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

Project Associate Professor	Susumu Nakae, Ph.D.	
Project Associate Professor	Beate Heissig, M.D.	
Project Assistant Professor	Riu Yamashita, Ph.D.	
Project Assistant Professor	Katsuyoshi Yamamoto, Ph.D.	
Project Assistant Professor	Kazumasa Yokoyama, Ph.D.	

特任准教授	農学博士	中　江　　進
特任准教授	医学博士	ハイジッヒ　ベアーテ
特任助教	ﾊﾞｲｵｻｲｴﾝｽ博士	山　下　理　宇
特任助教	理学博士	山　本　勝　良
特任助教	理学博士	横　山　一　剛

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‹Susumu Nakae Group›

To understand the molecular mechanism for development of allergic diseases, we investigate the role of cytokines, Th17 cells and mast cells in the diseases using gene-deficient mice.

1. IL-33 is a crucial amplifier of innate rather than acquired immunity

Keisuke Oboki[1], Tatsukuni Ohno[1], Naoki Kajiwara[2], Ken Arae[1], Hideaki Morita[1], Akina Ishii[1], Aya Nambu[2], Takaya Abe[3], Hiroshi Kiyonari[3], Kenji Matsumoto[1], Katsuko Sudo[4], Ko Okumura[2], Hirohisa Saito[1] and Susumu Nakae[5]: [1]Department of Allergy and Immunology, National Research Institute for Child Health and Development, Japan, [2]Atopy Research Center, Juntendo University, Japan, [3]Laboratory for Animal Resources and Genetic Engineering, Center for Developmental Biology (CDB), RIKEN Kobe, Japan, [4]Animal Research Center, Tokyo Medical University, Japan, [5]Frontier Research Initiative, The Institute of Medical Science, The University of Tokyo, Japan.

IL-33, a member of the IL-1-related cytokines, is considered to be a pro-allergic cytokine that is especially involved in Th2-type immune responses. Moreover, like IL-1α, IL-33 has been suggested to act as an "alarmin" that amplifies immune responses during tissue injury. In contrast to IL-1, however, the precise roles of IL-33 in those settings are poorly understood. Using IL-1- and IL-33-deficient mice, we found that IL-1, but not IL-33, played a substantial role in induction of T cell-mediated type IV hypersensitivity such as contact and delayed-type hypersensitivity and autoimmune diseases such as experimental autoimmune encephalomyelitis. Most notably, however, IL-33 was important for innate-type mucosal immunity in the lungs and gut. That is, IL-33 was essential for manifesta-

tion of T cell-independent protease allergen-induced airway inflammation as well as OVA-induced allergic topical airway inflammation, without affecting acquisition of antigen-specific memory T cells. IL-33 was significantly involved in the development of dextran-induced colitis accompanied by T cell-independent epithelial cell damage, but not in streptozocin-induced diabetes or concanavalin A-induced hepatitis characterized by T cell-mediated apoptotic tissue destruction. In addition, IL-33-deficient mice showed a substantially diminished LPS-induced systemic inflammatory response. These observations indicate that IL-33 is a crucial amplifier of mucosal and systemic innate, rather than acquired, immune responses.

2. Development of IL-17-mediated delayed-type hypersensitivity is not affected by down-regulation of IL-25 expression

Akina Ishii[1], Keisuke Oboki[1], Aya Nambu[2], Hideaki Morita[1], Tatsukuni Ohno[1], Naoki Kajiwara[2], Ken Arae[1], Hajime Sudo[2], Ko Okumura[2], Hirohisa Saito[1] and Susumu Nakae[3]: [1]Department of Allergy and Immunology, National Research Institute for Child Health and Development, Japan, [2]Atopy Research Center, Juntendo University, Japan, [3]Frontier Research Initiative, The Institute of Medical Science, The University of Tokyo, Japan.

IL-25, which is a member of the IL-17 family, induces Th2 cell differentiation and Th2 cytokine production, contributing to induction of Th2-type immune responses and diseases, as a result of which it suppresses Th1- and Th17-type immune responses. To elucidate the role of IL-25 in the pathogenesis of IL-17-mediated delayed-type hypersensitivity (DTH), IL-25-deficient mice were sensitized with methylated BSA (mBSA), and then a DTH reaction was induced by mBSA challenge. IL-25 expression was markedly reduced in local DTH lesions. However, mBSA-specific Th1, Th2 and Th17 cell induction, and the mBSA-induced DTH reaction were comparable in IL-25-deficient and wild-type mice. IL-25 is not essential for differentiation of Th1, Th2 and Th17 cells in the sensitization phase or induction of local inflammation in the elicitation phase of the mBSA-induced DTH reaction.

