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Rat Embryonic Stem Cells: Establishment and Their Use for Transgenesis

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1. Introduction

Rats (*Rattus norvegicus*) have been used more extensively than mice in the research fields of neuroscience, pharmacology and toxicology. There are more than 100 rat strains with various genetic backgrounds, including some useful models for human diseases. For example, the SHR (spontaneously hypertensive rat) strain and the BB (rats spontaneously developing insulin-dependent diabetes mellitus) strain are well-established models for studying cardiovascular diseases and endocrinopathy (Okamoto, 1969; Like et al., 1982). Because of the well-understood mapping of brain functions, rats are often used for physiological studies on memory and emotion (Wood et al., 1999; van Erp et al., 2000). Furthermore, experimental studies on mammary tumors require the use of rats in which symptoms of the disease are distinct from those caused by mouse mammary tumor viruses; mouse mammary tumor viruses cause tumors in the mammary glands of mice but not those in humans or rats (Carr et al., 1981; Gould, 1986). In addition, transgenic rats have been used as model animals for human diseases (e.g., Alzheimer's disease, autoimmunity and high-density lipoprotein [HDL] metabolism) and organ transplantation, and as animal bioreactors for protein production (Heideman, 1991; Charreau et al., 1996; Ganten, 1998). One advantage of using rats rather than mice in transgenic studies is the ease of continuous or repeated sample collection (e.g., of blood or urine) and surgery, due to their larger size, while litter size, gestation length, maturation rate, estrous cycle length, and life span of rats are all very similar to those of mice. Thus, the rat has the advantage of being a reasonably well-characterized and intermediate-sized rodent that can be maintained much more cheaply than larger animals and can often be manipulated much more easily than smaller rodents.

On the other hand, the reverse genetic approach using the rats (precise and conditional gene replacements [knock-in] or loss of gene function [knock-out] at the specific locus) was considered impossible because any protocols to establish embryonic stem (ES) cell lines conventionally used in mice were not applicable to the rats. However in 2008, functional germline-competent ES cell lines have been reported (Buehr et al., 2008; Li et al., 2008). Very recently, successful production of p53 gene knock-out rats by homologous recombination in the ES cells has been achieved at last (Tong et al., 2010). The present chapter deals with an overview of attempts at producing gene-modified rats (with or without using ES cells), followed by detailed protocols for establishment of rat ES cell lines, and for successful use of the rat ES cells in transgenesis via electroporation (Hirabayashi et al., 2010a; 2010b).

2. Approaches to produce gene-modified rats

Transgenic rats integrated with foreign gene into their genomes can be routinely produced by conventional pronuclear microinjection (Hammer et al., 1990; Hochi et al., 1990; Mullins et al., 1990) or by an intracytoplasmic sperm injection (ICSI)-mediated gene transfer protocol (Kato et al., 2004; Hirabayashi et al., 2005). As for production of rats genetically modified (knock-in or knock-out of endogenous gene function), the most convenient tool, ES cell line, was not available for this rodent. Despite of numerous efforts, all attempts at establishing rat ES cell lines have resulted in total failures (Brenin et al., 1997; Vassilieva et al., 2000; Buehr et al., 2003); some ES-like cell lines have been derivated, but phenotype of the ES-like cell origin was not contributed to chimera or was not transmitted to G1 generation offspring, even though they could proliferate while maintaining undifferentiated status and show multipotency under certain conditions for inducing differentiation. Ueda et al. (2008) reported the production of chimeric rats derived from blastocyst injection of transgenic ES cells (established by classical method), without data for germline transmission of the exogenous gene.

Therefore alternatively, sophisticated approaches were used to induce genetic mutations in the rats. Gene-modified rats have been successfully produced by the following three approaches. (1) The yeast-based screening assay of N-ethyl-N-nitrosourea (ENU) mutated G1 offspring (Zan et al., 2003; Chen & Gould, 2004) allows to produce serial allelic variations of targeted genes, in addition to knock-out mutants expected. In another words, targeted gene modifications at the specific locus (Scn1a; a missense mutantion presumptively responsible to epilepsy) are difficult to achieve in the ENU-mutagenesis (Mashimo et al., 2008). (2) Sleeping beauty (SB) transposon system was found effective to induce insertional mutatation in rats (Kitada et al., 2007) as well as in mice (Keng et al., 2005). This system includes the production of two independent transgenic lines carrying the SB transposon vector (eqipped with promoter trap system and poly-[A] trap system) and SB transposase, respectively, and the subsequent interline coupling to produce double-transgenic rats. Transposition events occurs not only on the same chromosome but also between different chromosomes. This SB transposon-tagged mutagenesis is disadvantageous in controlling the frequency of mutation at the specific locus, too. (3) Zinc-finger nucleases (ZFNs) can create genome-specific double-stranded breaks and therefore more likely to induce targeted gene mutation. Microinjection of DNA or mRNA that encodes specific ZFNs into pronuclear zygotes derived from green fluorescent protein (GFP) transgenic rats resulted in a high frequency of rat offspring that do not express the transgenic marker, due to a consequence of homologous recombination at the GFP locus (Geurts et al., 2009). Mutation in rat genome has been induced in interleukin 2 receptor gamma (IL2rγ) locus by this approach (Mashimo et al., 2010). One of the obstacles in the ZFNs technology is the difficult customization of the DNA/mRNA motif for ZFNs specific to the desired locus.

