Zygote **22** (May), pp. 182–186. © Cambridge University Press 2012
doi:10.1017/S0967199412000366 First Published Online 18 July 2012

Differences in chimera formation and germline transmission between E14 and C2J embryonic stem cells in mice

Yan Zhu[2], *Dun-Gao Li*[3,4], *Zhao-Gui Sun*[2], *Xue-Jin Chen*[3] *and Man-Xi Jiang*[1,3]

Key Laboratory of Contraceptive Drugs and Devices of National Population and Family Planning Committee, Shanghai Institute of Planned Parenthood Research, Shanghai; Department of Laboratory Animal Sciences, Shanghai Jiao Tong University School of Medicine, Shanghai; and College of Wildlife Resource, Northeast Forestry University, Harbin, China

Date submitted: 16.01.2012. Date revised/accepted: 05.05.2012

Summary

The goal of this project was to determine whether the originating strain of mouse embryonic stem (ES) cells affects the maintenance of their pluripotency under uniform culture conditions. ES cells from two strains of mice, E14 and C2J, were tested. Both ES cell lines were cultured in KOSR + 2i medium and then injected into C57BL/6J blastocysts. Our results demonstrate that this medium could support both E14 and C2J ES cells to keep their pluripotency, though E14 ES cells were found to have a higher chimeric rate than C2J ES cells. However, analysis by backcrossing revealed that C2J and E14 ES cells have the same ability for germline transmission. Our results demonstrate that ES cells derived from E14 and C2J cells have the same capacity for germline transmission when injected into C57BL/6J blastocysts; however, due to the limitation of mixed genetic background between E14 cells and host C57BL/6J embryos, C2J ES cells are preferable to E14 ES cells for use in gene-targeting and should become the cell line of choice for the generation of genetically engineered mutant mouse lines.

Keywords: Chimera formation, Embryonic stem cell, Germline transmission, Mouse

Introduction

It is essential for the production of genetically engineered mutant mice to maintain the pluripotency of mouse embryonic stem (ES) cells. If pluripotency decreases or disappears completely, then the application of those ES cells will be greatly restricted. Despite the importance of maintaining ES pluripotency, the mechanisms governing it are poorly understood, although are recognized to be complex (Brown *et al.*, 1992; Liu *et al.*, 1997). Rigorous procedures are currently undertaken to ensure the maintenance of ES cell pluripotency and germline transmission capability, including ES cell culture under optimal conditions, such as being grown on a fibroblast feeder layer or in suitable batch-tested serum (Elder *et al.*, 1998; Akiyama *et al.*, 2002; Harris *et al.*, 2002).

With regards to the latter point, the variable quality of serum between different batches of culture medium means that batch-testing is required; a time-consuming procedure complicated by the fact that well defined conditions for ES cell culture are lacking. The ideal solution to this problem is the culture of ES cells in synthetic serum, which can provide fully defined growth conditions, thereby greatly reducing the time required for ES cell culture, while maintaining efficient chimera production and germline transmission (Ward *et al.*, 2002). Recently, several groups have reported that the use of inhibitors of some signal transduction pathways could significantly improve both the derivation of new ES cell lines, including those from C57BL/6 mice, and their subsequent potential to contribute to chimeric mice and germline transmission (Chen *et al.*, 2006; Ying *et al.*, 2008; Kiyonari *et al.*, 2010). These studies established that KOSR + 2i media enabled

[1]All correspondence to: Man-Xi Jiang. Department of Laboratory Animal Sciences, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China Tel: +86 21 63846590 ext. 776638. e-mail: manxijiang2002@yahoo.com.cn
[2]Key Laboratory of Contraceptive Drugs and Devices of National Population and Family Planning Committee, Shanghai Institute of Planned Parenthood Research, Shanghai 200032, China.
[3]Department of Laboratory Animal Sciences, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China.
[4]College of Wildlife Resource, Northeast Forestry University, Harbin 150040, China.

https://doi.org/10.1017/S0967199412000366 Published online by Cambridge University Press

highly efficient generation of germline transmitting chimeras from targeted C57BL/6-derived ES cells.

