DATE: Day 2 Month May Year 2017

SUMMARY of
2016 RESEARCH RESULTS REPORT
For International Collaborative Research with IPR, Osaka University

Research Title		**Structures and functions of Dnmt1 mutants identified by genetic screen**
Applicant	**Name**	**Norimasa Iwanami**
	Affiliation	**Max Planck Institute of Immunobiology and Epigenetics**
	Present Title	**Project leader**
Research Collaborator (Host PI)		**Isao Suetake**

Summary

Dnmt1 is known as an epigenetic regulator indispensable for the maintenance of the status of DNA methylation. However, a possible tissue-specific function of Dnmt1 is not well understood. In our genetic screeens using zebrafish and medaka, we have identified *Dnmt1* missense mutations specifically affecting lymphocyte development. These mutants show overall hypomethylation of the genome. In order to understand molecular mechanisms for the above-mentioned tissue-specific effect of the *Dnmt* mutations, we started this international collaborative research aiming at biochemical and structural analyses of the mutant proteins.

In vitro DNA methylation assays showed that mouse Dnmt1 proteins with zebrafish and medaka mutant-type missense mutations exhibit reduced methylation activities of hemimethylated and unmethylated CpGs compared to wild-type Dnmt1 protein. These results offer a molecular explanation for the genome-wide DNA hypomethylation in zebrafish and medaka mutants.

For structural analysis of Dnmt1 mutant proteins, we replaced the relevant side chains in the crystal structure of wild type mouse Dnmt1 protein, supported by Dr Kohei Takeshita. In further collaboration with Professor Yasushige Yonezawa at Kindai University, we have started molecular dynamics simulations of Dnmt1 mutant proteins. The result of molecular dynamics using mouse Dnmt1 protein with zebrafish-type mutation offered structural evidence for the observed hypomethylation in mutants.

Our biochemical and structural analyses of mutant Dnmt1 protein in this international collaborative research identified molecular mechanisms of decreased methylation activities in the mutants. This collaboration enabled us to connect *in vivo* DNA hypomethylation caused by *Dnmt1* mutation with structural and functional defects in the mutant proteins. This collaboration positioned us at the start point of understanding tissue-specific role of the protein, in combination with ongoing phenotypic analysis of zebrafish, medaka, and mouse *Dnmt1* mutants.

In addition, we organized a Protein Institute Seminar "Epigenetics governing our life system" (Dec 20, 2016). This was a good opportunity to communicate the outcome of our collaboration to the scientific community, and we believe that it will facilitate our further collaboration.

***Deadline: May 19, 2017**
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