The human hepatic cell line HepaRG® as a possible cell source for the steady generation of humanized liver TK-NOG mice

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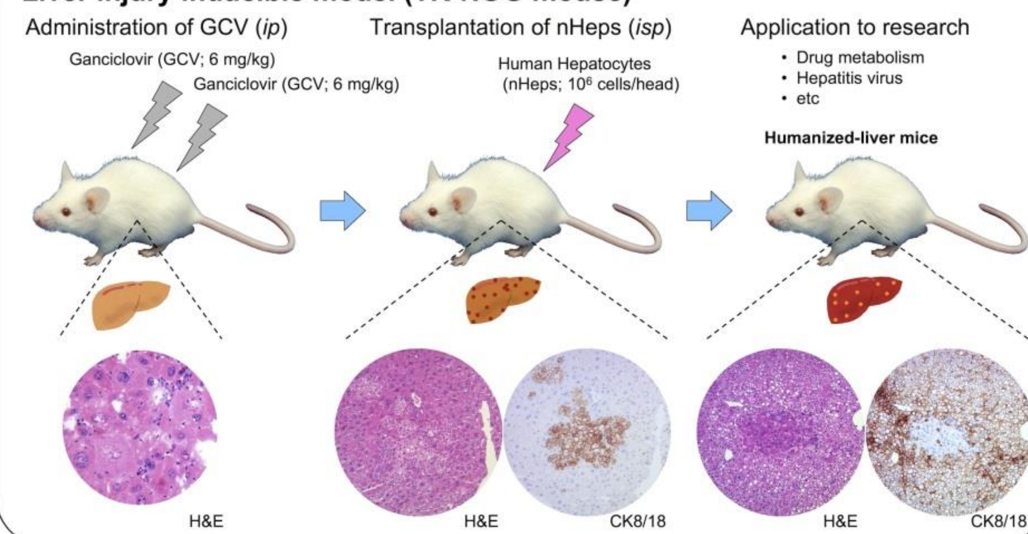
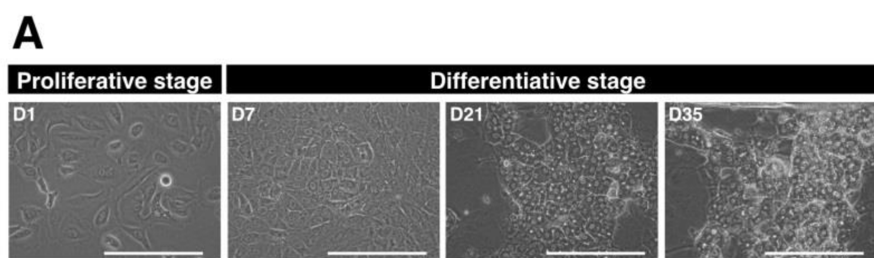
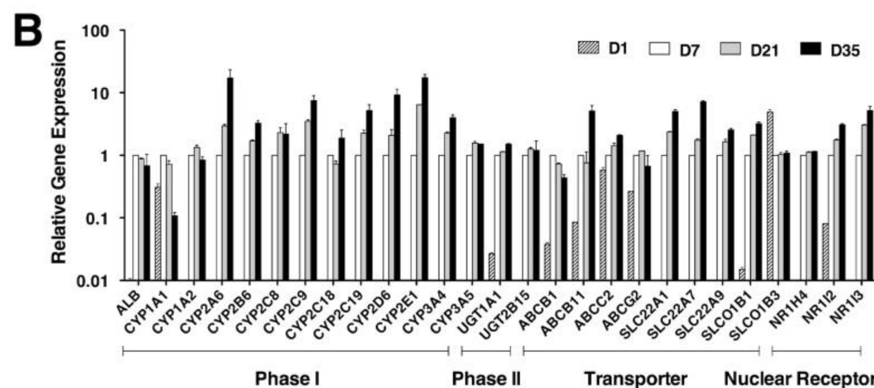
BACKGROUND:
Humanized-liver mice, in which the liver has been repopulated with human hepatocytes, have been used to study aspects of human liver physiology such as drug metabolism, toxicology, and hepatitis infection. However, the procurement of human hepatocytes is a major problem in producing humanized-liver mice because of the finite nature of the patient-derived resource. To overcome this issue, we tried to generate humanized-liver mice with the human bipotent liver progenitor cell line HepaRG® cells.

