Frontier Research Initiative
フロンティア研究拠点

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‹Tae-Hwa Chun Group›

Proteolytic tissue remodeling defines the progression of metabolic diseases, such as obesity, diabetes and cardiovascular diseases as well as cancer. A cohort of proteolytic enzymes uniquely regulates cell shape and function in three-dimensional (3-D) microenvironment. Our research efforts are focused on identifying the molecular mechanisms by which proteolytic machinery regulates 3-D cell shape and function during the progression of metabolic diseases.

1. Role of Pericellular Collagenolysis in Obesity Initiation

Tae-Hwa Chun, Mayumi Inoue[1]: [1]University of Michigan

Membrane-type matrix metalloproteinase (MT 1-MMP) is the major pericellular collagenase that plays a critical role in regulating the differentiation and morphogenesis of mesenchymal cells (Chun et al., Cell 2006; JCB 2004). To define the role of MT1-MMP as a potent modifier of adipose tissue mass and function, we employed high fat diet-induced obesity model. Upon high fat diet (45% fat) feeding for two weeks, both inguinal and perigonadal fat pads significantly increase in their sizes (2-2.5 folds) in wild-type, 3 months old, male C57BL/6 mice. At this stage, massive degradation of type I collagen fibers was detected. Interestingly, type I collagen degradation was coupled with increased de novo synthesis of type I and type III collagens reflecting active remodeling of collagenous scaffold in vivo. In MT1-MMP haploinsufficient (+/−) mice, however, high fat diet-induced increase of adiposity was suppressed by 50%. Concurrently, type I collagen cleavage was blocked almost completely. Scanning electron microscopy revealed disrupted thick type I collagen fibers that

enwrap adipocytes and dysmorphic adipocytes of variable diameters in wild-type mice, while intact collagen fibers and uniform appearance of adipocytes were noted in MT1-MMP$^{+/-}$ mice. Transcriptome analyses revealed that lipid biosynthetic process was overrepresented together with collagen catabolism and integrin signaling pathway in wild-type inguinal fat pads. Contrary to the functional coupling of collagen catabolism and lipid biosynthesis found in wild-type mice, gene expression profile of MT1-MMP$^{+/-}$ inguinal fat pads was uncoordinated and dispersed among diverse pathways. These results suggest a central role played by MT1-MMP in regulating adipose tissue function via pericellular collagenolysis at a very early stage of obesity progression. We are currently investigating the role of insulin-dependent signaling pathway acting at the downstream of collagenolysis in regulating adipose tissue size. Some of these results were presented in the symposia at Keystone Symposium, NIH Workshop, and Endocrine Society in 2008.

2. Epigenetic Regulation of Adipocyte Differentiation by Collagenolysis

Kaori Sato Kusubata, Tae-Hwa Chun

Our preliminary data suggest that MT1-MMP-dependent collagenolysis regulates both cell shape and tension in collagen-rich microenvironment. Meanwhile, nuclear shape and chromatin structure are known to be coordinated with cell shape and tension. As such, we hypothesize that MT1-MMP-dependent pericellular collagenolysis may regulate adipogenesis by modifying the dynamic linkage of cell shape to nuclear structure and function. Adipocyte precursor cells (3T3L1 preadipocytes) change their cell shape at an early stage of adipogenesis. We observed rapid nuclear condensation and the acetylation of histone H3 lysine 9 (H3K9) following the cell shape change. H3K9 acetylation triggers a cascade of adipogenic gene expression. This induction of H3K9 acetylation, however, was not affected in the presence of a broad spectrum metalloenzyme inhibitor (GM6001) when preadipocytes are cultured atop regular plastic plates. When cultured atop type I collagen fibers, preadipocytes were still able to undergo the same pattern of cell shape change and H3K9 acetylation. However, on top of collagen fibrils, H3K9 acetylation in response to adipogenic mix was markedly inhibited by GM6001. This inhibition was further recapitulated by an endogenous MMP inhibitor, TIMP-2, indicating MMPs as responsible modifiers of adipogenic histone acetylation in collagen-rich microenvironment. Moreover, underscoring the importance of MMP/type I collagen axis, when preadipocytes were cultured atop degraded type I collagen, the inhibitory effect of TIMP-2 was no longer observed. These results suggest that MMP family regulates the histone modification necessary for adipocyte differentiation via degradation of type I collagen fibers. We aim to further define the molecular mechanisms underlying the collagenolysis-dependent regulation of chromatin structure and function in collagen-rich microenvironment.

