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

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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 association study of HCV induced liver cirrhosis in the Japanese population identifies novel susceptibility loci at MHC region

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We performed a genome-wide association study (GWAS) of hepatitis C virus (HCV)-induced liver cirrhosis (LC) to identify predictive biomarkers for the risk of LC in patients with chronic hepatitis C (CHC). A total of 682 HCV-induced LC cases and 1,045 CHC patients of Japanese origin were genotyped by Illumina Human Hap 610-Quad bead Chip. Eight SNPs which showed possible associations ($P<1.0\times10(-5)$) in the GWAS stage were fur-

ther genotyped using 936 LC cases and 3,809 CHC patients. We found that two SNPs within the major histocompatibility complex (MHC) region on chromosome 6p21, rs910049 and rs3135363, were significantly associated with the progression from CHC to LC (P(combined) = $9.15 \times 10(-11)$ and $1.45 \times 10(-10)$, odds ratio (OR) = 1.46 and 1.37, respectively). We also found that HLA-DQA1 0601 and HLA-DRB1 0405 were associated with progression from CHC to LC ($P = 4.53 \times 10^{-4}$ and 1.54×10^{-4} with OR = 2.80 and 1.45, respectively). Multiple logistic regression analysis revealed that rs3135363, rs910049, and HLA-DQA1 0601 were independently associated with the risk of HCV-induced LC. In addition, individuals with four or more risk alleles for these three loci have a 2.83-fold higher risk for LC than those with no risk allele, indicating the cumulative effects of these variations. Our findings elucidated the crucial roles of multiple genetic variations within the MHC region as prognostic/predictive biomarkers for CHC patients.

2. The epigenetic regulation might play an important role in the expression of miR122 which is involved in Hepatitis C virus replication and interferon treatment response

Ryosuke Muroyama, Kaku Goto, Norie Kowatari, Wenwen Li, Ryo Nakagawa, Naoya Kato

MicroRNAs (miRNAs) are a family of small, non-coding RNAs, and regulate the gene expression level at posttranscriptional level. Among these miRNAs, miR122 is a highly abundant and liver-specific miRNA that accounts for about 70% of the total liver miRNA population, and was reported to enhance Hepatitis C virus replication and be associated with interferon treatment response and hepatocarcinogenesis. In this study, we investigated the regulation mechanism of miR122. At first, we examined the correlation between the expression level of miR122 and pri-miR122 which was primary transcript of miR122 in various hepatoma cell lines by real-time PCR. The result showed the positive correlation between miR122 and pri-miR122. Therefore, the expression level of pri-miR122 was considered to be a major factor which determined that of miR122. Next, we compared the amount of HNF1 A/3B/4A protein, which were recently reported to enhance the expression level of pri-miR122, among hepatoma cell lines by western blot. However, there was no correlation between the amount of HNF1A/3B/4A and the expression level of pri-miR122. Next, we examined the DNA methylation status, which was in the promoter region of pri-miR122 by sodium bisulfite DNA sequencing. The result showed that the promoter region of pri-miR122 was hypermethylated in the hepatoma cell lines which has low expression level of pri-miR122. Therefore, the epigenetic regulation might play an important role in the expression of miR122.

3. A small molecule screen for MICA regulation

Kaku Goto, Ryosuke Muroyama, Wenwen Li, Ryo Nakagawa, Norie Kowatari, Naoya Kato

MHC class I polypeptide-related sequence A (MICA) was found to be a genetic susceptibility factor for HCV-induced hepatocellular carcinoma (HCC) in our genome-wide association study. Lower levels of MICA expression heightened the risk of HCC development in patients, indicating preventive effects of MICA induction on hepatocarcinogenesis. Accordingly, we sought to identify small molecules for regulation of MICA expression. Treatment with sodium butyrate (NaB), a well-known HDAC inhibitor and a reported MICA inducer in multiple cell lines, significantly elevated the MICA mRNA level in Huh7 cells. We then constructed active luciferase reporters encoding MICA promoter sequences, whose signals were increased by NaB likewise. Subsequently, stable cell transformants harboring the reporters were isolated via antibiotics selection, finally demonstrating full luciferase activities, which were similarly enhanced by the NaB treatment in a dose-dependent fashion. All the data indicated that endogenous expression and induction of MICA mRNA were successfully monitored by the reporter system. Hitherto our screen system has detected the elevation of MICA transcriptional activity by several compounds including saturated fatty acids, and a further screen for a FDA-approved drug library is currently underway. Findings in the study are expected to serve to develop anticarcinogenic strategy in chronic hepatitis C.

