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

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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.

Paracrine IL-33 stimulation enhances lipopolysaccharide-mediated macrophage activation

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IL-33, a member of the IL-1 family of cytokines, provokes Th2-type inflammation accompanied by accumulation of eosinophils through IL-33R, which consists of ST2 and IL-1RAcP. We previously demonstrated that macrophages produce IL-33 in response to LPS. Some immune responses were shown to differ between ST2-deficient mice and soluble ST2-Fc fusion protein-

treated mice. Even in anti-ST2 antibody (Ab)-treated mice, the phenotypes differed between distinct Ab clones, because the characterization of such Abs (i.e., depletion, agonistic or blocking Abs) was unclear in some cases. To elucidate the precise role of IL-33, we newly generated neutralizing monoclonal Abs for IL-33. Exogenous IL-33 potentiated LPS-mediated cytokine production by macrophages. That LPS-mediated cytokine production by macrophages was suppressed by inhibition of endogenous IL-33 by the anti-IL-33 neutralizing mAbs.

Our findings suggest that LPS-mediated macrophage activation is accelerated by macrophage-derived paracrine IL-33 stimulation.

Alteration of immune responses by N-acetylglucosaminyltransferase V during allergic airway inflammation

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β-1,6-N-acetylglucosaminyltransferase V (Mgat 5 or GlcNac-TV), which is involved in the glycosylation of proteins, is known to be important for down-regulation of TCR-mediated T-cell activation and negatively regulates induction of contact dermatitis and experimental autoimmune encephalomyelitis. However, the role of Mgat5 in the induction of allergic airway inflammation remains unclear. To elucidate the role of Mgat5 in the pathogenesis of allergic airway inflammation, ovalbumin (OVA)-induced airway inflammation was induced in Mgat5-deficient mice. OVA-specific lymphocyte proliferation and production of IFN-γ and IL-10, but not IL-4, were increased in Mgat5-deficient mice, suggesting that Th2-type immune responses are seemed to be suppressed by increased IFN-γ and IL-10 production in these mice. However, Th2-type responses such as OVA-specific IgG1, but not IgE, and IL-5 levels in BAL fluids were increased in Mgat5-deficient mice. Meanwhile, the number of eosinophils was normal, but the numbers of neutrophils, macrophages and lymphocytes were reduced, in these mutant mice during OVA-induced airway inflammation. Thus, Mgat5-dependent glycosylation of proteins can modulate acquired immune responses, but it is not essential for the development of OVA-induced eosinophilic airway inflammation.

‹Beate Heissig Group›

Proteases can perform highly selective and limited cleavage of specific substrates including growth factors and their receptors, cell adhesion molecules, cytokines, apoptotic ligand and angiogenic factors. To understand the molecular mechanism underlying hematopoietic stem cell differentiation, we investigated the role of factors of the fibrinolytic pathway in the regulation of red blood cell production using gene deficient mice.

Plasminogen deficiency attenuates post-natal erythropoiesis in male C57BL/6 mice through decreased activity of the LH-testosterone axis

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Novel roles for the serine protease plasmin have been recently implicated in physiological and pathological processes. However, whether plasmin is involved in erythropoiesis, is not known. In the present study, we studied the consequences of plasminogen deficiency on erythropoiesis in plasminogen deficient (Plg KO) mice. Erythroid differentiation was attenuated in male Plg KO mice and resulted in erythroblastic accumulation within the spleen and bone marrow, with increased apoptosis in the former, erythrocytosis and splenomegaly, whereas similar erythropoietic defect was less prominent in female Plg KO mice. In addition, erythrocyte lifespan was shorter in both male and female Plg KO mice. Erythropoietin levels were compensatory increased in both male and female Plg KO mice, and resulted in a higher frequency of BFU-E within the spleen and bone marrow. Surprisingly, we found that male Plg KO mice but not their female counterparts exhibited normochromic normocytic anemia. The observed gender-linked erythropoietic defect was attributed

to decreased serum testosterone levels in Plg KO mice, as a consequence of impaired secretion of the pituitary luteinizing hormone (LH) under steady state condition. Surgical castration, causing testosterone deficiency and stimulating LH release, attenuated erythroid differentiation and induced anemia in WT animals, but did not further decrease the hematocrit levels in Plg KO mice. In addition, complementation of LH using human choriogonadotropin, which increases testosterone production, improved the erythropoietic defect and anemia in Plg KO mice. The present results identify a novel role for plasmin in the hormonal regulation of post-natal erythropoiesis by the LH-testosterone axis.

