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International Project-completion Report (FY2022 ver.)

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Principal Investigator	Position, Institution: Professor and Research Group Leader, Institute for Medical Virology and Epidemiology of Viral Diseases, University Hospital Tübingen, Germany
	Name: Daniel SAUTER
IMSUT Host Researcher	Division: Division of Systems Virology
	Name: Kei SATO
Project Title	Regulation of cellular gene expression by endogenous retroviruses in infectious and non-infectious diseases
Duration	From 04/01/2022 to 03/31/2023

Project Members

Name	Position, Institution
Daniel Sauter	Professor, University Hospital Tübingen, Germany
Kei Sato	Professor, IMSUT
Jumpei Ito	Postdoctoral fellow, IMSUT
Izumi Kimura	Postdoctoral fellow, IMSUT
Keiya Uriu	Graduate student, IMSUT
Shigeru Fujita	Graduate student, IMSUT
Martin Müller	Graduate student, University Hospital Tübingen, Germany
Adam Strange	Technician, IMSUT

Project-completion Report

*To proceed this collaborative project, Dr. Sauter visited the IMSUT from October 2022 to January 2023.

The initial goal of the project was to investigate the role of endogenous viral elements in infectious and non-infectious diseases. About 9% of the human genome consists of so-called endogenous retroviruses (ERVs). While these viral fossils are not infectious anymore, some of them can be reactivated by external stimuli, including viral infection. The project aimed at identifying those endogenous retroviruses that are activated upon HIV infection and other disorders, as well as the underlying molecular mechanisms. Furthermore, we aimed at determining the downstream effects

of endogenous retrovirus activity on cellular gene expression. Within the project, we were able to show that HIV-1 infection triggers the activation of a specific subfamily of HERVs, so-called solo-LTRs of the LTR12C and LTR12D families. Intriguingly, some of the endogenous retroviral elements were shown to act as promoters regulating the expression of cellular genes. Among the proteins expressed in an LTR12C- or LTR12D-dependent manner are the antiviral factors GBP2 and GBP5, as well as the p53-regulator DHRS2. Thus, our findings revealed that infection with an exogenous retrovirus results in the activation of ERVs regulating antiviral immunity and potentially cell cycle progression. Notably, infection with other viruses (e.g. Influenza A Virus) results in the activation of a similar set of ERVs. This suggests that activation of LTR12C/D elements is a phenomenon potentially observed in various infectious diseases. It is therefore tempting to speculate that our immune system has exapted these retroviral promoters to orchestrate immune responses against a variety of viral pathogens.

In response to the SARS-CoV-2/COVID-19 pandemic, all participating project members also used their expertise to decipher the evolution, immune evasion and replication strategies of SARS-CoV-2. The primary goal was to elucidate the mechanisms that SARS-CoV-2 has evolved to suppress or evade the human immune response. Furthermore, we aimed at deciphering the impact of naturally occurring mutations in SARS-CoV-2 on transmission, spread and pathogenicity of the virus. Finally, keeping the initial project idea in mind, the project team investigated whether endogenous retroviruses are activated upon SARS-CoV-2 infection and may interfere with the replication of this novel pathogen.

Among other things, we could identify a small viral protein, termed ORF3c, as a suppressor of IFNbeta production. We showed that ORF3c suppresses RIG-I- and MDA5-mediated immune activation and interacts with the signaling adaptor MAVS. This immunosuppressive activity of ORF3c is conserved among members of the subgenus sarbecovirus, including SARS-CoV and coronaviruses isolated from bats. Notably, however, the SARS-CoV-2 delta and kappa variants harbor premature stop codons in ORF3c demonstrating that this reading frame is not essential for efficient viral replication in vivo and likely compensated by other viral proteins. In agreement with this, disruption of ORF3c did not significantly affect SARS-CoV-2 replication in CaCo-2 or CaLu-3 cells. The results of this subproject were published on bioRxiv and are currently in revision.

