ID No.	K2003
Project Title	Establishment of a platform for development and evaluation of exon skipping based gene therapy tool by using human iPS cell
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Project Members IMSUT Host Researcher	Sumiko Watanabe （Project Prof./IMSUT）
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Report

Introduction of hiPSC lines which were established in Dr. Conklin's lab to IMSUT

Dr. Watanabe introduced several human iPSC lines, which had been established in Dr. Conklin's lab. These cells were genetic manipulated for several purposes, and the cells were established in Dr. Watanabe's lab with the help of Dr. Miyaoka. One of cells contains inducible expression cassette of retinal specific transcription factors, which enable cells to differentiate hiPSC to retinal cells efficiently and rapidly. Culture condition had been developed in collaboration of Dr. Conklin and Dr. Watanabe groups.

Establishment of exon skipping efficacy-evaluation system by using ddPCR method

To evaluate exon skipping vector efficiency to remove target exon, Dr. Watanabe's group established a platform of evaluation by combination of a series of experiments such as semi-quantitative PCR, sequence analysis by TIDE, ddPCR. Introduction of ddPCR technology was achieved by collaboration with Dr. Miyaoka.

Construction of CRISPR/Cas9 based exon skipping vector

CHM, which is frequently mutated in retinal inherited degenerative diseases, was chosen as a model system, CRISPR/Cas9 plasmids containing two gRNAs were constructed, and heir efficacy of exon skipping was evaluated by using cell lines.

Introduction of exon skipping vector to hiPSC for evaluation of its effects for RPE function

The exon skipping vectors were introduced into hiPSC to check exon skipping efficiency and recovery of function using RPE derived from hiPSC. For that purpose, condition of electroporation of plasmids into hiPSC was carefully examined upon advise of Dr. Conklin's lab members.

AI based cells differentiation evaluation system

Dr. Tabuchi and Dr. Watanabe groups developed prototype model of AI based cell differentiation stage evaluation system of hiPSC differentiation to the RPE.