Analysis on Xenograft Susceptibility in NOG and NOG-hairless Mice

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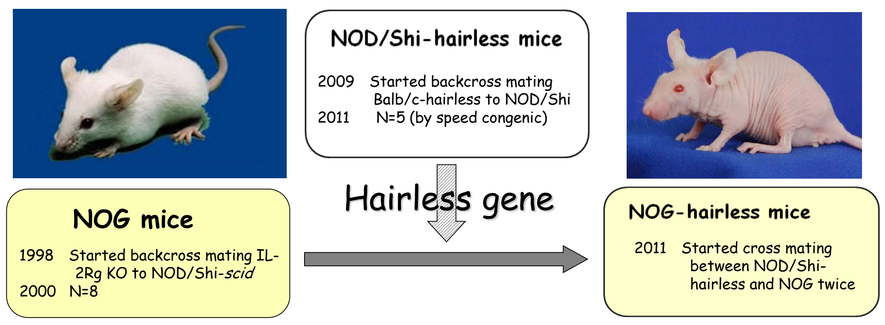
Introduction

NOG（NOD/Shi-*scid* IL2Rg^{null}） mice are used as a tool to study *in vivo* tumorigenicity for regenerative medicine/cell products due to their hyper immune-deficiency (Kusakawa *et al*, ISSCR 2013). We have introduced the hairless gene derived from BALB/c mice to produce NOG-hairless mice (NOD/Shi-*scid* IL2Rg^{null}-hr, NOG-hr), which are expected to be more useful for basic medical research (Figure 1).
In the present study, we conducted a qualitative comparison between NOG and NOG-hr mice by examined the TPD50 (tumor-producing dose at the 50% endpoint) using HeLa cells, which have been used as a positive control in the WHO TRS878 guideline to check tumorigenicity of *in vitro* substrates for the biologics medicine production. We also compared the differentiating ability of human CD34+ hematopoietic stem cells from umbilical cord blood in these mice.

Figure 1　Development of NOG and NOG-hr mice



Materials and Method

【Quantitative Analysis on Cell Engrafting Ability】 One hundred male NOG mice, 80 male NOG-hr mice and 40 male Nude mice were obtained at age of 6-weeks from CLEA Japan, Inc. (Tokyo, Japan) or our own breeding colony. After a one-week acclimatization, all mice were assigned to HeLa cell (ATCC, Washington DC, US) inoculated groups or HeLa cell + MG (Matrigel,LONZA Inc., NJ, US) inoculated groups (Table 1). Each mouse was subcutaneously inoculated once with a certain dose of material in the right abdomen. Daily clinical observations and weekly palpations to check tumor-formation were conducted until 16-weeks after inoculation. TPD50 was analyzed by 4-parameter logistics using Prism (ver. 6 GraphPad Software Inc., CA, US).

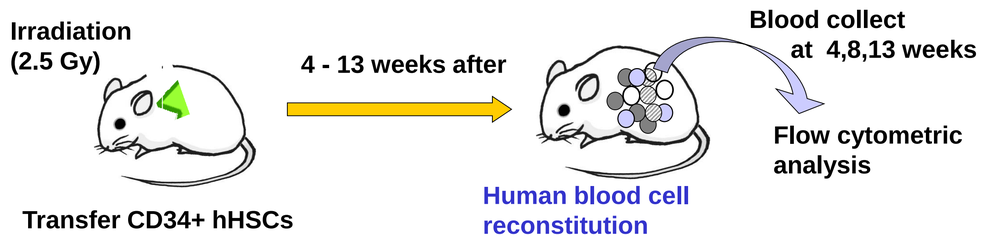
Table 1　Test groups

Stain	Group / No. of inoculated cells	0	1 x 10^1	1 x 10^2	1 x 10^3	1 x 10^4	1 x 10^5	1 x 10^6
Nude	**HeLa**	○	—	—	—	○	○	○
NOG	**HeLa**	○	—	○	○	○	○	—
	HeLa w/ MG	○	○	○	○	○	—	—
NOG-hr	**HeLa**	○	—	—	○	○	○	—
	HeLa w/ MG	○	○	○	○	—	—	—

○ : Test group, — : No test

【Human Hematopoietic Stem Cell (hHSC) Transfer and Cell Differentiating Analysis】 Eight weeks old NOG and NOG-hr mice were irradiated with 2.5 Gy at a day before cell transfer. Human CD34+ hematopoietic stem cells (hHSCs) from umbilical cord blood (AllCells,LLC. Alameda, CA.) were intravenously transplanted into irradiated mice (8.5x10^4 cells/ mouse). Peripheral blood (PB) was taken from the retro-orbital venous plexus at 4, 8 and 13 weeks after cell transplantation, and multicolor cytometric analysis was performed for comparison of the engraftment rate between NOG and NOG-hr mice using FACScalibur (Becton Dickinson, Franklin Lakes,NJ).
【Hematological Analysis of Mouse PB】 Blood was collected from retro-orbital venous plexus of intact NOG, NOG-hr and Nude (BALB/cA -nu/nu) mice under slight anesthesia of Isoflurene. Differential diagnosis of blood cells was performed with an automatic blood cell counter (XT-2000i, Sysmex, Osaka).

