Laboratory Animal Research Center
実験動物研究施設

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Our major research interests are to elucidate molecular mechanisms of pathogenicity and species specificity of negative and single strand RNA viruses (Mononegavirales), and to control viral diseases. For these purposes, we are studying virus replication and identifying viral and host factors important for the expression of pathogenicity using a novel reverse genetics technique. We are also developing new virus vaccines, virus vectors, and oncolytic virus by genetic engineering. In the animal research center, more than 30,000 mice, mainly transgenic or knockout, are kept for research of IMSUT, and the technical staff support their breeding, frozen storage of eggs and microbiological cleaning.

Possible role of the Nipah virus V protein in the regulation of the interferon beta induction by interacting with UBX domain-containing protein1.

Uchida S, Horie R, Sato H, Kai C, Yoneda M.

Nipah virus (NiV) is a highly pathogenic paramyxovirus that causes lethal encephalitis in humans. We previously reported that the V protein, one of the three accessory proteins encoded by the P gene, is one of the key determinants of the pathogenesis of NiV in a hamster infection model. Satterfield B.A. et al. have also revealed that V protein is required for the pathogenicity of henipavirus in a ferret infection model. However, the complete functions of NiV V have not been clarified. In this study, we identified UBX domain-containing protein 1 (UBXN1), a negative regulator of RIG-I-like receptor signaling, as a host protein that interacts with NiV V. NiV V interacted with the UBX domain of UBXN1 via its proximal zinc-finger motif in the C-terminal domain. NiV V increased the level of UBXN1 protein by suppressing its proteolysis. Furthermore, NiV V suppressed RIG-I and MDA5-dependent interferon signaling by stabilizing UBXN1 and increasing the interaction between MAVS and UBXN1 in addition to directly interrupting the activation of MDA5. Our results suggest a novel molecular mechanism by which the induction of interferon is potentially suppressed by NiV V protein via UBXN1.

Gene end-like sequences within the 3' non-coding region of the Nipah virus genome attenuate viral gene transcription.

Sugai, A., Sato, H., Yoneda, M. and Kai, C.

The regulation of transcription during NiV replication is poorly understood. Using a bicistronic minigenome system, we investigated the involvement of non-coding regions (NCRs) in the transcriptional re-initiation efficiency of NiV RNA polymerase. Reporter assays revealed that attenuation of NiV gene expression was not constant at each gene junction, and that the attenuating property was controlled by the 3' NCR. However, this regulation was independent of the gene-end, gene-start and intergenic regions. Northern blot analysis indicated that regulation of viral gene expression by the phosphoprotein (P) and large protein (L) 3' NCRs occurred at the transcription level. We identified

uridine-rich tracts within the L 3' NCR that are similar to gene-end signals. These gene-end-like sequences were recognized as weak transcription termination signals by the viral RNA polymerase, thereby reducing downstream gene transcription. Thus, we suggest that NiV has a unique mechanism of transcriptional regulation.

Efficacy of recombinant measles virus expressing highly pathogenic avian influenza virus (HPAIV) antigen against HPAIV infection in monkeys

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Highly pathogenic avian influenza virus (HPAIV) is a serious threat not only to domestic fowls but also to humans. Vaccines inducing long-lasting immunity against HPAIV are required. In the present study, we generated recombinant measles virus (MV) expressing the hemagglutinin protein of HPAIV without the multibasic site necessary for its pathogenicity in chickens using the backbone of an MV vaccine strain (rMV-Ed-H5HA) or a wild-type MV-derived mutant (rMV-HL-Vko-H5HA). We examined protective efficacy of the candidate vaccines in the monkey infection model by the challenge with a HPAIV (H5N1). Cynomolgus monkeys inoculated with the candidate vaccines produced both anti-H5 HA and anti-MV antibodies. They recovered earlier from influenza symptoms than unvaccinated monkeys after the challenge with the HPAIV strain. Chest radiography and histopathological analyses confirmed less severe pneumonia in the vaccinated monkeys. Vaccination tended to suppress viral shedding and reduced the interleukin-6 levels in the lungs. Furthermore, the vaccination with rMV-Ed-H5HA of monkeys with pre-existing anti-MV immunity induced the production of anti-H5 HA antibodies. These results suggest that both candidate vaccines effectively reduce disease severity in naïve hosts, and that rMV-Ed-H5HA is a particularly good candidate vaccine against HPAIV infection.

Publications

1. Uchida S, Horie R, Sato H, Kai C, Yoneda M. Possible role of the Nipah virus V protein in the regulation of the interferon beta induction by interacting with UBX domain-containing protein1. *Sci Rep.* 16; 8(1): 7682, 2018.

