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Lab Resource: Stem Cell Line

Derivation of hybrid ES cell lines from two different strains of mice

Ho-Tak Lau [a], Lizhi Liu [a], Chelsea Ray [a], Fong T. Bell [a], Xiajun Li [a,b,*]

[a] Black Family Stem Cell Institute, Department of Developmental and Regenerative Biology, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, New York, NY 10029, USA
[b] Department of Oncological Sciences, Graduate School of Biological Sciences, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, New York, NY 10029, USA

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ABSTRACT

Parental origin-dependent expression of the imprinted genes is essential for mammalian development. *Zfp57* maintains genomic imprinting in mouse embryos and ES cells. To examine the allelic expression patterns of the imprinted genes in ES cells, we obtained multiple hybrid ES clones that were directly derived from the blastocysts generated from the cross between mice on two different genetic backgrounds. The blastocyst-derived ES clones displayed largely intact DNA methylation imprint at the tested imprinted regions. These hybrid ES clones will be useful for future studies to examine the allelic expression of the imprinted genes in ES cells and their differentiated progeny.

Resource table

Name of stem cell construct	Not applicable
Institution	Icahn School of Medicine at Mount Sinai
Person who created resource	Ho-Tak Lau, Xiajun Li
Contact person and email	Xiajun Li, xiajun.li@mssm.edu
Date archived/stock date	December, 2011
Origin	Mouse blastocysts
Type of resource	Biological reagent: Mouse Embryonic Stem (ES) Cell Lines
Sub-type	129/DBA Hybrid ES cell lines
Key transcription factors	*Zfp57*
Authentication	Undifferentiated ES cell morphology confirmed (Fig. 1)
Link to related literature (direct URL links and full references)	http://www.ncbi.nlm.nih.gov/pubmed/18854139
Information in public databases	None

Resource details

Blastocyst-derived hybrid ES clones displayed typical ES cell characters

Parental origin-dependent mono-allelic expression of the imprinted genes is essential for mammalian development. Many imprinted genes are clustered and co-regulated by a cis-acting imprinting control region that harbors germline-derived differential DNA methylation. *Zfp57* is a master regulator in genomic imprinting and it maintains DNA methylation imprint at many imprinted regions in mouse embryos and ES cells (Li et al., 2008; Zuo et al., 2012). To facilitate allelic analysis of imprinting control regions and the imprinted genes, we have successfully established multiple wild-type ($Zfp57^{+/+}$) and *Zfp57* heterozygous ($Zfp57^{-/+}$) hybrid ES clones from the blastocysts generated from the cross between the wild-type male mice on the DBA/2 genetic background and $Zfp57^{+/-}$ heterozygous female mice on the 129 genetic background. These hybrid ES clones are on the 129/DBA hybrid genetic background containing one 129 allele and one DBA/2 allele at all loci of the entire genome, including approximately 150 known imprinted genes. The colonies of these 129/DBA hybrid ES clones exhibited typical undifferentiated ES cell morphology when they were grown on top of the feeder cells, as exemplified by one $Zfp57^{+/+}$ hybrid ES clone D1911 shown in Fig. 1A. The cells of these hybrid ES clones formed embryoid bodies (EBs) when grown in suspension on non-adherent Petri dish plates (Fig. 1B). These EBs differentiated into three germ layers based on semi-quantitative RT-PCR analysis (Fig. 1C). Indeed, expression of the markers for endoderm (*Foxa2*), mesoderm (*Mlc2a* and *Pdgfrα*) and ectoderm (*Ck18*) all appeared to be increased in the EB samples compared with the ES cell samples (Fig. 1C). We also counted the metaphase chromosome numbers in five hybrid ES clones (Table 1), with one metaphase chromosome spread as an example shown in Fig. 1D. At least 30% or more cells in these five hybrid ES clones appeared to be euploid cells with 40 chromosomes (Table 1). We also examined expression of OCT4 and NANOG, two pluripotency markers, in four $Zfp57^{+/+}$ and four $Zfp57^{-/+}$ hybrid ES clones (Fig. 2). Based on immunostaining, both OCT4 and NANOG are highly expressed in these hybrid ES clones (Fig. 2).

