Cell Medicine, Vol. 5, pp. 45–51, 2013
Printed in the USA.

2155-1790/13 $90.00 + .00
DOI: http://dx.doi.org/10.3727/215517913X666549
www.cognizantcommunication.com

Review

Bioimaging of Transgenic Rats Established at Jichi Medical University: Applications in Transplantation Research

Takumi Teratani and Eiji Kobayashi

Division of Development of Advanced Therapy, Center for Development of Advanced Medical Technology, Jichi Medical University, Shimotsukeshi, Tochigi, Japan

Research in the life sciences has been greatly advanced by the ability to directly visualize cells, tissues, and organs. Preclinical studies often involve many small and large animal experiments and, frequently, cell and organ transplantations. The rat is an excellent animal model for the development of transplantation and surgical techniques because of its small size and ability to breed in small spaces. Ten years ago, we established color-imaging transgenic rats and methods for the direct visualization of their tissues. Since then, our transgenic rats have been used throughout the various fields that are concerned with cell transplantation therapy. In this minireview, we summarize results from some of the groups that have used our transgenic rats at the bench level and in cell transplantation research.

Key words: Cell transplantation; Transgenic rat; Bioimaging; Human injury models

INTRODUCTION

The Japan Society for Organ Preservation and Medical Biology (JSOPMB) is a multidisciplinary assembly drawing from the fields of medicine, pharmacology, agricultural science, engineering, and biology for the purpose of the functional analysis and preservation of organs, tissues, and cells. This type of cross-disciplinary society is different from those that specialize by organ, and it has shown that collecting information across disciplines is extremely important.

For decades, the rat has been used as an animal model of human disease and in drug discovery research, but it is more difficult to use in transgenic studies than the mouse. Recently, rat embryonic stem cells have been established from the inner cell mass of blastocysts, and induced pluripotent stem cells have been produced from skin fibroblasts by using several inhibitors of cell metabolism containing histone modification and/or protein phosphorylation (5,21–23,31). Genome editing has been facilitated by the development of zinc finger nucleases and transcription activator-like effector nucleases (11,15). These new technologies have received attention for their usefulness in drug discovery and development. More than 10 years ago, we created rats genetically modified for superior imaging in studies of cell and organ transplantation (10,30), and now these fields have entered a new age with the validation of these approaches.

In cooperation with the JSOPMB, we were asked to share our transgenic (Tg) rats for cell transplantation research with the global scientific community (19,30). In this minireview, we summarize the studies that have used our green fluorescent protein (GFP)-Tg rats and luciferase-Tg rats so far.

RESEARCH USING GREEN FLUORESCENT PROTEIN (GFP)-Tg RATS

Stem cell transplantation is a promising therapeutic approach for several organ and tissue diseases. Research on the viability of transplantation grafts has been important for the development of both basic research and clinical applications. For example, at the basic research level, the monitoring of the kinetics of transplanted cells is important, and there is the need for stably labeled cells that can easily be imaged. We successfully developed Tg

Received June 1, 2012; final acceptance August 20, 2013. Online prepub date: August 29, 2013.
Address correspondence to Eiji Kobayashi, M.D., Ph.D., Division of Development of Advanced Therapy, Center for Development of Advanced Medical Technology, Jichi Medical University, 3311-1 Yakushiji, Shimotsukeshi, Tochigi 329-0498, Japan.
Tel: +81-285-58-7583; Fax: +81-285-44-6219; E-mail: eijikoba@jichi.ac.jp

inbred rats ubiquitously expressing GFP, which are an ideal donor source of stably labeled cells (10) for use in organ transplantation research (26). The GFP construct was expressed in the Wistar rat strain, chosen as the transgene carrier because of their high egg production (20). However, the intensity of GFP expression in the Tg rats could not be controlled. In collaboration with PhoenixBio (YS New Technology, Tochigi, Japan), we subsequently established the brightest GFP-Tg rat strain to date by using instead inbred Lewis (LEW) rats (14).

A group from Hannover Medical School (Hannover, Germany) has studied the therapeutic effect of bone marrow-derived progenitor cells on focal and segmental glomerulosclerosis (FSGS) by using enhanced GFP (eGFP)-Tg rat-derived bone marrow and wild-type rats (25). The loss of podocytes is a key event in the progression of FSGS, and so their potential replacement by bone marrow transplant is of interest. Female Wistar rats underwent whole-body irradiation followed by an eGFP-positive bone marrow transplant to replace the endogenous bone marrow destroyed by the irradiation, creating chimeric rats with eGFP-positive bone marrow cells. Three experimental rat models of FSGS were produced 8 weeks later, using three different forms of renal injury: puromycin aminoglycoside nephropathy (PAN), subtotal nephrectomy (SN), and uninephrectomy (UN). The PAN model induces primary damage to the podocytes, while the SN and UN models induce secondary damage. Segmental glomerulosclerosis (SGS) and global glomerulosclerosis (GGS) were observed in the PAN and SN models after 23 days, but no homing of the eGFP-positive bone marrow progenitor cells or podocyte lineage differentiation was observed, suggesting that if any new podocytes were created as a repair mechanism, they did not originate from the bone marrow.

