Lipoprotein profile and lipid metabolism of PXB-cells[®], human primary hepatocytes from liver-humanized mice: proposal of novel *in vitro* system for screening anti-lipidemic drugs

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ABSTRACT

We investigated lipid metabolism in PXB-cells, which are human primary hepatocytes isolated from liver-humanized mice, and HepG2 and HuH-7 human hepatoma cell lines. Lipoprotein levels were higher in PXB-cells than in the 2 other cell lines, and PXB-cells mainly released triglycerides and cholesterol as very low density lipoprotein (VLDL), similar to actual liver tissue, whereas the major lipoprotein released from the 2 hepatoma cell lines was LDL. RT-PCR analysis demonstrated that the gene expression levels of apolipoprotein B100 (ApoB100), the apolipoprotein of VLDL/LDL, were similar in PXB-cells and HepG2 cells, while the overexpression of ApoC2, ApoC3, and ApoE, which are components of VLDL, but not LDL, was observed in PXB-cells. A protein immunoassay revealed that ApoB100 levels secreted from PXB-cells and HuH-7 cells were similar; however, ApoC3 levels were higher in PXB-cells than in the two other cell lines. We also examined the anti-lipidemic activities of fenofibrate using this assay system. Fenofibrate suppressed lipoprotein production from PXB-cells in a dose-dependent manner mainly by activating the β -oxidation pathway. These results suggest that PXB-cells produce high levels of lipoproteins and are suitable for screening anti-lipidemic agents.

Serum-insoluble lipids circulate in the bloodstream as lipoproteins, which are macromolecular complexes of free cholesterol, cholesterol esters, triglycerides, phospholipids, and apolipoproteins (Apo). Lipoproteins mainly produced in intestinal epithelial tissues and the liver are separated into 4 major classes based on their particle size and density. Chylomicrons (>80 nm) released in intestinal epithelium cells are transported to the liver through lymphatic vessels after their conversion to chylomicron remnants by lipoprotein lipase. Very low density lipoprotein (VLDL, 30–80 nm) and LDL (16–30 nm) with lipids and apolipoprotein B100 (ApoB100) are mainly synthesized in the liver and transport serum lipids to each tissue. High density lipoprotein (HDL, 8–16 nm) with ApoA is an important lipoprotein for the collection and transport of excess serum cholesterol. High levels of VLDL and LDL and/or low levels of HDL cause dyslipidemic diseases, such as hypercholesterolemia and hypertriglyceridemia, and are associated with an increased risk of atherosclerotic disease (11, 13, 24).

The attenuating activities of crude drugs or food-stuffs on metabolic syndrome have been examined (7, 10). In many cases, experimental animals, such as dyslipidemic mice/rats, have been used to screen anti-metabolic syndrome activities; however, studies using experimental animals are very expensive, and

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

difficulties are associated with simultaneously evaluating many test samples. We previously developed a novel system for evaluating anti-lipidemic agents by assessing lipoprotein profiles secreted from HepG2 human hepatoma cells (6). The anti-lipidemic effects of water shield extracts were demonstrated in the assay system, and their effects were confirmed in experimental animals and a pilot clinical study (20). However, a number of issues need to be resolved, such as differences in the lipoprotein profiles of hepatoma cells and human primary hepatocytes (18). PXB-cells® are fresh hepatocytes isolated from humanized mouse livers repopulated completely with human hepatocytes by transplantation (14). Advances have been achieved in the application of PXB-cells to the study of drug metabolism and hepatitis virus infection (5, 25); however, their application to other fields, such as lipid/sugar metabolism, has progressed to a lesser extent. In the present study, we examined the lipoprotein profiles of PXB-cells and investigated whether they were suitable for screening anti-lipidemic activities *in vitro*. The results obtained suggest that PXB-cells produce high levels of lipoproteins and are suitable for screening anti-lipidemic agents.

