The 8th China-Japan Joint Laboratory Workshop

Pathogenesis, Gene Regulation and Signal Transduction

2011

21st November 2011

Meeting Room 9501

Institute of Biophysics

Chinese Academy of Sciences

Wishing further progress in the second term of China-Japan Collaboration

Entering into the second 5-year term (2010-2015) of China-Japan Research Collaboration on Emerging and Re-emerging Infections, not only are we following the collaboration scheme established in the first term of the program, but we are exploring further development. First, although the main focus of our project is on infectious diseases, we tried to include interdisciplinary presentations in this meeting. Some speakers outside the Japan-China joint laboratories in IBP and IM and some from IMSUT (the Institute of Medical Science of the University of Tokyo) are invited to help achieve a wider recognition of the meeting. In future, we hope to fix the timing of this annual joint laboratories meeting and invite scientists from the other countries than China and Japan so that the profile of this meeting will be well recognized among the scientific community. Secondly, we have been introducing gradual turnover of steering committee members as well. This turnover is a positive sign for the long-term successful continuation of the program: those who served in earlier days will be able to support this program from outside, raising awareness for this program; those joining afterwards will bring in fresh ideas for the research collaboration, strengthening the program. Lastly but not the least, the Japanese research team in the IM is now headed by new principal investigator Takaomi Ishida. Thanks to Ishida's research interest, the research activity of his team will be synergized with those of the laboratories in IM under the leadership of George Fu Gao and Li Huang. Furthermore, more interaction between the Joint Laboratories in IM and in IBP than before is anticipated.

We are holding this year's annual meeting reverberating with the above-mentioned changes and showing our high level of scientific activity. I am sure that the great success of this meeting will be a testimony to the friendly and strong interaction of the two countries in the field of infection research. May every participant enjoy and obtain something new.

Fall, 2011
Tadashi Yamamoto
Chair of the IMSUT Research Laboratories in CAS
Professor, IMSUT

The 8th China-Japan Joint Laboratory Workshop: Pathogenesis, Gene Regulation and Signal Transduction

Date: November 21 (Mon), 2011

Place: Conference Room 9501 (Building 9, 5F)
Institute of Biophysics, Chinese Academy of Sciences
15 Datun Road, Chaoyang District, Beijing

Registration 8:30 - 9:30

Session	Time	Speaker	Title
Opening Session	9:30 - 9:50	-	-
Photograph taking	9:50 - 10:00		
Session I (Chaired by Tadashi Yamamoto & Bin Gao)	10:00 - 10:30	**Aikichi Iwamoto** Institute of Medical Science, The University of Tokyo	1. Building clinic-bench network on HIV-1 via China-Japan joint laboratories
	10:30 - 11:00	**Rui-Ming Xu** Institute of Biophysics, Chinese Academy of Sciences	2. Structural Mechanisms of Histone Methylation
	11:00 - 11:30	**Kensuke Miyake** Institute of Medical Science, The University of Tokyo	3. Unc93B1 restricts systemic lethal inflammation by orchestrating TLR7- and TLR9-trafficking
	11:30 - 12:00	**Linqi Zhang** Comprehensive AIDS Research Center, Tsinghua University	4. Antibody profiling, epitope mapping and vaccine design based on yeast surface display system
Lunch	12:00 - 13:00		
Session II (Chaired by Aikichi Iwamoto & Rui-Ming Xu)	13:00 - 13:30	**Mifang Liang** National Institute for Viral Disease Control and Prevention, China CDC	5. A novel bunyavirus causing severe fever with thrombocytopenia syndrome in humans
	13:30 - 14:00	**Nobuo Sakaguchi** Graduate School of Medical Sciences, Kumamoto University	6. Regulation of AID/Apobec family molecule by RNA export factor GANP in immune responses and viral infection
Break	14:00 - 14:15		

Session	Time	Speaker	Title
Session III (Chaired by Nobuo Sakaguchi & Zhihai Qin)	14:15 - 14:30	**Yuhai Bi** Institute of Microbiology, Chinese Academy of Sciences	7. Novel genetic reassortants in H9N2 influenza A viruses and their diverse pathogenicity to mice
	14:30 - 14:45	**Shinya Yamada** Institute of Medical Science, The University of Tokyo	8. Adaptation of a duck influenza A virus in quail
	14:45 - 15:00	**Liu Dawei** Institute of Microbiology, Chinese Academy of Sciences	9. Single domain antibody multimer against Rabies infection
	15:00 - 15:15	**Akihisa Kato** Institute of Medical Science, The University of Tokyo	10. Phosphoproteomic analysis reveals an HSV-1 kinase-mediated phosphorylation event involved specifically in the regulation of viral neurovirulence
Break	15:15 - 15:30		
Session IV (Chaired by Kensuke Miyake & Xiyun Yan)	15:30 - 15:45	**Hirohito Ishikawa** Institute of Medical Science, The University of Tokyo	11. New strategy to develop a dual-functional split protein using self-associating split GFP
	15:45 - 16:00	**Yang Wang** Institute of Microbiology, Chinese Academy of Science	12. Rapid high throughput screening of drug resistance in HIV-HBV co-infected patients by PCR-SSOP Luminex assay
	16:00 - 16:15	**Noritaka Yamaguchi** Institute of Medical Science, The University of Tokyo	13. Functional analysis of cIAPs in RANK signaling in osteoclast precursor cells
Break	16:15 - 16:30		
Session V (Chaired by Takaomi Ishida & Wenjun Liu)	16:30 - 16:45	**Yongting Luo** Institute of Biophysics, Chinese Academy of Sciences	14. Recognition of CD146 as an ERM-binding protein offers novel mechanisms for melanoma cell migration
	16:45 - 17:00	**Mika Sakurai-Yageta** Institute of Medical Science, The University of Tokyo	15. The role of a cell adhesion molecule, CADM1, in human adult T-cell leukemia
	17:00 - 17:15	**Lin Chen** Institute of Biophysics, Chinese Academy of Sciences	16. S100A4-expressing Bone Marrow Derived-cells (BMDCs) contribute to Liver fibrosis and HCC via activation of hepatic stellate cells
Closing Session	17:15 - 17:30	-	-
Banquet	18:00 -	-	Place TBA

