Improvement of human hematopoiesis in c-kit mutant NOG mice transferred with human HSCs

(ヒト造血幹細胞移植c-kit変異NOGマウスにおけるヒト造血能の向上)

Ryoji Ito[1], Yunmei Mu[1, 2], Yuyo Ka[1], Yusuke Ohno[1], Motohito Goto[1], Riichi Takahashi[1], Mamoru Ito[1]

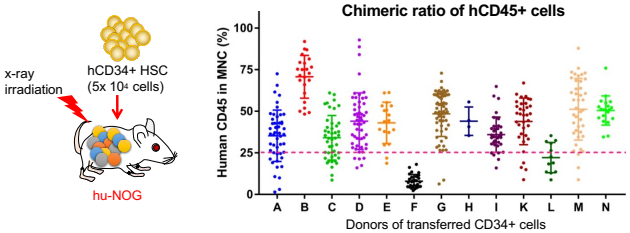
[1]Central Institute for Experimental Animals [2]Mayo Clinic

Abstract

Humanized mice, in which the human hematopoietic system is reconstituted in immunodeficient mice, are useful animal models to study human hematology and immunology. NOG or NSG mice are well-known recipients with transfer of human hematopoietic stem cells (HSCs). However, large amounts of enriched human CD34+ cells (at least 50,000 cells) and total body irradiation are needed for sufficient human hematopoiesis. In this study, therefore, we newly generated the NOG mouse strains which accompanied with a point mutation of the c-kit tyrosine kinase domain (W41 mutant; NOGW mice). Irradiated NOGW mice have shown high engraftment level of human CD45+ cells in PB, bone marrow (BM), and spleen even when transferred with 5,000 - 10,000 CD34+ HSCs. The efficient hematopoiesis was also observed in non-irradiated NOGW mice transferred with 20,000 - 40,000 HSCs. Serial BM transfer experiments revealed that the long-term human HSC was effectively sustained in NOGW mice compared to conventional NOG mice. We further generated NOGW-hIL-3/hGM-CSF Tg (NOGW-EXL) mice, and high engraftment level of human CD45+ cells was found in the non-irradiated NOGW-EXL mice when transferred with 5,000-10,000 HSCs. Human myelopoiesis especially granulocytes and platelets were significantly developed in NOGW-EXL than that in NOG-EXL mice. Thus, the c-kit mutant humanized NOG and NOG-EXL mice are advanced models in HSC-transferred humanized mice and may be approved for universal use in their high versatility.

Introduction

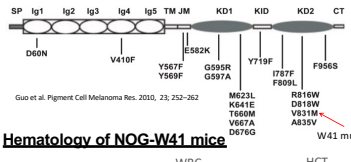
Donor dependency in the chimerism of humanized NOG mice



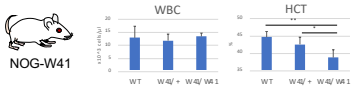
Thirteen lots of the commercially available CB-derived hCD34+ hematopoietic progenitor cells were transferred into x-ray-irradiated NOG mice, and chimeric ratio of hCD45+ cells in PB was evaluated by flowcytometry. Donor dependency in the hCD45+ ratio was observed.

1: Generation of c-kit mutant NOG-W41 mice

Mouse c-kit gene 145 kDa

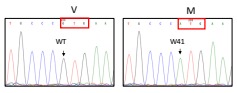


Hematology of NOG-W41 mice



NOG-W41 homozygous mice exhibited mild anemia which indicated slight decrease of hematocrit level. Otherwise, they showed no significant pathology.

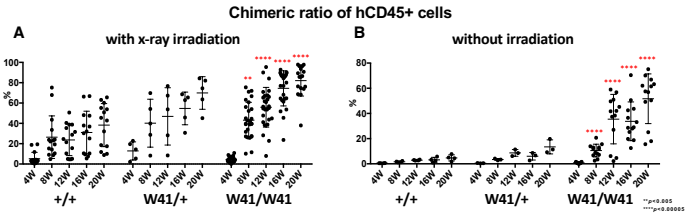
Targeting mutation in c-kit gene (V831M)



Kit-mutated NOG-W41 mice were established by genome editing using TALEN technique. Designed TALEN mRNA pairs (Forward; 5'-gtgttccgttctaggcac-3', and Reverse; 5'-atgctctctggtgccatc-3') and 100-bp single-strand oligonucleotide (ssOligo) containing G to A point mutation in the kinase domain of Kit locus were purchased from Thermo Fisher Scientific (Waltham, MA, USA). TALEN mRNA (4 ng/μl) and ssOligo (15 ng/μl) were mixed and injected into NOG mouse embryo to generate NOG-W41 mice.

