Poster Session

P-01 The metabolic function of RB in controlling mevalonate (MVA) pathway and cancer stem cells

Nobunari Sasaki, Chiaki Takahashi
(Division of Oncology and Molecular Biology, Cancer Research Institute, Kanazawa University, Kanazawa, Japan)

Mutation in RB gene is found at the initiation of limited human cancers, whereas RB protein inactivation is frequently found in much wider variety of cancers mainly during their progression. These findings suggest that RB exerts more roles than previously thought beyond its well-appreciated roles in controlling cell cycle and differentiation. We previously reported that RB controls protein isoprenylation via E2Fs and SREBPs transcription factors, thus regulates Ras maturation processes. Here we demonstrate that RB affects transcription of many enzymes involved in Mevalonate (MVA) pathway. MVA pathway is the upstream of many biosynthesis pathways including protein farnesylation, protein geranylgeranylation and cholesterol synthesis. We developed an in vitro cancer stem cell (CSC) model in which some of typical CSC-like features are induced in RB and p53-dependent manners. By employing specific enzyme inhibitors, we determined that these features are sensitive to drugs those antagonize the MVA pathway. We will discuss on which of MVA pathway products contributes to the development of CSC-like features, and also on the metabolic basis of RB and p53 functions in CSCs.

P-02 Essential role of Nucleostemin in the maintenance of malignant phenotypes in germ cell tumors

Noriyuki Uema[1], Takako Ooshio[1], Masako Oomura[1], Kenichi Harada[2], Yashuni Nakanuma[2], Akihiko Okuda[3], Tomoyoshi Soga[4], Atsushi Hirao[1]
([1]Divison of Molecular genetics, Kanazawa Univ., Cancer Res. Inst., [2]2nd Path., Kanazawa Univ., Med., [3]Division of Developmental Biology, Genomic Res. Ctr., Saitama Medical Univ., [4]Inst. for Advanced Biosciences, Keio Univ. Tsuruoka, Yamagata.)

Nucleostemin (NS) is a putative GTPase, which is involved in ribosomal biogenesis. In this study, we analyzed expression and function of NS in germ cell tumors. In human testicular germ cell tumors, expression of NS was observed in cells that expressed OCT3/4, which is a critical regulator of undifferentiated status in embryonic stem (ES) cells and germ cell tumors. Consistently, we found the co-expression of NS and Oct3/4 in mouse teratoma model derived from ES cells. The cells expressing NS were actively proliferating and showed undifferentiated characteristics in the teratoma model. The cells with high level of NS expression exhibited capacity for generating immature ES like colonies, whereas cells without NS did not. NS deficiency by tetracycline-inducible system lost undifferentiated characteristics in teratoma, resulting in defective tumor growth. These data demonstrated that NS is essential for maintenance of malignant phenotypes in germ cell tumors.

P-03 Effects of inflammation on the epithelial differentiation and tumorigenesis

Tomo-o Ishikawa, Hiroko Oshima and Masanobu Oshima
(Division of Genetics, Cancer Research Institute, Kanazawa University)

Tumor microenvironment including inflammation plays an important role in cancer development. Dedifferentiation is a distinctive feature of cancer progression. Dedifferentiation is a distinctive feature of cancer progression. However, effects of inflammation on the differentiation status of tumor cells have not been revealed. Using *K19-C2mE* gastritis mouse model and *Gan* gastritis-associated cancer model, we found that the expression of more than 500 genes was up-regulated by inflammation. This gene list was compared to the expression profile of *Lgr5* positive gastric epithelial stem cells, to elucidate the relationship between inflammation and differentiation status. Among 345 genes upregulated in gastric stem cells, 38 genes were upregulated in *K19-C2mE* and *Gan* mice. By siRNA knockdown screening of those genes for soft agar colony formation in gastric cancer cell lines, 2 genes were implicated in tumorigenicity. We considered those genes as candidates which implicate in epithelial differentiation and tumorigenesis.

