Elsevier Editorial System(tm) for Experimental Hematology
Manuscript Draft

Manuscript Number: 12- 210R2

Title: Effective expansion of engrafted human hematopoietic stem cells in bone marrow of mice expressing human Jagged-1

Article Type: Regular Submission

Keywords: Humanized mice; Jagged-1; Hematopoiesis; Bone marrow niche

Corresponding Author: Dr. Sonoko Habu, PhD, MD

Corresponding Author's Institution: Juntendo University School of Medicine

First Author: Naoko Negishi

Order of Authors: Naoko Negishi; Daisuke Suzuki; Ryoji Ito; Naoko Irie; Koichi Matsuo; Takashi Yahata; Kenichi Nagano; Kazuhiro Aoki; Keiichi Ohya; Katsuto Hozumi; Kiyoshi Ando; Norikazu Tamaoki; Mamoru Ito; Sonoko Habu, PhD, MD

Abstract: The human immune system can be reconstituted in experimental animals by transplanting human hematopoietic stem cells (hHSCs) into immunodeficient mice. To generate such humanized mice, further improvements are required, particularly to ensure that transplanted hHSCs are maintained in mice and proliferate long enough to follow prolonged immune responses to chronic diseases or monitor therapeutic effects. To prepare the relatively human bone marrow environment in mice, we generated non-obese diabetic/severe combined immunodeficiency/IL-2 receptor gamma chain null (NOG) mice expressing human Jagged1 (hJ1) in an osteoblast-specific manner (hJ1-NOG mice) to examine whether Notch signaling induced by hJ1 mediates hHSC proliferation and/or maintenance in mice. The established hJ1-NOG mice possess relatively larger bone marrow (BM) space and thinner cortical bone compared to non-transgenic littermates but the number of c-kit+ Sca-1+ lineage- (KSL) cells was not significantly different between hJ1-NOG and non-transgenic littermates. In the transplantation experiments of CD34+ cells obtained from human cord blood, CD34+CD38- cells (hHSCs) were more increased in hJ1-NOG recipient mice than in non-transgenic littermates in mouse BM environment. In contrast, the transplanted mouse KSL cells did not show significant increase in the same hJ1-NOG mice. These results suggest that hJ1-NOG mice could contribute to the growth of transplanted human CD34+ cells in a human specific manner and be useful to study the in vivo behavior and/or development of human stem cells including cancer stem cells and immune cells.

*Manuscript

Effective expansion of engrafted human hematopoietic stem cells in bone marrow of mice expressing human Jagged-1

Naoko Negishi[1*], Daisuke Suzuki[2,3*], Ryoji Ito[4], Naoko Irie[5], Koichi Matsuo[5], Takashi Yahata[6], Kenichi Nagano[7], Kazuhiro Aoki[7], Keiichi Ohya[7], Katsuto Hozumi[2], Kiyoshi Ando[6], Norikazu Tamaoki[4], Mamoru Ito[4] and Sonoko Habu[1]

Author Affiliations

[1]Department of Immunology, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan

[2]Department of Immunology, Tokai University School of Medicine, 143 Shimokasuya, Isehara, Kanagawa 259-1193, Japan

[3]Department of Animal Biology, University of Pennsylvania School of Veterinary Medicine, 3800 Spruce St., Room 161E Old Vet. Philadelphia, PA 19104, U.S.A.

[4]Central Institute for Experimental Animals, 3-25-12 Tonomachi, Kawasaki-ku, Kawasaki, Kanagawa 210-0821, Japan

[5]Laboratory of Cell and Tissue Biology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan

[6]Division of Hematopoiesis, Research Center for Regenerative Medicine and Department of Hematology, Tokai University School of Medicine, 143 Shimokasuya, Isehara, Kanagawa 259-1193, Japan

[7]Section of Pharmacology, Department of Bio-Matrix, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, 113-8549 Tokyo, Japan

* Negishi and Suzuki have contributed equally to this work.

Keywords

Humanized mice, Jagged-1, Hematopoiesis, Bone marrow niche

Corresponding author

Sonoko Habu, M.D. Ph.D., Department of Immunology, Juntendo University School of Medicine

2-1-1 Hongo, Bunkyo-ku, Tokyo, Japan, 113-8421

Tel: +81-3-5802-1591, Fax: +81-3-3813-5512

e-mail: sonoko-h@juntendo.ac.jp

Table of Contents category: Stem Cell

Word count (excluding references, figure legends, and tables): 2664 words

Abbreviations:

