J Toxicol Pathol 2025; 38: 183–189

Technical Report

Application of humanized mice to toxicology studies: properties of chimeric mice with humanized liver (PXB-mice) for hepatotoxicity

Nazneen Sultana[1], Takeshi Izawa[1]*, Tomomi Kamei[1], Sho Fujiwara[1], Yuri Ito[1], Yuki Takami[1], and Mitsuru Kuwamura[1]

[1]Laboratory of Veterinary Pathology, Osaka Metropolitan University, 1-58 Rinku-Orai-Kita, Izumisano, Osaka 598-8531, Japan

Abstract: Chimeric mice with humanized liver are considered a useful tool to predict drug pharmacokinetics and *in vivo* toxicity in humans. The PXB-mouse is one of such chimeric (humanized) mouse models with more than 70% of human hepatocytes in their liver, which can produce human albumin with human-type bile secretion and express human xenobiotic metabolizing enzymes. However, data are limited regarding the properties of such humanized mice in hepatotoxicity studies. This study aimed to explore the distinctive characteristics of chimeric PXB-mice with humanized liver that can influence susceptibility to hepatotoxicity. Morphologically, the PXB-mice have a diffuse hepatic macrovesicular and microvesicular steatosis in the transplanted human hepatocytes, which can be suppressed after human growth hormone treatment. The humanized liver of the PXB-mice has a metabolic zonation of glutamine synthetase, cytochrome P450 2E1, and argininosuccinate synthase 1, similar to normal liver in rodents and humans. The transplanted human hepatocytes in the PXB liver have a markedly decreased N-cadherin expression compared with normal human liver. Scanning electron microscopy revealed formation of septum-like structures encircling the transplanted human hepatocytes in the PXB liver, which consists of an accumulation of fibers in the space of Disse under transmission electron microscopy and is immunolabeled for laminin. Overall, the present report demonstrated the morphological and immunohistochemical characteristics of the PXB-mice with humanized liver along with some abnormalities in the cell adhesion of the transplanted human hepatocytes. These findings would be useful for hepatotoxicity studies using humanized animal models. (DOI: 10.1293/tox.2024-0092; J Toxicol Pathol 2025; 38: 183–189)

Key words: cell adhesion, humanized mouse, liver, morphology, metabolic enzyme

Drug-induced liver injury (DILI) represents a major health issue worldwide that results in substantial morbidity and mortality in humans[1, 2]. Due to the widespread use of prescribed and over-the-counter drugs, along with the rapid development of novel drug research, different types of clinical drugs which can be potentially cause DILI are increasing. As a result, the development of DILI has been concerning over the years[3]. Although the incidence of DILI is low amongst the general population, it can cause decreased liver functions. In the worst-case scenario, acute liver injury can lead to severe liver damage with a case fatality rate of up to 50%[4]. In that case, it might require liver transplant and can even lead to death[1]. While progress has been made in understanding the diagnosis, prevention, and treatment of viral, autoimmune, and hereditary liver diseases, such understanding of DILI has not been advanced as much. The diagnosis of DILI is difficult since it is based largely on the elimination of other possible etiologies. To date, there are no specific diagnostic markers for DILI. Based on one published case report, 447 (46%) have been linked in causing liver injury out of 971 prescription drugs described[5]. DILI is also one of the leading causes of drug development attritions, post market withdrawal, and boxed-warnings[6]. A deeper understanding of the pathogenesis of DILI would facilitate effective control strategies.

The mechanism of DILI involves multifactorial processes, and elucidating the complete mechanism of DILI is still extremely challenging[7]. Precisely predicting DILI in humans from preclinical safety test data remains difficult because of considerable species differences between animals and humans. Rodents, particularly mice and rats, are commonly used for testing the safety of drugs and chemical compounds. However, drug metabolism varies between rodents and humans[8, 9], such that although some drugs may not harm rodents, they can be toxic to humans[10]. Reportedly, regulatory toxicology studies conducted in rodent models failed to predict human DILI in approximately 45% of cases[11]. Thus, more sensitive and reliable preclinical models are urgently required to predict the development of DILI.

To date, a few chimeric mice with humanized livers have been developed for new drug development, drug metabolism and pharmacokinetics, and *in vivo* toxicology stud-

Received: 18 November 2024, Accepted: 23 January 2025
Published online in J-STAGE: 12 February 2025
*Corresponding author: T Izawa
(e-mail: takeshi.izawa@omu.ac.jp)

ies[12, 13]. The PXB (cDNA-uPA/SCID)-mouse is one of such chimeric (humanized) mouse models where more than 70% of endogenous murine hepatocytes are replaced by transplanted human hepatocytes[13]. PXB-mice produce human albumin with human-type bile secretion and express xenobiotic metabolizing enzymes and transporters that enable a high predictability for human drug pharmacokinetics[14]. Several studies have reported the utility of PXB-mice to detect human-specific toxicological responses in the liver[15–17], however, data are still limited regarding the properties of PXB-mice in hepatotoxicity studies. Therefore, the present study aimed to explore the distinctive characteristics of PXB-mice that can influence susceptibility to hepatotoxicity. All experimental procedures were approved by the Institutional Animal Care and Use Committee (code nos. 21-161, 22-143, 23-063, and 24-063) and was performed according to the Guidelines for Animal Experimentation at Osaka Metropolitan University. The PXB-mice were fed a diet containing vitamin C (300 mg/100 g diet; Oriental Yeast Co., Ltd., Tokyo, Japan) *ad libitum*, with no fasting performed before necropsy. Mice were euthanized under deep isoflurane anesthesia.