‹Riu Yamashita Group›

Construction of DBTSS version 8 with epigenomic information

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We have constructed the DBTSS (DataBase of Transcriptional Start Sites) which represnts exact positions of transcriptional start sites (TSSs) in the genome based on our unique experimentally validated TSS sequencing method, TSS-Seq. In this update, we included new TSS data, so that a major part of human adult and embryonic tissues are covered. DBTSS now contains 491 million TSS tag sequences for collected from a total of 20 tissues and 7 cell cultures. We also integrated our newly generated RNA-seq data of subcellular- fractionated RNAs and ChIP-Seq data of histone modifications, RNA polymerase II and several transcriptional regulatory factors in cultured cell lines. We also included recently accumulating external epigenomic data, such as chromatin map of the ENCODE project. We further associated those TSS information with public and original SNV data, in order to indentify single nucleotide variations (SNVs) in the regulatory regions. These data can be browsed in our new viewer which also supports versatile search conditions of users (http://dbtss.hgc.jp). We believe new DBTSS is helpful to understand biological consequences of the massively identified TSSs and identify human genetic valuations which are associated with disordered transcriptional regulations.

‹Katsuyoshi Yamamoto Group›

Interaction mechanism of integral membrane proteins that are required for activation of the yeast HOG MAPK pathway

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To cope with severe external high osmolarity, the budding yeast *Saccharomyces cerevisiae* activates the high osmolarity glycerol (HOG) MAP kinase (MAPK) pathway, which regulates synthesis and intracellular retention of the compatible osmolyte glycerol, as well as more general stress responses. The HOG pathway is composed of two functionally redundant upstream signaling pathways, termed the SLN1 branch pathway and the SHO1 branch pathway. Mutants that are defective in both the branches of the HOG pathway cannot survive in high osmolarity environments. A signal emanating from either branch pathway converges on the common Pbs2 MAPKK, which activates the Hog1 MAPK. In the SHO1 branch pathway, two putative single-span transmembrane (TM) osmosensors, Hkr1 and Msb2, detect osmotic stress, and, together with tetra-span TM protein Sho1, generate an intracellular signal that leads to activation of the Ste11 MAPKKK, which then activates Pbs2. Single-span TM protein Opy2 is also required for activation of the SHO1 branch pathway and indirectly recruits Ste11 to the membrane via scaffold protein Ste50, which is an essential component of the SHO1 branch pathway and binds both Ste11 and Opy2. We recently reported that dynamic regulation of Opy2-Ste50 interaction fine-tunes the yeast MAPK signaling network. It is unclear that how four TM proteins (Hkr1, Msb2, Sho1, and Opy2) interact to activate the SHO1 branch pathway.

We formerly isolated active *STE50* and *SHO1* mutants (*STE50-D146F*, *SHO1-R342G*, and *SHO1-G346S*) by mutant screening using active form of Ste11, Ste11-Q301P. Genetic and biochemical analysis of these active genes and proteins led to further understanding of activation mechanism of the SHO1 branch pathway. Based on this experience, we conducted isolation of active *OPY2* mutants using the same method. As a result, we succeeded in isolation of three *OPY2* mutants that led to activation of the SHO1 branch pathway in the presence of active Ste11-Q301P. Two of three *OPY2* mutants have a mutation in TM segment, and the other mutant has a nonsense mutation in cytoplasmic region to produce a C-terminal deleted Opy2. In-

terestingly, two Opy2 TM mutant proteins, Opy2-F96I and Opy2-A104V, may have different property, because Opy2-F96I A104V that contains both TM mutations activates the SHO1 branch pathway without active Ste11-Q301P. We investigated whether F96I and A104V mutations in Opy2 TM had an effect for binding affinity to other integral membrane proteins (Hkr1, Msb2, and Sho1). Analysis of interaction between Opy2 and Sho1 by GST pull-down assay resulted in that Opy2 A104V bound Sho1 more strongly than wild-type Opy2 did. Binding affinity between Opy2 A104V and Sho1 changed by a detergent that was used in an expreriment. These results suggest that Opy2 A104V bound Sho1 via TM helices. We propose a model in that interaction of integral membrane proteins, Opy2 and Sho1, may be induced by osmotic stress, and then may lead to activation of the SHO1 branch pathway. In order to prove our model, we are proceeding further genetic and biochemical analysis on TM interactions between Opy2 and Sho1.