J1 Jagged-1

HSC hematopoietic stem cell

KSL c-kit-positive, sca-1-positive, lineage marker-negative

NOD non-obese diabetes

SCID severe combined immunodeficiency

NOG NOD/Shi-*scid* IL2rγ^{null}

Abstract

The human immune system can be reconstituted in experimental animals by transplanting human hematopoietic stem cells (hHSCs) into immunodeficient mice. To generate such humanized mice, further improvements are required, particularly to ensure that transplanted hHSCs are maintained in mice and proliferate long enough to follow prolonged immune responses to chronic diseases or monitor therapeutic effects. To prepare the relatively human bone marrow environment in mice, we generated non-obese diabetic/severe combined immunodeficiency/IL-2 receptor gamma chain null (NOG) mice expressing human Jagged1 (hJ1) in an osteoblast-specific manner (hJ1-NOG mice) to examine whether Notch signaling induced by hJ1 mediates hHSC proliferation and/or maintenance in mice. The established hJ1-NOG mice possess relatively larger bone marrow (BM) space and thinner cortical bone compared to non-transgenic littermates but the number of c-kit^{+} Sca-1^{+} lineage^{-} (KSL) cells was not significantly different between hJ1-NOG and non-transgenic littermates. In the transplantation experiments of CD34^{+} cells obtained from human cord blood, CD34^{+}CD38^{-} cells (hHSCs) were more increased in

hJ1-NOG recipient mice than in non-transgenic littermates in mouse BM environment. In contrast, the transplanted mouse KSL cells did not show significant increase in the same hJ1-NOG mice. These results suggest that hJ1-NOG mice could contribute to the growth of transplanted human $CD34^+$ cells in a human specific manner and be useful to study the *in vivo* behavior and/or development of human stem cells including cancer stem cells and immune cells.

Introduction

Humanized animals generated by transplantation of human cells or tissues into immunodeficient mice have been used for studying human-specific diseases and therapeutic interventions. Among engrafted cells, human cord blood (CB)-derived $CD34^+$ have been widely employed to establish a human immune system in mice in order to examine the *in vivo* behavior of human immune cells, including long-term immune responses to infections and tumor growth [1]. Efficient engraftment has been achieved by selection of recipients and the hHSC injection route. However, the maintenance and proliferation of transplanted hHSCs in humanized mice have until now been insufficient to allow analysis of long-term processes including chronic diseases [2].

HSC self-renewal and pluripotency are regulated primarily in the bone marrow (BM) niche, in part via Notch signaling. Notch signaling induced by specific binding of the Notch ligands (Delta-like 1-2, 4, and Jagged1-2 in mammals) to their receptors (Notch1-4 in mammals) determines cell fate [3]. Since Jagged1 (J1) is expressed on BM stromal cells, HSCs, which express Notch, may receive Notch

signaling from the BM niche [4]. Many studies have revealed enhanced self-renewal capacity in HSCs expressing a constitutively active form of Notch or in those co-cultured with stromal cells expressing Notch ligands [5,6]. Moreover, increased HSC proliferation has been reported in a transgenic mouse expressing an active form of parathyroid hormone-related protein receptor in osteoblasts, which drives high J1 expression [7]. Based on these studies, we generated a transgenic mouse expressing human J1 (hJ1) in osteoblasts of non-obese diabetic (NOD/Shi)-*scid*-IL2rγ^{null} (NOG) mice (hJ1-NOG mice) in order to create highly acceptable recipients for human cells. In these transgenic mice, high proportions of engrafted hHSCs relative to total BM cells were present after transplantation of human CB-derived $CD34^{+}$ cells. Thus, hJ1-NOG mice may be useful as humanized mice for maintaining the stem cell pool.

Materials and Methods

Generation of hJ1 NOG mice

A DNA fragment containing the 2.3-Kb osteoblast-specific promoter region for the mouse α1(I) collagen (*Col1a1*) promoter [8] was provided by Dr. B. de Crombrugghe (University of Texas, Houston, TX). The chicken β-globin 5' HS4 insulator (1.2 kb) [9, 10] was provided by Dr. G. M. Lefevre (National Institutes of Health, Bethesda, MD). The DNA fragments of the *Col1a1* promoter, insulator, and human *J1* (3.7kb) were inserted into the pCMVb vector (Clontech Laboratories, Inc., Mountain View, CA) (Fig. 1A). To generate transgenic mice, linearized DNA was injected into NOD/Shi mouse embryos, and transgenic offspring were further backcrossed to NOG mice [11]. Experiments using mice maintained under specific pathogen-free conditions were approved by the Institutional Animal Care and Use Committee of the Central Institute for Experimental Animals.

Bone analysis

The histological analysis was described previously [12]. For RT-PCR analysis, the mRNA from femurs of hJ1-NOG and control non-transgenic NOG mice was

isolated by RNA mini kit (Qiagen, Hilden, Germany) and was transcribed with oligo-dT and Ominiscript reverse transcriptase (Qiagen). The oligonucleotides used to hJ1 and osteocalcin(OCN) were the following:

hJ1 sense, 5'-AGCTGTAAGGAGACCTCCCTG-3',

hJ1 antisense, 5'-TTCTGACACTGGCCAAGGCAGTC-3';

OCN sense, 5'-CTCTGTCTCTCTGACCTCACAG-3',

OCN antisense, 5'-GGAGCTGCTGTGACATCCATAC-3'.

Three-dimensional imaging by microcomputed tomography (micro-CT)

Each isolated mouse femur was scanned by CT scanner (eXplore Locus CT System, GE Healthcare, Buckinghamshire, U.K) operated at 80 kV and 450 μA using 400 projections over 88 min for half-scan reconstruction. Voxel resolution was 21 μm. The volume of bone marrow space was quantified using three-dimensional image analysis software TRI/3DBON (Ratoc System Engineering, Tokyo, Japan).

