The 9th China-Japan Joint Laboratory Workshop
Pathogenesis, Gene Regulation and Signal Transduction

2013

November 1, 2013

Meeting Room A102

Institute of Microbiology

Chinese Academy of Sciences

Wishing great progress in the China-Japan Collaboration

I am very happy to hold the China-Japan Joint Laboratories Workshop 2013, which will focus on Pathogenesis, Gene Regulation and Signal Transduction. Three principal investigators and 6 young scientists from each country, 18 scientists in total, will talk about their own research. I am eager to hear all the talks, but especially I hope to feel strong energy from the talks by the young scientists, whose future scientific breakthroughs, we hope, may create healthier and better lives for the world's peoples.

In March 2012, Tadashi Yamamoto, a key person in this China-Japan collaboration program, retired from the University of Tokyo. One of his tasks, mentoring PIs at the Joint Laboratories in Beijing, was passed along to me. Therefore, I have visited Beijing frequently to hold discussions with Zene Matsuda and Takaomi Ishida together with their young colleagues. During these discussions, I recognized that they have already made significant progress in their investigations. This progress leads me to expect that the Joint Laboratories could continue producing high quality science with appropriate support from both countries.

I cordially appreciate George Fu Gao, Li Huang (from IM), Zihe Rao, Tao Xu (from IBP), Aikichi Iwamoto, Tadashi Yamamoto, Motoharu Seiki and Hiroshi Kiyono (from IMSUT) for their ceaseless efforts in supporting the Joint Laboratories. In addition, I sincerely ask new Director-General of IM, Shuang-Jiang Liu, for supporting this promising collaboration. Such strong support together with the keen scientific interests of people in the Joint Laboratories could lead to the tremendous success of the China-Japan collaboration program.

Finally, great success in this meeting will be a testimony to the friendly and strong interaction between the two countries. Please enjoy!

Fall, 2013
Jun-ichiro Inoue
Chief, China-Japan Joint Laboratories in Beijing
Professor, IMSUT

The 9th China-Japan Joint Laboratory Workshop : Pathogenesis, Gene Regulation and Signal Transduction

Date：November 1st (Friday), 2013

Place: Conference Hall A102 (Building A, 1F), Institute of Microbiology, Chinese Academy of Sciences
No1 Beichengxi Road, Chaoynag District, Beijing, China.

Registration: 8:20 - 9:00

Session	Time	Speaker	Title
Opening Session	9:00 - 9:20	**Shuang-Jiang Liu** Director General, Institute of Microbiology Chinese Academy of Sciences. **Yoshinori Murakami** Vice-Dean, Institute of Medical Science, University of Tokyo. **Wataru Todoroki** First Secretary, Embassy of Japan in China.	
Group Photograph taking : 9:20 - 9:30			
Session I (Chaired by George Gao Fu & Junichiro Inoue)	9:30-10:00	**Yoshinori Murakami** Institute of Medical Science, University of Tokyo.	1. Dual roles of a cell adhesion molecule, CADM1, in human oncogenesis
	10:00-10:30	**Zhihai Qin** Instute of Biophsics, Chinese Academy of Sciences.	2. Macrophage derived S100A4 promotes liver fibrosis and hepatic cellular carcinoma
	Break 10:30-10:40		
	10:40-11:10	**Ai Kawana-Tachikawa** Institute of Medical Science, University of Tokyo.	3. Interaction between virus and host immune response during chronic HIV-1 infection
	11:10-11:40	**Takeshi Noda** Institute of Medical Science University of Tokyo.	4. Genome Packaging Mechanism of Influenza A Viruses
Lunch 11:40 - 12:45			
Session II (Chaired by Bin Gao & Aikichi Iwamoto)	12:45-13:15	**Wenjun Liu** Institute of Microbiology, Chinese Academy of Sciences.	5. Phosphorylations of M1 and NP of influenza virus
	13:15-13:45	**Taisheng Li** Division of Infectious Diseases, Pekin Union Medical College Hospital.	6.Overview of antiretroviral treatment in China：Advancement and challenges
Break 13:45 - 13:55			

