FIGURE LEGENDS

Figure 1. Flow cytometric analysis during the in vitro differentiation of cynomolgus embryonic stem cells (cyESCs). Undifferentiated cyESCs expressing green fluorescent protein were cultured on OP9 cells with multiple cytokines (see Materials and Methods). Cells on day 0 (**A**) and day 6 (**B**) are shown in bright (left) and dark fields (right). Cells on days 0, 4, 6, 12, and 18 were stained for CD34 (**C**), CD31 (**D**), vascular endothelial (VE)-cadherin (**E**), and CD45 (**F**). The vertical axis shows the fraction (%) of cells that were stained positive. In **C-F**, results of two or three independent experiments are shown. Although cells on day 0 already express low levels of vascular endothelial growth factor receptor (VEGFR)-2, a VEGFR-2^{high} population did not emerge until day 6 (**G**). Dot-plot profiles for VEGFR-2 and VE-cadherin expression indicate that cells positive for both VEGFR-2 and VE-cadherin emerged until day 6 (**H**). In **G** and **H**, representative results from three independent experiments are shown. The Scl gene expression was up-regulated on day 6 to a similar level to that in the cynomolgus fetal liver (cyFL) as assessed by RNA-polymerase chain reaction (**I**). Day-6 cells (putative hematopoietic precursors) were used for transplantation.

Figure 2. Tumor formation after the transplantation of cynomolgus embryonic stem cell (cyESC)-derived progenitor cells. Tumors formed in all three monkey fetuses transplanted with the day-6 cyESC-derived progenitor cells (putative hematopoietic precursors). A representative tumor in the thoracic cavity at 3 months after transplantation is shown (**A**, monkey No. 0841). The tumor was observed in bright and dark fields under a fluorescence scope (**B**).

Figure 3. Cynomolgus embryonic stem cell (cyESC)-derived hematopoiesis in vivo. Bone marrow, cord blood, and liver cells were harvested from newborn monkeys and placed in methylcellulose medium to produce clonogenic hematopoietic colonies (**A**). A cytospin specimen (stained with the May-Giemsa method) of plucked colonies reveals mature neutrophils (**B**). To identify cyESC-derived colonies, well-separated individual colonies were plucked and examined for the green fluorescent protein (GFP) sequence by polymerase chain reaction (PCR). Plucked methylcellulose (MeC) alone (not containing colonies) served as a negative control. PCR of the β-actin sequence in the same colonies was simultaneously performed as an internal control. Colony PCR was repeated at least twice. Representative colony PCR results for monkey No. 0021 are shown (**C**). *Bands positive for the GFP sequence. Abbreviations: M, molecular weight marker; CMK6G, positive control GFP-expressing cynomolgus cells; DW, distilled water.

Figure 4. Purging stage-specific embryonic antigen (SSEA)-4^+ cells from among cynomolgus embryonic stem cell (cyESC)-derived progenitor cells. Undifferentiated cyESCs (day 0) and cyESC-derivatives (day 6) were stained with anti-SSEA-4. The SSEA-4 expression (% of total) at day 0 and day 6 is shown (n = 8) (**A**). The Oct-4 expression at day 0 and 6 was also examined by RNA-polymerase chain reaction (**B**). Flow cytometric dot-plot profiles are shown for the SSEA-4 versus green fluorescent protein (GFP) expression at day 0 (**C** left), at day 6 before the purge (**C** middle), and at day 6 after the purge (**C** right). In **C**, six independent experiments were conducted and

similar results were obtained. No tumors were detected in any monkey after the transplantation of SSEA-4-negative day-6 cyESC-derivatives (E, a representative monkey, No. 0981).