Transplantation of CB-derived CD34^{+} cells

For human CD34^{+} cells transplantation, commercially available human CB-derived CD34^{+}cells (Cat. No. 2C-101A, Lonza, Switzerland) were used. The purity of

CD34$^+$ cells was more than 95% (Lot No. 0F4041, Certificate of analysis, www.lonza.com.). Method of human CD34$^+$ cell transplantation was described previously [12]. Briefly, after 2.5 Gy X-ray irradiation, 5 x 10^4 human CD34$^+$ cells were intravenously transplanted into hJ1-NOG and control non-transgenic NOG mice. Eighteen weeks after transplantation of human CD34$^+$ cells, BM and spleen cells were stained with anti-human monoclonal antibodies and analyzed by flow cytometry.

Transplantation of mouse c-kit$^+$ sca-1$^+$ lineage$^-$ (KSL) cells

For mouse c-kit$^+$ sca-1$^+$ lineage$^-$ cell transplantation, c-kit-positive, sca-1-positive, lineage marker (Lin: TER119, CD3, CD19, Mac-1, Gr-1)-negative cells (KSL cells) were isolated from fetal liver of E15.5 green fluorescent protein (GFP) mouse embryos (SLC, Japan) or BM of 4-week-old GFP mice by sorting using a FACSAria (BD Biosciences, San Jose, CA). Mouse GFP$^+$KSL cells (2 x 10^4 cells) were injected into hJ1-NOG and control non-transgenic NOG mice irradiated previously 24hr with 2.5 Gy of radiation. After eight weeks, their BM cells were analyzed by flow cytometry.

Serial transplantation in NOG mice

Human $CD34^+$ cells were isolated from BM of initial $CD34^+$ transplanted hJ1-NOG or control non-transgenic NOG mice, eighteen weeks after transplantation of human CB-derived $CD34^+$ cells (5×10^4 cells), by magnetic cell sorting (MACS) system using biotinylated anti-human CD34 antibody (BioLegend, San Diego, CA) and avidin-conjugated magnetic beads (Milteny Biotec, Bergisch Gladbach, Germany). For the serial transplantation, 2×10^5 human $CD34^+$ cells from an each initial recipient were intravenously transplanted into NOG mice with 2.5 Gy irradiation. Eight weeks later, their BM cells were analyzed by flow cytometry.

Co-culture assay with stromal cells

For establishment of OP9 cell lines expressing NotchL were obtained after transfection into the Plat-E ecotropic packaging cell line as described previously [13]. For co-culture, human $CBCD34^+$ cells were plated at $0.3〜1{\times}10^4$ cells on a monolayer of stromal OP9 transfectants prepared in six-well culture plates for 3〜7 weeks in the presence of recombinant cytokines: 50ng/mL hSCF, 50ng/mL hTPO and 50ng/mL Flt3L (Peprotech, London, UK). After two weeks of co-culture, half of the

cultured floating cells were re-cultured onto new OP9 monolayers with fresh medium containing growth factors weekly, to prevent overgrowth. After co-culture, growing cells were harvested for flow cytometric analysis.

Flow cytometry

Both BM and spleen cell preparation and flow cytometric analysis have been described [14]. Antibodies used included: anti-human CD45-allophycocyanin-Cy7 (APC-Cy7), anti-human CD33-fluorescein isothiocyanate (FITC) and anti-human CD38-FITC antibodies (BD Biosciences); anti-human CD19-phycoerythrin (PE) and CD3-PE-Cy7 antibodies (Beckman Coulter, Brea CA); anti-mouse c-kit-APC, anti-human lineage cocktail-PE, anti-mouse lineage cocktail-PE, anti-human CD34-PE-Cy7 antibodies, biotinylated anti-mouse Sca-1, and streptavidin-PE-Cy7 (BioLegend) and anti-mouse Sca-1-FITC antibody (eBioscience, San Diego, CA).

Immunohistochemistry

Femurs were fixed in 4% paraformaldehyde (PFA) and decalcified with 20% EDTA in PBS. Bones were then embedded in paraffin and cut at 4-μm thickness. Sections were stained with anti-HA (Serotec, Kidlington, U.K), anti-mouse

osteocalcin (Enzo Life Sciences, Farmingdale, NY) and anti-human CD34 (DakoCytomation, Glostrup, Denmark). Secondary antibodies were horseradish peroxidase-labeled polymer-conjugated anti-rabbit IgG (Nichirei, Tokyo, Japan). For color development, these sections were incubated with 0.02% 3,3'-diaminobenzidine (DAB; Dojindo, Kumamoto, Japan) substrate solution containing 0.006% H_2O_2. Immunostained sections were counterstained with hematoxylin (Sakura Finetek, Tokyo, Japan) for visualization of nuclei.

Statistical analysis

Mean values and standard deviations were computed using Excel (Microsoft, Redmond, WA). Significant differences were calculated by Student's *t*-test.