Session	Time	Speaker	Title
Session III (Chaired by Zene Matsuda & Wenjun Liu)	13:55-14:10	**Mimmin Liang** Institute of Biophisics, Chinese Academy of Sciences.	7. Magnetoferritin nanoparticles for targeting and visualizing tumour tissues
	14:10-14:25	**Kazuhiko Maeda** Graduate School of Medical Science, Kumamoto University.	8. GANP regulates transcription and nucleosome occupancy at the *immunoglobulin variable region* for AID-accession
	14:25-14:40	**Chen Ni** Institute of Biophisics, Chinese Academy of Sciences.	9. IFN-gamma safeguards blood-brain barrier by stabilizing endothelial tight junctions during experimental autoimmune encephalomyelitis
	14:40-14:55	**Atsuo Kanno** Institute of Medical Science, University of Tokyo.	10. Essential role for Toll-like receptor 7 (TLR7)-unique cysteines in an intramolecular disulfide bond, proteolytic cleavage and RNA sensing
	14:55-15:10	**Toshihiro Uchihashi** Institute of Medical Science, University of Tokyo.	11. A new therapeutic strategy for oral squamous cell carcinoma using oncolytic herpes simplex virus G47Δ
	15:10-15:25	**Yuu Taguchi** Institute of Medical Science, University of Tokyo.	12. Stimulation-dependent internalization of RANK is required for the cell-cell fusion during osteoclastogenesis
Break15:25 - 15:45			
Session IV (Chaired by Ai Kawana-Tachikawa &Takeshi Noda)	15:45-16:00	**Tomoko Kuwahara** Institute of Medical Science, University of Tokyo.	13. Identification and Characterization of Host Factors that Interact with Influenza Virus M2 Protein
	16:00-16:15	**Shengyan Gao** Institute of Microbiology, Chinese Academy of Sciences.	14.Differential cellular localization of nuclear export protein (NEP) of influenza A virus correlates with viral replication and virulence
	16:15-16:30	**Jun Arii** Institute of Medical Science, University of Tokyo.	15. Non-Muscle Myosin Heavy Chain IIA and IIB Associate with Herpes Simplex Virus 1 Envelope Glycoprotein B and Mediates Viral Entry.
	16:30-16:45	**Zhang Ge** Institute of Microbiology, Chinese Academy of Sciences.	16. Retargeting NK-92 for anti-melanoma activity by a TCR-like single-domain antibody
	16:45-17:00	**Shuhei Nakane** Institute of Biophisics, Chinese Academy of Sciences.	17.V4 and V5 loops of HIV-1 gp120 are tolerant to insertion of foreign protein
	17:00-17:15	**Shuo Guan** Institute of Microbiology, Chinese Academy of Sciences.	18. Human immunodeficiency virus type-1 subtype and co-receptor usage in treatment-naive patients in a Chinese cohort study
Closing Session	17:15-17:30	**Junichiro Inoue** Institute of Medical Science, University of Tokyo	
Banquet	18:00	眉州東坡・国奥村店	

Dual roles of a cell adhesion molecule, CADM1, in human oncogenesis

Takeshi Ito, Mika Sakurai-Yageta, Daisuke Matsubara, and *Yoshinori Murakami

Division of Molecular Pathology, Institute of Medical Science, the University of Tokyo, JAPAN

The aberration of cell adhesion is a critical step in the invasion and metastasis of human cancer. A tumor suppressor gene, *CADM1*/*TSLC1*, was originally identified in non-small cell lung cancer (NSCLC) by functional complementation. *CADM1* encodes an immunoglobulin superfamily cell adhesion molecule homologous to nectins. CADM1 is expressed in the brain, testis, lung and many other epithelial tissues. In contrast, CADM1 is inactivated in 30-60% of various cancers in advanced stages, including NSCLC, breast cancer, renal cancer and bladder cancer. CADM1 is primarily involved in the formation of an epithelial cell structure and associates with an actin-binding protein, 4.1B, and the membrane associated guanylate kinase homologs, providing a novel tumor suppressor cascade. Lung tumor development in the *Cadm1* gene-deficient mice indicates that this cascade is essential for lung tumor suppression. Further analyses of lung tumors in the *Cadm1*$^{-/-}$ mice showed that loss of another CADM-family cell adhesion molecule, CADM4, and 4.1N, as well as activation of several tyrosine kinases, is involved in tumor formation. On the other hand, CADM1 is ectopically expressed in adult T-cell leukemia (ATL) cells and could promote tumor invasion. In ATL cells, the cytoplasmic domain of CADM1 directly interacted with the PDZ domain of Tiam1, a guanine nucleotide exchange factor of Rac, and induced lamellipodia formation through Rac activation. Furthermore, overexpression of CADM1 was frequently observed in small cell lung cancer (SCLC). The distinct roles of CADM1 in the oncogenesis, a tumor suppressor in lung cancer and an oncoprotein in ATL or SCLC, would be due to the distinct down-stream cascade specific to each tissue. Unique features of CADM1-mediated cell adhesion in oncogenesis will be discussed.

