IMSUT International Joint Usage/Research Center
International Project-completion Report (FY2022 ver.)

Date of submission: **Month** / **Date** / **Year**

Principal Investigator	Position, Institution: Member, Memorial Sloan Kettering Cancer Center Name: Omar Abdel-Wahab
IMSUT Host Researcher	Division: Hematopoietic Disease Controlr Name: Yasuhito Nannya
Project Title	Clonal hematopoiesis and a variety of related disorders in the aged population
Duration	From 04/01/2022 to 03/31/2023

Project Members

Name	Position, Institution
Omar Abdel-Wahab	Member, Memorial Sloan Kettering Cancer Center
Yasuhito Nannya	Professor, IMSUT
Daichi Inoue	Professor, Foundation of Biomedical Research and Innovation at Kobe/Institute of Biomedical Research and Innovation
Toshio Kitamura	Professor Emeritus, University of Tokyo/Pharmaceutical Sciences

Project-completion Report

It is now known that blood clones harboring one or two leukemia-related mutations are identified in 10% of healthy aged people, which is called clonal hematopoiesis (CH). To investigate the me chanisms by which a small CH clone leads to inflammation and development of various diseases, we investigate ASXL1-mutant knock-in (ASXL1-MT-KI) mice and relate the results to human CH and the associated diseases.

1. ASXL1-MT-KI/STAG2-KO combined mice developed various hematological malignancies inc luding MDS (50~60%), MDS-AML (20%), and B- or T-ALL (20%). Among them, we first i nvestigated how ASXL1-MT-KI and STAG2-KO collaborate in inducing MDS, while ASXL1-MT-KI or STAG2-KO alone did not induce MDS and the mice survived longer than 18 months. Anayses of these mice using CHIPseq, ATAC-seq and HiC revealed that ASXL1-MT reduced Histone H3K27me3 and that addional KO of STAG2 induced the changes i n combination of promoters and enhancers, leading to derepression of polucomb-regula ted genes, including HoxA9. The paper including these results are now in preparation.
2. Like ASXL1-MT-KI/STAG2-KO mice, newly established ASXL1-MT-KI/BCOR-KO combined mi ce developed MDS-like disease. We have started to investigate these mice.
3. To investigate if ASXL1-MT enhanced the atherosclerosis of LDLR-KO mice fed by high fat high cholesterol diet (HFHCD), we transplanted bonemarrow (BM) cells of ASXL1-MT -KI mice or normal mice to LDLR-KO mice, followed by HFHCD. We found that the mi ce transplanted with ASXL1-MT-KI BM cells developed more severe atherosclerosis whe n compared with those transplanted with normal BM cells. As for the molecular basis, we did not find the increased expression of IL-1 like TET2-KO mice. Instead, we found that wt-ASXL1 bound the downstream molecule of IL-1R/TLR including TRAF6, IRAK1, a nd TAK1, and attenuate the activation of this pathway associated with profound reduc

tion of K63-polyubiquitination of TAK1, which is a hallmark of the activation of this pathway. On the other hand, ASXL1-MT did not inhibit the activation of this pathway, associated with the increased phosphorylation of IRAK1 and TAK1 as well as stable or increased levels of K63-polyubiquitination of TAK1, leading to the activation of the downstream pathway including NFkB, p38MAPK and JUNK. The activation of this pathway was inhibited by IRAK inhibitors. Moreover, the enhanced atherosclerosis induced by ASXL1-MT-KI cells but not the regular atherosclerosis of LDLR-KO mice fed by HFHCD was inhibited by IRAK inhibitors. These reluts together demonstrate ASXL1-MT activates downstream of IL-1R/TLR, leading to the enhanced atherosclerosis. The paper is now underrevision.

<Publications>

None

<Patent Applications>

None

Days of visits to IMSUT

Name	Position, Institution	Sex	Age	Visits to IMSUT (Days)
Omar Abdel-Wahab	Member, Memorial Sloan Kettering Cancer Center	Male	**40 or older**	
Yasuhito Nannya	Professor, IMSUT	Male	**40 or older**	One Zoom meeting with Dr. Abdel-Wahab
Daichi Inoue	Professor, Foundation of Biomedical Research and Innovation at Kobe	Male	**40 or older**	Zoom meetings with Dr. Abdel-Wahab, 5 times. E-mails many.
Toshio Kitamura	Professor Emeritus, University of Tokyo	Male	**40 or older**	More than 50 days One Zoom meeting with Dr. Abdel-Wahab. E-mails with Dr. Abdel-Wahab, 8 times.
Name	**Position, Institution**	**Sex**	**Age**	**Online Meetings (Days)**
		Pull-down▼	Pull-down▼	

		Pull-down▼	Pull-down▼	
		Pull-down▼	Pull-down▼	
		Pull-down▼	Pull-down▼	
Name	**Position, Institution**	**Sex**	**Age**	**Discussions via E-mail, Slack, etc. (Days)**
		Pull-down▼	Pull-down▼	
		Pull-down▼	Pull-down▼	
		Pull-down▼	Pull-down▼	
		Pull-down▼	Pull-down▼	

Usage of Facilities/Equipment			
Name of Facility	**Equipment**	**Number of Use (Times)**	**Usage time (Hours)**
FACS Core Laboratory	e.g.) FACS Aria (BD)	15	5
Medical Proteomics Laboratory	e.g.) Orbitrap QSTAR Elite	none	
Imaging Core Laboratory	e.g.) Zeiss Multiphoton Microscopy（LSM710NLO)	none	
Gene Manipulated Mouse Section	Creation and cryopreservation embryo of Knockout mouse	none	
Human Genome Center	Supercomputer	10	5
Amami Laboratory of Injurious Animals	Experimental lab	none	
Other		none	

Usage of Scientific Resources	
Name of Scientific Resource	**Number of Samples/Lines**
Serum (BioBank Japan)	0
DNA (BioBank Japan)	0
Knockout mouse	0
Pathogenic bacteria	0

Other	0
Usage of Database	
Name of Database	**Number of Use (Times)**
Single-cell genome database	10