3. Amphiregulin is dispensable for induction of contact hypersensitivity

Akiko Yagami[1], Naoki Kajiwara[2], Keisuke Oboki[1], Tatsukuni Ohno[1], Hideaki Morita[1], Susan W. Sunnarborg[3], Ko Okumura[2], Hideoki Ogawa[2], Hirohisa Saito[1] and Susumu Nakae[4]: [1]Department of Allergy and Immunology, National Research Institute for Child Health and Development, Japan, [2]Atopy Research Center, Juntendo University, Japan, [3]Department of Biochemistry and Biophysics, School of Medicine, University of North Carolina, USA, [4]Frontier Research Initiative, The Institute of Medical Science, The University of Tokyo, Japan.

Amphiregulin (AR) is expressed in Th2 cells, rather than Th1 cells, and plays important roles in Th2 cell/cytokine-mediated host defense against nematodes. We also found earlier that AR mRNA expression was strongly upregulated in the inflamed tissue during Th2 cell/cytokine-mediated fluorescein isothiocyanate (FITC)-induced contact hypersensitivity (CHS), suggesting a contribution of AR to the induction of those responses. To elucidate the role of AR in the induction of FITC- or dinitrofluorobenzene (DNFB)-induced CHS, AR-deficient mice were sensitized and/or challenged with FITC or DNFB epicutaneously. DC migration and FITC-specific lymph node cell proliferation and cytokine production were normal in the AR-deficient mice. Ear swelling, tissue MPO and EPO activities and FITC-specific serum Ig levels were also similar in AR-deficient and -sufficient mice. Therefore, amphiregulin is not essential for the induction of FITC- or DNFB-induced CHS responses in mice.

4. Amphiregulin is not essential for ovalbumin-induced acute airway inflammation in mice

Naoki Kajiwara[1], Keisuke Oboki[2], Tatsukuni Ohno[2], Akina Ishii[1], Susan W. Sunnarborg[3], Ko Okumura[1], Hirohisa Saito[2] and Susumu Nakae[4]: [1]Atopy Research Center, Juntendo University, Japan, [2]Department of Allergy and Immunology, National Research Institute for Child Health and Development, Japan, [3]Department of Biochemistry and Biophysics, School of Medicine, University of North Carolina, USA, [4]Frontier Research Initiative, The Institute of Medical Science, The University of Tokyo, Japan.

The number of amphiregulin (AR)-positive mast cells in the bronchial mucosa and the levels of AR in sputum from asthmatic patients were reported to be increased. In addition, AR can promote mucin gene expression in human epithelial cells, suggesting that AR contributes to the pathogenesis of allergic asthma. To elucidate the role of AR in the pathogenesis of

asthma, we immunized AR-deficient mice with ovalbumin (OVA) and then induced airway inflammation in them after OVA inhalation. The OVA-induced airway inflammation was assessed on the basis of the lung histology, number of leukocytes in the bronchoalveolar lavage (BAL) fluid, Th2 cytokine levels in the BAL fluid and OVA-specific IgG1 and IgE levels in the serum and compared between AR-sufficient and -deficient mice. However, the OVA-induced airway inflammation was comparable in the AR-sufficient and -deficient mice. Therefore, Amphiregulin is not essential for induction of acute airway inflammation by OVA in mice.

‹Beate Heissig Group›

Proteases perform highly selective and limited cleavage of specific substrates including growth factors and their receptors, cell adhesion molecules, cytokines, chemokines, apoptotic ligands and angiogenic factors. Our research efforts are focused on identifying the molecular mechanisms by which the proteolytic machinery regulates cell migration during cancer progression and tissue regeneration.

Plasmin inhibitor reduces lymphoid tumor growth by suppressing matrixmetallproteinase-9 dependent CD11b＋/F4/80＋ myeloid cell recruitment

Makoto Ishihara[1], Chiemi Nishida[1], Yoshihiko Tashiro[1], Ismael Gritli, Jeanette Rosenkvist, Makiko Koizumi[1], Ryo Yamamoto[1], Hideo Yagita[2], Ko Okumura[3], Momoko Nishikori[4], Keiko Wanaka[5], Yuko Tsuda[6], Yoshio Okada[6], Hiromitsu Nakauchi[1], Koichi Hattori[1,3], Beate Heissig: [1]Center for Stem Cell Biology and Regenerative Medicine, Institute of Medical Science at the University of Tokyo, Japan, [2]Dep. of Immunology, Juntendo University School of Medicine, Japan, [3]Atopy (Allergy) Research Center, Juntendo University School of Medicine, Japan, [4]Dep. of Hematology and Oncology, Kyoto University, Japan, [5]Kobe Research Projects on Thrombosis and Haemostasis, Japan, [6]Faculty of Pharmaceutical Sciences, Kobe Gakuin University, Japan.