Results and Discussion

Generation and characteristics of transgenic mice expressing hJ1

To establish the experimental system that allows human hematopoietic stem cells (hHSCs) to grow efficiently in mouse BM, we first examined the *in vitro* system in which human CB-derived $CD34^+$cells were co-cultured on mouse OP9 stromal cells expressing human and mouse Notch ligands in the presence of growth factors for maintenance of HSCs. In pre-culture, the purity of $CD34^+$ cells was more than 95% and among them, the proportion of $CD34^+CD38^-$cell was about 10%. The co-culture of $CD34^+$ cells on OP9 showed much greater proliferation of $CD34^+CD38^-$ cells (termed hHSCs in this study) in conditions employing hJ1 than in those with mouse J1 (Table 1). Based on this *in vitro* experimental result, we generated transgenic mice expressing hJ1 cDNA in osteoblasts under the control of the *Col1a1* promoter (Fig. 1A) in order to examine the *in vivo* effect of hJ1 in the mouse BM niche on hHSC. Of two transgenic lines (#115 and #116) we established, #116 expressing higher levels of hJ1 protein was bred on an immuno-deficient NOG mouse (hJ1-NOG mice) for further experiments of human cell transplantation.

In hJ1-NOG mice, hJ1 expression was detectable in femur by RT-PCR (Fig. 1B) in addition to the mouse J1 expression respectively (data not shown). The immunohistochemical analysis showed hJ1 positive cells in both metaphyseal trabecular and diaphyseal cortical areas in hJ1-NOG mice but not in non-transgenic NOG mice (Fig. 1C). In the same site of serial sections, osteocalcin positive cells were detectable (Fig. 1D). Bone sections of hJ1-NOG mouse seem to have relatively larger marrow space and thin trabecular bone in comparison to non-transgenic NOG littermates (Fig. 1C), which was confirmed in micro-CT analysis showing the increase of marrow volume and of M/T ratio (marrow vs. total volume) (Fig. 1E) and the decrease of trabecular bone volume fraction (BV/TV: bone volume vs. trabecular volume) (Supplemental Fig. 1A). In fact, tissue mineral density (TMD) of cortical area in hJ1-NOG mouse was decreased (Supplemental Fig. 1A). Although the marrow space of hJ1-NOG mouse was enlarged, mouse total BM cells and c-kit^{+} sca-1^{+} lineage^{-} (KSL) cells were not particularly increased (Fig. 1F). These characteristics of hJ1-NOG mouse suggest that hJ1 expression on osteoblasts does not seem to enhance mouse stem cell proliferation. In addition to

hJ1 expression, hJ1- NOG mice showed other BM phenotypes such as lower bone density and the increase of both osteoblasts and osteoclasts (Supplemental Fig. 1B), which might also be related to the enlargement of BM space and warrant further investigation.

Preferential increase of hHSC in hJ1-NOG mice post engraftment of CB-derived cells.

These hJ1-NOG mice were then used to study whether hHSCs are efficiently maintained and/or proliferated in the mouse BM niche due to hJ1 expression. For that, human CB-derived $CD34^+$ cells were transplanted into hJ1-NOG and non-transgenic NOG mice, and then BM cells in the recipients were analyzed 18 weeks after transplantation. Immunohistochemical staining showed a higher proportion of $CD34^+$ cells in the marrow space (Fig. 2A). As shown in Fig.2B and C, the proportion and cell number of $CD34^+$ cells and $CD34^+38^-$ cells in $CD45^+$ cells were clearly increased in hJ1-NOG mice while the number of human $CD45^+$ cells in BM was not significantly different between hJ1-NOG and non-transgenic NOG mouse. Since both recipients were equally injected with $5x10^4$ human $CD34^+$ cells

containing around 10% of CD34$^+$CD38$^-$ cells, the mathematical estimation from Fig.2B indicates 20 fold-increase of CD34$^+$CD38$^-$ cells in hJ1-NOG mice and about 4 folds in non-transgenic NOG mice. These results suggest that hHSC can be promoted to expand in hJ1 expressing BM niche, which is consistent with the observation in Table 1 showing that human CD34$^+$ cells greatly increased in co-culture with hJ1$^+$ stromal cells (Table 1). At the same time, it may also be possible that the increased proportion of CD34$^+$ cells in BM may result from the suppression for the developing pathway from CD34$^+$ cells into CD34$^-$ cells by hJ1, but this possibility is remained to be clear because our aim of this study is to establish a model mouse in which human CD34$^+$ cells grow efficiently.

In hJ1-NOG mouse spleen, lymphoid cells such as T and B cells and granulocytes were comparable in number and proportion between hJ1-NOG mice and non-transgenic NOG littermates (Fig. 2D), indicating that developing cells belonging to lineage committed cells may be released equally from BM comparably in both recipients.

To validate whether the above experimental results by transplantation of human

CB-derived $CD34^+$ cells substantially occurs specifically in hHSC, the mouse hematopoietic cells instead of human cells were transplanted into the same hJ1-NOG mice and were examined their growth. To distinguish transplanted cells from recipient BM cells, donor cells were prepared from GFP transgenic mice. Then, the isolated GFP^+KSL cells were injected into non-GFP hJ1-NOG and non-transgenic NOG recipients. No significant difference was found in total GFP^+ cells and in the proportion of GFP^+KSL cells in both recipients, indicating that hJ1 expression did not significantly contribute to the growth of transplanted mouse KSL in BM (Table 2).

