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Lab Resource: Stem Cell Line

Generation of a Bag1 homozygous knockout mouse embryonic stem cell line using CRISPR/Cas9

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ABSTRACT

Bag1 transcribes a multifunctional protein that participates in many important biological processes such as cell apoptosis, proliferation, differentiation and embryo development. Despite numerous published studies, the role of *Bag1* in the context of embryonic stem (ES) cells, has not been explored. To investigate the function of *Bag1* in ES cells, we generated mutant *Bag1*$^{-/-}$ ES cells using the CRISPR/Cas9 system. We established that the *Bag1* double knockout ES cell line maintained their pluripotency, possessed a normal karyotype and the ability to differentiate into all three germ layers.

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Resource table.

Name of stem cell line	Bag1KO-mESC
Institution	Chinese University of Hong Kong
Person who created resource	Chengcheng Tang
Contact person and email	Kenneth Ka Ho Lee, kaholee@cuhk.edu.hk
Date archived/stock date	Jan 10, 2017
Origin	C57BL/6 mice
Type of resource	Genetically modified mouse embryonic stem cell line
Sub-type	cell line
Key transcription factors	N/A
Authentication	Identity and purity of cell line are confirmed in Fig. 1
Link to related literature	N/A
Information in public databases	N/A
Ethics	The mice and cell lines used have been approved by the Hong Kong Department of Health and the Chinese University of Hong Kong Animal Ethic Committees

Resource details

We have generated a double knockout *Bag1* ES cell line by targeting the exon 2 of *Bag1* gene using the CRISPR/Cas9 gene editing system (Fig. 1A). Mouse ES cells were transfected with pSpCas9(BB)-2A-GFP plasmids which contained sgRNA targeting the exon 2 of *Bag1*. The transfected cells were cultured for two days and then the presence of GFP expressing cells were sorted and selected by fluorescence flow cytometry. The sorted GFP^{+} cells were reseeded onto 0.1% gelatin-coated culture dishes to obtain single cell derived ES cell clones. Fifty-two individual ES cell clones were picked, isolated and expanded for further analysis by DNA sequencing of targeted regions. Twenty homozygous mutant ES cell clones were obtained and a bi-allelic 34-nucleotide-deleted and 38-nucleotide-deleted clone, *Bag1*KO-mESC (Fig. 1B,C), were chosen for further characterization.

The *Bag1*KO-mESC line maintains a classical dome-shaped ES cell morphology and expressed high levels of ES-associated protein such as, alkaline phosphatase (Fig. 1D). Immunofluorescent staining for the three key pluripotent stem cell markers Oct4, Sox2 and Nanog revealed that the *Bag1*KO-mESC line was positive for these all three stemness markers (Fig. 1E). Karyotype analysis also confirmed that the CRISPR/Cas9 gene editing process did not introduce any chromosomal abnormality in the *Bag1*KO-mESC line (Fig. 1F). Furthermore, the *Bag1*KO-mESC line still maintained the potential to differentiate into all three germ layers as determined by their ability to form teratoma in vivo and histological examinations (Fig. 1G).

In summary, our *Bag1*KO-mESC line has been determined to be pluripotent and possess a normal karyotype. This mutant cell line would be a good model for investigating the biological function of *Bag1* during development.

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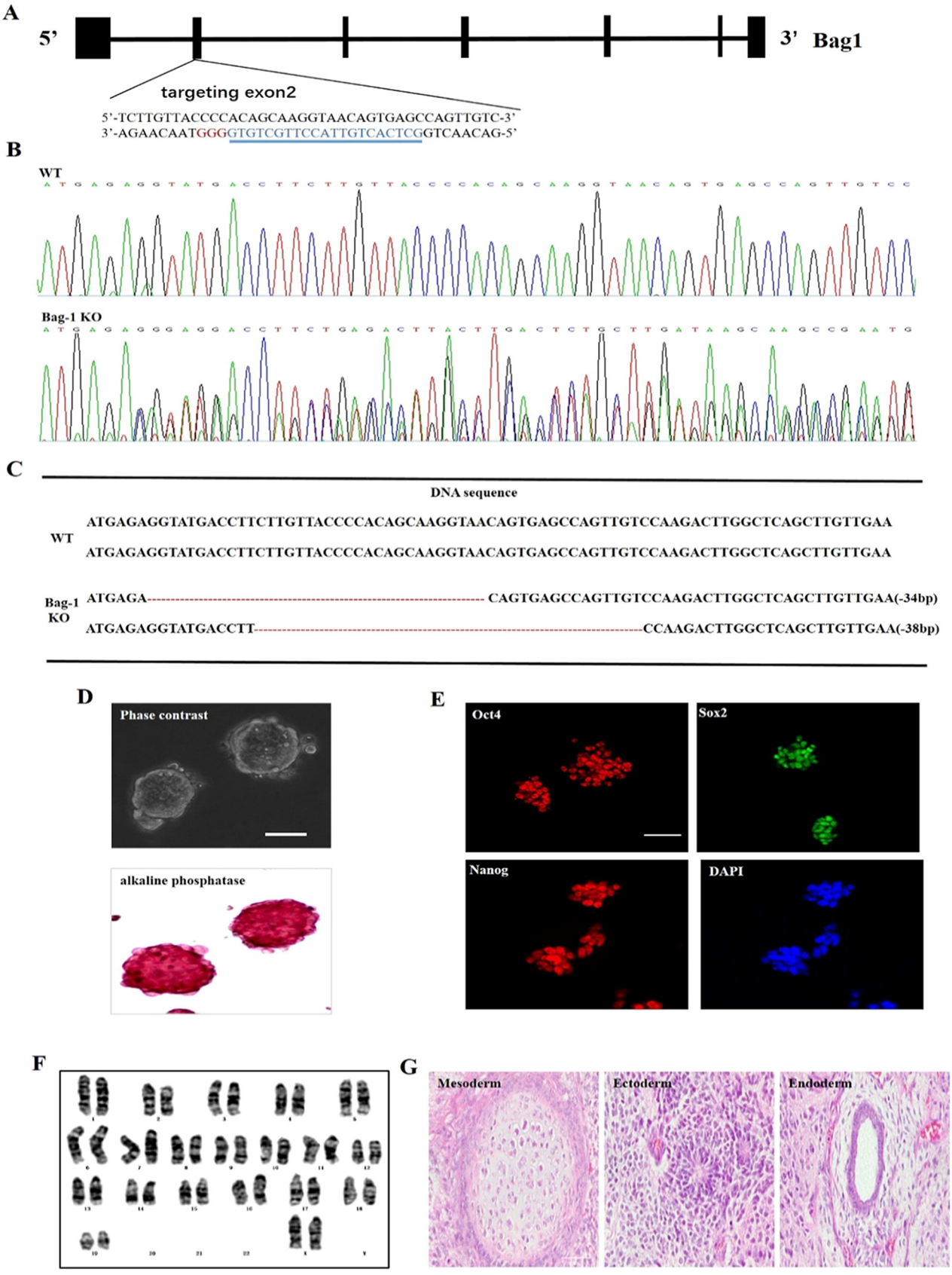