‹Kazumasa Yokoyama Group›

Analysis of the NYAP family-mediated signaling pathway in neurons

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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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‹Beate Heissig Group›

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‹Riu Yamashita Group›

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‹Katsuyoshi Yamamoto Group›

No publications

‹Kazumasa Yokoyama 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

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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. Genome-wide association study identifies a susceptibility locus for hepatitis C virus-induced hepatocellular carcinoma

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To identify the genetic susceptibility factor(s) for hepatitis C virus-induced hepatocellular carcinoma (HCV-induced HCC), we conducted a genome-wide association study using 432,703 autosomal SNPs in 721 individuals with HCV-induced HCC (cases) and 2,890 HCV-negative controls of Japanese origin. Eight SNPs that showed possible association ($P < 1 \times 10^{-5}$) in the genome-wide association study were further genotyped in 673 cases and 2,596 controls. We found a previously unidentified locus in the 5′ flanking region of MICA on 6p21.33 (rs2596542, $P_{combined} = 4.21 \times 10^{-13}$, odds ratio = 1.39) to be strongly associated with HCV-induced HCC.

Subsequent analyses using individuals with chronic hepatitis C (CHC) indicated that this SNP is not associated with CHC susceptibility ($P = 0.61$) but is significantly associated with progression from CHC to HCC ($P = 3.13 \times 10^{-8}$). We also found that the risk allele of rs 2596542 was associated with lower soluble MICA protein levels in individuals with HCV-induced HCC ($P = 1.38 \times 10^{-13}$).

2. A genome wide association study of hepatitis C virus induced liver cirrhosis in the Japanese population identifies novel susceptibility loci at MHC region

Yuji Urabe[1], Vinod Kumar[1], Ryosuke Muroyama[2], Norie Kowatari[2], Kaku Goto[2], Wenwen Li[2], Ryo Nakagawa[2], Motoyuki Otsuka[3], Ryosuke Tateishi[3], Masao Omata[3], Kazuhiko Koike[3], Michiaki Kubo[4], Kazuaki Chayama[5], 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; [4]Center for Genomic Medicine, RIKEN; [5]Departments of Medical and Molecular Science, Graduate School of Biomedical Sciences, Hiroshima University

To identify a prognostic factor(s) for patients with chronic hepatitis C (CHC), we conducted a genome-wide association study (GWAS) using 682 hepatitis C virus (HCV)-induced liver cirrhosis (LC) cases and 1,045 CHC patients in Japan. Eight SNPs which showed possible associations ($P < 1.0 \times 10\text{-}5$) in the GWAS stage were further 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 rs 3135363, were significantly associated with the progression from CHC to LC (Pcombined = $9.15 \times 10\text{-}11$ and $1.45 \times 10\text{-}10$, 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 ($P = 4.53 \times 10\text{-}4$ and $1.54 \times 10\text{-}4$ with OR = 2.80 and 1.45, respectively). Multiple logistic regression analysis revealed that rs 3135363, 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. Conclusion; SNPs rs3135363 and rs910049 were significantly associated with progression from CHC to LC. Multiple genetic variations within the MHC region would be prognostic/predictive biomarkers for CHC patients.