Chimeric PXB-mice at the age of 18–21 weeks, which had received transplantation of human hepatocytes at the age of 3 weeks, were investigated in this study, using formalin-fixed paraffin-embedded sections and primary antibodies as listed in Table 1. The results were compared with those of normal liver in humans, mice (C57BL/6, 6–10 weeks old), and rats (F344, 6–10 weeks old). Immunohistochemical images of normal human liver were obtained from Human Protein Atlas (http://www.proteinatlas.org/)[18]. Macroscopically, the humanized mouse liver was diffusely pale in color (Fig. 1A). Histopathologically, the human hepatocytes had small and round to ovoid nuclei (Fig. 1B, 1C), while in the residual murine hepatocytes, the nuclei were larger and the cytoplasm was more eosinophilic with more variations in their size (Fig. 1D, indicated by arrows). The human hepatocytes were characterized by diffuse macrovesicular and microvesicular steatosis (Fig. 1B, 1C). Cytoplasmic vacuolation was absent or minimal in the residual mouse hepatocytes (Fig. 1D, indicated by arrows). The hepatic steatosis of the humanized liver can be suppressed by the administration of recombinant human growth hormone (hGH; Fujifilm Wako, Osaka, Japan) at 2.5 mg/kg/day via osmotic pump (Model 1002, Alzet Corporation, PaloAlto, CA, USA) for 14 days (Fig. 1E, 1F), as reported elsewhere[19]. The influence of GH on hepatotoxicity should be considered in the preclinical study using humanized mice with GH administration, since GH is reported to reduce liver injury in metabolic dysfunction-associated steatotic liver disease[20]. The hepatic steatosis can also be suppressed by treatment with hGH analogue (Sogroya; Bagsvaerd, Denmark) at 1 mg/kg/day via osmotic pump (Fig. 1G) or its repeated intraperitoneal injection at 5 mg/kg 3 times per week for 14 days (Fig. 1H). Immunohistochemistry for adipophilin, a protein specifically localized to the lipid droplet surfaces[21], can delineate lipid vacuoles of the human hepatocytes in the humanized liver using paraffin sections (Fig. 1I). The clone AP125 antibody

Table 1. Antibodies Used for Immunohistochemistry on the Humanized Liver

Antibody	Company	Catalogue #	Pretreatment	Dilution	Reactivity in FFPE sections
Adipophilin/perilipin-2	Progen, Heidelberg, Germany	690102S	Autoclaving in 10 mM citrate buffer (pH6.0)	1:200	Human (+) Mouse (–) Rat (+)
Glutamine synthetase (GS)	Merck, Darmstadt, Germany	MAB302	Autoclaving in 10 mM citrate buffer (pH6.0)	1:200	Human (+) Mouse (+) Rat (+)
Cytochrome P450 2E1 (CYP2E1)	Merck	HPA009128	Autoclaving in 10 mM Tris-1 mM EDTA (pH9.0)	1:200	Human (+) Mouse (+) Rat (+)
argininosuccinate synthase 1 (ASS1)	Abcam, Cambridge, UK	ab170952	Autoclaving in 10 mM citrate buffer (pH6.0)	1:1,000	Human (+) Mouse (+) Rat (+)
E-cadherin	BD Biosciences, Franklin Lakes, NJ, USA	610181	Autoclaving in 10 mM Tris-1 mM EDTA (pH9.0)	1:500	Human (+) Mouse (+) Rat (+)
N-cadherin	Thermo Fisher Scientific, Waltham, MA, USA	33-3900	Autoclaving in10 mM Tris-1 mM EDTA (pH9.0)	1:500	Human (+) Mouse (+) Rat (+)
β-catenin	Thermo Fisher Scientific	13-8400	Autoclaving in 10 mM Tris-1 mM EDTA (pH9.0)	1:500	Human (+) Mouse (+) Rat (+)
Laminin	Cosmo Bio, Tokyo, Japan	LB-1013	100 μg/mL proteinase K, 30 °C, 10 min	1:1,000	Human (+) Mouse (+) Rat (+)

FFPE: formalin-fixed paraffin-embedded; EDTA: Ethylenediaminetetraacetic acid.

is useful to evaluate the degree of steatosis suppression after hGH treatment, since it does not react with murine hepatocytes (Fig. 1J). Periodic acid-Schiff (PAS) reaction revealed diffuse accumulation of glycogen in the human hepatocytes in hGH-treated groups, which was more prominent than in the residual murine hepatocytes (Fig. 1K). The glycogen accumulation did not change after hGH treatment (Fig. 1L).