Cloning by somatic cell nuclear transplantation (SCNT) is an alternative approach to produce gene-modified rats. Successful production of cloned rats with somatic cells was first reported by Zhou et al. (2003). However, the reproducibility of the data is still questionable (Hirabayashi & Hochi, 2006). As to reasons why rat SCNT is difficult, rat oocytes are likely to activate spontaneously in vitro after recovery from the oviductal ampullae (Zernicka-Goetz, 1991; Ito et al., 2005). The male germline stem (GS) cell line, which is capable of culturing and differentiating in vitro, is also a source of cells for gene targeting and production of knockout animals via ICSI or other microinsemination techniques (Feng et al., 2002; Toyooka et al., 2003; Geijsen et al., 2004). When rat spermatogonial stem cells were

transplanted into busulfan-treated nude mouse testes, microinsemination of the retrieved rat spermatids and spermatozoa into rat oocytes resulted in viable rat offspring (Shinohara et al., 2006). The SCNT/GS cell technologies combined with homologous recombination-based gene targeting will become a powerful tool for production of knock-out rats, once they can be established and widespread.

Breakthrough in establishing rat ES cell lines was appeared at the end of 2008 year (26th December, 2008). Functional germline-competent ES cell lines have been reported by using a few inhibitors for fibroblast growth factor (FGF) receptor, mitogen activated protein kinase kinase (MEK) and glycogen synthase kinase 3 (GSK3) in differentiation-related signaling pathways (Buehr et al., 2008; Li et al., 2008). This protocol, the so-called 3i/2i culture system, was originated from ES cell research in mouse (Ying et al., 2008) and was proved reproducible even after slight modificatons were added to the culture system (Hirabayashi et al., 2010a; Kawamata & Ochiya, 2010). The modification made by Hirabayashi et al. (2010a) is to replace MEK activation inhibitor PD1843521 with MEK inhibitor PD325901 and to add rat leukemia inhibitory factor (LIF) instead of LIF-secreting feeder cells in the 3i system, while that made by Kawamata & Ochiya (2010) is to add fetal bovine serum (FBS), ß-mercaptoethanol, rat LIF, and inhibitors for Rho-associated coiled-coil kinase (ROCK) and transforming growth factor-beta (TGF-ß) type-I receptor ALK5 kinase to the 2i culture medium containing MEK and GSK3 inhibitors. Very recently, successful production of p53 gene knock-out rats by homologous recombination in the ES cells has been achieved (Tong et al., 2010). The p53 is tumour suppressor gene located on rat chromosome 10, and mutations in the p53 gene are highly associated with genetic lesions in human cancers. Soon or later, the homologous recombination-based gene targeting in rat ES cell lines will allow for practical production and analysis of individual rats with targeted mutation in every research fields, as routinely done in mice.

3. Establishment of rat ES cells

Development of a methodology for reverse genetic research has been long desired, because the rat is the most widely used animal model in biological research. The most convenient method for production of knockout rats is to use gene-targeted ES cells in germline chimera production. Although rat ES cell lines have not been established despite of numerous efforts, recent application of 2i/3i culture system enabled the derivation of germline-competent rat ES cell lines (Buehr et al., 2008; Li et al., 2008). In this section, rat ES cell lines that can participate in germline chimeras at high efficiency (Hirabayashi et al., 2010a; with a few additional data) are described.

3.1 Isolating undifferentiated colonies from blastocysts

Blastocysts at E4.5 (E0 was defined as the initiation of coupling) were recovered by uterine flushing of Wistar females couplated with a homogenous CAG/venus transgenic male rat. Green fluorescence of the venus gene was used as the transgenic marker. Their zonae pellucidae were removed in acid tyrodes solution (5 sec at an ambient temperature), and then each blastocyst was placed on mitomycin (MMC)-treated mouse embryonic fibroblasts (MEF; concentraton of the feeder cells, 3 to 4 x 10^5 cells/ml; see below note-1) in a well of 4-well dish. The culture medium consisted of 2 μM FGF receptor inhibitor, SU5402 (Calbiochem), 1 μM MEK inhibitor, PD0325901 (Stemgent), 3 μM GSK3 inhibitor, CHIR99021 (Axon), and 1,000 U/ml rat LIF (ESGRO®, Millipore) in N2B27 medium (see below note-2). After 7 days culture at 37˚C in a humidified atmosphere of 5% CO_2 in air, the