MATERIALS AND METHODS

Cell lines. PXB-cells were isolated from humanized

murine livers 16 weeks after transplantation according to a previously described procedure (Fig. 1), 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 μ g/mL L-proline, 0.25 μ g/mL insulin, 50 nM dexamethasone, 44 mM NaHCO₃, 5 ng/mL EGF, 0.1 mM ascorbic acid 2-phosphate, 2% DMSO and antibiotics. The HepG2 and HuH-7 human hepatoma cell lines were from the RIKEN Cell BANK and maintained in DMEM containing 10% FBS and antibiotics.

Lipid assay. PXB-cells on Day 13, or hepatoma cells precultured for 20 h in collagen-coated 24-well microplates (4×10^5 cells, respectively), were incubated in 500 μ L William's E medium supplemented with Hepatocyte Maintenance Supplement Pack (CM-4000; Thermo Fisher Scientific, Waltham, MA, USA) for 2 days. Intracellular cholesterol and triglyceride levels were assessed using the Cholestest Cho Kit and TG Kit (Sekisui Medical, Tokyo, Japan), respectively, according to the laboratory procedure described in each manual. Culture media were subjected to a lipoprotein assay using LipoSEARCH® (22).

Real-time RT-PCR. Total RNA was isolated using the QuickGene RNA cultured cell kit S (Fujifilm

Table 1 Primer sequences used in the present study

Gene name	Forward	Reverse
ACC	5'-TGATGTCAATCTCCCCGAGC-3'	5'-TTGCTTCTTCTCTGTTTTCTCCCC-3'
ACOX1	5'-TCAACCCGAGCTGCTTAC-3'	5'-GCTGGAAGTCTGGGTGCTT-3'
ACSL1	5'-AGCGGCGCCTTAAATAGCA-3'	5'-CATGGGCTTGCATTGTCCT-3'
Angptl3	5'-CCAGAACACCCAGAAGTACT-3'	5'-TCTGTGGGTTCTTGAATACTGATC-3'
Angptl4	5'-GCCTATAGCCTGCAGCTCAC-3'	5'-CAAGTGGAGAAGGGTACGGA-3'
Angptl8	5'-TTCCATCCAGGCAGATTC-3'	5'-AGGTCTTAAAGGCTCACG-3'
Albumin	5'-ACTGCATTGCCGAAGTGG-3'	5'-GCAGCACGACAGAGTAATCA GGA -3'
ApoA1	5'-TGTGTACGTGGATGTGCTCAAAGA-3'	5'-TGTGTACGTGGATGTGCTCAAAGA-3'
ApoA2	5'-GCAGCAACTGTGCTACTCCACA-3'	5'-GCAAAGAGTGGGTAGGGACAGG-3'
ApoA5	5'-ACGCACGCATCCAGCAGAAC-3'	5'-TCGGAGAGCATCTGGGGGTC-3'
ApoB100	5'-TCGCCTGCCAACTGCTTC-3'	5'-CATTGGTGCCTGTGTTCCATT-3'
ApoC2	5'-GAATCTCTCTCCAGTTACTGGG-3'	5'-GAATCAGGCTAGAGTTGGGAG-3'
ApoC3	5'-TACATGAAGCAGCCACCAAG-3'	5'-AGCCACGGCTGAAGTTGGTC-3'
ApoE	5'-GGGCGCTTTTGGGATTAC-3'	5'-CAACTCCTTCATGGTATCG-3'
CACT	5'-GCTATCCCCAGCTTTTTTGCA-3'	5'-AGCACTTGATCCGTTCTCCA-3'
CYP1A2	5'-AACAAGGGACACAACGCTGAAT-3'	5'-GGAAGAGAAACAAGGGCTGAGT-3'
CYP2C9	5'-CCTCTGGGGCATTATCCATC-3'	5'-ATATTTGCACAGTGAAACATAGGA-3'
CYP2B6	5'-ATGGGGCACTGAAAAAGACTGA-3'	5'-AGAGGCGGGACACTGAATGAC-3'
CYP3A4	5'-CCAAGCTATGCTCTTCACCG-3'	5'-TCAGGCTCCACTTACGGTGC-3'
DGAT1	5'-GGCCTTCTTCCACGAGTACC-3'	5'-GGCCTCATAGTTGAGCACG-3'
DGAT2	5'-AGTGGCAATGCTATCATCAT-3'	5'-GAGGCCTCGACCATGGAAGAT-3'
FAS	5'-CAGCAGTTCACGGACATGGAG-3'	5'-CGGCACGCAGCTTGTAGTAGA-3'
GAPDH	5'-GCACCGTCAAGGCTGAGAAC-3'	5'-TGGTGAAGACGCCAGTGGA-3'
GPAM	5'-AACCCCAAGTATCCCGTCTTT-3'	5'-CAGTCACATTGGTGGCAAAC-3'
HMGCoAR	5'-TACCATGTGAGGGGTACGTC-3'	5'-CAAGCCTAGAGACATAATCATC-3'
HTGL	5'-TTCAGAAAACACAGATGACCTACTTCT-3'	5'-CTGATCTTTCGCTTTGATGATGTTTACAG-3'
Lipin1	5'-TGAAAAGGGGCTCTGTGGAC-3'	5'-ACTACAGAGCTGCTTGACGG-3'
MCAD	5'-GGTGCTGTTGGATTAGCACAAAGA-3'	5'-TTTCGACGACCAAGATCAACCTC-3'
MGAT1	5'-CAGGGCCGATGTCCATTGG-3'	5'-GGGTATGCCAGTCAAAGTAAAGC-3'
MGAT2	5'-CCTTCGGGGAGAATGACCTAT-3'	5'-GAGGGAGATGCCCATGATCTT-3'
MGAT3	5'-ATGGGAGTTGCCACAACCC-3'	5'-CAGAGTGACGTGAAGAGGAGG-3'
MTTP	5'-TCTCTACTCGGGTTCTGGCATTCTA-3'	5'-GCTGCGATTAAGGCTTCCAGTC-3'
PPAR α	5'-GCAATGGAAGTGGATGACAGTGA-3'	5'-TGCTCTGCAGGTGGAGTCTGA-3'
PPAR γ	5'-TGGAATTAGATGACAGCGACTTGG-3'	5'-CTGGAGCAGCTTGCCAAACA-3'
SCD	5'-TGAACAGTGCTGCCACCTC-3'	5'-CGGCCATGCAATCAATGAAG-3'
SREBP1c	5'-GGCTCCTGCCTACAGCTTCT-3'	5'-CAGCCAGTGGATCACCACA-3'
SREBP2	5'-CTATGGAGCAGCCTCAACGTCA-3'	5'-CCGTAGCGACAGTAGCAGGTCA-3'

