al.[49] reported that newborn intestine possessed attractive and promising potential as a donor graft in the field of intestinal transplantation, even when cryopre-
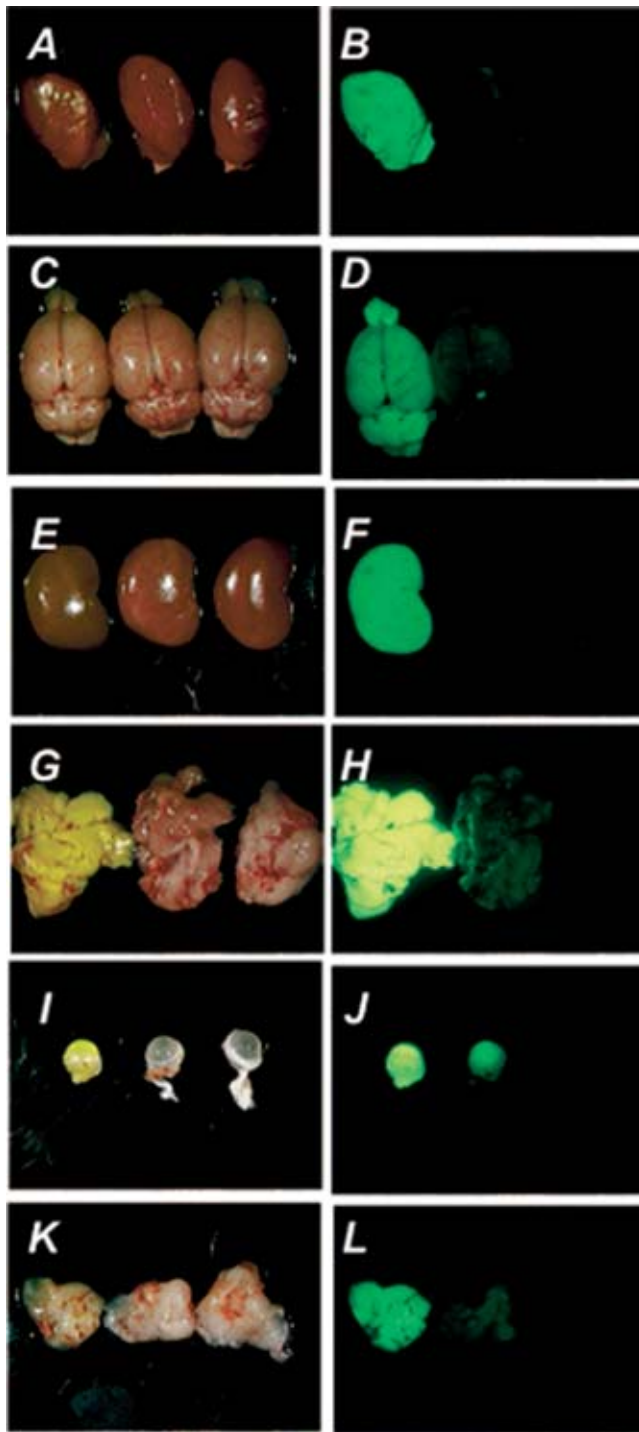
**Fig. 1** Differential GFP-expression pattern between GFP-Wistar and GFP-LEW Tg rats. Representative organs [heart (a) and (b), brain (c) and (d), kidney (e) and (f), small intestine (g) and (h), eye (i) and (j), and thymus (k) and (l)] were removed from CAG/GFP-LEW Tg (left in each panel), CAG/GFP-Wistar Tg (middle in each panel), and wild-type LEW rats (right in each panel), and examined under visible [(a), (c), (e), (g), (i), and (k)] or 489-nm excitation light [(b), (d), (f), (h), (j), and (l)].
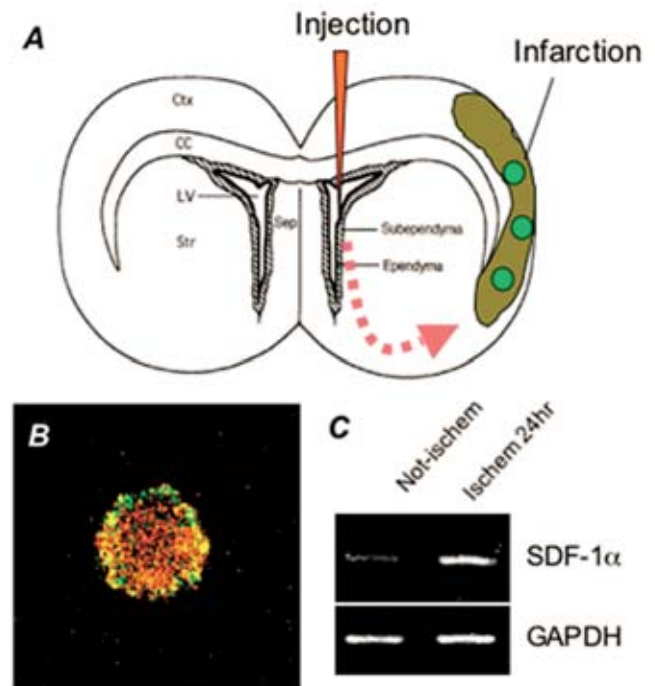