3. MMP-Dependent Regulation of Mesenchymal Stem Cell Differentiation in 3-D Microenvironment

Tae-Hwa Chun, Jennfer H. Elisseeff[1], Shuichi Takayama[2]: [1]Johns Hopkins University, [2]University of Michigan

Recent advance in stem cell biology has widened the prospect of regenerative medicine for the treatment of chronic and devastating diseases. The behavior of stem cells in 3-D microenvironment, however, has not been defined. When human bone marrow stem cells are cultured in 3-D collagen matrix, we observed MMP plays a key role in determining the cell differentiation and gene expression. We hypothesized that extracellular tensile force and intracellular contractile force demine the cell shape and differentiation. As such, incorporating the dynamic cell-ECM interaction into static artificial nanomaterials, we should be able to expand the impact of tissue engineering on stem cell-based regenerative medicine. Our collaborative efforts with researchers in the field of tissue engineering are now providing us new insights into the roles of physical 3-D parameters that define MMP-dependent stem cell differentiation. We aim to identify the molecular mechanisms for stem cell function and differentiation in 3-D microenvironment and apply the knowledge to the development of proteolytically interactive 3-D nano-scaffold that maximizes the regenerative function of stem cells in the body.

‹Susumu Nakae Group›

Role of CCRL2 in IgE-dependent inflammation

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We generated novel monoclonal antibodies (mAb) specific for the orphan G-protein coupled receptor (GPCR) mCCRL2, and demonstrated that freshly isolated mouse peritoneal mast cells selectively express the receptor. mCCRL2-deficient mice displayed no overt phenotype and had normal numbers of mast cells in the tissues analyzed. When tested *in vitro*, bone marrow-derived cultured mast cells (BMCMCs) of mCCRL2-deficient mouse origin exhibited responses to IgE and specific antigen-mediated crosslinking of FceRI that were statistically indistinguishable from those of wild type BMCMCs. In IgE-dependent passive cutaneous anaphylaxis reactions *in vivo*, a model of IgE-mediated local anaphylaxis, mast cell-expressed CCRL2 was not required for the development of cutaneous inflammatory responses in mice sensitized with a high dose of antigen-specific IgE. However, mast cell-expressed CCRL2 was required for the development of optimal cutaneous tissue swelling and leukocyte infiltrates in mice sensitized with a low dose of antigen-specific IgE. We also identified CCRL2 ligands "chemerin" as a novel, non-signaling protein ligand for both human and mouse receptors. As opposed to chemokine interceptors that serve as a sink for chemokines upon their ligand-induced internalization, chemerin is not rapidly internalized by CCRL2; rather, CCRL2 concentrates the chemoattractant and increases local chemerin concentrations available to interact with chemokine like receptor-1 (CMKLR1). Therefore, CCRL2 is a novel attractant receptor, serving to focus chemerin localization *in vivo* and contribute to CMKLR1-mediated processes that in turn regulate pathways leading to increased vascular permeability, tissue swelling, and leukocyte recruitment.

‹Beate Heissig Group›

Stem cells can differentiate into tissue-specific cells to repair damaged tissue, a process controlled in part by proteases. The major focus of our laboratory is to understand how stem cells are regulated and how one can apply this knowledge for stem cell-based regenerative medicine. We studied the mechanism how the fibrinolytic pathway regulates myeloid-cell dependent neoangiogenesis during tissue regeneration (1) and examined the role proteinase inhibitors for the in vitro generation of embryonic cell -derived platelets (2).