Abbreviations: ACC, acetyl-CoA carboxylase; ACOX, peroxisomal acyl-coenzyme A oxidase; ACSL, long chain acyl-CoA synthase; Angptl, angiopoietin-like protein; Apo, apolipoprotein; CACT, carnitine acylcarnitine translocase; CYP, cytochrome P450; DGAT, diacylglycerol acyltransferase; FAS, fatty acid synthase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GPAM, glycerol-3-phosphate acyltransferase mitochondrial; HMGCoAR, HMG-CoA reductase; HTGL, hepatic triglyceride lipase; MCAD, medium-chain acyl-coenzyme A dehydrogenase; MGAT, monoacylglycerol acyltransferase; PPAR, peroxisome proliferator-activated receptor; SCD, stearoyl-CoA desaturase; SREBP, sterol regulatory element-binding protein

Wako, Osaka Japan). Template cDNA synthesis was performed with 5 μ g of total RNA using the PrimeScript RT reagent Kit (Takara Bio, Kusatsu, Japan). In a fluorescent temperature cycler (CFX Connect™ Real-Time PCR Detection System; Bio-Rad Laboratories, Hercules, CA, USA), 2.5% of each RT reaction solution was amplified in 25 μ L of 1 $\times$ SYBR Premix Ex Taq (Takara Bio) containing 0.2 μ M of

each primer. Samples were incubated in the thermal cycler for an initial denaturation at 95°C for 10 s, followed by 40 PCR cycles. Each cycle consisted of 95°C for 5 s and 60°C for 30 s. The relative expression level of each mRNA was normalized by the amount of glyceraldehyde-3-phosphate dehydrogenase mRNA. The primer sequences used in the present study were shown in Table 1.