3. MICA variation and soluble MICA are possible prognostic biomarkers for hepatitis B virus-induced hepatocellular carcinoma

Vinod Kumar[1], Yuji Urabe[1], Ryosuke Muroyama[2], Norie Kowatari[2], Kaku Goto[2], Wenwen Li[2], Ryo Nakagawa[2], Motoyuki Otsuka[3], Ryosuke Tateishi[3], Masao Omata[3], Kazuo Koike[3], Katsushi Tokunaga[4], Yasuhito Tanaka[5], Masashi Mizokami[6], Michiaki Kubo[7], 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; [4]Department of Human Genetics, Graduate School of Medicine, University of Tokyo; [5]Department of Clinical Molecular Informative Medicine, Graduate School of Medical Sciences, Nagoya City University; [6]The Research Center for Hepatitis and Immunology, National Center for Global Health and Medicine; [7]Center for Genomic Medicine, RIKEN

Hepatitis B virus (HBV) infection still remains to be one of the dominant risk factor for hepatocellular carcinoma (HCC). Both genetic as well as environmental factors influence the progression of chronic hepatitis B (CHB) to HCC. MHC class I polypeptide-related chain A and B (MICA and MICB) molecules are induced in response to viral infection as well as various stresses. We have recently revealed that a SNP in MICA promoter was significantly associated with HCV-induced HCC risk as well as lower soluble MICA (sMICA) level in serum. To investigate the possible involvement of MICA in HBV-induced HCC, we tested the role of MICA gene polymorphisms and the levels of sMICA in HBV-induced HCC patients. The genetic association analysis revealed a nominal association at rs2596542 in which G allele was associated with susceptibility to HBV-induced HCC ($P = 0.029$ with odds ratio of 1.19). We found a significant elevation of sMICA in of HBV-induced HCC cases. Moreover, genotypes of SNP rs2596542 located in the upstream of MICA promoter, was significantly associated with sMICA levels. Further analysis the clinicopathological data of the patients revealed that sMICA level is associated with overall survival probability ($p = 0.008$). Thus, our results highlight the importance of MICA genetic variations and sMICA as predic-

tive biomarkers for HBV-induced HCC and a possibility that sMICA may be a potent biomarker for HBV-induced HCC prognosis.

4. A small molecule screen for MICA induction

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

Recently our genome-wide association study (GWAS) identified MHC class I polypeptide-related sequence A (MICA) as a genetic susceptibility factor for HCV-induced hepatocellular carcinoma (HCC). In addition lower levels of MICA expression hightened the risk of HCC development in patients, which indicated preventive effects of MICA induction against hepatocarcinogenesis. Accordingly we strove to develop a screen system to identify small molecules for the upregulation of MICA expression. Treatment of Huh7 cells with valproic acid (VPA) and hydroxyurea (HU), reported MICA inducers in leukemic cell lines, elevated MICA mRNA leveles 5 times. Then we constructed luciferase reporters encoding MICA promoter sequences, with their activities increased by VPA and HU likewise. Subsequently stable cell transformants carrying those reporters were isolated via antibiotics selection, and they finally demonstrated full luciferase acitivities, which were similarly enhanced by the VPA/HU treatment in a dose-dependent fashion. All the data indicated that the endogenous expression and induction of MICA mRNA were to be successfully monitored in the reporter system. Hitherto our screen system has detected the elevation of MICA transcriptional activity by several compounds including saturated fatty acids and HDAC inhibitors consistent with actual mRNA levels' increase. Thus the new reporter system is such a suitable platform for upcoming small molecule/drug screens for MICA expression inducers, eventually contributing to the development of anticarcinogenic strategy in chronic hepatitis C.

5. Hepatitis C virus (HCV) core amino acid 70 mutant ratio is associated with response to pegylated-interferon/ribavirin treatment in HCV genotype 1b patients

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

HCV core amino acid (AA) 70 substitution (Arg to Gln) has been proved to be associated with null virological response (NVR) to pegylated-interferon (PEG-IFN)/ribavirin (RBV) treatment and HCV-related hepatocellular carcinoma (HCC) in HCV 1b infected patients. However, the mechanism remains undefined. We previously developed a Taqman realtime PCR system for monitoring the viral dynamics in HCV 1b patients who received PEG-IFN/RBV treatment, and also found that core 70 mutant (70M) ratio of pre-treatment could predict the treatment response. However, this effect was attenuated when host factor (IL28B polymorphism) was introduced into multivariate logistic regression analyses. During monitoring viral responses to the treatment, we found that in relapsed patients, 70M appeared to be predominant during the early stage of relapse, which indicated that 70M-infected liver cells probably got "resistance" and hence be more difficult to be eliminated. Moreover, we could detect a higher level of Bcl-xL mRNA in 70M transfected HepG2 cells compared to 70W (core 70 wild type). This may partly explain that the HCV 70 M could enhance cell survival via altering apoptosis pathway, and probably further participated in hepatocarcinogenesis. Further evaluating for the effect of 70M on BCL-xL is ongoing.