1. Tissue type plasminogen activator regulates myeloid-cell dependent neoangiogenesis during tissue regeneration

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Here, we report that tissue-type plasminogen activator (tPA), by activating the extracellular protease plasmin and matrix metalloproteinase-9 (MMP-9) mobilized $CD11b^+$ myeloid cells, a process dependent on kit ligand and vascular endothelial growth factor (VEGF) signaling. tPA mobilized $CD11b^+$ cells readily incorporated into ischemic tissues during ischemic recovery. Transplantation of BM-derived tPA-mobilized $CD11b^+$ cells, and $VEGFR1^+$ cells, but not carrier -mobilized cells or CD11b- cells accelerated neovascularisation in part by augmenting the angiogenic factor VEGF-A. Inhibition of VEGF signaling suppressed tPA-induced neovascularisation in a model of hindlimb ischemia. tPA might be useful to induce therapeutic neovascularisation in the growing field of regenerative medicine.

2. Metalloproteinase regulation improves in vitro generation of efficacious platelets from mouse embryonic stem cells

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Here, we describe the generating of mouse embryonic stem cells-derived platelets (ESPs)

that contribute to hemostasis in vivo. We show that administration of metalloproteinase inhibitors during differentiation increased the expression of GPIb alpha, improving both thrombogenesis in vitro and posttransfusion recovery in vivo. Thus, the regulation of metalloproteinases in culture could be useful for obtaining high-quality, efficacious ESPs as an alternative platelet source for transfusions.

‹Riu Yamashita Group›

Analysis of multiple and alternative promoters using huge number of 5'end cDNA sequences

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In order to study transcriptional regulation, we have constructed a database, DataBase of Transcriptional Start Sites (DBTSS: http://dbtss.hgc.jp), which includes a number of 5'-end sequences produced by oligo-capping method. Recently, the database contains not only 1,540,411 mapped sequences from conventional cDNA clones, but also 296.5 million tags from eight kinds of cells (15 kinds of experimental conditions).

From these data, we could obtain 14,831,367 TSSs. After removing the TSS with less than 1 ppm tags, the number of TSSs decreased to 341,049 TSSs. Clustering these TSSs with 500 bp windows, we could obtain 75,918 promoters. These promoters could be classified into 36,251 gene regions and 39,667 intergenic regions. The promoters in gene region are in 14,307 genes. Among them, 5,428 genes have one promoter, and 8,879 genes have multiple promoters. This gives that 2.5 promoters per one gene.

To analyze the usage of promoters, we defined the promoters with the largest number of tags as the '1st promoters' and the 2nd highest promoters as the '2nd promoters' for each gene. For example, tumor protein p53 (TP53) has four promoters at around 7,531,532 (AP1: 41.4 ppm), 7,521,906 (AP4: 1.1 ppm), 7,521,565 (AP2: 9.8 ppm), 7,513,176 (AP3: 1,2 ppm) on chromosome 17. Comparing between HEK293 and ES cells, we observed that the ratio of tags for AP1/AP2 are 54.5 and 0.542, respectively. In total, 1,027 genes (14.1%) had different 1st and 2nd promoters, and 6,236 genes had the same 1st and the 2nd promoter. Then we analyzed whether the 1st promoter and the 2nd promoter are the same among different samples. Among the other cell types, the average percentage of the inversion was 28.3%, and 9.6% of difference for promoters expressed in the same cell types with different conditions. These numbers may correspond to potential alternative promoters which are regulated in the specific cells.

‹Katsuyoshi Yamamoto Group›

Negative feedback regulation in the SHO1 branch of the yeast HOG osmoregulatory MAPK pathway by induced dissociation of the Ste50 adaptor protein from the Opy2 membrane anchor

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To cope with severe external high osmolarity, the budding yeast (*Saccharomyces cerevisiae*) activates the HOG MAPK pathway, which regulates synthesis and intracellular retention of the compatible osmolyte glycerol, as well as more general stress responses. The HOG pathway is composed of two functionally redundant upstream signaling pathways, termed the SLN1 branch and the SHO1 branch. Mutants that are defective in both the branches of the HOG pathway cannot survive in high osmolarity environments.

