Department of Microbiology and Immunology

Division of Molecular Virology
ウイルス病態制御分野

Professor	Yasushi Kawaguchi, D.V.M., Ph.D.	教　授　博士(獣医学)	川　口　　寧
Associate Professor	Akihisa Kato, Ph.D.	准教授　博士(医学)	加　藤　哲　久
Assistant Professor	Naoto Koyanagi, Ph.D.	助　教　博士(生命科学)	小　柳　直　人
Assistant Professor	Yuhei Maruzuru, Ph.D.	助　教　博士(生命科学)	丸　鶴　雄　平
Assistant Professor	Kousuke Takeshima, Ph.D.	助　教　博士(医学)	竹　島　功　高

In our laboratory, we are promoting strategic fundamental research aimed at developing a novel method of viral infection control by elucidating the mechanism underlying viral proliferation/pathology. Moreover, using viruses as a biological probe, we are also challenging next-generation virology to reconsider viruses as a homeostasis factor and explore their significance, in addition to unraveling cells and physiological control mechanisms that cannot be elucidated by research on normal human hosts.

1. Herpes simplex virus 1 evades APOBEC1-mediated immunity via its uracil-DNA glycosylase in mice

Akihisa Kato, Hayato Harima, Yuji Tsunekawa, Manabu Igarashi, Kouichi Kitamura, Kousho Wakae, Tomoaki Nishiyama, Satoru Morimoto, Toru Suzuki, Hiroko Kozuka-Hata, Masaaki Oyama, Daisuke Motooka, Mizuki Watanabe, Kousuke Takeshima, Yuhei Maruzuru, Naoto Koyanagi, Hideyuki Okano, Toshifumi Inada, Takashi Okada, Masamichi Muramatsu and Yasushi Kawaguchi

Herpes simplex virus 1 (HSV-1) is the most common cause of viral encephalitis, which can be lethal or result in severe neurological defects despite antiviral therapy. The apolipoprotein B messenger-RNA editing enzyme, catalytic polypeptide-like (APOBEC) group of proteins can act as viral restriction factors. How HSV-1 evades this intrinsic immune mechanism is unclear. Here, using human carcinoma HEp-2 cells, we find that phosphorylation and therefore activation of HSV-1 uracil-DNA glycosylase counteracts mouse APOBEC1 DNA-editing activity on HSV-1 genomes. This protects viral genomes, promotes viral replication and encephalitis in the central nervous system of mice. Presence of Apobec1 improved encephalitis outcomes in mice challenged with HSV-1 carrying a mutation in the phosphorylation site of uracil-DNA glycosylase. Treatment with an UNG inhibitor, adeno-associated virus vector expressing UGI, protected wild-type HSV-1-infected mice from lethal encephalitis. These findings identify uracil-DNA glycosylase as a viral factor enabling evasion from intrinsic antiviral immunity mediated by APOBEC1 in the central nervous system and suggests a potential therapeutic approach to treat HSV-1 encephalitis.

2. Regulatory mimicry of cyclin-dependent kinases by a conserved herpesvirus protein kinase

Naoto Koyanagi, Kowit Hengphasatporn, Akihisa Kato, Moeka Nobe, Kosuke Takeshima, Yuhei Maruzuru, Katsumi Maenaka, Yasuteru Shigeta and Yasushi Kawaguchi

Herpesviruses encode conserved protein kinases (CHPKs) that target cellular cyclin-dependent kinase (CDK) phosphorylation sites; thus, they are termed viral CDK-like kinases. Tyrosine 15 in the GxGxxG

motifs of CDK1 and CDK2, whose phosphorylation down-regulates their catalytic activities, is conserved in the corresponding motifs of CHPKs. We found that CHPK UL13, the corresponding Tyr-162 in herpes simplex virus 2 (HSV-2), was phosphorylated in HSV-2-infected cells. Mutational analyses of HSV-2 UL13 Tyr-162 suggested that phosphorylation of UL13 Tyr-162 reduced the phosphorylation of all UL13 substrates tested in HSV-2-infected cells. These findings suggested that HSV-2 UL13 mimicked the regulatory mechanism of CDKs and that this CHPK has regulatory and functional mimicry with CDKs. Furthermore, phosphorylation of HSV-2 UL13 Tyr-162 was suggested to be required for the downregulation of viral replication and pathogenicity, specifically in the brains of mice, and for efficient viral recurrence in guinea pigs. These findings highlight the dual impact of the regulatory mimicry of CDKs by CHPK on the fine-tuned regulation of lytic and latent HSV-2 infections in vivo.