Altogether, transplantation experimental results of human and mouse hematopoietic stem cells clearly indicated that that hJ1-expressing niche greatly contributes to the growth of transplantated hematopoietic precursor cells in a human cell specific manner.

Previous studies reported that both human and mouse Notch ligands support mouse KSL cell growth in the co-culture system [15,16]. However, mouse KSL cell growth was not sufficiently enhanced in hJ1-NOG mice compared to control NOG mice, whereas hHSCs are clearly increased in the same BM niche. This disparity could be

due, at least in part, to experimental differences between *in vivo* and *in vitro* co-cultures, which may employ various types of HSC growth factors. Also, the pairing of combinations of Notch receptors and ligands in distinct species likely influences HSC growth because, as mentioned in the above-described culture system, hHSCs grew more rapidly in combination with hJ1 than with the mouse counterpart (Table 1).

Next, we examined whether the intra-marrow expanded human $CD34^{+}CD38^{-}$ cells in hJ1-NOG mouse still maintain the original growing potentials of hHSC without retardation. For that, human $CD34^{+}$ cells grown in the first hJ1-NOG or in non-transgenic NOG recipient were isolated from each BM respectively, and then, the same number of them was transplanted into the secondary recipient non-transgenic NOG mice but not hJ1-NOG mice. The flow cytometric analysis of BM cells from the secondary non-transgenic NOG recipient showed that the number and proportion of human $CD34^{+}38^{-}$ cells derived from hJ1-NOG mice were not statistically different although they seem to be slightly enhanced in hJ1 NOG derived case (Table 3). These results suggest that enhancement of human $CD34^{+}$ cell growth is transiently induced

as long as hJ1 exists, but restores without abrogation of their intrinsic growing potential in the absence of hJ1.

In conclusion, our established hJ1-NOG mouse is of use as an *in vivo* model to study human immune responses to various stimulants such as pathogens or cancer cells, particularly of a chronic status, which needs continuous and sufficient development of immune cells supplied by enhanced expansion and increased survival of hHSCs. At the same time, due to the longer maintenance and enhanced expansion of hHSCs in the mouse BM niche, the hJ1-NOG mouse could be a powerful tool for directly investigating the characterization of human-derived hematopoietic cancer stem cells and leukemogenesis and for developing therapeutic strategies.

Funding disclosure

This work was supported by a Grant-in-Aid for Scientific Research (B and S) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

Acknowledgements

We thank Tamaki Sasou of the Educational and Research Support Center, Tokai University School of Medicine, Dr. Toshimi Sato and Mariko Takahashi of Tokyo Medical and Dental University for experimental support.

Conflict of interest disclosure

No financial interest/relationships with financial interest relating to the topic of this article have been declared.

References

[1] Hiramatsu H, Nishikomori R, Heike T, et al. Complete reconstitution of human lymphocytes from cord blood CD34+ cells using the NOD/SCID/gammacnull mice model. Blood. 2003;102:873-880.

[2] Ito M, Kobayashi K, Nakahata T. NOD/Shi-scid IL2rgamma(null) (NOG) mice more appropriate for humanized mouse models. Curr Top Microbiol Immunol. 2008;324:53-76.

[3] Radtke F, Wilson A, Mancini SJ, MacDonald HR. Notch regulation of lymphocyte development and function. Nat Immunol. 2004;5:247-253.

[4] Weber JM, Calvi LM. Notch signaling and the bone marrow hematopoietic stem cell niche. Bone. 2010;46:281-285.

[5] Karanu FN, Murdoch B, Gallacher L, et al. The notch ligand jagged-1 represents a novel growth factor of human hematopoietic stem cells. J Exp Med. 2000;192:1365-1372.

[6] Varnum-Finney B, Brashem-Stein C, Bernstein ID. Combined effects of Notch signaling and cytokines induce a multiple log increase in precursors with

lymphoid and myeloid reconstituting ability. Blood. 2003;101:1784-1789.

[7] Calvi LM, Adams GB, Weibrecht KW, et al. Osteoblastic cells regulate the haematopoietic stem cell niche. Nature. 2003;425:841-846.

[8] Rossert J, Eberspaecher H, de Crombrugghe B. Separate cis-acting DNA elements of the mouse pro-alpha 1(I) collagen promoter direct expression of reporter genes to different type I collagen-producing cells in transgenic mice. J Cell Biol. 1995;129:1421-1432.

[9] Chung JH, Whiteley M, Felsenfeld G. A 5' element of the chicken beta-globin domain serves as an insulator in human erythroid cells and protects against position effect in Drosophila. Cell. 1993;74:505-514.

[10] Potts W, Tucker D, Wood H, Martin C. Chicken beta-globin 5'HS4 insulators function to reduce variability in transgenic founder mice. Biochem Biophys Res Commun. 2000;273:1015-1018.

[11] Ito M, Hiramatsu H, Kobayashi K, et al. NOD/SCID/gamma(c)(null) mouse: an excellent recipient mouse model for engraftment of human cells. Blood. 2002;100:3175-3182.