Building clinic-bench network on HIV-1 via China-Japan joint laboratories

*Aikichi Iwamoto[1], Zene Matsuda[2], Takaomi Ishida[3], Noriaki Hosoya[1], Lijun Gu[3], Hiroo Hoshino[4], Hong Li[5], Huanliang Liu[6], Taisheng Li[7], Akihisa Shimizu[1], Shuya Fukai[8], Ai Kawana-Tachikawa[1], Yi Shi[9], George F. Gao[9]

1 Division of Infectious Diseases, The Institute of Medical Science, The University of Tokyo;2 China-Japan Joint Laboratory of Structural Virology and Immunology (LSVI), Institute of Biophysics, Chinese Academy of Sciences;3 China-Japan Joint Laboratory of Molecular Immunology and Molecular Microbiology (LMIMM), Institute of Microbiology, Chinese Academy of Sciences;4 Gunma University;5 Yunnan CDC;6 Sun Yatsen University;7 Peking Union Medical College Hospital, Chinese Academy of Medical Sciences;8 Institute of Molecular and Cellular Biosciences, The University of Tokyo;9 CAS Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Sciences.

I would like to present two topics (HIV-1 tropism and CTL/HIV-1 interaction) through which I have been trying to set up a research network on HIV-1 between clinics/laboratories in China and Japan.

HIV-1 uses either CD4/CXCR4 or CD4/CCR5 as cellular receptors to initiate infection. Dr Matsuda's group in Beijing invented dual-reporter assay for cell fusion called Dual-Split Protein (DSP) system. Using NP2 glioma cell line expressing CD4, my group in Tokyo established stable cell lines which express a part of DSP (DSP_{1-7}) together with CXCR4 (N4X4) or CCR5 (N4R5), respectively. An expression vector (PRE-11) was constructed for the subcloning of HIV-1 env gene. PRE-11 contains also the rest of DSP (DSP_{8-11}). After the transfection of PRE-11_{env} into 293FT cells, they are co-cultured with N4X4 and N4R5 cells, respectively. HIV-1 co-receptor usage (tropism) can be measured by the luciferase or fluorescent activities reconstituted in the fused cells. This phenotypic tropism assay system (DSP-pheno) is highly time-saving (minimum turn-around time: 5days) and safe in handling. DSP-pheno has been characterized further in my laboratory and an effort to improve DSP is progressing in LSVI. We wish to study the tropism of HIV-1 circulating in Asia using DSP-pheno and to compare with the nucleotide sequence variations. The collaboration would be performed in PUMC Hospital, Yunnan CDC, Sun Yat-sen University, LMIMM, LSVI and my lab in Tokyo.

Another interest of my group is host vs HIV-1 interaction in the infected individuals and population. HIV-1 prevalence is still very low in the general population of Japan, however, highly focused infection is increasing in men who have sex with men. HLA Class I molecules are less diverse in Japanese as compared with Caucasian and African. Almost 70% of Japanese express HLA-A*2402 (A24). HIV-1-specific CTLs recognize peptides bound to HLA Class I molecules. Nef138-10 is an immune-dominant CTL epitope presented by A24. HIV-1 with the wild type Nef138-10 sequences (RYPLTFGWCF) is removed quickly from the circulation in the HLA-A24-positive individuals. HIV-1 with a major escape mutation, Nef138-10(2F) (RFPLTFGWCF) is selected and is circulating in Japan. We are analyzing the molecular interaction between T cell receptor (TCR) of the Nef138-10 specific CTL clones and the peptide-A24 (pMHC) complexes. The collaboration has been going on between Dr Gao's group in CAS Key Laboratory of Pathogenic Microbiology and Immunology and my group in Tokyo.

Structural Mechanisms of Histone Methylation

Rui-Ming Xu

National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, 15 Datun Road, Beijing 100101, China

Epigenetic inheritance involves DNA methylation and post-translational modifications of histone proteins. Histone methylation provides versatile epigenetic information owing to the complex pattern of lysine and arginine methylations. Lysine residues on the N-terminal tails of histone H3 and H4 are methylated by SET domain proteins with one, two or three methyl groups attached, and arginine residues can be symmetrically or asymmetrically methylated. The structures of a number of SET domain histone methylases and asymmetric arginine dimethylases have greatly contributed to our understanding of the molecular mechanism of histone methylation. However, little is known about how the enzymatic activities of SET domain proteins are regulated, and no structural information of symmetric arginine dimethylases is available. I will present our work on the structure of histone H3K36 methylase NSD1, which reveals an autoregulatory mechanism of the SET domain protein, and the structure of the first arginine symmetric dimethylase, PRMT5, which methylates H4R3 and has been implicated in global repression of gene expression.