P-04 CCL3-CCR5 axis regulates progression of fibrosis occurring as a result of chronic colitis in mice

Soichiro Sasaki, Tomohisa Baba, Naofumi Mukaida
(Div. Molec. Bioregulation, Cancer Res. Inst., Kanazawa Univ., Kanazawa)

Patients with ulcerative colitis are sometimes complicated with colon carcinoma. This carcinogenesis can be recapitulated in mice by the combined treatment with azoxymethane (AOM)-dextran sulfate sodium (DSS). Collagen type I- and α-smooth muscle actin (SMA)-positive cells accumulated in the course of carcinogenesis process of wild-type (WT) mice with intracolonal fibrocytes/fibroblasts expressing CCL3 and its specific receptor, CCR5. Moreover, *CCL3* ablation decreased collagen type I- and α- SMA-positive cell numbers and eventually reduced the numbers and sizes of colon tumors, compared with WT mice. Likewise, reduction in fibrosis and tumor incidence was observed in mice deficient in CCR5. Furthermore, WT mice transplanted with CCL3 or CCR5-deficient mouse-derived bone marrow cells, developed significantly fewer tumors after AOM/DSS treatment, compared with WT mice. Thus, chronic colitis-associated fibrosis and subsequent carcinogenesis can be regulated by the CCL3/CCR5-expressing bone-marrow-derived cells. Thus, blockade of the CCL3-CCR5 axis can be a potential therapeutic target against colon cancer, particularly one that is associated with fibrosis.

P-05 Evaluation of the inhibition of NF-κB activation by NF-κB decoy transfection in M2 like macrophages using mannose-modified bubble lipoplexes with ultrasound exposure

Yusuke Kono[1], Shigeru Kawakami[1], Yuriko Higuchi[1], Fumiyoshi Yamashita[1], Mitsuru Hashida[1,2]
([1]Department of Drug Delivery Research, Graduated School of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan. [2]Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto University, Kyoto, Japan)

Tumor-associated macrophages (TAM) enhance tumor growth by secretion of cytokines and growth factors. Recently, we have reported that inhibition of NF-κB activation by NF-κB decoy changes the properties of cytokines production from M2-like macrophages that cultured in cancer cells conditioned medium to M1 macrophages. Therefore, it is necessary to develop TAM-selective targeting system. Previously, we have developed efficient and cell-selective gene transfection method to macrophages using mannose-modified bubble lipoplexes with ultrasound (US) exposure. Here, we developed TAM-selective targeting system and the effect of NF-κB decoy transfection into M2-like macrophages on the immune response of macrophages was evaluated. The cell-selective NF-κB decoy delivery to TAM was observed after intratumoral injection of mannose-modified bubble lipoplexes with US exposure. When NF-κB decoy was transfected into M2-like macrophages by mannose-modified bubble lipoplexes *in-vitro*, IL-10 production that is Th2 cytokine was significantly reduced and the secretion of Th1 cytokines was significantly increased. Vascular endothelial growth factor (VEGF) and arginase mRNA expression that are secreted from M2-like macrophages was also significantly suppressed by NF-κB decoy transfection. These results suggest that NF-κB decoy transfection using mannose-modified bubble lipoplexes with US exposure could change the properties of cytokines production from M2-like macrophages to M1 macrophages.

P-06 The therapeutic administration of TLR2 ligand leads to tumor retardation through CTL activation in mice

Masahiro Azuma, Ryoko Sawahata, Misako Matsumoto, Tsukasa Seya
(Department of Microbiology and Immunology, Graduate School of Medicine, Hokkaido University.)

Many clinical trials using TLR ligand for tumor immunotherapy are being performed. The use of TLR2 ligand, e.g. BCG-CWS, have been examined in various clinical applications and got a positive outcome. On the other hand, TLR2 signal in the tumor cells is reported to promote a tumorigenesis and a metastasis and correlate with a poor prognosis. We previously showed that prophylactic administration of TLR2 ligand, Pam2CSK4, leaded to exacerbation of melanoma through Treg expansion and IL-10 production. In this study, we investigated whether the therapeutic administration of TLR2 ligands restricts the tumor progression. When EG7 cells (OVA-expressing EL4 thymoma) were inoculated on back and treated with TLR2 ligand (MALP2s) after tumor formation, EG7 was clearly eradicated by the administration of Ag and MALP2s in a CTL-dependent manner. At the time, the numbers of Tregs were almost the same among all four groups of mice treated with PBS, OVA, MALP2s and OVA/MALP2s. And the same was true in IL-10-producing cell number. Furthermore, IFN- γ -producing $CD4^+$ T cells had a tendency to expand in the group of mice treated with OVA/MALP2s. Collectively, these data suggest that therapeutic administration of TLR2 ligands have a potential to suppress a tumor progression via CTL activation.