Macrophage derived S100A4 promotes liver fibrosis and hepatic cellular carcinoma

Lin Chen [1], Jinhua Zhang [1], Zhihai Qin *.

1 Key Laboratory of Protein and Peptide Pharmaceuticals, Institute of Biophysics, China
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S100A4 (also known as Fibroblast specific protein 1) is a member of the S100 calcium-binding family which was first cloned in metastatic cells and fibroblasts. Here, we discovered a novel myeloid cell population ($CD11b^{+}S100A4^{+}$), accumulating in the liver during progression of liver fibrosis and liver cancer in mice. Selective depletion of these cells attenuated liver fibrosis and cancer progression. The S100A4 secreted by these cells activates HSCs and stimulates their expression of α-SMA. S100A4 deficiency correlated with impaired liver fibrosis. Blocking S100A4 expression in vivo eliminated the progression of liver fibrosis. Most importantly, increased S100A4 levels in both liver tissue and serum correlated also positively with liver fibrosis in humans. The results above demonstrate that S100A4 secreted by a novel macrophage population promotes liver fibrosis by activation of HSCs, which may represent an interesting target for anti-fibrotic strategies. The mechanism of liver cancer promotion effect of S100A4 and the clinical relationship will be further investigated.

Interaction between virus and host immune response during chronic HIV-1 infection

Ai Kawana-Tachikawa

Division of Infectious Diseases, Advanced Clinical Research Center, the Institute of Medical Science, the University of Tokyo, Japan

During chronic HIV-1 infection, host immune system fights continuously against productive HIV-1 infection, which causes characteristic immunopathogenesis in HIV-1 infection.

HIV-1-specific cytotoxic T lymphocytes (CTLs) are strongly induced during HIV-1 infection. An amino acid substitution around CTL epitope allows the virus to evade the CTL response. We identified an amino acid substitution driven by CTL selection pressure on an epitope, which generates another *de novo* CTL epitope presented by the cognate HLA class I molecule. Although strong CTL responses were induced against the new epitope, additional escape mutation from the CTL occurred in most of the patients.

Although T cell dysfunction is another notable feature during chronic HIV-1 infection, the molecular mechanism of the T cell damage is still not fully understood. We assessed cytokines expression after T cell stimulation in HIV-1 infected subjects with different clinical status, and found that IL-2 expression was significantly lower in non-controllers than viremic controllers. We also revealed that the specific CpG site located within the regulatory elements in *IL2* promoter region was hyper-methylated in non-controllers.

Genome Packaging Mechanism of Influenza A Viruses

*Takeshi Noda[1], Yoshihito Kawaoka[1,2,3]

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The influenza A virus genome comprises eight single-stranded negative-sense RNA (vRNA) segments. Each vRNA binds to multiple copies of viral nucleoproteins together with a heterotrimeric polymerase complex to form a rod-like ribonucleoprotein complex (RNP), which serves as a minimum functional unit responsible for transcription and replication of the viral genome.

While genome segmentation provides advantages such as genetic reassortment, which contributes to the emergence of novel strains with pandemic potential, it complicates the genome packaging process of progeny virions. Here, by using several electron microscopic techniques, we examined how the RNPs are incorporated into each progeny virion and how they are organized within the virion. Electron microscopy of progeny virions budding from cells suggests that each virion selectively packages a full set of 8 vRNAs, which would contribute to maintenance of the integrity of the viral genome during repeated cycles of replication.

Phosphorylations of M1 and NP of influenza virus

Shanshan Wang, Weinan Zheng and Wenjun Liu

Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China.

Phosphorylations of viral proteins play important roles in influenza A virus (IAV) life cycle. Eleven of the fourteen viral proteins encoded by the genome of IAV are described as phosphorylated proteins, including the most abundant protein, the matrix protein M1, and the vRNP component nucleoprotein NP in virions. By using mass spectrometry, we identified tyrosine 132 (Y132) as a phosphorylation site of the matrix protein (M1) of the influenza virus A/WSN/1933(H1N1). Phosphorylation at this residue is essential to the process of virus replication by controlling the nuclear import of M1.The present data indicate that the phosphorylated tyrosine is crucial for the binding of M1 to the nuclear import factor importin-α1, since any substitutions at this site severely reduce this protein-protein interaction and damage the importin-α1-mediated nuclear import of M1. Additionally, the tyrosine phosphorylation which leads to the nuclear import of M1 is blocked by a Janus kinase inhibitor. These results reveal a pivotal role of this tyrosine phosphorylation in the intracellular transportation of M1, which controls the process of viral replication.