Previous studies have demonstrated that the efficiency of the establishment of ES cell lines is different among different mouse strains; however, no research has been conducted to systematically compare the pluripotency of ES cell lines that are derived from different strains of mice (Kawase *et al.*, 1994). With this in mind, in the present study we have compared the differences in germline transmission between ES cells derived from 129 mice (E14) and ES cells derived from C57BL/6J-Tyrc-2J mice (C2J), cultured in KOSR + 2i medium. We successfully produced healthy mouse chimeras by using C2J and E14 ES cells with transmission to the germline; however, we believe that C2J cells are a superior model because they have the same genetic backgrounds as the C57BL/6J host embryos.

Materials and methods

Ethics statement

Mice were maintained and used for experiments according to the ethical permit issued by the Animal Care guidelines for Use of Animals of Department of Laboratory Animal Sciences, Shanghai Jiao Tong University School of Medicine.

Animals

C57BL/6J mice were used as embryo hosts for ES cell injection, and CD-1 females were used as pseudopregnant recipients. Animals were maintained on a 12 h light/dark cycle and provided with food and water *ad libitum*, in individually ventilated units in the specific-pathogen-free facility of the Laboratory Animal Sciences, Shanghai Jiao Tong University School of Medicine, China.

ES cell culture

E14 (Thompson *et al.*, 1989) and C2J (Millipore, Billerica, MA, USA) ES cells were routinely grown at 37°C in 95% air and 5% CO_2 on mouse embryonic fibroblast feeder layers treated with mitomycin-C (Sigma-Aldrich Co., St Louis, MO, USA). For KOSR + 2i culture, we used Knockout-DMEM (KO-DMEM, Invitrogen, Life Technologies Co., Grand Island, NY, USA) supplemented with 0.1 mM non-essential amino acids, 0.1 mM 2-mercaptoethanol, 4 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 500 U/ml leukemia inhibitory factor (all from Millipore), 15% KnockOut™ Serum Replacement (Invitrogen), 1 μM of the mitogen-activated protein kinase inhibitor PD 98059 (Cayman Chemical, Ann Arbor, MI, USA) and 3 μM of the glycogen synthase kinase-3 inhibitor BIO (Cayman Chemical).

Chimera generation and evaluation

Blastocyst injection was performed according to Papaioannou's protocol (Papaioannou & Johnson, 2000). Briefly, embryos were collected at embryonic day 3.5 from pregnant C57BL/6J mice which had been superovulated. After ES cell injection, blastocysts were transferred into the uteri of 2.5-days post-coitum pseudopregnant CD-1 females who had been previously mated with vasectomized males. Evaluation of chimera grades were judged by the coat colour of pups aged up to 2 weeks old. For both E14 and C2J ES cell lines, the percentage of chinchilla or white fur on the black coat background was 50% (grade A), 15–50% (grade B), 1–15% (grade C) or <1% (grade D).

Backcross of E14 and C2J chimeras

All chimeras were weaned at 3 weeks of age and used for natural breeding at 4 to 8 weeks. Chimeras were tested for the ability of germline transmission to the next generation by backcrossing with C57BL/6 mice of the opposite gender. For E14 and C2J chimeras, their positive F1 offspring owned chinchilla and white coat colour respectively, allowing easy identification of chimeras with successful germline transmission.