Activation of the fibrinolytic system during lymphoma progression has been described. But the mechanism by which the fibrinolytic system can modulate lymphoma progression has been elusive. The main fibrinolytic enzyme, plasminogen (Plg)/plasmin (Plm), can activate matrix metalloproteinases (MMPs), like MMP-9, which has been linked to various malignancies. We report that blockade of Plg reduces lymphoma growth by inhibiting MMP-9-dependent recruitment of $CD11b^{+}F4/80^{+}$ myeloid cells locally within the lymphoma tissue. Genetic plasminogen deficiency and drug-mediated Plm blockade delayed lymphoma growth and diminished MMP-9 dependent $CD11b^{+}F4/80^{+}$ myeloid cell infiltration into lymphoma tissues. A neutralizing antibody against CD11b inhibited lymphoma growth *in vivo*, which indicates that CD $11b^{+}$ myeloid cells play a role in lymphoma growth. Plg deficiency in lymphoma-bearing mice resulted in reduced plasma levels of the growth factors vascular endothelial growth-A and Kit ligand, both of which are known to enhance myeloid cell proliferation. Therefore, specific blockade of Plg represents a promising approach for the regulation of lymphoma growth.

‹Riu Yamashita Group›

Classification and characterization of bi-directional promoters in human and mouse cells.

Riu Yamashita[1,2], Yutaka Suzuki[3], Sumio Sugano[3], Kenta Nakai[2]: [1]Frontier Research Initiative, Inst. of Med. Science, Univ. of Tokyo, [2]Human Genome Center, Inst. of Med. Science, Univ. of Tokyo, [3]Department of Medical Genome Sciences, Graduate School of Frontier Sciences, Univ. of Tokyo.

We have constructed the DataBase of Transcription Start Sites (DBTSS: http://dbtss.hgc.jp/) to provide information of accurate transcription start sites (TSSs) based on experimentally determined 5′-end clones. Recently we have updated this database, and now DBTSS has 328 million short sequences generated by massively sequencing the 5′ end of oligo-cap selected cDNAs in humans and mice. Using this database, we focused on bi-directional promoters, which has TSSs on both plus and minus strand closely. We obtained 10,031 TSSs for DLD-1 cell lines. Among these TSSs, the distribution of frequency of the distance between a transcript's TSS and the closest anti-transcript TSS showed significant two peaks. The first peak corresponded to for 707 TSSs on −160: −100 (upstream anti-sense transcript promoters: upASTPs), and the second corresponded to 237 TSSs 0:＋40 (downstream anti-sense transcript promoters: downASTPs). We also defined 2166 TSSs which did not have any anti-transcript within 10 kb as no anti-sense transcript promoters (noASTPs). According to the Gene Ontology analysis, we found that a GO term 'signal transduction' is overrepresented in noASTPs. On the other hand, 'translation factors' is overrepre-

sented in downASTPs. Around TSS region, noASTPs and upASTPs had pyrimidine-purine bases on −1:+1, which can be widely observed on TSSs (5). We also observed significant characteristics difference in the downASTPs: namely, GC poor, CpG islands poor, and disordered nucleosome structures. Interestingly, even though we observed highly ordered nucleosome structures in both downASTPs and noASTPs, the noASTPs showed more asymmetrical nucleosome structures. These phenomena could be observed in not only other human cell lines (Hek293, MCF7, TIG), but also mouse a cell line (3T3). These results indicate that we could classify promoters into three classes based on their anti-transcript, and these classes showed biologically different features.

‹Katsuyoshi Yamamoto Group›

Control of the yeast MAPK pathways by dynamic regulation of Ste50 scaffold protein-Opy2 membrane anchor interaction

Katsuyoshi Yamamoto, Kazuo Tatebayashi[1], Keiichiro Tanaka[1], and Haruo Saito[1]: [1]Division of Molecular Cell Signaling, Institute of Medical Science, University of Tokyo.

