Development of humanized nonalcoholic steatohepatitis model using chimeric mice with highly repopulated humanized livers

Chise Tateno[1,2], Go Sugahara[1], Keishi Kisoh[1], Yuji Ishida[1,2], Yasumi Yoshizane[1], Suzue Furukawa[1] and Michinori Kohara[3]

[1]R&D Department, PhoenixBio Co., Ltd., [2]Research Center for Hepatology and Gastroenterology, Hiroshima University, [3]Department of Microbiology and Cell Biology, Tokyo Metropolitan Institute of Medical Science

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広島大学

公益財団法人 東京都医学総合研究所 Tokyo Metropolitan Institute of Medical Science

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Metabolism, alcohol and toxicity Chise Tateno

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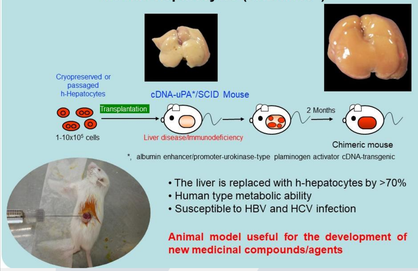
Introduction

Nonalcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease. Although a significant number of new drugs for NASH are already in the development pipeline, there are no animal models of human (h-) NASH to estimate the efficacy and safety of potential new drugs. We have been producing humanized chimeric mice (PXB-mice) with livers highly repopulated with h-hepatocytes (h-heps). These mouse (m-) models are used to study drug metabolism, pharmacokinetics, toxicity of new drugs, and efficacy of anti-hepatitis B and anti-hepatitis C therapeutic agents. In the present study, to develop the h-NASH model, we tried a choline-deficient, L-amino acid-defined, high-fat diet (CDAHFD) (A06071302; Research Diets, Inc.) in PXB-mice.

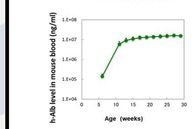
Stable mass production of chimeric mice with human hepatocytes (PXB-mice®)



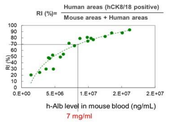
- The liver is replaced with h-hepatocytes by >70%
- Human type metabolic ability
- Susceptible to HBV and HCV infection

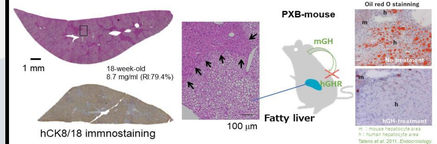
Animal model useful for the development of new medicinal compounds/agents

Human albumin (hAlb) in mouse blood



h-Alb level and replacement index (RI) of chimeric mice



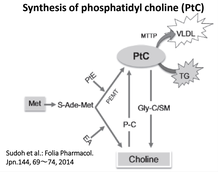


PXB-mouse liver is a fatty liver because of human growth hormone deficiency, but no inflammation or fibrosis are observed in the PXB-mouse liver.

NAFLD/NASH Diet

- Methionine/choline deficient diet（MCD）
- Choline deficient amino acid diet CDAA）
- Choline-deficient, L-amino acid-defined, high-fat die（CDAHFD）
- High fat, High cholesterol diet

Synthesis of phosphatidyl choline (PtC)

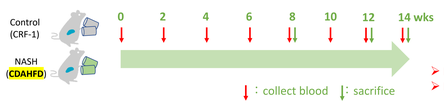


Sudoh et al.: Folia Pharmacol. Jpn.144, 69～74, 2014

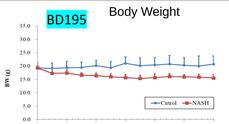
Materials & Methods and Results

Experimental design

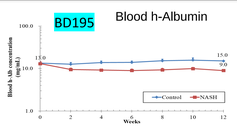
- PXB-mice (expected RI by hAlb levels >90%), male, 13-18-week-old
- Donor cells (BD195: 2-year-old Hispanic girl, JFC: 1-year-old, Caucasian boy)
- NASH group: Choline-deficient, L-amino acid-defined, high-fat diet (CDAHFD) (Research Diets, Inc)
- Control group: CRF-1 (CHARLES RIVER LABORATORIES JAPAN, INC.)
- Treatment period： 12 or 14 weeks