P-07 Analysis of the role of RUNX3 in gastric cancer cell lines

Xiaoli Ju[1], Tomo-o Ishikawa[1], Hiroko Oshima[1], Kosei Ito[2], Yoshiaki Ito[3], Masanobu Oshima[1]
([1]Division of Genetics, Cancer Research Institute, Kanazawa University, [2]Graduate School of Biomedical Sciences, Nagasaki University, [3]Cancer Science Institute of Singapore, National University of Singapore)

It has been demonstrated that RUNX3 functions as a tumor suppressor in various cancers. It has also been shown that RUNX3 can attenuate the transactivation potential of β-catenin/TCFs in colon cancer cell lines. In order to examine the function of RUNX3 in Wnt signal pathway in gastric cancer cell lines, we knocked down RUNX3 expression in AZ521 gastric cancer cells that express endogenous RUNX3, and Wnt activity was monitored by TOPflash assay. Notably, Wnt activity was significantly increased by RUNX3 down-regulation in AZ521 cells, which is consistent with previous reports. On the other hand, however, RUNX3 overexpression in Kato III gastric cancer cells that lack RUNX3 expression by methylation resulted in significant increase of Wnt activity. These results were confirmed by QRT-PCR. We previously found that Wnt activity is oscillating in Kato III cells. Interestingly, we found that RUNX3 increased Wnt activity through suppression of Wnt oscillation. We also found that RUNX3 increased Wnt activity by Wnt ligand dependent mechanism. Although Wnt is activated, proliferation and soft agar colony formation were suppressed in Kato III cells by RUNX3 expression. Accordingly, it is possible that RUNX3 inhibits tumorigenicity of Kato III cells, although Wnt is activated.

P-08 Paracrine HGF-induced as well as constitutive Met phosphorylation promotes peritoneal carcinomatosis in gastric cancer

Lu Zhao[1], Kazuo Yasumoto[1], Atsuhiro Kawashima[2], Takayuki Nakagawa[1], Shinji Takeuchi[1], Tadaaki Yamada[1], Kunio Matsumoto[3], Osamu Yoshie[4], Kouji Matsushima[5], Kazuhiko Yonekura[6], Seiji Yano[1]
([1]Division of Medical Oncology, Cancer Research Institute, Kanazawa University, [2]Kanazawa Medical Center, [3]Division of Tumor Dynamics and Regulation, Cancer Research Institute, Kanazawa University, [4]Department of Microbiology, Kinki University Faculty of Medicine, [5]Department of Molecular Preventive Medicine, Graduate School of Medicine, The University of Tokyo, [6]Tsukuba Research Center, Taiho Pharmaceutical CO., LTD.,

Met plays an important role in tumorigenesis. Here, we investigated whether the Met/hepatocyte growth factor (HGF) signaling pathways are involved in the development of peritoneal carcinomatosis from gastric cancer, which is the most frequent cause of death in gastric cancer. Human gastric cancer cell lines, which were highly efficient in generating peritoneal metastases in nude mice after i.p. inoculation, expressed Met and showed phosphorylation of Met. In particular, NUGC4 cells, without *Met* amplification, developed peritoneal carcinomatosis in mice whose condition mimic human this condition. HGF induced migration of all human gastric cancer cells was examined. HGF enhanced proliferation and rapid increase in phosphorylation of Met, protein kinase B/Akt, and extracellular signal-regulated kinase of NUGC4 cells, but not MKN45 cells with *Met* amplification. Interestingly, HGF proteins was markedly expressed by human normal fibroblasts in a paracrine manner. Furthermore, we showed that Met kinase inhibitors crizotinib and TAS-115 exhibited marked antitumor effects in gastric cancer xenografts negative as well as positive for *Met* amplification, accompanied by inhibition of Met phosphorylation in disseminated tumors in nude mice. Collectively, our results strongly suggest that the Met/HGF axis play an important role in the development of peritoneal carcinomatosis from gastric cancer.

P-09 Preparation and culture of primary cancer cells, and its application to gastric cancer

Yumi Sato[1,2], Hiroaki Okuyama[2], Hiroko Endo[2], Masahiro Inoue[2]
([1]Department of Molecular Virology and Oncology, Cancer Research Institute, Kanazawa University, Depertment of Biochemistry, [2]Osaka Medical Center for Cancer and Cardiovasclar Disease)

Primary culture of cancer cells could be a powerful tool for investigating cancer biology and predicting chemosensitivity for individual patient.