In addition, we identified several of phosphorylated residues of NP including two reported residues and four of them are important for virus growth. Such as the mutation of Y296 to-E impairs the nuclear export of NP, while Y296F mutation restores it to the wild-type. The data demonstrated that the phosphorylation of Y296 damages the interaction of NP and nuclear export factor CRM1, consequently, NP with phosphorylated Y296 was detained in the nucleus after the initial infection. Also, we measured influenza polymerase complex activity of each NP mutation and determined that effect of each substitution of S165, Y296 and Y385 to-E on enzymatic activity. Furthermore, we identified that the Y-to-E mutation of Y296 and Y385 reduce the oligomerisation of NP, which is crucial for influenza polymerase activity. Our results suggest that phosphorylation of NP affects virus replication by regulating its nuclear-cytoplasmic trafficking, oligomerisation and vRNP activity.

Overview of antiretroviral treatment in China : Advancement and challenges

Taisheng LI

Department of Infectious Diseases, Peking Union Medical College Hospital, China

To respond the HIV/AIDS epidemic in China, the national AIDS control policy, "Four Frees and One Care" was announced since 2003. There were a lot of difficulties in complementing the national free antiretroviral treatment (ART) program, including lack of qualified human resources, inadequate access to laboratory monitoring and limited class of available antiretroviral drugs. Overcoming these difficulties, China has made significant strides in the fight against HIV/AIDS. By 2012, Over 180 000 adult persons had received first-line HAART thus far and mortality significantly decreased to 4 or 5 deaths per 100 person years. However many challenges remain and well-coordinated efforts will be needed for continued progress. Increased funding, development of the health care system, and greater attention to side effects and comorbid conditions will help China achieve its future HIV-treatment goal.

Magnetoferritin nanoparticles for targeting and visualizing tumour tissues

Minmin Liang, Kelong Fan, Xiyun Yan

Key Laboratory of Protein and Peptide Pharmaceutical, National Laboratory ofBiomacromolecules,CAS-University of Tokyo Joint Laboratory of Structural Virology andImmunology, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China.

Abstract

Engineered nanoparticles have been used to provide diagnostic, therapeutic and prognostic information about the status of disease. Nanoparticles developed for these purposes are typically modified with targeting ligands (such as antibodies, peptides or small molecules) or contrast agents using complicated processes and expensive reagents. Moreover, this approach can lead to an excess of ligands on the nanoparticle surface, and this causes nonspecific binding and aggregation of nanoparticles, which decreases detection sensitivity17−20. Here, we show that magnetoferritin nanoparticles (M-HFn) can be used to target and visualize tumour tissues without the use of any targeting ligands or contrast agents. Iron oxide nanoparticles are encapsulated inside a recombinant human heavy-chain ferritin (HFn) protein shell, which binds to tumour cells that overexpress transferrin receptor 1 (TfR1). The iron oxide core catalyses the oxidation of peroxidase substrates in the presence of hydrogen peroxide to produce a colour reaction that is used to visualize tumour tissues.We examined 474 clinical specimens from patients with nine types of cancer and verified that these nanoparticles can distinguish cancerous cells from normal cells with a sensitivity of 98% and specificity of 95%.

GANP regulates transcription and nucleosome occupancy at the *immunoglobulin variable region* for AID-accession

Kazuhiko Maeda*, Shailendra Kumar Singh, Mohammed Mansour Abbas Eid, Sarah Ameen Almofty, and Nobuo Sakaguchi

Department of Immunology, Graduate School of Life Sciences, Kumamoto University, Japan