Unc93B1 restricts systemic lethal inflammation by orchestrating TLR7- and TLR9-trafficking

Kensuke Miyake

Division of Infectious Genetics, Department of Microbiology and Immunology; Laboratory of Innate Immunity, Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minatoku, TOKYO1208-8639, Japan.

TLR7 and 9, innate immune sensors for microbial RNA or DNA, have been implicated in autoimmune diseases such as psoriasis, arthritis, and systemic lupus erythematosus (SLE). Autoimmune response is restricted by sequestration of self nucleic acids from endolysosomes, the site for nucleic acid-sensing by TLR7 and 9. Whereas extracellular self nucleic acids are normally degraded, microbial nucleic acids are protected by bacterial cell walls or viral particles, and transported to endolysosomes. Aberrant transportation of self nucleic acids to endolysosomes exacerbates autoimmunity. Upon activation, TLR7 and 9 are transported to endolysosomes from the endoplasmic reticulum (ER) by an ER-resident protein Unc93B1. We previously reported that TLR9 competes with TLR7 for Unc93B1-dependent transportation and predominates over TLR7. Little is known, however, about the consequences of Unc93B1-dependent TLR9 skewing *in vivo*. We now show that TLR9-skewing is essential for restricting TLR7-dependent, systemic inflammation. The skewing can be reversed to TLR7 by a single D34A mutation in Unc93B1, rendering TLR7 hyper-responsive and TLR9 hypo-responsive[12]. Mice harbouring the D34A mutation showed monocytosis, splenomegaly, liver necrosis, thrombocytopenia, nephritis, and premature death. Dendritic cells, macrophages, and B cells all showed TLR7-skewed response despite unaltered expression of TLR7 and 9. Massive inflammation was abolished by the lack of TLR7, but not TLR9. $CD4^+$ T cells showed marked differentiation towards Th1 or Th17 subsets. Mature B cells expanded and showed strengthened B cell receptor signalling. Depletion of B cells abolished T cell activation/differentiation and systemic inflammation. Therefore, opposing actions of TLR7 and 9 are balanced by Unc93B1 to restrict systemic lethal inflammation. TLR7 exacerbates SLE, whereas TLR9 ameliorates SLE by antagonizing TLR7. Aberrant TLR7-skewing may underlie the pathology associated with SLE and other autoimmune diseases.

Antibody profiling, epitope mapping and vaccine design based on yeast surface display system

Teng Zuo, Xuanling Shi, Hua Wang, Chen Yao, Linqi Zhang*

Comprehensive AIDS Research Center, School of Medicine, Tsinghua University, Beijing, China 100084

Host antibody response is a crucial defense against pathogenic infection. Here, we report a novel technique allowing quantitative profiling, epitope mapping and vaccine design based on yeast surface display system. This involves expression of a combinatorial library of target proteins from a candidate pathogen on the surface of yeast *Saccharomyces cerevisiae.* After mixing with serum/plasma from infected or immunized subjects or monoclonal antibodies, positive yeast clones are identified and obtained by fluorescence-activated cell sorting (FACS) followed by sequencing and structural analysis. Using this technique, we have studied mice immunized serum with recombinant hemagglutinin (HA) protein from a human influenza H5N1 strain (A/Anhui/1/2005) and convalescent plasma from an infected human in China. We have also mapped minimal antigenic domains recognized by several potent human broadly neutralizing monoclonal antibodies (bnmAb) against HIV-1. Our technique has identified novel antigenic domains targeted by serum/plasma or bnmAb and allowed calculation of the relative proportion of the antibody response against each domain. We believe such systematic measurement of an antibody response is unprecedented. Further studies on the immunogenicity of these protein fragments in vivo and the application of the technique to study other pathogens, bnmAbs and their germline precursors will improve our understanding of protective immunity and guide rational design and development of vaccines and therapeutics.

A novel bunyavirus causing severe fever with thrombocytopenia syndrome in humans

Mifang Liang，Chongjing，Jiandong Li，Yulan Sun, Quanfu Zhang，Chuna Li, Wu Wei, Qing Wang Fushun Zhang, Wen Gu, Shiwen Wang, Dexin Li* and the Group members

State Key Laboratory for Molecular Virology and Genetic Engineering, National Institute for Viral Disease Control and Prevention, Beijing 102206，China
e-mail: lidx@chinacdc.cn

Heightened surveillance of acute febrile illness in China since 2009 led to the identification of a new infectious disease characterized of severe fever with thrombocytopenia syndrome (SFTS) with unknown etiology. A novel virus now named SFTS bunyavirus (SFTSV) (Yu and Li et al, NEJM March 16, 2011) was isolated from blood samples of patients who presented clinical signs of high fever, thrombocytopenia, leucopenia, and multi-organ dysfunction. RNA sequence analysis revealed that the virus was a new member of the genus Phlebovirus in the family Bunyaviridae. Electron microscopic examination revealed virions with typical morphology of a bunyavirus. Viral RNA and/or specific antibodies against the virus were detected in blood samples of 171 confirmed SFTS patients from six provinces in China. Serological assays demonstrated a virus-specific immune response in all 35 pairs of sera collected from patients during the acute and convalescent phases. Inoculation of SFTSV isolated from patients into mice induced a significant thrombocytopenia in the acute phase after the virus infection. Histological analysis revealed marked hepatocellular degeneration and renal glomerular fibrosis in virus infected mice, which coinside with elevated liver transaminase levels and proteinuria observed in some SFTS patients. The animal data further confirmed that SFTSV was the etiological agent of the newly emerging infectious disease, SFTS.