**Fig. 2** Migration of neural progenitor-derived cells to the cerebral infarction area. (a) Representative scheme of stereotactic microinjection and accumulation of GFP+ neural progenitor-derived cells to the cerebral infarction area. (b) Neural progenitor cells from CAG/GFP-LEW Tg rats express substantial levels of GFP under a 489-nm excitation light (green). Expression of the chemokine receptor CXCR4 was visualized by immunostaining using antirat CXCR4 antibodies (red) ($\times 40$ magnification). (c) RT-PCR analysis demonstrates that cerebral infarction enhances chemokine SDF-1a/CXCL12 expression. GAPDH represents an internal control.

served in liquid nitrogen. The regeneration process of newborn intestine was highly implicated in neoangiogenesis in the host, and newborn intestine can revascularize and mature following subcutaneous transplantation without surgical vascular anastomosis.[49] This phenomenon was specifically observed shortly after birth, where the maturation ability of newborn intestinal grafts gradually decreased with time (this regeneration ability peaked within 24 h after birth). Furthermore, they demonstrated surprisingly that the newborn intestinal graft was capable of promoting the survival of a 10-day-old intestinal graft that lacked regenerating potential when both grafts were adhered to each other in parallel [Figs. 3(a)–3(d)].

In their effort to determine whether maturation-incompetent 10-day-old grafts could survive even in the tissue aggregates, 10-day-old intestinal grafts of GFP-LEW rats were processed by chopping, followed by subcutaneous transplantation with processed newborn grafts of wild-type Lewis rats [Fig. 3(e)]. As shown in Fig. 3(f), the control GFP+ newborn grafts showed morphological reconstitution following transplantation, and regenerated epithelium gave rise to a green fluorescence under excitation light at 14 postoperation days. The 10-day-old intestinal grafts from GFP-LEW Tg rats were able to survive in the presence of chopped newborn grafts, and some mucus had accumulated in the lumen [Figs. 3(g) and 3(h)]. These findings demonstrate that the incompetent intestinal graft from tissue aggregates could reconstitute
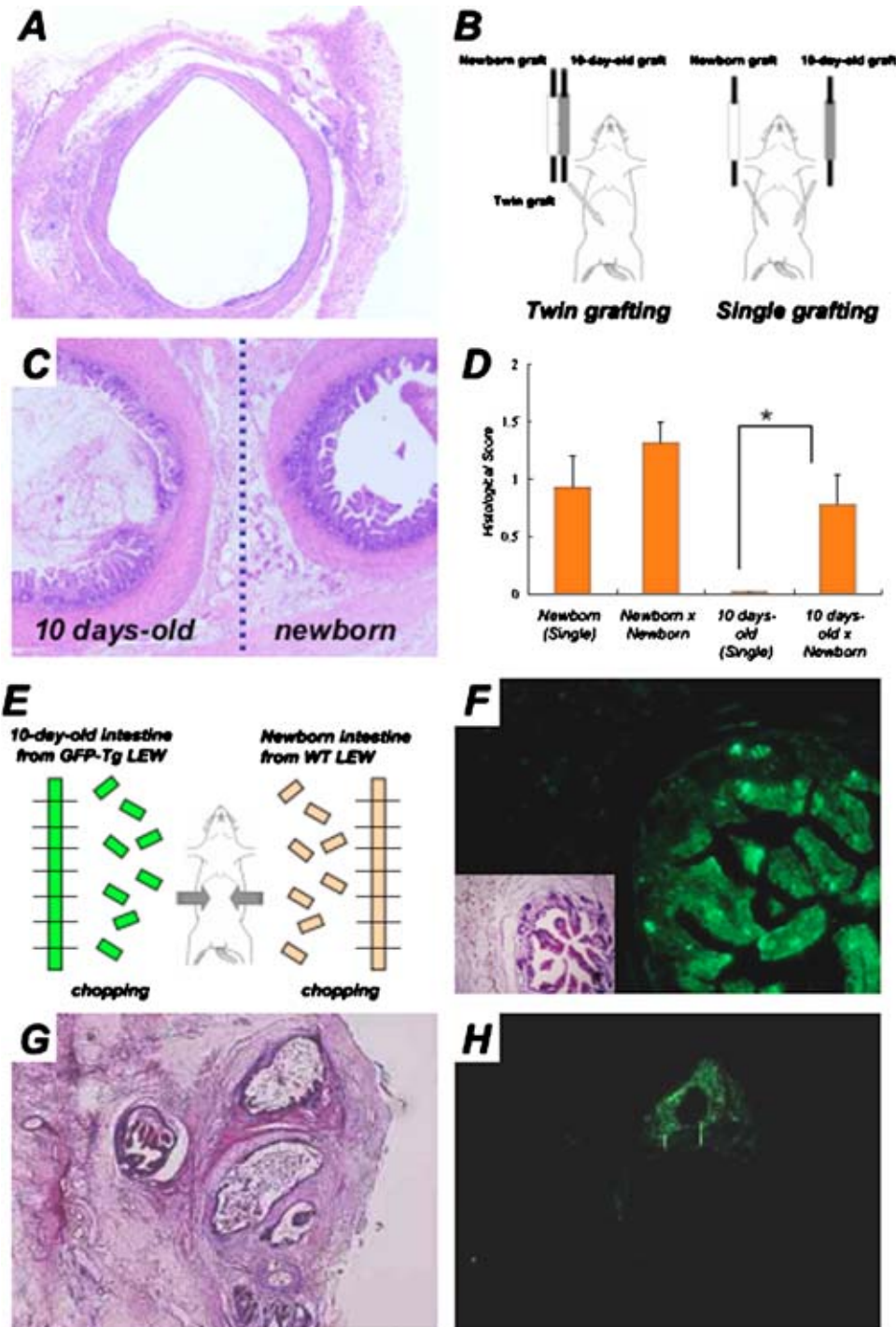
**Fig. 3** Regeneration potential of the rat newborn intestine. (a) A fresh 10-day-old intestinal graft was transplanted in the subcutaneous space of syngeneic rats, and then removed at 14 days post-transplantation [hematoxylin and eosin (H&E), ×20 magnification]. Notably, the mucosal epithelium and villi were stripped away. (b) Representative scheme of the twin grafting. (c) Histological recovery of the 10-day-old intestinal graft in the presence of a newborn graft (twin grafting: H&E, ×20 magnification). The mucosal epithelium and villi were maturated in the 10-day-old intestinal graft. (d) Average histological score of graft. Data represent mean±SED ($n$=13). $^*P$=0.006 versus 10-day-old×newborn. (e) Representative scheme of histological reconstitution of a 10-day-old GFP+ graft from tissue aggregates. (f) Neonatal intestinal grafts from GFP-transgenic Lewis rats were chopped at random, and transplanted into the subcutaneous space of wild-type Lewis rats. A representative specimen is shown at 14 days post-transplantation (H&E, ×40 magnification). (g) 10-day-old intestinal grafts from GFP-transgenic Lewis rats were chopped at random and subcutaneously transplanted with similarly chopped newborn grafts from wild-type Lewis rats. A representative specimen is shown at 14 days post-transplantation (H&E, ×20 magnification). (h) GFP expression of the specimen in (g) (under a 489-nm excitation light, ×20 magnification). Note the substantial expression of GFP from the 10-day-old intestinal graft.