Results

Effects of ES cell difference on chimera production

In order to evaluate the pluripotency of E14 and C2J ES cell lines, both of them were injected into blastocysts of C57BL/6J mice. Two hundred and forty-one blastocysts were injected with E14 ES cells yielding 56 chimeras, and 268 blastocysts were injected with C2J ES cells, yielding 21 chimeras. The phenotype of chimeric mice from the two ES cell lines examined were visually distinct, examples of which are shown in Fig. 1. The grade of chimerism was determined for each ES cell line and the data are summarized in Table 1. Blastocyst injection of E14 ES cells resulted in a 92.8% rate of chimerism, of which 46.4% were grade A, 19.6% were grade B, and 26.8% were grade C. In contrast, blastocyst injection of C2J ES cells resulted in only a 61.9% rate of chimerism, of which 23.8% were grade A, 14.3% were grade B, and 23.8% were grade C. Thus, these data indicate that E14 ES cells have a greater capability to form chimeric mice than C2J ES cells.).

https://doi.org/10.1017/S0967199412000366 Published online by Cambridge University Press

Table 1 Grading of chimeras generated from E14 and C2J ES cells

			Chimera rates of different grade (%)			
	Blastocyst injection (*n*)	Offspring (*n*)	A	B	C	D (Black)
E14	241	56	26 (46.4)	11 (19.6)	15 (26.8)	4 (7.1)
C2J	268	21	5 (23.8)	3 (14.3)	5 (23.8)	8 (38.1)

Table 2 Results from backcross of E14 and C2J ES cells derived from chimeric mice

	Percentage of positive F1 from different grade (%)			
Cell lines	A	B	C	D (Black)
E14	26/36 (72.2)	14/45 (31.1)	6/40 (15.0)	0/32 (0)
C2J	23/31 (74.2)	6/14 (42.9)	9/45 (20.0)	0/28 (0)

