Proposal for
collaborative research using unique genetically modified non-human primates

Shiga University of Medical Science (SUMS) is recruiting new research collaboration using genetically modified non-human primates (NHP) from around the world. We are highly expecting collaborative research with universities abroad, and are introducing facility and researchers.

About RCALS

SUMS have Research Center for Animal Life Science (RCALS) which is the joint-use facility for animal experimentation performed in accordance with international regulations. RCALS is mainly focused on generating and using NHP as model animal for human diseases. There are about 700 cynomolgus monkeys (*Macaca fascicularis*) in RCALS which is the largest NHP facility in Japan.

RCALS has two unique techniques for artificial breeding and genetic transformation to generate *M. fascicularis*. RCALS have generated *M. fascicularis* such as:

1. *M. fascicularis* with homozygous Major Histocompatibility Complex (MHC) haplotype which is useful in the research field of regenerative medicine using iPS cell.
2. *M. fascicularis* model of some diseases including dementia and some unpublished diseases.

RCALS is now the satellite of the Institute for Advanced Synthesis of Human Biology (ASHBi), Kyoto University which is funding from the MEXT provided under its FY2018 World Premier International Research Center Initiative (WPI) program.

As you may know, exporting gene modified *M. fascicularis* to US is very hard by the Convention on Biological Diversity (CBD), Access to genetic resources and Benefit Sharing (ABS), etc. So it is better for researchers of universities abroad to visit to SUMS to carry out collaborative research.

Contact
Academia-Industry Collaboration Section, Research Strategy Promotion Office,
Research Administration Office,
Shiga University of Medical Science

Seta Tsukinowa-cho, Otsu, Shiga 520-2192, JAPAN
Tel: +81-77-548-2936
E-mail: sangaku[a]belle.shiga-med.ac.jp (Replace [a] to @ when sending email)

Researchers in Medical Research using Cynomolgus Monkeys

Kazumasa Ogasawara (Professor, Div. Pathology and Disease Regulation, Director of Academic Affairs and Research and Vice President of SUMS)

Research Interest:

He is interested in establishment of disease models of cynomolgus monkeys for preclinical studies. Mice are used as disease models, but all mice disease models do not reproduced human disease. Thus, he focuses on establishment of disease models in none but cynomolgus monkeys. In addition, he is interested in transplantation using cynomolgus monkeys, which are identified for major histocompatibility complex (MHC). Indeed, the MHC-identified cynomolgus monkeys were used in the transplantation experiments of differentiated iPS cells, such as neurons, retinal pigment epithelial cells and cardiomyocytes.

Articles:

1. Sugita S, Iwasaki Y, Makabe K, Kamao H, Mandai M, Shiina T, Ogasawara K, Hirami Y, Kurimoto Y, Takahashi M. Successful Transplantation of Retinal Pigment Epithelial Cells from MHC Homozygote iPSCs in MHC-Matched Models. *Stem Cell Reports* **7(4)**, 635-648 (2016), doi: 10.1016/j.stemcr.2016.08.010
2. Kawamura, T, Miyagawa S, fukushima S, Maeda A, Kashiyama N, Kawamura A, Miki K, Okita K, Yoshida Y, Shiina T, Ogasawara K, Miyagawa S, Toda K, Okuyama H, Sawa Y. Cardiomyocytes derived from MHC-homozygous induced pluripotent stem cells exhibit reduce allogeneic immunogenicity in MHC-matched non-human primates. *Stem Cell Reports* **6(3)**, 312-320 (2016), doi: 10.1016/j.stemcr.2016.01.012
3. Morizane A, Kikuchi T, Hayashi T, Mizuma H, Takara S, Doi H, Mawatari A, Glasser MF, Shiina T, Ishigaki H, Itoh Y, Okita K, Yamasaki E, Doi D, Onoe H, Ogasawara K, Yamanaka S, Takahashi J. MHC matching improves engraftment of iPSC-derived neurons in non-human primates. *Nat Commun* **8(1)**, 385 (2017), doi: 10.1038/s41467-017-00926-5
4. Ishigaki H, Maeda T, Inoue H, Akagi T, Sasamura T, Ishida H, Inubushi T, Okahara J, Shiina T, Nakayama M, Itoh Y, Ogasawara K. Transplantation of iPS-derived tumor cells with a homozygous MHC haplotype induces GRP94 antibody production in MHC-matched macaques. *Cancer Res* **77(21)**, 1-10 (2017), doi: 10.1158/0008-5472.CAN-17-0775
5. Ishigaki H, Shiina T, Ogasawara K. MHC-identical and transgenic cynomolgus macaques for preclinical studies. *Inflamm Regen* **38**, 30 (2018), doi: 10.1186/s41232-018-0088-3

Yasushi Itoh (Associate professor, Div. Pathology and Disease Regulation)
Research Interest:

He has been developed two disease models using cynomolgus monkeys: influenza and premature aging. Various influenza viruses including seasonal and highly pathogenic avian influenza viruses cause clinical signs of diseases in macaques as seen in humans. The premature aging model of genome-edited macaques is expected to show age-related metabolic disorders and tumors. Since macaques' immunity and metabolism are similar to humans', the macaque model is suitable to evaluate the efficacy of vaccines and drugs as preclinical studies.