6. Roles of the AMPK-related kinase SNARK in hepatitis C virus replication and pathogenesis

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Host cellular cofactors supporting hepatitis C virus (HCV) infection are now being recognized as attractive antiviral targets because of their independence from viral sequence. Through genome-wide RNAi screen of host cellular cofactors, we found that a stress-activated kinase, 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 replication and in turn HCV-mediated modulation of SNARK. Knockdown of SNARK reduced levels of HCV replication by twofold in both OR6 replicon and JFH1 infection systems. Overexpression of siRNA-resistant wild type SNARK rescued the suppressed viral replication. However, siRNA-resistant SNARK with a kinase-deficient mutation or a mutation that abolishes phosphorylation of SNARK failed to rescue the viral replication. Reciprocally, SNARK mRNA levels were found to be induced by JFH1 infection. We speculate that viral induction and exploitation of this proviral kinase in-

volved in cellular metabolisms and signaling pathways promotes HCV pathogenesis. Further studies are underway to explore the possibilities.

7. Fusion HBx translated from hepatitis B virus integrant is a responsible molecule for hepatocarcinogenesis and could be a universal treatment target

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

Epidemiological studies have demonstrated that chronic infection with hepatitis B virus (HBV) is a major risk factor associated with hepatocellular carcinoma (HCC), and HBV X protein (HBx) has been suggested to play an important role in 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 a human hepatoma cell line, and investigated its role in hepatocarcinogenesis. We could identify 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.

In KD cells, cell proliferation and invasion ability was reduced. I addition, KD cells could not develop any visible tumor in nude mice when we injected KD cells subcutaneously into 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. 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. In microarray analysis for miRNAs, the expression level of four miRNAs (miR-193b, 363, 376a, 376c) was changed in KD cells compared with Hep3B cells. Not HBx but fusion HBx translated from HBV integrant played an important role in hepatocarcinogenesis. 5. Fusion HBx could be an universal treatment target for HBV-related HCC.

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

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

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third leading cause of cancer-related death worldwide. More than three-quarters of all HCC cases were induced by chronic HBV infection. Mounting evidence had shown that some viral factors such as HBV genotype C infection, HBV X or S gene mutations could highly affect the disease outcome. However, the results for genotype specific mutations were still conflictive. Here we performed a large-scale analysis for HCC susceptive mutations in HBV infected patients, we focused on the most oncogenic X gene and HBV genotype C, which was also an independent risk factor for HCC. The aims of this study were: 1. To identify specific X point mutations associated with HBV genotype C infected HCC. 2. To see if the severity of diseases in HBV genotype C patients were accompanied with increasing X mutations. Results: 1) 5380 HBV sequences were screened from online databases, and finally 495 full-length X sequences collected from 15 countries were enrolled (human sera origin, HCC: 153; Non-HCC: 342). 2) Twenty HCC suspect nucleotides mutations were identified including 6 also located in overlapped Enhancer 2 region and 14 in Core Promoter region. 3) X mutations occurred significantly higher in HCC groups. (HCC: 10.6 ± 1.98, Non-HCC: 8.8 ± 2.2, $p < 0.01$) 4) Multivariate analysis showed that A1383 C (OR: 1.996, 95%CI: 1.075-3.706), A1479C/G/T (OR: 2.93, 95%CI: 1.49-5.79; OR: 2.79, 95%CI: 1.32-5.90) OR: 6.70, 95%CI: 2.81-15.99), C1485T (OR: 2.63, 95%CI: 1.50-4.60), C1653T (OR: 1.95, 95%CI: 1.15-3.31), A1762T (OR: 1.85, 95%CI: 1.01-3.40) were independent risk factors for HBV genotype C-related HCC.