outgrowths of the blastocysts (Fig. 1a) were disaggregated by gentle pipetting and transferred to the same MEF/3i conditions (first passage). However, time required until the first passage (5 days to 2 weeks) may depend on rat strains for blastocysts. When ES cell-like colonies were emerged (Fig. 1b), they were trypsinized in 0.25% trypsin/0.6 mM EDTA solution (Sigma) and then expanded (Fig. 1c). The tentative ES cell lines were maintained in MEF/3i conditions, with medium exchange every other day and trypsinization/expansion (passage) every three days. Otherwise, the ES cell lines were cryopreserved to prevent senescence (see below note-3). It was checked whether the derivated cells are alkaline phosphatase (AP)-positive (Fig. 1d). Nine ES cell lines (69.2%) were established from 13 transgenic blastocysts. Among them, two lines with excellent growth rate (rESWIv3i-1 and rESWIv3i-5) were selected, and both lines were found female by PCR analysis to detect rat *Sry* gene. Attachment of ES cell colonies with the feeder cells was not so strong, and morphological appearance of the rat ES cell colonies was similar to that of mouse ES cells. As the passage number of the ES cells increased, signs of differentiation into extraembryonic cell-like cells were observed in cultures, especially when the culture medium was exchanged every other day rather than every day (Fig. 1e and 1f).

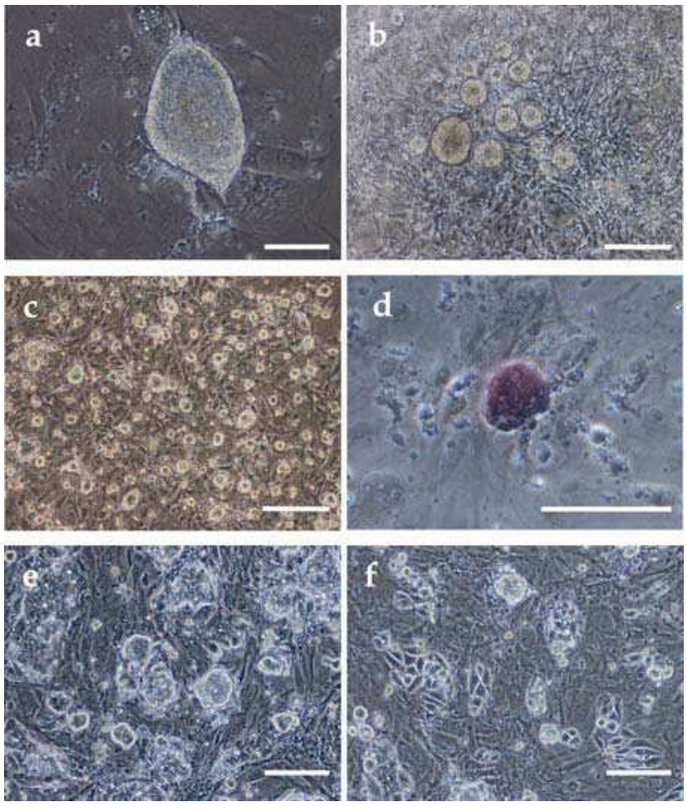

Fig. 1. Establishment of rat ES cell line. (a) Outgrowth of a blastocyst on MEF feeders 7 days after plating. (b) Formation of colonies 3 days after the first passage. (c) Expanded ES cell colonies 2 days after fourth passage. (d) Alkaline phosphatase-positive colony at passage 12. (e) The ES cells at passage 17. Under the daily exchange of culture medium, very little cells showed any signs of differentiation. (f) The ES cells at passage 17, maintained with medium exchange every other day. Differentiated extra-embryonic cells were observed. Scale bars: a, d, e and f; 100-μm, b and c; 500-μm

Notes: (1) MEF Preparation; One-ml of neomycin (G418)-resistant MEF cell cryostock (2 to 3 x 10^7 cells/vial, Kitayama Labes) were thawed in 37˚C waterbath, and resuspended in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS (ES cell qualified). The cell suspension was centrifuged for 190 x *g* for 5 min at 4˚C, resuspended in DMEM/10% FBS, and then cultured for 2 to 3 days. Confluently proliferated cells were liberated by trypsin/EDTA treatment, centrihuged for 190 x *g* for 5 min at 4˚C, resuspended, and replated (passage). After several passages, the cells were treated with 10 μg/ml MMC for 2 h at 37˚C in 5% CO_2 in air. After washing twice by centrifugation, the MCC-treated MEFs were used as the feeder cells. The DMEM and FBS were purchased from Gibco. (2) N2B27 medium; A 1:1 mixture of DMEM/F12 (1:1) and Neurobasal medium was supplemented with B-27 serum-free supplement, N-2 serum-free supplement, 2 mM L-glutamine, 50 U/ml penicillin, and 50 mg/ml streptomycin. Media and reagents for N2B27 medium were all purchased from Gibco. (3) Cryopreservation of ES cells; ES cells were liberated by the trypsin/EDTA treatment for 5 min at 37˚C in 5% CO_2 in air, and were collected in a 15 ml conical tube after gentle pipetting. After centrifugation for 190 x *g* for 5 min at 4˚C, the cell pellet was resuspended with 1 ml of CellBanker®. The cryovials (1 x 10^6 cells/ml/cryovial) were transferred into a BICELL®, and kept overnight in a –80˚C deep-freezer. The cryovials were then stored in a liquid nitrogen tank until use.