Epigenetic modifications are currently known to regulate expression of various functional molecules in the cell-lineage specific manner. *Immunoglobulin (Ig)* gene is a most well characterized locus for the highly stage-specific expression and for the somatic gene rearrangements, the generation of *variable (V)-region* specific mutations and class switch recombination during immune responses. We have found a molecule named GANP that regulates the *IgV-region* somatic hypermutation in B-cells. Mutant mice of both GANP-deficiency and GANP-overexpression in B-cell demonstrated that GANP is involved in generation of high-affinity antigen-specific B-cells against T-cell-dependent antigens. Somatic hypermutation in B-cells is initiated by activation-induced cytidine deaminase (AID) catalyzed C to U deamination at *IgV-regions*. GANP selectively binds to the AID and is involved in its recruitment into the nucleus and to the transcription active *IgV-region* gene in B-cells. Here we investigate the role of the GANP in enhancing the access of AID to *IgV-regions*. We also show that the GANP regulates the chromatin modification at rearranged *IgV-loci*, and its activity requires a histone acetyltransferase domain. GANP interacts with the transcription stalling protein Spt5 and facilitates RNA polymerase-II recruitment to *IgV-regions*. Germinal center B-cells from GANP-transgenic mice showed a higher AID occupancy at the *IgV-regions*, whereas B-cells from conditional GANP-knockout mice exhibit a lower AID accessibility. These findings suggest that GANP-mediated chromatin modification promotes transcription complex recruitment and positioning at *IgV-loci t*o favour AID targeting. Collectively, GANP is a selective regulator of epigenetic modification.

1) Proc. Natl. Acad. Sci. USA, 2000
2) Proc. Natl. Acad. Sci. USA, 2002
3) J. Biol. Chem., 2010
4) Nat. Commun., 2013

Interferon-γ safeguards blood-brain barrier by stabilizing endothelial tight junctions during experimental autoimmune encephalomyelitis

Chen Ni[1,2,*], Chunhui Wang[1,2], Liwei Qu[1,2], Xiaoman Liu[1], Yu Lu[1,2], Wei Yang[1,2], Jingjing Deng[1,2], Jingjing Zhang[4,5], Pan Gao[3], Xiyun Yan[1], Ingolf E. Blasig[5] and Zhihai Qin[1]

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The function of the blood-brain barrier (BBB) is often disrupted during the progressing of multiple sclerosis and its animal model, experimental autoimmune encephalomyelitis (EAE). Interferon-γ (IFNγ), a pleiotropic pro-inflammatory cytokine, plays a critical role in these diseases; however, its regulation on BBB function is still not known. Here, we show that the expression of IFNγR on endothelial cells protected mice from their brain but not spinal cord damage during EAE. IFNγ stabilized the integrity of brain endothelium and prevented the infiltration of leukocytes into the brain. Further analysis revealed that IFNγ increased the expression of tight junction proteins ZO-1 and OCLN, and enhanced membranous distribution of claudin-5 in brain endothelial cells. Silencing claudin-5 eliminated the IFNγ-mediated improvement on endothelial cell integrity. The results demonstrate that IFNγ prevents brain inflammation by stabilizing endothelial tight junctions and provide evidence for explaining the contradictory effects of IFNγ in EAE.

Essential role for Toll-like receptor 7 (TLR7)-unique cysteines in an intramolecular disulfide bond, proteolytic cleavage and RNA sensing

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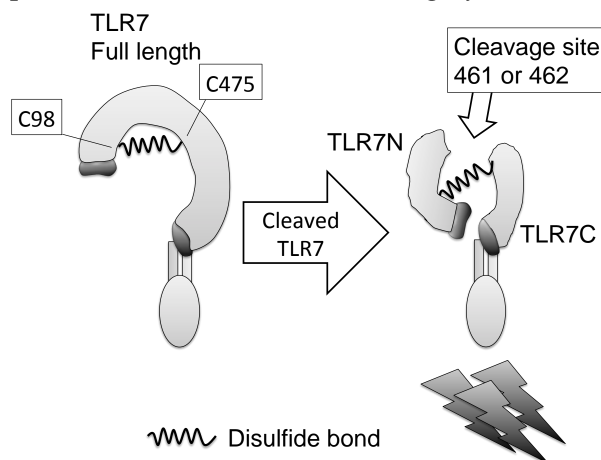
Toll-like receptor 7 (TLR7) is an innate immune sensor for microbial RNA, which erroneously responds to self-derived RNA. To avoid autoimmune responses, TLR7 is suggested to maintain silence until the N-terminal half of the TLR7 ectodomain (TLR7N) is cleaved off. Resultant truncated TLR7 (TLR7C) is thought to signal microbial RNA. We here show that TLR7N remains associated with TLR7C through a disulfide bond. By N-terminal amino acid sequencing, TLR7C was found to start at 461E or 462A. The newly established monoclonal anti-TLR7N showed that endogenous TLR7 in bone marrow-derived dendritic cells was almost all cleaved and cleaved TLR7N localized outside endoplasmic reticulum in steady state. TLR7N outside endoplasmic reticulum was linked with TLR7C by a disulfide bond. In contrast, TLR9 did not have a disulfide bond between TLR9N and TLR9C fragments. Among the cysteines unique to the ectodomain of TLR7 but not TLR9 (Cys98, Cys445, Cys475 and Cys722), Cys98 in TLR7N and Cys475 in TLR7C were required for an intramolecular disulfide bond. These cysteines were also needed for proteolytic cleavage of RNA sensing by TLR7, but not for TLR7 trafficking from endoplasmic reticulum to endosomes. No response was seen in TLR7 mutants lacking the proteolytic cleavage site. These results demonstrate requirement for proteolytic cleavage and TLR7N in TLR7 responses and indicate RNA sensing by TLR7N + TLR7C.