Regulation of AID/Apobec family molecule by RNA export factor GANP in immune responses and viral infection

Nobuo Sakaguchi[1], Shailendra Kumar Singh[1], Almofty Sarah Ameen[1], Mohammed Mansour Abbas Eid[1], Kazuhiko Kuwahara[1], Terumasa Ikeda[2], Atsushi Koito[2], Phuong Pham[3], Myron F. Goodman[3], and Kazuhiko Maeda[1],

[1]Department of Immunology, [2]Department of Retrovirology and Self-Defense, Graduate School of Medical Sciences, Kumamoto University, Japan, [3]Departments of Biological Sciences and Chemistry, University of Southern California, Los Angeles, CA 90089-2910, USA

Cytidine deaminases of AID/Apobec family is composed of 11 members in human that play important functions in various cell types by targeting to single stranded and double stranded DNA and RNA molecules. Particularly, activation induced cytidine deaminase AID plays a critical roles in the diversification of immunoglobulin (Ig) V-region genes and class switch recombination in antigen (Ag)-driven B cells in peripheral lymphoid organs. Lack of GANP in B cells caused a severe impairment of generation of high-affinity antibody (Ab) against T cell-dependent Ag in mice. On the contrary, transgenic over-expression of GANP in B cells enormously augmented the generation of high affinity Ab production. These results determined that GANP is an important regulator of generation of somatic hypermutation (SHM) in IgV-region gene and selection of high affinity B cells in germinal centers of peripheral lymphoid organs. On the other hand, Apobec3G is involved in the prevention of HIV-1 infectivity in activated T-cells in the absence of Vif. These results predicted the existence of molecules involved in the regulation of AID/Apobec family molecules in human.

As a specific regulator of AID in activated B cells, we demonstrated that GANP, a member of mRNA export complex of TREX2, is involved in the recruitment of AID into the nucleus of activated B cells and then recruit AID selectively to the rearranged IgV-region gene, which provided an important information to account the molecular mechanism how SHM occur specifically to the rearranged IgV-region (1-3). Here, we show that GANP is a host component to regulate Apobec3G and is also involved in regulation of HIV-1 infectivity. GANP in interaction with Apobec3G may account the regulation of HIV-1 infectivity and the regulation of immune responses during the long-term HIV-1 infection.

1. K. Kuwahara, et al. Germinal center-associated nuclear protein contributes to affinity maturation of B cell antigen receptor in T cell-dependent responses. Proc. Natl. Acad. Sci. U. S. A. 27:1010-1015, 2004.
2. K. Maeda, et al. GANP-mediated recruitment of activation-induced cytidine deaminase to cell nuclei and to immunoglobulin Variable region DNA. *J. Biol. Chem.* 285(31): 23945-53, 2010.
3. N. Sakaguchi, et al. Molecular mechanism of immunoglobulin V-region diversification regulated by transcription and RNA metabolism in antigen-driven B cells. *Scand. J. Immunol.* 73(6): 520-526, 2011.

Novel genetic reassortants in H9N2 influenza A viruses and their diverse pathogenicity to mice

Yuhai Bi[a1] *, Lulu[a,b1], Jing Li[a], Yanbo Yin[c], Yi Zhang[e], Huijie Gao[e], Zhuoming Qin[d], Basit Zeshan[a], Jinhua Liu[e], Wenjun Liu[a]†, Lei Sun[a]†

[a] Center for Molecular Virology, Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China.
[b] Graduate University of Chinese Academy of Sciences, Beijing 100101, China.
[c] College of Animal Science and Veterinary Medicine, Qingdao Agriculture University, Qingdao 266109, China.
[d] Institute of Animal Science and Veterinary Medicine, Shandong Academy of Agricultural Sciences, Jinan, Shandong 250100, China.
[e] Key Laboratory of Zoonosis of Ministry of Agriculture, College of Veterinary Medicine, China Agricultural University, Beijing 100193, China.
[1] These authors contributed equally to this work.
† Corresponding author at: Center for Molecular Virology, CAS Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Sciences, Beichenxi Road, Chaoyang District, Beijing 100101, China. Tel.: +86 10 64807497; fax: +86 10 64807503.