Immunohistochemistry for glutamine synthetase (GS; specifically expressed by zone-3 hepatocytes surrounding the central vein), cytochrome P450 2E1 (CYP2E1; expressed by zone 3 to 2 hepatocytes), and argininosuccinate synthase 1 (ASS1; expressed by zone 1 to 2 hepatocytes)[22] was performed in order to investigate the "metabolic zonation" (zone specificity of the metabolic enzymes) in the humanized liver of PXB-mice. The distribution of these enzymes was similar between the humanized liver of PXB-mice (Fig. 2B, 2F, 2J) and normal liver of humans (Fig. 2A, 2E, 2I), mice (Fig. 2C, 2G, 2K), and rats (Fig. 2D, 2H, 2L), suggesting that metabolic zonation is formed in the humanized liver. The reproductivity of the metabolic zonation in the humanized liver may be beneficial for *in vivo* preclinical models to predict human hepatotoxicity.

Immunohistochemistry for E-cadherin, N-cadherin, β-catenin, and laminin was performed in order to characterize cell adhesion properties of PXB-mice. Immunolabeling for E-cadherin was diffuse and strong in the cell membrane of the human hepatocytes in PXB-mice (Fig. 3B), similar to normal human liver (Fig. 3A). The membranous E-cadherin immunolabeling was stronger in the zone 1 than in the zone 3 in mice (Fig. 3C) and to a lesser extent in rats (Fig. 3D). Membranous immunolabeling for N-cadherin was very faint in the human hepatocytes of PXB-mice (Fig. 3F), whereas it was diffuse and strong in normal human hepatocytes (Fig. 3E). In the PXB-mice, membranous N-cadherin immunolabeling was present in the residual murine hepatocytes (Fig. 3F, asterisk). In contrast to E-cadherin, the membranous immunolabeling for N-cadherin was stronger in the zone 3 than in the zone 1 in mice (Fig. 3G) and to a lesser extent in rats (Fig. 3H), indicative of "cadherin zonation" in the rodent liver but not in humans. Membranous immu-