In yeast, activation of three functionally distinct MAPK pathways (the mating pathway, the filamentous growth/invasive growth (FG/IG) pathway, and the osmoregulatory HOG pathway) shares the essential Ste11 MAPKKK. Nonetheless, they are differentially activated by distinct stimuli. In all these pathways, Ste11 is phosphorylated and activated by the PAK like kinase Ste20. Because active Ste20 is anchored onto the plasma membrane via membrane-bound small G protein Cdc42, localization of Ste11 to the membrane is crucial for its activation. In the mating pathway, the scaffold protein Ste5 directly recruits Ste11 to the membrane. In the FG/IG and the HOG pathways, the membrane protein Opy2 indirectly recruits Ste11 to the membrane via Ste50, which binds both Ste11 and Opy2. We found that Opy2 has two major (CR-A and CR-B), and one minor (CR-D), binding sites for Ste50. CR-A binds Ste50 constitutively and can transmit signals to both the Hog1 MAPK (component of the HOG pathway) and Fus3/Kss1 MAPKs (components of the mating and the FG/IG pathways). CR-B binds Ste50 only when Opy2 is phosphorylated by casein kinase I (CKI) Yck1/Yck2 under glucose-rich conditions and transmits the signal preferentially to the Hog1 MAPK.

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/Kss1 MAPKs. The phosphorylated form of Ste50 had much lower affinity to Opy2 in *in vitro* binding assays. The yeast cells expressing the Ste50-2-6A 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. Furthermore, Ste50 phosphorylation, together with MAPK specific protein phosophatases, reduces the basal activity of the HOG and the mating pathways. Thus, dynamic regulation of Ste50-Opy2 interaction fine-tunes the yeast MAPK signaling network.

‹Kazumasa Yokoyama Group›

Analysis of the NYAP family-mediated signaling pathway in neurons

Kazumasa Yokoyama[1,2], Tadashi Yamamoto[2]: [1]Frontier Research Initiative, [2]Division of Oncology, Institute of Medical Science, University of Tokyo, Japan.

The Src family of protein tyrosine kinases is implicated in various neural functions such as neuronal development, myelination, and synaptic plasticity. To elucidate roles for 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 kinases, we performed solid-phase phosphorylation screening and identified a novel NYAP family of phosphoproteins. We demonstrated that NYAPs regulate both upstream and downstream of the phosphoinositide 3-kinase (PI3K) signaling in developing neurons. Upon stimulation of Contactin family membrane proteins, NYAPs were tyrosine phosphorylated by Fyn, thereby providing the major binding sites for PI 3K in neurons. Disruptions of NYAPs decreased Rho, Rac, PI3K and Akt activity. Furthermore, NYAPs associated with the WAVE1 complex (i.e., Nap1, Sra1, and WAVE1) which is an essential link between Rac1 activation and actin polymerization. Rac1 is activated by PI3K-produced PIP_3. Interestingly, we found that WAVE1 is associated with PI3K p85 in the developing brain, while this association is not observed at all in non-neuronal cells such as oligodendrocytes. More importantly, this association in neurons completely depends on NYAPs: that is, WAVE1 is not associated with PI3K p85 in the brain of NYAPs triple knockout mice. Thus, the roles for NYAPs are 1) activation of

PI3K, and 2) recruitment of effector proteins which are activated downstream of PI3K such as WAVE1. 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, PI3K, and WAVE1. Physiological significance of the NYAP family was revealed through the analysis of the triple knockout mice. There, the brain size is reduced, neurite outgrowth is less prominent and loses their sensitivity to Contactin, and mating and nurturing behaviors are compromised.

Many proteins containing phospho-Tyr-x-x-Met (YxxM) motifs have been reported to bind with and activate PI3K 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 p 85-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.

Publications

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‹Riu Yamashita Group›

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Global COE Program of University of Tokyo

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.
Project Assistant Professor Ryosuke Muroyama, M.D., Ph.D.

特任准教授 医学博士 加藤直也
特任助教 医学博士 室山良介

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. A Genome-Wide Study Identifies Susceptibility Loci for Hepatitis C Virus-Induced Hepatocellular Carcinoma

Vinod Kumar[1], Ryosuke Muroyama[2], Norie Kowatari[2], Wenwen Li[2], Motoyuki Otsuka[3], Ryosuke Tateishi[3], Kazuhiko Koike[3], Yusuke Nakamura[1], Koichi Matsuda[1], Naoya Kato[2]: [1]Laboratory of Molecular Medicine, Human Genome Center, IMSUT, [2]Unit of Disease Control Genome Medicine, IMSUT, [3]Department of Gastroenterology, Graduate School of Medicine, University of Tokyo.