Background: H9N2 influenza A viruses have undergone extensive reassortments in different host species, and could lead to the epidemics or pandemics with the potential emergence of novel viruses. **Methods:** To understand the genetic and pathogenic features of early and current circulating H9N2 viruses, 15 representative H9N2 viruses isolated from diseased chickens in northern China between 1998 and 2010 were characterized and compared with all Chinese H9N2 viruses available in the NCBI database. Then, the representative viruses of different genotypes were selected to study the pathogenicity in mice with the aim to investigate the adaptation and the potential pathogenicity of the novel H9N2 reassortants to mammals. **Results:** Our results demonstrated that most of the 15 isolates were reassortants and generated four novel genotypes (B62-B65), incorporated the gene segments from Eurasian H9N2 lineage, North American H9N2 branch, and H5N1 viruses. It was noteworthy that the newly identified genotype B65 has been prevalent in China since 2007, and more importantly, different H9N2 influenza viruses displayed a diverse pathogenicity to mice. The isolates of the 2008-2010 epidemic (genotypes B55 and B65) were lowly infectious, while representatives of two viral genotypes (B0 and G0) from the late 1990s were highly pathogenic to mice. In addition, Ck/SD/LY-1/08 (genotype 63, containing H5N1-like NP and PA genes) was able to replicate at high titers in mouse lungs but with mild clinical signs. **Conclusion:** Several lines of evidence indicated that the H9N2 influenza viruses constantly change their genetics and pathogenicity. Thus, the genetic evolution of H9N2 viruses and their pathogenicity to mammals should be closely monitored to prevent the emergence of novel pandemic viruses.

Adaptation of a duck influenza A virus in quail

Shinya Yamada[1], Kyoko Shinya[3], Ayato Takada[4], Toshihiro Ito[5],Takashi Suzuki[6,8], Yasuo Suzuki[7,8], Quynh Mai Le[9], Masahito Ebina[10], Noriyuki Kasai[11], Hiroshi Kida[12,13], Taisuke Horimoto[1], Pierre Rivailler[14], Li Mei Chen[14], Ruben O. Donis[14]and Yoshihiro Kawaoka[1,2,3,15,16]

1.Division of Virology, Department of Microbiology and Immunology, 2.International Research Center for Infectious Diseases, Institute of Medical Science, University of Tokyo, Japan, 3.Department of Microbiology and Infectious Diseases, Kobe University, Japan 4.Department of Global Epidemiology, Research Center for Zoonosis Control, Hokkaido University, Japan, 5.Department of Veterinary Public Health, Faculty of Agriculture, Tottori University, Japan, 6.Department of Biochemistry, School of Pharmaceutical Sciences, University of Shizuoka, Japan, 7.Health Science Hills, College of Life and Health Sciences, Chubu University, Japan, 8.Global COE Program for Innovation in Human Health Sciences, University of Shizuoka, Japan, 9.National Institute of Hygiene and Epidemiology (NIHE), Vietnam, 10.Department of Respiratory Medicine, Tohoku University Graduate School of Medicine, Japan, 11.Institute for Animal Experimentation, Tohoku University Graduate School of Medicine, Japan, 12.Laboratory of Microbiology, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Japan, 13.Research Center for Zoonosis Control, Hokkaido University, Japan, 14.Centers for Disease Control & Prevention, USA, 15.Department of Pathobiological Sciences, University of Wisconsin-Madison, USA 16.ERATO Infection-Induced Host Responses Project, Japan

Quails are thought to serve as intermediate hosts of influenza A viruses between aquatic birds and terrestrial birds, such as chickens, due to their high susceptibility to aquatic bird viruses, which then adapt to replicate efficiently in their new hosts. However, does replication of aquatic bird influenza viruses in quails similarly result in their efficient replication in humans? Using sialic acid-galactose linkage-specific lectins, we found both avian- (sialic acid-α2-3-galactose [Siaα2-3Gal] linkages on sialyloligosaccharides) and human-(Siaα2-6Gal) type receptors on the tracheal cells of quail, consistent with previous reports. We also passaged a duck H3N2 virus in quails 19 times. Sequence analysis revealed that eight mutations accumulated in hemagglutinin during these passages. Interestingly, many of the altered HA amino acids found in the adapted virus are present in human seasonal viruses but not in duck viruses. We also found that stepwise stalk deletion of neuraminidase occurred during passages, resulting in reduced neuraminidase function. Despite some hemagglutinin mutations near the receptor-binding pocket, appreciable changes in receptor specificity were not detected. However, reverse genetics-generated viruses that possessed the hemagglutinin and neuraminidase of the quail-passaged virus replicated significantly better than the virus possessing the parent HA and neuraminidase in normal human bronchial epithelial cells, whereas no significant difference in replication between the two viruses was observed in duck cells. Further, the quail-passaged, but not the original duck virus replicated in human bronchial epithelial cells. These data indicate that quails can serve as intermediate hosts for aquatic bird influenza viruses to transmit to humans.