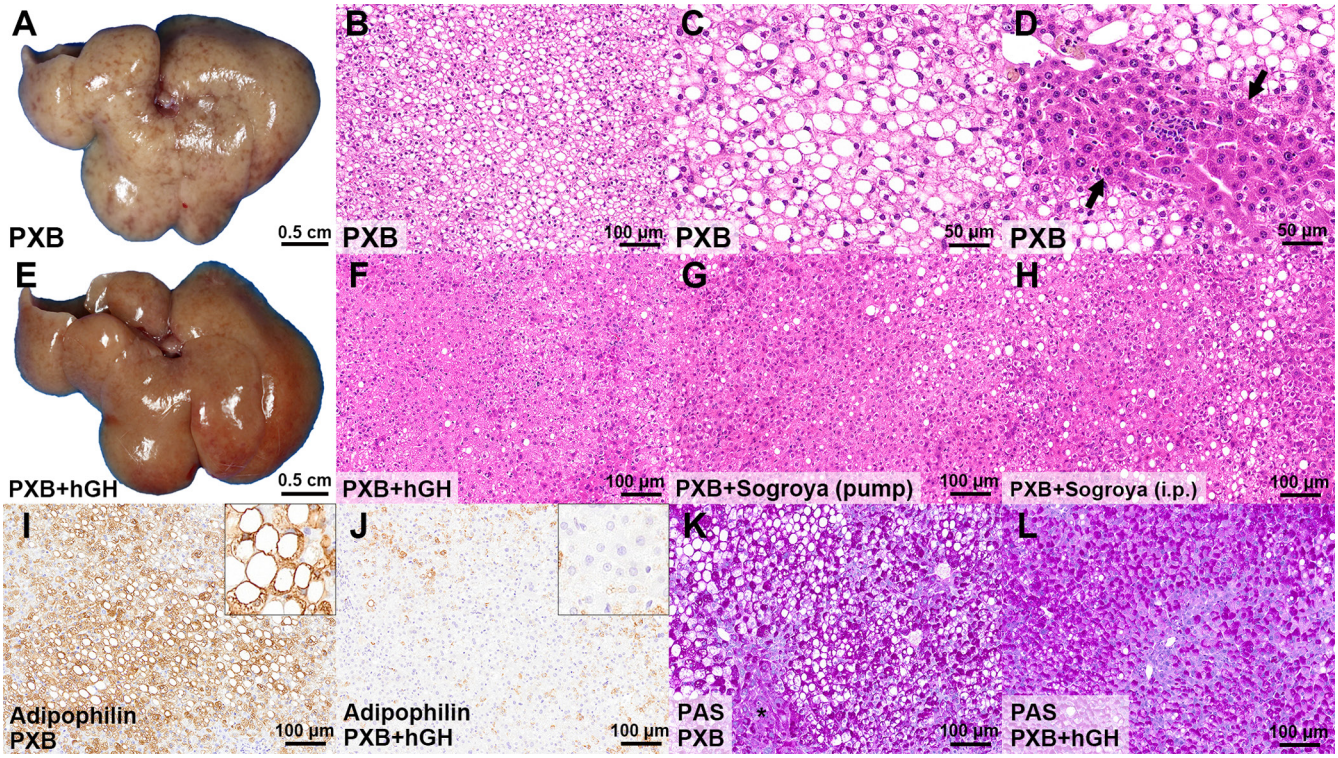

Fig. 1. Pathological phenotypes of chimeric mice with humanized liver (PXB-mice). (A) Grossly, the humanized liver is diffusely pale, suggestive of fatty liver. (B–D) Histopathology of the humanized liver. The liver is characterized by a diffuse macrovesicular and microvesicular steatosis in the transplanted human hepatocytes. The human hepatocytes have small and round to ovoid nuclei while the residual murine hepatocytes, indicated by arrows in D, have large nuclei and eosinophilic cytoplasm with more variations in their size. (E, F) The hepatic steatosis of the humanized liver is suppressed by treatment with human growth hormone (hGH) using an osmotic pump for 14 days. (G, H) The hepatic steatosis is also suppressed by treatment with hGH analogue (Sogroya) via osmotic pump (G) or repeated intraperitoneal injection (H) for 14 days. (I, J) Adipophilin immunohistochemistry using paraffin sections visualizes macrovesicular and microvesicular vacuoles in the humanized liver without hGH treatment (I) and reveals suppression of the lipid vacuoles after hGH treatment via osmotic pump (J). (K, L) Periodic acid-Schiff reaction reveals a diffuse glycogen accumulation in the human hepatocytes without hGH treatment (K), which is more prominent than in the murine hepatocytes (asterisk). The degree of the glycogen accumulation does not change after hGH treatment (L).