4. Roles of AMPK-related kinase SNARK in chronic hepatitis C

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Host cellular cofactors supporting hepatitis C virus (HCV) infection are now being recognized as attractive antiviral targets due to their independence from viral sequence. Our genome-wide RNAi screen for host cellular cofactors identified that sucrose, non-fermenting 1/AMP-activated protein kinase-related kinase (SNARK) positively regulated HCV replication. Here we sought to clarify the mechanisms of SNARK-mediated regulation of HCV and in turn HCV-mediated modulation of SNARK. Knockdown of SNARK reduced levels of HCV replication in both OR6 replicon and JFH1 in-

fection systems. Overexpression of siRNA-resistant wild type SNARK rescued the suppressed viral replication, which was abolished by either a kinase deficiency or phospho-deficient mutation. Reciprocally, SNARK mRNA level was elevated by HCV infection, deranging cellular signaling. These SNARK-mediated effects on both virus and host were abrogated by a SNARK kinase inhibitor. Hence viral induction/exploitation of the proviral kinase was speculated to promote HCV pathogenesis. We are presently investigating physiological targets and direct substrates of the kinase to explore the possibilities.

5. Soluble MICA and a MICA variation as possible prognostic biomarkers for HBV-induced hepatocellular carcinoma

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MHC class I polypeptide-related chain A (MICA) molecule is induced in response to viral infection and various types of stress. We recently reported that a single nucleotide polymorphism (SNP) rs 2596542 located in the MICA promoter region was significantly associated with the risk for hepatitis C virus (HCV)-induced hepatocellular carcinoma (HCC) and also with serum levels of soluble MICA (sMICA). In this study, we focused on the possible involvement of MICA in liver carcinogenesis related to hepatitis B virus (HBV) infection and examined correlation between the MICA polymorphism and the serum sMICA levels in HBV-induced HCC patients. The genetic association analysis revealed a nominal association with an SNP rs2596542; a G allele was considered to increase the risk of HBV-induced HCC ($P = 0.029$ with odds ratio of 1.19). We also found a significant elevation of sMICA in HBV-induced HCC cases. Moreover, a G allele of SNP rs2596542 was significantly associated with increased sMICA levels ($P = 0.009$). Interestingly, HCC patients with the high serum level of sMICA (>5 pg/ml) exhibited poorer prognosis than those with the low serum level of sMICA (≤5 pg/ml) ($P = 0.008$). Thus, our results highlight the importance of MICA genetic variations and the significance of sMICA as a predictive biomarker for HBV-induced HCC.

6. Specific HBV X gene mutations between HBV genotype C infected patients with and without hepatocellular carcinoma

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Hepatitis B virus (HBV) genotype C is an independent risk factor of Hepatocellular carcinoma (HCC). However, the mechanism is unclear. The multifunctional HBx protein, encoded by HBV X region, has been found to be heavily involved in hepatocarcinogenesis. Previous studies have proved that mutations in this region usually associated with severe disease progression including HCC. We therefore suppose that there exist genotype C-specific HCC-susceptive mutations in HBV X region. A large-scale sequence analysis was performed for identifying genotype-specific HCC mutations. In order to obtain sufficient X sequences, we firstly searched online HBV databases. Thousands of X sequences were downloaded and then screened by series of criteria. After screening, enrolled genotype C X sequences were divided into two groups (HCC, Non-HCC) by the attached diagnosis information. Each nucleotide position was then compared by Chi-square test between the two groups. Moreover, the correlated protein sequences and overlapped cis-elements region were also analyzed. Finally, logistic regression was performed to evaluate the effects of mutations on HCC risk. Five thousand three hundred and eighty X sequences were downloaded from Hepatitis Virus Database (HVDB) and Genbank. After screening, four hundred and ninety-five complete genotype C X sequences (HCC: 153; Non-HCC: 342) were finally extracted for analysis. 1) HCC group showed 20 nucleotide differences compared with the Non-HCC group. 45 % (9/20) positions showed a high mutant ratio (>50 % mutated) in both groups. 2) HCC group showed more mutations than Non-HCC groups. (Mean ± SD, HCC: 10.6 ± 2.0, Non-HCC: 8.8 ± 2.2, $p<0.05$), in particular those highly mutated positions, the ratio of HCC patients who displayed all the ten mutations (including G1764A) was twice higher than in Non-HCC (56%/28%, $p<0.0001$). 3) Among the 20 point mutations, six were also included in Enhancer (Enh) 2 region (nt 1636-1744), with 4 highly mutated positions; 14 in core promoter (CP) region (nt 1742-1849) with 4 highly mutated positions. 4) Logistic regression showed that mutations A1383C (OR: 1.996, 95% CI: 1.075-3.706), A1479C/G/T (OR: 2.932, 95% CI: 1.485-5.791; OR: 2.793, 95% CI:

