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A New Generation Humanized Liver Mouse Model Hu-URG®

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1.Introduction

Human hepatocyte chimeric liver mouse models are valuable in studies on hepatitis viruses, liver-targeted drug development as well as drug metabolism and toxicology. We report a new mouse model, URG, which is engineered to overexpress uPA specifically in liver under Tet-On system regulation, and to be *Rag2* and *Il2rg* gene knockout so that allows human hepatocyte engraftment. The URG mice are healthy and normal in breeding. When inducing with Dox， liver injury occurs in the mice, as indicated by the elevated ALT/AST and pale appearance of the livers. Transplanting 1 million primary human hepatocytes into Dox-induced URG mice achieved a high chimeric rate of liver, with up to 10 mg/mL of human albumin in serum, and 90% human CK-18 antibody positive stain area in liver sections. This mouse model was named Hu-URG. With continuous Dox inducing, the human albumin concentration in the mouse serum peaked at around 8 weeks post transplantation and sustained for at least 6 months.
Some functional proteins specifically secreted by human hepatocytes, such as ApoCIII, Apo(a), PCSK9, Angptl3, C1INH etc., which are hot targets for new drug development, were also detected in the serum of models. When being inoculated with live HBV isolates obtained from either clinic or supernatant of HBV expressing cell cultures, a high virus titer close to that of hepatitis B patients could be detected. Hu-URG mice also exhibited different drug metabolism characteristics from URG mice , which are more similar to human’s. Therefore, URG is a useful mouse strain to establish humanized liver models.

2.Phenotype

2.1 Liver damage can be induced at any point of the lifetime of URG mice

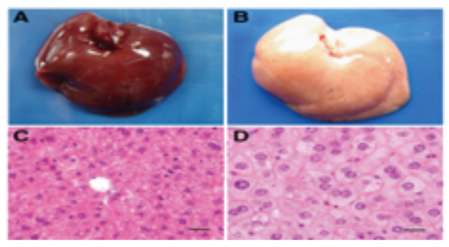


Figure1. The mouse liver morphology after Dox inducing uPA expression in URG. The liver with uPA expression was pale and HE staining showed swelling and vacuolation of hepatocytes.

2.2 Serological indices of liver injury in URG mice

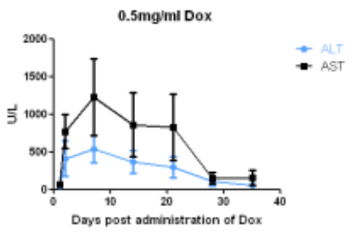


Figure2. Levels of AST and ALT change in URG mice after continuous inducing with Dox drinking water

2.3 Human hepatocytes evenly distributed in each liver lobe in Hu-URG mice

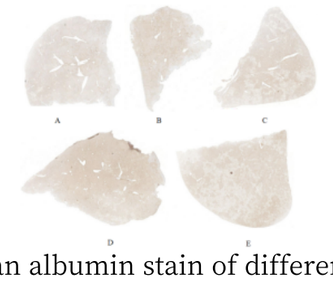
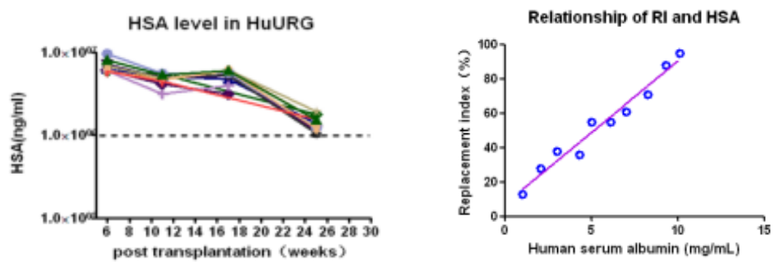


Figure3. Human albumin stain of different liver lobes of Hu-URG mice. (A-E) IHC staining with human albumin antibody showed that human hepatocytes evenly distributed in 5 lobes of the chimeric liver.

2.4 The human albumin levels in Hu-URG serum and the relation with the replacement rate of human hepatocytes in the mouse liver



A B

Figure4. The human albumin level in the Hu-URG mice and the relationship between HSA level and human hepatocyte replacement rate. (A) HSA levels could reach 10 mg/mL after 6 weeks of reconstruction, and the high HSA level (>1 mg/mL) lasted up to 26 weeks. (B) The HSA level was positively correlated with the proportion of human hepatocytes in the liver of Hu-URG as determined by ICH stain of liver tissue sections.

2.5 Normal expression of human liver cell specific genes and proteins in the liver of Hu-URG mice

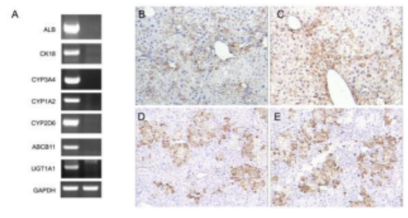


Figure5. Analysis of human liver gene expression in the liver tissues of Hu-URG mice after 6 weeks of transplantation. (A) RT-PCR analysis of human liver specific genes; (B,C,D,E) IHC staining showed human Albumin, CK18, CYP3A4, and CYP1A2 were positive.

