Differentiation of a hepatic phenotype after heterotropic transplantation of heart, kidney, brain, and skin tissues into liver in F344 rats

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Abstract

While organ-specific stem cells with roles in tissue injury repair have been documented, their pathogenic significance in diseases and the factors potentially responsible for their activation remain largely unclear. In the present study, heart, kidney, brain, and skin samples from F344 transgenic rats carrying the *GFP* gene were transplanted into normal F344 rat liver one day after an intraperitoneal injection (i.p.) of carbon tetrachloride (CCl_4) to test their differentiation capacity. The transplantation was carried out by female donors to male recipients, and vice versa. One week after transplantation, GFP antigen-positive cells with phenotypic characteristics of hepatocytes were noted. After two weeks, their extent increased, and at 4 weeks, large areas of strongly GFP-stained cells developed. All recipient livers had GFP antigen-positive hepatocyte cells. PCR analysis coupled with laser capture micro-dissection (LCM) revealed those cells to contain GFP DNA. Thus, our results indicate that tissue stem cells have multipotential ability, differentiating into hepatocytes when transplanted into an injured liver.

Key words: differentiation; hepatocyte; transplantation; stem cells; liver; GFP; transgenic rat; CCl_4

Introduction

Characterization of the cell populations involved in tissue repair allows for a new understanding of the mechanisms that are responsible for organ homeostasis. Mesenchymal cells are a well-differentiated population of bone marrow-derived non-hemopoietic cells with multipotent properties. The medical interest of such multipotency rests in the potential of such cells to repair damaged tissue. Several recent reports suggest that there is far more plasticity than previously believed in the developmental potential of many different adult cell types. Adult stem cells have been reported from several tissue sources, including the central nervous system (1-3), bone marrow (4-17), retina (18), brain (19), hair follicle (20, 21), inner ear (22), adipotic tissues (23, 24), oral mucosal epithelium (25), liver (26-28), skeletal muscle (29, 30), and skin (31). However, the mechanisms regulating stem cell differentiation and self-renewal are largely unknown at present.

Our ultimate goal is to regulate somatic stem cell differentiation and proliferation. There is growing evidence that injury to a target organ can be sensed by tissue-specific adult stem cells that migrate to a damaged site, undergo differentiation, and promote structural and functional repair. Here, we developed a model experimental system with a rat carrying a GFP transgene for testing the differentiation capacity of adult stem cells by engraftment with adult tissues into an injured liver. Albumin-positive GFP-containing cells localized in the context of the damaged liver after engraftment with heart, kidney, brain, and skin from adult animals, indicating that organ-specific stem cells engraft with a damaged liver and differentiate into hepatocytes, thereby restoring liver structure and function.

Materials and Methods

Donor animals

Irrespective of the approach used to obtain stem cells, the fate of adult stem cells following tissue transplantation can be traced by labeling donor cells with green fluorescence protein GFP, a non-secreting cytosolic protein that appears green under UV light. The plasmid construct carrying GFP is driven by a CAG promoter. The fate of any cell expressing GFP is easy to investigate by UV light or by immunohistochemistry in combination with an anti-GFP antibody. The *Eco*RI fragment of full-length EGFP cDNA was introduced into a pCAGGS expression vector containing the chicken beta-actin promoter and the cytomegalovirus enhancer, beta-actin intron, and bovine globin poly-adenylation signal (32). Transgenic rat lines were produced by injecting the purified linearized pCAG-EGFP plasmid DNA into F344/DuCrj (Fisher) rat-fertilized eggs. In total, 320 DNA-injected eggs were transplanted into pseudo-pregnant mice, resulting in 46 newborns. The incorporation of the transgene was examined by placing 1-day-old pups under a fluorescent microscope. Five of these were found to be transgenic. All rats were prepared by Oriental Yeast Co., Tokyo, Japan.

Recipients

Both sexes of F344/DuCrj rats, 6 weeks of age at commencement, were purchased from Charles River and housed five to a polycarbonate cage under constant conditions of temperature (24±2℃) and relative humidity (55±10%) with a 12:12-hour light-dark cycle. The animals were maintained according to the "Guide for the Care and Use of Laboratory Animals" established by Hiroshima University. All rats were fed a commercial diet (MF; Oriental Yeast Co., Tokyo, Japan).