Apart from this, we investigated the evolution of SARS-CoV-2 Omicron subvariants and characterized the BQ.1.1 variant. *In silico* sequence analyses suggest that Omicron subvariants acquired mutations increasing viral fitness several times independently, via convergent evolution. Moreover, BQ.1.1 turned out to be less sensitive to breakthrough BA.2/5 infection sera than BA.5. We also characterized the SARS-CoV-2 XBB variant, demonstrating that it represents a recombinant virus that most likely emerged in summer 2022. While XBB Spike is more more fusogenic than BA2.75 Spike, the pathogenicities of XBB and BA2.75 in hamsters are similar. The results of these subprojects were recently published in two independent papers in Nature Communication.

Overall, the project has not only provided important insights into the (patho)physiological roles of endogenous retroviruses during infection, but also shed light on the evolution and immune evasion activities of SARS-CoV-2.

Research Results from the Project

<Publications>

(1) Tomokazu Tamura, Jumpei Ito, Keiya Uriu, Jiri Zahradnik, Izumi Kida, Yuki Anraku, Hesham Nasser, Maya Shofa, Yoshitaka Oda, Spyros Lytras, Naganori Nao, Yukari Itakura, Sayaka Deguchi, Rigel Suzuki, Lei Wang, MST Monira Begum, Shunsuke Kita, Hisano Yajima, Jiei Sasaki, Kaori Sasaki-Tabata, Ryo Shimizu, Masumi Tsuda, Yusuke Kosugi, Shigeru Fujita, Lin Pan, Daniel Sauter, Kumiko Yoshimatsu, Saori Suzuki, Hiroyuki Asakura, Mami Nagashima, Kenji Sadamasu, Kazuhisa Yoshimura, Yuki Yamamoto, Tetsuharu Nagamoto, Gideon Schreiber, Katsumi Maenaka, The Genotype to Phenotype Japan (G2P-Japan) Consortium, Takao Hashiguchi, Terumasa Ikeda, Takasuke Fukuhara, Akatsuki Saito, Shinya Tanaka, Keita Matsuno, Kazuo Takayama, Kei Sato; Virological characteristics of the SARS-CoV-2 XBB variant derived from recombination of two Omicron subvariants, Nature Communications; in press.

(2) Martin Müller, Alexandra Herrmann, Shigeru Fujita, Keiya Uriu, Carolin Kruth, Adam Strange, Jan E. Kolberg, Markus Schneider, Jumpei Ito, Armin Ensser, The Genotype to Phenotype Japan (G2P-Japan) Consortium, Kei Sato, Daniel Sauter; SARS-CoV-2 ORF3c suppresses immune activation by inhibiting innate sensing; bioRxiv, 2023.

<Patent Applications>

None

Days of visits to IMSUT

Name	Position, Institution	Sex	Age	Visits to IMSUT (Days)
Daniel Sauter	Professor, University Hospital Tübingen, Germany	Male	**40 or older**	89
Name	**Position, Institution**	**Sex**	**Age**	**Online Meetings (Days)**
N/A	N/A	Pull-down▼	Pull-down▼	N/A
Name	**Position, Institution**	**Sex**	**Age**	**Discussions via E-mail, Slack, etc. (Days)**
Daniel Sauter	Professor, University Hospital Tübingen, Germany	Male	**40 or older**	60
Martin Müller	Graduate student, University Hospital Tübingen, Germany	Male	**35 or younger**	5

Usage of Facilities/Equipment

Name of Facility	Equipment	Number of Use (Times)	Usage time (Hours)
FACS Core Laboratory	e.g.) FACS Aria (BD)	N/A	N/A
Medical Proteomics Laboratory	e.g.) Orbitrap QSTAR Elite	N/A	N/A
Imaging Core Laboratory	e.g.) Zeiss Multiphoton Microscopy（LSM710NLO)	N/A	N/A

Gene Manipulated Mouse Section	Creation and cryopreservation embryo of Knockout mouse	N/A	N/A
Human Genome Center	Supercomputer	N/A	N/A
Amami Laboratory of Injurious Animals	Experimental lab	N/A	N/A
Other		N/A	N/A

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

Usage of Database	
Name of Database	**Number of Use (Times)**
N/A	N/A