Western blotting. Cell lysates were prepared using RIPA buffer (Nacalai Tesque, Kyoto, Japan). Cell lysates (10 µg/lane) were separated on 4–15% gradient SDS-polyacrylamide gels and transferred onto polyvinylidene fluoride membranes. After blocking with a Blocking Reagent (Nacalai Tesque), each membrane was incubated with anti-acyl-CoA synthetase long chain 1 (ACSL1, 1 : 3,000; ab89854; Abcam, Cambridge, MA, USA), anti-monoglyceride acyl transferase-1 (MGAT1, 1 : 3,000; ab38857; Abcam), anti-glycerol-3-phosphate acyltransferase 1, mitochondrial (GPAM, 1 : 8,000; sc-398135; Santa Cruz Biotechnology, Dallas, TX, USA), or anti-β-actin antibodies (1 : 3,000; IMG-5142A; IMGENEX, San Diego, CA, USA). The membrane was incubated with horseradish peroxidase-conjugated anti-mouse IgG (1 : 30,000; W4021; Promega, Madison, WI, USA) or anti-rabbit IgG (1 : 30,000; W4011; Promega) as secondary antibodies at appropriate concentrations. The enzyme reaction was detected using an ECL prime Western Blotting Reagent (GE Healthcare Bio-Sciences, Piscataway, NJ, USA). Chemiluminescent signals were detected using a Light-Capture II imaging system (AE-6982/C/FC; ATTO, Tokyo, Japan) and CS Analyzer (ver. 3.0 software; ATTO).

ELISA. Angiopoietin-like proteins (Angptls), albumin, ApoB100, and ApoC3 in cultured media were measured using the Angptl3, 4, or 8 ELISA Kit (Immuno-Biological Laboratories, Fujioka, Japan), LZ-test “Eiken” U-ALB (Eiken Chemical, Tokyo, Japan), human ApoB100 assay kit (Immuno-Biological Laboratories), and human APOC3 ELISA Kit (Abcam), respectively, according to the laboratory procedure described in each manual.

Statistical analysis. Data are expressed as the mean ± standard deviation (SD). The significance of differences was analyzed using the Kruskal-Wallis test with Steel (Tables 3 and 5) and Steel-Dwass (Figs. 2 and 5) multiple comparison tests and the Mann-Whitney U-test (Table 4 and Fig. 7). A value of $P < 0.05$ was considered to be significant.

RESULTS & DISCUSSION

Hepatic properties of PXB-cells

To clarify the differentiation stages of PXB-cells and the 2 hepatoma cell lines, we briefly examined the expression of 5 hepatic markers at the mRNA and protein levels. The RT-PCR analysis revealed higher expression levels of albumin and 4 cytochrome P450 genes in PXB-cells than in the HepG2

Table 2 Comparison of gene expression levels in PXB-cells and human hepatoma cell lines