Recently we established a novel method of primary culture of colorectal cancer cells, in which cell-cell contact is maintained throughout the preparation. The tumor fragments formed spheroid efficiently within a short time and we termed these spheroid as cancer tissue-originated spheroid (CTOS).

The CTOS method was already applied to lung and urothelial cancer, therefore we next tried gastric cancer culture. It was difficult to obtain pure cancer CTOSs because of the contamination of fibroblast and/or normal epithelial cells. To overcome this problem we transplanted small pieces of primary tumor to NOD/SCID mice. Xenograft tumors were obtained from 43% of the cases and CTOS could be prepared from all these xenografts. All CTOSs had tumor forming capacity. These results suggest that the xenograft tumor formation facilitates the preparation of CTOS from human gastric cancer. These gastric cancer CTOSs could be cryopreserved and 6 out of 7 lines could grow after thaw. With these 6 CTOS lines, we can further investigate the culture conditions, chemosensitivity, and pathwayactivation in gastric cancer.

P-10 Estrogen-mediated enhancement of epithelial monolayer disruption by *Helicobacter pylori* CagA oncoprotein

Yui Kashiba, Naoko Kamiya, Masanori Hatakeyama
(Department of Microbiology, Graduate School of Medicine, University of Tokyo.)

Helicobacter pylori cagA-positive strains are closely associated with gastric carcinoma. The CagA protein is delivered into gastric epithelial cells via type IV secretion. Delivered CagA interacts specifically with several host proteins and thereby deregulates multiple cell signaling pathways, which contributes to the development of gastric carcinoma. Scirrhous gastric cancer, a poorly differentiated adenocarcinoma that often occurs in relatively young female with *cagA*-positive *H. pylori* infection, is characterized by strong infiltration into the mucosal wall. Since estrogen has been associated with various carcinomas, we investigated the effect of estrogen on cancer-associated activity of CagA. Polarized MDCK epithelial cells are known to expel from the monolayer upon CagA expression. Following inhibition of estrogen receptor β (ER β) expression in polarized MDCK cells, extrusion of CagA-expressing cells was robustly enhanced by estrogen, which was followed by massive disruption of the epithelial monolayer. This study indicates that aberrant estrogen signaling markedly potentiates the ability of CagA to disorganize normal epithelial monolayer, which may be associated with the development of scirrhous cancer of the stomach.

P-11 Anti-CXCL13 antibody can protect against gastric lymphoid follicles induced by *Helicobacter* infection

Koji Yamamoto, Yang Lin, Takeshi Azuma
(Division of Gastroenterology, Department of Internal Medicine, Kobe University Graduate School of Medicine)

【Abstract】

Helicobacter (H.) suis infects the stomachs of various animals including humans, and can induce gastric MALT lymphomas in 100% of C57BL6J mice. It is known that CXC chemokine ligand 13 (CXCL13) is highly expressed in the *Helicobacter*-infected mice and gastric MALT lymphoma patients, but the relationship between the activation of CXCL13 and the formation of gastric MALT lymphomas remain unclear.

In this study, we examined the role of CXCL13 in the formation of gastric MALT lymphoma after *H. suis* infection, and then determined whether CXCL13 neutralization can attenuate the formation of gastric lymphoid follicles. As a result, the number of gastric lymphoid follicles was significantly reduced by anti-CXCL13 antibody treatment. Moreover, the expression of genes associated with the lymphoid follicle formation was effectively suppressed by anti-CXCL13 antibody treatment. These results suggest that the up-regulation of CXCL13 plays an important role in the development of gastric MALT lymphomas, and highlight the potential of anti-CXCL13 antibody for protection against *Helicobacter*-induced gastric diseases.