its own tissue in the presence of newborn grafts, and provide new insights into the regenerative role of newborn intestinal grafts.

How are the intestinal stem cells of the graft activated in the recipient (host)? There are two possible explanations that account for the graft and host interaction: 1. host-derived enterocytes are stimulated in the intestinal graft,[50] and 2. bone marrow-derived cells repair damaged epithelia of the graft.[51] Although Tahara et al.[49] also observed some cellular migration of host cells, they were unable to determine which host-derived cells contributed to tissue reconstruction. An initial neoangiogenic event during the early days may stimulate the stem cells toward their maturation.

## 3  DsRed2-Tg Rats

### 3.1  Development of the Liver-Specific Reporter Tg Rat

Recent studies have shown that bone marrow-derived cells (BMDCs) are a potential source for liver regeneration.[52–55] While spontaneous cell fusion is one hypothesis for adaptation of BMDCs into mature hepatocytes,[56,57] it still remains unclear how BMDCs could generate or differentiate into mature cells. As serum albumin is a characteristic protein produced in significant amounts by mature hepatocytes, the albumin gene promoter provides attractive machinery for the reporting of cellular events during liver specification and terminal differentiation. Sato et al.[58] developed a liver-specific reporter Tg rat (Alb-DsRed2) [Fig. 4(a)], in which DsRed2 derived from a red coral (*Discosoma*)[59] is expressed as a reporter protein under the control of the mouse albumin enhancer and promoter.[60]

In accordance with albumin production from hepatoblasts (early progenitor cells), liver-specific expression of DsRed2 was observed from 14.5 embryonic days (ED) in the developmental stage of Tg rats [Figs. 4(b)–4(e)]. DsRed2 expression was observed in the adult liver, but in BMDCs, lymphocytes, and granulocytes (not shown).

### 3.2  Differentiation of BMDCs to Albumin-Producing Cells in the Damaged Liver

In an effort to examine the Alb-DsRed2 Tg rat for liver regeneration studies, we utilized a chemically induced liver damage model using carbon tetrachloride ($CCl_4$). In the acute liver injury model with $CCl_4$ and 2-actylaminofluorene (2-AAF), no DsRed2-expressing cells were observed at 19 days after portal injection of BMDCs. However, a few DsRed2^{+} small cells were recognized in each lower power view section around 60 days after BMDCs injection ($1.5 \pm 1.6$ cells) [Fig. 5(f)]. Immunohistochemical studies also revealed albumin expression in DsRed2^{+} cells, indicating that donor-derived BMDCs migrated and differentiated into the albumin-producing cells in the host liver.

On the other hand, Sato et al.[58] also evaluated the contribution of BMDCs in the chronic liver injury model with $CCl_4$. $CCl_4$ was repeatedly administrated into the subcutaneous space of rats. Several DsRed2^{+} cells were easily identified at 30 and 60 days after portal injection of BMDCs (lower magnification) [Fig. 5(g)]. Different sizes of DsRed2^{+} cells were observed at 60 days after portal injection, and their number also increased in comparison to the acute liver injury model ($89.3 \pm 17.6$ cells versus $1.5 \pm 1.6$ cells). It is unlikely that differentiated BMDCs simply adhered to the damaged liver, since significant DsRed2 expression was not observed in BMDCs or the peripheral blood of Tg rats. They therefore concluded that BMDCs differentiated into DsRed2^{+} albumin-producing cells in conditions involving a damaged liver.