[12] Ito R, Negishi N, Irie N, et al. Osteosclerosis and inhibition of human hematopoiesis in NOG mice expressing human Delta-like 1 in osteoblasts. Exp Hematol. 2012;40:953-963.

[13] Hozumi K, Abe N, Chiba S, et al. Active form of Notch members can enforce T lymphopoiesis on lymphoid progenitors in the monolayer culture specific for B cell development. J Immunol. 2003;170:4973-4979.

[14] Hozumi K, Negishi N, Suzuki D, et al. Delta-like 1 is necessary for the generation of marginal zone B cells but not T cells in vivo. Nat Immunol. 2004;5:638-644.

[15] Jones P, May G, Healy L, et al. Stromal expression of Jagged 1 promotes colony formation by fetal hematopoietic progenitor cells. Blood. 1998;92:1505-1511.

[16] Han W, Ye Q, Moore MA. A soluble form of human Delta-like-1 inhibits differentiation of hematopoietic progenitor cells. Blood. 2000;95:1616-1625.

Figure Legends

Figure 1. Generation and characterization of hJ1-NOG mice. (A) Schematic diagram of the hJ1 transgene. (B) RT-PCR analysis of hJ1 and OCN in femurs of 8-week-old hJ1-NOG (J1-Tg) mice and non-transgenic NOG (nTg) littermates. (C) H&E staining of femurs of J1-Tg mice and nTg littermates (each of left, scale bars, 200 μm.), and higher magnification of immunohistochmical staining with anti-HA antibody of metaphyseal trabecular area (top) and diaphyseal cortical area (bottom). Scale bars, 100 μm. (D) Immunohistochemical staining of serial sections of femurs (metaphyseal trabecular regions: left and diaphyseal cortical regions: right) from nTg and J1-Tg mice using anti-osteocalcin antibody (α-OCN; top) and anti-HA antibody (α-HA; bottom). Arrowheads indicate positive cells. Scale bars, 20 μm. (E) Measurements of the BM cavity of femurs J1Tg and nTg mice (n=9 each). Total volume (red and blue), bone volume (blue), marrow volume (red) were quantified as

described in Materials and Methods, and based on them, marrow/total (M/T) ratio were calculated. Error bars represent SEM. *$P<0.05$ and **$P<0.01$ vs. nTg. Scale bars, 2mm. (F) Average number of total BM cells (left) of femurs in nTg (n=3) and J1-Tg mice (n=4) was counted under microscopy. The frequency (middle) and average number (right) of KSL cells in both mice. KSL cells were determined by staining BM cells with monoclonal antibodies to mouse lineage cocktail, c-Kit and Sca-1. KSL cell number was analyzed by flowcytometry. Error bars represent SEM (*$P<0.05$).

Figure 2. Characterization of hHSCs post transplantation in hJ1-NOG mice.

Eighteen weeks after human $CD34^+$ cell transplantation ($5x10^4$ cells) into hJ1-NOG (J1-Tg) mice and non-transgenic NOG (nTg) littermates, BM obtained from the femurs of each recipient was analyzed by immunohistochemistry and flowcytometry. (A) Immunohistochemical staining for human CD34 in femurs of J1-Tg and nTg mice. Arrowheads indicate positive cells. Scale bars; 100 μm. (B) Representative flowcytometric profiles of $CD34^+CD38^+$ and $CD34^+CD38^-$ cell proportion in $CD45^+$ BM cells. Numbers indicate the relative percentages of cells within indicated areas.

(C) Histograms showed the number of engrafted $CD45^+$ cells in total BM cells, the proportion of $CD34^+$ cells and $CD34^+CD38^-$ cells in $CD45^+$ cells in five hJ1-Tg and nTg mice each. Error bars represent SEM (n=5, *P<0.05). Total cell numbers of $CD34^+CD38^-$ cells (hatched bar), $CD34^+CD38^+$ cells (grey bar) and $CD34^+$ cells (hatched bar + grey bar) in J1-Tg and nTg mice. Error bars represent SEM (n=5, *P<0.05). (D) Human-derived immune cells in spleen cells. Average number of total spleen cells (left), human CD45, CD3, CD19, and CD33 cells (right) in J1-Tg and nTg mice was determined by flow cytometer.

Table1

Table1. Number of total cells and HSCs after co-culture of CB-derived $CD34^+$ cells on OP9 stromal cell lines

	stromal cell	No. of $CBCD34^+$ cells (day 0) ($\times 10^4$ cells)	No. of total cells ($\times 10^6$ cells)			No. of $CD34^+$ $CD38^-$ cells ($\times 10^6$ cells)		
			3W	4W	7W	3W	4W	7W
Exp. 1	Control OP9	0.3	10.1	14.1	72.3	1.7	2.2	5.8
	hD1/OP9	0.3	4.6	11.5	60.8	1.5	4.2	22.7
	hJ1/OP9	0.3	10.6	28.6	232.0	4.9	4.9	62.9
Exp. 2	Control OP9	1.0	77.0	93.5	5667.0	6.8	3.9	561.6
	mD1/OP9	1.0	10.0	21.6	2100.0	0.1	1.7	59.4
	hD1/OP9	1.0	6.6	22.3	1123.0	1.2	1.6	112.6
	mJ1/OP9	1.0	99.0	115.5	3346.0	8.4	20.5	657.2
	hJ1/OP9	1.0	112.2	151.8	57024.0	8.9	30.7	16286.0

hD1, human Delta-like-1; hJ1, human Jagged-1; mD1, mouse Delta-like-1; mJ1, mouse Jagged-1

Human $CBCD34^+$ cells were cultured for 3, 4, or 7 weeks (W) on a monolayer of OP9-derived transfectants expressing hD1, hJ1, mD1 or hJ1 in the presence of hSCF, hTPO and Flt3L. Each week, after washing the floating cells were re-cultured on a monolayer of OP9 transfectans. After culture, growing cells were harvested for flow cytometric analysis.