Single domain antibody multimer against Rabies infection

Liu Dawei[1], Bhargavi M. Boruah[1, 2*], Bin Gao[1]

[1]CAS Key Laboratory for Pathogenic Microbiology and Immunology (CASPMI), Center for Cellular Immunology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, PR China;

[2]Graduate University of Chinese Academy of Sciences, Beijing, PR China

Abstract:

Rabies, occurring in over 150 countries and territories causes about 55,000 deaths worldwide each year. As 40% of the affected patients are children, the years of life lost by this disease makes rabies the seventh most important infectious disease worldwide. Current vaccine and antisera are effective in most cases but they have pitfalls since antisera are human or animal origin. Here we report that the isolation of several specific sdAbs derived from naïve Llama library against Rabies virus (RABV) capable of recognizing the primary surface antigen, Glycoprotein (G), of RABV. Single domain antibodies (sdAbs) is the smallest functional unit of antibodies and have found various applications in both therapy and diagnostics. To increase the avidity of sdAbs they have been given a pentavalent format by fusion with a coiled-coil peptide derived from human cartilage oligomeric matrix protein (COMP48). *In-vitro* assays to test the neutralizing potencies of the monomer and pentamer format of the sdAbs have revealed encouraging results, whereby the recombinant antibodies displayed high neutralizing potencies against rabies pseudotypes as were comparable to the prevalent Human Rabies Immunoglobulin (HRIgG), currently used in the post-exposure treatment for RABV. The results show that the multimerization strategy adopted in our study can prove to be a powerful anti-viral tool for diagnostics and possible treatment for RABV, as well as for studies relating to mechanisms during viral infection, that are still poorly understood.

Phosphoproteomic analysis reveals an HSV-1 kinase-mediated phosphorylation event involved specifically in the regulation of viral neurovirulence

*Akihisa Kato[1], Masaaki Oyama[2], Hiroko Kozuka-Hata[2], Takahiko Imai[1], Yasushi Kawaguchi[1]

1 Division of Viral Infection, International Research Center for Infectious Diseases, The Institute of Medical Science, The University of Tokyo, Japan
2 Medical Proteomics Laboratory, The University of Tokyo, Japan

Protein phosphorylation is one of the most common and effective modifications, which regulate a variety of cellular and viral functions. Phosphorylation events in herpesvirus-infected cells are of particular interest since herpesviruses encode viral specific protein kinase(s) unlike most of the other viruses. However, although some of biological consequences and mechanisms of the phosphorylation events in herpesvirus-infected cells have been gradually elucidated, our knowledge of them remains to be limited and fragmented. In the present study, for closing the knowledge gap, we carried out phosphoproteomic analysis of titanium dioxide affinity chromatography-enriched phosphopeptides from HSV-1-infected cells by using high-resolution mass spectrometry (MS). We identified more than 3,000 unique phosphopeptides covering 366 unique phosphorylation sites in 392 distinct cellular and viral proteins. To demonstrate the significance of these screening results, we focused on a viral dUTPase encoded by UL50 gene of HSV-1. Our results are as follows. (i) An HSV-1 kinase Us3 directly phosphotylated the HSV-1 dUTPase in vitro and mediated the phosphotylation of the viral enzyme in infected cells. (ii) In agreement with a previous report elsewhere, a null-mutation in UL50 significantly attenuated both the neurovirulence in mice following intracrebral inoculation and the pathogenic manifestations in mice following peripheral inoculations such as corneal and intravaginal inoculations. (iii) In contrast, alanine substitution in the Us3 phosphorulation site of the dUTPase identified by the MS analysis, followed by biochemial analyses, significantly impaired the neurovirulence in mice following intracerebral inoculation but not the pathogenic manifestations in mice following corneal and intravaginal inoculations. In addition, a phosphomimeric mutation at the phosphorylation site in the dUTPase in part restored the neurovirulence in mice. These results suggested that the Us3-mediated phosphorylation of the dUTPase specifically regulated the viral neurovirulence in vivo.

New strategy to develop a dual-functional split protein using self-associating split GFP

Hirohito Ishikawa*[1, 3], Naoyuki Kondo[4], Yufei Long[3], Kosuke Miyauchi[5], Fanxia Meng[3], Aikichi Iwamoto[1, 2] and Zene Matsuda[1, 3].

1 Research Center for Asian Infectious Diseases, Institute of Medical Science, The University of Tokyo, Japan.
2 Division of Infectious Diseases, Advanced Clinical Research Center, Institute of Medical Science, The University of Tokyo, Japan.
3 China-Japan Joint Laboratory of Structural Virology and Immunology, Institute of Biophysics, Chinese Academy of Sciences, China.
4 Department of Pediatrics, Emory University School of Medicine, USA.
5 AIDS Research Center, National Institute of Infectious Diseases, Japan.

Split proteins have a wide variety of biomedical applications. Designing a pair of split proteins, especially the selection of appropriate split points, however, is rather serendipitous. Furthermore, some split proteins are incapable of self-association. We have recently designed a pair of split *Renilla* luciferase (RL) by incorporating a self-associating split GFP module to compensate the weak self-association of split RL. The resultant pair regains dual function, RL and GFP activities, upon self-association; hence we call it dual split protein (DSP). Extending our DSP work, here we describe a new methodology to generate a split protein of interest with split GFP as a self-association module. In this study, we inserted the whole GFP into a target protein at one of several candidate split points, and chose clones retaining a high activity of the original protein as well as GFP signal. Since the N- and C-termini of GFP are close to each other in tertiary structure, when GFP is inserted, it is expected to bulge from the target protein to preserve its function. Once candidate sites were identified, a final pair of split proteins was obtained by simply splitting the GFP-inserted, chimeric protein within the GFP domain. The final pair consists of two proteins: N-split target protein-split GFP-C; N-split GFP-split target protein-C (N and C represent N-terminus and C-terminus of proteins, respectively). Employing this strategy, we identified a new split point within RL, which had a four times higher RL activity than the previous pair. This simple method of screening will facilitate the designing of split proteins which are capable of self-association via the split GFP portions.