Our GFP-Tg LEW rat has also been used in the field of neuroscience. A Freiburg (Germany) group has investigated the potential of these rats as donors of tissue for neural stem cell transplantation (19). A single-cell suspension of ventral mesencephalon (VM) was obtained from GFP-Tg or wild-type LEW rat embryos on days 12.5 and 14.5. The cells were plated on culture dishes with several concentrations of the neurotoxin 6-hydroxydopamine. Survival was similar between GFP-Tg and wild-type VM cells. Next, GFP-Tg rat VM cells were transplanted into a 6-hydroxydopamine-induced unilateral medial forebrain bundle lesion rat model of Parkinson's disease. There was no significant difference in either graft volume or fiber density between one recipient group that was immunosuppressed and another one that was not. In the grafts, 13.3% of the cells were positive for glial fibrillary acidic protein (GFAP), 56.1% were positive for neuronal nuclear antigen (NeuN), and 1.9% were positive for tyrosine hydroxylase (TH). Moreover, 70.6% of the GFAP-positive cells, 86.9% of the NeuN-positive cells, and 80.1% of the TH-positive cells in the grafts coexpressed GFP. This provides evidence that our GFP-Tg LEW rat can be used as a cell donor to precisely investigate the fate of transplanted neuronal stem cells in models of neurodegenerative diseases such as Parkinson's disease.

An Aachen (Germany) group has presented the successful use of Perimaix (PM; a collagen-based microstructured scaffold) for the repair of rat sciatic nerve defects using our GFP-Tg LEW rat (4). A GFP-Tg rat-derived Schwann cell (SC) suspension was seeded on the PM scaffold and cultured for 16–24 h. The scaffold was then placed into the epineurial tube of wild-type LEW rats to bridge the proximal and distal sciatic nerve stumps so that the implant abutted both nerve stumps where the nerve fascicle was detached. At 6 weeks after transplantation, GFP-positive SCs were detected on the PM scaffold by using confocal laser scanning microscopy. Axon regeneration was also observed. This study shows that SCs seeded on a PM scaffold are useful for the repair of peripheral nerve defects.

In previous studies, nerve defects were created in recipient rats by transplanting eGFP-Tg rat nerve grafts that either had or had not been pretreated by freeze–thaw cycles into wild-type rats (2,18). Sciatic nerve specimens were examined under excitation light at 1, 2, and 3 weeks after transplantation. The eGFP-positive area clearly expanded both proximally and distally beyond the transplant site in the transplanted nerve grafts (Fig. 1). Antegrade and retrograde migration of columns of eGFP-positive cells that had formed in the degenerated graft was observed in the recipient nerve. SCs originating from the graft were S100-positive/eGFP-positive cells (Fig. 2A), and these double-positive cells were seen in the regenerating axons of the recipient nerve (Fig. 2B). Thus, we were able to visualize how grafted cells could contribute to axonal regeneration beyond a peripheral nerve anastomosis.

RESEARCH USING LUCIFERASE (LUC)-Tg RATS

Transplantation research involving the use of stem cells requires an appropriate in vivo visualization system to monitor cellular fate. A new field of in vivo imaging is being developed that utilizes luminescence biotechnology for the real-time visualization of complex cellular processes in living animals. We created inbred Tg LEW rats expressing firefly luciferase that did not show an immune response to the luciferase (9).

Mesenchymal stem cells (MSCs) have potential as cell therapy for several organ and tissue diseases (3,6–8,13,28,29). We used MSCs derived from Luc-Tg LEW rats to explore the impact of the cells against warm liver ischemia/reperfusion (I/R) injury and hepatectomy (16). Initially 70% partial ischemia was induced in wild-type