## 4  Double-Reporter Tg Rat: From Red to Green

### 4.1  DsRed2/GFP Double-Reporter Tg Rat

Advanced genetic manipulation has progressed to a lesser degree in the rat than the mouse. However, generations of cloned rats and the mutagenesis-based gene knockout rat yield enormous promise.[7,8] We introduce the rat Cre/LoxP system,[61] which allows examination of a particular gene function in a temporal and tissue-specific manner by means of conditional gene recombination.

The double-reporter Tg rats possess DsRed2 cDNA flanking LoxP sites at both ends as a stuffer and GFP downstream of the DsRed2 [Fig. 5(a)]. Their expression is controlled by a ubiquitous CAG promoter, and DsRed2 is expressed in the rat before Cre/LoxP site-specific excision. Skeletal muscle, the pancreas, heart myocardium, and bronchus show relatively strong DsRed2 expression in adult Tg rats (data not shown). During the developmental stage, DsRed2 expression was detected in embryos at the 2-cell stage (1.5 embryonic days) under 560-nm wavelength excitation light [Figs. 5(b) and 5(c)], showing that the CAG promoter functions in rats in many tissues and from early embryonic development.

### 4.2  Spatio-Temporal Control of Gene Expression by the Cre/LoxP Rat System

To examine functional excision of the DsRed2 stuffer gene by Cre recombinase, we first mated DsRed2/GFP double-reporter Tg rats with the other NCre Tg rats expressing Cre recombinase. In mating a heterozygous DsRed2/GFP double-reporter male Tg rat with a homozygous NCre Tg female rat, blastocysts at 5.5 embryonic days did not express red fluorescence (DsRed2), but showed green fluorescence (GFP), especially in the inner cell mass [Figs. 5(d) and 5(e)]. Newborns of double-Tg (DsRed2/GFP × NCre) rats also displayed ubiquitous green fluorescence [Figs. 5(f) and 5(g)]. These results show that the Cre/LoxP rat system is already functioning during the developmental stage. Sato et al.[61] confirmed that the DsRed2 stuffer gene in the double-reporter Tg rat was completely excluded by Cre recombinase by polymerase chain reaction (PCR) analysis.

This binary double-reporter system allows us to control spatio-temporal gene expression. For example, targeted tissue delivery of the Cre-expressing vector is a promising strategy for the regulation of gene expression. We then introduced Cre-expressing adenovirus (AdV-Cre) into the muscle of adult double-reporter Tg rats by intramuscular injection. Local infection of the Adv-Cre into the muscle changed red-fluorescent muscle fibers expressing DsRed2 to green-fluorescent fibers expressing GFP,[61] implying excision of the DsRed2 stuffer gene.

To enhance genetic control of a specific target organ, we also developed a catheter-based gene transfer system.[61] For
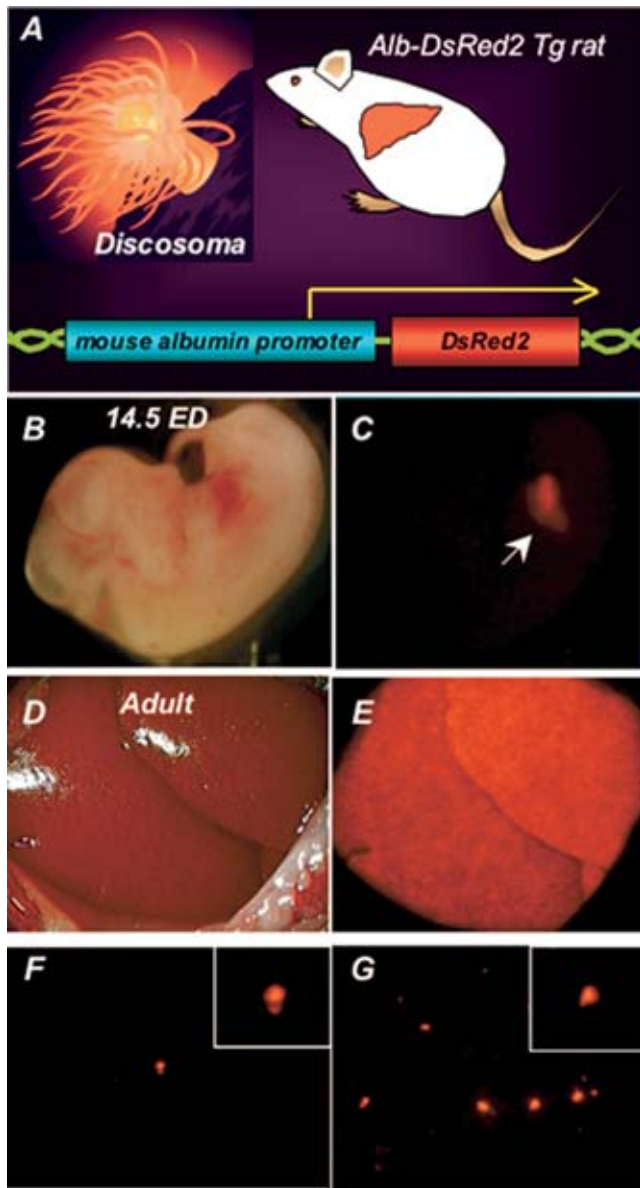
**Fig. 4** The Alb-DsRed2 transgenic rats. (a) Representative scheme of liver-specific DsRed2 expression in the Alb-DsRed2 Tg rat and the mouse albumin/enhancer promoter. (b) A representative embryo of an Alb-DsRed2 Tg rat at 14.5 embryonic days after gestation. (c) Liver-specific DsRed2 expression was observed in (b) under 560-nm excitation light. (d) A representative adult liver (4 weeks old) of an Alb-DsRed2 Tg rat. (e) DsRed2 expression in (b) under 560-nm excitation light. (f) Differentiation of BMDCs derived from Alb-DsRed2 Tg rats into albumin-producing cells with acute liver injury using $CCl_4$ and 2-AAF ($\times$400 magnification). The upper-right panel represents a high-power view ($\times$1000 magnification). (g) Chronic liver injury by repeated administration of $CCl_4$-induced differentiation of BMDCs from Alb-DsRed2 Tg rats into albumin-producing cells ($\times$400 magnification). The upper-right panel represents a high-power view ($\times$1000 magnification).