3.2 Confirmation of characteristics essential for ES cells

In addition to AP activity of the tentative ES cells (Fig. 1d), expression of stem cell marker genes, such as Oct-4, Nanog, Fgf-4, or Rex-1, should be confirmed by reverse transcription (RT)-PCR analysis. Each primer sets were designed as shown in Table 1. Total RNA was prepared using the RNeasy mini Kit (Qiagen), and the RT-PCR was performed using the SuperScript™III One-Step RT-PCR System with Platinum®Taq High Fidelity kit (Invitrogen™), with the reaction conditions for cDNA synthesis (55°C for 30 min and 94°C for 2 min) and amplification (30 cycles at 94°C for 15 sec, 55°C for 30 sec, and 68°C for 60 sec). As shown in Fig. 2, both rESWIv3i-1 and rESWIv3i-5 lines expressed Oct-4, rNanog, Fgf-4 and Rex-1, indicating that these cell lines had undifferentiated characteristics. Weakly detectable bands of Gata6 may be due to the presence of differentiated cells in ES cell cultures (Fig. 1f).

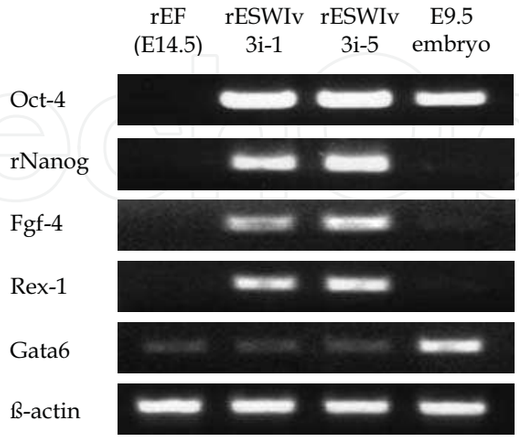

Fig. 2. Reverse transcription-PCR analysis for rat embryonic fibroblast cells at E14.5 (rEF; negative control for stem cell markers), rat embryos at E9.5 (positive control for Gata6), and two rat ES cell lines (rESWIv3i-1 and rESWIv3i-5). Both ES cell lines expressed stem cell markers, including Oct-4, rNanog, Fgf-4 and Rex-1

Furthermore, multipotency of the tentative ES cells (whether the cells can differentiate into endoderm, mesoderm, and ectoderm) should be confirmed. The tentative rESWIv3i-1 line (2.5 x 10^5 cells) has been subcutaneously transplanted into an adult male F344 nude rat. Five weeks after the transplntation, a tumor was observed (Fig. 3a), and its histological section was evaluated ater hematoxylin-eosin staining. As shown in Fig. 3b, the tumor was found to be a teratoma with various tissues including gut-like epithelium or hepatic cells (endoderm), bone, cartilage or muscle (mesoderm), and neural tissues (edctoderm).

Marker gene		Primer sequence	Product size
Oct-4	forward	5′- GGGATGGCATACTGTGGAC-3′	412 bp
	reverse	5′- CTTCCTCCACCCACTTCTC-3′	
rNanog	forward	5′- GCCCTGAGAAGAAAGAAGAG-3′	356 bp
	reverse	5′- CGTACTGCCCCATACTGGAA-3′	
Fgf-4	forward	5′- CGGGGTGTGGTGAGCATCTTC-3′	202 bp
	reverse	5′- CCTTCTTGGTCCGCCCGTTC-3′	
Rex-1	forward	5′- TTCTTGCCAGGTTCTGGAAGC-3′	297 bp
	reverse	5′- TTTCCCACACTCTGCACACAC-3′	
Gata6	forward	5′- TCATCACGACGGCTTGGACTG-3′	467 bp
	reverse	5′- GCCAGAGCACACCAAGAATCC-3′	
ß-actin	forward	5′- CATGGCATTGTGATGGACT-3′	427 bp
	reverse	5′- ACGGATGTCAACGTCACACT-3′	