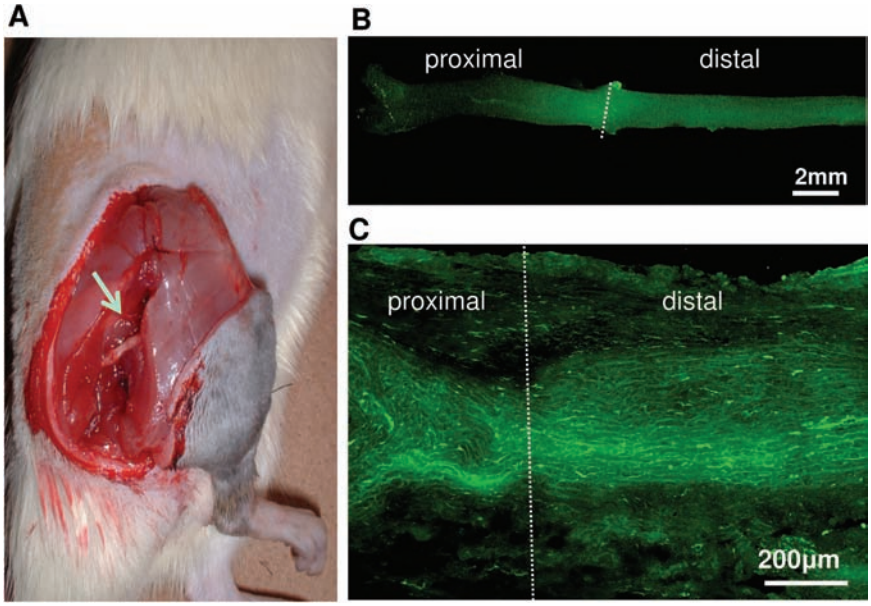

Figure 1. Imaging of an enhanced green fluorescent protein (eGFP)-transgenic (Tg) rat-derived lower extremity transplantation. (A) Transplanted lower extremity in a wild-type Lewis (LEW) rat. Arrow indicates the location of the nervus ischiadicus suture. (B) Macroscopic image. (C) Fluorescence microscopy shows that transplanted eGFP-Tg LEW rat-derived Schwann cells extended throughout the area of the nervus ischiadicus. Broken lines indicate suture site. Figure reprinted from Ajiki et al. (1), courtesy of the Japanese Society for Surgery of the Hand.

LEW rats by clamping the portal vein, the hepatic artery, and the bile duct supplying the median and left lateral lobes of the liver. Reperfusion was initiated after 40 min, and the nonischemic lobes were resected. Subsequently, 200 μl phosphate-buffered saline (placebo) or 1×10^6 Luc-Tg rat-derived MSCs were administered via the portal vein. In vivo luminescence imaging demonstrated peak luciferase activity of transplanted Luc-Tg rat-derived MSCs in the remnant liver, 24 h after reperfusion, which gradually declined over time. The injury-induced rise in

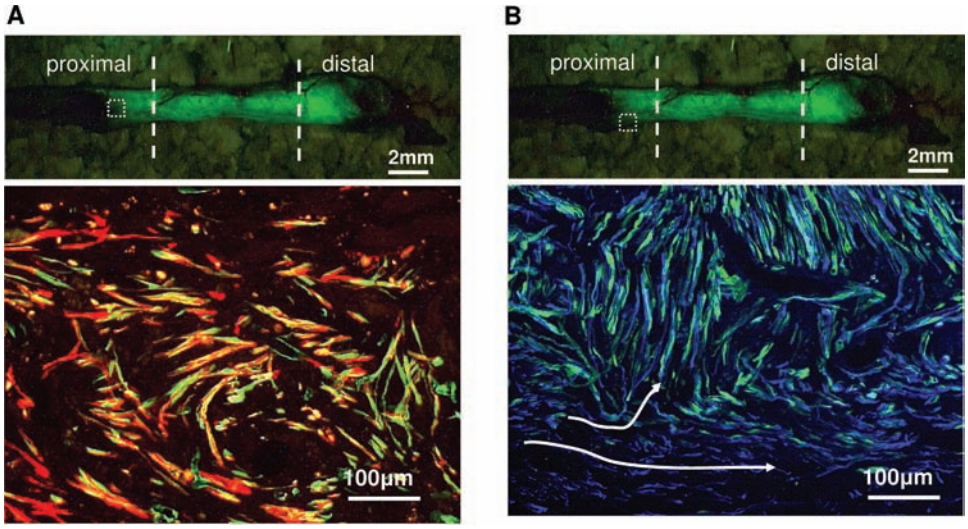

Figure 2. Fluorescence imaging of transplanted eGFP-Tg LEW rat-derived nervus ischiadicus in wild-type LEW rat. (A) Macroscopic evaluation: transplanted eGFP-Tg LEW rat neural cells migrated into the area of the recipient's wild-type LEW rat neural cells (top). Broken lines indicate suture sites; broken square shows the area stained with an anti-S100 (Schwann cell-specific marker) antibody (red; bottom). (B) The transplanted eGFP-Tg LEW rat-derived nervus ischiadicus is shown again for comparison (top). Host neural cells were stained with an anti-neurofilament antibody (blue; bottom). Arrows indicate areas where Schwann cells (green) induced the extension of host neural cells. Figure reprinted from Ajiki et al. (1), courtesy of the Japanese Society for Surgery of the Hand. Figure 2B reproduced from Kimura et al. (18), with the permission of Elsevier.