P-12 Interferon-γ induces the formation of gastric lymphoid follicles after *Helicobacter suis* infection

Yang Lin, Koji Yamamoto, Takeshi Azuma
(Division of Gastroenterology, Department of Internal Medicine, Kobe University Graduate School of Medicine)

【Abstract】

H. suis, which belongs to the *Helicobacter* family just like *H. pylori*, is spiral-shaped gram-negative bacterium, and is found in the stomachs of various animals including cats, dogs, pigs, and humans. *H. suis* infection can induce gastric MALT lymphoma in 100% of mice. Our recent study revealed that the formation of gastric lymphoid follicle was detected in *H. suis*-infected mice accompanied by the high expression level of IFN- γ . The gastric lymphoid follicles were also consisted of B cells, $CD4^+$T cells, DCs, and FDCs. However, the formation of gastric lymphoid follicles was not observed in *H. suis*-infected IFN- γ KO mice, suggesting that IFN- γ is important for the *H. suis*-evoked formation of gastric lymphoid follicles. Next, TCR KO mice were infected with *H. suis*, because T cell is known as the inducer for IFN- γ , but the formation rate of gastric lymphoid follicles in the *H. suis*-infected TCR KO mice was as same as that of the *H. suis*-infected mice. Thus, these results raise the possibility that the induction of IFN- γ after *H. suis* infection is essential for the formation of gastric lymphoid follicles, which may be induced by B cells, DCs or FDCs except T cells as IFN- γ -producing cells.

P-13 Hypermutation of human papillomavirus 16 viral DNA by APOBEC3 proteins

Zhe Wang[1], Kouichi Kitamura[1], Kosho Wakae[1], Iwao Kukimoto2, Miki Koura[1], Masamichi Muramatsu[1]
([1]Department of Molecular Genetics, Graduate School of Medical Science, Kanazawa University, [2]Center for Pathogen Genomics, National Institute of Infectious Diseases of Japan)

Cervical cancer is the second most common cancer in women worldwide. Human papillomavirus 16 (HPV16) and 18 are the most important high risk viruses to cause cervical cancer. Epidemiology investigation shows that integration of the human papillomavirus (HPV) genome into the host chromatin is a characteristic step in cervical carcinogenesis. However, the mechanism of integration is still poorly understood. Recent studies reveal a novel antiviral activity of apolipoprotein B mRNA editing enzyme catalytic polypeptide 3 (APOBEC3) proteins in Human immunodeficiency virus (HIV) and Human hepatitis B virus (HBV). It was reported that APOBEC3 proteins introduce C-to-U conversion in these viral DNA. Here, we asked whether APOBEC3 proteins play any role in HPV integration. We found that overexpression of A3s resulted in hypermutation of HPV episomal DNA in HPV episome positive cell line (W12). Moreover, endogenous APOBEC3s expression were induced in W12 cells by Interferon (IFN) and IFN initiated hypermutation on viral DNA. Conversely, siRNA against A3s abrogated IFN mediated hypermutation. Overexpression of A3s might result in host genome instability. Since base excision repair pathway can generate the DNA strand breaks, our observation suggests that APOBEC3 proteins may involve in integration of HPV DNA by generating DNA strand breaks.

P-14 Augmented productions of inflammatory cytokines by novel mechanisms in HCV-infected liver

Yuko Shimizu[1], Hironori Nishitsuji[1], Hiroyuki Marusawa[2], Kenji Funami[3], Tsukasa Seya[3], Saneyuki Ujino[1], Ritsuko Shina[1], Hiromi Yamamoto[1], Atsuko Tsukimoto[1], Kunitada Shimotohno[1]
([1]The Research Center for Hepatitis and Immunology, National Center for Global Health and Medicine. [2]Graduate School of Medicine, Kyoto University. [3]Graduate School of Medicine, Hokkaido University)

Chronic inflammation caused by HCV is a necessary step for developing hepatocellular carcinoma. However, the underlying mechanism that develops chronic hepatitis by HCV remains elusive. Here, we report two mechanisms that increase inflammatory cytokines in HCV-infected cells as well as liver specimens in HCV-infected patients.

One is an increase in IL-8 production through APOBEC1. We found aberrant APOBEC1 expression in some specimens of liver cirrhosis. APOBEC1 was also induced in hepatocytes treated with antibiotics such as tunicamycin and doxorubicin. Ectopically expressed APOBEC1 associated with IL8 mRNA and increased IL8 production in hepatocytes. IL8 mRNA seems to be sustained its half-life through the interaction, which results in increase of IL8 production.

The other is an increase in MIP1β production through a communication between hepatocytes and hepatic stellate cells. Co-culture of HCV-infected hepatocytes with stellate cells augmented MIP1β production in hepatocytes, which was initiated with IL-1α from stellate cells. Meanwhile, TGFβ, which is secreted from HCV-infected hepatocytes and activate stellate cells, augmented MIP1β expression. Collectively, it is suggested that cellular communication via cytokines contributes to MIP1β augmentation.