Articles:

1. Itoh Y, Shinya K, Kiso M, Watanabe T, Sakoda Y, Hatta M, Muramoto Y, Tamura D, Sakai-Tagawa Y, Noda T, Sakabe S, Imai M, Hatta Y, Watanabe S, Li C, Yamada S, Fujii K, Murakami S, Imai H, Kakugawa S, Ito M, Takano R, Iwatsuki-Horimoto K, Shimojima M, Horimoto T, Goto H, Takahashi K, Makino A, Ishigaki H, Nakayama M, Okamatsu M, Takahashi K, Warshauer D, Shult PA, Saito R, Suzuki H, Furuta Y, Yamashita M, Mitamura K, Nakano K, Nakamura M, Brockman-Schneider R, Mitamura H, Yamazaki M, Sugaya N, Suresh M, Ozawa M, Neumann G, Gern J, Kida H, Ogasawara K, Kawaoka Y. *In vitro* and *in vivo* characterization of new swine-origin H1N1 influenza viruses. *Nature* **460**, 1021-1025 (2009), doi: 10.1038/nature08260
2. Kitano M, Itoh Y, Ishigaki H, Nakayama M, Ishida H, Pham VL, Arikata M, Shichinohe S, Tsuchiya H, Kitagawa N, Kobayashi M, Yoshida R, Sato A, Le QM, Kawaoka Y, Ogasawara K. Efficacy of repeated intravenous administration of peramivir against highly pathogenic avian influenza A (H5N1) virus in cynomolgus macaques. *Antimicrob Agents Chemother* **58(8)**, 4795-4803 (2014), doi: 10.1128/AAC.02817-14
3. Itoh Y, Yoshida R, Shichinohe S, Higuchi M, Ishigaki H, Nakayama M, Pham VL, Ishida H, Kitano M, Arikata M, Kitagawa N, Mitsuishi Y, Ogasawara K, Tsuchiya H, Hiono T, Okamatsu M, Sakoda Y, Kida H, Ito M, Le QM, Kawaoka Y, Miyamoto H, Ishijima M, Igarashi M, Suzuki Y, Takada A. Protective efficacy of passive immunization with monoclonal antibodies in animal models of H5N1 highly pathogenic avian influenza virus infection. *PLoS Pathog* **10(6)**, e1004192 (2014), doi: 10.1371/journal.ppat.1004192
4. Itoh Y, Shichinohe S, Nakayama M, Igarashi M, Ishii A, Ishigaki H, Ishida H, Kitagawa N, Sasamura T, Shiohara M, Doi M, Tsuchiya H, Nakamura S, Okamatsu M, Sakoda Y, Kida H, Ogasawara K. Emergence of H7N9 influenza A virus resistant to neuraminidase inhibitors in nonhuman primates. *Antimicrob Agents Chemother* **59(8)**, 4962-4973 (2015), doi: 10.1128/AAC.00793-15
5. Morizane A, Kikuchi T, Hayashi T, Mizuma H, Takara S, Doi H, Mawatari A, Glasser MF, Shiina T, Ishigaki H, Itoh Y, Okita K, Yamasaki E, Doi D, Onoe H, Ogasawara K, Yamanaka S, Takahashi J. MHC matching improves engraftment of iPSC-derived neurons in non-human primates. *Nat Commun* **8(1)**, 385 (2017), doi: 10.1038/s41467-017-00926-5

Masatsugu Ema (Professor, Dept. Stem Cells and Human Disease Models, Director of RCALS)
Research Interest:

Nonhuman primates (NHPs) are considered one of the most valuable animal models, because NHPs are closer to humans in organ size and anatomical structure. So far, he has established techniques to create transgenic and genome editing cynomolgus monkeys. By using these techniques, he has explored an intractable human disease, Autosomal dominant polycystic kidney disease (ADPKD) with CRISPR/Cas9 technique, and demonstrated that targeted disruption of PKD1, a causative gene for ADPKD can recapitulate the human ADPKD pathology. He believes that disease modeling with genetically modified-cynomolgus monkey will open the way for the elucidation of molecular mechanism of human diseases and new therapeutic approaches.