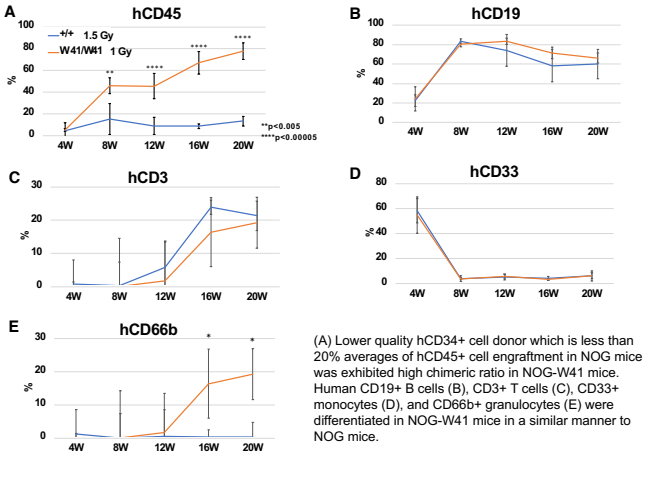
2: Human hematopoiesis in NOG-W41 mice

HSC transplantation into NOG-W41 mice



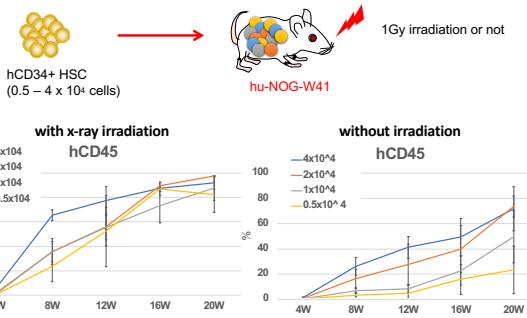
4-5 x10^4 hCD34+ cells were transferred into NOG or NOG-W41 heterozygous (W41/+) and homozygous (W41/W41) mice with (A) or without 1 Gy x-ray irradiation (B). Time course of hCD45+ chimeric ratio was shown. NOG-W41 mice allowed higher engraftment of hCD45+ cells compared to NOG or W41/+ mice in both with and without irradiation.

Reconstitution of human cells by the lower quality donor of hCD34+ cells in NOG-W41 mice



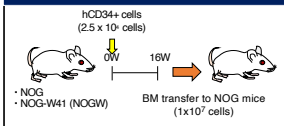
(A) Lower quality hCD34+ cell donor which is less than 20% averages of hCD45+ cell engraftment in NOG mice was exhibited high chimeric ratio in NOG-W41 mice. Human CD19+ B cells (B), CD3+ T cells (C), CD33+ monocytes (D), and CD66b+ granulocytes (E) were differentiated in NOG-W41 mice in a similar manner to NOG mice.

3: In vivo limiting dilution assay of hCD34+ cells

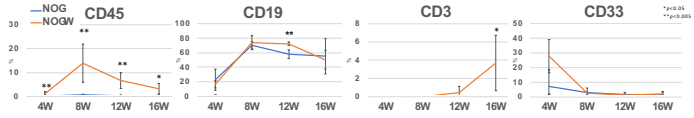


In vivo limiting dilution assay of hCD34+ cells was performed. 0.5, 1, 2, or 4 x 10^4 hCD34+ cells was transferred into NOG-W41 mice with or without irradiation. hCD45+ cells in PB of the mice were analyzed by flowcytometry. Enough amount of CD45+ cell engraftment was observed even in 0.5 and 1 x10^4 cell-transferred irradiated NOG-W41 mice. Whereas at least 2 x 10^4 cells were needed for the engraftment in non-irradiated NOG-W41 mice.