METHOD:
To create a niche for liver reconstitution with transplanted cells, an inducible hepatic injury was achieved by the targeted expression of the HSVtk in the liver of NOG mice (TK-NOG). To induce liver damage, adult 8-10-week-old male TK-NOG mice were injected intraperitoneally with ganciclovir (GCV, 6 mg/kg) seven days prior to transplantation. The optimally damaged animals were selected by measuring the serum alanine aminotransferase (ALT) levels on the day before transplantation. For differentiation of HepaRG® cells, the cells seeded onto 6-well plates at a density of $2x10^4$ cells/cm² were cultured in maintenance medium until the 7th day after plating, when the medium was replaced with HepaRG® differentiation medium containing 1.7% DMSO. A total of $1×10^6$ differentiated HepaRG® cells in 40 μl of Hanks' Balanced Salt Solution were intrasplenically injected to TK-NOG mice.



In vitro differentiation of HepaRG® cells.

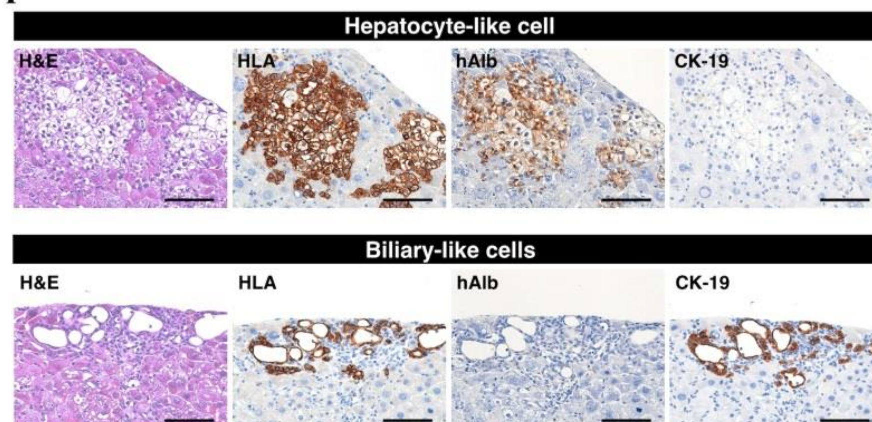




A. Phase-contrast photographs of HepaRG® cells at the proliferative stage (D1: low density culture), and the differentiative stage (D7: confluent culture; D21 and D35: differentiation culture with 1.7% DMSO). D1, 7, 21 and 35 in HepaRG® stage indicate the number of days after seeding. Bar = 100 μm. **B.** The relative expression of 26 human drug metabolism-related mRNAs on day 1 (D1), 7 (D7), 21 (D21), or 35 (D35) in the HepaRG® cells was assessed by qPCR. Each bar represents the average of two independent determinations, and the standard error is shown.

RESULTS:
We demonstrate that, *in vivo*, transplanted confluent culture or differentiated HepaRG® cells proliferated and differentiated toward both hepatocyte-like and biliary-like cells within the recipient liver. In contrast, proliferative HepaRG® cells could engraft TK-NOG mouse liver but could differentiate only toward biliary-like cells. The differentiation to hepatocyte-like cells was characterized by the detection of human albumin in the recipient mouse serum and was confirmed by immunohistochemical staining for human leukocyte antigen (HLA), human albumin, cytochrome P450 3A4 (CYP3A4) and multidrug resistance-associated protein 2 (MRP2). Biliary-like cells were characterized by positive staining for cytokeratin-19 (CK-19).

