Project number 88

Role of taurine modification of mitochondrial tRNAs in normal and stressed erythropoiesis

［1］**Research group**
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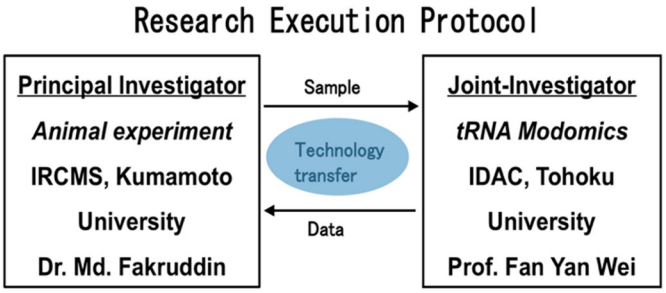
Consumables 100,000 YEN

［2］**Research setup**

Erythropoiesis is an essential process that generates enucleated erythrocytes from hematopoietic stem/ progenitor cells to meet the staggering demand of red blood cells (RBC), an oxygen supplier (~2x1012 per day). Erythroid differentiation is a complex and multistep process with tightly controlled stepwise transition from pro-erythroblasts to enucleated reticulocytes. Erythropoiesis related disorders such as different forms of anemia affects significant populations worldwide and effective therapeutic approaches are still not available due to limited understanding of metabolic and epigenetic regulation of erythroid differentiation. In this study, we will elucidate the role of MTO1 (Mitochondrial Translation Optimization-1) which mediates taurine modification of mitochondrial tRNAs in fetal erythropoiesis using our mitochondria specific tRNA modifier deficient mouse model and using the state-of-art technologies through international multidisciplinary collaborations.

Dynamics of mitochondrial tRNA modifications during hematopoiesis and erythropoiesis is not clearly understood yet. Through this joint-research program, the dynamics of tRNA modifications (taurine modification as well as other modifications of mitochondrial tRNAs) during terminal erythroid differentiation will be analyzed. We expect to uncover interesting changes in tRNA modifications through this joint research program.



［3］**Research outcomes**

（3－1）**Methods and Results**

Hematopoiesis specific MTO1 knock out mice has been generated by the principal investigator at Kumamoto University and immunophenotypic analysis has been finished. To understand how MTO1-mediated taurine modification is involved in the process of hematopoiesis, we isolated early hematopoietic cells using FACS Canto II and FACS Aria III flow cytometers. Fluorescence-conjugated antibodies against c-Kit, Sca-1, CD34, Flt3, CD150, CD48, and CD16/32, were used to isolate each cell population.

The sorted erythroblast cells were stored in -80 degree and sent to Dr. Wei's laboratory for mass spectrometry analysis. Briefly, the cells were homogenized using TRIzol reagent followed by RNA precipitation. Purified RNA was subject to RNase digestion sing Nuclease P1. RNA modifications were separated on a C-18 column and were analyzed using Tandem Quadrupole Mass Spectroscopy (Shimazu LCMS8050). The area of peak corresponding to taurine modification was calculated using LabSolution software.

According to the previously curated database, the expression level of taurine modification enzyme

Mto1 is the highest in erythroid lineage especially in early promyelocyte, with the expression level gradually declines toward the maturation. The mass spectrometry analysis of mitochondrial tRNA taurine modification revealed that the modification pattern was highest in a particular stage during hematopoiesis, which is in line with the periodic expression pattern of MTO1. By contrast, the taurine modification was not detected in hematopoietic cells isolated from MTO1 knockout mice. We have previously reported that the constitutive MTO1 knockout mice were embryonic lethal. Given the potential role of MTO1 in the hematopoiesis, it is conceivable that the defective taurine modification is responsible for the defective hematopoiesis, leading to the death of embryo.

（3－2）Future perspectives

The collaboration between my laboratory and Dr. Wei's laboratory revealed an important role of mitochondrial tRNA taurine modification during hematopoiesis. Using mass spectrometry, we found that mitochondrial tRNA modification is dynamically regulated during development, and the defective modification can cause a catastrophic pathological consequence. Taurine modification has been implicated in the development of mitochondrial disease, which is a genetic disease characterized by neuromuscular dysfunction due to mitochondrial defects. Interestingly, decrease of blood cells is also reported in the patients but the molecular mechanism has been unclear. Our results suggest that loss of taurine modification is likely underlying the defective hematopoiesis, which pave a way to treatment the disease.

［4］List of research achievements

Based on these results, we are currently writing a manuscript and hopefully it will be accepted in the coming fiscal year.