J. Biol. Macromol., **21** (1), 51-52 (2021)

Letter to Editor

PXB-cells, fresh primary hepatocytes from humanized mouse livers, exhibit nonalcoholic fatty liver like properties, including large very low density lipoprotein

Sayaka Tomatsu [1], Masaki Takahashi [2], Masakazu Kakuni [2], Gen Toshima [3], Fumiko Kimura [3], Junichiro Takahashi [3], Yui Umekawa [1], Akira Sasaki [1], Keishi Hata [1,*]

[1]Akita Research Institute of Food & Brewing, Akita 010-1623, Japan, [2]PhoenixBio Co., Ltd., Hiroshima 739-0046, Japan, [3]Skylight-Biotech Inc, Akita 011-0911, Japan

Received June 1, 2020; Accepted August 17, 2020

Dear Editor,

We previously examined lipoprotein profile in PXB-cells, fresh primary human hepatocytes from humanized mouse livers (Fig. 1), and demonstrated that they are suitable for screening anti-lipidemic agents [1)]. The accumulation of numerous oil droplets － a hallmark of non-alcoholic fatty liver disease (NAFLD) －was observed in PXB-cells immediately after their isolation from humanized murine livers 16 weeks after transplantation, but markedly decreased in an *in vitro* culture of more than 10 days. Some studies demonstrated that the size of very low density lipoproteins (VLDL) in the plasma of patients NAFLD or non-alcoholic steatohepatitis significantly increased [2, 3)], suggesting that increases in the size of VLDL have potential as a minimally invasive biomarker for the diagnosis and/or progression of NAFLD. To clarify whether hepatocytes with numerous oil droplets actually secret larger size of VLDL, we investigated intra- and extracellular lipid levels and lipoprotein profiles of PXB-cells isolated at several post-transplantation durations, which seem to be closely related to cytosolic oil droplet accumulations.

Microscopic observations revealed the accumulation of numerous large oil droplets in PXB-cells isolated 16 and 19 weeks (16W and 19W), but not 5 weeks (5W), after transplantation. The accumulation of cytosolic large oil droplets in the humanized livers of chimeric mice increased with longer post-transplantation durations due to a deficiency in circulating human growth factor [4)], suggesting that oil droplet levels in PXB-cells depend on the post-transplantation duration. We measured intra- and extracellular lipid levels in 5W, 16W, and 19W using LipoSEARCH [5)] (Table 1). The results obtained showed that intracellular triglyceride levels markedly increased with longer post-transplantation durations, and intracellular triglyceride levels in 16W and 19W were 3.7- and 3.2-fold those in 5W. However, extracellular triglyceride levels were similar in 5W and 16W, and the intracellular accumulation of oil droplets did not affect lipoprotein levels in the culture medium of PXB-cells. We calculated the particle size of VLDL released from PXB-cells using lipoprotein profiles, and confirmed that their size increased with longer post-transplantation durations. These results suggest that the post-transplantation duration affects intracellular triglyceride levels in PXB-cells, and the accumulation of intracellular oil droplets may be associated with the size of VLDL secreted from cells. Increases in the size of VLDL have potential as a minimally invasive plasma biomarker for the diagnosis and/or progression of NAFLD.

* Corresponding author.
E-mail: hata@arif.pref.akita.jp

Conflict of interest

There is no conflict of interest.

References

1. Hata K., Tomatsu S., Takahashi M. et al. (2020) *Biomed Res-Tokyo*, **31**, 33-42.
2. Fujita K., Nozaki Y., Wada K. et al. (2009) *Hepatol.*, **50**, 772-780.
3. Rinella M. E, Sanyal A.J. (2015) *Nat. Rev. Gastroenterol. Hepatol.*, **12**, 65-66.
4. Tateno C., Kataoka M., Utoh R. et al. (2011) *Endocrinology*, **152**: 1479-1491.
5. Toshima G, Iwama Y., Kimura F. et al. (2013) *J. Biol. Macromol.*, **13**, 21-32.
6. Yamasaki C., Kataoka M., Kato Y. et al. (2010) *Drug Metab. Pharmacokinet.*, **25**, 539-550.