A signal emanating from either branch converges on the common Pbs2 MAPKK, which activates the Hog1 MAPK. In the SHO1 branch, two putative transmembrane osmosensors, Msb2 and Hkr1, detect osmotic stress, and, together with the membrane anchor protein Sho1, generate an intracellular signal that leads to activation of the Ste11 MAPKKK, which then activates Pbs 2.

The adaptor protein Ste50, which binds constitutively to Ste11, and the transmembrane protein Opy2 are essential components in the SHO1 branch. Our results have suggested that Opy2 binds Ste50 to recruit the Ste50-Ste11 complex to the plasma membrane, and that the membrane localization of Ste50 is essential for activation of the SHO branch. More recently, we found that Ste50 was phosphorylated at multiple sites following osmotic stress. Interestingly, Ste50 was phosphorylated not only by the Hog1 MAPK but also by the Fus3 and Kss1 MAPKs, which are components of other yeast MAPK signaling pathways activated by the mating factor or by glycosylation defects. The phosphorylated form of Ste50 had much lower affinity to Opy2 in *in*

vitro binding assays. The yeast cells expressing the Ste50-23456A mutant protein, in which all the phosphorylation sites (Ser or Thr) were mutated to Ala, showed a prolonged Hog1 MAPK phosphorylation after osmotic stress. The same mutant was also more sensitive to activation of the HOG pathway by hyperactive Hkr1/Msb2 osmosensor mutants. These and other data suggest that the Ste50 phosphorylation following osmotic stress or mating factor stimulation leads to dissociation of the Ste50-Ste11 complex from Opy2 to negatively regulate the HOG pathway.

‹Kazumasa Yokoyama Group›

Analysis of the NYAP family-mediated signaling pathway in neurons

The Src family protein tyrosine kinases are implicated in various neural functions such as neuronal development, myelination, and synaptic plasticity. To analyze roles of Src family kinases, we have been trying to identify binding partners and substrates of the kinases in the brain. To identify substrates of Src family tyrosine kinases in the brain, we performed solid-phase phosphorylation screening and identified a novel protein family, which we termed NYAP. We demonstrated that the NYAP family regulates both upstream and downstream of the PI3 K signaling in developing neurons. Upon stimulation of Contactin family membrane proteins, NYAP family proteins were tyrosine phosphorylated by Fyn, thereby providing the major binding sites for phosphoinositide 3-kinase (PI3 K) in neurons. Disruptions of Nyap family members decreased Rho, Rac and Akt activity, and affected neuronal morphology and nurturing behaviors. Furthermore, NYAP family proteins associated with the NCKAP1/CYFIP complex which is an essential link between Rac and actin polymerization. Thus, we proposed that the NYAP family is the central scaffold of PI3K, tightening a molecular link between cell surface Contactin family proteins and neuronal morphogenesis through Fyn and PI3K. Many proteins containing phospho-Tyr-x-x-Met (YxxM) motifs have been reported to bind with and activate PI 3K p85, but their relative contributions to PI3K activation have not been studied. In this study, we revealed that the NYAP family accounts for almost all of PI3K p85-binding phosphoproteins in the brain. This means that previously reported YxxM proteins have relatively small contributions to PI3K activation in spatially- and temporally-restricted situations in neurons. Previous models of PI3K activation and function, therefore, would be refined to take into account contributions of NYAP family proteins in neurons.

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Global Centar of Excellence (GCOE) Program

Center of Education and Research for Advanced Genome-Based Medicine: For personalized medicine and the control of worldwide infectious diseases Unit of Disease Control Genome Medicine

ゲノム情報に基づく先端医療の教育研究拠点 オーダーメイド医療の実現と感染症克服を目指して 疾患制御ゲノム医学ユニット

Project Associate Professor Naoya Kato, M.D., Ph.D.　特任准教授 医学博士 加 藤 直 也

Our major goal is to establish personalized medicine for patients with infectious diseases or cancers, especially those of gastrointestinal and hepatic fields, based on human or pathogenic microbe genome information.