A new therapeutic strategy for oral squamous cell carcinoma using oncolytic herpes simplex virus G47Δ

Toshihiro Uchihashi[1,2]*, Hirokazu Nakahara[3], Yasushi Ino[1], Hiroshi Fukuhara[1], Mikihiko Kogo[2] and Tomoki Todo[1]

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Herpes simplex virus type 1(HSV-1)-based oncolytic viruses have shown to be effective in killing a variety of tumor cells. G47Δ is a genetically engineered oncolytic HSV-1 with triple mutations, that has realized augmented viral replication in tumor cells, induction of enhanced systemic antitumor immunity, while maintaining safety in normal tissues. Clinical trials of G47Δ are ongoing in patients with glioblastoma, prostate cancer, or olfactory neuroblastoma at the University of Tokyo. We intend to extend the clinical application of G47Δ to oral squamous cell carcinoma (OSCC).

The cytopathic effect and the viral replication capability of G47Δ were studied in several OSCC cell lines. Most human OSCC cell lines were susceptible to G47Δ and showed high virus yields *in vitro*. In *in vivo* studies, G47Δ was effective in inhibiting the growth of subcutaneous tumors in mice compared with mock. In an orthotopic tongue tumor model, G47Δ treatment significantly prolonged the survival of athymic mice bearing human OSCC and decreased the number of neck lymph node metastases. In both athymic mice and immnocompetent mice, G47Δ injected into primary tongue tumors was detected in tumors metastatic to the neck lymph nodes by immunohistochemistry and real time PCR. Additionally, in a lung metastases model, the progression of lung lesions was almost totally suppressed by systemic G47Δ administration.

Oncolytic virus therapy using G47Δ was efficacious for both primary and metastatic lesions in mouse OSCC models. The results indicate the use of G47Δ may be an effective new therapeutic approach for OSCC.

Stimulation-dependent internalization of RANK is required for the cell-cell fusion during osteoclastogenesis

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* Presenting author

Many patients of infectious diseases have clinical complications involving bone disorders, such as osteoporosis in HIV-infected patients, osteodystrophy deformans of Paget's disease in paramyxoviruses-infected patients, and bone destruction caused by periodontitis. Such bone disorders are caused by excess formation and activation of osteoclasts, multi-nucleated cells that degrade bone matrix. Therefore, it is important to elucidate molecular mechanisms of osteoclastogenesis for developing methods and drugs to cure such diseases sufficiently.

Activation of RANK signaling by RANK ligand is sufficient to trigger osteoclastogenic signal. The RANK signaling activates MAPKs/NF-κB/Ca^{2+} pathways leading to the induction of NFATc1, a master transcriptional factor for osteoclastogenesis. We have previously identified a functional domain in the cytoplasmic tail of RANK, named HCR. The HCR functions as a platform for formation of signal complex, and emanates sustained RANK signaling, which is essential for NFATc1 induction. However, the role of HCR remains to be elucidated.

Here we report that HCR is required for the stimulation-dependent internalization of RANK. Our immunofluorescence microscopic analysis revealed that RANK became localized to peri-nuclear region from plasma membrane after stimulation in an HCR-dependent manner. Moreover, we found that treatment of precursor cells with Dynasore, an inhibitor of Dynamin-dependent endocytosis and receptor internalization, resulted in the inhibition of RANK-internalization. More interestingly, the Dynasore treatment blocked formation of multinucleated cells while it scarcely affected NFATc1 activation and expression of TRAP, an osteoslast-specific gene. Taken together, our results strongly suggest that the RANK-internalization is necessary to emanate the cell-cell fusion signal during osteoclastogenesis.