These mechanisms could play a role in attraction of inflammatory cells such as neutrophil and cytotoxic T cells to the micro-environment where HCV-infected cells reside.

P-15 Modification of Immune/Inflammatory System by EBV and Its Contribution to Cancer

Takayuki Murata, Shinichi Saito, Tatsuya Tsurumi
(Division of Virology, Aichi Cancer Center Research Institute)

Being a human oncogenic herpesvirus that establishes a lifelong infection mainly in B cells, Epstein-Barr virus (EBV) has acquired many genes that can modify various aspects of immune system, after co-evolution with the host. To take some examples, LMP1 is a viral oncogene that mimics and constitutively activates CD40/TNFR signaling. LMP2 is a mimic of BCR, BCRF1 is a viral homolog of IL-10, and BNLF2a downregulates MHC. Because EBV encodes >80 genes and most of them have not been clearly characterized yet, we are now determined to identify novel genes that can modify immune/inflammatory system. To this end, we are currently doing preliminary experiment by focusing on a viral gene BPLF1.

BPLF1 has recently been reported to have deubiquitination/deneddylation activity (Gastaldello et al, Nat Cell Biol 2010), although targets and functions of the factor are still elusive. We here newly found that BPLF1 interacts and deubiquitinates TRAF6 during lytic infection, and thereby suppresses NF-κB signaling. BPLF1-deficient recombinant EBV exhibited higher NF-κB activity, and lower viral lytic DNA replication than the wild-type. Expression of NF-κB target genes, including AGT, CCL2, ICAM1 and IL-8, was markedly elevated in cells infected with BPLF1-deficient virus. Physiological significance of this action will be discussed.

P-16 Cleavage of hepatocyte growth factor activator inhibitor-1 by membrane-type MMP-1 stimulates tumor cell invasion and metastasis

Takahiro Domoto[1], Takahisa Takino[2], Toshinari Minamoto[1], Hiroshi Sato[2]
([1]Division of Translational and Clinical Oncology, [2]Division of Molecular Virology and Oncology, Cancer Research Institute, Kanazawa University.)

Membrane-type matrix metalloprotease-1 (MT1-MMP) cleaves multiple proteins in the pericellular milieu, and promotes cancer invasion and metastasis. We previously reported that MT1-MMP activates a membrane-type serine protease matriptase through the cleavage of hepatocyte growth factor activator inhibitor-1 (HAI-1), which contributes in collaboration with MT1-MMP to the invasive growth of squamous carcinoma-derived HSC-4 cells (*Cancer Sci.*, 2012). In this study, we designed HAI-1 mutant, which is resistant to cleavage by MT1-MMP, and compared with wild-type HAI-1 for migration and invasion of fibrosarcoma HT1080 cells. HT1080 cells do not express matriptase. Wild-type HAI-1 was cleaved and inactivated by endogenous MT1-MMP, and did not show significant effect, however, MT1-MMP-resistant HAI-1 mutant suppressed cell migration and invasive growth in collagen gel. On Chick Chorioallantoic Membrane (CAM) assay, MT1-MMP-resistant HAI-1 also suppressed cell metastasis. Another HAI-1 mutant, which lost protease-inhibitor function, had no effect. These results confirmed that MT1-MMP regulates serine protease activity by digesting HAI-1, and suggest that pericellular proteolysis by not only MT1-MMP but also serine protease(s) is essential for tumor cell migration and invasion.

P-17 Functional analysis of histone-modifying enzymes in epithelial-mesenchymal transition of cancer cells

Dulamsuren Oktyabri, Zanabazar Enkhbaatar, Shoichiro Tange, Minoru Terashima, Akihiko Ishimura, Takeshi Suzuki
(Division of Functional Genomics, Cancer Research Institute, Kanazawa University)

Epithelial to mesenchymal transition (EMT) facilitates tissue remodelling during embryonic development and is observed as an essential early step in tumor metastasis through a variety of mechanisms. Aberrant expression of histone-modifying enzymes has been implicated in the course of tumor initiation and progression. Using retroviral insertional mutagenesis in mice, we have identified most of histone lysine methyltransferase and demethylase genes as candidate oncogenes or tumor suppressor genes. Recently, we have found that PLU1 H3K4 demethylase and DOT1L H3K79 methyltransferase are involved in the malignant progression such as cell invasion and epithelial-mesenchymal transition. To uncover the molecular function of these enzymes in tumor progression, we have investigated the downstream target genes regulated by them using a digital expression profile, and identified several important target genes. In this study we will discuss about the detailed mechanism by which these methyl-modifying enzymes would affect the functions of the target genes in the process of EMT.