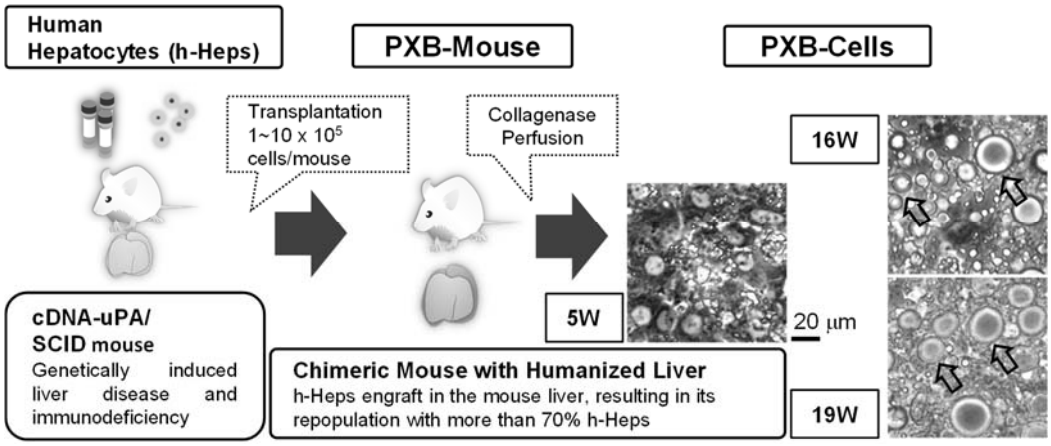

Fig. 1 Preparation of PXB-cells from urokinase-type plasminogen activator/severe combined immunodeficiency (uPA/SCID) mice with humanized livers (PXB-mouse)

PXB-cells were isolated from humanized murine livers 5, 16 and 19 weeks after transplantation (5W, 16W, and 19W) according to a previously described procedure [6]. **Note:** numerous large oil droplets (arrows) were observed in 16W and 19W.

Table 1. Intra- and extracellular lipid contents in NAFL-like PXB-cells

	PXB-cells		
	5W	16W	19W
Cell number (× 10^5 cells)	3.0±0.1	3.0±0.1	2.8±0.1
Cholesterol (μg/10^6 cells)			
intracellular	50.0±1.8	34.9±0.8*	49.6±2.4
extracellular	4.9±0.2	5.1±0.2	4.9±0.1
VLDL fraction (30-80 nm)	4.2±0.2	4.4±0.2	4.3±0.1
LDL fraction (16-30 nm)	0.5	0.5	0.5
HDL fraction (8-16 nm)	0	0.2	0.2
Triglycerides (μg/10^6 cells)			
intracellular	66.8±3.3	248.0±15.4*	216.7±31.5*
extracellular	68.8±3.4	71.6± 2.8	96.0± 2.4*
VLDL fraction (30-80 nm)	62.6±1.0	66.7± 2.7	88.8± 2.5*
LDL fraction (16-30 nm)	5.6±0.2	4.3± 0.3	6.4± 0.1*
HDL fraction (8-16 nm)	0.6±0.1	0.7	0.9
VLDL size (diameter, nm)	43.0±0.1	46.7± 0.4*	51.2± 0.2*

PXB-cells seeded at 4 × 10^5 cells in collagen-coated 24-well microplates (Day 0), and maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% FBS, 20 mM HEPES, 15 μg/mL L-proline, 0.25 μg/mL insulin, 50 nM dexamethasone, 44 mM $NaHCO_3$, 5 ng/mL EGF, 0.1 mM ascorbic acid 2-phosphate, 2% DMSO, and antibiotics for 6 days. Cells on Day 6 were incubated in 500 μl William's E medium supplemented with CM-4000 (Thermo Fisher Scientific) for 2 days. Intracellular cholesterol and triglyceride levels were assessed using the Cholestest Cho Kit and TG Kit (Sekisui Medical), respectively, according to the laboratory procedure described in each manual. Culture media were subjected to a lipoprotein assay and the sizes of VLDL were measured by LipoSEARCH. Standard samples for calibrating particle diameter were latex beads with 25 nm and 37 nm in diameter (Magsphere Inc) and thyroglobulin, 17 nm; ferritin, 12.2 nm; catalase, 9.2 nm; albumin, 7.1 nm; ovalbumin, 6.1 nm (High-molecular-weight standards, Pharmacia Biotech). Data represent means ± standard deviations (n=3). *P<0.05 vs 5W analyzed using the Kruskal-Wallis test with Steel multiple comparison tests (BellCurve for Excel, Social Survey Research Information).

Funding

The study was supported in part by "Subsidies for projects to promote the enhancement of manufacturing technology for small and medium enterprises" from the Japan Ministry of Economy, Trade and Industry.

Communicated by Kikuzaki Hiroe