1.323-5.899; OR: 6.699, 95% CI: 2.807-15.988), C1485 T (OR: 2.626, 95% CI: 1.498-4.602), C1653T (OR: 1.947, 95% CI: 1.145-3.311), and A1762T (OR: 1.849, 95% CI: 1.005-3.404) were independent risk factors for genotype C HBV-related HCC. Moreover, these nucleotide mutations also induced the substitution of related amino acids which may further change the structure and functions of HBx or polymerase protein.HBV genotype C HCC has specific nucleotide mutations in X region. These mutations may contribute to the higher incidence of HCC in HBV genotype C infected patients.

7. Establishment of New Drug Screening System Based on the Essential Role of HBx Transactivation Activity in HBV Replication

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Due to the long half-life of nuclear covalently closed circular HBV-DNA (cccDNA) and the limitation of current drugs, chronic HBV-infected patients have to accept long-time therapy and the high incidence of drug resistance. New drugs which could suppress HBV replication and are possible to eradicate cccDNA are therefore needed. The HBV genome is a 3.2kb-long, circular and partially double-stranded DNA with four overlapped open reading frames (ORFs) termed S, C, P and X ORF. HBx, a multi-functional transactive protein encoded by X ORF, has been proved to be essential for HBV replication probably by its interaction with cccDNA. Moreover, the transactivation function of HBx is also important in augmenting replication process. Therefore, inhibition of HBx transcriptional transactivity should be a promising target for developing anti-HBV drugs, based upon which We sought to establish drug screening system. Firstly, we've constructed genotype C HBx encoding plasmid and then checked the effects of HBx on main signal pathways such as NF-kappaB, AP-1, and SRE) via luciferase reporters. We selected the most stimulated signal pathway (NF-kappaB) by the transcriptional transactivity of HBx as candidate. Secondly, we're establishing hepatoma cell line stably transfected by NF-kappaB luc reporter (pLUC NF-kappaB) via antibiotics selection. Meanwhile a Tet-on system-controlled HBx-encoding plasmid is also under construction, which contains a different antibiotics selecting element from pLUC NF-kappaB. Our primary data of pLUC NF-kappaB clones demonstrated dose-dependent luciferase activities when treated with TNF-alpha, which indicated that our stable cell line was effective and could be evaluated by luciferase reporter system. In order to identify a relatively ideal clone, the screening work is now still ongoing. Thirdly, we'll establish a double stable hepatoma cell line with both NF-kappaB and HBx Tet-on system using different antibiotics selection. Finally, drug libraries including FDA-approved drug library, and the one at the open innovation center for drug discovery in the University of Tokyo will be used for drug screenings.