2.6 Functional human liver proteins present in the circulation of Hu-URG

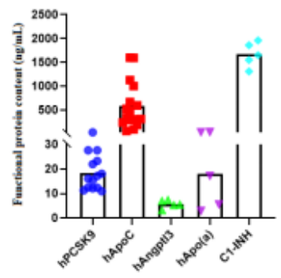


Figure6. Analysis of functional proteins specifically secreted by human hepatocytes in Hu-URG. The results showed that the humanized liver are functional and content various human proteins, many of which are hot targets of new drug development.

2.7 Similarity of liver gene expression profiles between Hu-URG mice and human

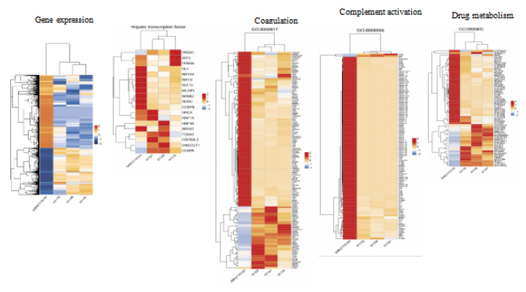


Figure7. RNAseq analysis of humanized liver tissues of Hu-URG and comparing with human liver tissue. The results showed the global gene expression profile and the expression of hepatic transcript factors, genes related to coagulation, complement activation as well as drug metabolism processes.

2.8 Hu-URG mice can be infected by human hepatitis B virus (HBV)

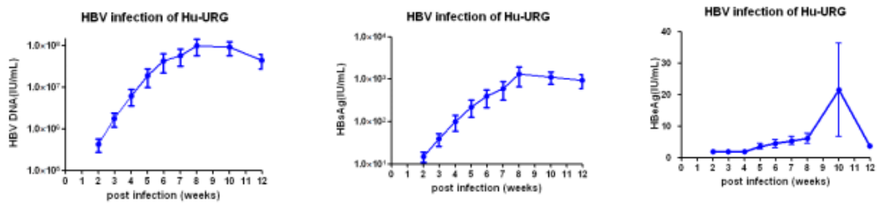


Figure8. HBV DNA, HBsAg, HBeAg content of Hu-URG infected with HBV (type A) at different time.

3.Applications

3.1 Efficacy studies of Anti-HBV drugs (ETV)

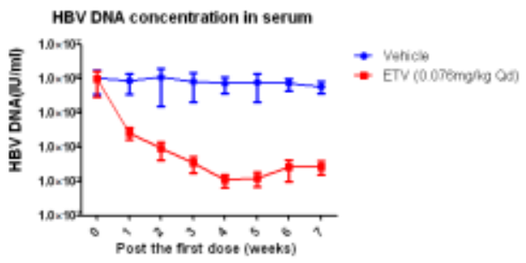


Figure9. Anti-HBV efficacy of ETV in Hu-URG infected with HBV. The results showed that ETV could decrease HBV DNA content in serum of HBV-Hu-URG, indicating that Hu-URG is a good pharmacological model.

3.2 Drug metabolism study

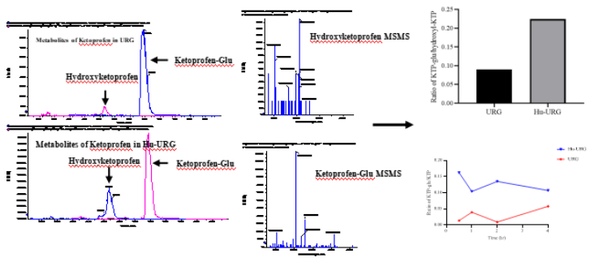


Figure10. Hu-URG exhibited different drug metabolism characteristics from URG mice, but more similar to human. Hu-URG mice and URG mice were given the same dose of Ketoprofen. The results showed that Hu-URG could produce more metabolite of Ketoprofen like human and this model is ideal to study drug metabolism in human.

4.Conclusion

Hu-URG, the chimeric mice with humanized liver, can be used for:

- Establishment of human specific pathogen, such as HBV (type A, B, C, D), HCV, HDV, malaria, etc.
- Drug metabolism study
- Drug safety evaluation
- Human liver function study
- *In vivo* study of induced hepatocyte-like cells[1]
- Efficacy study of gene or immuno-therapies targeting human hepatocytes[2-4]
- Study of human liver diseases, such as fatty liver, MASH, hepatocellular carcinoma etc.

5.Reference

[1] Li Z, et al. Generation of qualified clinical-grade functional hepatocytes from human embryonic stem cells in chemically defined conditions. Cell Death Dis. 2019, 10:763.
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[4] Ren F, et al. Sphondin efficiently blocks HBsAg production and cccDNA transcription through promoting HBx degradation. J Med Virol. 2023, 95:e28578.

6.Acknowledgements

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