P-18 Dysfunction of polycomb group cbx protein is possibly involved in tumor malignancy

Katsuya Sakai[1], Takahiro Nakamura[1], Yoshinori Suzuki[1], Takeshi Suzuki[2], Seiji Yano[3], Chiaki Takahashi[4], Kunio Matsumoto[1]
(Division of [1]Tumor Dynamics and Regulation, [2]Functional Genomics, [3]Medical Oncology, and [4]Oncology and Molecular Biology, Cancer Research Institute, Kanazawa University)

Cancer, traditionally seen as a genetic disease, is now realized to involve epigenetic abnormalities along with genetic alterations. However, how these epigenetic abnormalities are accumulated during cancer development, and what kind of epigenetic abbrations contribute to the initiation and progression cancer are still not well understood.

Polycomb group proteins (PcG) form chromatin-modifying complexes that contribute transcriptional silencing essential for normal development and maintenance of tissue-specific gene expression. Here we show that PcG-mediated repression of matrix metalloproteinase-2 (MMP-2) was lost in invasive mesothelioma cells. We specified a chromobox homolog protein (CBX) responsible for MMP-2 silencing. CBXs are components of polycomb repressive complex 1, and interact with methylated histones and RNA. We found chromatin interaction of the CBX was globally reduced in invasive tumor cells. In addition, CBXs were precluded from polycomb complex in invasive tumor cells. Our findings suggest that the global loss of CBXs' function may participate in a malignant progression of tumor cells.

P-19 Regulatory interactions between NBS1 and DNMT1 for epigenetical control

Naoyuki Hayashi, Masahiko Kobayashi, Ken-ichi Yamamoto
(Division of Molecular Pathology, Cancer Research Institute, Kanazawa University)

NBS1 plays crucial role for DNA damage repair and signal transmission via interactions to diverse factors, such as Mre11/Rad50 complex, ATM, ATR, TopBP1 and so on To investigate the NBS1 function in DNA replication checkpoint we chose DNMT1 in the isolated candidates of NBS1 binding factors, which was isolated from 2-hybrid screening.. DNMT1. The isolated DNMT1 cDNA encoded its C-terminal part containing the catalytic region as DNA methyltransferase. Their binding was observed in the condition of replication stall by HU treatment in vivo. Our deletion experiment of their binding regions showed that N-terminus of NBS1 including the FHA domain and Target Recognition Domain in DNMT1 were mapped as their binding regions. On the other hands, we examined DNA methylation in NBS patient cells to investigate epigenetic control of NBS1. The chromatin structure at the *survivin* promoter is regulated by p53 responding to replication stall for repression to allow apoptosis. Either DNA methylation or heterochromatin formation was not observed there in NBS patient cells, and consistently DNMT1 was not recruited there.

P-20 A direct role for NBS1 in ATR activation pathway induced by DNA replication stall

Masahiko Kobayashi, Naoyuki Hayashi, Ken-ichi Yamamoto
(Division of Molecular Pathology, Cancer Research Institute, Kanazawa University)

NBS1 functions as a damage sensor acting upstream of ATM in response to DNA double strand break. We found that Chk1 phosphorylation and FancD2 ubiquitination induced by various DNA replication-stalling agents were diminished in the Nbs1-knockout DT40 cells but not in the conditional Mre11-knockout cells. However, Chk1 phosphorylation and FancD2 ubiquitination induced by ionizing radiation were equally diminished in both knockout cells. Furthermore, we found that the N-terminal half of NBS1 but not the C-terminal one activates ATR by an in vitro ATR kinase assay. In addition, while ATR and TopBP1 colocalized with NBS1 after hydroxyurea treatment in the NBS1-complemented GM07166 (Nbs1+) cells, focus formation and colocalization of ATR and TopBP1 were not detected in the GM07166 (Nbs1-) cells. In Rad17-knockout DT40 cells, which are deficient in Chk1 phosphorylation in response to DNA replication stall, the expression of the N-terminal region of NBS1 fused to PCNA induced Chk1 phosphorylation. These results suggest that NBS1 is directly involved in the ATR pathway.