1. Hepatitis C virus and innate immunity

Naoya Kato

It has been estimated that more than 170 million people worldwide are chronically infected with hepatitis C virus (HCV). The most important sequel of chronic HCV infection is progressive liver fibrosis leading to cirrhosis, and hepatocellular carcinoma (HCC), which is responsible for significant morbidity and mortality throughout the world. HCV is a positive-sense, single-stranded RNA virus, consisting of an approximately 10-kb genome containing a large open reading frame, encoding a polyprotein precursor of 3010-3033 amino acids and an untranslated region at the 5′ and 3′ ends. The putative organization of the HCV genome includes the 5′-untranslated region, 3-4 structural proteins (core, E1, and E2/p7), 6 non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B), and the 3′-untraanslated region.

Viral infection intrinsically activates host innate immunity and induces host anti-viral system such as interferon (IFN) production. Innate immune system suppresses viral replication and finally leads to viral clearance. In case of HCV infection, the battle between HCV and innate immunity may be associated with persistent infection of this virus and may contribute to the

pathogenesis of hepatitis C. Thus, we have been investigating interaction between HCV and innate immunity.

IFN-beta plays a leading role in innate immune system as an antiviral response against HCV. To investigate the influence of HCV on innate immunity, we examined the effect of viral proteins on IFN-beta induction. Among HCV proteins, only NS5B, a viral RNA-dependent RNA polymerase, activated the IFN-beta promoter. NS5B actually phosphorylated IFN regulatory factor (IRF)-3 and activated IFN-beta promoter through Toll-like receptor (TLR)-3 and TIR domain-containing adaptor protein (TRIF) by its polymerase activity. On the other hand, NS3, NS4A, NS4B, and NS5A efficiently inhibited this activation. This may contribute toward the persistence of this virus. We then analyzed the mechanism of inhibition of IFN-beta activation by NS3. TRIF and two protein kinases, TBK 1 and IKK-epsilon, play essential roles in TLR3-mediated IFN-beta production. We found that NS3 interacted directly with TBK1, and that this binding resulted in the inhibition of the association between TBK1 and IRF-3, which led to the inhibition of IRF-3 activation. This is one of the mechanisms of the inhibition of the innate immune responses of HCV infection by NS3.

MxA/PKR/OAS is the most studied IFN stimulated genes. We therefore examined their effect on HCV replication. First, we examined the expression of MxA/PKR/OAS in Huh7 cells. In our Huh7 cells, MxA/PKR was weakly expressed in innate state and strongly induced by IFN. However, OAS was hardly expressed both in innate state and by IFN. Thus, we established MxA/PKR knocked-down (KD) cells, and examined the effect on HCV replication. HCV replicated better in MxA/PKR KD cells than in control cells. Furthermore, HCV replication was suppressed by MxA/PKR overexpression. Although IFN-alpha treatment was less efficient against HCV in MxA KD cells compared to control cells, IFN-alpha treatment was similarly efficient against HCV in PKR KD cells and control cells. As well as MxA/PKR, OAS overexpression suppressed HCV replication. However, IFN-alpha treatment was fully efficient in our Huh7 cells expressing almost no OAS. In conclusion, although all of PKR/MxA/OAS suppressed HCV replication, MxA played an important role in IFN treatment whereas PKR/OAS did not.

Finally, 6 OAS SNPs were examined and analyzed in 409 patients with chronic HCV infection using TaqMan PCR genotyping method. The relationship of genotypes of different SNP and clinical manifestations of the patients was analyzed. Patients with genotype A/A, A/G and G/G of a nonsynonymous SNP of OAS at the exon 3 of its coding sequence was at a gradient increased risk of suffering from higher serum ALT, higher degree of liver fibrosis, and occurrence of liver cirrhosis. In experiments with Huh7 cells, plasmid imitating G/G type showed lower ability of clearance of virus comparing with plasmid imitating A/A type. The SNP of OAS at the exon 3 of its coding sequence was associated with progression of disease in HCV infected patients. Patients with lower ability to inhibit HCV replication may permit disease progression of hepatitis C.