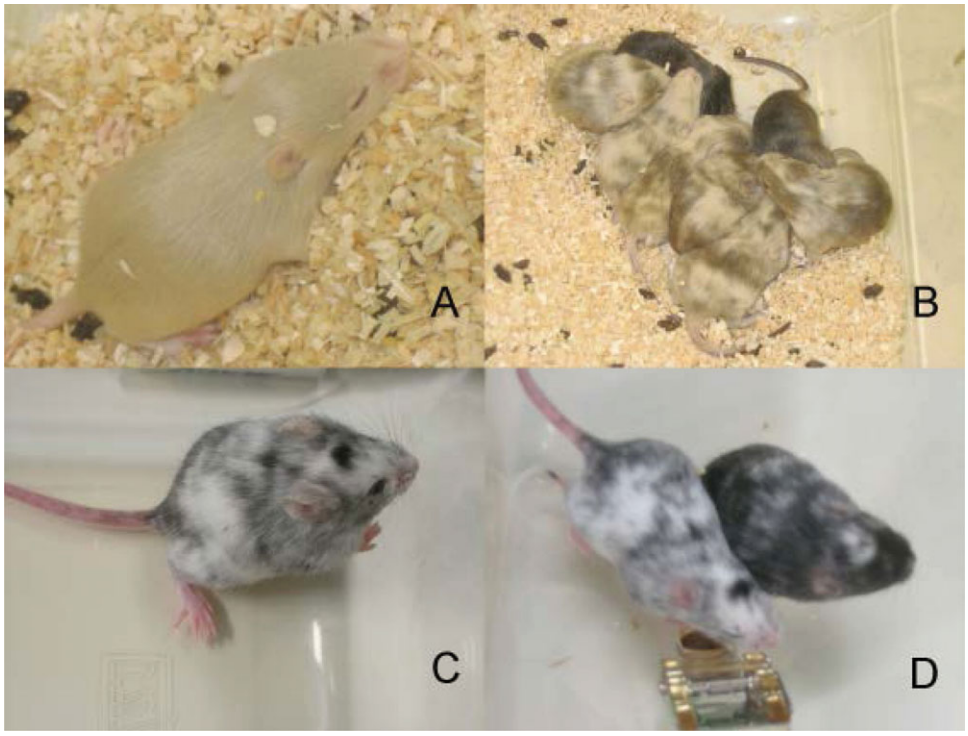


Figure 1 Representative photographs of chimeras obtained from E14-derived ES cells (*A*, *B*) and C2J-derived ES cell lines (*C*, *D*). Panel A shows an example of a grade A chimera obtained from E14 ES cells, showing an animal with a 100% chinchilla colored coat. Panel B shows a mix of grade A and grade B chimeras derived from E14 ES cells. Panel C shows an example of a grade A chimera obtained from C2J ES cells, with a coat that is >50% white. Panel D shows a comparison image of a grade A chimera (left) and grade C chimera (right) derived from C2J ES cells. (See online for colour version of this figure.)

Result of backcrossing E14 and C2J chimeric mice

All of the chimeric mice generated from E14 and C2J ES cells were mated with C57BL/6J mice to check the rate of germline transmission, and to compare this rate between the two ES cell lines. As summarized in Table 2, the result of this backcrossing experiment revealed that the percentage of positive F1 mice from grade A C2J chimeras was almost identical to that from grade A E14 chimeras (74.2 versus 72.2%, respectively). However, only 31.1% of positive F1 mice were produced from grade B E14 chimeras, whereas 42.9% positive F1 mice were generated from grade B C2J chimeras. With regard to grade C chimeras, the results were comparable, with E14 and C2J grade C chimeras producing 15.9% and 20.0% of positive F1 offspring,

https://doi.org/10.1017/S0967199412000366 Published online by Cambridge University Press

respectively. Taken together, our results suggest that when comparing the highest grade of chimeras (grade A), C2J and E14 ES cells are comparable in their rate of germline transmission; however, E14 cells retain a higher capability for forming chimeras.).

Discussion

The aim of this study was to compare the ability of two different mouse ES cells lines (E14 and C2J) in their chimera forming and germline transmission capability. Chimeric mice were generated by injecting both E14 and C2J ES cell clumps into host blastocysts; the resultant chimeric embryos were transferred into the uterus of recipient females leading to live born chimeric offspring which were assessed for their chimeric grade and successful germline transmission.

Previous research has suggested that growth of ES cells in synthetic serum could provide a more efficient method for the production of chimeric mice, and that fully defined culture conditions would allow the investigation of factors involved in ES cell self-renewal, differentiation, and germline transmission (Ward *et al.*, 2002). More recently, Gertsenstein *et al.* confirmed that KOSR + 2i medium enabled highly efficient generation of germline transmitting chimeras from targeted C57BL/6-derived ES cells (Gertsenstein *et al.*, 2010). Consistent with this report, our data show that the efficiency of germline transmission of ES cells in KOSR + 2i medium was high, and that the KOSR + 2i medium could support both C2J and E14 ES cells to keep their pluripotent capability.

Previous studies have indicated that B6-derived ES cells appear to be less efficient than other strains in generating germline transmitting chimeras; for example, in a recent large study only about 43% of B6-derived chimeras had successful germline transmission, versus 81% of 129S5 ES cells (Ware *et al.*, 2003; Ward *et al.*, 2004; Hansen *et al.*, 2008). In our work, we have examined the chimera forming capability of E14 and C2J mouse ES cell lines, finding that E14 ES cells had a higher capability to form chimeras than C2J ES cells. We assume that as C57BL/6-Tyrc-2J/J (B6-albino) is a substrain of C57BL/6J mice that carry a mutation in the tyrosinase gene, thus the same genetic background between C57BL/6J and B6-albino is probably the main reason for the lower capability for forming chimeras in the C2J ES cells. Despite the lower rate of chimera formation in the C2J ES cells, our backcrossing experiment revealed that grade A C2J chimeras and grade A E14 chimeras were comparable in their rates of germline transmission. This result suggested that C2J cells have same germline transmission capability as E14 ES cells, despite their lower ability to form chimeras.