Hepatitis C virus (HCV) infection is a major risk factor for developing hepatocellular carcinoma (HCC). The host genetic factors that are involved in the development of HCC in patients with HCV infection remain to be investigated. To identify the genetic susceptibility factor(s) for HCV-induced HCC, a genome-wide association study (GWAS) was conducted using 432,703 autosomal SNPs in 721 HCV-induced HCC cases and 2,890 HCV-negative controls of Japanese origin. As progression from chronic hepatitis C (CHC) to HCC is strongly affected by age and gender, we performed logistic regression analysis by including age and gender as covari-

ates at all tested loci in our analyses. Single nucleotide polymorphisms (SNPs) which showed possible association ($P<1\times10^{-5}$) in the GWAS were further genotyped in 673 cases and 2,596 controls. We identified 8 independent loci showing possible association in the GWAS. After replication, we found a novel locus (SNP) in the 5 prime flanking region of gene MK [$P_{combined}=4.21\times10^{-13}$, odds ratio=1.39] to be strongly associated with HCV-induced HCC, whereas the remaining 7 SNPs failed to replicate the association. Subsequent analyses using patients with CHC indicated that this SNP is not associated with CHC susceptibility ($P=0.61$), but significantly associated with progression from CHC to HCC ($P=3.13\times10^{-8}$). We also found that risk allele of SNP was associated with lower serum MK levels in HCV-induced HCC patients ($P=1.38\times10^{-13}$). Our results highlight the importance of gene MK genetic variations as a predictive biomarker for HCV-induced HCC.

2. Core Mutant Ratio is associated with Response to Pegylated-Interferon/Ribavirin Treatment in HCV Genotype 1b Patients

Wenwen Li[1], Ryosuke Muroyama[1], Zhongjie Hu[1], Norie Kowatari[1], Tadashi Goto[2], Qiang Li[3], Kazuhiko Koike[2], Naoya Kato[1]: [1]Unit of Disease Control Genome Medicine, IMSUT, [2]Department of Gastroenterology, Graduate School of Medicine, University of Tokyo, [3]Division of Liver Disease, Jinan Infectious Disease Hospital, Shandong University, Jinan, China.

Mutant type of amino acid (aa) 70 (Gln/His) in genotype 1b HCV core sequence was reported to be a significant and independent factor of non-virological response (NVR), however, its mechanism remains unclear. The aim of this study was to clarify how mutant strain responds and whether mutant ratio/quantity is related to pegylated-interferon (PEG-IFN)/ribavirin (RBV) treatment response in HCV genotype 1b patients. Total 36 genotype 1b hepatitis C patients who received 48-72 week PEG-IFN/RBV treatment were enrolled. Baseline (before treatment) mutant ratio/quantity of each patient was examined by real-time RT-PCR with aa70 wild (W) and mutant (M) specific probes. Mutant nucleotide (nt) sequence of full-length core in each patient was determined. Moreover, treatment response of each 70W/M and relationship between mutant ratio/quantity and treatment response were analyzed. At baseline, 9 patients were infected with 70W only. 26 patients were double infected with both 70W and 70M. 1 patient was infected with 70M only. According to mutant ratio, patients were divided into 2 groups: Major/All mutant (6/36, 17%) and Minor/No mutant (30/36, 83%). Mutant sequence analyses showed that there were 2 significant nt differences between these two groups. They caused no aa changes in the core protein (nt 351/aa117 and nt381/aa127) while they were possible to change F protein. During treatment, 70M had similar response as 70W. However, lower Mutant ratio patients were significantly easier to achieve early virological response ($P=0.003$) and sustained virological response ($P<0.001$) while mutant quantity showed no effect on antiviral responses. In conclusion, high mutant ratio of HCV genotype 1b core aa70 is associated with NVR to PEG-IFN/RBV treatment. Quantitive mutant ratio of core aa70 should be a more accurate predicator for PEG-IFN/RBV treatment. Functional difference between 70W core/F and 70M core/F will be further analyzed.

3. Fusion HBx translated from HBV integrant is a responsible molecule for hepatocarcinogenesis and could be a universal treatment target

Ryosuke Muroyama, Norie Kowatari, Wenwen Li, Naoya Kato

Hepatitis B virus (HBV) is a major risk factor for HCC, and HBV X protein (HBx), which is the pleiotropic transactivator, is reported to be associated with the hepatocarcinogenesis. However, HBV asymptomatic carriers, expressing a large amount of HBx, rarely develop HCC. In this study, we identified fusion HBx (3′-trancated HBx+human peptides) from HBV integrant in Hep3B cells established from HBV-related HCC, and investigated its role in hepatocarcinogenesis. We could identified fusion HBx translated from HBV integrant in Hep3B cells, which consisted of 3′-trancated HBx following 61 amino acids translated from human sequences, and established stably HBx knocked-down (KD) cells by siRNA. The expression of fusion HBx in KD cells disappeared in immunofluorescence, and cell proliferation and invasion ability were significantly reduced in KD cells. We injected KD cells subcutaneously into nude mice, and monitored tumor growth. KD cells could not develop any visible tumor in nude mice although Hep3B cells could. We constructed the plasmids expressing wild HBx and fusion HBx, and compared anchorage-independent growth ability and transactivation ability of NFκB, AP-1, Wnt/β-catenin, and androgen receptor pathway by soft agar assay and luciferase assay, respectively. Although fusion HBx had significantly decreased transactivation