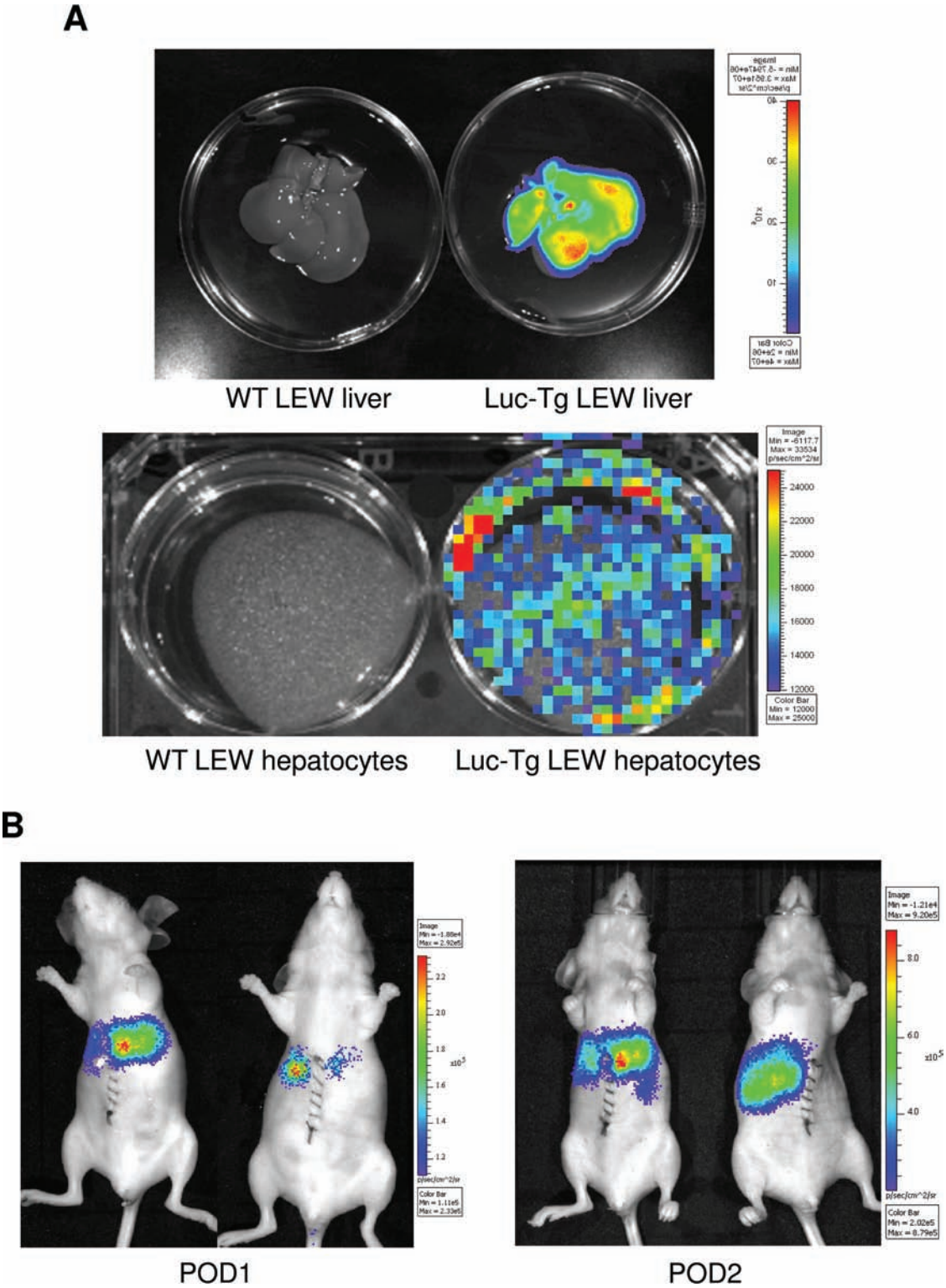

Figure 3. Visualization of relative photon intensity in Luc-Tg rat with an in vivo bioimaging system. (A) Comparison of whole liver (top) and cultured hepatocytes (bottom) from wild-type (WT) and luciferase transgenic (Luc-Tg) LEW rats. (B) Evaluation of relative photon intensity in carbon tetrachloride (CCl_4)-treated nude mice after transplantation of Luc-Tg LEW rat-derived hepatocytes.

serum alanine transaminase levels significantly declined following MSC infusion. The degree of vacuolar degeneration was also significantly reduced, while liver mass significantly increased implying accelerated remnant liver regeneration in the MSC group. In addition, significantly fewer TdT-mediated dUTP-biotin nick end-labeling (TUNEL)-positive cells were observed in the MSC group compared with the controls. The report suggests that MSCs could be potentially beneficial in protecting against warm I/R injury-induced hepatocyte apoptosis, besides enhancing liver regeneration.