Articles:

1. Seita Y, Tsukiyama T, Azami T, Kobayashi K, Iwatani C, Tsuchiya H, Nakaya M, Tanabe H, Hitoshi S, Miyoshi H, Nakamura S, Kawauchi A, Ema M, Comprehensive evaluation of ubiquitous promoters suitable for the generation of transgenic cynomolgus monkeys. *Biol Reprod.* ioz040 (2019), doi: 10.1093/biolre/ioz040
2. Seita Y, Iwatani C, Tsuchiya H, Nakamura S, Kimura F, Murakami T, Ema M, Poor second ovarian stimulation in cynomolgus monkeys (*Macaca fascicularis*) is associated with the production of antibodies against human follicle-stimulating hormone. *J Reprod Dev*, Mar 7 (2019), doi: 10.1262/jrd.2018-156
3. Azami T, Waku T, Matsumoto K, Jeon H, Muratani M, Kawashima A, Yanagisawa J, Manabe I, Nagai R, Kunath T, Nakamura T, Kurimoto K, Saitou M, Takahashi S, Ema M, *Klf5* maintains the balance of primitive endoderm versus epiblast specification during mouse embryonic development by suppression of *Fgf4*. *Development* **144(20)**, 3706-3718 (2017), doi: 10.1242/dev.150755
4. Seita Y, Tsukiyama T, Iwatani C, Tsuchiya H, Matsushita J, Azami T, Okahara J, Nakamura S, Hayashi Y, Hitoshi S, Itoh Y, Imamura T, Nishimura M, Tooyama I, Miyoshi H, Saitou M, Ogasawara K, Sasaki E, Ema M, Generation of transgenic cynomolgus monkeys that express green fluorescent protein throughout the whole body. *Sci Rep.* **6**, 24868 (2016), doi: 10.1038/srep24868

Ikuo Tooyama (Professor, Dept. Diagnostics and Therapeutics for Brain Diseases, Director of Molecular Neuroscience Research Center (MNRC))

Research Interest:

He is interested in Alzheimer's disease (AD) and related disorders. His research team have developed several novel ligands for amyloid imaging or tau-imaging using ^{19}F-MRI, such as Shiga-X and Shiga-Y. Using these chemicals he has succeeded in amyloid and tau imaging in the brain of transgenic mouse models such as Tg2576, APP/PS1 and rTg4510. Some of them have therapeutic potentials in transgenic mouse models of AD. However, it is well known that these rodent models do not show the same neuropathology as human Alzheimer's disease. Thus, he has collaborated with Professor Ema in RCALS and developed monkey models over-expressing mutant APP gene. These monkey models should provide us valuable tools to evaluate diagnostic and/or therapeutic effects as well as toxicity of chemicals targeting AD.

Articles:

1. Yanagisawa D, Ibrahim NF, Taguchi H, Morikawa S, Kato T, Hirao K, Shirai N, Sogabe T, Tooyama I. Fluorine-19 magnetic resonance imaging probe for the detection of tau pathology in female rTg4510 mice. *J Neurosci Res* **96(5)**, 841-851 (2018), doi: 10.1002/jnr.24188
2. Tooyama I, Yanagisawa D, Taguchi H, Kato T, Hirao K, Shirai N, Sogabe T, Ibrahim NF, Inubushi T, Morikawa S. Amyloid imaging using fluorine-19 magnetic resonance imaging (^{19}F-MRI). *Ageing Res Rev* **30**, 85-94 (2016), doi: 10.1016/j.arr.2015.12.008
3. Yanagisawa D, Ibrahim NF, Taguchi H, Morikawa S, Hirao K, Shirai N, Sogabe T, Tooyama I. Curcumin derivative with the substitution at C-4 position, but not curcumin, is effective against amyloid pathology in APP/PS1 mice. *Neurobiol Aging* **36(1)**, 201-210 (2015), doi: 10.1016/j.neurobiolaging.2014.07.041
4. Yanagisawa D, Taguchi H, Ibrahim NF, Morikawa S, Shiino A, Inubushi T, Hirao K, Shirai N, Sogabe T, Tooyama I. Preferred features of a fluorine-19 MRI probe for amyloid detection in the brain. *J Alzheimer's Dis* **39(3)**, 617-631 (2014), doi: 10.3233/JAD-131025
5. Yanagisawa D, Amatsubo T, Morikawa S, Taguchi H, Urushitania M, Shirai N, Hirao K, Shiino A, Inubushid T, Tooyama I. *In vivo* detection of amyloid β deposition using ^{19}F magnetic resonance imaging with a ^{19}F-containing curcumin derivative in a mouse model of Alzheimer's disease. *Neuroscience* **184**, 120-127 (2011), doi: 10.1016/j.neuroscience.2011.03.071