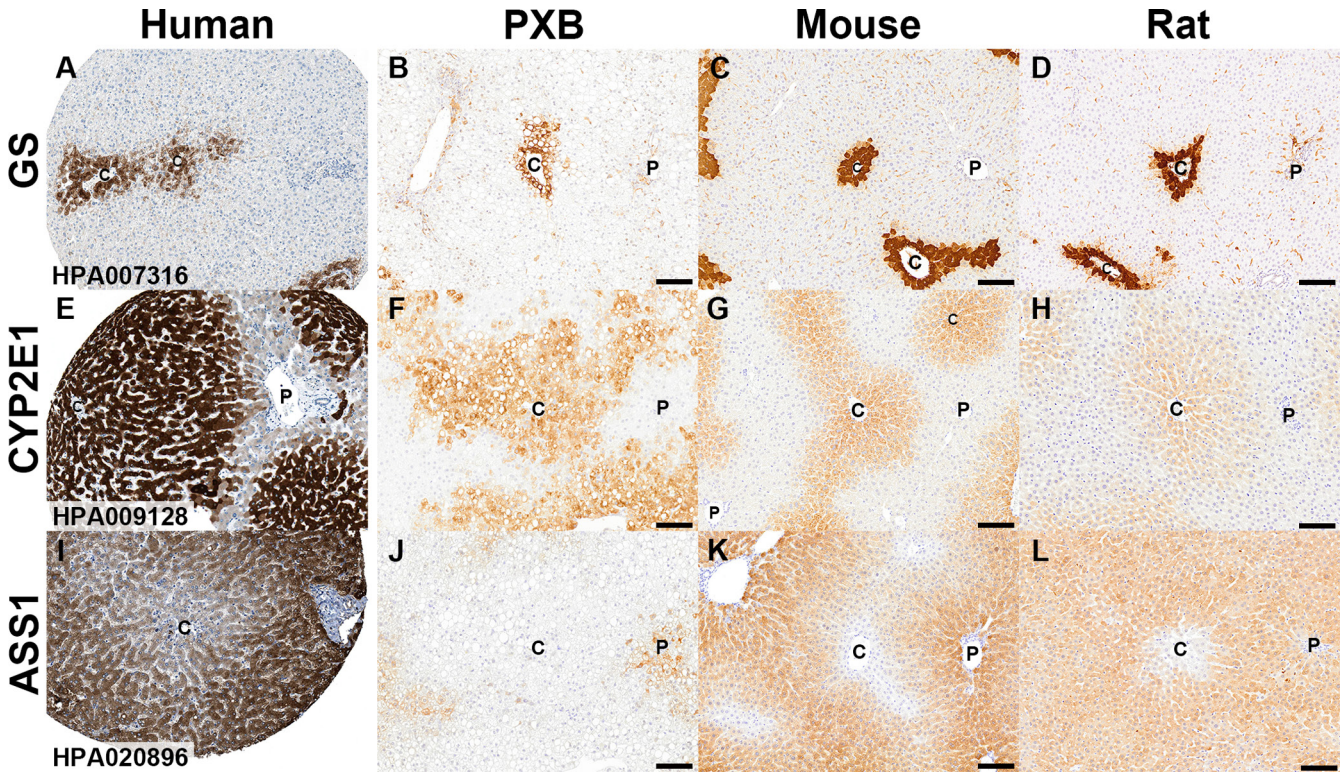

Fig. 2. Distribution of xenobiotic metabolizing enzymes in the humanized liver. Representative images of immunohistochemistry for glutamine synthetase (GS; A–D), cytochrome P450 2E1 (CYP2E1; E–H), and argininosuccinate synthase 1 (ASS1; I–L) in the humanized PXB-mice (B, F, J), humans (A, E, I), normal mice (C, G, K), and normal rats (D, H, L). The humanized liver has a similar distribution of GS and CYP2E1 to normal liver of humans and rodents: GS and CYP2E1 are expressed by zone 3 and zone 3 to 2 hepatocytes, respectively. ASS1 is weakly expressed by zone 1 hepatocytes in PXB-mice while it is expressed broadly by zone 1 to 2 hepatocytes of normal humans and rodents. Images of immunohistochemistry for GS (https://www.proteinatlas.org/ENSG00000135821-GLUL/tissue/liver; antibody HPA007316), CYP2E1 (https://www.proteinatlas.org/ENSG00000130649-CYP2E1/tissue/liver; antibody HPA009128), and ASS1 (https://www.proteinatlas.org/ENSG00000130707-ASS1/tissue/liver; antibody HPA020896) in the human liver were obtained from Human Protein Atlas. C: central vein; P: portal vein. Bars=100 μm.

nolabeling for β-catenin was diffusely seen in hepatocytes of PXB-mice, humans, mice, and rats (Fig. 3I–3L). These results suggest an altered balance of cadherin expression in the humanized liver of PXB-mice. In addition, as shown in the E-cadherin and β-catenin immunohistochemistry, the contour of the human hepatocytes was irregular, corrugated, and jagged in the PXB-mice while the contour was smooth and polygonal in normal hepatocytes of humans, mice, and rats (Fig. 3, insets).

Immunolabeling for laminin was irregularly and strongly seen along the sinusoids adjacent to the human hepatocytes (Fig. 3N), while it was almost absent in the sinusoids of the murine hepatocyte region in the PXB- mice and of normal liver in humans, mice, and rats (Fig. 3M, 3O, 3P). Electron microscopy analysis was performed in order to further characterize the morphology of the humanized liver in the PXB-mice. Scanning electron microscopy revealed formation of septum-like structures encircling the transplanted human hepatocytes in the PXB- mice (Fig. 4B, 4C), which was not observed in the normal mouse liver (Fig. 4A). Transmission electron microscopy revealed a widening of the space of Disse with an accumulation of fibers in the PXB-mice (Fig. 4D). These results suggest that the humanized liver has an abnormal formation of septum-like structures, containing laminins, around the transplanted human hepatocytes. To the best of our knowledge, this is the first report showing alterations in the expression of cell adhesion molecules and the structure of sinusoidal wall in the humanized liver[12, 13]. Since the sinusoidal wall is located between hepatocytes and the circulating blood, its structural change can influence hepatocyte responses to xenobiotics. Further investigation is needed in order to elucidate the significance of such alterations in the evaluation of hepatotoxicity study.

The present study reported the morphological and immunohistochemical characteristics of the PXB-mice with humanized liver, and some abnormalities in the cell adhesion of the transplanted human hepatocytes. These findings would be useful for hepatotoxicity studies using humanized animal models.

Disclosure of Potential Conflicts of Interest: The authors declare that they have no potential conflicts of interest.

