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Lab Resource: Stem Cell Line

Zfp57 mutant ES cell lines directly derived from blastocysts

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ABSTRACT

Zfp57 is a master regulator of genomic imprinting in mouse embryos. To further test its functions, we have derived multiple *Zfp57* mutant ES clones directly from mouse blastocysts. Indeed, we found DNA methylation imprint was lost at most examined imprinting control regions in these *Zfp57* mutant ES clones, similar to what was observed in *Zfp57* mutant embryos in the previous studies. This result indicates that these blastocyst-derived *Zfp57* mutant ES clones can be employed for functional analyses of *Zfp57* in genomic imprinting.

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	November, 2010
Origin	Mouse blastocysts
Type of resource	Biological reagent: Mouse Embryonic Stem (ES) Cell Lines
Sub-type	*Zfp57* mutant 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 ES clones display typical properties of ES cells

Zfp57 is a maternal-zygotic effect gene and it has both maternal (M) and zygotic (Z) functions (Li et al., 2008; Shamis et al., 2015). Eight $Zfp57^{+/-}$ (M^+Z^+) and three $Zfp57^{-/-}$ (M^+Z^-) ES clones were derived from the blastocysts generated from the cross between $Zfp57^{+/-}$ heterozygous female mice and $Zfp57^{-/-}$ homozygous male mice, whereas five $Zfp57^{-/-}$ (M^-Z^-) ES clones were derived from the cross between $Zfp57^{-/-}$ homozygous female mice and $Zfp57^{-/-}$ homozygous male mice. The genotypes for these ES clones were confirmed by PCR-based genotyping (see Fig. 3A below). These ES clones displayed typical ES cell morphology as exemplified by one ES clone for each genotype shown in Fig. 1A, suggesting that undifferentiated ES clones can be properly established without maternal (M^-) or zygotic (Z^-) *Zfp57*. They formed embryoid bodies (EBs) when grown in suspension (Fig. 1A). Based on semi-quantitative RT-PCR analysis, the expression of the markers for endoderm (*Foxa2*), mesoderm (*Mlc2a*), and ectoderm (*Ck18*) seems to be increased in the EB samples compared with the ES cell samples in three tested blastocyst-derived ES clones (Fig. 1B). We also counted the metaphase chromosome numbers in five ES clones (Table 1), as exemplified by one metaphase chromosome spread (Fig. 1C). All five ES clones appeared to have relatively normal chromosome numbers, with over 60% or more euploid cells with 40 chromosomes in these ES clones (Table 1). Based on immunostaining, these five ES clones express OCT4 and NANOG, two pluripotency markers (Fig. 2).

Maintenance of maternally inherited DNA methylation imprint

We also analyzed DNA methylation imprint inherited on the maternal chromosomes at four imprinted regions (*Peg1*, *Peg3*, *Snrpn*, and *Igf2r*) in these ES clones. Similar to what had been observed in $Zfp57^{+/-}$ (M^+Z^+) and $Zfp57^{-/-}$ (M^-Z^-) mouse embryos in our previous study (Li et al., 2008), DNA methylation imprint was lost at these four imprinted regions in five $Zfp57^{-/-}$ (M^-Z^-) ES clones (M4–M8 of Fig. 3B) in comparison with eight $Zfp57^{+/-}$ (M^+Z^+) ES clones (H1–H8 of Fig. 3B). DNA methylation imprint was also lost at these four imprinted regions in three $Zfp57^{-/-}$ (M^+Z^-) ES clones (M1–M3 of Fig. 3B), whereas partial loss of DNA methylation was observed at