Table 1. Embryonic stem (ES) cell-derived hematopoiesis and tumor formation

Animals	Animal number	Transplanted cells	Purging SSEA-4$^+$ cells	Cell number per fetus (x10^6)	Donor-derived CFU in recipients[a] at birth (donor/total colony number)	Tumor formation	Observation period (months)
Monkeys	0031	Undifferentiated ES cells	-	3.90	ND	+	3
	2311		-	0.16	ND, Dead	+	2
	0321		-	0.21	ND, Dead	+	2
	0841	Day-6 ES-derived cells	-	10	4.1% (2/49)	+	3
	1551		-	46	ND, Dead	+	2.5
	0021		-	46	4.7% (4/85)	+	3
	0691	Day-6 ES-derived cells	+	0.16	3.2% (2/62)	-	3
	0381		+	1.40	5.0% (4/80)	-	3
	0022		+	0.17	2.3% (2/86)	-	3
	0981		+	0.31	4.1% (3/73)	-	3
	0051		+	0.31	ND, Dead [b]	-	3

	1552		+	0.75	4.4% (2/45)	-	4
Sheep[c]	57	Day-6 ES-derived cells	-	50	1.1% (1/91)	-	18
	55		-	50	1.1% (1/91)	-	26
	141		-	78	1.1% (1/91)	-	26
	182		-	14	1.6% (1/63)	-	21

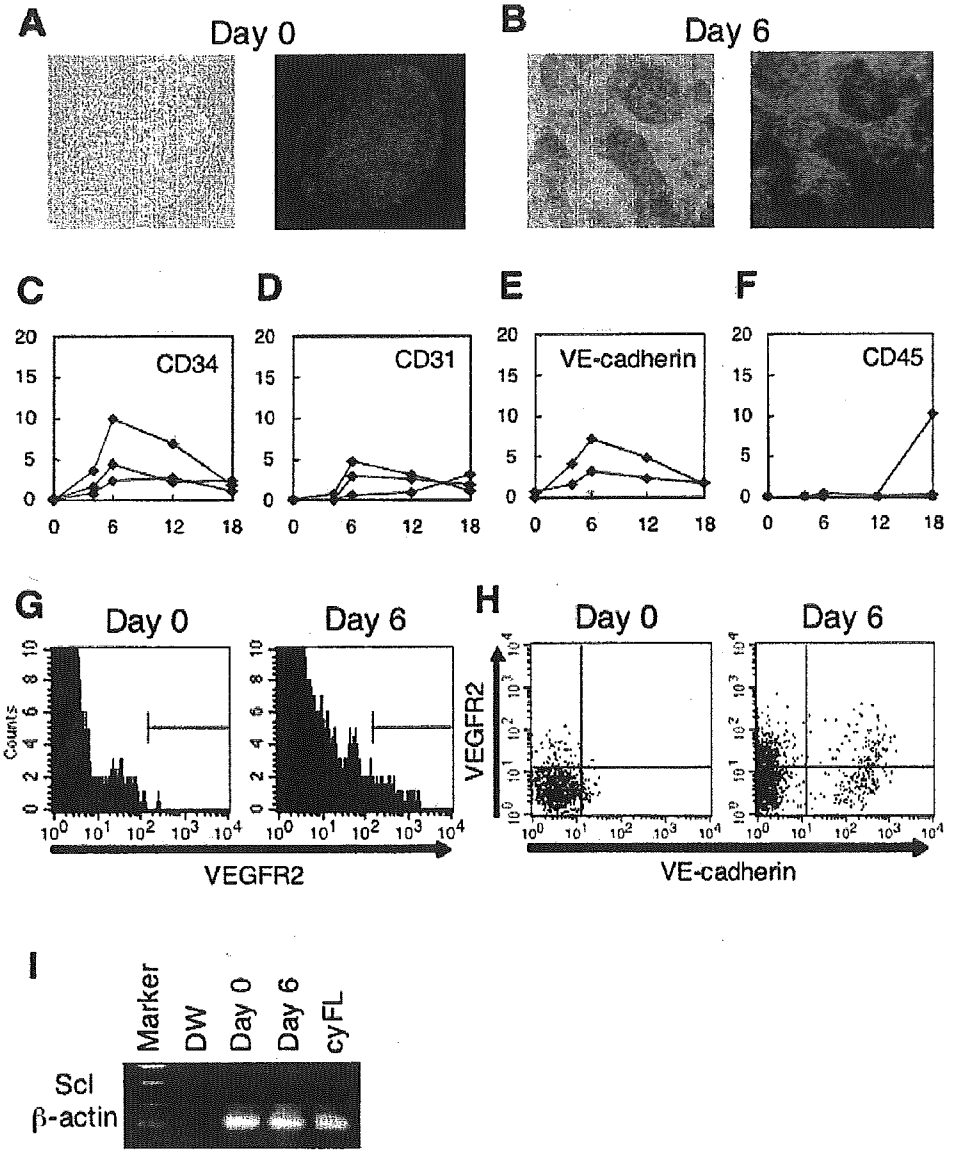
[a]Percent donor-derived colony-forming units (CFU) was calculated by dividing the number of CFU positive for the green fluorescent protein gene sequence by the number of CFU positive for the β-actin gene sequence. Donor-derived CFU were analyzed at delivery.