example, to target the pancreas of living animals, we successfully performed retrograde intrapancreatic duct injection of the adenovirus vector using a fine catheter. As a control delivery, *in vivo* luciferase activity was evaluated following AdV-luciferase infection, indicating pancreas-selective gene
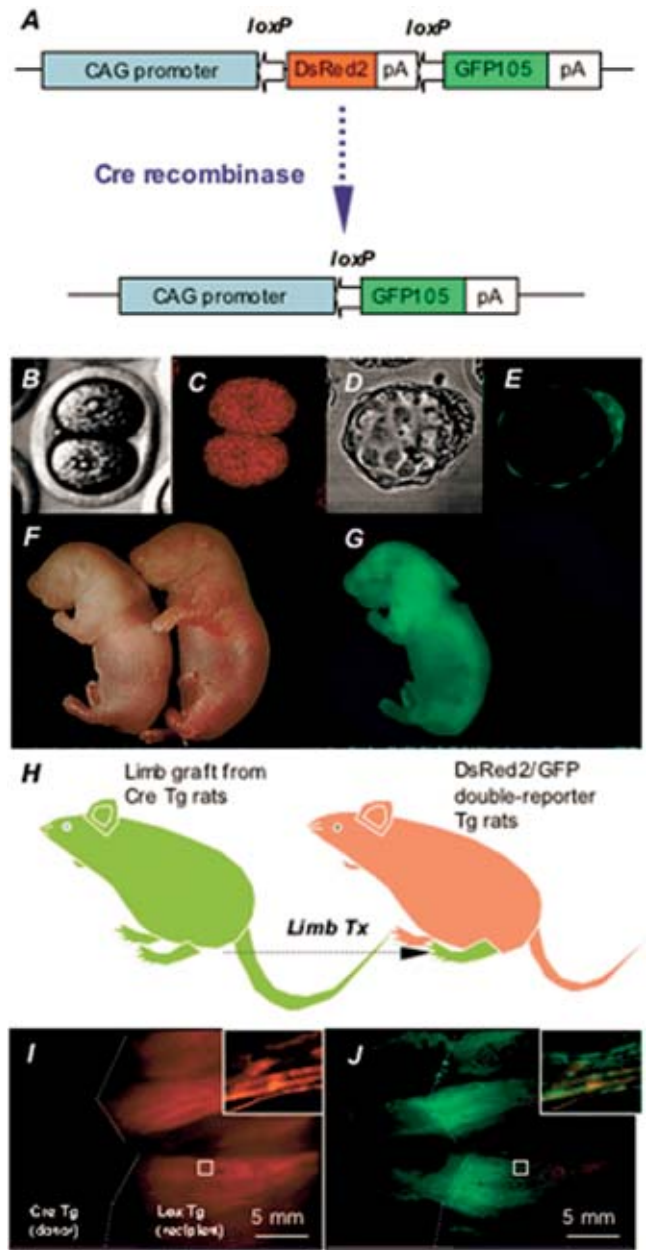


**Fig. 5** The Cre/LoxP-mediated color-conditioning Tg system and cell fusion. (a) Schematic representation of the DsRed2/GFP double expressing gene and Cre recombinase-mediated LoxP site-specific recombination. (b) DsRed2 expression at the 2-cell stage (1.5 embryonic days). (c) DsRed2 expression in (b) was observed under 560-nm excitation light. (d) and (e) By mating a heterozygous DsRed2/GFPdouble-reporter male Tg rat with a homozygous Cre-expressing female Tg rat, blastocysts at 5.5 embryonic days expressed green fluorescence (GFP), especially in the inner cell mass. (e), (f) and (g) A representative newborn of a double Tg (DsRed2/GFP$\times$NCre, left) rat, but not that of a NCre Tg rat (right), displayed ubiquitous green fluorescence (see left newborn). (h) Schematic representation of rat limb transplantation. A hind limb of DsRed2/GFP double-reporter Tg rats was orthotopically transplanted to NCre Tg rats. To prevent rejection, 1 mg/kg of FK506 (kindly provided by Fujisawa Pharmaceutical Company, Osaka, Japan) was injected intramuscularly for 14 days after transplantation. GFP-positive muscle fibers were detected 4 weeks after limb transplantation. (i) DsRed2 and (j) GFP expression were observed under 560- and 489-nm excitation light, respectively. The upper-right corner panel in (i) and (j) represents a high-power magnification ($\times$100) of the indicated square.

expression after AdV administration. This methodology is limited to the pancreas, but it is capable of being applied to other organs (e.g., liver and limbs) for specific gene expression in living animals,[61–63] and leads to the easy control of spatio-temporal gene expression.