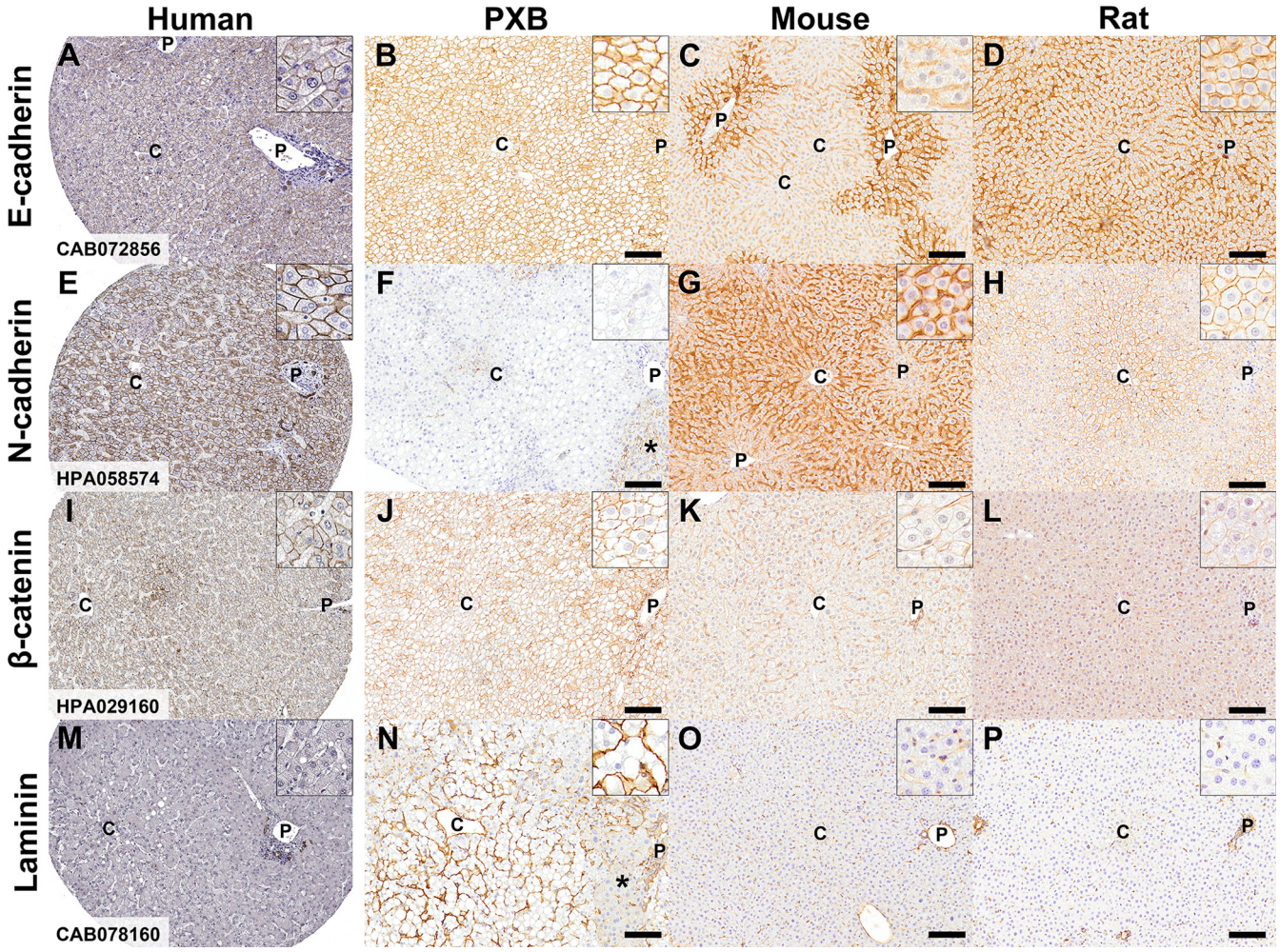

Fig. 3. Distribution of cell adhesion molecules in the humanized liver. Representative images of immunohistochemistry for E-cadherin (A–D), N-cadherin (E–H), β-catenin (I–L), and laminin (M–P) in humanized PXB-mice (B, F, J, N), humans (A, E, I, M), normal mice (C, G, K, O), and normal rats (D, H, L, P). Membranous expression for E-cadherin in hepatocytes is zone-1 specific in mice (C), diffuse but zone-1 associated in rats (D), and is diffuse in PXB-mice (B) and humans (A). Membranous N-cadherin expression in hepatocytes is zone-3 (to zone-2) specific in mice (G) and rats (H), diffuse in humans (E), and is rare and faint in PXB-mice (F). Membranous β-catenin expression in hepatocytes is diffuse without zone specificity in PXB-mice (J), humans (I), mice (K), and rats (L). Laminin expression is absent in the sinusoids of normal liver in humans (M), mice (O), and rats (P) while aberrant laminin expression is present in the sinusoids of PXB-mice (N). Images of immunohistochemistry for E-cadherin (https://www.proteinatlas.org/ENSG00000039068-CDH1/tissue/liver; antibody CAB072856), N-cadherin (https://www.proteinatlas.org/ENSG00000170558-CDH2/tissue/liver; antibody HPA058574), β-catenin (https://www.proteinatlas.org/ENSG00000168036-CTNNB1/tissue/liver; antibody HPA029160), and laminin (https://www.proteinatlas.org/ENSG00000172037-LAMB2/tissue/liver; antibody CAB078160) in the human liver were obtained from Human Protein Atlas. C: central vein; P: portal vein; asterisk: residual murine hepatocytes. Asterisks indicate a region of residual murine hepatocytes. Bars=100 μm.

Acknowledgment: We would like to express our deepest gratitude to PhoenixBio Co., Ltd. (https://phoenixbio.co.jp) for providing animals and their advice on our studies. This work was partly supported by JSPS KAKENHI Grant Number 24K21921.

