IMSUT International Joint Usage/Research Center

International Project-completion Report (FY2022 ver.)

Date of submission: **05 / 12 / 2023**

Principal Investigator	Position, Institution: Professor, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences Name: Miguel Esteban
IMSUT Host Researcher	Division: Division of Stem Cell Pathology Name: Yasuhiro Yamada
Project Title	Dissecting the molecular roadmap of *in vivo* reprogramming
Duration	From 04/01/2022 to 03/31/2023

Project Members

Name	Position, Institution
Yasuhiro Yamada	Professor, Division of Stem Cell Pathology, Center for Experimental Medicine and Systems Biology, Institute of Medical Science, University of Tokyo
Jumpei Taguchi	Project Assistant Professor, Division of Stem Cell Pathology, Center for Experimental Medicine and Systems Biology, Institute of Medical Science, University of Tokyo
Miguel Esteban	Professor, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences
Yiwei Lai	Postdoctoral researcher, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences

Project-completion Report on achievements/progress through the entire project period

To elucidate molecular basis of cancer development and the acquisition of totipotency induced by the reprogramming factors (OCT4, SOX2, KLF4 and c-MYC; OSKM) *in vivo*, we performed single-cell RNA sequencing of kidney tumors developed in reprogrammable mice. Samples analyzed in this study are; normal kidney, kidney tumors, ES cells, primordial germ cells, and placental tissues. Using the UMAP method, we succeeded in clearly clustering each cell type contained in the above samples. Moreover, we were able to detect the expression of representative genes in each cell clusters. Further analysis revealed that some of the kidney tumor cells were positive for genes (Oct4 etc.) which are representatively expressed in pluripotent stem cells, suggesting transcriptionally dedifferentiation of kidney cells *in vivo*. In addition, cells expressing primordial germ cell marker genes were observed in some of the kidney tumor cells. The Japanese group has reported that acquisition of totipotency during *in vivo* reprogramming requires passing through transient gene expression patterns of fetal germ cells (*Nat Commun.* 2021). This result suggests that we could detect the reprogramming process from somatic cells to totipotent cells *in vivo* at the single cell level. In the future, the underlying mechanisms of tumor formation and the reprogramming to totipotency may be clarified by analyzing sequential changes in OSKM-expressing kidney cells that have already been sampled. In this collaborative project, we have established a pipeline to perform scRNA-seq from solid tissues (collecting and freezing samples, nuclear extraction and sc-RNA analysis after transportation etc.) and confirmed that the procedure can yield scRNA-seq data with the better quality. Taken together, we were able to demonstrate the technical feasibility of the scRNA-seq from solid tissues in this collaboration.

Research Results from the Project during FY2022

<Publications>

No paper related to this grant project has not been published yet.

<Patent Applications>

No patent related to this grant project has not been filed yet.

Days of visits to IMSUT during FY2022

Name	Position, Institution	Sex	Age	Visits to IMSUT (Days)
		Pull-down▼	Pull-down▼	
		Pull-down▼	Pull-down▼	
		Pull-down▼	Pull-down▼	
		Pull-down▼	Pull-down▼	
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)**
Miguel Esteban	Professor, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences	Male	**40 or older**	**Discussions via E-mail (5 days)**
Yiwei Lai	Postdoctoral researcher, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences	Male	**35 or younger**	**Discussions via E-mail (5 days)**
		Pull-down▼	Pull-down▼	
		Pull-down▼	Pull-down▼	

Usage of Facilities/Equipment during FY2022

Name of Facility	Equipment	Number of Use (Times)	Usage time (Hours)
FACS Core Laboratory	e.g.) FACS Aria (BD)	0	
Medical Proteomics Laboratory	e.g.) Orbitrap QSTAR Elite	0	
Imaging Core Laboratory	e.g.) Zeiss Multiphoton Microscopy（LSM710NLO)	0	
Gene Manipulated Mouse Section	Creation and cryopreservation embryo of Knockout mouse	10	
Human Genome Center	Supercomputer	0	
Amami Laboratory of Injurious Animals	Experimental lab	0	
Other		0	

Usage of Scientific Resources *Please enter'0' or 'N/A' if you have not used any.

Name of Scientific Resource	Number of Samples/Lines
Serum (BioBank Japan)	0
DNA (BioBank Japan)	0

Knockout mouse	1
Pathogenic bacteria	0
Other	0
Usage of Database *Please enter‘0' or ‘N/A’ if you have not used any.	
Name of Database	**Number of Use (Times)**
	0