Figure 2　Cell Differentiating Analysis



Results

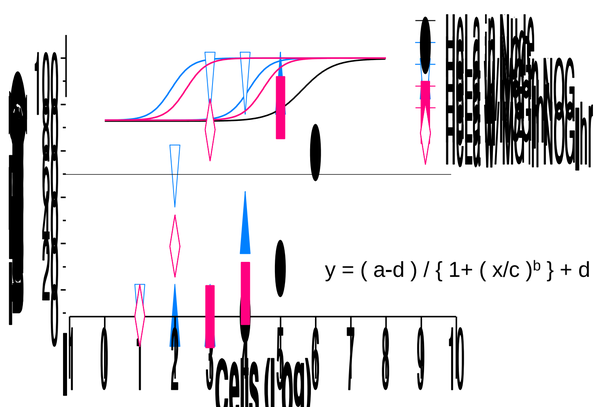
Table 2　Tumor Formation Rate at 16-week after Inoculation

Stain	Group/No. of inoculated cells	0	1 x 10^1	1 x 10^2	1 x 10^3	1 x 10^4	1 x 10^5	1 x 10^6
Nude	**HeLa**	**0/10[a]**	**-**	**-**	**-**	**0/10**	**2/10**	**7/10**
NOG	**HeLa**	**0/10**	**-**	**0/10**	**0/10**	**4/10**	**10/10**	**-**
	HeLa w/ MG	**0/10**	**0/10**	**6/10**	**10/10**	**10/10**	**-**	**-**
NOG-hr	**HeLa**	**0/10**	**-**	**-**	**0/10**	**1/10**	**9/10**	**-**
	HeLa w/ MG	**0/10**	**0/10**	**3/10**	**8/10**	**-**	**-**	**-**

a : No.of tumor bearing mice/No.of test mice, - : No test

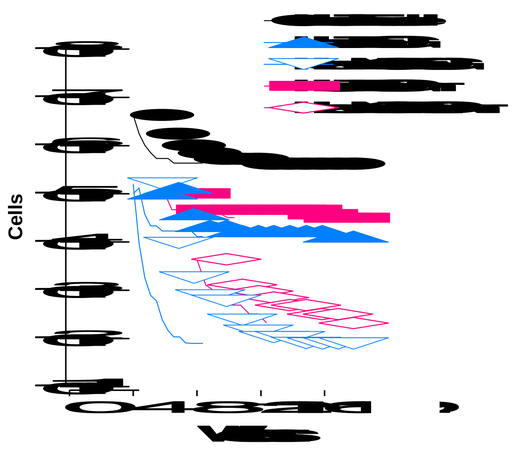
The order of tumor formation rates was Nude mice < NOG-hr mice < NOG mice. A remarkable increase in engrafting ability was observed when MG was mixed with HeLa cells in both NOG and NOG-hr mice.

Figure 3　Logistic Regression Analysis on Tumor Formation Rate at 16-week after Inoculation



a : Bottom, b : HillSlope, c : EC50(TPD50), d : Top

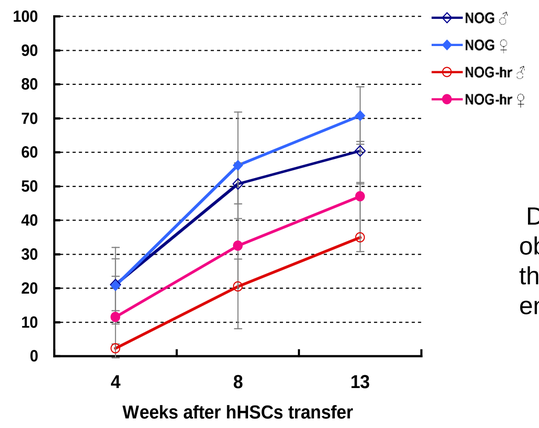
Figure 4　Changes in TPD50



TPD50 in all groups showed a time-dependent decrease. It stabilized within 16-weeks of inoculation, except for the HeLa with MG group in NOG-hr mice.