* Corresponding author at: Black Family Stem Cell Institute, Department of Developmental and Regenerative Biology, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, New York, NY 10029, USA.
E-mail address: xiajun.li@mssm.edu (X. Li).

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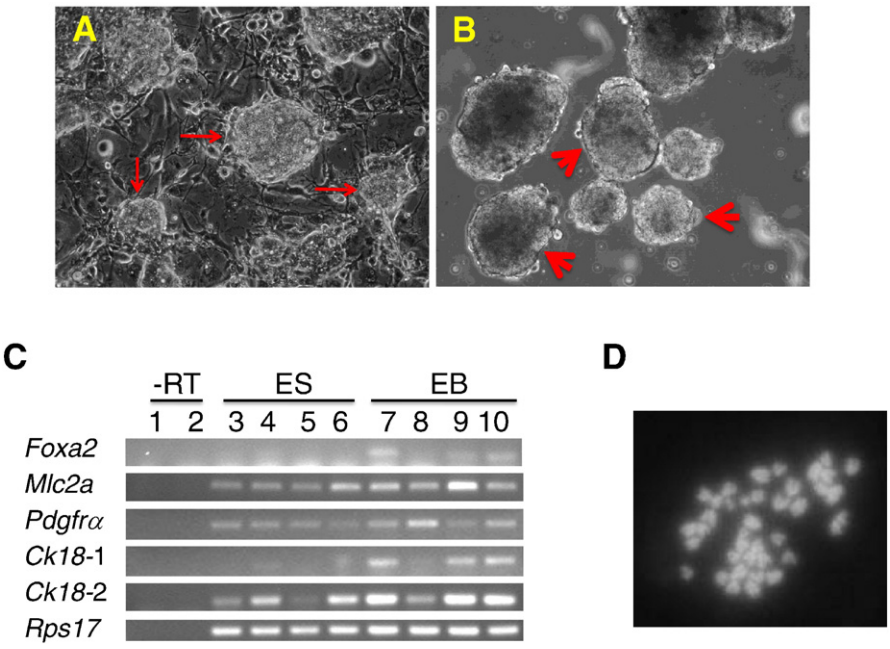

Fig. 1. Blastocyst-derived 129/DBA hybrid ES clones displayed typical properties of ES cells. A, one *Zfp57*$^{+/+}$ 129/DBA hybrid ES clone (D1911) was shown as an example that exhibited typical undifferentiated ES cell colonies (red arrows) when grown on the SNL feeder cells. B, the cells of D1911 hybrid ES clone formed embryoid bodies (EBs, arrowheads) when cultured on non-adherent Petri dish plates. C, RT-PCR expression analysis of the marker genes in four hybrid ES clones and the EBs derived from these hybrid ES clones after growing in suspension culture for 6 or 7 or 9 days. Lanes 1–2, negative control without reverse transcription (−RT) of the same total RNA samples in Lanes 4 and 5, respectively. Lanes 3–6, ES cell samples. Lanes 7–10, EB samples. Lane 3, D1903 *Zfp57*$^{+/+}$ 129/DBA hybrid ES clone. Lane 4, D1911 *Zfp57*$^{+/+}$ 129/DBA hybrid ES clone. Lane 5, D1902 *Zfp57*$^{-/+}$ 129/DBA hybrid ES clone. Lane 6, D1904 *Zfp57*$^{-/+}$ 129/DBA hybrid ES clone. Lane 7, day 7 EBs of D1911 *Zfp57*$^{+/+}$ 129/DBA hybrid ES clone. Lane 8, day 7 EBs of D1902 *Zfp57*$^{-/+}$ 129/DBA hybrid ES clone. Lane 9, day 6 EBs of D1904 *Zfp57*$^{-/+}$ 129/DBA hybrid ES clone. Lane 10, day 9 EBs of D1911 *Zfp57*$^{+/+}$ 129/DBA hybrid ES clone. *Mlc2a* and *Pdgfrα* are markers for mesoderm, whereas *Foxa2* and *Ck18* are the lineage markers for endoderm and ectoderm, respectively. A house-keeping gene *Rps17* was similarly amplified as the loading control. *Rps17* was subjected to 25 cycles of PCR amplification, whereas 30 PCR cycles were applied to *Mlc2a* and *Pdgfrα*. *Ck18* was amplified for 25 PCR cycles (*Ck18*–1) or 30 PCR cycles (*Ck18*–2). D, an example is shown here for DAPI-stained metaphase chromosome spread of a cell of D1911 *Zfp57*$^{+/+}$ 129/DBA hybrid ES clone.