### 4.3 *Visualization of Cell Fusion Using the DsRed2/ GFP Double-Reporter Tg Rat*

We employed a method based on Cre/LoxP recombination to detect cell fusion events in the muscles. We transplanted a hind limb of DsRed2/GFP double-reporter Tg rats orthotopically to NCre-Tg rats [Fig. 5(h)]. Four weeks after limb transplantation, GFP[+] muscle fibers were detected in the proximal side of the recipient limb [Figs. 5(i) and 5(j)]. Chromosomal translocation in the anastomosis site was also confirmed by PCR. These results substantially demonstrate that GFP expression resulted from fusion events between donor and recipient myofibers.

## 5 LacZ Tg Rats: From Staining to Luminescent Imaging

### 5.1 *LacZ Expression Profiling and Luminescent Imaging*

As with the fluorescent proteins, cell marking with a LacZ (beta-galactosidase) reporter gene is still widely used in the study of cell lineage and differentiation because LacZ staining has taken advantage of the histological analysis and the easy staining procedure. Apart from the authentic LacZ-staining procedure, we shortly introduce LacZ luminescent imaging in living animals (rats).

Two types of LacZ-Tg rats have been available from our group: one is derived from a DA rat (CAG/LacZ-DA; MHC haplotype: RT1[a]) driven under the CAG promoter,[64] and the other is derived from LEW (Rosa/LacZ-LEW: RT1[l]) driven under the ROSA26 promoter.[42] Both promoters provide the potential for ubiquitous expression. However, their characteristics are a little different. Various organs were removed from transgenic animals and their expression pattern and intensity were determined. Our investigation of LacZ-LEW transgenic rats involved a comparison of LacZ expression of the previously established LacZ-DA Tg (CAG/LacZ-DA) line[64] 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 [Figs. 6(a) and 6(d)]. In contrast, ROSA/LacZ-LEW rats showed superior expression in the liver (hepatocytes) [Figs. 6(b) and 6(e)] and skin (epidermis and hair follicles) [Figs. 6(c) and 6(f)], compared to CAG/LacZ-DA rats.[42] Detailed expression patterns of LacZ and intensity are described in the recent literature by Inoue et al.[42] It is notable that bone marrow cells were not stained by this histological staining procedure (beta-gal staining), but their expression was visualized by a LacZ bioluminescent system.

The most representative bioluminescent probe is luciferase,[25] but the current chemical luminescent technology allows us to visualize LacZ-tagged cells. Beta-Glo™ (Promega, Madison, Wisconsin) was originally developed for *in vitro* assay system to quantify photons from LacZ-mediated
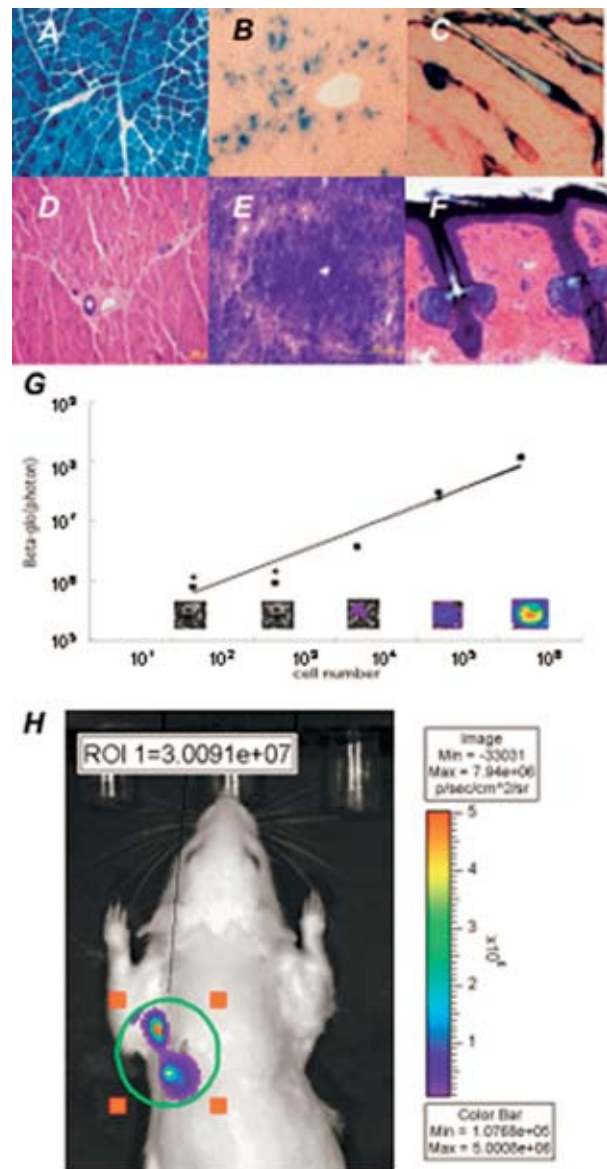