2. Fusion HBx from HBV integrant in human hepatoma cell line is implicated in the development and progression of hepatocellular carcinoma

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Hepatitis B virus (HBV) is a major risk factor for HCC, and HBx has been suggested to play an important role in hepatocarcinogenesis. However, the molecular mechanism of HBx in the pathogenesis of HCC remains unclear. In this study, we identified fusion HBx from HBV integrant in human hapatoma cell line, and investigated its role in the development and progression of HCC.

1) Using cassette-ligation-mediated PCR and 3′-/5′-RACE, we determined viral-host flanking sequences and full-length mRNA from HBV integrant in Hep3B cells, which had been established from HBV-related HCC. We identified full-length mRNA from HBV integrant with the length of 3725bp containing 1877bp human sequences at 3′ end, and fusion HBx (3′-truncated HBx+human peptides) was supposed to be translated. 2) We silenced the expression of mRNA from HBV integrant with siRNA in Hep 3B cells, and established stably knocked-down clones (KD cells). Using KD cells, we investigated the expression of fusion HBx by immunofluorescence staining. Nearly 90% reduction of fusion mRNA expression by siRNA was confirmed using Realtime PCR and Northern blot analysis, and fusion HBx was disappeared in KD cells by immunofluorescence staining. 3) Using KD cells, we evaluated the effect of fusion HBx on cell growth and invasiveness by MTT/BrdU assay and matrigel invasion assay. KD cells demonstrated significant reduction in cell proliferation and invasion ability compared to parental cells. 4) We compared the transactivation activity and the ability to transform cells

between wild HBx and fusion HBx by luciferase assay and soft agar assay. Although fusion HBx had significantly decreased transactivation ability compared to wild HBx, fusion HBx had anchorage-independent growth ability in soft agar whereas wild HBx did not have.

Fusion HBx from HBV integrant may play an important role in the development and progression of hepatocellular carcinoma.

Center for Asian Infectious Diseases

IMSUT Research Center for Infectious Diseases in China

中国における感染症研究拠点

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The Institute of Medical Science, University of Tokyo (IMSUT) has established Japan-China joint laboratories for research on emerging and re-emerging infectious diseases in Asia, in collaboration with the Chinese Academy of Sciences and Chinese Academy of Agricultural Sciences. In the laboratories, Japanese and Chinese scientists conduct research on the viral pathogenicity, the genetic variation of viruses in the field, structure of viral and host proteins, and so on.

BACKGROUND

Historically, China is a very important neighbor of Japan. Official diplomatic delegations were first sent from Japan during the Sui dynasty some 1400 years ago. Since late 20th century, geopolitical and economical interdependence between Japan and China has developed substantially and will deepen further in the future. China is an enormous country often symbolically referred to as the dragon. While China is developing and transforming rapidly in the coastal regions, its rural areas have been left far behind. With regard to infectious diseases, China is beset with problems ranging widely from those of a developing country to those of dense urban environments. No one can discuss emerging and re-emerging infectious diseases (ERID) without mentioning China. Severe acute respiratory syndrome (SARS) emerged in Guangdong and shocked the world in 2003. With Lake Qinghai as a reference point, avian influenza expanded westward in the Eurasian continent in 2005 and reached Africa in February 2006. The carrier rate of hepatitis viruses is very high and HIV infection is rapidly increasing.

Given these situations, academic collaboration on research in infectious diseases would be beneficial to both countries, facilitate mutual understanding, and help strengthen the stable long-term relationship between the two peoples. Establishing joint research laboratories in China is particularly important because this would allow Japanese scientists access to possible emerging pathogens and to have an opportunity to fight against possible emerging infections. Supported by a contract research fund from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) (*Japan-China Collabora-*

tion on Emerging and Re-emerging Infectious Diseases; MEXT Project Director: Aikichi Iwamoto), IMSUT has established two joint laboratories in Beijing in collaboration with the Institute of Biophysics and Institute of Microbiology, Chinese Academy of Sciences (IBPCAS and IMCAS, respectively); a collaborative research program with Harbin Veterinary Research Institute (HVRI), the Chinese Academy of Agricultural Science; and IMSUT's project office in Beijing.