Rapid high throughput screening of drug resistance in HIV-HBV co-infected patients by PCR-SSOP Luminex assay

Lijun Gu[1,2,3], Noriaki Hosoya[3,5], Ai Tachikawa-Kawana[5], Jin Gohda[1,2,3], Yang Wang[1,2], Yijing Ma[1,2], Yang Han[4], Taisheng Li[4], George Fu Gao[2], Aikichi Iwamoto[3,5], Takaomi Ishida[1,2,3].

1. China-Japan Joint Laboratory of Molecular Immunology and Molecular Microbiology, Institute of Microbiology, Chinese Academy of Science. Beijing, China.
2. Chinese Academy of Science Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology. Beijing, China.
3. Research Center for Asian Infectious Diseases, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.
4. Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, China.
5. Division of Infectious Diseases, Advanced Clinical Research Center, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

The highly active antiretroviral therapy (HAART) has significantly prolonged the lifespan of HIV-1 infected individuals. However, the emergence of drug resistant HIV-1 is becoming a major cause of treatment failure. Therefore, the detection of the emergence of drug resistant HIV-1 has a profound merit for successful treatment. Furthermore, such a screening will provide a vital epidemiological data on the spread of drug resistant HIV-1. The combination of the reverse transcriptase inhibitors (RTI) including Lamivudine (Lamivudine, Nevirapine, and Zidovudine or Sanilvudine) is the first choice for HAART in resource limited countries. Lamivudine, also active against HBV, is the only available drug for HBV in resource limited countries. HBV and HIV share the similar transmission routes, and HBV surface antigen positive carriers are found in 10 to 20% of HIV-1 infected individuals in the high HBV endemic countries such as China. Therefore, if lamivudine resistant HBV is induced during HAART, it can be quite problematic. For these reasons, we established a strategy to detect major lamivudine-resistant mutations in both HIV-1 RT gene (K103N and M184V) and in HBV Polymerase/RT gene (M204I/V and L180M) using a PCR amplification-sequence-specific oligonucleotide probes (SSOP) protocol. The readout was obtained by Luminex 100 technology. Plasma samples obtained from patients infected only with HIV-1 (111 samples) and coinfected with HIV-1 and HBV (20 samples) were analyzed. In HIV-1 RT, K103N and M184V mutations were found in 9 (8.1%) and 24 subjects (21.6%), respectively. For HBVRT, M204 (1 sample, 5%), M204V(1 sample, 5%), and L180M (2 samples,10%) were detected. We were unable to characterize 7 (6.3%) subjects for K103N, and 2 (1.8%) subjects for M184V HIV-1 RT, respectively. We validated the PCR-SSOP-Luminex results, by comparing with those obtained by cloning and DNA sequencing. We are going to analyze samples from HIV-1 and HBV dual infected patients in China by this rapid testing method.

Functional analysis of cIAPs in RANK signaling in osteoclast precursor cells

*Noritaka Yamaguchi[1], Mami Yokota[1], Yuu Taguchi[1], Hiroko Kozuka-Hata[2], Masaaki Oyama[2], Jin Gohda[3], and Jun-ichiro Inoue[1]

1 Division of Cellular & Molecular Biology, Institute of Medical Science, The University of Tokyo, Japan
2 Medical Proteomics Laboratory, Institute of Medical Science, The University of Tokyo, Japan
3 Research Center for Asian Infectious Diseases, Institute of Medical Science, The University of Tokyo, Japan

HIV infection and highly active anti-retroviral therapy (HAART) are known to induce osteoporosis in HIV patients. One of the major causes of osteoporosis is excess formation or activity of osteoclasts, the responsible cells for bone resorption. It is thus crucial to understand molecular mechanisms underlying osteoclast differentiation for development of therapies against osteoporosis in HIV patients.
Osteoclast is known to be formed by the fusion of hematopoietic cells of the monocyte-macrophage lineage. One of the essential signaling molecules for osteoclast differentiation is RANK, a member of the TNF receptor superfamily expressing on the surface of the precursor cells. Binding of RANK to its ligand activates NF-κB and MAP kinases and finally induces expression of NFATc1, a master transcription factor of osteoclastgenesis. Recently, emerging evidence indicates that cellular inhibitor of apoptosis proteins (cIAPs) are involved in the signaling of the TNF receptor superfamily. cIAPs have ubiquitin E3 ligase activity and induce K63-linked polyubiquitinaiton of RIP1 to activate NF-κB in the TNF signaling. In the CD40 signaling, cIAPs induce K48-linked polyubiquitination and degradation of TRAF3, and its degradation leads to activation of MAP kinases. Although RANK is a member of the TNF receptor superfamily, significance of cIAPs in the RANK signaling is not clear. To investigate the function of cIAPs in this signaling, we performed overexpression and knockdown experiments using Raw264.7, a murine monocytic cell line. A critical regulatory role of cIAPs in RANK signaling will be discussed.