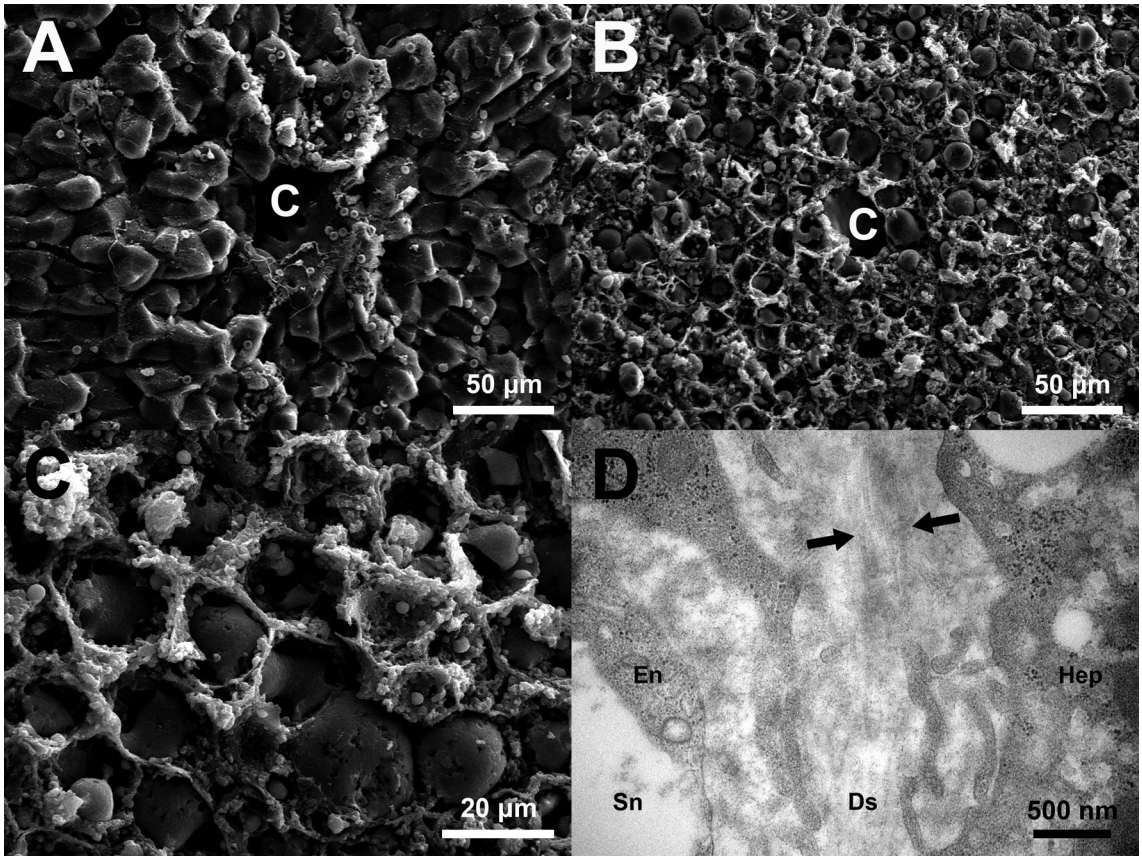

Fig. 4. Ultrastructure of the humanized liver. (A–C) Representative images of scanning electron microscopy. Regularly-arranged hepatic cords, radiated from the central vein, is observed in normal mice (A), while septum-like structures are formed around the transplanted human hepatocytes in the PXB-mice (B, C), which causes an indistinct hepatic cord structure. C, central vein. (D) Representative image of transmission electron microscopy of the humanized liver. The space of Disse is widened with an accumulation of fibers (arrows) in the PXB-mice. Ds: space of Disse; En: sinusoidal endothelium; Hep: hepatocyte; Sn: sinusoids.