MSCs are able to differentiate into several cell types of mature tissues and organs, and this differentiation is defined by their interaction with the stem cell niche. By using Luc-Tg LEW rats, we established a novel technique that uses a donor developmental program to prompt endogenous MSCs to differentiate into mature erythropoietin (EPO)-producing cells (24). In this technique, immature metanephros are transplanted into a host omentum to provide a stem cell niche that recruits endogenous cells during its development and stimulates their differentiation into kidney resident cells. We showed that injected Luc-Tg rat-derived MSCs are recruited and begin to express rat EPO and detection of photon intensity in the mouse-derived metanephros. This suggests that injected MSCs differentiate into EPO-producing cells only in the developing metanephros as a result of interaction with the stem cell niche.

Research into hepatocyte transplantation therapy is also being undertaken. Luc-Tg rat-derived hepatocytes isolated by using a collagenase perfusion protocol were observed with an in vivo imaging system and seen to have photon intensity levels comparable with those seen in whole liver (Fig. 3A). Nude mice treated with carbon tetrachloride (CCl_4; a fulminant hepatitis model) were injected with Luc-Tg rat-derived hepatocytes through the portal vein at 1.0×10^6 cells/mouse, and changes were assessed over time (Fig. 3B). Luc-Tg LEW rats were able to provide sufficient numbers of hepatocytes.

The use of native organs as scaffolds for the regeneration or engineering of new organs has also been attempted. We successfully developed a strategy to create engineered chimeric livers (CLs) with autologous or allogeneic hepatocytes by using xenogeneic scaffolds (12). First, hepatocytes were isolated from Luc-Tg LEW rats and transplanted into albumin enhancer/promoter-driven urokinase-type plasminogen activator/severe combined immunodeficient (uPA/SCID) mice to produce CLs. To assess the repopulation of the transplanted rat hepatocytes in the uPA/SCID mice, the spreading of Luc-positive areas was observed by using in vivo bioluminescence imaging. Next, the CLs were harvested from the uPA/SCID mice and connected to the jejunum of recipient wild-type rats, with the recipient's liver being left intact. This was followed by immunosuppression with tacrolimus for 4 months. The significant increase in cells positive for the cell proliferation marker Ki-67 in the CL and in the remnant native liver at 2 days after left lobectomy in the CL recipients compared with prehepatectomy samples provide support for a proliferative potential of transplanted CLs.

Recently, we evaluated several organ preservation solutions by using isolated islet cells (27). Primary islets were obtained by handpicking them from Luc-Tg LEW rat pancreata. Relative photon intensity measures were used to determine the viability and living condition of islets preserved with different preservation solutions.