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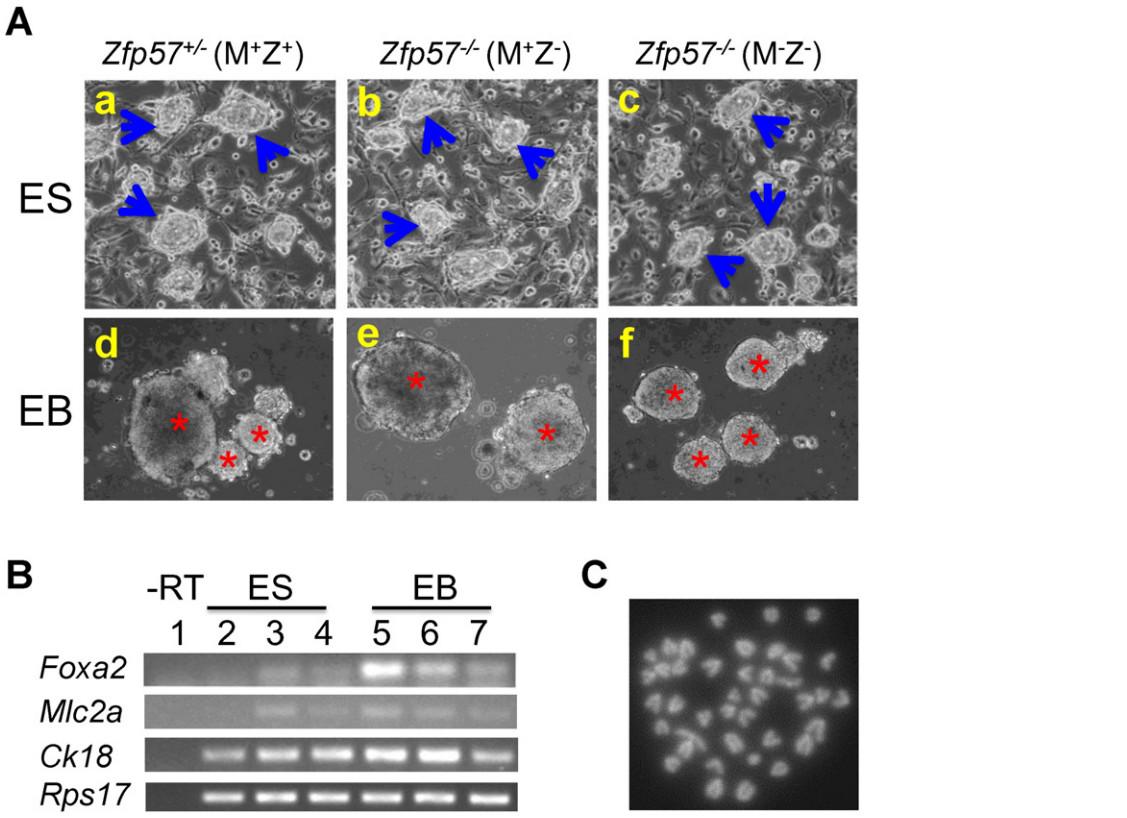

Fig. 1. Blastocyst-derived ES clones display normal characters of ES cells. (A) ES clones on feeder cells (a–c) or EBs on non-adherent Petri dish plates (d–f). One *Zfp57*$^{+/-}$ (M^+Z^+) ES clone (a, d) and one *Zfp57*$^{-/-}$ (M^+Z^-) ES clone (b, e) were shown here as the examples for the ES clones derived from the blastocysts generated from the cross between *Zfp57*$^{+/-}$ heterozygous female mice and *Zfp57*$^{-/-}$ homozygous male mice, whereas one *Zfp57*$^{-/-}$ (M^-Z^-) ES clone (c, f) shown here was an example for the ES clones derived from the cross between *Zfp57*$^{-/-}$ homozygous female mice and *Zfp57*$^{-/-}$ homozygous male mice. Blue arrows in a–c, undifferentiated ES cell colonies grown on feeder cells. Red asterisks in d–f, embryoid bodies (EBs) in suspension formed by ES cells plated on non-adherent Petri dish plates. (B) RT-PCR expression analysis of the marker genes in three ES clones and the EBs derived from these three ES clones after growing in suspension culture for 7–8 days. Lane 1, negative control without reverse transcription (−RT) of the same total RNA sample in lane 4. Lane 2, ES cells of one *Zfp57*$^{-/-}$ (M^-Z^-) ES clone. Lanes 3–4, the ES cells of two *Zfp57*$^{+/-}$ (M^+Z^+) ES clones. Lane 5, EBs of the *Zfp57*$^{-/-}$ (M^-Z^-) ES clone. Lanes 6–7, EBs of two *Zfp57*$^{+/-}$ (M^+Z^+) ES clones. *Foxa2*, *Mlc2a*, and *Ck18* are markers for endoderm, mesoderm, and ectoderm, respectively. *Rps17* is a housekeeping gene that was used as the loading control. (C) DAPI-stained metaphase chromosome spread derived from a cell of one *Zfp57*$^{-/-}$ (M^-Z^-) ES clone. An example is provided here for the metaphase chromosome spread of five ES clones.