Identification and Characterization of Host Factors that Interact with Influenza Virus M2 Protein

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Influenza virus relies heavily on the cellular machinery to replicate in host cells. Therefore, to better understand the influenza virus life cycle, we must identify which host proteins are important and how they function during virus replication. In this study, we focused on the multi-functional viral protein M2 and attempted to identify the host proteins that interact with it. To identify M2-interacting host proteins, we used coimmunoprecipitation and mass spectrometry; siRNA interference was also used to analyze the functions of the host proteins. We identified almost 400 M2-interacting host proteins; of these, we focused on G protein pathway suppressor 1 (GPS1). GPS1 plays a major role in the MAPK pathway by regulating the AP-1 transcription factor. Down-regulation of GPS1 expression reduced influenza virus replication by more than two log units. The viral polymerase activity was also reduced in GPS1 down-regulated cells. In view of these results, we hypothesize that the interaction between GPS1 and M2 influences influenza virus replication by regulating the transcription and replication of viral RNAs.

Differential cellular localization of nuclear export protein (NEP) of influenza A virus correlates with viral replication and virulence

Shengyan Gao*, Shan Wang, Shuai Cao and Wenjun Liu

CAS Key laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China.

Influenza viruses are major causative agents of epidemic and pandemic infections and are responsible for the death of at least half a million worldwide each year. The genome of influenza A viruses comprises eight negative-sense, single-stranded RNA encapsidated by viral nucleoprotein (NP) encoding 13 proteins. NS1 and NS2 (NEP) are derived from alternatively spliced mRNAs that are transcribed from the eighth segment. NEP was proved playing crucial role during the nuclear export of newly assembled viral ribonucleoprotein complexes (vRNPs). In the present study we identified that NEP of A/H1N1/WSN/33 and A/H1N1/California/04 both diffused into the nuclear in an energy-independent manner and exited through the CRM1-mediated nuclear export pathway. Two functional leucine-rich, CRM1-dependent nuclear export signals (NESs) were found on the N- terminal domain of both proteins. However, NEP from three strains showed differential cellular localization pattern either when overexpressed in cells or during infection. The NEP from CA04 strain preferred to form aggregates in the nuclear and exhibited reduced binding activity to CRM1 compared to the NEP from WSN. Through sequence alignment and site-directed mutagenesis, we identified the amino acids that determined these differences. Our research would help to understand the mechanism of the pandemic 2009 (H1N1) influenza A virus's adaption to humans and virulence.

Non-Muscle Myosin Heavy Chain IIA and IIB Associate with Herpes Simplex Virus 1 Envelope Glycoprotein B and Mediates Viral Entry

Jun Arii[1, 2], Akihisa Kato[1, 2] and Yasushi Kawaguchi[1, 2]

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Our group reported that non-muscle myosin heavy chain IIA (NMHC-IIA) function as a herpes simplex virus 1 (HSV-1) entry receptor by interacting with viral envelope glycoprotein B (gB). Vertebrates have three genetically distinct isoforms of the NMHC-II, designated NMHC-IIA, NMHC-IIB and NMHC-IIC. In these, NMHC-IIB is highly expressed in neural tissue which is one of the most important targets for HSV-1 infection in vivo. COS cells, which are readily infected by HSV-1, do not express NMHC-IIA but do express NMHC-IIB. This observation prompted us to investigate whether NMHC-IIB might associate with HSV-1 gB and be involved in an HSV-1 entry like NMHC-IIA. In these studies, we show that: (i) NMHC-IIB co-precipitated with gB in COS-1 cells upon HSV-1 entry; (ii) overexpression of NMHC-IIB in IC21 cells significantly increased their susceptibility to HSV-1 infection; (iii) knock-down of NMHC-IIB in COS-1 cells inhibited HSV-1 infection as well as cell-cell fusion mediated by HSV-1 envelope glycoproteins; and (iv) a specific inhibitor of myosin light chain kinase, which has been reported to induce cell surface expression of NMHC-IIA, inhibited both cell surface expression of NMHC-IIB in COS-1 cells upon HSV-1 entry and HSV-1 infection. These results supported the hypothesis that, like NMHC-IIA, NMHC-IIB associated with HSV-1 gB and mediated HSV-1 entry. Future clarification of the precise roles of NMHC-IIA and NMHC-IIB in vivo should account for the HSV-1 tropism and pathogenicity.

