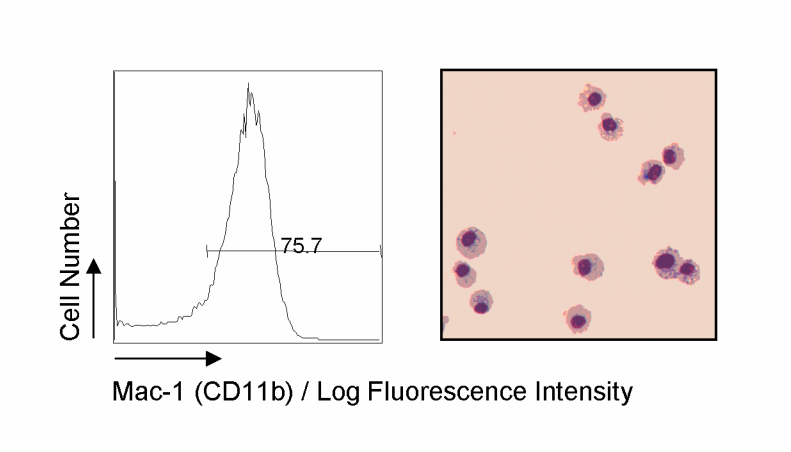
Supplementary Materials



Supplementary Figure 1. *BEX*-transduced colonies. Left panel, immunophenotyping of cells from colonies transduced by *BEX*. Right panel, Wright-Giemsa-stained cytospin preparation of cells isolated from colonies. Original magnification ×20.

Materials and Methods

Primers for RT-PCR

Smad4: forward, 5'-TGGCC-TGATCTTCACAAAAA-3',

Reverse, 5'-CACAGTGTTAATCCTGAGAGAT-3';

Gapdh: forward, 5'-TAAAGGGCATCCTGGGCTACACT-3',

Reverse, 5'-TTACTCCTTGGAGGCCATGTAGG-3';

Nup98-Hoxa9: forward, 5'-AGCTCCTCCACCACTAATTCAGGCTTT-3',

Reverse, 5'-AGAGAAGGCGCCTTCGCTGGGTTG-3';

Hoxa9: forward, 5'-CTACGTGCACCCCCAGGCGC-3',

Reverse, 5'-CTTCTGGCCGACAGCGGTTCAGGTTTA-3'.

Bone Marrow Harvesting and Culture

C57B/6-Ly-5.2 mice were injected intraperitoneally with 150 mg/kg body weight 5-FU (Sigma) dissolved in sterile phosphate-buffered saline (PBS). Four days later, mice were killed, and bone marrow was harvested from the femurs and tibias by using a 25-gauge needle and sterile PBS. After a single cell suspension was obtained, red cells were lysed for 7 min on ice in 1.0 mL ACK (8.3 g ammonium chloride and 1.0 g potassium bicarbonate in 1 L distilled deionized water) per mouse equivalent of bone marrow. Cells were then washed in PBS, filtered, and then resuspended in Dulbecco modified eagle medium (DMEM; Gibco) supplemented with 10% heat-inactivated fetal calf serum (FCS; Gibco), penicillin 100 IU/mL, streptomycin 100 IU/mL (Gibco), 1× nonessential amino acids, 1× sodium pyruvate (1 mM), 1×L-glutamine (2 mM; Gibco) and 50 μM β-mercaptoethanol (BME; Sigma). To induce stem cell cycling, cytokines were added to the culture medium as follows: 5 ng/mL IL-6 (R&D Systems), 50 ng/mL stem cell factor (R&D Systems) and 1x leukemia inhibitory factor (ESGRO). Cells were prestimulated for 24 h in a sterile incubator at 37°C in 5% CO_2.

Retrovirus Production and Transduction

Retrovirus-packaging BOSC23 cells were transfected by standard calcium-phosphate transfection. After 24 h of transfection, the cells were irradiated (3000 rads) with a Cobalt irradiator. The prestimulated bone marrow cells were then plated on top of the irradiated, retroviral producer cells along with 8 μg/mL polybrene (hexadimethrine bromide; Sigma). Transduced cells were then sorted in the core facility after 48 h.

Methylcellulose Colony-Forming Assay

Cells were sorted into Iscove's modified Dulbecco's medium (IMDM) supplemented with 10% FCS and then were plated into methylcellulose by using MethCult 3434 medium (Stem Cell Technologies), which contains the recombinant cytokines, stem cell factor, IL-3, IL-6 and erythropoietin (EPO). Seven days later, colonies of more than 50 cells were counted and examined for BEX expression by using an inverted fluorescent microscope. Representative colonies were picked, and cells were cytospun onto microscopic glass slides. Wright-Giemsa staining was performed on the slides to examine cell morphology.

ChIP Assay

NIH/3T3 cells were seeded in 100-mm plates at 40% confluency and were transfected with pcDNA3, pRK5-Smad2-flag, pRK5-Smad4-flag and Hoxa9-luc promoter. After transfection, cells were infected with virus-containing supernatant for 18-24 h with 8 μg/mL polybrene. Fresh medium was used. After 24 h, cells were cross-linked with 1% formaldehyde for 25 min at room temperature, and glycine (125 mM) was added for 10 min at room temperature to quench the formaldehyde. The cells were washed twice with ice-cold PBS and lysed in 500 mL of lysis buffer (5 mM PIPES, pH 8.0, 85 mM KCl, 0.5% NP-40, protease inhibitors) for 30 min on ice in 0.4 mL nuclear lysis buffer (50mM Tris-HCl, pH 8.0, 10 mM EDTA, 1% SDS, protease inhibitors). The samples were sonicated four times for 10 sec (60% duty cycle). The average DNA fragments ranged from 300 to 1000 bp. The lysates were then clarified by centrifugation at max speed for 10 min at 4°C. 2% of the samples were saved as input control and the rest were diluted 10 times in ChIP dilution buffer (15 mM Tris-HCl, pH 8.0, 1% Triton X-100, 0.01% SDS, 1 mM

EDTA, 150 mM NaCl, and protease inhibitors). Samples were then incubated with antibodies overnight at 4°C. Immunoprecipitation was carried out by using Protein G-sepharose beads for 1 h at 4°C. Immune complexes were washed consecutively for 10 min with each of the following solutions: low-salt wash buffer (20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% Triton X-100, 0.10% SDS and 2 mM EDTA), high-salt wash buffer (20 mM Tris-HCl, pH 8.0, 500 mM NaCl, 1% Triton X-100, 0.10% SDS and 2 mM EDTA), LiCl wash buffer (0.25 M LiCl, 1% NP-40, 1% sodium deoxycholate, 1 mM EDTA and 1% SDS) and twice with TE. Complexes were then eluted twice at 65°C for 10 min in elution buffer (50 mM Tris-HCl, pH 8.0, 10 mM EDTA, and 1% SDS). Immunoprecipitated DNA was reversed cross-linked at 65°C overnight and purified by using a PCR purification kit (Qiagen). Two microliters of purified DNA were subjected to PCR for 30 cycles. PCR products were dissolved in 2% agarose gel containing EB.

Primers used for amplication of *Hoxa9* promoter were:

Forward, 5'-ACCCCATCGTAGAGCGGCACGA-3',

Reverse, 5'-GGAAGTACAGTCACCTAATAAGTTGCCG-3'.