Preparation of the donor tissues and transplantation procedures

The outline of our transplantation strategy is shown in Fig. 1. Carbon tetrachloride (CCl_4) was diluted with olive oil (carbon tetrachloride: olive oil 3:2) and administered at the rate of 1.5ml/kg body weight by i.p. injection. Twenty-four hours post-CCl_4-injection, the 4 different donor tissues of heart, kidney, skin with shaved hairs, and brain, were minced and injected into the largest lobe of the liver in the recipient using a 16-gauge needle. The injected volume was 0.5 ml/liver, including 0.25g minced tissue. As a control, 0.5 ml of Minimum Essential Medium (MEM, Invitrogen) was injected in the same manner. Tissue from a male donor was transplanted into 5 female recipients, and that from a female donor was transplanted into 5 male recipients. The

liver autopsies were performed at 1, 2, and 4 weeks after the transplantation. In total, 150 recipient rats were used for transplantation. The harvested livers were fixed in 10% buffered formalin and routinely embedded in paraffin and serially sectioned at 3 μm. Sections were stained with hematoxylin and eosin and by the periodic acid-Schiff (PAS)-alcian blue.

Immunohistochemistry

Three-μm-thick sections were treated for 30 min at room temperature with 2% BSA and incubated with anti-GFP antibody (diluted 1:200; Abcom), monoclonal mouse anti-proliferating cell nuclear antigen (PCNA, diluted 1:50; Dako), and polyclonal antibody to human albumin (DBS, Ca) for 1 hr at room temperature. In each case, negative controls were performed on serial sections, whereby incubation with the primary antibody was omitted. All slides were then exposed to the secondary antibody, biotinylated horse anti-universal-monkey IgG (Vectastain Universal Quick Kit, Vector Laboratories, CA,) and peroxidase conjugated streptoavidin complexes. Peroxidase activity was visualized by treatment with H_2O_2 and diaminobenzidine for 5 min. At the final step, the sections were counterstained with hematoxylin for 1 min.

Detection of transgene in recipients

GFP-positive cells in the liver engrafted with heart, kidney, brain, and skin tissue were harvested using an LM200 PixCell LCM System (Arcturus Bioscience, Inc., Mountain View, CA). Tissue was collected on TF-100 caps (Arcturus) containing transfer film from two or three sections per sample. DNA was extracted using a SepaGene solution (Sanko Jyunyaku Co., Ltd.) according to the manufacturer's protocol. PCR detection of EGFP cDNA was performed with the TaKaRa Ex TaqTM (TaKaRa SHUZO Co., Ltd.) following standard procedures and using the primers recommended by Clontech: forward, 5 CGT CGC CGT CCA GCT CGA CCA G 3; reverse, 5 CAT GGT CCT GCT GGA GTT CGT G 3. As a control, the quality of DNA was assessed by β-actin primers: forward, 5 AGA GCA AGA GAG AGG TAT CCT G 3; reverse, 5 AGA GCA TAG CCC TCG TAG AT 3.

Results and Discussion

Implantation of somatic tissues into injured liver

To allow testing of whether regenerating rat liver might offer a favored site for the growth of stem cells from other tissues, we developed a rat strain carrying a GFP

transgene as a model experimental system to allow the determination of cell derivation. Samples of heart, kidney, brain, and skin obtained from male F344 rats carrying the *GFP* gene were transplanted into the GFP-negative livers of female F344 rats after induction of liver injury with CCl_4 (Fig. 1). The implant sites were then harvested, and tissue sections were analyzed by hematoxylin and eosin and immunohistochemical GFP staining.

Appearance of GFP-positive cells

One and two weeks after transplantation, the sinusoids were enlarged (Fig. 2a), and macrophage-like cells and mitoses increased and then decreased. In addition, most of the transplanted tissues had disappeared from the liver parenchyma. Some remnants were observed, however, especially with skin grafts. To monitor donor derived-cells, tissue sections were analyzed for the presence of GFP by immunohistochemical staining. GFP antigen-positive cells with hepatocyte-like morphology were detected 1 week after implantation with heart, kidney, brain, and skin tissues. Two weeks later, their sizes had increased (Fig. 2b), and larger areas of GFP-positive hepatocyte-like cells were apparent at 4 weeks (Fig. 2c). GFP-positive hepatocyte-like cells were observed in all recipients. The morphological characteristics of the GFP-positive cells are identical to the adjacent host hepatocytes, with pale staining for PAS (Fig. 2d). In addition, both GFP-positive and -negative hepatocytes demonstrated strong binding of the polyclonal antibody against human albumin. These results were reproducibly observed when organs were transplanted from male donor to female recipients, moreover; they were also transplanted from female donor to male recipients.

Characterization of the GFP-positive cells

To further elucidate whether these hepatocyte-like cells are immunohistochemically positive for GFP originated from donor tissues of F344/DuCrj EGFP rats, PCR analysis coupled with LCM was performed. As shown in Fig. 2e, hepatocytes immunohistochemically positive for GFP were found to carry the GFP transgene.