[b]Death due to ablation of placentae. Other deaths were presumably tumor-related.

[c]As published by Sasaki et al. [13].

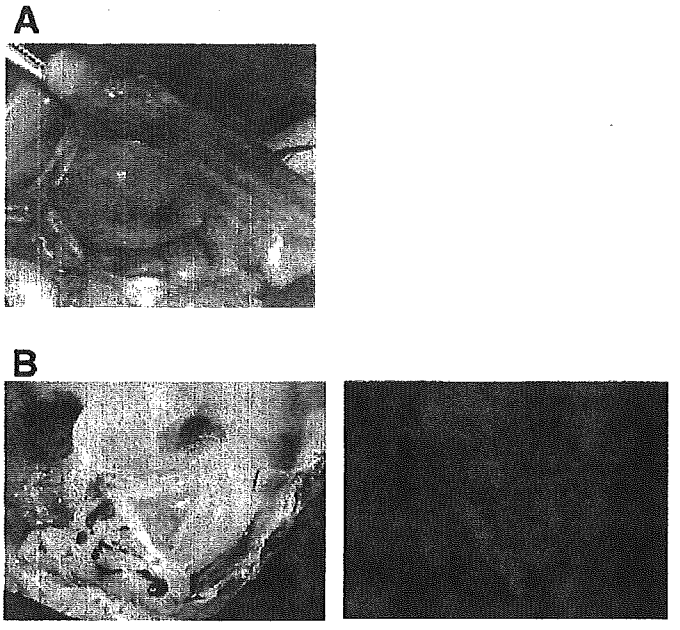
Abbreviations: SSEA, stage-specific embryonic antigen; ND, not done.