9. Type 2 diabetes and hepatocellular carcinoma: a case-control study in patients with chronic hepatitis B

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Type 2 diabetes has been suggested as an independent risk factor for the development of hepatocellular carcinoma (HCC). However, the role of Type 2 diabetes on the development of HCC in the presence of chronic hepatitis B (CHB) remains inconclusive. We conducted this hospital-based case-control study to evaluate the roles of Type 2 diabetes in HCC development in patients with CHB. From January 2004 to December 2008, a total of 6,275 eligible consecutive patients with chronic hepatitis B virus (HBV) infection were recruited. A total of 1,105 of them

were patients with HBV-related HCC and 5,170 patients were CHB but without HCC. We used multivariate logistic regression models to investigate the association between Type 2 diabetes and HCC risk. The prevalence of Type 2 diabetes is higher among HCC patients without cirrhosis than among those with cirrhosis (12.1% vs. 6.7%, p = 0.003). Type 2 diabetes was associated with a significantly high risk of HCC in female patients after adjusting for age, family history of HCC, city of residence, hepatitis B e antigen and cirrhosis with an odds ratio (95% confidence interval, CI) of 1.9 (1.1-3.4). Restricted analyses among female patients without cirrhosis indicated that Type 2 diabetes was strongly associated with HCC risk with adjusted odds ratio (95% CI) of 5.6 (2.2-14.1). In conclusion, Type 2 diabetes is independently associated with the increased risk of HCC in female CHB patients. Female CHB patients with Type 2 diabetes are of a high HCC risk population and should be considered for HCC close surveillance program.

10. ABO blood group and the risk of hepatocellular carcinoma: a case-control study in patients with chronic hepatitis B

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Studies have observed an association between the ABO blood group and risk of certain malignancies. However, no studies of the association with hepatocellular carcinoma (HCC) risk are available. We conducted this hospital-based case-control study to examine the association with HCC in patients with chronic hepatitis B (CHB). From January 2004 to December 2008, a total of 6275 consecutive eligible patients with chronic hepatitis B virus (HBV) infection were recruited. 1105 of them were patients with HBV-related HCC and 5,170 patients were CHB without HCC. Multivariate logistic regression models were used to investigate the association between the ABO blood group and HCC risk. Compared with subjects with blood type O, the adjusted odds ratio (AOR) for the association of those with blood type A and HCC risk was 1.39 [95% confidence interval (CI), 1.05-1.83] after adjusting for age, sex, type 2 diabetes, cirrhosis, hepatitis B e antigen, and HBV DNA. The associations were only statistically significant [AOR (95%CI) = 1.56(1.14-2.13)] for men, for being hepatitis B e antigen positive [AOR (95%CI) = 4.92(2.83-8.57)], for those with cirrhosis [AOR (95%CI), 1.57(1.12-2.20)], and for those with HBV DNA $\leq$ 10(5) copies/mL [AOR (95%CI), 1.58(1.04-2.42)]. Stratified analysis by sex indicated that compared with those with blood type O, those with blood type B also had a significantly high risk of HCC among men, whereas, those with blood type AB or B had a low risk of HCC among women. The ABO blood type was associated with the risk of HCC in Chinese patients with CHB. The association was gender-related.

11. CD26 in hepatocellular carcinoma

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CD26 is a pleiotropic transmembrane protein with dipeptidyl peptidase IV (DPPIV) activity critically involved in major diseases including type 2 diabetes mellitus (T2M). Growing amount of evidence demonstrated druggable oncostatic/oncopromoting roles of CD26 in various cancers, and we aimed to characterize its oncorelated properties in the liver tissues and hepatocytes for the prevention of liver cancer. CD26 expression was profiled in multiple tissues including the normal liver and malignant liver tumors using tissue microarray analysis. Hepatocellular CD26 expression was investigated by cDNA array, western blotting, immunohistochemistry, and fluorescence activated cell sorting (FACS) analyses. Effects of humanized CD26 antibody, DPPIV inhibitor, and letiviral vector-mediated CD26 knockdown on cell proliferation were monitored by MTT assay in HCC cell lines. CD26 was abundantly expressed in the liver tissue with zonal distribution, which was dysregulated in HCC though the expression level of CD26 was not remarkably altered in HCC patient samples. Huh7 and HepG2 cell lines expressed CD26 well, but neither stimulation nor inhibition of CD26/DPPIV affected MTT assay-monitored cell proliferation. Significance of the topographically proper organization of CD26 expression was underscored for the normal liver, and intercellular/intertissue communications in addition to transmembrane/intracellular signalings may explain development of HCC. All the data and discussion are important prerequisites for the development of potential application of clinically accepted CD26/DPPIV modulator to anti-HCC treatment.