BD195 Body Weight



BD195 Blood h-Albumin



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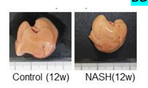
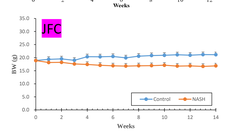
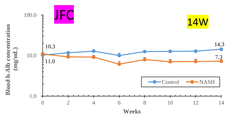


JFC



JFC 14W



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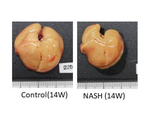
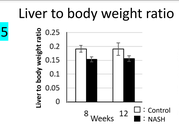
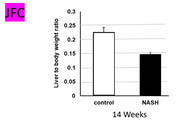
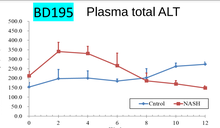


Liver to body weight ratio





BD195 Plasma total ALT



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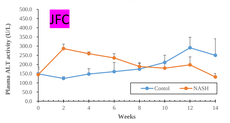
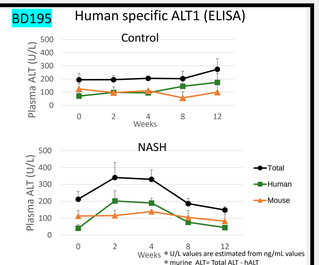


BD195 Human specific ALT1 (ELISA)

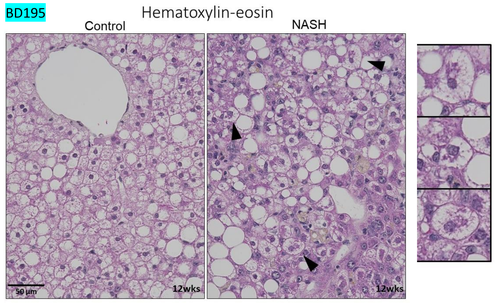


* U/L values are estimated from ng/mL values
* murine ALT= Total ALT - hALT

➢ Mean body weight was lower in CDAHFD group than that in the control group, maintaining about 80% of the initial level.
➢ Both liver weight to body weight and hAlb levels in m-blood in the CDAHFD group were lower compared to those in the control group at 8 and 12 (or 14) weeks.

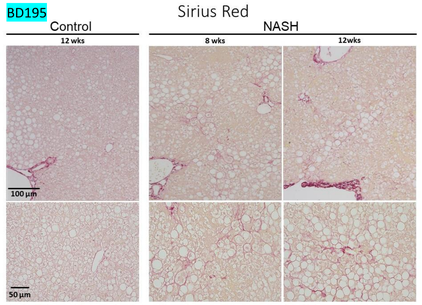
➢ Increase in ALT activity and hALT1 level were observed in m-plasma at 2 and 4 weeks, which were recovered to normal in the CDAHFD group.

BD195 Hematoxylin-eosin



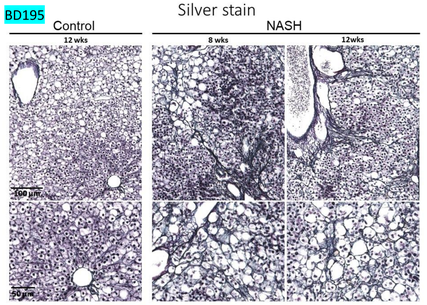
➢ Ballooning of h-heps and Mallory body-like structures in h-heps were observed only in the CDAHFD group at 12 weeks.

BD195 Sirius Red

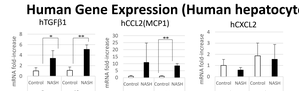


➢ Perisinusoidal/pericellular fibrosis were observed with Sirius red staining and silver staining with increase in Sirius red-positive areas in the CDAHFD group compared with the control group at 12 weeks.