Top



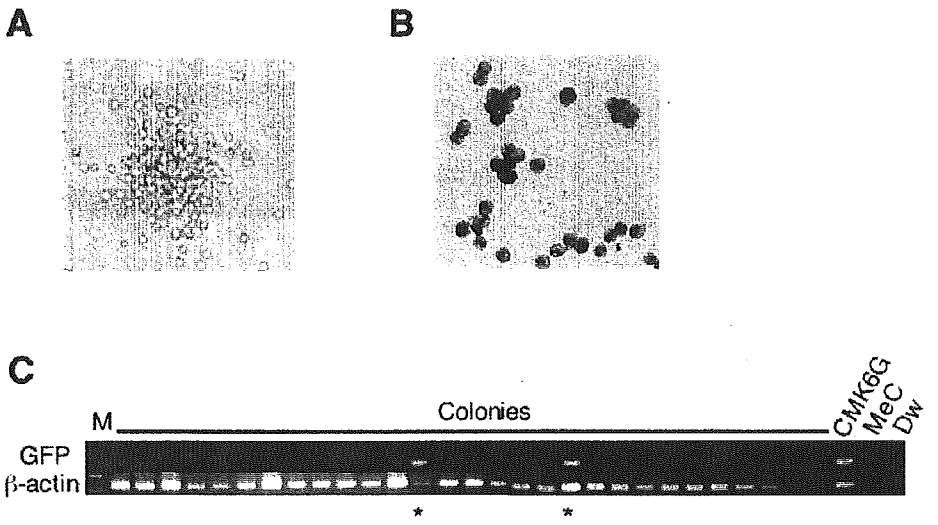
Hanazono Y, Figure 1

Top



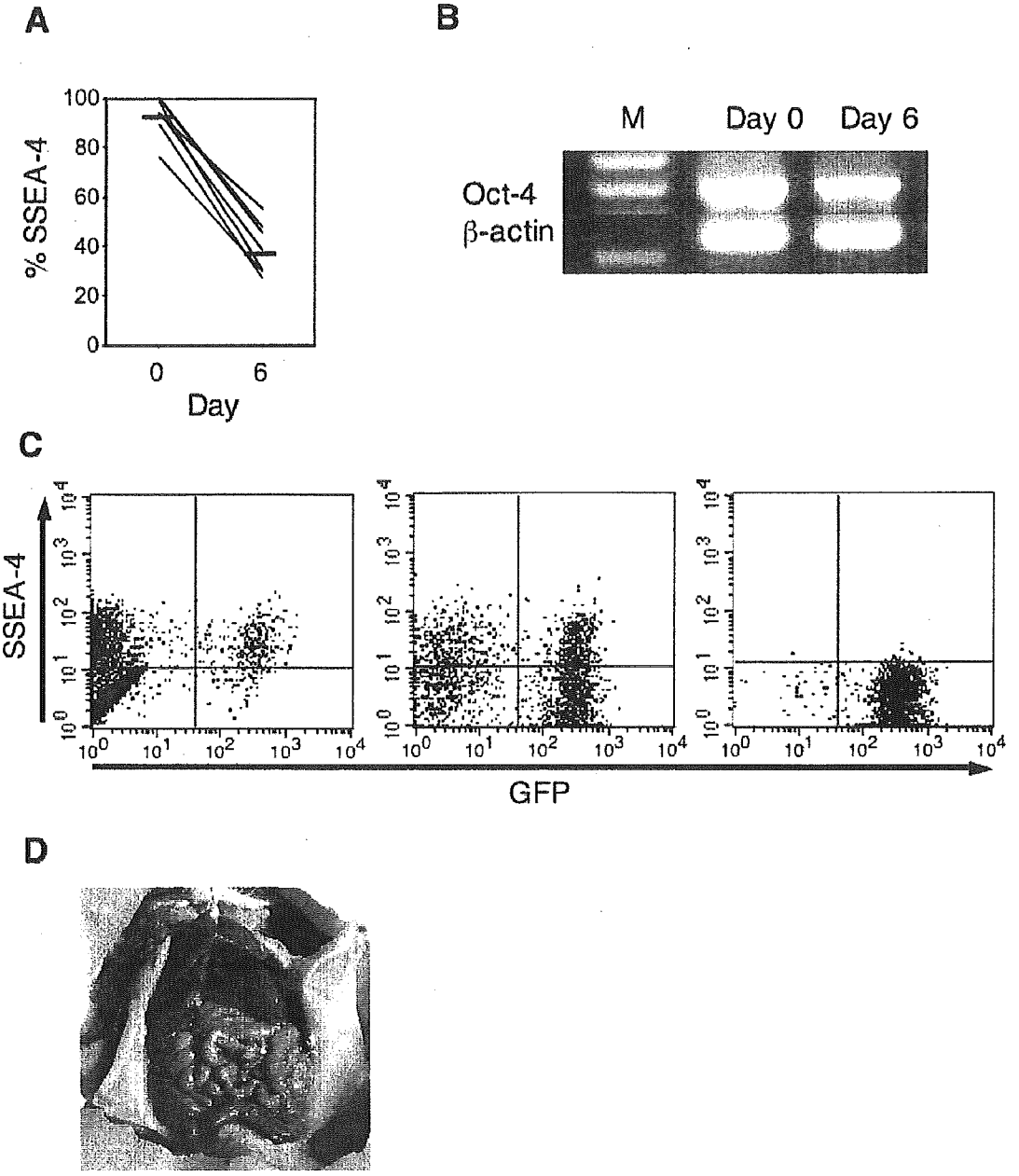
Hanazono Y, Figure 2

Top



Hanazono Y, Figure 3

Top



Hanazono Y, Figure 4