In this report, we have documented evidence of differentiation of stem cells from heart, kidney, brain, and skin samples to cells with a hepatic phenotype when grafted into livers during injury-dependent stimulation of cell division. Previously, we reported that esophagus, trachea, and bladder grafts transplanted into glandular stomach and duodenum differentiated into gastric and duodenal mucosa, respectively, suggesting that

stem cells have multipotential ability to respond to differentiation factors from the surrounding environment in the gastrointestinal tract. We also observed that pieces of heart transplanted into a kidney, immediately after the other kidney was removed, differentiated into renal tissue (unpublished observation). Other examples of extensive plasticity include the *in vivo* differentiation of a bone marrow population enriched for hematopoietic stem cells into mature hepatocytes in the liver of rodents (12, 33); after radiation-induced myeloablation (34), such differentiation of bone marrow cells into mature cells of the liver was also reported to occur in humans (35, 36). Krause et al. (13) reported that adult bone marrow cells had tremendous differentiation capacity for transforming into epithelial cells of liver, lung, GI tract, and skin. Furthermore, Suzuki et al. (27, 28) reported that self-renewing pluripotent stem cells exist in the developing liver, which differentiates into pancreatic ductal and acinar cells or intestinal epithelial cells when transplanted into pancreas or the duodenal wall. Recently, Amoh et al. suggested that hair-follicle stem cells may provide an accessible, autologous source of undifferentiated multipotent stem cells (22). Together with the data presented here, the available findings indicate that mammalian stem cells persist in various organs and that such cells can be induced to become cells of other organ differentiation with an appropriate microenvironment. Our experimental system, with its unique feature of a GFP marker, has clear advantages over other animal models.

Cells are known to change identity through fusion; in other words, molecules from one fusion partner re-program gene expression in the genome of another. Fusion can occur spontaneously *in vitro* (37), and there is considerable evidence for such a mechanism *in vivo* (38-40). On the other hand, human stem cells from human bone marrow and umbilical cord blood (41), human umbilical cord blood-derived mesenchymal stem cells (42), and mouse bone marrow mononuclear cells (43) can differentiate into functional hepatocyte-like cells without apparent fusion *in vitro*. Furthermore, Sato et al. recently reported that human mesenchymal stem cells xenografted directly into rat liver differentiate into hepatocytes without fusion (44). The situation with our model is unclear, but an understanding of how stem cells generate liver cells is clearly of great importance if the full potential of transplants for the treatment of genetic disorders is to be realized. In the future, we should demonstrate the presence of the rat Y chromosome because no such proof is currently available.

In conclusion, our results indicate that organ-specific stem cells in a wide range of tissues, including the heart, kidney, brain, and skin, may be able to differentiate into hepatocytes when transplanted into livers under conditions of strong stimulation of cell growth and division.

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References

[1] B.A. Reynolds, S. Weiss, Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system, Science 255 (1992) 1707–1710.

[2] F.H. Gage, Mammalian neural stem cells, Science 287(2000) 1433–1438.

[3] D.L. Clarke, C.B. Johansson, J. Wilbertz, B. Veress, E. Nilsson, H. Karlström, U. Lendahl, J. Frisén, Generalized potential of adult neural stem cells, Science 288(2000) 1660-1663.

[4] D.J. Prockop, Marrow stromal cells as stem cells for nonhematopoietic tissues, Science 276 (1997) 71–74.

[5] I.L Weissman, Translating stem and progenitor cell biology to the clinic: barriers and opportunities, Science 287 (2000) 1442–1446.

[6] M.F. Pittenger, A.M. Mackay, S.C. Beck, R.K. Jaiswal, R. Douglas, J.D. Mosca, M.A. Moorman, D.W. Simonetti, S. Craig, D.R. Marshak, Multilineage potential of adult human mesenchymal stem cells. Science 284 (1999) 143–147.

[7] S.Y. Corbel, A. Lee, L. Yi, J. Duenas, T.R. Brazelton, H.M Blau, F.M. Rossi. Contribution of hematopoietic stem cells to skeletal muscle. Nature Med 9(2003) 1528-1532.

[8] F.D. Camargo, R. Green, Y. Capetenaki, K.A. Jackson, M.A. Goodell, Single hematopoietic stem cells generate skeletal muscle through myeloid intermediates, Nature Med 9 (2003) 1520-1527.

[9] R. Doyonnas, M.A. LaBarge, A. Sacco, C. Charlton, H.M. Blau, Hematopoietic contribution to skeletal muscle regeneration by myelomonocytic precursors, Proc Natl Acad Sci USA 101 (2004) 13507-13512.