3. Direct relationship between protein expression and progeny yield of herpes simplex virus 1

Moeka Nobe, Yuhei Maruzuru, Kosuke Takeshima, Fumio Maeda, Hideo Kusano, Raiki Yoshimura, Takara Nishiyama, Hyeongki Park, Yoshitaka Kozaki, Shingo Iwami, Naoto Koyanagi, Akihisa Kato, Tohru Natsume, Shungo Adachi and Yasushi Kawaguchi

Although viral protein expression and progeny virus production were independently shown to be highly heterogeneous in individual cells, their direct relationship, analyzed by considering their heterogeneities, has not been investigated to date. To elucidate the direct relationship between viral protein expression and progeny virus production, we constructed a reporter herpes simplex virus 1 (HSV-1) by tagging Venus to the late protein Us11. We then separated the HSV-1-infected cell population into multiple subpopulations according to the fluorescence intensity of Venus—which reflected the expression of L proteins, largely constituting virion structural proteins—and titrated virus yields and performed electron microscopic analysis in each subpopulation. Our results revealed that infectious progeny virus production, as well as nucleocapsid maturation, was triggered only when L protein expression exceeded a specific threshold. This suggested the existence of a rate-limiting step in progeny virus production, with nucleocapsid maturation potentially being one such step.

4. Identification of viral activators of the HSV-2 UL13 protein kinase

Naoto Koyanagi, Kousuke Takeshima, Saori Shio, Yuhei Maruzuru, Akihisa Kato and Yasushi Kawaguchi.

Although previous studies reported that the herpes simplex virus 2 (HSV-2) UL13 protein kinase mediates the phosphorylation of elongation factor 1δ (EF-1δ) in infected cells, we found here that individual expression of UL13 was insufficient to induce phosphorylation of EF-1δ in mammalian cells. This led us to hypothesize that HSV-2 UL13 requires viral cofactors for full kinase activity and prompted us to identify such cofactors. Our results were as follows. (i) Co-expression of UL13 with UL55 or Us10 significantly enhanced phosphorylation of EF-1δ compared to UL13 alone. (ii) UL13 was co-precipitated with UL55 or Us10 upon co-expression, and its kinase activity was significantly increased in their presence, as demonstrated by in vitro kinase assays. (iii) In HSV-2-infected cells, UL13 was co-precipitated with Us10 and UL55. (iv) The UL55-null mutation significantly reduced phosphorylation of EF-1δ in HSV-2-infected cells, whereas the Us10-null mutation had little effect; however, the double-null mutation further decreased the phosphorylation compared to the UL55-null mutation alone. (v) The UL55-null mutation, but not the Us10-null mutation, significantly reduced HSV-2 replication and cell-cell spread in U2OS cells to levels comparable to those observed with the UL13 kinase-dead mutation. These results suggest that UL55 acts as a principal activator of UL13 in HSV-2-infected cells, whereas Us10 serves as an auxiliary activator. Moreover, the role of UL13 kinase activity in HSV-2 replication and cell-cell spread in U2OS cells appears to be largely dependent on UL55.

5. Impact of the changes in substrate specificity of herpes simplex virus 1 protein kinase Us3 on viral infection in vitro and in vivo

Saori Shio, Akihisa Kato, Jurika Kawasaki, Kosuke Takeshima, Yuhei Maruzuru, Naoto Koyanagi, Hayato Harima and Yasushi Kawaguchi

A serine-threonine protein kinase (PK), Us3, encoded by herpes simplex virus 1 (HSV-1), shares substrate specificity with host cellular PKs, protein kinase A (PKA), and AKT. Many Us3 substrates have been identified, and it is thought that during HSV-1 infection, Us3 fine-tunes the phosphorylation levels of individual substrates within their repertoire. However, the significance of this regulatory fine-tuning by Us3 during HSV-1 infection is poorly understood. Here, we found alanine at position 326 (Ala-326) in Us3 was required for the proper fine-tuning of Us3-mediated phosphorylation across the target repertoire in HSV-1-infected cells. Using recombinant viruses in which Us3 Ala-326 was replaced with valine (A326V) or isoleucine (A326I), we showed these mutations selectively altered the phosphorylation of only a subset of at least 14 Us3 target proteins tested in HSV-1-in-

fected cells, with each mutation generally affecting different targets. Of note, (i) both mutations significantly reduced plaque sizes without affecting viral replication in cell cultures; (ii) the Us3 A326I mutation impaired viral replication in the brains of mice and improved survival following intracranial infection, whereas the Us3 A326V mutation had little effect; and (iii) the Us3 A326V mutation reduced ocular pathogenic manifestations and viral replication in the trigeminal ganglia and brains of mice, thereby improving survival following ocular infection. Taken together, these results suggest that the proper fine-tuning of Us3-mediated phosphorylation across its target repertoire is required for efficient cell-to-cell spread of HSV-1 in vitro, and its replication and pathogenicity in vivo.