	Relative intensity/GAPDH (-fold)		
	PXB-cells	HepG2 cells	HuH-7 cells
Hepatic marker			
Albumin	21.6*	1.0	3.3*
CYP1A2	2451.9*	1.0	0.8
CYP2C9	7160.7*	1.0	86.3*
CYP2B6	1.6*	1.0	0.1*
CYP3A4	1363.6*	1.0	15.2*
Lipid metabolism			
ApoA1	3.1*	1.0	2.0*
ApoA2	0.1*	1.0	0.7
ApoA5	2.6*	1.0	<0.1*
ApoB100	2.2*	1.0	1.7*
ApoC2	7.4*	1.0	0.4*
ApoC3	81.1*	1.0	7.3*
ApoE	2.6*	1.0	<0.1*
MTTP	4.9*	1.0	2.3*
SREBP1c	18.1*	1.0	17.9*
FAS	2.5*	1.0	2.4*
SCD	1.8*	1.0	1.5
ACSL1	45.7*	1.0	3.0*
ACC	1.5	1.0	1.1
MGAT1	16.7*	1.0	1.1
MGAT2	3.6*	1.0	0.1*
MGAT3	1.0	1.0	0.8
DGAT1	3.1*	1.0	3.3*
DGAT2	6.0*	1.0	3.6*
GPAM	13.4*	1.0	1.2
Lipin1	2.2*	1.0	1.1
SREBP2	1.6*	1.0	0.9
HMGCoAR	0.8*	1.0	1.1

Data are expressed as the mean ± standard deviation ($n = 4$,

* $P < 0.05$ vs HepG2 cells).

and HuH-7 cell lines (Table 2). PAS staining showed the marked accumulation of glycogen, an important function in livers, in PXB-cells, and extracellular albumin levels were 11.1-fold higher in PXB-cells than in HepG2 cells ($P < 0.01$) and 5.0-fold higher than in HuH-7 cells ($P < 0.01$) (Fig. 2). These results suggested that PXB-cells maintained mature hepatocyte properties more stably than the 2 hepatoma cell lines.

Lipid-producing ability of PXB-cells

We previously developed an *in vitro* assay system to assess lipoprotein profiles from the human hepatoma cell line, HepG2 and used the assay system to screen anti-dyslipidemia activities in drugs and foodstuffs. We examined whether PXB-cells are more suitable for assessing lipoprotein profiles than hepatoma cells. Intra- and extracellular cholesterol and tri-

Fig. 2 Hepatic properties of PXB-cells and hepatoma cell lines. **A:** PAS staining of PXB-cells (a), HepG2 cells (b), and HuH-7 (c) cells. Note that the marked accumulation of glycogen is observed in PXB-cells than in hepatoma cell lines. **B:** Extracellular albumin contents of PXB-cells (open bar), HepG2 cells (hatched bar), and HuH-7 cells (closed bar).

glyceride levels in the 3 hepatic cell lines were summarized in Table 3, and Fig. 3 shows the lipoprotein profiles in culture media. Intra- and extracellular cholesterol levels in PXB-cells were similar to those in HuH-7 cells and higher than those in HepG2 cells. When the gene expression levels of sterol regulatory element-binding protein2 and HMG CoA reductase — a transcriptional factor and key enzyme in cholesterologenesis — were examined, they in PXB-cells and HuH-7 cells were similar to those in HepG2 cells. The intracellular triglyceride content of PXB-cells was 5.8- and 1.2-fold higher than those of HepG2 and HuH-7 cells. The extracellular triglyceride content of PXB-cells was also higher, and, thus, we examined the expression levels of genes involved in triglyceride synthesis to clarify why PXB-cells possess a high capacity to produce triglycerides. The gene expression levels of fatty acid

synthase and stearoyl-CoA desaturase, which are enzymes involved in the fatty acid synthesis pathway, were higher in PXB-cells than in HepG2 cells, but similar to those in HuH-7 cells (Table 2). The gene expression levels of ACSL1, MGAT1, and GPAM in PXB-cells were markedly different from those in the other 2 cell lines (>10-fold vs both cells). The results of a Western blotting analysis showed that PXB-cells more strongly expressed these proteins, particularly ACSL1, than the 2 other hepatoma cells (Fig. 4). These results suggested that the transacylation step, but not fatty acid synthesis, is key for the higher triglyceride synthesis in PXB-cells.