**Fig. 6** LacZ-expression pattern in CAG/LacZ-DA and ROSA/LacZ-LEW Tg rats and its immunogenicity. Tissues [skeletal muscle (a) and (d), liver (b) and (e), and skin (c) and (f)] were removed from CAG/LacZ-DA [(a), (b), and (c)] and ROSA/LacZ-LEW Tg rats [(d), (e), and (f)]. Specimens were fixed with 0.2%-glutaraldehyde and stained with a beta-gal staining solution (original magnification ×100). (g) *In vitro* visualization of LacZ-expressing BMDCs. The number of BMDC cells from the ROSA/LacZ-LEW Tg rat was analyzed by an *in vivo* bioimaging system using Beta-glo™ (Promega). Photons were correlated with cell number ($y = 1.3 \times 10^5$, $x^r$, $r = 0.53$). (h) *In vivo* LacZ imaging of the skin graft from the ROSA/LacZ-LEW Tg rat at 60 days post-transplantation.

reactions, in which the substrate (6-O-beta-galactopyranosyl luciferin) is cleaved by beta-galactosidase to yield free luciferin that is used in a reaction catalyzed by luciferase to generate a luminescent signal to the amount of beta-galactosidase present.[65] Inoue et al.[42] showed that the LacZ luminescent system was available to anesthetized living rats, and they successfully demonstrated *in vivo* LacZ luminescent imaging using an *in vivo* bioimaging system (Xenogen,
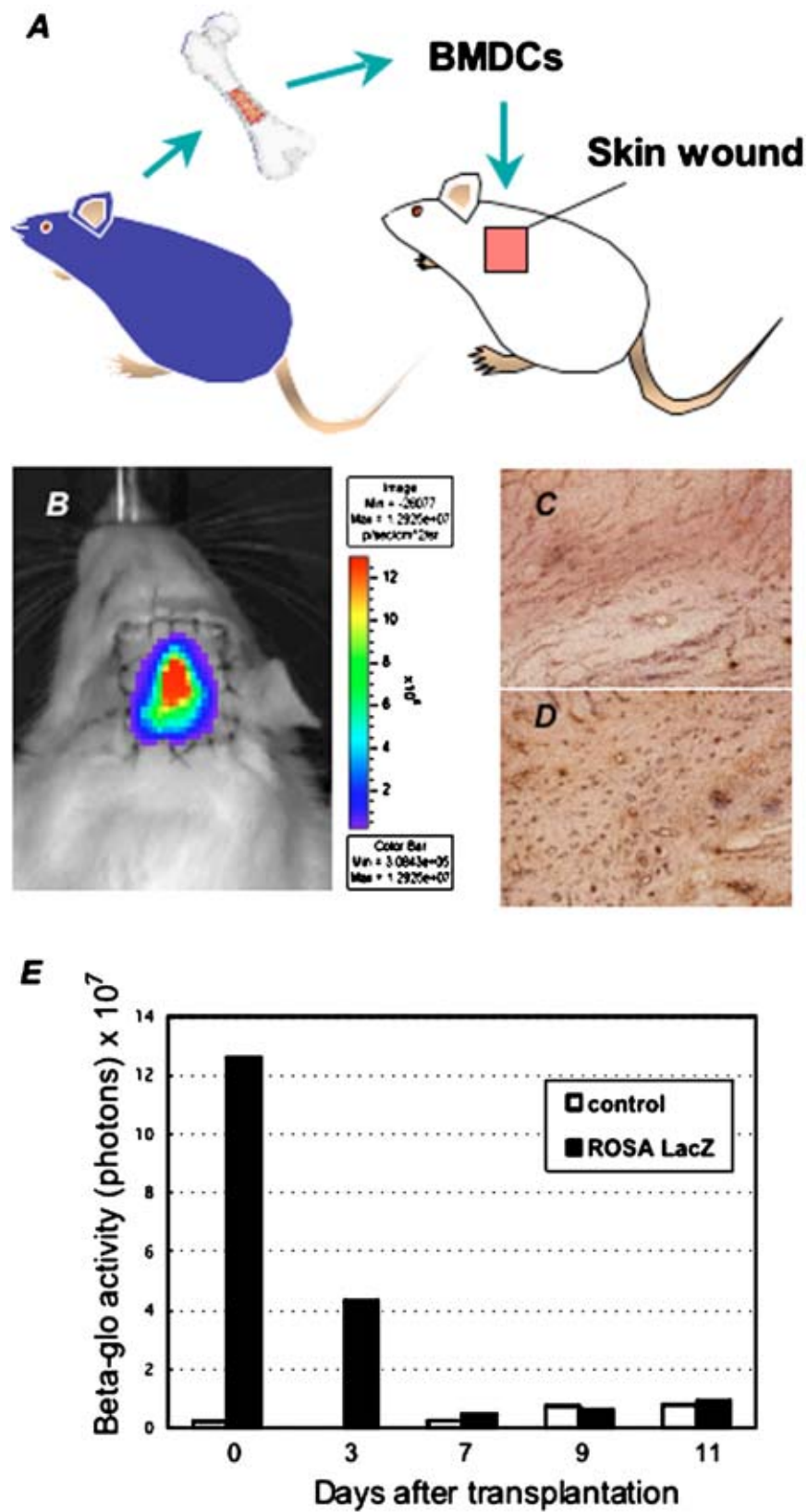
**Fig. 7** Role of BMDCs on skin wound healing. (a) Schematic representation of administration of ROSA/LacZ Tg-derived BMDCs to the skin wound. (b) *In vivo* imaging of BMDCs from the ROSA/LacZ-LEW Tg rat at 2 days post-transplantation. Full-thickness skin defects ($20 \times 20$ mm) were made on the 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. (c) and (d) Immunohistochemistry of the specimens with (c) mock or (d) treatment with BMDCs using antibodies against the von Willebrand factor (vWF) (at day 20 post-treatment). (e) Time-course quantification of LacZ-expressing BMDCs. The BMDC-treated animals were analyzed using the *in vivo* bioimaging system every other day.

