論　文　概　要

○ 論文題目　Generation of humanized liver in rats
（ヒト化肝臓ラットの作成）

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Abstract

Purpose

For precisely mimicking the human metabolism and testing the drug efficacy and toxicity, animal models, as well as human liver microsome are wildly used over the past decades. Moreover, humanized liver generated from rodents showed superior advantages for a great variety of applications. To data, liver-humanized mice have been developed by transplanting primary human hepatocytes to the livers of mice with severe immunodeficiency and liver injury. Since rats are much bigger and have been proved more similar to humans in terms of various physiological and pathological aspects, the generation of liver-humanized rats would be preferred over mice in drug development and liver disease modeling, thus deserve great promises.

Material and methods

To generate the *Fah*$^{-/-}$*Rag2*$^{-/-}$*IL2rg*$^{-/-}$ (FRG) rat model, CRISPR/Cas9 technique was used to knock out the targeted genes. To determine the immunodeficiency of FRG rats, proportion of T, B and NK cells, and immunoglobulin level in the peripheral blood were analyzed by flow cytometry and ELISA. To confirm the 2-(2-Nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC) -depended liver failure, FRG rats were administrated with or without NTBC, followed by examination of liver function and survival rate. To identify the capability for hepatic xenotransplantation, FRG rats were transplanted with wild-type (WT) rat and mouse hepatocytes, followed by assessment of liver repopulation efficiency by FAH staining. Humanized livers were generated following transplantation of primary human hepatocytes under optimized NTBC controlling. In term of characterization of the

humanized livers, the dynamic change of human albumin secretion was monitored by ELISA; the repopulation rate was determined by hNuclei/hALB staining; the metabolism-related gene expression pattern was analyzed by qPCR and RNA sequencing; the human-like metabolic function was estimated by *in vivo* pharmacokinetics of a UGT2B7-mediated drug following oral administration.

Results

The FRG rat model with triple genes knockout of *Fah*, *Rag2*, and *IL2rg* was generated, in which the progressive liver failure could be controlled by treatment of NTBC. Transplantation of hepatocytes from WT rats could repopulate over 90% of the FRG rat livers after 2 months, and rescue the survival rate under the withdrawal of NTBC. Moreover, FRG rats showed extremely low level of $CD3^+$ T cells, $CD45RA^+$ B cells, and $CD161a^+$ NK cells, which offered a superior tolerance for xenogeneic transplantation. Under bodyweight-based NTBC controlling, xenotransplantation of WT mouse hepatocytes could repopulate 62% of the FRG rat livers with significantly improved liver function. In light of the high mortality of FRG rats due to NTBC withdrawal-induced liver injury, an optimized NTBC cycling model was established, which enabled prolonged survival period while maintaining chronic liver failure. Under the optimized controlling, primary human hepatocytes could efficiently repopulate the livers of FRG rats, reaching 31% ± 4% repopulation rate and 1.7 ± 0.3 mg/ml human albumin secretion 7 months post transplantation. Meanwhile, over 10% human hepatocytes sustained the expression of Ki67 *in vivo*, indicating consistent proliferative potential. Finally, the humanized livers *in vivo* displayed the human liver-like metabolic zonation and gene expression patterns, and notably shared the drug metabolism features of human specific.

Discussion

As a breakthrough in existing mice models with humanized liver, in this study, liver humanization was achieved for the first time in rat models with *Fah*, *Rag2*, and *IL2rg* triple genes knockout. Mimicking the human liver-like function and metabolism is of great significance for clinical and industrial applications, particularly in drug testing and disease modeling, deserving the most careful evaluation on the humanized livers generated from animal models. Indeed, similar to that reported in mouse models, the humanized liver generated in rats also displayed the human liver-like metabolic zonation and metabolism-related gene expression patterns. Interestingly, compared to mice, discrepancy was found in rats with gene sets uniquely enriched in pathways involving small molecule catabolic and alcohol metabolic, suggesting their potential advantages for studying on drugs with low molecular weight and alcohol-related liver diseases. More importantly, liver-humanized rats displayed human-specific metabolism features *in vivo*; together with their advantage in sequential blood sampling, the liver-humanized rats will undoubtedly serve as the preferred model in pharmaceutical industry.

However, the current liver-humanization efficiency in FRG rats was relatively lower than in mice models. Firstly, despite the depletion of T, B, and NK cells, the resident macrophages were supposed to eliminate the xenogeneic human hepatocytes. In mouse models with non-obese diabetic background, the ‘‘don’t eat me’’ signal communicated between mouse Sirpα and human CD47 was found significantly suppressed the phagocytosis and promoted the humanization. It’s reasonable to believe that if human SIRPα knock-in is established in FRG rats, the compatibility for human hepatocytes will markedly enhanced in rat livers. Secondly, the bodyweight-normalized

cell transplantation dose was much lower in this study due to the high cost of primary human hepatocytes. With the rapid development of producible and low-cost human hepatic source generated from human pluripotent stem cells or by chemical induction, the transplantation of a higher cell dose may become available in near future. Finally, human hepatocyte-secreted complement factors were reported to attack host tissues, particularly kidney, causing high risk of mortality in a mouse model. Additional treatment with anti-complement drug may further improve the survival and humanization efficiency in this rat model.

With these further improvements, humanized FRG rats will be a superior alternative to the existing mouse models for prediction of the human-specific drug metabolism, and liver disease modeling.

Conclusion

The first liver-humanized rat model in the world was successfully established, which resembled human liver functions, particularly in metabolism. With further improvements in humanization efficiency, this model is expected to greatly benefit the regenerative medicine and pharmacological studies, as well as industrial applications in near future.

Key words: FRG rat, Humanized liver, NTBC controlling, Xenotransplantation, Repopulation rate, Drug metabolism