8. Profiling miRNA in $CD4^+$ T cells of autoimmune liver disease

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Primary biliary cirrhosis (PBC) is chronic inflammatory liver disease by abnormality of autoimmunity and often the cause of cirrhosis. $CD4^+$ T cells are suggested to play an important role in the pathogenesis of PBC. However, the molecular mechanisms are still unknown. Recently, microRNA (miRNA) was reported to be involved in autoimmune disorders and the loss of function in immune cells was shown to facilitate systemic autoimmune disease. In this study, we examined the molecular and etiological mechanisms of $CD4^+$ T cells in PBC by profiling the miRNA expressions. 7 PBC patients and 7 healthy controls were studied. $CD4^+$ T cells were purified from PBMCs by immunomagnetic beads. We assessed miRNA comprehensive profile by microarray analysis on $CD4^+$ T cells. Compared with healthy control, 2 miRNAs were increased, and 13 miRNAs were decreased in PBC. ($p<0.05$, Fold change>1.2) Furthermore, the quantitative real-time PCR (qRT-PCR) validated that miR-361-5p, miR-374b and miR-425 were decreased in PBC. ($p< 0.01$, $p<0.01$, $p<0.05$). By bioinformatics, 912 mRNAs were identified as the target genes of 3 miRNAs. Among them, TNFAIP3, IL-10 and TNFSF9 were significantly increased in $CD4^+$ T cells of PBC in qRT-PCR ($p<0.05$). These 3 target genes are related to immunological function such as interleukins and signal transduction factors. Therefore, we suggest that the change of miRNA expression might have effects on the function of $CD4^+$ T cell and contribute to the pathogenesis of PBC.

9. IL28B minor allele is associated with a younger age of onset of hepatocellular carcinoma in patients with chronic hepatitis C virus infection

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IL28B polymorphisms were shown to be associated with response to peg-interferon based treatment in chronic hepatitis C (CHC) and spontaneous clearance. However, little is known about how this polymorphism affects the course of CHC, including the development of hepatocellular carcinoma (HCC). The aim of this study is to evaluate the influence of IL28B polymorphisms on hepatocarcinogenesis in CHC patients. We genotyped the rs 8099917 single-nucleotide polymorphism in 351 hepatitis C-associated HCC patients without history of IFN-based treatment, and correlated the age at onset of HCC in patients with each genotype. The mean ages at onset of HCC for TT, TG, and GG genotypes were 69.9, 67.5 and 66.8, respectively. In multivariate analysis, IL28B minor allele (TG and GG genotypes) was an independent risk factor for younger age at onset of HCC ($p = 0.02$) in males ($p < 0.001$) with higher body mass index (BMI; $p = 0.009$). IL28B minor allele was also associated with a lower probability of having aspartate aminotransferase-to-platelet ratio index (APRI)>1.5 (minor vs. major, 46.7% vs. 58.6%; $p = 0.01$), lower AST (69.1 vs. 77.7 IU/l, $p = 0.02$), lower ALT (67.8 vs. 80.9 IU/l, $P = 0.002$), higher platelet count (12.8 vs. 11.2×104/μl, $p = 0.002$), and higher prothrombin time (79.3% vs. 75.4%, $p = 0.002$). In conclusion, IL28B minor allele was associated with lower inflammatory activity and less progressed fibrosis of liver, however, constituted a risk factor for younger-age onset of HCC in CHC patients.

10. Patatin-like phospholipase-3 (rs738409 C>G) polymorphism is associated with the development of hepatocellular carcinoma in patients with chronic hepatitis C infection

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An isoleucine to methionine substitution at position 148 in the PNPLA3 gene (p.I148M, rs738409) has recently been identified as a susceptibility factor for liver damage in steatohepatitis. However, little is known about the influence of this polymorphism on hepatocarcinogenesis. The aim of this study is to assess the impact of PNPLA3 polymorphism on the development of hepatocellular carcinoma (HCC) which is thought to be one of the major steatosis-related complications in patients with chronic hepatitis C. We genotyped the rs738409 single-nucleotide polymorphism (SNP) in 358 hepatitis C-related HCC patients, and correlated the age at onset of HCC and the duration between the hepatitis C virus (HCV) infection and the development of HCC. The median age at onset of HCC for the GG genotype was significantly younger compared to for non-GG genotypes (67.81 *vs.* 69.87 years, $P < 0.001$), and the median interval between HCV infection and the development of HCC was significantly shorter in patients with the GG genotype (39.96 *vs.* 40.85 years, $P = 0.008$). PNPLA3 GG genotype was also associated with a higher AST level (69.5 *vs.* 59.0 IU/l, $P = 0.02$), lower prothrombin time (73.0% *vs.* 78.0%, $P = 0.008$), and a higher prevalence of histological steatosis (40.0% *vs.* 22.2%, $P = 0.01$) at the time of HCC onset. In conclusion, PNPLA3 genotype independently influence the hepatocarcinogenesis in patients with chronic hepatitis C, probably through the mediation of inflammatory activity and fibrosis progression derived from steatosis.