ability compared to wild HBx, only fusion HBx had anchorage-independent growth ability in soft agar whereas wild HBx did not. Using the protein-synthesis inhibitor cycloheximide, we determined and compared the half-life of wild HBx and fusion HBx. There was no difference in the half-life of wild HBx and fusion HBx, which was estimated to be about 40 min. We transfected wild HBx and fusion HBx-expressing plasmids into Huh7 cells, and compared the gene-expression profiles using the whole genome microarray. In microarray analysis, fusion HBx showed different gene-expression profiles from wild HBx. Not HBx but fusion HBx translated from HBV integrant played an important role in hepatocarcinogenesis. Fusion HBx could be a universal treatment target for HBV-related HCC.

4. Specific Mutations in Genotype C HBx Associated with Hepatocellular Carcinoma

Wenwen Li[1], Ryosuke Muroyama[1], Norie Kowatari[1], Qiang Li[2], Naoya Kato[1]: [1]Unit of Disease Control Genome Medicine, IMSUT, [2]Division of Liver Disease, Jinan Infectious Diseases Hospital, Shandong University, Jinan, China.

HBx is considered to be oncogenic and play a key role in hepatocarcinogenesis. We aim to compare genotype C HBx sequences between HCC and Non-HCC patients and identify genotype specific HCC mutations in this region. 4223 HBx sequences were downloaded mainly from online databases. Sequences finally enrolled should be: 1) contain full-length X region, no insert or deletion; 2) genotype C; 3) came from human sera. 500 Non-HCC and 96 HCC sequences were finally analyzed. We found that: 1) the average mutation number of each sequence was 7.80 ± 3.73 in Non-HCC group and 7.72 ± 2.98 in HCC ($p>0.05$). 2) Genotype C, especially subgenotype C2 HBx was highly conserved in BCP mutation and some binding sites which participate in carcinogenesis. 3) HCC group showed 22 significant point mutations compared to Non-HCC. After logistic regression, 9 point mutations (1383C, 1479Y, 1485T, 1626A, 1653T, 1719T, 1726C, 1762T, 1800C) were proved to be independent factors for risk of HCC (8 aa of HBX were changed but no substitution in Polymerase). 4) A new significant combined mutation was identified: 1630A-1719T-1721G-1762T-1764A (Non-HCC, 39.4%; HCC, 70.8%; $p<0.01$). 5) Subgenotype C2 showed 16 significantly point mutations (8 also contained in Polymerase, 6 in Enh2, 2 in CP). The mutations included 2 new sites (G1511A, C1631T) which were different from those of genotype C, moreover, nt1458 here showed different mutant pattern according to genotype C. In conclusion, genotype C/C2 had specific mutations in HCC patients. These mutation patterns may facilitate the hepatocarcinogenesis.

5. CD26 in hepatocellular carcinoma

Norie Kowatari[1], Ryosuke Muroyama[1], Wenwen Li[1], Toshihiro Okamoto[2], Kei Ohnuma[2], Chikao Morimoto[2], Naoya Kato[1]: [1]Unit of Disease Control Genome Medicine, [2]Division of Clinical Immunology, Advanced Clinical Research Center, IMSUT.

CD26, also known as dipeptidyl peptidase IV (DPP IV), is a 110-kDa cell surface antigen having an important role in tumor development. To explore the possibility of anti-CD26 treatment against HCC, expression of CD26 was firstly examined in HCC and hepatoblastoma cell lines and also in normal liver cells by FACS and Western blotting of cell lysates. Normal liver cells, HLE, HLF, SK-Hep1, and HepG2 cells expressed almost no CD26. On the other hand, Hep3B, PLC/PRF/5, and Huh7 cells expressed CD26. CD26 expression pattern was also determined by immunohistochemistry and not membranous but cytoplasmic staining of CD26 was observed in liver cells expressing CD26. Secondly, immunostaining for CD26 was performed using liver tissue microarray containing HCC and noncancerous liver samples of chronic hepatitis and cirrhosis. Although there was no difference in degree of CD26 staining between HCC and non-HCC, zonal CD26 distribution in normal hepatic acinus (intense in zones 2/3 and weak in zone 1) was almost lost in HCC. Addition of anti-CD26 monoclonal antibody to the medium of Huh7 cells expressing CD26 had no effect on cell growth. Moreover, addition of DPP IV inhibitor to the medium of Huh7 cells also had no effect on cell growth. Evaluating effect of CD26 knocking down against HCC growth or invasion is ongoing.