Table 1. Primers used for PCR amplification

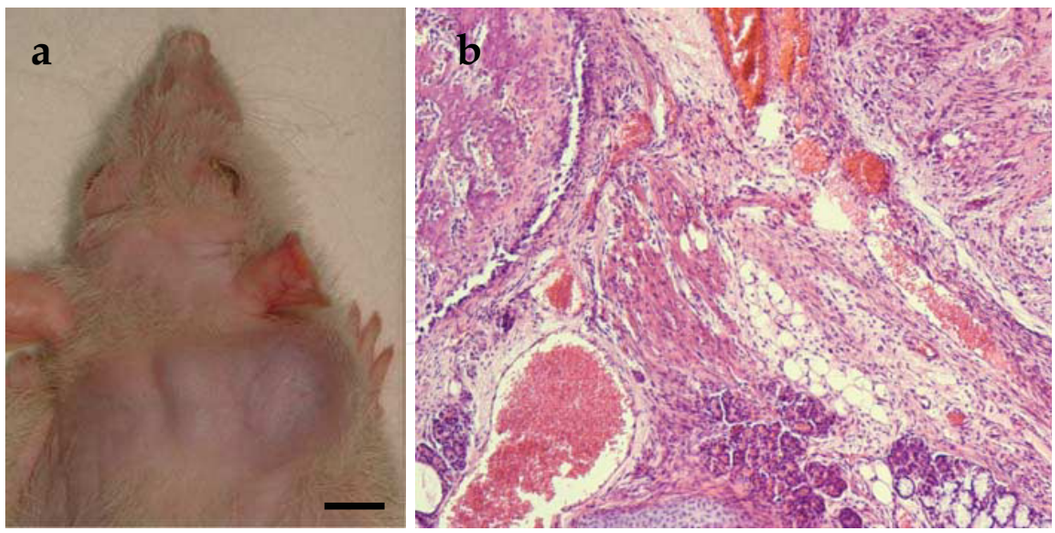

Fig. 3. Tumor observed 5 weeks after subcutaneous injection of rESWIv3i-1line ES cells into a nude rat (a), and histological section of the tumor stained with hematoxylin-eosin (b). The tumor was found to be a telatoma with various tissues including gut-like epithelium or hepatic cells (endoderm: bottom right), bone, cartilage or muscle (mesoderm: center), and neural tissues (ectoderm: upper right). Scale bar; 1-cm

3.3 Production of germline chimera rats by blastocyst injection

To generate ES cell-derived chimeras, host blastocysts at E4.5 derived from Wistar females or Wistar x Dark-Agouti F_1 females were microinjected with 10 ES cells each at passage 6 or 8 (Fig. 4a and 4b). Briefly, using blunt-ended injection pipette with an outer diamether of 15 μm, the zona pellucida of host blastocysts was penetrated by a piezo-pulse. The pipette tip was forwarded to inner cell mass (ICM) through thin junction area between the two trophoblastic cells (without any piezo force), and then the ES cells were deposited. Collapsed blastocysts (Fig. 4c) were re-blasturated 1 to 2 h after the microinjection, and allowed to develop to fetus (E15.5) or full-term pups in pseudopregnant Wistar recipients. All of the E15.5 fetuses (100%; Fig. 4d) and majority of the newborn pups (81.8 to 100%) were chimeric and expressed the venus gene (Table 2). The characteristics of the ES cells was successfully transmitted to their next generations in both lines (Fig. 5); Among 7 chimeras derived from passage-6 rESWIv3i-1 cells and developed to adult, three were female. Two of the 3 female chimeras transmitted the Venus gene through the germline (4/15 and 2/14, respectively). Among 5 chimeras derived from passage-8 rESWIv3i-5 cells and developed to adult, only one rat was female. This female chimera also transmitted the transgene to the next generations (1/26, two litters). The ability of the ES cells to participate in chemeras was still high (78.6 to 100%) at advanced passage numbers (17 or 18; Table 2). Overall efficiency of producing chimeric rats (50.3%, 94 chimeras/187 injected embryos) was higher than 8.2% (20/245) as reported in Buehr et al. (2008) and 11.0% (26/237) in Li et al. (2008). This higher efficiency of chimera rat production is probably due to the rat strain combination used for donor (ES cells) and host (blastocysts) and/or modification of culture medium.