[10] K. Ojima, A. Uezumi, H. Miyoshi, S. Masuda, Y. Morita, A. Fukase, A. Hattori, H. Nakauchi, Y. Miyagoe-Suzuki, S. Takeda, Mac-1(low) early myeloid cells in the bone marrow-derived SP fraction migrate into injured skeletal muscle and participate in muscle regeneration, Biochem Biophys Res Commun 321(2004) 1050-1061.

[11] Y. Zhao, D. Glesne, E.A. Huberman, Human peripheral blood monocyte-derived subset acts as pluripotent stem cells. Proc Natl Acad Sci USA 100 (2003) 2426-2431.

[12] E. Lagasse, H. Connors, M. Al-Dhalimy, M. Reitsma, M. Dohse, L. Osborne, X. Wang, M. Finegold, I.L. Weissman, M. Grompe, Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. Nature Med 5 (2000) 1229-1234.

[13] D.S. Krause, N. Theise, M. Collector, O. Henegariu, S. Hwang, R. Gardner, S. Neutzel, S. Sharkis, Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell, Cell 105 (2001) 369-77.

[14] G. Ferrari, G. Cusella-, De Angelis, M. Coletta, E. Paolucci, A. Stornaiuolo, G. Cossu, F. Mavilio**,** Muscle regeneration by bone marrow-derived myogenic progenitors, Science 279 (1998) 1528–1530.

[15] K.A Jackson, T. Mi, M.A, Hematopoietic potential of stem cells isolated from murine skeletal muscle Proc Natl Acad Sci 96 (1996) 14482–14486.

[16] C.R. R. Bjornson, R.L. Rietze, B.A. Reynolds, M.C. Magli, A.L. Vescovi, Turning brain into blood: a hematopoietic fate adopted by adult neural stem cells in vivo, Science 283 (1999) 534–537.

[17] M.A. Eglitis, E. Mezey E, Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice, Proc Natl Acad Sci USA 94 (1997) 4080–4085.

[18] V. Tropepe, B.L. Coles, B.J. Chiasson, D.J. Horsford, A.J. Elia, R.R. McInnes, D. Kooy, Retinal stem cells in the adult mammalian eye, Science 287 (2000) 2032–2036.

[19] Y. Jiang, B. Vaessen, T. Lenvik, M. Blackstad, M. Reyes, C.M. Verfaillie, Multipotent progenitor cells can be isolated from postnatal murine bone marrow, muscle, and brain, Exp Hematol 30 (2002) 896-904.

[20] C. Blanpain, W.E. Lowry, A. Geoghegan A, L. Polak, E.Fuchs, Self-renewal, multipotency, and the existence of two cell populations within an epithelial stem cell niche, Cell 118 (2004) 635-648.

[21] H. Li, H. Liu, S. Heller, Pluripotent stem cells from the adult mouse inner ear, Nature Med 9 (2003) 1293-1299.

[22] Y. Amoh, L. Li, K. Katsuoka, S. Penman, R.M. Hoffman, Multipotent nestin-positive, keratin-negative hair-follicle bulge stem cells can form neurons, Proc Natl Acad Sci U S A 102 (2005) 5530-5534.

[23] S.S Tholpady, A.J. Katz, R.C. Ogle, Mesenchymal stem cells from rat visceral fat exhibit multipotential differentiation in vitro, Anat Rec Part A 272A (2003) 398-402.

[24] P.A. Zuk, M. Zhu, H. Mizuno, J. Huang, J.W. Futrell, A.J. Katz, P. Benhaim, H.P. Lorenz, M.H. Hedrick, Multilineage cells from human adipose tissue: implications for cell-based therapies, Tissue Eng 7 (2001) 211-228.

[25] K. Nishida, M. Yamato, Y. Hayashida, K. Watanabe, K. Yamamoto, E. Adachi, S. Nagai, A. Kikuchi, N. Maeda, H. Watanabe, T. Okano, Y. Tano, Corneal reconstruction with tissue-engineered cell sheets composed of autologous oral mucosal epithelium, New Eng J Med 351 (2004) 1187-1196.

[26] A. Suzuki, H. Nakauchi, Identification and propagation of liver stem cells, Semin Cell Develop Biol 13 (2002) 455-461.

[27] A. Suzuki, Y. Zheng, S. Kaneko, M. Onodera, K. Fukao, H. Nakauchi, H. Taniguchi, Clonal identification and characterization of self-renewing pluripotent stem cells in the developing liver, J Cell Biol 156 (2002) 173-184.