Retargeting NK-92 for anti-melanoma activity by a TCR-like single-domain antibody

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The efficacy of immunotherapy based on natural killer (NK) cells is hampered by intrinsic non-specific cytotoxicity and insufficient activation of NK cells. Here, we confer the T cell receptor-like (TCR-like) specificity on NK cells, taking advantage of both the innate and adaptive immune arms of the immune response to generate enhanced anti-melanoma activity. The TCR-like antibody GPA7 was selected against melanoma-associated gp100/HLA-A2 complex and then fused to intracellular domain of CD3-ζ chain. This fusion construct was incorporated into NK-92MI cell line and expressed as a chimeric antigen receptor on the surface of the cell. The anti-tumour activity of the transgenic NK-92MI-GPA7-ζ cell line was assessed against melanoma *in vitro* and *in vivo*. The engineered NK-92MI-GPA7-ζ cells could recognize melanoma cells in the context of HLA-A2 and showed enhanced killing of both melanoma cell lines and primary melanoma. Furthermore, adoptively transferred NK-92MI-GPA7-ζ cells significantly suppressed the growth of human melanoma in a xenograft model in mice. Collectively, these results demonstrate that the TCR-like antibody, GPA7, could redirect NK cells to target the intracellular antigen gp100 and enhance anti-melanoma activity providing a promising immunotherapeutic strategy to prevent and treat melanoma.

V4 and V5 loops of HIV-1 gp120 are tolerant to insertion of foreign protein

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AIDS is caused by Human Immunodeficiency Virus type 1 (HIV-1). HIV-1 is an enveloped virus and its envelope glycoprotein (Env) is essential for attachment, membrane fusion, and entry for HIV-1. HIV-1 Env is a major target of anti-HIV-1 vaccine. HIV-1 Env consists of a trimer of non-covalently associated gp120 and gp41: the gp120 domain recognizes receptors/coreceptors, whereas gp41 mediates membrane fusion. The gp120 domain contains five variable loops (V1 to V5) that manifest great sequence heterogeneity. One of them, V3, harbors a major coreceptor binding site. V1/V2 region is also involved in coreceptor recognition and manifests diversity in its sequence as well as length. No known major function is assigned to the V4 and V5 regions. To further investigate the structure-function relationship of these variable loops, we performed an insertional mutagenesis using GFP as an insert. We inserted the GFP variant called GFPopt into codon optimized HXB2 Env and examined expression, fusogenicity, virus incorporation, and intracellular distributions of the mutated Env. Out of 18 constructs, 11 GFP-inserted Env (GFP-Env) retained fusogenicity in the cell-cell fusion assay. The rest of fusion-incompetent mutants showed a defective proteolytic processing of gp160 into gp120 and gp41. Among fusion-competent GFP-Envs, mutants with GFP in V4 and V5 loops showed better virus-cell fusion activities as compared with the other mutants. Our results suggested that each variable region has different sensitivity to insertion of foreign protein. As the coreceptor recognition site, V3 was intolerant to insertion. Despite its great diversity in sequence and length in natural isolates, V1/V2 was more sensitive to insertional mutagenesis. Since some of the GFP-Envs are functional in several assays, these will become a valuable tool to analyze the intracellular trafficking and fate of post-entry HIV-1 gp120.

Human immunodeficiency virus type-1 (HIV-1) subtype and coreceptor-usage in treatment-naive patients in a Chinese cohort study

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Information about the subtype and coreceptor-usage (R5- or X4-usage) of HIV-1 is important for better understanding and treatment of the infection: in subtype B, X4-virus is found in up to 50% of cases and may be associated with advanced disease. We examined specimens from 473 treatment-naïve patients enrolled in a Chinese cohort study, including no injecting-drug users, (National Key Technologies R&D Program for the 11th Five-year Plan) for the prevalence of subtypes and coreceptor-usage. Almost three quarters of participants were male, with median age 36 (range: 18-65). Sexual contact comprised 76.7%; 44.8% were men having sex with men. Blood transfusion accounted for 9.3%; 14.0% were not accounted for. The CD4+ T cell counts of the participants were less than 350 cells/μl. We used nucleotide sequences of V3 region of the envelope gene (from directly sequencing RT-PCR samples). Subtyping (from a phylogenetic tree) showed that 41.0% of the specimens were CRF01_AE, 32.2% were B or B', and 26.8% were CRF07 or 08_BC. The genotypic coreceptor-usage analysis (Geno2pheno algorithm) showed X4 virus was found more frequently in CRF01_AE than other subtypes: the prevalence of X4 or dual usage was 63.9% in CRF01_AE; 38.3% in B or B'; and only 6.0% in CRF07 or 08_BC. These findings provide basic data on the subtype and coreceptor-usage of HIV-1 from this cohort study, but with non-B subtypes, there remains some ambiguity of coreceptor-usage based only on DNA sequence. We are comparing coreceptor-usage between the current genotypic analysis and a phenotypic analysis using Dual-Split-reporter protein system.

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