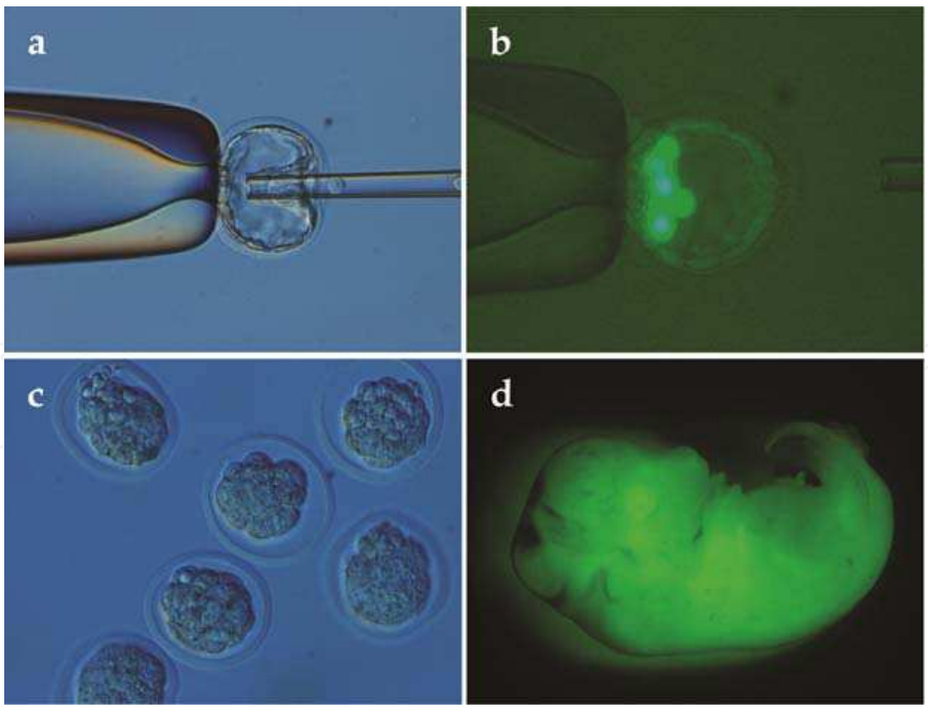

Fig. 4. Microinjection of 10 ES cells into a blastocoele of E4.5 blastocyst (a). Semi-bright, fluorescent image of venus-positive ES cells in the blastocyst (b). Collapsed blastocysts immediately after microinjection (c). Venus-positive fetal rat at E15.5 (d)

Cell line	Passage number	No. (%) of embryos			No. (%) of fetus or pups	
		Injected	Developed to fetus	Developed to pups	Analyzed	Identified as chimera
	6	17	13 (76.5)	—	13	13 (100)
rESWIv	6	34	—	21 (61.8)	17	17 (100)
3i-1	17	28	17 (60.7)	—	17	14 (82.4)
	17	28	—	14 (50.0)	14	11 (78.6)
	8	12	9 (75.0)	—	9	9 (100)
rESWIv	8	13	—	11 (84.6)	11	9 (81.8)
3i-5	18	22	7 (31.8)	—	7	7 (100)
	18	33	—	14 (42.4)	14	14 (100)

Table 2. Contribution of rat ES cells for chimera production

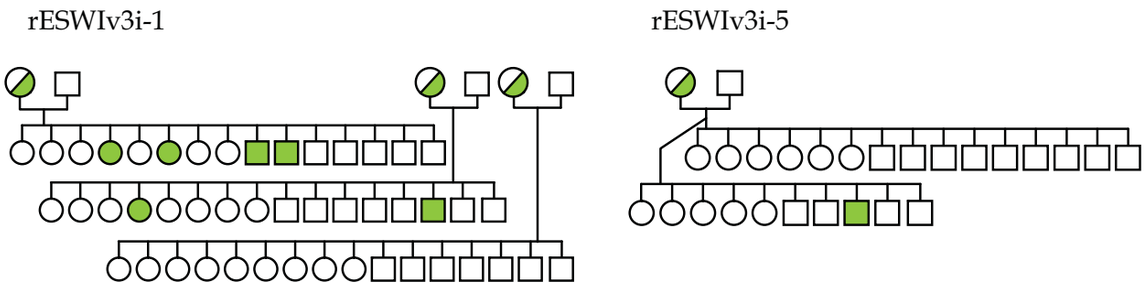

Fig. 5. Progeny test for germline transmission from rESWIv3i-1 or rESWIv3i-5-derived chimeric rats (half-green circle; female). Green circle and square indicate germline-transmitted (venus-positive) females and males, respectively

4. Transgenesis via rat ES cells

Functional germline-competent rat ES cell lines have been established by applying 2i/3i culture system, and the minimal essential materials for conducting transgenic studies including reverse genetic approaches, are now ready for this rodents. In this section, the production efficiency of chimeric rats by blastocyst injection of ES cells electroporated with a humanized Kusabira-Orange (huKO) gene and the germline transmission of the huKO gene from the chimeras to next generation (Hirabayashi et al., 2010b; with a few additional data) are described.

4.1 Electroporation of ES cells with foreign gene

Rat ES cell lines were newly established from E4.5 blastocysts derived from Brown-Norway (BN) females (couplated with BN males), as described above, by applying 2i culture system. The culture medium used for establishment of ES cell lines from BN rats was in N2B27

medium supplemented with 2i (MEK inhibitor PD0325901, 1 μM; GSK3 inhibitor CHIR99021, 3 μM) and rat LIF (1,000 U/ml ESGRO). One of the established lines, named as rESBN2i-4, was derived from a male embryo, based on a PCR analysis using a primer set to detect the rat *Sry* gene. At passage 8, the ES cells in N2B27 medium supplemented with 10% FBS (1 x 10^6 cells/0.5 ml) were electroporated with 25 μg huKO gene (CAG/huKO-neo plasmid; 4.5 kb) using the Bio-Rad Genepulser-II apparatus at 800 V, 10 μF. The electroporated ES cells were plated into a 60 mm petri dish containing 3 ml of 2i medium + 5% FBS (passage 9), and the next day the medium was changed to serum-free 2i medium. Two days after electroporation, 200 μg/ml G418 was added to the medium. The number of neomycin-resistant colonies (passage 10) was counted 8 days after electroporation (Fig. 6). The huKO-positive ES cells were passaged twice in G418-free medium.