[28] S.S. Thorgeirsson, J.W. Grisham, Overview of recent experimental studies on liver stem cells, Semin Liver Dis 23 (2003) 303-312.

[29] A. Asakura, M. Komaki, M. Rudnicki, Muscle satellite cells are multipotential stem cells that exhibit myogenic, osteogenic, and adipogenic differentiation, Differentiation 68 (2001) 245-253.

[30] M.R. Wada, M. Inagawa-Ogashiwa, S. Shimizu, S. Yasumoto, N. Hashimoto, Generation of different fates from multipotent muscle stem cells, Development 129 (2001) 2987-2995.

[31] J.G. Toma, M. Akhavan, K.J.L. Fernandes, F. Barnabé-Heider, A. Sadikot, D.R.

Kaplan, F.D. Miller, Isolation of multipotent adult stem cells from the dermis of mammalian skin, Nat Cell Biol 3 (2001) 778-784.

[32] H. Niwa, K. Yamamura, J. Miyazaki, Efficient selection for high-expression transfectants with a novel eukaryotic vector, Gene 108 (1991) 193-199.

[33] B.E. Petersen, W.C. Bowen, K.D. Patrene, W.M. Mars, A.K. Sullivan, N. Murase, S.S. Boggs, J.S. Greenberger, J.P. Goff**,** Bone marrow as a potential source of hepatic oval cells, Science 284 (1999) 1168–1170.

[34] N.D. Theise, S. Badve , R. Saxena , O. Henegariu , S. Sell , J.M. Crawford , D.S. Krause, Derivation of hepatocytes from bone marrow cells in mice after radiation-induced myeloablation, Hepatology 31 (2000) 235–240.

[35] N.D. Theise, M. Nimmakayalu , R. Gardner, P.B. Illei , G. Morgan , L. Teperman , O. Henegariu, D.S. Krause, Liver from bone marrow in humans, Hepatology 32 (2000) 11–16.

[36] M.R. Alison, R. Poulsom, R. Jeffery, A.P. Dhillon, A. Quaglia, J. Jacob, M. Novelli, G. Prentice, J. Williamson, N.A. Wright, Hepatocytes from non-hepatic adult stem cells, Nature 406(2000) 257.

[37] X. Wang, H. Willenbring, Y. Akkari, Y. Torimaru, M. Foster, M. Al-Dhalimy, E. Lagasse, M. Finegold, S. Olson, M. Grompe, Cell fusion is the principal source of bone-marrow-derived hepatocytes, Nature 422 (2003) 897-901.

[38] G. Vassilopoulos, P.R. Wang, D.W. Russell, Transplanted bone marrow regenerates liver by cell fusion, Nature 422 (2003) 901-904.

[39] N. Terada, T. Hamazaki, M. Oka, M. Hoki, D.M. Mastalerz, Y. Nakano, E.M. Meyer, L. Morel, B.E. Petersen, E.W. Scott, Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion, Nature 416(2002) 542-545.

[40] O.L. Ying, J. Nichols, E.P. Evans, A.G. Smith, Changing potency by spontaneous fusion, Nature 416(2002) 545-548.

[41] K.D. Lee, T.K Kuo, J. Whang-Peng , Y.F. Chung, C.T. Lin , S.H. Chou , J.R. Chen,

Y.P. Chen, O.K. Lee, In vitro hepatic differentiation of human mesenchymal stem cells, Hepatology 40 (2004) 1275-1284.

[42] S.H. Hong, E.J. Gang, J.A. Jeong, C. Ahn, S.H. Hwang, I.H. Yang, H.K. Park, H. Han, H. Kim, In vitro differentiation of human umbilical cord blood-derived mesenchymal stem cells into hepatocyte-like cells, Biochem Biophys Res Commun 330(2005) 1153-1161.

[43] X. Shi, Y. Qiu, X. Wu, T. Xie, Z. Zhu, L. Chen, L. Li, Y. Ding, In vitro differentiation of mouse bone marrow mononuclear cells into hepatocyte-like cells, Hepatol Res 31(2005) 223-231.

[44] Y. Sato, H. Araki, J. Kato, K. Nakamura, Y. Kawano, M. Kobune, T. Sato, K. Miyanishi, T. Takayama, M. Takahashi, R. Takimoto, S. Iyama, T. Matsunaga, S. Ohtani, A. Matsuura, H. Hamada, Y. Niitsu, Human mesenchymal stem cells xenografted directly to rat liver differentiated into human hepatocytes without fusion, Blood 106 (2005) 756-763.