11. Percutaneous ethanol injection for hepatocellular carcinoma: 20-year outcome and prognostic factors

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Ethanol injection is the best-known image-guided percutaneous ablation for hepatocellular carcinoma (HCC) and a well-tolerated, inexpensive procedure with few adverse effects. However, there have been few reports on its long-term results. We report a 20-year consecutive case series at a tertiary referral centre. We performed 2147 ethanol injection treatments on 685 primary HCC patients and analysed a collected database. Final computed tomography demonstrated complete ablation of treated tumours in 2108 (98.2%) of the 2147 treatments. With a median follow-up of 51.6 months, 5-, 10- and 20-year survival rates were 49.0% [95% confidence interval (CI) = 45.3-53.0%], 17.9% (95% CI = 15.0-21.2%) and 7.2% (95% CI = 4.5-11.5%) respectively. Multivariate analysis demonstrated that age, Child-Pugh class, tumour size, tumour number and serum alpha-fetoprotein level were significant prognostic factors for survival. Five-, 10- and 20-year local tumour progression rates were 18.2% (95% CI = 15.0-

21.4%), 18.4% (95% CI = 15.2-21.6%) and 18.4% (95 % CI = 15.2-21.6%) respectively. Five-, 10- and 20-year distant recurrence rates were 53.5% (95% CI = 49.4-57.7%), 60.4 (95% CI = 56.3-64.5%) and 60.8% (95% CI = 56.7-64.9%) respectively. There were 45 complications (2.1%) and two deaths (0.09%). Ethanol injection was potentially curative for HCC, resulting in survival for more than 20 years. This study suggests that new ablation therapies will achieve similar or even better long-term results in HCC.

Publications

Kumar V, Yi Lo PH, Sawai H, Kato N, Takahashi A, Deng Z, Urabe Y, Mbarek H, Tokunaga K, Tanaka Y, Sugiyama M, Mizokami M, Muroyama R, Tateishi R, Omata M, Koike K, Tanikawa C, Kamatani N, Kubo M, Nakamura Y, Matsuda K. Soluble MICA and a MICA variation as possible prognostic biomarkers for HBV-induced hepatocellular carcinoma. PLoS One. 2012; 7: e44743

Shiina S, Tateishi R, Imamura M, Teratani T, Koike Y, Sato S, Obi S, Kanai F, Kato N, Yoshida H, Omata M, Koike K. Percutaneous ethanol injection for hepatocellular carcinoma: 20-year outcome and prognostic factors. Liver Int. 2012; 32: 1434-1442

Urabe Y, Ochi H, Kato N, Kumar V, Takahashi A, Muroyama R, Hosono N, Otsuka M, Tateishi R, Lo PH, Tanikawa C, Omata M, Koike K, Miki D, Abe H, Kamatani N, Toyota J, Kumada H, Kubo M, Chayama K, Nakamura Y, Matsuda K. A genome-wide association study of HCV induced liver cirrhosis in the Japanese population identifies novel susceptibility loci at MHC region. J Hepatol. 2013 [Epub ahead of print]

Center for Asian Infectious Diseases

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中国における感染症研究拠点

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

SUT 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 Science; and IMSUT's project office in Beijing. The collaborating Chinese institutions are conducting highly advanced research on infections in their characteristic ways. This five-year project (fiscal 2005-2009) successfully ended in March 2010 (the academic activities are summarized in a brochure available from the Project Office at IMSUT or Beijing) and entered into the second term (fiscal 2010-2014) from April 2010.

During fiscal 2012, the third year of the second term, the ongoing collaborations in Beijing and Harbin have been further promoted and developed. Besides molecular studies of HIV-1, some members at IMCAS and IMSUT are preparing for and conducting collaborative studies of clinical specimens from areas including southern China. Cooperation between Kumamoto University and the joint laboratory at IBPCAS continues as before. A group of IMSUT faculty members is conducting some studies to support the two joint laboratories in Beijing. Meetings scheduled for September-October 2012 (*the 9th China-Japan Joint Laboratory Workshop: Pathogenesis, Gene Regulation and Signal Transduction*; the 11th Steering Committee Meeting; and a bi-monthly joint-lab seminar of the two laboratories) had to be deferred because of the sudden change of political/social environment in China. MEXT carried out the midterm evaluation of the project in July 2012.