The genome of C57BL/6J mice has been sequenced and well characterized, making this strain the preferred choice for generating mutant mice. However, the combination of using 129 ES cells and a C57BL/6J host leads to the production of mixed-background chimeras, which may not be suitable for all experimental applications. As such, using ES cell lines of the same genetic background as the host mouse model simplifies analyses and reduces the time and cost involved in producing appropriate mouse models. For this reason, some laboratories are trying to use C57BL/6J original ES cell instead of 129 original ES cell for gene targeting (Keskintepe *et al.*, 2007; Gertsenstein *et al.*, 2010). However, B6-derived ES cells have been shown to be more difficult to maintain in culture and have a greater tendency to lose their pluripotency when cultured in standard 15% FBS and LIF-supplemented ES cell medium, compared with 129-dervied ES cells (Brook & Gardner, 1997; Auerbach *et al.*, 2000; Sharova *et al.*, 2007). We have shown that despite a reduced rate of chimerism, B6-derived ES cells retain a high capacity for germline transmission.

In summary, we found that KOSR + 2i medium could support both E14 and C2J ES cells to keep their pluripotency. Moreover, C2J cells have a poor capability for forming chimeras compared with E14 ES cells, though they seem to possess a similar ability for germline transmission. As such, we believe it will be possible for C2J-derived ES cells to be used as replacements for E14-derived ES cells, and promote the widespread use of this ES cell line as a superior choice for gene targeting in mice.

Acknowledgement

The study was supported by the grants from the Natural Science Foundation of Shanghai (11ZR418800) and National Natural Science Foundation of China (81170756/H0713). This manuscript was proofread by an English speaking professional with a science background at Elixigen Corporation

References

Akiyama, T.E., Sakai, S., Lambert, G., Nicol, C.J., Matsusue, K., Pimprale, S., Lee, Y.H., Ricote, M., Glass, C.K., Brewer, H.B. Jr & Gonzalez, F.J. (2002). Conditional disruption of the peroxisome proliferator-activated receptor gamma gene in mice results in lowered expression of ABCA1, ABCG1, and apoE in macrophages and reduced cholesterol efflux. *Mol. Cell. Biol.* **22**, 2607–19.

Auerbach, W., Dunmore, J.H., Fairchild-Huntress, V., Fang, Q., Auerbach, A.B., Huszar, D. & Joyner, A.L. (2000). Establishment and chimera analysis of 129/SvEv- and C57BL/6-derived mouse embryonic stem cell lines. *Biotechniques* **29**, 1024–32.

https://doi.org/10.1017/S0967199412000366 Published online by Cambridge University Press

Brook, F.A. & Gardner, R.L. (1997). The origin and efficient derivation of embryonic stem cells in the mouse. *Proc. Natl. Acad. Sci. USA* **94**, 5709–12.

Brown, D.G., Willington, M.A., Findlay, I. & Muggleton-Harris, A.L. (1992). Criteria that optimize the potential of murine embryonic stem cells for *in vitro* and *in vivo* developmental studies. *In Vitro Cell. Dev. Biol.* **28A**, 773–8.

Chen, S., Hilcove, S. & Ding, S. (2006). Exploring stem cell biology with small molecules. *Mol. Biosyst.* **2**, 18–24.

Elder, R.H., Jansen, J.G., Weeks, R.J., Willington, M.A., Deans, B., Watson, A.J., Mynett, K.J., Bailey, J.A., Cooper, D.P., Rafferty, J.A., Heeran, M.C., Wijnhoven, S.W., van Zeeland, A.A. & Margison, G.P. (1998). Alkylpurine-DNA-N-glycosylase knockout mice show increased susceptibility to induction of mutations by methyl methanesulfonate. *Mol. Cell. Biol.* **18**, 5828–37.

Gertsenstein, M., Nutter, L.M., Reid, T., Pereira, M., Stanford, W.L., Rossant, J. & Nagy, A. (2010). Efficient generation of germ line transmitting chimeras from C57BL/6N ES cells by aggregation with outbred host embryos. *PLoS One* **5**, e11260.

