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

In this project, we aimed to establish a method for manufacturing a large-scale human iPS-derived hepatocytes (iPS-Heps) in rats. Since rats are about ten times larger than mice, they expected to be suitable for the humanization. So far, we have already generated severe combined immunodeficiency (SCID) rats by knockout of the *Il2rg* and *Rag2* genes in F344 rats, using CRISPR/Cas9 system. In this project, we have 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 in IMSUT. 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 started in Kyushu University by Dr. Kazuki Takeishi. We are conducting the pre-experiment of inducing the apoptosis to the hepatocytes of *iCasp9*-modified rats in IMSUT. We also started to generate another liver-specific gene knockin rats harboring different liver-specific promoter to improve the efficiency.

These experiments including both in vitro assay and in vivo assay, which results 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 still very difficult. Instead of visiting IMSUT, we had online regular meetings with other project members to share the progress.