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), IBPCAS

We (Z. Matsuda's group in LSVI) have been studying the envelope protein (Env) of human immunodeficiency virus type 1 (HIV-1). To elucidate the structure-function relationship of HIV-1 Env in membrane fusion, we performed a systematic insertional mutagenesis of the entire gp41 subunit and analyzed its effects on membrane fusion. The fusion activity of mutants was evaluated with a new pair of reporter proteins called dual split proteins (DSPs) whose sensitivity was recently enhanced in our laboratory. The results showed that only the portion called the membrane-proximal external region is tolerant against the introduced mutations. In collaboration with Dr. Sakaguchi's group at Kumamoto University, we analyzed cDNA of several monoclonal antibodies raised against the fusion intermediates of HIV-1 Env protein.

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

In LMIMM we (T. Ishida's research group) have been focusing on two research areas on HIV-1 (and/or hepatitis viruses) infections: molecular epidemiology and cell biology. In epidemiology research we analyzed the subtype and co-receptor usage of HIV-1 currently circulating in China by using the samples from Chinese patients enrolled in a nationwide cohort study, in collaboration with Professor T. Li of Peking Union Medical College Hospital. The genotype analysis of the co-receptor usage revealed that the prevalence of X4 or dual tropism was as high as 69.3% in subtype CRF01_AE; 38.3% in subtype B or B'; and only 6.0% in subtype CRF07 or 08_BC. In cell-biology research we tested osteoclasts that were differentiated in vitro from CD14+ cells for their susceptibility to a pseudotyped HIV. The osteoclasts were found susceptible to the pseudo-virus infection, suggesting that they serve as a novel target for HIV-1 infection in humans.

c. Collaborative research program with HVRI

In 2009, the novel influenza "pandemic (H1N1) 2009 (pdmH1N1)" emerged and spread rapidly throughout the world. In addition, since 2003, highly pathogenic avian H5N1 influenza viruses have continued to cause unprecedented global outbreaks with high case fatality rates in humans. For these reasons, HVRI (Director, Xiangang Kong) has been conducting collaborative research on influenza virus isolates from all over Asia.

HVRI focuses on avian influenza viruses (AIVs) that are circulating in Chinese wild waterfowl and domestic poultry. Specifically, we (Y. Kawaoka's group) study type A influenza viruses from wild birds, waterfowl, poultry, swine, and horses, with an emphasis on viral pathogenicity in various hosts, viral evolution, and viral prevalence.

Our major findings for this year include the following: 1) We showed that the amino acids at 271 of PB2 and 226 of HA are both important for the adaptation and transmission of pdmH1N1 virus in humans. Our findings provide important insights into monitoring and evaluating the pandemic po-

tential of field influenza viruses. 2) To study the role of quail as an intermediate host of influenza A viruses, we passaged a duck H3N2 influenza virus in quail and analyzed it. The quail-passaged, but not the original duck virus, replicated in human bronchial epithelial cells, indicating that quail can serve as an intermediate host for aquatic bird influenza virus transmission to humans. 3) To better understand the pathogenic potential of pdmH1N1 viruses, we characterized two Norwegian isolates, A/Norway/3568/2009 (Norway3568; from a mild case) and A/Norway/3487-2/2009 (Norway 3487; from a severe case). We observed more efficient replication in cultured cells and delayed virus clearance from ferret respiratory organs for Norway3487 virus compared with those for Norway3568. Moreover, Norway3487 caused somewhat more severe lung damage in nonhuman primates than did Norway 3568. Our data suggest that the distinct replicative and pathogenic potentials of these two viruses may result from differences in their biological properties.

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 H. Kiyono, A. Iwamoto, L. Huang, and T. Xu. The collaborative program in Harbin was implemented by the steering committee consisting of H. Kiyono, Y. Kawaoka, X. Kong, and H. Chen.

Publications

Ishikawa H, Meng F, Kondo N, Iwamoto A, Matsuda Z. Generation of a dual-functional split-reporter protein for monitoring membrane fusion using self-associating split GFP. Protein Eng Des Sel 25: 813-820, 2012.