Fig. 1. Generation and characterization of the *Bag1* homozygous knockout mouse embryonic stem cell line. (A) Schematic overview of the gene targeting strategy for *Bag1* using CRISPR/Cas9. The Cas9/sgRNA target site is *underlined*. (B) Sanger-sequencing result of targeted regions. (C) Showing the base sequences of wild type and Bag1 double mutant alleles. (D) Phase contrast microscopic image of *Bag1*KO-mESC cell line maintained on 0.1% gelatin coated 4-well-plate (Left). The cells are positively stained for the marker, alkaline phosphatase (Right). Scale bar = 50 μm. (E) *Bag1*KO-mESCs are immunofluorescently stained with pluripotent markers Oct4, Sox2 and Nanog. Scale bar = 50 μm. (F) Karyotype analysis of the *Bag1*KO-mESC cell line. (G) Hematoxylin and eosin staining of teratomas derived from the *Bag1*KO-mESC. Examination of the histological sections revealed that the tissues formed in the teratomas are derived from all three germ layers. Scar bar = 50 μm.

Materials and methods

ES cell culture

The ES cells were produced and validated according to published protocols (Bryja et al., 2006; Gertsenstein et al., 2010). The isolated ES cells were cultured on 0.1% gelatin-coated plastic culture dishes and maintained in normal ES medium (DMEM/F12 (Sigma-Aldrich), 15% ESQ (Hyclone), 2 mM Glutamine (Gibco), 0.1 mM non-essential amino acids (Gibco), 0.1 mM β-mercaptoethanol (Sigma-Aldrich), 1 mM Sodium Pyruvate (Gibco) 100 U/ml penicillin/streptomycin (Invitrogen), 1000 U/ml LIF (Millipore), 1 μM PD0325901 (Sigma-Aldrich) and 3 μM CHIR99021 (Sigma-Aldrich).

Bag1$^{-/-}$ ES cell production by CRISPR/Cas9 system

The sgRNA Oligos were synthesized using protocol as previously described (Ran et al., 2013; Wettstein et al., 2016). The sgRNA were introduced into pSpCas9(BB)-2A-GFP plasmids (Addgene, 48138). 1 μg of pSpCas9(BB)-2A-GFP plasmids containing sgRNAs were digested using *Bbs*I restriction enzymes (NEB) and then transfected into mouse ES cells with Lipofectamine 2000 (Life Technologies, 11668027). The transfected cells were cultured for two days and then all successfully transfected GFP^{+} cells were sorted and selected by fluorescence flow cytometry. The purified cells were then introduced into 0.1% gelatin-coated 10 cm dish in the presence of normal ES medium (3000 cells/10 cm dish). Cell colonies started to appear approximately 7 days after seeding. The ES cell clones were then picked and expanded for further analysis by DNA sequencing of the targeted region.

Alkaline phosphatase (AP) staining

ES cells were fixed in 4% paraformaldehyde for 5 min and then washed with phosphate buffered saline Tween-20 (PBST) buffer. This was followed by incubating with the AP substrate solution (Alkaline Phosphatase Kit II, Stemgent) in the dark for 10 min. After staining, the cells were washed with PBS buffer and photographed using a microscope with an attaching spot flex camera.

Immunofluorescent staining

The stem cells were fixed, washed, permeabilized and blocked. The treated cells were incubated with primary antibodies overnight at 4 °C. The primary antibodies were diluted before use: rabbit polyclonal anti-Oct4 antibody (ab-18976 Abcam) at 1:100, rabbit monoclonal anti-Sox2 antibody (ab-92494 Abcam) at 1:100 and rabbit polyclonal anti-Nanog antibody (ab-80892 Abcam) at 1:100. The samples were then washed 3 times in PBS and then incubated with the appropriate fluorescently labelled secondary antibody, Alex flor 555 (Invitrogen, 1:300) for 1 h at room temperature. The nuclei were counter-stained with DAPI for 10 min. Images were photographed using an Olympus FV1200 confocal laser scanning microscope.

Karyotyping analysis

The G banding technique was used for karyotyping analysis. Twenty metaphase chromosome spreads were counted and the G bands resolution was between 300 and 500.

Teratoma assay

10^6 ES cells were injected subcutaneously into three 8-week-old female nude mice. Three weeks after injection, the teratomas were harvested and used for histological examination by hematoxylin and eosin (H&E) staining

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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