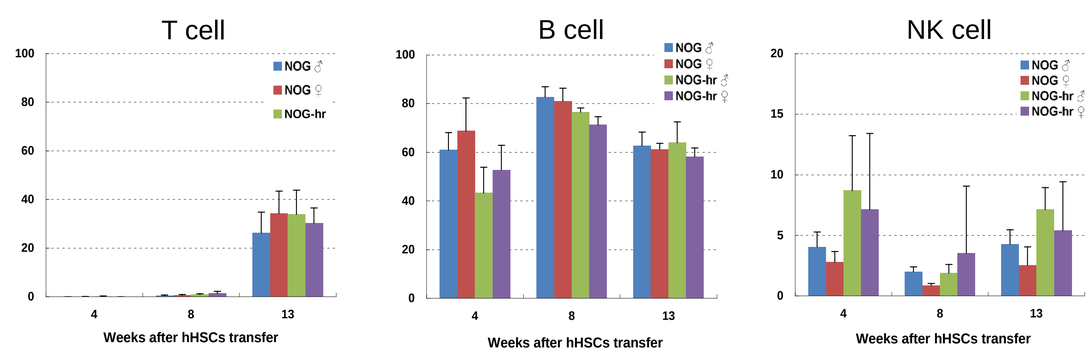
Figure 5　Human HSCs Engraftment Rate and Differentiation

a.) Change of human CD45+ cell ratio in all (mouse+human) CD45+ cells from peripheral blood of NOG and NOG-hr mice.



Differentiation of human CD45+ cells was observed in NOG and NOG-hr mice. But there was significant difference between engraftment rates in those strains.

b.) The ratio of human CD3+, CD19+ and CD56+ cell in NOG and NOG-hr mice (% of human CD45+ cells).



There was no significant difference in the characteristic of cell differentiation from human CD34+ cells between NOG and NOG-hr mice.

Table 3　TPD50 and Comparison Data Among Groups

Stain	Group	TPD50	fold	Power of MG.
Nude	**HeLa**	**4.2×10^5**	**1**	
NOG	**HeLa**	**1.3×10^4**	**1/33**	**1**
	HeLa w/ MG	**7.8×10**	**1/5431**	**×165**
NOG-hr	**HeLa**	**3.2×10^4**	**1/13**	**1**
	HeLa w/ MG	**2.1×10^2**	**1/2040**	**×152**

TPD50 of the HeLa cells or HeLa cells mixed with MG in NOG mice were 1/33 or 1/5431 of that in Nude mice, respectively. In NOG-hr mice, the ratios were 1/13 or 1/2040 of that in Nude mice, respectively. These results show that the engrafting activity of xenografts in NOG mice was higher than that in NOG-hr mice.

Table 4　Hematological Values of Mouse PB

		WBC	RBC	HGB	HCT	MCV	MCH	MCHC	PLT	NEUT	LYMPH	MONO	EO	BASO
	N=3	x10^2 /μl	x10^4 /μl	g/dl	%	fl	pg	g/dl	x10^3 /μl	%	%	%	%	%
NOG	Male	10.20 ±1.212	916.00 ±34.77	13.93 ±0.55	44.93 ±2.11	49.03 ±0.50	15.20 ±0.00	31.00 ±0.30	1542.33 ±125.16	73.40 ±3.05	19.73 ±4.95	5.93 ±1.86	0.93 ±0.86	0.00 ±0.00
	Female	9.37 ±3.79	892.67 ±16.17	13.63 ±0.35	42.60 ±0.87	47.70 ±0.35	15.27 ±0.15	32.00 ±0.36	1287.00 ±76.15	78.13 ±4.45	19.13 ±2.75	1.80 ±1.64	0.93 ±0.83	0.00 ±0.00
NOG-hr	Male	21.80 ±4.68	825.67 ±28.31	13.20 ±0.56	42.03 ±1.50	50.90 0.17	16.00 ±0.26	31.40 ±0.56	1397.67 ±237.51	82.00 ±3.32	13.67 ±2.51	3.13 ±0.92	1.20 ±0.26	0.00 ±0.00
	Female	25.60 ±21.08	855.67 ±135.27	14.70 ±1.85	44.13 ±6.63	51.63 ±0.61	17.23 ±0.78	33.40 ±1.14	976.00 ±258.22	84.37 ±8.30	1.03 ±0.49	7.20 ±1.48	7.40 ±8.60	0.00 ±0.00
Nude	Female	18.77 ±6.67	956.00 ±28.58	14.90 ±0.26	42.93 ±0.90	44.90 ±0.53	15.57 ±0.31	34.70 ±0.61	871.00 ±172.88	43.67 ±2.50	49.90 ±3.86	4.13 ±0.55	2.30 ±0.90	0.00 ±0.00

The WBC values of NOG-hr mice were significantly higher (2-2.5 fold) than that of NOG mice.

Conclusion

Engrafting ability in NOG and NOG-hr mice were much higher than that in Nude mice. However, a difference between NOG and NOG-hr mice, the former power was higher than latter's, was shown .
Following this, CD34+ hHSCs differentiation rates in NOG was faster than that in NOG-hr although there was no qualitative difference.
We consider that this difference might be caused by an insufficiency of backcrossing, so that further trial is going on.

Acknowledgments

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