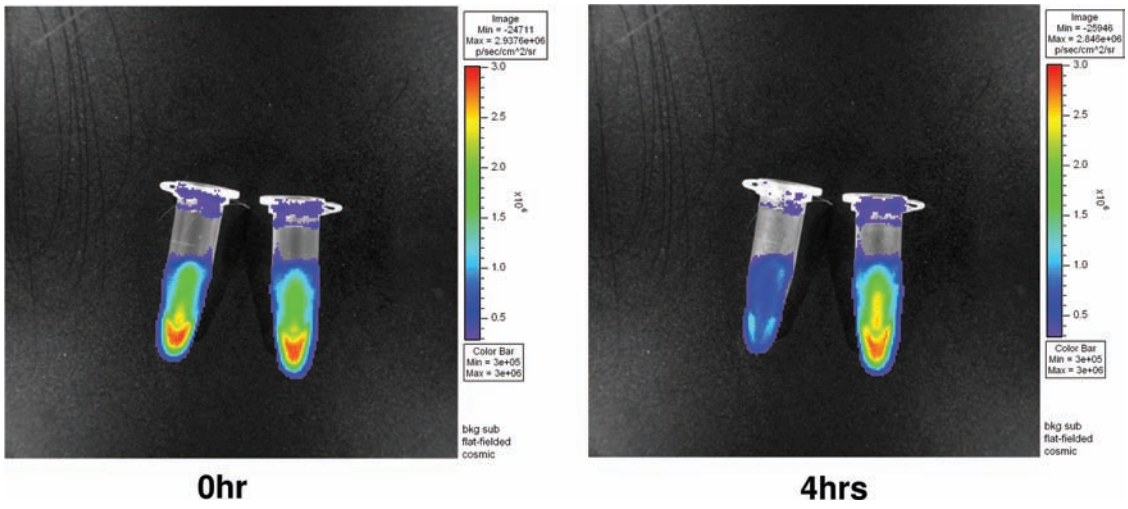

Figure 4. Localization of Luc-Tg rat-derived islets. Isolated islets were preserved in organ preservation solution [extracellular-type trehalose-containing Kyoto solution (ET-K)] at 37°C for 4 h. The left and right panels show relative photon intensity at 0 and 4 h, respectively. In each panel, the sample tube on the right contains ET-K solution and mesenchymal stem cell-secreted factors, while the tube on the left contains ET-K solution only.

Islets preserved for 1 day in extracellular-type trehalose-containing Kyoto (ET-K) or University of Wisconsin (UW) solution were significantly more viable than cells preserved in other solutions based on bioluminescence imaging and confirmatory trypan blue staining. The islets were then transplanted into the renal capsule of streptozotocin-induced type 1 diabetic nonobese diabetic-severe combined immunodeficient (NOD-scid) mice. Again, the islets preserved in ET-K or UW solution appeared to be more effective than those preserved in other solutions, since glucose injection-induced hyperglycemia declined, and the glucose level returned to normal levels in mice transplanted with these islets. This demonstrated that the 24-h preservation of isolated islets with ET-K reduced cold ischemic damage and resulted in viable cells that could restore glycemic control in diabetic mice.

We also found that preserved islets can be activated by protein fractions secreted by MSCs (17). A conditioned medium was obtained by culturing adipose tissue MSCs (derived from wild-type LEW rats) for 2 days in serum-free medium. Organ preservation solution was added to Luc-Tg rat-derived islets, which were then incubated at 4°C with various molecular weight fractions of the conditioned medium. After 4 days, some fractions increased the relative photon intensities (which are representative of the ATP levels of the cold-preserved islets) to over 150% of their initial values. We also evaluated the photon intensity of fresh Luc-Tg rat-derived islets that were cultured in ET-K solution only or in ET-K solution containing MSC-secreted factors at 37 C (Fig. 4). After 4 h, islets cultured only in ET-K solution showed decreased photon intensity compared to those with MSC-secreted factors.

CONCLUSION

We believe that our Tg rats are a powerful tool for small animal studies, promising to facilitate discoveries and the development of new and innovative techniques. Our Tg rats are useful in the fields of regenerative medicine, cell/tissue engineering, drug discovery research, and cell medicine.

ACKNOWLEDGMENTS: We collaborated with Professors G. Nikkhah (Freiburg, Germany), J. Becker (Hannover, Germany), and R. H. Tolba (Aachen, Germany), who used the GFP-Tg LEW rats. We also collaborated with Professors Q. F. Li (Shanghai, People's Republic of China) and F. C. Wei (Taipei, Taiwan), who used the Luc-Tg LEW rats. Our Tg rats are available from the National BioResource Project for the Rat (nbrprat@anim.med.kyoto-u.ac.jp) in Japan and from the Rat Resource and Research Center (RRRC, http://www.rrrc.us/) in the US. Eiji Kobayashi is the Chief Scientific Advisor for Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan. There are no patents, products in development, or marketed products to declare. The position held by E. Kobayashi does not alter the author's adherence to all of Cell Medicine*'s policies regarding the sharing of data and materials, as described in detail online in the guide for authors. Takumi Teratani declares no competing financial interests.*