Kobayashi-Ishihara M, Yamagishi M, Hara T, Matsuda Y, Takahashi R, Miyake A, Nakano K, Yamochi T, Ishida T, Watanabe T. HIV-1-encoded antisense RNA suppresses viral replication for a prolonged period. Retrovirology 9: 38, 2012.

Ahlenstiel CL, Lim HG, Cooper DA, Ishida T, Kelleher AD, Suzuki K. Direct evidence of nuclear Argonaute distribution during transcriptional silencing links the actin cytoskeleton to nuclear RNAi machinery in human cells. Nucleic Acids Res 40: 1579-1595, 2012.

Saito M, Kato Y, Ito E, Fujimoto J, Ishikawa K, Doi A, Kumazawa K, Matsui A, Takebe S, Ishida T, Azuma S, Mochizuki H, Kawamura Y, Yanagisawa Y, Honma R, Imai J, Ohbayashi H, Goshima N, Semba K, Watanabe S. Expression screening of 17q12-21 amplicon reveals GRB7 as an ERBB2-dependent oncogene. FEBS Lett 586: 1708-1714, 2012.

Yamaguchi N, Yokota M, Taguchi Y, Gohda J, Inoue J. cIAP1/2 negatively regulate RANKL-induced osteoclastogenesis through the inhibition of NFATc1 expression. Genes Cells 17: 971-981, 2012.

Shibata Y, Oyama M, Kozuka-Hata H, Han X, Tanaka Y, Gohda J, Inoue J. p47 negatively regulates IKK activation by inducing the lysosomal degradation of polyubiquitinated NEMO. Nat Commun 3: 1061, 2012.

Taguchi Y, Kiga Y, Gohda J, Inoue J. Identification and characterization of anti-osteoclastogenic peptides derived from the cytoplasmic tail of receptor activator of nuclear factor kappa B. J Bone Miner Metab 30: 543-553, 2012.

Sanada T, Kim M, Mimuro H, Suzuki M, Ogawa M, Oyama A, Ashida H, Kobayashi T, Koyama T, Nagai S, Shibata Y, Gohda J, Inoue J, Mizushima T, Sasakawa C. The Shigella flexneri effector OspI deamidates UBC13 to dampen the inflammatory response. Nature 483: 623-626, 2012.

Uraki R, Kiso M, Shinya K, Goto H, Takano R, Iwatsuki-Horimoto K, Takahashi K, Daniels RS, Hungnes O, Watanabe T, Kawaoka Y. Virulence determinants of pandemic A (H1N1)2009 virus in a mouse model. J Virol, in press.

Muramoto Y, Noda T, Kawakami E, Akkina R, Kawaoka Y. Identification of novel influenza A virus proteins translated from PA mRNA. J Virol, in press.

Sakabe S, Takano R, Nagamura-Inoue T, Yamashita N, Nidom CA, Quynh Le MT, Iwatsuki-Horimoto K, Kawaoka Y. Differences in cytokine production in human macrophages and in virulence in mice are attributable to the acidic polymerase protein of highly pathogenic influenza A virus