Legends

Figure 1

Schematic representation of transplantation procedures. Transgenic rats carrying EGFP cDNA (F344/DuCrj EGFP) driven by a CAG promoter (32) were prepared and monitored with an *in vivo* imaging system (IVIS, Xenogen, Inc., USA). The fate of cells expressing GFP could be followed under UV light or by immunohistochemistry in combination with an anti-GFP antibody. Carbon tetrachloride was diluted with olive oil (carbon tetrachloride: olive oil 3:2) and administered at the rate of 1.5ml/kg body weight via i.p. injection. Twenty-four hours post-injection, minced samples of donor heart, kidney, skin with shaved hairs, and brain were injected into the largest liver lobes of recipient F344/DuCrj rats (6-week-old) using 16-gauge needles. Tissues from male donors were transplanted into female recipients, and those from female donors were transplanted into male recipients.

Figure 2a

Histological appearance of HE-stained implanted tissues. Implants of minced tissues from heart one week after transplantation. There were no signs of necrosis or bleeding. Mitoses were observed in parenchymal cells and sinusoids, in which there were macrophage-like cells and enlarged Kupffer cells. The scale bar indicates 100 μm.

Figure 2b

Appearance of donor-derived hepatocytes immunohistochemically positive for GFP two weeks after brain tissue transplantation. The scale bar indicates 100 μm.

Figure 2c

GFP immunohistochemical staining, 4 weeks after heart tissue transplantation. The scale bar indicates 100 μm.

Figure 2d

Periodic acid-Schiff (PAS)-alcian blue staining 4 weeks after heart tissue transplantation. The scale bar indicates 100 μm. The GFP-positive cells showed pale staining with PAS, unlike those in Figure 2c.

Figure 2e

DNA from GFP-positive cells in the liver engrafted with tissues from heart, kidney,

brain, and skin was harvested using an LM200 PixCell LCM System and subjected to PCR analysis for the detection of the GFP gene.

Fig. 1 Watanabe *et al*.