6. Monoclonal antibodies against human TMPRSS2 prevent infection by any SARS-CoV-2 variant

Michishige Harada, Takehisa Matsumoto, Mizuki Yamamoto, Jin Goda, Akiko Idei, Kenichi Ohtaki, Natsuki Kojima, Natsumi Yoneda, Kosuke Miyauchi, Kazushige Katsura, Mariko Ikeda, Kazuharu Hanada, Yoshiko Ishizuka-Katsura, Toshiaki Hosaka, Tamao Hisano, Toshie Kaizuka, Takako Yamamoto, Masashi Matsuda, Manabu Nakayama, Akiko Sugimoto-Ishige, Machie Sakuma, Rina Hashimoto, Kazuo Takayama, Misako Nakayama, Cong Thanh Nguyen, Hirohito Ishigaki, Yasushi Itoh, Yoshinobu Hashizume , Minoru Yoshida, Yasushi Kawaguchi, Makoto Takeda, Haruhiko Koseki, Mikako Shirouzu, Jun-ichiro Inoue and Takashi Saito

The transmembrane serine protease 2 (TMPRSS2) plays a critical role in SARS-CoV-2 infection by priming the viral Spike (S) protein for host cell entry and thus represents a potential target for COVID-19 therapy. Here monoclonal antibodies (mAbs) against human TMPRSS2 were established for therapeutic application. In vitro infection by SARS-CoV-2 of cell lines and human lung organoids was strongly inhibited by the TMPRSS2 mAbs. These mAbs inhibited infection of all SARS-CoV-2 variants tested including omicron. mAbs recognized epitopes different from the enzymatic active site and did not inhibit protease activity, suggesting blockade of steric interactions of S protein-ACE2/TMPRSS2. The inhibitory activity of the mAbs in vivo was examined in human ACE2/TMPRSS2-double knock-in mouse and macaque models. Analysis of viral titers and histopathological analysis of the lung in these infected animals indicated that the TMPRSS2 mAb effectively suppressed viral titers and induction of inflammation in vivo.

7. ESCRT-III is recruited by human herpesvirus 6A nuclear egress complex to promote nuclear egress of the nucleocapsid

Aila Gulijiahani, Jun Arii, Vuk Isakovic, Jing Rin Huang, Yasushi Kawaguchi and Yasuko Mori

Herpesviruses replicate their genomes and package them into capsids within the host cell nucleus. These capsids must then translocate from the nucleus to the cytoplasm through a process designated nuclear egress. The virus-encoded nuclear egress complex (NEC), consisting of a nuclear matrix protein and a nuclear membrane protein, plays a crucial role in this process. Although the role of NEC for nucleo-cytoplasmic transport of capsids is conserved in Herpesviridae, some of the binding partners of the NEC components are specific for individual viruses. The NECs of alpha- and gammaherpesviruses recruit the Endosomal Sorting Complex Required for Transport III (ESCRT-III) to the inner nuclear membrane for efficient nuclear egress of capsids. In contrast, the role of ESCRT-III for nuclear egress of betaherpesviruses, including human cytomegalovirus (HCMV) and human herpesvirus 6A (HHV-6A), has not been elucidated. Here, we show that ESCRT-III is recruited to the nuclear rim in cells expressing the NEC of HCMV or of HHV-6A. Inhibition of ESCRT-III impaired HHV-6A replication and nuclear egress of the capsids. Mechanistically, ESCRT-III adaptor ALIX interacts with HHV-6A NEC membrane protein U34 and thus contributes to HHV-6A replication. From these observations, we conclude that, like at least some alpha- and gammaherpesviruses, HHV-6A NEC recruits ESCRT-III through ALIX to promote viral capsid nuclear egress.

8. Unveiling the impact of simulated microgravity on HSV-1 infection, neuroinflammation, and endogenous retroviral activation in SH-SY5Y cells

Seyedesomaye Jasemi, Elena Rita Simula, Yasushi Kawaguchi & Leonardo Antonio Sechi

Microgravity (μg) during spaceflight affects cellular and molecular functions of both human cells and microbial pathogens, influencing viral replication and the host immune system. This study aimed to investigate the effects of simulated μg on Herpes Simplex Virus-1 (HSV-1) replication, host pro-inflammatory cytokine, and human endogenous retrovirus (HERV) activation in human neuroblastoma SH-SY5Y cells. Our results show that μg has a negative impact on HSV-1 replication, leading to significantly reduced viral titers and lower expression levels of HSV-1 early genes (ICP0, ICP4, and ICP27) compared to 1 gravity (1 g) conditions. Interestingly, despite lower viral titers and HSV-1 gene expressions under μg condition,

we observed higher levels of HERVs and pro-inflammatory cytokine gene expression. In addition, there was a significant correlation between HSV-1 immediate-early genes with HERVs and pro-inflammatory cytokine gene expression, with stronger correlations observed under μg conditions. Taken together, μg reduces HSV-1 replication and increases host pro-inflammatory and HERVs gene expression, which demands further investigation for human health protection in space.

Publications

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