2019年度　技術交流助成　成果報告（日本留学）

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1.　留学中に実施した研究テーマ

Molecular mechanisms by which *DDX41* gene mutations cause myeloid malignancies.

2.　留学期間中の研究成果

Current comprehensive sequence approaches led to the identification of rare but reproducible somatic gene mutations in myeloid malignancies. These gene mutations include not only those that were known to be mutated in the past, such as *RUNX1*, *FLT3* and *CEBPA*, but also many previously unrecognized genes to be mutated. Among which, there are somatic mutations in the *DDX41* gene encoding a DEAD box type ATP-dependent helicase. They are detected in 1-2% of AML/MDS patients. The germline mutations of *DDX41* are basically a kind of loss-of-function type of cells, while somatic mutation is highly concentrated at c.G1574A position (p.R525H) in the conserved motif VI, located at the C-terminus of the RecA-like helicase core domain where ATP interacts and is hydrolyzed. It is likely that the p.R525H mutation in the DDX41 protein disturbs ATPase activity in dominant negative manner. In a previous paper from our laboratory, we showed that the hematopoietic cells enforcedly expressed with DDX41 p.R525H mutant showed slow growth, along with impaired processing and production of ribosomal RNA (Kadono M et al. Exp. Hematol. 2016). In addition, in that study we performed mRNA-sequencing analysis of CD34-positive tumor cells of 23 patients with myeloid malignancies and found that the patients with a DDX41 p.R525H somatic mutation exhibited acute myeloid leukemia with bone marrow failure phenotype, i.e., pancytopenia with lower blast counts. Based on these observations, in this study, we hypothesized that changes in ribosome biogenesis may lead to changes in translation of mRNA and that DDX41 is involved in tumor development or growth through this regulation.

We found that the proliferation of DDX41 knockdown cells was suppressed as compared with the control cells, along with the increase of apoptotic cells and the accumulation of G2/M phase by cell

cycle analysis. We also performed a comprehensive translation analysis, also known as ribosome profiling, using a next generation sequencer (NGS) to compare the association between the changes in transcription and translation. The overall translation changes were correlated with transcription changes to some extent, but the correlation coefficient was not so high. Remarkably, when we picked up transcripts that were transcriptionally or translationally changed for more than 2-fold or less than 0.5-fold, translation of many genes was found to be differently changed from transcriptional changes. We then performed a GSEA analysis and a Gene ontology analysis for ribosome-profiling data. These analyses suggested that genes involved in ribosome biogenesis and translation were translationally increased by DDX41 knockdown. This suggests that DDX41 negatively regulates ribosome biogenesis at translation level. This result was orally presented at the 82nd annual meeting of the Japanese Society of Hematology (the title of the presentation was “Translational regulation of a subset of genes by DDX41”), and the paper is currently being prepared for submission.

3. 今後の研究計画

RNA helicases are generally known to play multiple roles, including translation, transcription, RNA splicing, RNA export and so on, and it is supposed that many RNA helicases have more than dual functions. Although we have elucidated the roles of DDX41 in the regulation of translation in this study, we also recently found that the molecule is involved in RNA splicing. Furthermore, we noticed that the inhibition of DDX41 accumulates DNA damages in hematopoietic cells as well. We would like to clarify these mechanisms in our future study.

4. その他と謝辞（日本での生活・交流の様子など）

I would like to thank the Nakatani Foundation for supporting me through its Technology Exchanges Grant for Study program in Japan. Second and foremost, I gratefully thank Professor Hirotaka Matsui and everyone in Molecular Laboratory Medicine, Kumamoto University School of Medicine. I also express my sincere appreciation to Dr. Oyundelger Norov in Center of Hematology and Bone Marrow Transplantation and Dr. Enkhjargal Tserennadmid in Public Health Institute, Ministry of Health of Mongolia.

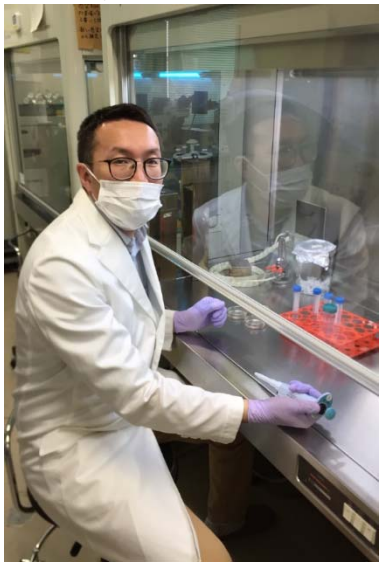

Image: me working in the laboratory.

I spend most of the time in the laboratory for culturing cells and performing experiments. It is of great pleasure for me to acquire knowledge and experimental techniques for molecular and cellular biology.