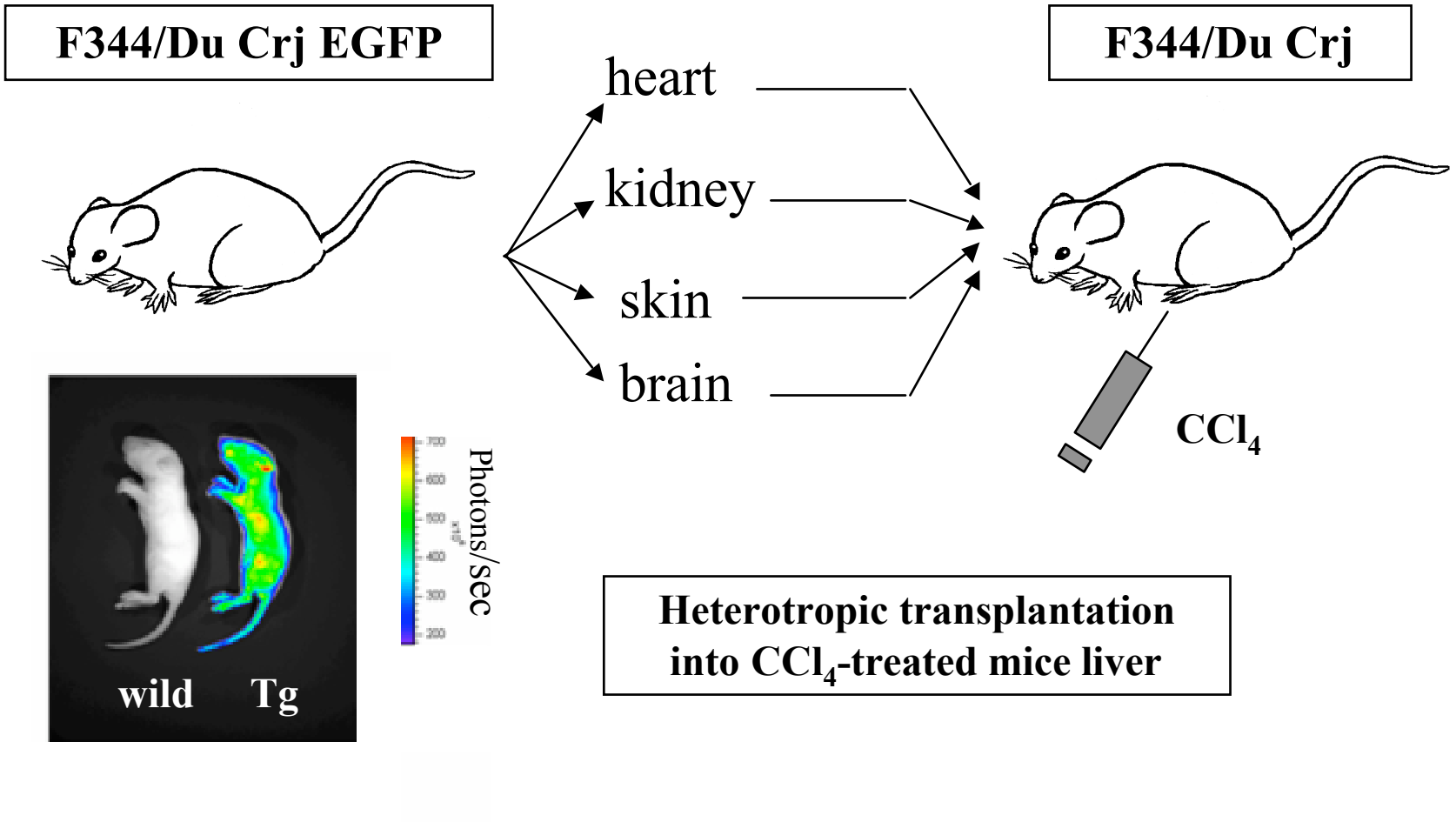
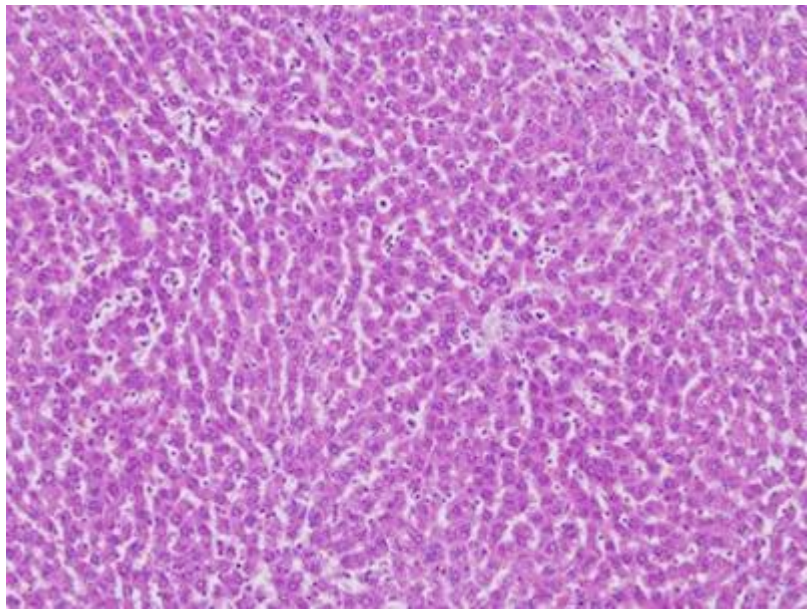


Fig.2 Watanabe *et al*

2a



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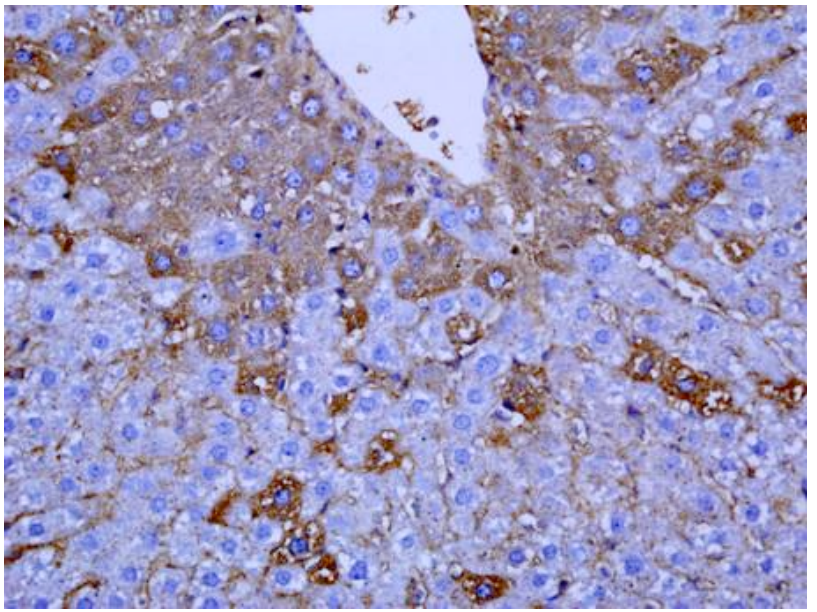
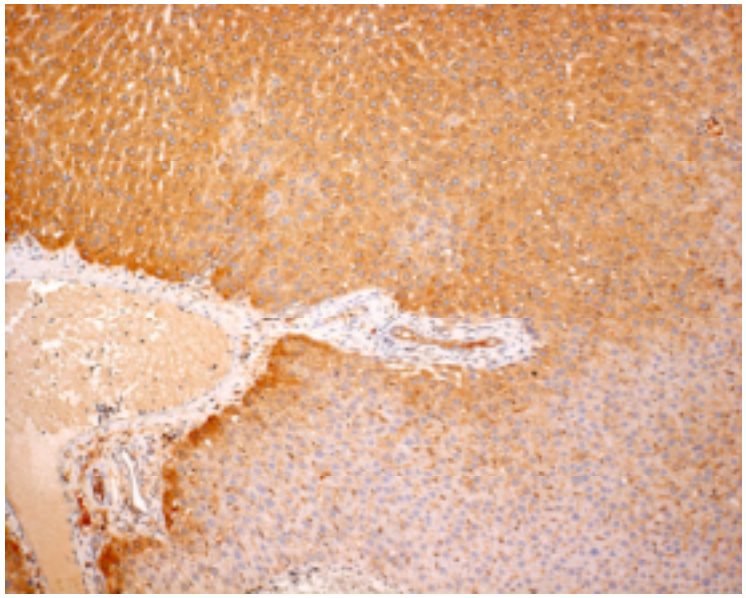