6. Type 2 Diabetes and Hepatocellular Carcinoma: A Prospective Case-Control Study in Patients with Chronic Hepatitis B

Qiang Li[1], Wenwen Li[2], Naoya Kato[2]: [1]Division of Liver Disease, Jinan Infectious Disease Hospital, Shandong University, Jinan, China, [2]Unit of Disease Control Genome Medicine, IMSUT.

Type 2 diabetes has been suggested as an independent risk factor for the development of HCC. However, the role of type 2 diabetes on

the development of HCC in the presence of chronic hepatitis B (CHB) has not been well documented. We aim to investigate the association between type 2 diabetes and HCC risk. A prospective case-control study was performed, which included 1410 eligible consecutive hospitalized patients with HBV-related HCC and 2820 consecutive hospitalized controls with CHB but without HCC. Multivariate logistic regression models were established to assess HCC risk factors. No differences were found in the overall prevalence of type 2 diabetes between HCC and control patients. After adjusting the age, family history of HCC, and cirrhosis, significantly high risk of HCC in female patients were found in type 2 diabetes (OR, 2.0; 95% CI, 1.1-3.9) and alcohol consumption (OR, 7.0; 95% CI, 1.7-29.5). Restricted analyses among female patients without cirrhosis indicated that type 2 diabetes and alcohol consumption were strongly associated with HCC risk with adjusted OR (95% CI) of 6.5 (2.2-19.2) and 54.3 (6.7-443.4), respectively. In contrast, male patients without cirrhosis and in female patients with cirrhosis, no association between type 2 diabetes and HCC risk was observed. In male patients with cirrhosis, a negative association between type 2 diabetes and HCC risk was observed. In conclusion, type 2 diabetes and alcohol consumption are independently associated with the increased risk of HCC in female CHB patients. Gender differences in HCC risk and known risk factors are substantial and warrant further study.

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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

Professor	Aikichi Iwamoto, M.D., D.M.Sc.	教　授	医学博士	岩　本　愛　吉
Professor	Tadashi Yamamoto, Ph.D.	教　授	理学博士	山　本　　雅
Professor	Yoshihiro Kawaoka, D.V M , Ph.D.	教　授	獣医学博士	河　岡　義　裕
Project Professor	Kunito Yoshiike, D.Sc.	特任教授	理学博士	吉　池　邦　人
Project Professor	Mitsue Hayashi, Ph.D.	特任教授	人類学博士	林　　光　江
Project Professor	Yoshihiro Kitamura, M.D., Ph.D.	特任教授	医学博士	北　村　義　浩
Project Professor	Zene Matsuda, M.D., Ph.D., D.Sc.	特任教授	医学博士	松　田　善　衛
Project Associate Professor	Takaomi Ishida, Ph.D.	特任准教授	医学博士	石　田　尚　臣
Project Assistant Professor	Fumihiro Yagyu, Ph.D.	特任助教	保健学博士	柳　生　文　宏

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 Collaboration on Emerging and Re-emerging Infectious Diseases*; *MEXT Project Director*: *Aikichi Iwamoto*), IMSUT established in 2006 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 the Harbin Veterinary Research Institute (HVRI), the Chinese Academy of Agricultural Sciences; and IMSUT's project office in Beijing. The collaborating Chinese institutions are conducting highly advanced research on infections in their characteristic ways. The first term of the project (fiscal 2005-2009) ended in March 2010, and the entire academic activities were summarized in a brochure published in the same month (available from the Project Office at IMSUT or Beijing).

After evaluating the first term, the MEXT decided to extend this successful project, and thus the collaboration entered into the second term (fiscal 2010-2014) from April 2010. Now, the ongoing collaborations in Beijing and Harbin are being further promoted and developed, and furthermore, new collaborative research in southern China will be started soon. Cooperation with Kumamoto University and Kobe University continues as has been done in the first term. In addition, several IMSUT faculty members have formed a core group to promote the China-Japan joint research. The group's activities are focused on pathogens and the host factors affecting the pathogenicity.

China contains hot spots for emerging and re-emerging infections, as exemplified by the high carrier rate of hepatitis virus, rapidly increasing HIV/AIDS, the occurrence of SARS, and epidemics of avian influenza. For various reasons, China is also at risk of new influenza pandemics. The outcome of the joint research conducted within this region should provide a useful basis for treating and preventing some of those diseases and for predicting their possible pandemics not only in China but for all of Asia.