Major lipoprotein classes from PXB-cells

We previously reported that human primary hepatocytes mainly secreted VLDL-sized lipoproteins, and hepatoma cell lines mainly produced LDL-sized li-

Table 3 Intra- and extracellular lipid contents of PXB-cells and 2 hepatoma cell lines

	PXB-cells	HepG2 cells	HuH-7 cells
Cell number ($\times 10^6$ cells)	0.3	0.9	0.5
Cholesterol ($\mu\text{g}/10^6$ cells)			
intracellular	41.5 ± 0.5	12.3 ± 0.1	41.0 ± 0.9
extracellular	5.0 ± 0.1	0.5	4.9 ± 0.1
VLDL fraction (> 30 nm)	4.1 ± 0.1	0.1	1.6
LDL fraction (16–30 nm)	0.5 ± 0.1	0.2	2.2
HDL fraction (8–16 nm)	0.3	0.3	1.1 ± 0.1
Triglycerides ($\mu\text{g}/10^6$ cells)			
intracellular	163.1 ± 4.6	28.2 ± 2.4	137.2 ± 3.6
extracellular	53.8 ± 1.0	1.2	12.1 ± 0.2
VLDL fraction (> 30 nm)	50.2 ± 1.0	0.1	5.3 ± 0.2
LDL fraction (16–30 nm)	3.1	0.5	6.3 ± 0.2
HDL fraction (8–16 nm)	0.5	0.6 ± 0.1	0.4

Hepatic cells at a density of 4×10^5 cells/ml (1 mL) were precultured in collagen-coated 24-well microplates for 20 h and then incubated in William's E medium with CM4000 for 48 h. Triglyceride and cholesterol levels in cells and culture media were measured.

Fig. 3 Lipoprotein profiles from 3 hepatic cell lines. Three hepatic cell lines seeded on collagen-coated 24-well microplates were cultured in William's E medium supplemented with CM-4000 for 48 h, and the levels of triglycerides and cholesterol in culture media were measured. Data are representative of 4 independent experiments.

Fig. 4 Expression of enzymes involved in the transacylation of PXB-cells. Proteins (10 $\mu\text{g}/\text{lane}$) in 3 hepatic cell lysates were separated by SDS-PAGE, followed by Western blotting with anti-ACSL1, MGAT1, and GPAM antibodies.

poproteins (18). PXB-cells mainly released cholesterol and triglycerides in the VLDL fraction (Table 2 and Fig. 3) because these cells are primary hepatocytes. To clarify whether the major lipoproteins released from PXB-cells and the 2 hepatoma cell lines were VLDL and LDL, respectively, we measured the gene expression levels of Apo in the 3 cell lines. Apo were classified into 5 major classes, ApoA to ApoE (8, 17). ApoB100, which is encoded by the ApoB gene, is synthesized in hepatic tissues and a

structural component of VLDL and LDL. ApoB100 gene expression levels were 2.2-fold and 1.7-fold higher in PXB-cells and HuH-7 cells, respectively, than in HepG2 cells, while its extracellular levels were 10.3- ($P < 0.01$) and 12.3-fold ($P < 0.01$) higher. The results of an ELISA analysis on ApoB100 in cultured media also supported the high VLDL/LDL contents of this lipoprotein in PXB-cells and HuH-7 cells. Three Apo — ApoC2, C3, and E — which were distributed in VLDL, but not LDL, were strongly expressed in PXB-cells and weakly in HepG2 and HuH-7 cells at the mRNA level (Table 3). We then measured extracellular ApoC3 levels in the 3 hepatic cell lines (Fig. 5). The results obtained showed that the extracellular ApoC3 contents of PXB-cells were 302.6-fold ($P < 0.01$) and 60.0-fold ($P < 0.01$) higher than those of HepG2 and HuH-7 cells. These

Fig. 5 ApoB100 and ApoC3 levels secreted from PXB-cells. Extracellular ApoB and ApoC contents of 3 hepatic cell lines were measured using the respective ELISA kits.

results suggested that PXB-cells and HuH-7 cells release triglyceride-rich lipoproteins; however, PXB-cells produce both VLDL and LDL, similar to liver tissues, whereas the 2 other hepatoma cell lines synthesized LDL, but not VLDL.