References

1. Björnsson ES, and Björnsson HK. Mortality associated with drug-induced liver injury (DILI). Transl Gastroenterol Hepatol. **2**: 114. 2017. [Medline] [CrossRef]
2. Suk KT, and Kim DJ. Drug-induced liver injury: present and future. Clin Mol Hepatol. **18**: 249–257. 2012. [Medline] [CrossRef]
3. Regev A. Drug-induced liver injury and drug development: industry perspective. Semin Liver Dis. **34**: 227–239. 2014. [Medline] [CrossRef]
4. Hosack T, Damry D, and Biswas S. Drug-induced liver injury: a comprehensive review. Therap Adv Gastroenterol. **16**: 17562848231163410. 2023. [Medline] [CrossRef]
5. Hoofnagle JH, and Björnsson ES. Drug-induced liver injury—types and phenotypes. N Engl J Med. **381**: 264–273. 2019. [Medline] [CrossRef]
6. Chipangura JK, Ntamo Y, Mohr B, and Chellan N. A review of challenges and prospects of 3D cell-based culture models used for studying drug induced liver injury during early phases of drug development. Hum Exp Toxicol. **42**: 9603271221147884. 2023. [Medline] [CrossRef]
7. Kuna L, Bozic I, Kizivat T, Bojanic K, Mrso M, Kralj E, Smolic R, Wu GY, and Smolic M. Models of drug induced liver injury (DILI)—current issues and future perspectives. Curr Drug Metab. **19**: 830–838. 2018. [Medline] [CrossRef]
8. Bogaards JJ, Bertrand M, Jackson P, Oudshoorn MJ, Weaver RJ, van Bladeren PJ, and Walther B. Determining the best animal model for human cytochrome P450 activities: a comparison of mouse, rat, rabbit, dog, micropig, monkey and man. Xenobiotica. **30**: 1131–1152. 2000. [Medline] [CrossRef]
9. Takahashi Y, Soejima Y, and Fukusato T. Animal models of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. World J Gastroenterol. **18**: 2300–2308. 2012. [Medline] [CrossRef]
10. Atkins JT, George GC, Hess K, Marcelo-Lewis KL, Yuan Y, Borthakur G, Khozin S, LoRusso P, and Hong DS. Preclinical animal models are poor predictors of human toxicities in phase 1 oncology clinical trials. Br J Cancer. **123**: 1496–1501. 2020. [Medline] [CrossRef]
11. Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, Lilly P, Sanders J, Sipes G, Bracken W, Dorato M, Van Deun K, Smith P, Berger B, and Heller A. Concordance of the toxicity of pharmaceuticals in humans and in animals. Regul Toxicol Pharmacol. **32**: 56–67. 2000. [Medline] [CrossRef]

12. Luo Y, Lu H, Peng D, Ruan X, Eugene Chen Y, and Guo Y. Liver-humanized mice: a translational strategy to study metabolic disorders. J Cell Physiol. **237**: 489–506. 2022. [Medline] [CrossRef]
13. Tateno C, and Kojima Y. Characterization and applications of chimeric mice with humanized livers for preclinical drug development. Lab Anim Res. **36**: 2. 2020. [Medline] [CrossRef]
14. Miyamoto M, Iwasaki S, Chisaki I, Nakagawa S, Amano N, and Hirabayashi H. Comparison of predictability for human pharmacokinetics parameters among monkeys, rats, and chimeric mice with humanised liver. Xenobiotica. **47**: 1052–1063. 2017. [Medline] [CrossRef]
15. Foster JR, Jacobsen M, Kenna G, Schulz-Utermoehl T, Morikawa Y, Salmu J, and Wilson ID. Differential effect of troglitazone on the human bile acid transporters, MRP2 and BSEP, in the PXB hepatic chimeric mouse. Toxicol Pathol. **40**: 1106–1116. 2012. [Medline] [CrossRef]
16. Nihira K, Nan-Ya KI, Kakuni M, Ono Y, Yoshikawa Y, Ota T, Hiura M, and Yoshinari K. Chimeric mice with humanized livers demonstrate human-specific hepatotoxicity caused by a therapeutic antibody against TRAIL-receptor 2/death receptor 5. Toxicol Sci. **167**: 190–201. 2019. [Medline] [CrossRef]
17. Yamada T. Application of humanized mice to toxicology studies: evaluation of the human relevance of the mode of action for rodent liver tumor formation by activators of the constitutive androstane receptor (CAR). J Toxicol Pathol. **34**: 283–297. 2021. [Medline] [CrossRef]
18. Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson Å, Kampf C, Sjöstedt E, Asplund A, Olsson I, Edlund K, Lundberg E, Navani S, Szigyarto CA, Odeberg J, Djureinovic D, Takanen JO, Hober S, Alm T, Edqvist PH, Berling H, Tegel H, Mulder J, Rockberg J, Nilsson P, Schwenk JM, Hamsten M, von Feilitzen K, Forsberg M, Persson L, Johansson F, Zwahlen M, von Heijne G, Nielsen J, and Pontén F. Proteomics. Tissue-based map of the human proteome. Science. **347**: 1260419. 2015. [Medline] [CrossRef]
19. Tateno C, Kataoka M, Utoh R, Tachibana A, Itamoto T, Asahara T, Miya F, Tsunoda T, and Yoshizato K. Growth hormone-dependent pathogenesis of human hepatic steatosis in a novel mouse model bearing a human hepatocyte-repopulated liver. Endocrinology. **152**: 1479–1491. 2011. [Medline] [CrossRef]
20. Kineman RD, Del Rio-Moreno M, and Waxman DJ. Liver-specific actions of GH and IGF1 that protect against MASLD. Nat Rev Endocrinol. **21**: 105–117. 2025. [Medline] [CrossRef]
21. Walther TC, and Farese RV Jr. Lipid droplets and cellular lipid metabolism. Annu Rev Biochem. **81**: 687–714. 2012. [Medline] [CrossRef]
22. Ben-Moshe S, and Itzkovitz S. Spatial heterogeneity in the mammalian liver. Nat Rev Gastroenterol Hepatol. **16**: 395–410. 2019. [Medline] [CrossRef]