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

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Autoimmune liver disease (ALD), which contains autoimmune hepatitis (AIH) and primary biliary cirrhosis (PBC), is chronic inflammatory liver disease by abnormality of autoimmunity and often the cause of cirrhosis. $CD4^+$Tcells are suggested to play an important role in the pathogenesis of ALD. However, the molecular mechanisms are still well unknown. Recently, microRNAs (miRNAs) were reported to be involved in autoimmune disorders and their loss of function in immune cells was shown to facilitate systemic autoimmune disease. In this study, we examined the molecular and etiological mechanisms of $CD4^+$ Tcells in ALD by profiling these miRNA expression.

14 patients (7 AIH, 7 PBC) 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 34 miRNAs were decreased in ALD. In addition, 19 miRNAs were differentially expressed between AIH and PBC.

The change of miRNA expression might have effects on the function of $CD4^+$ T cell and contribute to the pathogenesis of ALD.

13. IL28B minor allele is associated with an early 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 rs8099917 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 early 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 $\times$ 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 early-age onset of HCC in CHC patients.

14. 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, rs 738409) 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 473 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 ages at onset of HCC for CC, CG, and GG genotypes were 69.9, 69.3, and 67.9, respectively. The rs738409 GG genotype was an

independent risk factor for early age at onset of HCC ($p = 0.008$) in males ($p < 0.001$) with higher body mass index ($p = 0.009$). In a dominant model analysis, the duration between the HCV infection and the development of HCC was significantly short in G allele carriers (CG or GG genotypes) ($p = 0.04$, n = 214) compared to CC genotype. The rs738409 GG genotype was also associated with a higher probability of having severe fibrosis/cirrhosis (F4 stage, OR = 1.78, $p = 0.047$) and higher ALT level (65.5 vs. 59.0 IU/l, $p = 0.04$) at the time of HCC onset. In conclusion, PNPLA3 genotype independently influenced the hepatocarcinogenesis in patients with chronic hepatitis C, probably through the mediation of inflammatory activity and fibrosis progression derived from steatosis.

Publications

Kumar V, Kato N, Urabe Y, Takahashi A, Muroyama R, Hosono N, Otsuka M, Tateishi R, Omata M, Nakagawa H, Koike K, Kamatani N, Kubo M, Nakamura Y, Matsuda K. Genome-wide association study identifies a susceptibility locus for HCV-induced hepatocellular carcinoma. Nat Genet. 2011; 43: 455-458.

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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

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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*), 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 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 2011, the second year of the second term, the ongoing collaborations in Beijing and Harbin have been further promoted and developed. T. Ishida (as new PI) and J. Gohda joined the joint laboratory at IMCAS, from which Y. Kitamura resigned as PI in March 2011. The new members are preparing for collaborative research using clinical specimens from areas including southern China. Cooperation with Kumamoto University and Kobe University continues as in 2010. In addition, the several IMSUT faculty members (a core group to promote the China-Japan joint research) are focusing on pathogens and the host factors affecting the pathogenicity. The 2011 annual joint laboratories meeting (*The 8th China-Japan Joint Laboratory Workshop: Pathogenesis, Gene Regulation and Signal Transduction*) was successfully held in IBPCAS in November 2011, which was joined by speakers from IMSUT, IMCAS, IBPCAS, Kumamoto University, Tsinghua University, and the China CDC.