BD195 Silver stain

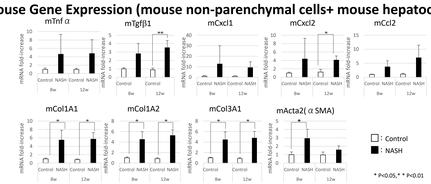


BD195 **Human Gene Expression (Human hepatocytes)**



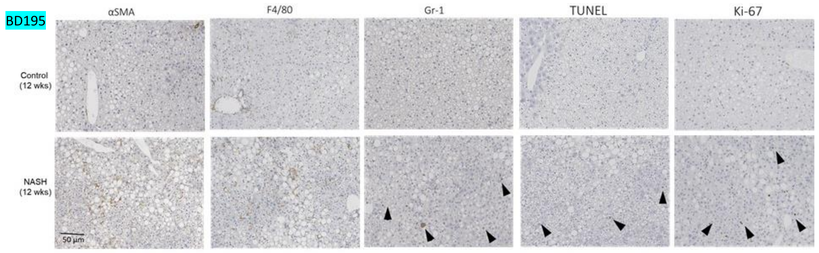
Under detection limit
hCOL1A2
hCOL3A1
hTNF-α
hCXCL1

Mouse Gene Expression (mouse non-parenchymal cells+ mouse hepatocytes)

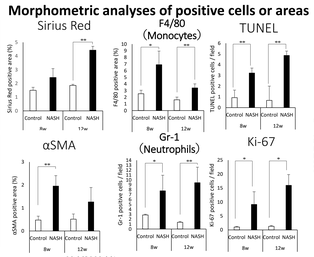


➢ CDAHFD group compared with the control group at 12 weeks. qPCR revealed higher levels of h-TGFbeta1, h-CCL2, m-Tgfbeta1, m-Cxcl2, m-Col1a1, m-Col1a2, m-Col3a1, and m-Acta2 mRNA expressions in the CDAHFD group than the control group.

BD195

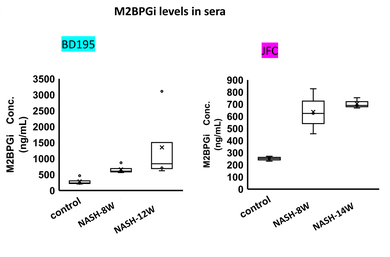


Morphometric analyses of positive cells or areas



M2BPGi levels in sera

BD195

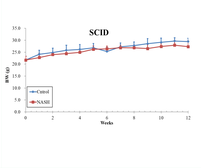


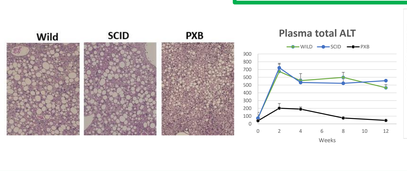
JFC

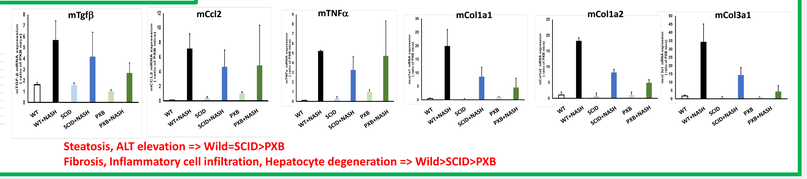
➢ Increase in α-smooth muscle actin-positive stellate cell area, tunnel-positive h-heps, Ki-67 positive h-heps, Gr-1 positive neutrophils, and F4/80-positive macrophages was noted in the CDAHFD group compared with those in the control group at 8 and/or 12 weeks.

➢ A marker of fibrosis, M2BPGi were higher in m-plasma at 8 and12 weeks in the CDAHFD group than in control group.

Wild/SCID/PXB mouse comparison







Steatosis, ALT elevation => Wild=SCID>PXB
Fibrosis, Inflammatory cell infiltration, Hepatocyte degeneration => Wild>SCID>PXB

Conclusion

We successfully developed humanized NASH model using h-hep chimeric mice with CDAHFD diet.

References

1) Tateno C et. al. (2015) Generation of Novel Chimeric Mice with Humanized Livers by Using Hemizygous cDNA-uPA/SCID Mice. PLoS One 10:e0142145.

Disclosure

Chise Tateno, Go Sugahara, Keishi Kisoh, Yuji Ishida, Yasumi Yoshizane, and Suzue Furukawa are employees of PhoenixBio, Co., ltd.

Contact Information

Chise Tateno, chise.mukaidani@phoenixbio.co.jp.