Table2

Table 2. Engrafted mouse GFP^+ KSL cells in BM of hJ1-NOG or non-transgenic NOG recipient mice

Mouse no.	Recipient	in recipient mice				
		No. of total cells in BM (x10^5)	% of GFP^+ cells in total cells	No. of GFP^+ cells in BM (x10^5)	% of KSL cells in GFP^+ cells	No. of GFP^+KSL cells in BM (x10^5)
1	J1-Tg	121.5	73.3	89.1	2.9	2.6
2	J1-Tg	69.3	73.7	51.1	4.9	2.5
3	J1-Tg	40.5	55.7	22.6	1.3	0.3
4	J1-Tg	102.6	96.5	99.0	3.3	3.3
5	J1-Tg	52.0	88.3	45.9	0.7	0.3
6	J1-Tg	73.0	94.0	68.9	2.3	1.6
mean±SD		76.5±30.6	80.3±15.6	72.8±28.6	2.6±1.5	1.8±1.3
7	non-Tg	104.4	55.3	57.7	1.9	1.1
8	non-Tg	137.7	86.4	119.0	1.4	1.7
9	non-Tg	63.0	89.2	56.2	0.2	0.1
10	non-Tg	62.5	95.0	59.4	2.5	1.5
11	non-Tg	71.0	94.3	67.0	2.4	1.6
12	non-Tg	55.0	73.5	40.4	0.2	0.1
mean±SD		82.3±32.2	82.3±15.3	66.6±27.1	1.4±1.0	1.0±0.7

Isolated BM KSL cells (2x10^4) from GFP mice were transplanted into hJ1-NOG (J1-Tg) or non transgenic NOG (nTg) mice, and 8weeks later, the number of BM cells, the percentage and the number of GFP^+ cells and GFP^+ KSL cells in BM of the recipient mice were estimated based on the flow cytometric analysis.

KSL: Lineage-negative,c-kit-positive, sca-1-positove cells.

Table3

Table 3. Human HSCs grown in hJ1-NOG or non-Tg mice in the second non-Tg recipients

Mouse no.	Doner	Recipient	Injected $CD34^+$ cells ($x10^5$)	In the recipent NOG mice transplanted after 8weeks: % of $CD45^+$cells in BM cells	Number of $CD45^+$ cells ($x10^5$)	% of $CD34^+$cells in $CD45^+$ cells	Number of $CD34^+$cells ($x10^4$)	% of $CD34^+CD38^-$ cells in $CD45^+$ cells	Number of $CD34^+CD38^-$ cells ($x10^3$)
1	J1-Tg	nTg	2.0	5.8	3.4	3.5	1.2	2.0	6.9
2	J1-Tg	nTg	2.0	3.0	1.9	0.5	0.1	0.4	0.7
3	J1-Tg	nTg	2.0	5.5	3.5	0.3	0.1	0.1	0.4
4	J1-Tg	nTg	2.0	8.5	6.3	1.4	0.9	1.1	6.8
5	J1-Tg	nTg	2.0	17.7	6.5	5.0	3.3	0.6	4.0
6	J1-Tg	nTg	2.0	1.4	0.7	2.9	0.2	0.1	0.1
mean±SD				7.0±5.8	3.7±2.3	2.3±1.9	1.0±1.2	0.7±0.7	3.1±3.2
7	nTg	nTg	2.0	2.0	1.0	2.9	0.3	1.1	1.2
8	nTg	nTg	2.0	37.5	26.7	1.1	2.9	0.4	10.4
9	nTg	nTg	2.0	3.0	1.8	1.7	0.3	0.2	0.3
10	nTg	nTg	2.0	1.8	1.0	1.0	0.1	0.2	0.2
11	nTg	nTg	2.0	3.3	1.3	0.8	0.1	0.3	0.4
12	nTg	nTg	2.0	10.3	3.4	1.8	0.6	0.8	2.8
mean±SD				9.7±14.0	5.9±10.2	1.5±0.8	0.7±1.1	0.5±0.4	2.6±4.0

Human $CD34^+$ cells ($2x10^5$) obtained from the first recipients, hJ1-NOG (J1-Tg) or non-transgenic NOG (nTg) mice, were transplanted into the nTg NOG second recipients. The transplanted $CD34^+$ cells derived from hJ1-Tg and nTg contained $CD34^+CD38^-$cells in average 10.3% and 9.9%, respectively. Eight weeks later, the percentage and the number of human $CD45^+$ and of $CD34^+$ or $CD34^+CD38^-$ cells in $CD45^+$ cell in BM were analyzed by flowcytometry. Difference between engrafted cells derived from J1-Tg and non-Tg was no significant.

