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| ID No. | K2005 |
| Project Title | Generation of iPS-derived human hepatocytes in rat liver |
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Report

In this project, we are going to establish a method for manufacturing a large-scale human iPS-derived hepatocytes (iPS-Heps) in model animals. In current, we have already generated severe combined immunodeficiency (SCID) rats by knockout of the *Il2rg* and *Rag2* genes in F344 rats, using CRISPR/Cas9 system. Furthermore, we have also generated the liver-specific *iCasp9* gene knockin in the F344 rats. The *iCasp9*-modified SCID colony is breeding and extending in SPF breeding room of animal facility. The suicide *iCasp9* gene can regulate these rats' hepatocytes to apoptosis, therefore, we can proliferate human iPS-Heps in the rats' liver and harvest large-scale human hepatocytes without rat cells. The preliminary experiment of transplanting human hepatocytes into the SCID rats has already started in Kyushu University by Dr. Kazuki Takeishi. In the meantime, we are also conducting the pre-experiment of inducing the apoptosis to the hepatocytes of *iCasp9*-modified rats in IMSUT. This pre-experiment including both in vitro assay and in vivo assay, which results will greatly help us to establish the optimal protocol of proliferating the human iPS-Heps in rat liver and isolating the human hepatocytes with high efficiency.

Due to the prevalence of COVID-19, the travel from America to Japan is very difficult. Instead of visiting IMSUT, we held several online meetings with other project members to communicate current problems and the future plan. By the regular communications, we adjust the protocols constantly, including the breeding of the SCID rats to ensure their survival and quality in different institutes, the optimal age and body weight of the animals for transplantation, examining the engrafted of human hepatocytes in the recipient rats, inducing the apoptosis to the hepatocytes of the iCasp9-modified rats in vivo, etc. To ensure our project progressing smoothly, we will continue the regular meeting to exchange information and the visits for technology supports will be started after the outbreak of COVID-19.