Evaluation of anti-lipidemic agents by lipoprotein profiles of PXB-cells

The present study demonstrated that PXB-cells produce high levels of lipoproteins, similar to liver tissues; however, the responses of PXB-cells to anti-lipidemic agents remain unclear. Peroxisome proliferator-activated receptor alpha (PPAR α) ligands, such as fenofibrate, have been shown to reduce plasma triglyceride levels by stimulating β -oxidation in the liver (2, 4). We examined the effects of fenofibrate on the lipoprotein profile of PXB-cells in order to clarify whether these cells are useful for screening anti-lipidemic activities in drugs. Table 4 and Fig. 6 show that fenofibrate reduced triglycerides and cholesterol levels in culture media in a dose-dependent manner. A real-time RT-PCR analysis demonstrated that fenofibrate at 50 μ M did not affect the expression levels of genes involved in fatty acid synthesis and transacylation steps, such as FAS and diacylglycerol acyltransferases; however, it markedly increased those of peroxisomal acyl-coenzyme A oxidase 1, carnitine acylcarnitine translocase, and medium-chain acyl-CoA dehydrogenase, which are PPAR α -regulated enzymes in β -oxidation, without affecting PPAR α gene expression levels (Table 5). Furthermore, fenofibrate markedly increased the gene expression levels of ApoA2 and ApoA5, but not those of ApoB, ApoC2, or ApoC3. These results demon-

Table 4 Effects of fenofibrate on intra- and extracellular lipid contents of PXB-cells

	Fenofibrate (μ M)			
	untreated	10	50	100
Cell number ($\times 10^6$ cells)	0.3	0.3	0.3	0.3
Cholesterol (μ g/ 10^6 cells)				
intracellular	26.0 $\pm$ 4.5	25.5 $\pm$ 1.9	21.5 $\pm$ 4.2	25.8 $\pm$ 1.2
extracellular	5.8 $\pm$ 0.3	5.4 $\pm$ 0.3	4.5 $\pm$ 0.3 ^a	3.9 $\pm$ 0.2 ^a
VLDL fraction	4.7 $\pm$ 0.3	4.3 $\pm$ 0.3	3.4 $\pm$ 0.2 ^a	2.6 $\pm$ 0.2 ^a
LDL fraction	0.8	0.7	0.7	0.8
HDL fraction	0.4 $\pm$ 0.1	0.4	0.4	0.5 $\pm$ 0.1
Triglycerides (μ g/ 10^6 cells)				
intracellular	175.7 $\pm$ 26.7	182.4 $\pm$ 23.5	185.0 $\pm$ 26.9	189.9 $\pm$ 29.2
extracellular	68.4 $\pm$ 2.9	61.0 $\pm$ 4.1	41.5 $\pm$ 2.8 ^a	27.7 $\pm$ 2.3 ^a
VLDL fraction	62.7 $\pm$ 2.8	55.4 $\pm$ 3.8 ^a	36.9 $\pm$ 2.7 ^a	23.6 $\pm$ 2.0 ^a
LDL fraction	5.1 $\pm$ 0.2	5.2 $\pm$ 0.3	4.3 $\pm$ 0.1 ^a	3.7 $\pm$ 0.2 ^a
HDL fraction	0.6 $\pm$ 0.1	0.5 $\pm$ 0.1	0.4	0.4

Data are expressed as the mean $\pm$ standard deviation ($n = 4$, ^a $P < 0.05$ vs untreated group).

Fig. 6 Effects of fenofibrate on the lipoprotein profile of PXB-cells. Following the treatment of PXB-cells with fenofibrate at concentrations of 10–100 μM for 48 h, a lipoprotein assay was performed by LipoSEARCH®.

strated that PXB-cells responded to fenofibrate in a known manner (16).