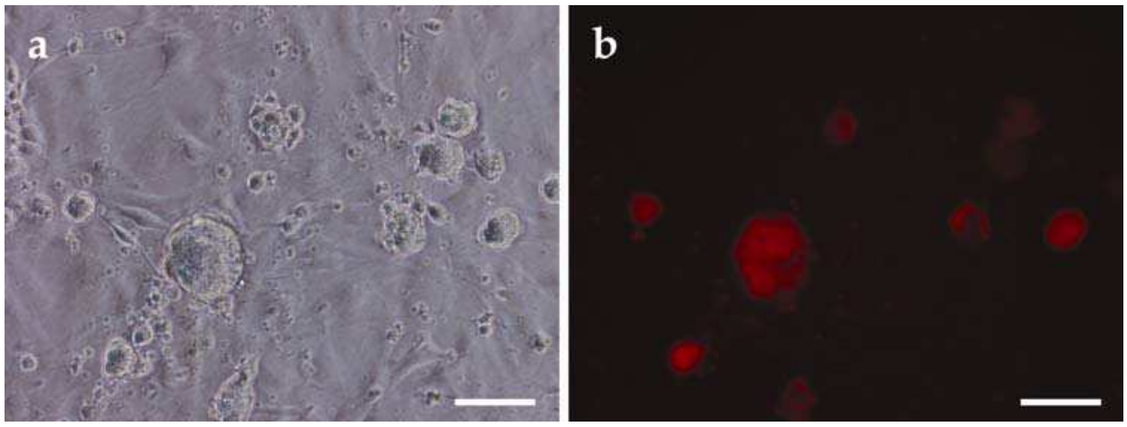

Fig. 6. Rat ES cell colonies stably expressing huKO gene, generated by electroporation, selection with neomycin, and the subsequent expansion. Bright field (a) and fluorescent image (b). Scale bars; 100-μm

4.2 Production of G1 generation transgenic rats

Host blastocysts derived from Wistar/ST or Wistar-Hannover females were microinjected with 10 each of the G418-resistant huKO-positive ES cells, and allowed to develop to full-term in pseudopregnant Wistar recipients. As shown in Table 3, transfer of 116 and 97 blastocysts resulted in 31 and 44 new-born offspring (26.7 and 45.4%), and 22 (70.9%; male 12, female 10) and 34 (77.3%; male 15, female 17, not-identified 2) out of the offspring were judged as chimeras by their coat color, respectively (Fig, 7a). Using non-electroporated control ES cells, similar offspring rate (37.5%, 9/24) and chimera production efficiency (88.9%, 8/9) were obtained. Rat strain for host blastocysts may be a factor influencing the the overall efficiency of chimera production, due to different preference for full-term development. Some male chimeras (n=9) were coupled with wild-type Wistar females, resulting in the birth of total 118 G1 offspring. Germline transmission of the CAG/huKO-neo gene was confirmed in 6 out of 25 G1 offspring (Fig. 7b and 7c) derived from 1 chimeric male with >95% brown-colored coat. Thus, integration of exogenous DNA into rat ES cells did not affect the production efficiency of chimera offspring. In addition, the result described in this section (slightly expanded from Hirabayashi et al., 2010b) achieved the first successful production of transgenic rats via electroporated ES cells, followed by Kawamata & Ochiya (2010) and Tong et al. (2010).

Transfected DNA	Rat strains for host blastocysts	No. (%) of embryos		No. (%) [%]* of pups
		Injected	Developed to pups	Identified as chimera
CAG/huKO	Wistar/ST	116	31 (26.7)	22 (70.9) [19.0]
	Wistar-Hannover	97	44 (45.4)	34 (77.3) [35.1]
Control (No DNA)	Wistar/ST	24	9 (37.5)	8 (88.9) [33.3]

Table 3. Effect of electroporation of rESBN2i-4 cells with huKO gene on the production of chimeric rats.
* Calculated from injected embryos