Alameda, California). LacZ-expression of BMDCs from ROSA/LacZ-LEW rats was visualized in the presence of a luminescent substrate with at least 2000 cells contributing to successful imaging for the *in vitro* analysis (5 $\times 10^6$ photons) [Fig. 6(g)]. For *in vivo* luminescent analysis, skin grafts of ROSA/LacZ-LEW rats were transplanted onto parental LEW rats (dorsal skin), and the graft imaging was followed by the local injection of LacZ luminescence substrate (50 $\mu$l of the reagent/animal) [Fig. 6(h)]. Although adverse effects by the systemic administration remain to be considered, these results demonstrate that LacZ-tagged cells, coupled with appropriate substrates, are visualized as *in vivo* real-time imaging.

### 5.2 Evaluation of the Contribution of BMDCs to Skin Wound Healing

Considering the sensitive luminescence assay for the expression of LacZ, Inoue et al.[42] evaluated the contribution of BMDCs to skin wound healing [Fig. 7(a)]. Full-thickness skin defects (2 $\times$ 2 cm) were made on the head of wild-type LEW rats, and BMDCs ($10^7$ cells/100 $\mu$l) from ROSA/LacZ-LEW rats with the artificial dermis (Terudermis®) were transplanted into the skin defects of these rats (artificial dermis plus PBS 100 $\mu$l was grafted as the experimental control). Although substantial luminescent images were only obtained for a few days [Figs. 7(b) and 7(c)], it was clear that BMDCs contributed to skin wound healing, as also recently demonstrated by Yamaguchi et al.[66] The wound area reduction rate was 9.6±2.7% in the BMDC-administered group, and 12.9±6.9% in the control group. Neovascularization was enhanced by administration of BMDCs [Fig. 7(d)], and BMDCs administered to a wound area shortened the healing period. Nonetheless, their cellular signals were equivalent to the background level after 4 days postgrafting, and cell fate was not monitored throughout the healing period [Fig. 7(c)]. These results therefore suggest that BMDCs can indeed enhance skin wound healing, but their contribution may be low and perhaps transitory.

Investigations in this arena were undertaken by Takahashi et al.,[64] who evaluated the contribution of BMDCs to myocardial injury (by a cryoinjury) in BMDC (from LacZ Tg rats) transplanted (BMT) rats, in which their results also demonstrate that the contribution of BMDCs is still low. Thus, the contribution of administered BMDCs to damaged tissues may perhaps involve an appropriate supply of certain kinds of beneficial growth factors.

## 6 Conclusion

In this work we highlight remarkable features in a color-engineered rat system and potential application of LacZ *in vivo* imaging: 1. CAG/GFP-LEW Tg expressed GFP ubiquitously and strongly in all of the tissues we examined, the cellular source from GFP-LEW Tg rats provides a high-performance tool for an investigation of cellular fate, especially in immune privilege sites (e.g., brain and testis), and the GFP-Wistar line is also useful in leukocyte-trafficking studies (shortly described by Sato et al.[67]); 2. the Alb-DsRed 2Tg rat was capable of revealing the role played by BMDCs during liver regeneration studies; 3. DsRed2/GFP double-reporter Tg rats allowed the control of gene expression under Cre/LoxP

site-specific recognition, providing effective materials for the elucidation of the cellular fusion process; and 4. ROSA/LacZ-Tg was strongly expressed in the liver, small intestine, cartilage, and skin (the CAG/LacZ-DA Tg line expressed LacZ in significant amounts in the heart and skeletal muscle), and they still represent attractive animal tools, because sensitive LacZ luminescent technology is now available for the visualization of *in vivo* cellular fate in living animals.

Since the rat is larger than the mouse, studies using the former animal make available various physiological techniques. Synergized with modern advances in fluorescent and luminescent imaging, this transgenic rat system provides innovative animal tools and a new platform for a better and profound understanding in new biomedical research, such as regeneration medicine. The spatio-temporal information obtained using the rat Tg system can accelerate the development of experimental therapeutic strategies.

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