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学位論文題目	Generation of humanized liver in rats （ヒト化肝臓ラットの作成）
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論文の内容の要旨
Abstract of thesis

In this doctoral dissertation, Ge Jianyun describes the generation of humanized liver in rats. The summary is as follows:

（**目的　Purpose**）

For precisely mimicking the human metabolism and test 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.

（**対象と方法　Materials 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 WT rat and mouse hepatocytes, followed by assessment of liver repopulation rate 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.

（**結果　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 rescued 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 after 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.

（**考察　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 mice models, the humanized liver generated in rats also displayed the human liver-like metabolic zonation and metabolism-related gene expression patterns. Interestingly, compared to mouse, discrepancy was found in rat model 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 samplings compared with mice, 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. With further improvement in human hepatocyte repopulation, humanized FRG rats will be a superior alternative to the existing mice models in prediction of the human-specific drug metabolism, and liver disease modeling.

審査の結果の要旨
Abstract of assessment result

（**批評　General Comments**）

The author generated a severe immunodeficient FRG rat in which liver failure was controllable by treatment of NTBC. He provided the effective condition for hepatic xenotransplantation in FRG rats. He further revealed that the humanized liver in FRG rats displayed the human liver-like metabolic zonation and gene expression patterns, and notably shared the drug metabolism features of human specific. FRG rats would make significant contributions to the regenerative medicine and pharmacological studies. The research is original and appropriately designed. Data are clear and convincing and the discussion is reasonable. The thesis paper is carefully written with a suitable style.

（最終試験の結果　**Assessment**）

The final examination committee conducted a meeting as a final examination on Dec 23, 2020. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

（結論　**Conclusion**）

The final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Medical Sciences.