Year 2008 was the year of the Olympic Games in Beijing. Preparation for the historic national event caused some inconvenience for the laboratories, particularly limited supplies of some research materials. Nonetheless, the year 2008 was highly productive thanks to the hardworking scientists from China and Japan.

LABORATORIES AND PROJECT OFFICE

a. Laboratory of Structural Virology and Immunology (LSVI)

In LSVI, Z. Matsuda's group is studying the primate lentiviruses (HIV and SIV), aiming to analyze the structure-function relationship of the viral proteins. In order to solubilize insoluble HIV-1 proteins and facilitate their crystallization, his group fused tag proteins derived from*Thermus thermophilus (T.th)* to the target viral proteins, since *T.th* proteins are known to crystallize easily. They successfully identified several *T.th* tags that solubilized Vpr and gp41, insoluble HIV-1 proteins. To study the molecular mechanism of the membrane fusion induced by the HIV-1 envelope protein (Env), they engineered two sets of split reporter proteins that regained the reporter activities through reassociation. Membrane fusion was monitored by detecting the signals produced during the fusion of two cells each expressing one of the pair of the split proteins. One set of the reporter used a pair of complementary split GFPs and the other, the combination of split GFP and split luciferase (called dual split proteins). Both reporters allowed a real-time monitoring of the Env-induced membrane fusion.

b. Laboratory of Molecular Immunology and Molecular Microbiology (LMIMM)

LMIMM was founded in May 2006, set to work in October 2006 in IMCAS, and moved to an IM's new building in the CAS Olympic Science Park in January 2007. It is an important member of the CAS Key Laboratory of Pathogenic Microbiology and Immunology, which was newly established in December 2008. Y. Kitamura's group in LMIMM is currently focusing on hepatitis C virus (HCV) and human immunodeficiency virus (HIV), to obtain a better understanding of mechanisms of the viral replication and to develop antiviral drugs. Using the human cell line/infectious HCV RNA system, they are characterizing the adapted virus population and in particular, are testing whether some host genes stimulated by interferon have anti-HCV functions. To investigate the pathogenicity of the viruses in the field, they are analyzing clinical samples from HIV-infected individuals and chronic hepatitis C patients treated in some collaborating hospitals in Beijing. They are also collaborating with Chinese scientists to study some zoonotic viruses such as avian influenzavirus.

c. Collaborative research program with HVRI

HVRI (Director, Xiangang Kong) serves as a research center that focuses on the study of avian influenza viruses in domestic fowl and wild waterfowl in China. Japanese scientists from IMSUT (headed by Y. Kawaoka), together with Chinese scientists at HVRI (headed by Hualan Chen), studied the pathogenesis of the highly pathogenic avian influenza H5N1 viruses in mammals at a BSL3 laboratory at HVRI. They showed that an H5N1 virus, isolated from a human in China, replicated in the lower respiratory tract of rhesus macaques, causing severe lung damage. Using a mouse model, they also determined the molecular basis of the differences in pathogenicity between two H5N1 isolates from pigs in Fujian Province in southern China. In addition, the scientists affiliated with this program have conducted molecular surveillance studies of H5N1 viruses isolated in Asian countries.

d. IMSUT Project Office

The office (M. Hayashi and K. Yoshiike) is supporting the activities of the two joint laboratories in Beijing and one program in Harbin. It serves as Secretariat for Steering Committee Meeting and files MOU and Minutes. It helps scientists visiting the joint laboratories and program for collaborative research. It is gathering the information about emerging infections in China from the Chinese mass media and official announcements, and the gathered information (in Japanese) is presented and updated on the website of the Project (www.rcaid.jp).

IMPLEMENTATION OF COLLABORATION

The collaboration is implemented, being based on MOU and Minutes of Meeting between IM-

SUT and the Chinese institutes. For the joint laboratories the implementation was controlled by the steering committee consisting of Motoharu Seiki, A. Iwamoto, George Fu Gao, and Tao Xu. The collaborative program in Harbin was implemented by the steering committee consisting of M. Seiki, Y. Kawaoka, X. Kong, and H. Chen.

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