subtype H5N1. J Infect Dis, in press.
Takano R, Kiso M, Igarashi M, Le QM, Sekijima M, Ito K, Takada A, Kawaoka Y. Molecular mechanisms underlying oseltamivir resistance mediated by an I117V substitution in the NA of H5N1 avian influenza viruses. J Infect Dis, in press.
Shoemaker JE, Fukuyama S, Eisfeld AJ, Muramoto Y, Watanabe S, Watanabe T, Matsuoka Y, Kitano H, Kawaoka Y. Integrated network analysis reveals a novel role for the cell cycle in 2009 pandemic influenza virus-induced inflammation in macaque lungs. BMC Syst Biol 6: 117, 2012.
Das SC, Hatta M, Wilker PR, Myc A, Hamouda T, Neumann G, Baker JR Jr, Kawaoka Y. Nanoemulsion W (80) 5EC improves immune responses upon intranasal delivery of an inactivated pandemic H1N1 influenza vaccine. Vaccine 30: 6871-6877, 2012.
Imai M, Watanabe S, Kawaoka Y. The cytoplasmic tail domain of influenza B virus hemagglutinin is important for its incorporation into virions but is not essential for virus replication in cell culture in the presence of compensatory mutations. J Virol 86: 11633-11644, 2012.
Shimojima M, Kawaoka Y. Cell surface molecules involved in infection mediated by lymphocytic choriomeningitis virus gycoprotein. J Vet Med Sci 74: 1363-1366, 2012.
Shimojima M, Ströher U, Ebihara H, Feldmann H, Kawaoka Y. Identification of cell surface molecules involved in dystroglycan-independent Lassa virus cell entry. J Virol 86: 2067-2078, 2012.
Victor ST, Watanabe S, Katsura H, Ozawa M, Kawaoka Y. A replication-incompetent PB2-knockout influenza A virus vaccine vector. J Virol 86: 4123-4128, 2012.
Nidom CA, Yamada S, Nidom RV, Rahmawati K, Alamudi MY, Kholik, Indrasari S, Hayati RS, Iwatsuki Horimoto K, Kawaoka Y. Genetic characterization of H5N1 influenza viruses isolated from chickens in Indonesia in 2010. Virus Genes 44: 459-465, 2012.
Shinya K, Gao Y, Cilloniz C, Suzuki Y, Fujie M, Deng G, Zhu Q, Fan S, Makino A, Muramoto Y, Fukuyama S, Tamura D, Noda T, Eisfeld AJ, Katze MG, Chen H, Kawaoka Y. Integrated clinical, pathologic, virologic, and transcriptomic analysis of H5N1 influenza virus-induced viral pneumonia in the rhesus macaque. J Virol 86: 6055-6066, 2012.
Kikuchi T, Iwatsuki-Horimoto K, Adachi E, Koga M, Nakamura H, Hosoya N, Kawana-Tachikawa A, Koibuchi T, Miura T, Fujii T, Kawaoka Y, Iwamoto A. Improved neutralizing antibody response in the second season after a single dose of pandemic (H1N1) 2009 influenza vaccine in HIV-1-positive adults. Vaccine 30: 3819-3823, 2012.
Watanabe T, Imai M, Watanabe S, Shinya K, Hatta M, Li C, Neumann G, Ozawa M, Hanson A, Zhong G, Fukuyama S, Kawakami E, Simmons HA, Schenkman D, Brunner K, Capuano SV 3rd, Weinfurter JT, Kilander A, Dudman SG, Suresh M, Hungnes O, Friedrich TC, Kawaoka Y. Characterization in vitro and in vivo of pandemic (H1 N1) 2009 viruses isolated from patients. J Virol 86: 9361-9368, 2012.
Zhang Y, Zhang Q, Gao Y, He X, Kong H, Jiang Y, Guan Y, Xia X, Shu Y, Kawaoka Y, Bu Z, Chen H. Key molecular factors in HA and PB2 contribute to the efficient transmission of the 2009 H1N1 pandemic influenza virus. J Virol 86: 9666-9674, 2012.
Inagaki A, Goto H, Kakugawa S, Ozawa M, Kawaoka Y. Competitive incorporation of homologous gene segments of influenza A virus into virions. J Virol 86: 10200-10202, 2012.
Das SC, Watanabe S, Hatta M, Noda T, Neumann G, Ozawa M, Kawaoka Y. The arginine residues at positions 76-78 of influenza A virus matrix protein M1 play an important role in viral replication. J Virol 86: 1522-1530, 2012.
Murakami S, Horimoto T, Ito M, Takano R, Katsura H, Shimojima M, Kawaoka Y. Enhanced growth of influenza vaccine seed viruses in Vero cells mediated by broadening the optimal pH range for virus membrane fusion. J Virol 86: 1405-1410, 2012.
Yamada S, Shinya K, Takada A, Ito T, Suzuki T, Suzuki Y, Le QM, Ebina M, Kasai N, Kida H, Horimoto T, Rivailler P, Chen LM, Donis RO, Kawaoka Y. Adaptation of a duck influenza A virus in quail. J Virol 86: 1411-1420, 2012.
Ginting TE, Shinya K, Kyan Y, Makino A, Matsumoto N, Kaneda S, Kawaoka Y. Amino acid changes in hemagglutinin contribute to the replication of oseltamivir-resistant H1N1 influenza viruses. J Virol 86: 121-127, 2012.