these four imprinted regions in *Zfp57*$^{-/-}$ (M^+Z^-) zygotic mutant embryos lacking just zygotic *Zfp57* (Li et al., 2008). These results suggest that maternal *Zfp57* appears to have more lasting effect on the maintenance of DNA methylation imprint inherited on maternal chromosomes in mouse embryos than in blastocyst-derived ES cells.

Maintenance of paternally inherited DNA methylation imprint

We examined paternally inherited DNA methylation imprint at three imprinted regions (*Dlk1–Dio3*, *H19*, and *Rasgrf1*) in these ES clones by COBRA. Similar to what was found in *Zfp57*$^{+/-}$ (M^+Z^+) and *Zfp57*$^{-/-}$ (M^-Z^-) mouse embryos in our previous study (Li et al., 2008), DNA methylation imprint was lost at the *Dlk1–Dio3* imprinted region but not at the *H19* imprinted region in five *Zfp57*$^{-/-}$ (M^-Z^-) ES clones (M4–M8 of Fig. 4A) in comparison to eight *Zfp57*$^{+/-}$ (M^+Z^+) ES clones (H1–H8 of Fig. 4A). Unlike partial loss of DNA methylation imprint observed in *Zfp57*$^{-/-}$ (M^+Z^-) zygotic mutant embryos lacking just zygotic *Zfp57* in our previous study (Li et al., 2008), DNA methylation imprint was almost completely lost at the *Dlk1–Dio3* imprinted region but not at the *H19* imprinted region in three *Zfp57*$^{-/-}$ (M^+Z^-) ES clones (M1–M3 of Fig. 4A), indicating that maternal *Zfp57* has more lasting effect on the maintenance of DNA methylation imprint inherited on paternal chromosomes in mouse embryos than in blastocyst-derived ES cells. We also found that DNA methylation imprint was similarly lost at the *Rasgrf1* imprinted region in three *Zfp57*$^{-/-}$ (M^+Z^-) and five *Zfp57*$^{-/-}$ (M^-Z^-) ES clones in comparison with eight *Zfp57*$^{+/-}$ (M^+Z^+) ES clones (Fig. 4A). This is the first experimental evidence demonstrating that DNA methylation imprint at the *Rasgrf1* imprinted region is lost without *Zfp57*.

DNA methylation at the IAP repeats

Previously, we did not observe any loss of DNA methylation at the *IAP* repeat regions in mouse embryos lacking *Zfp57* (Li et al., 2008).

Table 1
Counting of metaphase chromosome spreads of five ES clones.

ES clone	6909	6911	406	631	634
Genotype	*Zfp57*$^{+/-}$ (M^+Z^+)	*Zfp57*$^{+/-}$ (M^+Z^+)	*Zfp57*$^{-/-}$ (M^+Z^-)	*Zfp57*$^{-/-}$ (M^-Z^-)	*Zfp57*$^{-/-}$ (M^-Z^-)
No. of counted metaphase spreads	16	20	20	20	20
No. of spreads with 40 chromosomes	10	12	14	14	13
% of euploid cells	62.5	60	70	70	65