Recognition of CD146 as an ERM-binding protein offers novel mechanisms for melanoma cell migration

Yongting Luo[1,2], Chaogu Zheng[1,2], Jinbin Zhang[1,2], Di Lu[1,2], Jie Zhuang[1,2], Shu Xing[1,2], Jing Feng[1,2], Dongling Yang[1,2] and Xiyun Yan[1,2]

[1] National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China
[2] Chinese Academy of Sciences-University of Tokyo Joint Laboratory of Structural Virology and Immunology, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China

Tumor cell migration is a well-orchestrated multistep process that drives cancer development and metastasis. Previous data indicated that CD146 expression correlates with malignant progression and metastatic potential of human melanoma cells. However, the exact molecular mechanism of how CD146 promotes melanoma cell migration still remains poorly understood. Here, we report that CD146 physically interacts with actin-linking ezrin-radixin-moesin (ERM) proteins and recruits ERM proteins to cell protrusions, promoting the formation and elongation of microvilli. Moreover, CD146-promoted melanoma cell migration is linked to RhoA activation and ERM phosphorylation. CD146 recruits Rho guanine nucleotide dissociation inhibitory factors 1 (RhoGDI1) through ERM proteins and thus sequesters RhoGDI1 from RhoA, which leads to upregulated RhoA activity and increased melanoma cell motility. CD146-activated RhoA also promotes further ERM phosphorylation and activation through Rho-phosphatidylinositol-4-phosphate-5-kinase-phosphatidylinositol 4,5-biphosphate pathway, which reinforces CD146/ERM association. Thus, our results provide a mechanistic basis to understand the role of CD146 in regulating human melanoma cell motility.

The role of a cell adhesion molecule, CADM1, in human adult T-cell leukemia

Mika Sakurai-Yageta[1,*], Mari Masuda[2], Toshiki Watanabe[3], and Yoshinori Murakami[1]

[1]Division of Molecular Pathology, Department of Cancer Biology, Institute of Medical Science, The University of Tokyo, Japan
[2]Genetics Division, National Cancer Center Research Institute, Japan
[3]Laboratory of Tumor Cell Biology, Department of Medical Genome Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Japan

ATL is a neoplastic disease of CD4-positive T lymphocytes that is etiologically associated with infection of human T-cell leukemia virus type I (HTLV-I). ATL develops in 3-5% of HTLV-I-infected individuals after an extended latent period of ~40-60 years, yet it remains an aggressive disease with poor prognosis and a median survival time of 11-13 months reported even in patients treated with the most effective first line combination chemotherapy. ATL is well known for its propensity of infiltrating leukemic cells into various organs and tissues, such as the skin, lung, liver, gastrointestinal tract, central nervous system, lymph nodes, and bone. We have previously demonstrated in collaborative studies that a cell adhesion molecule, CADM1, is up-regulated over 30-fold in acute-type primary ATL cells by DNA microarray (1).

CADM1 is an immunoglobulin superfamily cell adhesion molecule expressed in epithelial and neuronal tissues and mediates cell-cell adhesion through *trans*-interaction in neighboring cells. CADM1 was originally identified as a tumor suppressor in lung cancer and loss of CADM1 expression was frequently observed in various cancers derived from epithelial cells such as lung and kidney (2, 3). By contrast, ectopic expression of CADM1 was observed in primary ATL and HTLV-1-infected cells, although CADM1 expression was absent in normal $CD4^+$ T-cells. We have shown that in ATL cells, CADM1 is associated with Tiam1 (T-lymphoma invasion and metastasis 1), a guanine nucleotide exchange factor (GEF) specific for Rac, through class II PDZ binding motif. Tiam1-mediated activation of Rac induced lamellipodia formation of ATL cells attached on endothelial cells, suggesting that CADM1-Tiam1-Rac pathway may be involved in an invasive phenotype of leukemic cells in ATL patients (4). We are currently investigating the role of CADM1 in trans-endothelial migration of ATL cells, which is required for extravasation of ATL cells to tissue invasion. Possible applications of CADM1 to a diagnostic marker and a therapeutic target for ATL will be discussed.

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S100A4-expressing Bone Marrow Derived-cells (BMDCs) contribute to Liver fibrosis and HCC via activation of hepatic stellate cells

Lin Chen[1,*], Jie Li [1], Jinhua Zhang[1] and Zhihai Qin[1]

1 Institute of Biophysics, Chinese Academy of Sciences, 15 Datun Road, Beijing 100101, China.

Hepatic cirrhosis and hepatocellular carcinoma (HCC) are the most common causes of death in patients with chronic liver disease. It has been known that activation of hepatic stellate cells can promote progression of hepatic fibrosis and HCC. However, the role of bone marrow derived cells (BMDCs) in this process is unclear and double-edged. Here, we found that one subpopulation of BMDCs which were $S100A4^{+}CD11b^{+}$, accumulated in liver during the development of liver fibrosis and HCC. The progression of liver fibrosis and HCC can be retarded after selective depletion of these cells. This correlated with impaired activation of hepatic stellate cells. S100A4 secreted by these cells can stimulate hepatic stellate cells and up-regulate the expression of MMPs by hepatic stellate cells *in vitro*. These results indicate that S100A4-expressing BMDCs promote the development of liver fibrosis and HCC via activation of hepatic stellate cells in liver.

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