Figure 1

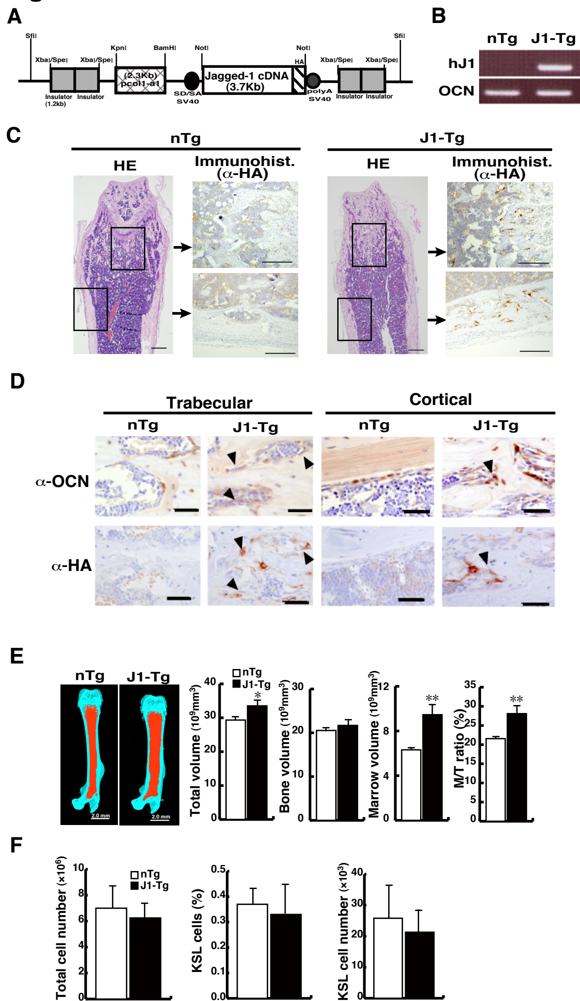
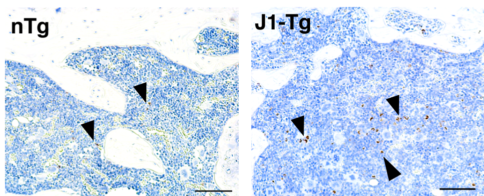
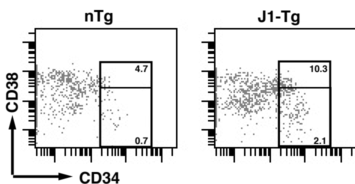
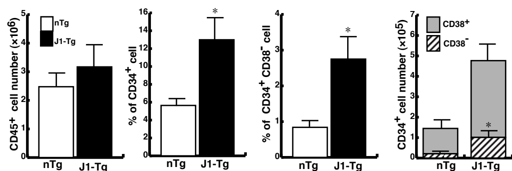


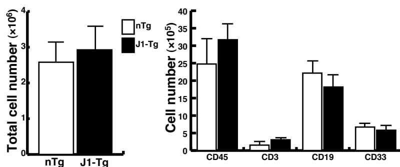
Figure 2

Figure 2

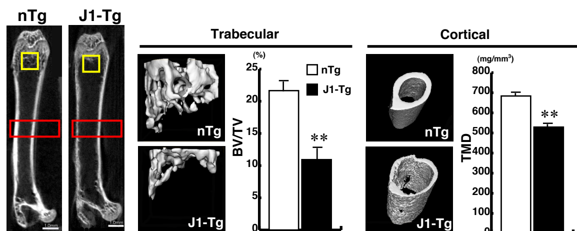


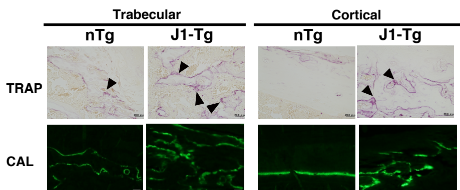






Supplemental Figure 1





Supplemental Figure 1. Bone phenotypes of hJ1-NOG mice.

(A) Three-dimensional microCT imaging (left) of femurs from 10-week-old female hJ1-NOG mice (J1-Tg) and non-transgenic NOG littermates (nTg). Middle and right show three-dimensional reconstructions of metaphyseal trabecular regions and diaphyseal cortical regions. Trabecular bone volume fraction (BV/TV) and tissue mineral density (TMD) were calculated using MicroView software. Yellow and red boxes indicate measurement regions corresponding to trabecular and cortical, respectively. Error bars represent SEM. **P<0.01 for nTg vs. J1-Tg mice (n=9 each). (B) Histological analysis of femurs from 10-week-old female nTg and J1-Tg mice. Histological sections of trabecular (left) and cortical (right) bones. Top panels show TRAP staining (TRAP) indicative of osteoclast. Arrowheads indicate TRAP positiveosteoclasts. Bottom panels show high-power fluorescent microscopy images of undecalcified femur sections labeled with calcein (CAL) indicative of the newly formed bone. Scale bars: 20 μm.