Angptls are a family of proteins that are structurally similar to angiopoietins, and its members are involved in angiogenesis, similar to angiopoietins (15). Since Angptl3, 4, and 8 from the liver are responsible for regulating the activities of plasma lipoprotein lipase/hepatic triglyceride lipase, and a deficiency (overexpression) in any one of these causes dyslipidemia, their roles in lipid metabolism have attracted a great deal of attention (1, 12, 19). The transcriptional regulation of these members is well known (3, 27), and Angptl4 (fasting-induced adipose factor, Fiaf) in mouse hepatocytes and human hepatoma cells is up-regulated by PPAR α ligands (9); therefore, we herein examined the levels of the 3 Angptl members in fenofibrate-treated PXB-cells (Fig. 7). Fenofibrate at 50 μM induced Angptl4, as reported previously, at both the mRNA and extracellular protein levels. This agent had a negligible effect on Angptl3 and Angptl8 gene expression levels, and increased extracellular Angptl3 levels by 1.7-fold from those in untreated cells. It currently remains unclear whether fenofibrate up-regulates Angptl3 protein production and/or its secretion from PXB-cells. Further studies are needed to reveal including the involvement of Angptl members in post-secretory modifications to lipoprotein.

In the present study, we revealed some of the

Table 5 Effects of fenofibrate on the expression of genes involved in lipid metabolism in PXB-cells

Gene name	Relative intensity/GAPDH (fold)	
	untreated	fenofibrate-treated
<i>β-Oxidation</i>		
PPAR α	1.0	1.1 $\pm$ 0.1
ACOX1	1.0 $\pm$ 0.2	3.6 $\pm$ 0.3*
CACT	1.0 $\pm$ 0.1	2.0 $\pm$ 0.1*
MCAD	1.0 $\pm$ 0.1	1.7 $\pm$ 0.2*
<i>Apolipoprotein</i>		
ApoA1	1.0 $\pm$ 0.1	1.0 $\pm$ 0.1
ApoA2	1.0 $\pm$ 0.1	1.3 $\pm$ 0.1*
ApoA5	1.0 $\pm$ 0.1	1.7 $\pm$ 0.2*
ApoB100	1.0 $\pm$ 0.1	0.9 $\pm$ 0.1
ApoC2	1.0 $\pm$ 0.2	0.8 $\pm$ 0.2
ApoC3	1.0 $\pm$ 0.1	0.9 $\pm$ 0.2
ApoE	1.0 $\pm$ 0.2	0.8 $\pm$ 0.2
<i>Lipid synthesis</i>		
PPAR γ	1.0 $\pm$ 0.2	1.0 $\pm$ 0.1
SREBP1c	1.0 $\pm$ 0.1	1.0 $\pm$ 0.1
FAS	1.0 $\pm$ 0.1	1.1 $\pm$ 0.3
SCD	1.0 $\pm$ 0.1	0.9 $\pm$ 0.1
MGAT2	1.0 $\pm$ 0.1	1.1 $\pm$ 0.1
DGAT1	1.0 $\pm$ 0.1	1.2 $\pm$ 0.2
DGAT2	1.0	1.0
GPAM	1.0 $\pm$ 0.1	1.1 $\pm$ 0.1
SREBP2	1.0	1.3 $\pm$ 0.1*
HMGCoAR	1.0	1.1 $\pm$ 0.1

PXB-cells were treated with or without 50 μM fenofibrate for 48 h, and RNA expression levels were analyzed. Data are expressed relative to untreated control cells and represent the mean $\pm$ SD ($n = 4$, * $P < 0.05$ vs the untreated group).

Fig. 7 Effects of fenofibrate on the expression of Angptls. PXB-cells were treated with (hatched bar) or without (open bar) fenofibrate for 48 h, and the gene and protein expression levels of Angptls were analyzed by RT-PCR and ELISA. The basal levels of extracellular Angptl3, 4, and 8 released from untreated cells were 107.4 ± 3.4 , 3.4 ± 0.1 , and 3.0 ± 0.4 ng/ 10^6 cells, respectively.

properties of lipid metabolism in PXB-cells using comparisons with well-differentiated hepatoma cells, and demonstrated that PXB-cells are suitable for lipid metabolism research, such as screening novel antilipidemic agents.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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