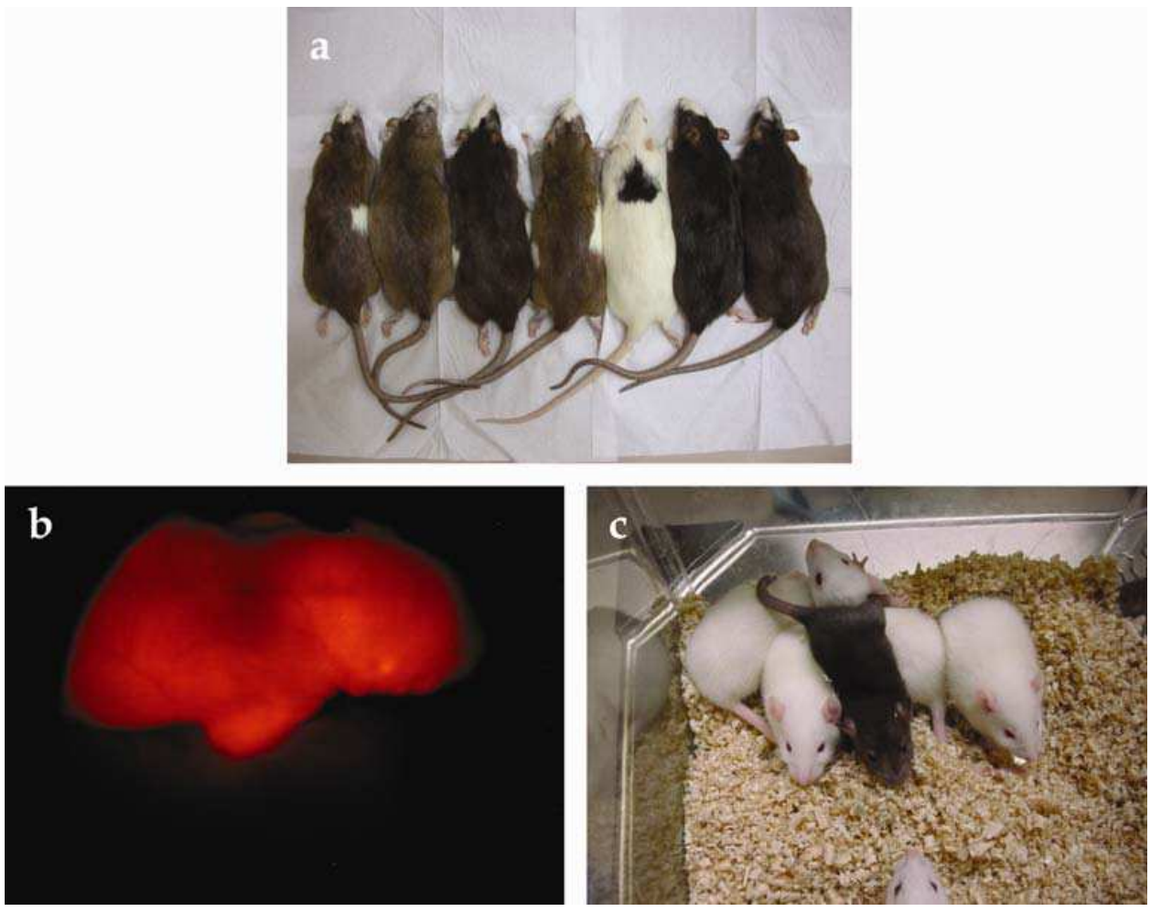

Fig. 7. Chimeric rats with different contribution of brown-colored coat at G0 generation, 17-days old (a), a huKO transgenic newborn young at G1 generation, 3-days old (b), and a huKO transgenic offspring (brown-colored) with huKO-negative littermates, 3-weeks old (c)

5. Conclusion

Functional germline-competent rat ES cell lines have been first reported in 2008 December (Buehr et al., 2008; Li et al., 2008). The difficulty in establishing rat ES cells until those time has been overcome by using a few inhibitors for FGF receptor, MEK and GSK3 in differentiation-related signaling pathways. Reproducibility of this 2i/3i culture system in establishing rat ES cells has been proved by two independent groups (Hirabayashi et al., 2010a; Kawamata & Ochiya, 2010) and both groups successfully applied their own cell lines for producing transgenic rats (Hirabayashi et al., 2010b; Kawamata & Ochiya, 2010). Very recently, the production of p53 gene knock-out rats via homologous recombination in ES cells has been reported by Tong et al. (2010). Although knockout rats were also successfully produced by ENU-induced, SB transposon-tagged, or ZFNs-based mutagenesis, such successes may not give impacts on creating rat models for human diseases due to limitation in genome modification by these technologies. Thus, the homologous recombination-based gene targeting technology in „ES cells" would provide a practical breakthrough for wide range of biomedical research in the laboratory rats.

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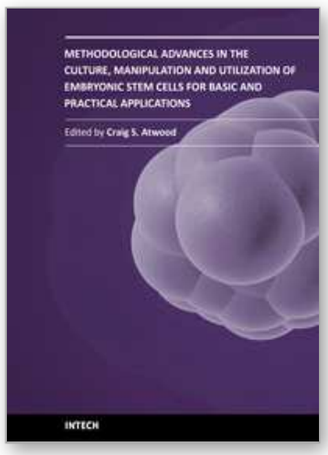

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Pluripotent stem cells have the potential to revolutionise medicine, providing treatment options for a wide range of diseases and conditions that currently lack therapies or cures. This book describes methodological advances in the culture and manipulation of embryonic stem cells that will serve to bring this promise to practice.

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