REFERENCES

1. Ajiki, T.; Hagiwara, H.; Horii, N.; Hagiwara, H.; Hoshino, Y. Movement of Schwann cells in the peripheral nerve suture. J. Jpn. Soc. Surg. Hand 25(4):68–71; 2009.
2. Ajiki, T.; Kimura, A.; Sato, Y.; Murakami, T.; Hakamata, Y.; Kariya, Y.; Hoshino, Y.; Kobayashi, E. Composite tissue transplantation in rats: Fusion of donor muscle to the recipient site. Transplant. Proc. 37:208–209; 2005.
3. Banas, A.; Teratani, T.; Yamamoto, Y.; Tokuhara, M.; Takeshita, F.; Osaki, M.; Kawamata, M.; Kato, T.; Okochi, H.; Ochiya, T. IFATS collection: In vivo therapeutic potential of human adipose tissue mesenchymal stem cells after transplantation into mice with liver injury. Stem Cells 26:2705–2712; 2008.
4. Bozkurt, A.; Lassner, F.; O'Dey, D.; Deumens, R.; Böcker, A.; Schwendt, T.; Janzen, C.; Suschek, C. V.; Tolba, R.; Kobayashi, E.; Sellhaus, B.; Tholl, S.; Eummelen, L.; Schügner, F.; Damink, L. O.; Weis, J.; Brook, G. A.; Pallua, N. The role of microstructured and interconnected pore channels in a collagen-based nerve guide on axonal regeneration in peripheral nerves. Biomaterials 33:1363–1375; 2012.
5. Buehr, M.; Meek, S.; Blair, K.; Yang, J.; Ure, J.; Silva, J.; McLay, R.; Hall, J.; Ying, Q. L.; Smith, A. Capture of authentic embryonic stem cells from rat blastocysts. Cell 135:1287–1298; 2008.
6. Busch, S. A.; van Crutchen, S. T. J.; Deans, R. J.; Ting, A. E. Mesenchymal stromal cells as a therapeutic strategy to support islet transplantation in type 1 diabetes mellitus. Cell Med. 2:43–53; 2011.
7. Ciccocioppo, R.; Bernardo, M. E.; Sgarella, A.; Maccario, R.; Avanzini, M. A.; Ubezio, C.; Minelli, A.; Alvisi, C.; Vanoli, A.; Calliada, F.; Dionigi, P.; Perotti, C.; Locatelli, F.; Corazza, G. R. Autologous bone marrow-derived mesenchymal stromal cells in the treatment of fistulising Crohn's disease. Gut 60: 788–798; 2011.
8. Duijvestein, M.; Vos, A. C.; Roelofs, H.; Wildenberg, M. E.; Wendrich, B. B.; Verspaget, H. W.; Kooy-Winkelaar, E. M.; Koning, F.; Zwaginga, J. J.; Fidder, H. H.; Verhaar, A. P.; Fibbe, W. E.; van den Brink, G. R.; Hommes, D. W. Autologous bone marrow-derived mesenchymal stromal cell treatment for refractory luminal Crohn's disease: Results of a phase I study. Gut 59:1662–1669; 2010.
9. Hakamata, Y.; Murakami, T.; Kobayashi, E. "Firefly rats" as an organ/cellular source for long-term in vivo bioluminescent imaging. Transplantation 27:1179–1184; 2006.
10. Hakamata, Y.; Tahara, K.; Uchida, H.; Sakuma, Y.; Nakamura, M.; Kume, A.; Murakami, T.; Takahashi, M.; Takahashi, R.; Hirabayashi, M.; Ueda, M.; Miyoshi, I.; Kasai, N.; Kobayashi, E. Green fluorescent protein-transgenic rat: A tool for organ transplantation research. Biochem. Biophys. Res. Commun. 286:779–785; 2001.
11. Hamra, F. K. Gene targeting: Enter the rat. Nature 467:161–163; 2010.
12. Hata, T.; Uemoto, S.; Fujimoto, Y.; Murakami, T.; Tateno, C.; Yoshizato, K.; Kobayashi, E. Transplantation of engineered chimeric liver with autologous hepatocytes and xenobiotic scaffold. Ann. Surg. 257:542–547; 2013.
13. Ho, S. T. B.; Tanavde, V. M.; Hui, J. H.; Lee, E. H. Upregulation of adipogenesis and chondrogenesis in MSC serum-free culture. Cell Med. 2:27–41; 2011.
14. Inoue, H.; Ohsawa, I.; Murakami, T.; Kimura, A.; Hakamata, Y.; Sato, Y.; Kaneko, T.; Takahashi, M.; Okada, T.; Ozawa, K.; Francis, J.; Leone, P.; Kobayashi, E. Development of new