LABORATORIES AND PROJECT OFFICE

a. Laboratory of Structural Virology and Immunology (LSVI)

In LSVI, Z. Matsuda's group has been studying the envelope protein of HIV-1. His group has engineered a pair of new reporters for membrane fusion called dual split proteins (DSPs). DSP is a chimera between split *Renilla* luciferase and split GFP. It allows a continuous live cell monitoring of membrane fusion. Together with A. Iwamoto's group at IMSUT, a DSP-based tropism assay system for HIV-1 envelope protein has been established. This assay can determine the tropism within a week. His group has revealed that the membrane-spanning domain (MSD) of the gp41 is an alpha-helix that crosses lipid bilayer once. The DSP assay revealed that the mutations in the MSD manifest an allosteric negative effect upon the conformational changes of envelope protein during membrane fusion. Alterations of the phase of the MSD alpha-helix have resulted in the reduced membrane fusion activity of HIV-1 envelope proteins. The altered phase also affects the intracellular transport of the HIV-1 envelope proteins. These findings have shown that the MSD of gp41 plays a critical role in the lifecycle of HIV-1. This may account for the fact that the MSD of gp41 is highly conserved among the different HIV-1 isolates.

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

LMIMM was founded in May 2006 and set to work in October 2006 in IMCAS. It is an important member of the CAS Key Laboratory of Pathogenic Microbiology and Immunology, which was established in December 2008. Kitamura's group at LMIMM is currently focusing on hepatitis C virus (HCV) 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 some anti-HCV host genes stimulated by interferon. They developed a new reporter HCVs to screen candidate antiviral drugs more easily. The new viruses have been filed for patents in Japan and China. To investigate the pathogenicity of the viruses in the field, they are analyzing clinical samples from 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 Porcine Reproductive and Respiratory Syndrome Virus.

c. Collaborative research program with HVRI

In 2009, the novel influenza "pandemic (H1N1) 2009" emerged and had spread rapidly throughout the world. On the other hand, H5N1 highly pathogenic avian influenza viruses have continued to cause unprecedented global outbreaks since 2003, and many human cases with a high fatality rate have been reported. For these reasons, a joint research program at HVRI (Direc-

tor, Xiangang Kong) has been conducted on influenza viral isolates from all over Asia.

HVRI focuses on avian influenza viruses (AIVs) that are circulating in Chinese wild waterfowl and domestic poultry. Specifically, we study type A influenza virus from wild birds, waterfowl, poultry, swine and horses, with an emphasis on viral pathogenicity in various hosts, viral evolution, and viral prevalence in China. Replication of AIVs in dogs may facilitate their adaptation to humans; however, the data to date on H5N1 influenza virus infection in dogs are conflicting. To elucidate the susceptibility of dogs to this pathogen, we infected two groups of 6 beagles with 10^6 50% egg-infectious dose of H5N1 AIV A/barheaded goose/Qinghai/3/05 (BHG/QH/3/05) intranasally (i.n.) and intratracheally (i.t.), respectively. The dogs showed disease symptoms, including anorexia, fever, conjunctivitis, labored breathing and cough, and one i.t. inoculated animal died on day 4 post-infection. Virus shedding was detected from all the 6 animals inoculated i.n. and one inoculated i.t. Virus replication was detected in all the animals that were euthanized on day 3 or day 5 post-infection and in the animal that died on day 4 post-infection. Our results demonstrate that dogs are highly susceptible to H5N 1 AIV and may serve as an intermediate host to transfer this virus to humans.

In addition, we have conducted molecular surveillance studies of pandemic (H1N1) 2009 viruses and H5N1 viruses isolated throughout Asia. Pigs have long been considered potential intermediate hosts in which AIVs can adapt to humans. To determine whether this potential exists for pigs in Indonesia, we conducted surveillance during 2005-2009. We found that 52 pigs in 4 provinces were infected during 2005-2007 but not 2008-2009. Phylogenetic analysis showed that the viruses had been introduced into the pig population in Indonesia on at least 3 occasions. One isolate had acquired the ability to recognize a human-type receptor. No infected pig had influenza-like symptoms, indicating that influenza A (H5N1) viruses can replicate undetected for prolonged periods, facilitating avian virus adaptation to mammalian hosts. Our data suggest that pigs are at risk for infection during outbreaks of influenza virus A (H5N1) and can serve as intermediate hosts in which this avian virus can adapt to mammals.

d. IMSUT Project Office

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

IMPLEMENTATION OF COLLABORATION

The collaboration was implemented, being based on the renewed MOUs between IMSUT and the Chinese institutes. For the joint laboratories the implementation was controlled by the steering committee consisting of M. Seiki, A. Iwamoto, G.F. Gao, L. Huang, and T. 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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