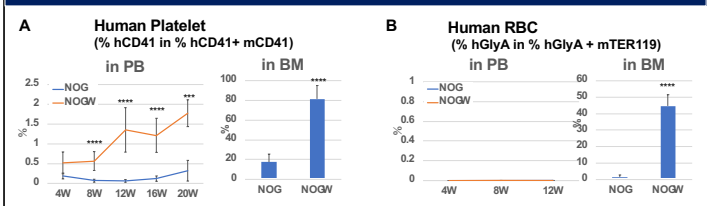
4: BM secondary transplantation



Secondary BM transplantation was performed to compare the HSC potential between NOG and NOGW mice. 2.5 x 10^4 hCD34+ cells was transferred into irradiated NOG or unirradiated NOG-W41 mice. After 16 weeks, 1x10^7 BM cells from NOG or NOGW mice were serially transferred into irradiated NOG mice. Engrafted hCD45 level was significantly higher in NOG mice reconstituted by NOGW BM cells than by NOG BM cells. B cells, T cells, and myeloid cells were differentiated in them.

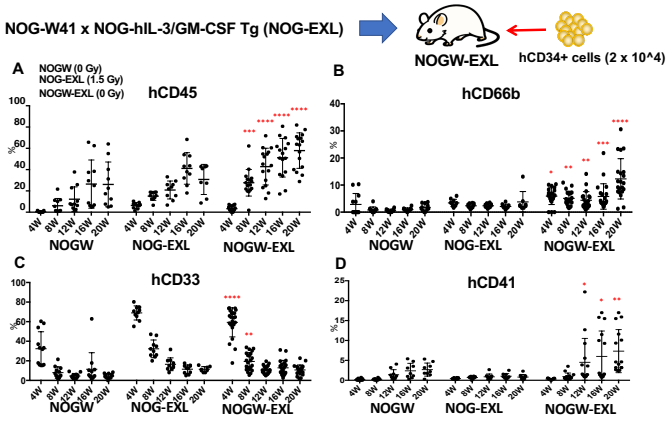


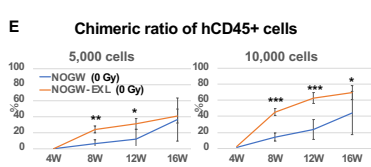
5: Human PLT/RBC engraftment in NOG-W41 mice



Human PLT and RBC were analyzed in CD34+ cell-transferred NOG or NOG-W41 mice. (A) The frequency of hCD41+ PLT was significant high level from 8 to 20 weeks in PB and markedly increased in BM at 20 weeks. (B) hGlycophorine A+ RBC was not detected in PB but significantly higher in the BM

6:Generation of NOG-W41-EXL mice





NOG-W41-hIL-3/GM-CSF Tg (NOGW-EXL) mice were generated by crossing NOGW with NOG-EXL mice. (A-D) 2 x10^4 hCD34+ cells were transferred into NOGW, NOG-EXL, or NOGW-EXL mice Time course (4-20W) of hCD45+, hCD66b+, hCD33+, and hCD41+ cell frequency was shown. Improved engraftment levels of hCD45+ and those myeloid lineage cells were observed. (E) The frequency of hCD45+ cells in unirradiated NOGW or NOGW-EXL mice transferred with 5,000 or 10,000 CD34+ cells was shown. 10,000 cells may be sufficient to reconstitute human cells in NOGW-EXL even without irradiation.

Conclusions

- **In this study, we generated NOG-W41 mice which showed high engraftment of human hematopoietic cells after transfer of hCD34+ cells even without x-ray irradiation.**
- **Serial transplantation of BM cells from NOG-W41 mice was exhibited improved human hematopoiesis in the secondary recipient NOG mice.**
- **Engraftment of platelet was improved in BM and PB, whereas RBC engraftment was significantly increased in BM but not in PB of NOG-W41 mice.**
- **Highest engraftment model of humanized mice, NOG-W41-EXL, has been generated.**

The authors have no conflicts of interest (COI) to declare.