Fig.2 c-e Watanabe *et al*

2c



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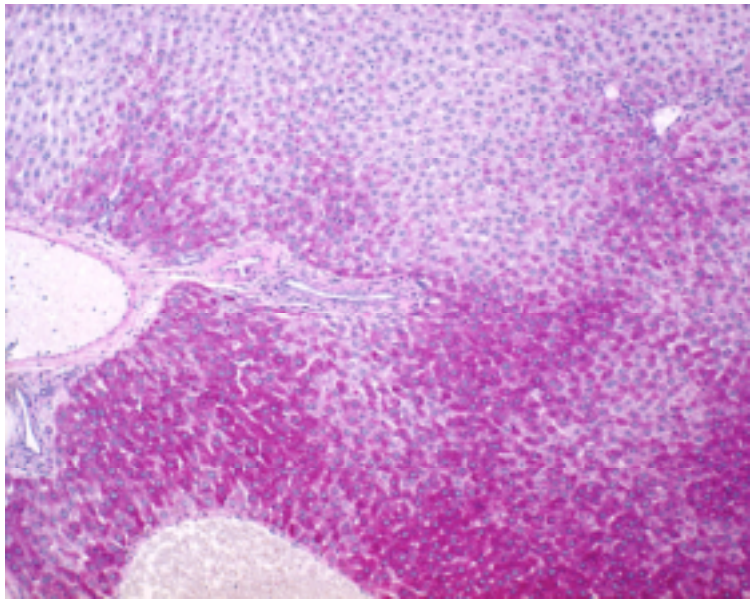

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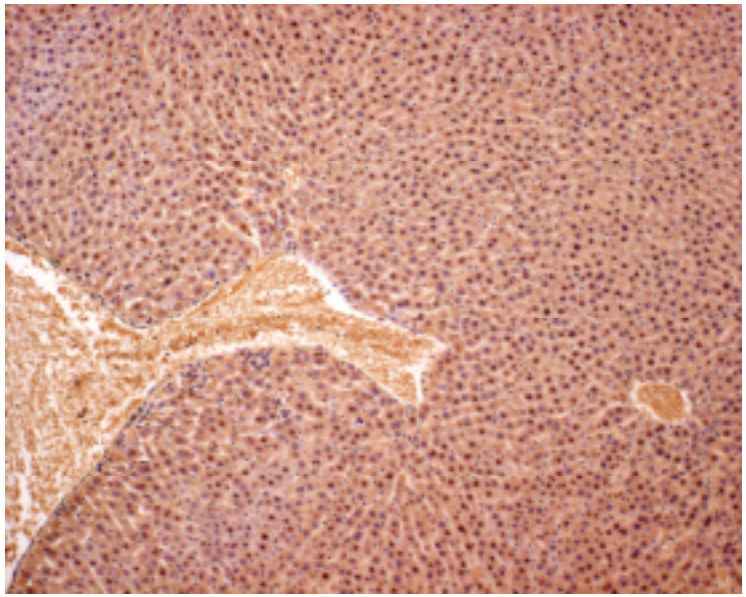

Fig. 2f Watanabe *et al*