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

In LSVI we (Z. Matsuda's research group) have been studying the mechanism of membrane fusion by the HIV-1 envelope protein. We engineered a pair of new reporter proteins called dual split proteins (DSPs) that allows real-time monitoring of membrane fusion; this method was applied, by A. Iwamoto's group at IMSUT, to a rapid tropism assay for HIV-1 clinical isolates. The DSP activity was further improved hundredfold by protein engineering. Despite high variation in HIV-1 proteins, the membrane-spanning domain (MSD) of the gp41 subunit of HIV-1 envelope protein is highly conserved. One of the highly conserved residues in the gp41 MSD is arginine, a rather rare amino acid in a hydrophobic transmembrane domain. Lysine is the only rare mutation observed in the field isolates. We studied the structure-function relationship of this arginine by mutagenesis. Any substitutions other than lysine resulted in reduced efficiency of membrane fusion. Thus, the highly conserved arginine residue in the gp 41 MSD is required for efficient membrane fusion. In collaboration with N. Sakaguchi's group at Kumamoto University, we prepared several monoclonal antibodies against the fusion intermediate of HIV-1 envelope protein. These antibodies will be valuable reagents to investigate the mechanism of membrane fusion by HIV-1.

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

In LMIMM we (T. Ishida's research group, established in 2011) have been focusing on the studies of clinical materials: molecular epidemiology of infections with HIV-1 and/or hepatitis viruses; and cell biology of the host cell functions influenced by HIV-1 infection. We have established the methodology to detect the drug resistant mutations in both HIV-1 and HBV in one tube using the Luminex technology. Since more than 10% of HIV-1 infected individuals in China are HBVsAg (HBV surface antigen)-positive HBV carriers, this system will provide vital epidemiological information on the spread of drug resistant HIV and HBV. Since the non-B subtypes of HIV-1 (subtype B' also known as Thai B subtype; CRF07_BC and CRF01_AE) are predominant viruses circulating in China, we developed a DSP-based co-receptor tropism assay for the non-clade B HIV-1 in collaboration with A. Iwamoto (IMSUT) and Z. Matsuda (LSVI). To investigate the influence of HIV-1 infection on host cell functions, we developed *in vitro* systems for differentiation of human monocyte into osteoclast.

c. Collaborative research program with HVRI

In 2009, the novel influenza "pandemic (H1N 1) 2009" emerged and spread rapidly throughout the world, while, since 2003, H5N1 highly pathogenic avian influenza viruses have continued to cause unprecedented global outbreaks with high case fatality rates in humans. For these reasons, a joint research program at HVRI (Director, Xiangang Kong) has been conducted 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 research group) study type A influenza virus from wild bird, waterfowl, poultry, swine, and horses, with an emphasis on viral pathogenicity in various hosts, viral evolution, and viral prevalence.

In China, up to 4 billion ducks are reared annually, often in open fields with no biosecurity measures. Vaccination coverage of H5N1 avian influenza in these ducks (＜30%) is much lower than that in chickens (about 70%), and therefore huge numbers of ducks remain susceptible and are serving as reservoirs for H5N1 viruses. We established a system to generate a duck enteritis virus (DEV; a herpesvirus) vaccine strain by using the transfection of overlapping fosmid DNAs. Using this system, we constructed recombinant viruses in which the hemagglutinin (HA) gene of the H5N1 virus A/duck/Anhui/1/06 was inserted and stably maintained within the DEV genome. Then, we demonstrated that the recombinant DEV was suitable for use as a bivalent live attenuated vaccine, providing rapid protection against both DEV and H5N1 virus infection in ducks.

In addition, we assessed the impact of the oseltamivir-resistance mutation NA N294S on the pathogenicity of human H5N1 viruses isolated in Vietnam by using mouse and ferret models. Although NA N294S-possessing H5N1 viruses were attenuated in mice and ferrets compared to their oseltamivir-sensitive counterparts, one of the infected ferrets died from systemic infection, demonstrating the potential lethality in ferrets of oseltamivir-resistant H5N1 viruses with the NA N294S substitution. The efficacy of oseltamivir, but not that of zanamivir, against an NA N294S-possessing virus was substantially impaired both in ferrets and *in vitro*. These results demonstrate the considerable pathogenicity of NA N294S-possessing H5N1 viruses and underscore the importance of monitoring the emergence of the NA N294S mutation in circulating H5N1 viruses.

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.

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