Hansen, G.M., Markesich, D.C., Burnett, M.B., Zhu, Q., Dionne, K.M., Richter, L.J., Finnell, R.H., Sands, A.T., Zambrowicz, B.P. & Abuin, A. (2008). Large-scale gene trapping in C57BL/6N mouse embryonic stem cells. *Genome Res.* **18**, 1670–9.

Harris, S.P., Bartley, C.R., Hacker, T.A., McDonald, K.S., Douglas, P.S., Greaser, M.L., Powers, P.A. & Moss, R.L. (2002). Hypertrophic cardiomyopathy in cardiac myosin binding protein-C knockout mice. *Circ. Res.* **90**, 594–601.

Kawase, E., Suemori, H., Takahashi, N., Okazaki, K., Hashimoto, K. & Nakatsuji, N. (1994). Strain difference in establishment of mouse embryonic stem (ES) cell lines. *Int. J. Dev. Biol.* **38**, 385–90.

Keskintepe, L., Norris, K., Pacholczyk, G., Dederscheck, S.M. & Eroglu, A. (2007). Derivation and comparison of C57BL/6 embryonic stem cells to a widely used 129 embryonic stem cell line. *Transgenic Res.* **16**, 751–8.

Kiyonari, H., Kaneko, M., Abe, S. & Aizawa, S. (2010). Three inhibitors of FGF receptor, ERK and GSK3 establish germline-competent embryonic stem cells of C57BL/6N mouse strain with high efficiency and stability. *Genesis* **48**, 317–27.

Liu, X., Wu, H., Loring, J., Hormuzdi, S., Disteche, C.M., Bornstein, P. & Jaenischm, R. (1997). Trisomy eight in ES cells is a common potential problem in gene targeting and interferes with germline transmission. *Dev. Dyn.* **209**, 85–91.

Papaioannou, V.E. & Johnson, R. (2000). Production of chimeras by blastocyst and morula injection of targeted ES cells. In *Gene Targeting: A Practical Approach* (ed. A.L. Joyner), pp. 133–75 New York: Oxford University Press Inc.

Sharova, L.V., Sharov, A.A., Piao, Y., Shaik, N., Sullivan, T., Stewart, C.L., Hogan, B.L. & Ko, M.S. (2007). Global gene expression profiling reveals similarities and differences among mouse pluripotent stem cells of different origins and strains. *Dev. Biol.* **307**, 446–59.

Thompson, S., Clarke, A.R., Pow, A.M., Hooper, M.L. & Melton, D/W. (1989). Germ line transmission and expression of a corrected *HPRT* gene produced by gene targeting in embryonic stem cells. *Cell* **56**, 313–21.

Ward, C.M., Stern, P., Willington, M.A. & Flenniken, A.M. (2002). Efficient germline transmission of mouse embryonic stem cells grown in synthetic serum in the absence of a fibroblast feeder layer. *Lab. Invest.* **82**, 1765–7.

Ward, C.M., Barrow, K.M. & Stern, P.L. (2004). Significant variations in differentiation properties between independent mouse ES cell lines cultured under defined conditions. *Exp. Cell. Res.* **293**, 229–38.

Ware, C.B., Siverts, L.A., Nelson, A.M., Morton, J.F. & Ladiges, W.C. (2003). Utility of a C57BL/6 ES line versus 129 ES lines for targeted mutations in mice. *Transgenic Res.* **12**, 743–6.

Ying, Q.L., Wray, J., Nichols, J., Batlle-Morera, L., Doble, B., Woodgett, J., Cohen, P. & Smith, A. (2008). The ground state of embryonic stem cell self-renewal. *Nature* **453**, 519–23.

https://doi.org/10.1017/S0967199412000366 Published online by Cambridge University Press