inbred transgenic strains of rats with LacZ or GFP. Biochem. Biophys. Res. Commun. 329:288–295; 2005.
15. Kaiser, J. Breakthrough of the year. Whiplash for stem cell researchers. Science 330:1609; 2010.
16. Kanazawa, H.; Fujimoto, Y.; Teratani, T.; Iwasaki, J.; Kasahara, N.; Negishi, K.; Tsuruyama, T.; Uemoto, S.; Kobayashi, E. Bone marrow-derived mesenchymal stem cells ameliorate hepatic ischemia reperfusion injury in a rat model. PLoS One 6:e19195; 2011.
17. Kasahara, N.; Teratani , T.; Doi, J.; Iijima, Y.; Maeda, M.; Uemoto, S.; Fujimoto, Y.; Sata, N.; Yasuda, Y.; Kobayashi, E. Use of mesenchymal stem cell-conditioned medium to activate islets in preservation solution. Cell Med. 2013 in press.
18. Kimura, A.; Ajiki, T.; Takeuchi, K.; Hakamata, Y.; Murakami, T.; Hoshino, Y.; Kobayashi, E. Transmigration of donor cells involved in the sciatic nerve graft. Transplant. Proc. 37:205–207; 2005.
19. Krause, M.; Ganser, C.; Kobayashi, E.; Papazoglou, A.; Nikkhah, G. The Lewis GFP transgenic rat strain is a useful cell donor for neural transplantation. Cell Transplant. 21:1837–1851; 2012.
20. Kulbatski, I.; Mothe, A. J.; Keating, A.; Hakamata, Y.; Kobayashi, E.; Tator, C. H. Oligodendrocytes and radial glia derived from adult rat spinal cord progenitors: Morphological and immunocytochemical characterization. J. Histochem. Cytochem. 55:209–222; 2007.
21. Li, P.; Tong, C.; Mehrian-Shai, R.; Jia, L.; Wu, N.; Yan, Y.; Maxson, R. E.; Schulze, E. N.; Song, H.; Hsieh, C. L.; Pera, M. F.; Ying, Q. L. Germline competent embryonic stem cells derived from rat blastocysts. Cell 135:1299–1310; 2008.
22. Li, W.; Wei, W.; Zhu, S.; Zhu, J.; Shi, Y.; Lin, T.; Hao, E.; Hayek, A.; Deng, H., Ding, S. Generation of rat and human induced pluripotent stem cells by combining genetic reprogramming and chemical inhibitors. Cell Stem Cell 4:16–19; 2009.
23. Liao, J.; Cui, C.; Chen, S.; Ren, J.; Chen, J.; Gao, Y.; Li, H.; Jia, N.; Cheng, L.; Xiao, H.; Xiao, L. Generation of induced pluripotent stem cell lines from adult rat cells. Cell Stem Cell 4:11–15; 2009.
24. Matsumoto, K.; Yokoo, T.; Matsunari, H.; Iwai, S.; Yokote, S.; Teratani, T.; Gheisari, Y.; Tsuji, O.; Okano, H.; Utsunomiya, Y.; Hosoya, T.; Okano, H. J.; Nagashima, H.; Kobayashi, E. Xenotransplanted embryonic kidney provides a niche for endogenous mesenchymal stem cell differentiation into erythropoietin-producing tissue. Stem Cell 30:1228–1235; 2012.
25. Meyer-Schwesinger, C.; Lange, C.; Bröcker, V.; Agustian, P. A.; Lehmann, U.; Raabe, A.; Brinkmeyer, M.; Kobayashi, E.; Schiffer, M.; Büsche, G.; Kreipe, H. H.; Thaiss, F.; Becker, J. U. Bone marrow-derived progenitor cells do not contribute to podocyte turnover in the puromycin aminoglycoside and renal ablation models in rats. Am. J. Pathol. 178:494–499; 2011.
26. Mothe, A. J.; Kulbatski, I.; van, Bendegem, R. L.; Lee, L.; Kobayashi, E.; Keating, A.; Tator, C. H. Analysis of green fluorescent protein expression in transgenic rats for tracking transplanted neural stem/progenitor cells. J. Histochem. Cytochem. 53:1215–1226; 2005.
27. Negishi, K.; Teratani, T.; Iwasaki, J.; Kanazawa, H.; Kasahara, N.; Lefor, A. T.; Uemoto, S.; Fujimoto, Y.; Kobayashi, E. Luminescence technology in preservation and transplantation for rat islet. Islets 3:111–117; 2011.
28. Seo, J. H.; Jang, I. K.; Kim, H.; Yang, M. S.; Lee, J. E.; Kim, H. E.; Eom, Y. W.; Lee, D. H.; Yu, J. H.; Kim, J. Y.; Kim, H. O.; Cho, S. R. Early immunomodulation by intravenously transplanted mesenchymal stem cells promotes functional recovery in spinal cord injured rats. Cell Med. 2:55–67; 2011.
29. Tan, J.; Wu, W.; Xu, X.; Liao, L.; Zheng, F.; Messinger, S.; Sun, X.; Chen, J.; Yang, S.; Cai, J.; Gao, X.; Pileggi, A.; Ricordi, C. Induction therapy with autologous mesenchymal stem cells in living-related kidney transplants: A randomized controlled trial. JAMA 307:1169–1177; 2012.
30. Teratani, T.; Kobayashi E. In vivo bioimaging rats for translational research in cell and tissue transplantation. Cell Med. 3:3–11; 2012.
31. Ying, Q. L.; Wray, J.; Nichols, J.; Batlle-Morera, L.; Doble, B.; Woodgett, J.; Cohen, P.; Smith A. The ground state of embryonic stem cell self-renewal. Nature 453:519–523; 2008.