Amami Laboratory of Injurious Animals
奄美病害動物研究施設

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The Amami Laboratory of Injurious Animals was established in 1965 at Setouchi-cho in Amami-oshima Island in order to study on endemic diseases involving parasite, arthropods, and venomous snakes in the tropics or subtropics.
The Amami-oshima Island belongs to the Nansei (Southwest) Islands and the fauna is quite different from that in other islands of Japan. Since establishment of the laboratory, trials have been carried out to utilize small mammals found unique in the Amami islands as experimental animals in addition to studies on prevention of Habu bites. As well known, successful eradication of filariasis from this island is one of the monumental works of the laboratory. Our present works are as follows:

1. Research on the Habu control

Shinichi Yokota, Shosaku Hattori, Motonori Ohno[1], Naoko Oda-Ueda[2], Takahito Chijiwa[1], Aichi Yoshida[3], Yoshihiro Hayashi[4], Tomohisa Ogawa[5], and Hiroki Shibata[6],: [1]Department of Applied Life Science, Faculty of Bioscience, Sojo University, [2]Department of Biochemistry, Faculty of Pharmaceutical Science, Sojo University, [3]School of Health Science, Faculty of Medicine, Kagoshima University, [4]National Museum of Nature and Science, Tokyo,[5]Faculty of Agriculture, Tohoku University, [6]Medical Institute of Bioregulation, Kyushu University

Snake bites by the venomous snake Habu, *Protobothrops flavoviridis*, have been reported annually about 60 cases in the population of 100,000 in the Amami Islands. Moreover, there is no indication that the population of the Habu itself has decreased, despite a campaign for capture of snakes by the Kagoshima Prefectural Government. Rat-baited box traps have been introduced to catch the snakes and found to be quite effective. However, maintenance of live rats requires man power and its cost is expensive. Therefore, our effort has been focused on the development of attractant for Habu. The attractant extracted from rats seems ineffective if compared with use of live rats.

It was known that the Habu survived the injection of the Habu venom since early times, because some proteins in the serum of the Habu blood combine to the elements of the Habu venom. The research of these binding proteins has been initiated with an objective of clinical trials. Phospholipase A_2 and its isozymes isolated from Habu venom have myonecrotic activity and hemorrhagic activity, and metal protease has hemorahagic activity. The binding proteins isolated from serum of Habu inhibit myonecrotic activity of phospholipase A_2 and its isozymes. We found that protein-HSF and peptide-AHP isolated from the Habu serum effectively control the hemorrhage caused by venom of the Habu, *Ovophis okinavensis, Agkistrodon blomhoffi brevicaudus, Calloselasma rhodostoma, Bitis arietans, Bothrops asper*, and, *Trimeresurus stejnegeri.*

Further, a statistics analysis and the simulation were done with the snakes captured by the Government, and the analysis of population dynamics of Habu was attempted. As a result of investigating the individual measurement data of the captured Habu over 9 years, we were able to obtain the generous age composition of the Habu. From analyzing of the age pyramid of the Habu and the result of questionnaire surveys for the inhabitant in the Amami-oshima Island, the total population of the Habu which lives in this island was estimated at about 80,000. By the analysis of the measured data

of last nine years, the snake sizes were miniaturized, and the population of young snakes decreased. According to these investigations, the population of the Habu is expected to decrease in the near future.

These studies are supported by grants from the Ministry of Land, Infrastructure and Transport and the Kagoshima Prefectural Government.

2. Reproduction of squirrel monkeys and owl monekys.

Shinichi Yokota, Shosaku Hattori, Kumiko Ikeda, and Chieko Kai

The squirrel monkey (*Saimiri boliviensis*) and the owl monkey (*Aotus lemurinus griseimenbra*) were widely distributed in the tropical rainforest in Central and South America. The advantage of using both species for medical researches resides in its small size and gentle behavior. In this laboratory, squirrel monkeys have a breeding season between winter and early spring. They are polygamy. Their puberty is 3-4 years old in females and 4-5 years old in males. Their gestation period is about 150 days. In contrast, the owl monkey is annual breeding animals. They are monogamy. Their puberty is 3 years old for both sex. Their gestation period is about 130 days. Three newborns were given in reproductive groups of squirrel monkeys in 2018, and were nursed by laboratory staffs because of neglect of their mothers.

3. Estimation of the therapeutic effect of transplantation of DFAT cells sheet on corneal epithelial defects in squirrel monkey (*Saimiri boliviensis*)

Shinichi Yokota, Shosaku Hattori, Akane Tanaka[10], Hiroshi Matsuda[10], and Chieko Kai: [10]Division of animal life science, Institute of agriculture, Tokyo University of Agriculture and Technology

Dedifferentiated fat (DFAT) cells are seemed to be a good candidate source of adult stem cells in regenerative medicine, because these cells exhibit multilineage potential as adipose-derived stem/stromal cells (ASCs). We isolated squirrel monkey DFAT cells from a small amount of adipose tissue by the ceiling culture method, and estimated the therapeutic effect of transplantation of DFAT cells sheet on corneal epithelial defects. We are currently preparing to submit the results of this study to the scientific journal.

Publications

Shibata H, Chijiwa T, Oda-Ueda N, Nakamura H, Yamaguchi K, Hattori S, et al.: 2018 The habu genome reveals accelerated evolution of venom protein genes. Sci Rep. 26: 11300.

Yokota SI, Ando M, Nakamura K, Shibata S: 2018 Combined effect of shortened photoperiod and low crude protein diet on liver triglyceride accumulation and lipid-related gene expression in quail. Livest Sci. 214 68-72.

Yokota SI, Nakamura K, Ando M, Haraguchi A, Omori K, Shibata S: 2019 A low-protein diet eliminates the circadian rhythm of serum insulin and hepatic lipid metabolism in mice. J Nutr Biochem. 63: 177-185.