Reconstitution of human liver structures from differentiated HepaRG® cells *in vivo.*



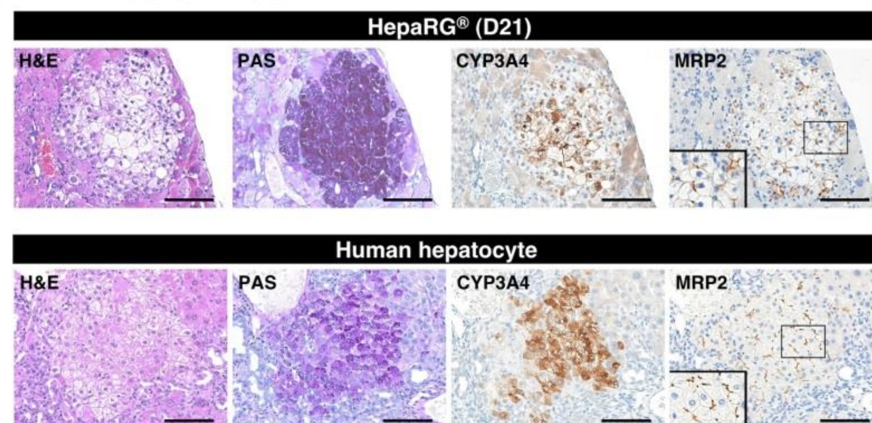
Hepatocyte-like colonies (upper panel) and biliary-like colonies (lower panel) in a TK-NOG mouse liver that was transplanted with differentiation D35 HepaRG® cells were subjected to immunohistochemical analysis. Serial liver sections were stained for H&E, HLA, human Albumin (hAlb) and CK-19. Bar = 100 μm

Colony-forming ability of various differentiation stages of HepaRG® cells in TK-NOG livers.

Experiment	HepaRG® stage	Mouse ID	BW (g)	hAlb (μg/mL)	Area (cm²)	Hepatocyte-like colony		Biliary-like colony	
						Number	Colonies/cm²	Number	Colonies/cm²
#1	D35	#2	15.2	N.D.	3.6	0	0	0	0
		#3	24.0	N.D.	4.3	6	1.4	5	1.2
		#4	26.5	N.D.	3.7	1	0.3	0	0
		#5	25.7	N.D.	4.0	0	0	1	0.3
		#8	27.0	N.D.	4.0	7	1.8	1	0.3
#2	D1	#2	28.6	N.D.	3.3	0	0	0	0
		#3	28.0	N.D.	4.9	0	0	31	6.3
	D7	#1	27.9	6.9	4.5	24	5.3	43	9.5
		#5	28.9	N.D.	4.0	3	0.7	23	5.7
	D21	#9	26.9	14.2	3.8	40	10.5	26	6.8
		#14	26.5	N.D.	2.9	21	7.4	37	13.0
	D35	#10	25.2	N.D.	2.8	2	0.7	1	0.4
		#12	27.7	N.D.	3.0	4	1.3	4	1.3

D1, 7, 21 and 35 in HepaRG® stage indicate the number of days after seeding. BW: body weight. The human serum albumin (hAlb) level of each animal is shown. N.D.: not detected by ELISA. Area: observed cross sections were measured and indicated as cm². Colonies/cm²: number of colonies per area of observation.

Expression of functional liver markers in reconstituted livers from TK-NOG mice.



Hepatocyte-like colonies in a TK-NOG mouse liver that was transplanted with differentiation D21 HepaRG® cells (upper panel) and human hepatocyte colonies in a TK-NOG mouse liver that was transplanted with cryopreserved human hepatocytes (HEP187170; 26 years, female)(lower panel) were assessed for functionality by histochemical and immunohistochemical analyses. Serial liver sections were stained for H&E, PAS, CYP3A4 and MRP2. Bar = 100 μm

CONCLUSION:
These results indicated that the differentiated HepaRG® cells are a possible cell source for generating humanized-liver mice, which are a useful model for *in vivo* studies of liver physiology.