Most hybrid ES clones exhibited intact DNA methylation imprint

The genotypes of *Zfp57* for these hybrid ES clones were confirmed by a PCR-based approach that were used in our previous studies (Li et al., 2008; Takikawa et al., 2013), as exemplified by six hybrid ES clones shown in Fig. 3A. To test if these 129/DBA hybrid ES clones are suitable for allelic expression analysis of imprinted genes in ES cells and their differentiated progeny in future studies, we analyzed DNA methylation imprint of six hybrid ES clones at multiple imprinted regions. We found genomic imprinting was maintained in most 129/DBA hybrid ES clones based on COBRA analysis of DNA methylation at multiple imprinted regions. The COBRA result for the *Snrpn* imprinted region was provided as an example (Fig. 3B). Except for partial loss of DNA methylation imprint observed in one *Zfp57*$^{+/+}$ hybrid ES clone (D1903, Lane 3 of Fig. 3B), DNA methylation imprint at the *Snrpn* imprinted region was largely intact at two other *Zfp57*$^{+/+}$ hybrid ES clones (D1908 and D1911, Lanes 6–7 of Fig. 3B) and three *Zfp57*$^{-/+}$ hybrid ES clones (D1902, D1904 and D1906 in lanes 2, 4–5 of Fig. 3B). This indicates that these hybrid ES clones will be useful for allelic analyses in genomic imprinting in future research.

Table 1
Chromosome numbers of five hybrid ES clones.

Hybrid ES clone	D1902	D1903	D1904	D1906	D1911
Genotype	*Zfp57*$^{-/+}$	*Zfp57*$^{+/+}$	*Zfp57*$^{-/+}$	*Zfp57*$^{-/+}$	*Zfp57*$^{+/+}$
# of counted metaphase spreads	20	15	6	10	20
# of spreads with 40 chromosomes	7	7	2	4	10
% of euploid cells	35	46.7	33.3	40	50

Materials and methods

Mouse maintenance

The mouse strains were maintained in the animal facility of Icahn School of Medicine at Mount Sinai in compliance with the animal care protocol approved by IACUC of the institution. The wild-type DBA/2 mice were obtained from the Jackson Laboratories. The *Zfp57* heterozygous (*Zfp57*$^{+/-}$) mice on the 129 genetic background were generated from our previously published study (Li et al., 2008).

Derivation of the 129/DBA hybrid ES clones from blastocysts

Mouse blastocytsts were isolated from the timed pregnancy mating between the wild-type male mice on the DBA/2 genetic background and *Zfp57*$^{+/-}$ heterozygous female mice on the 129 genetic background. Hybrid ES clones were derived from these blastocysts based on the previously published protocol (Meissner et al., 2009). First, blastocysts were added onto the feeder cells to allow outgrowth of the ES colonies. The colonies with ES cell colony-like morphology were picked individually to a well of 24-well plate seeded with feeder cells. Then these clones were passaged to a new well with feeder cells until stable ES cell lines were established. These established ES cell lines are on the 129/DBA hybrid genetic background with one 129 allele and one DBA/2 allele at all loci of the genome including the imprinted regions.

ES cell culture

The ES cell growth medium was made of high-glucose DMEM medium supplemented with 15% fetal bovine serum (FBS). The ES cells were cultured with the ES cell growth medium on top of the irradiated SNL feeder cells (McMahon and Bradley, 1990). Before they became too confluent, ES cells were split by trypsin digestion and passaged onto new