P-21 Rae1 associates with NuMA preventing aneuploidy formation

Nana Yamakoshi, Richard Wong
(Sch. of Nat. Sys., Col of Sci.and Eng .Kanazawa Univ)

In eukaryotic cells, the faithful segregation of daughter chromosomes during cell division depends on formation of a microtubule (MT)-based bipolar spindle apparatus. The Nuclear Mitotic Apparatus protein (NuMA) is recruited from interphase nuclei to spindle MTs during mitosis. We have identified a mitotic-specific interaction between Rae1 and NuMA and have explored the relationship between Rae1 and NuMA in spindle formation. We have mapped a specific binding site for Rae1 on NuMA that would convert a NuMA dimer to a "tetravalent" crosslinker of MTs. In mitosis, reducing Rae1 or increasing NuMA concentration would be expected to alter the valency of NuMA toward MTs; the "density" of NuMA-MT crosslinks in these conditions would be diminished, even though a threshold number of crosslinks sufficient to stabilize aberrant multipolar spindles may form. Likewise, we found that overexpression of the specific Rae1-binding domain of NuMA in HeLa cells led to aberrant spindle formation. These data point to the Rae1-NuMA interaction as a critical element for normal spindle formation in mitosis. To further investigate the Rae1-NuMA relationships in spatial and temporal approaches, we are generating GFP-NuMA stable cell lines for the high resolution live cell imaging in near future.

P-22 Nuclear pore protein RAE1 contributes to NUP98-mediated leukemogenesis.

Akiko Kobayashi[1], Tatsuyoshi Funasaka[1], Yu Wu[2,3], Chieko Hashizume[1], Ling Gu[4], Pengbo Zhou[5], Malcolm A.S. Moore[6], Hiroshi Sato[7], Richard Wong[1]
([1]Laboratory of Molecular and Cellular Biology, Department of Biology, Faculty of Natural Systems, Institute of Science and Engineering, Kanazawa University, [2]Department of Hematology Research Laboratory, State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, [3]Laboratory of Genome Stability, Development and Stem Cell Institute, [4]Laboratory of Hematology, Department of Pediatric Hematology, West China Second university hospital, Sichuan University, [5]Department of Pathology and Laboratory Medicine, Weill Medical College and Graduate School of Medical Sciences of Cornell University, [8]Cell Biology Program, Memorial Sloan-Kettering Cancer Center, Department of Molecular Virology and Oncology, Kanazawa University.)

Chromosomal translocations involving chimeric fusions of the nucleoporin NUP98 protein have often been described in acute myelogenous leukemia (AML). All the fusion proteins have an identical NUP98 N terminus, which contains the GLEBS motif for interaction with the mRNA export factor RAE1 and FG repeats that associate with the transcription factors HDAC1 and p300. It is virtually unknown whether these interaction partners affect leukemogenesis. We previously showed that RAE1 depletion caused aneuploidy, which enhanced tumorigenesis. Recently, we speculated that RAE1 may also be directly involved in NUP98 fusion-mediated leukemogenesis. We show that RNA interference (RNAi)-mediated knockdown of NUP98 caused severe chromosome segregation defects and disrupted RAE1 but not HDAC1 expression and localization. Next, we performed rescue experiments to confirm that the RAE1-NUP98 complex orchestrates proper chromosome segregation. Our cellular interpretations were further confirmed by NUP98-HOXA9 transgenic mice and the NUP98-HOXA9 AML patient. Now we are generating another Nup98 fusion mutant mice, we plan to further investigate the role of Rae1 in Nup98-mediated leukemogenesis..

P-23 Regulation of autophagy by nuclear pore protein Tpr

Eriko Tsuka[1], Tatsuyoshi Funasaka[1], Richard W. Wong[1,2]
([1]Laboratory of Molecular and Cellular Biology, Department of Biology, Faculty of Natural Systems, nstitute of Science and Engineering, Kanazawa University. [2]Bio-AFM Frontier Research Center, Kanazawa University.)

The nuclear pore complex consists of a conserved set of ~30 different proteins, termed nucle)porins, and serves as a gateway for the exchange of materials between the cytoplasm and nucleus. Tpr (translocated promoter region) is a component of NPC that presumably localizes at intranuclear filaments. Here, we show that T pr silencing caused a severe reduction in the number of nuclear pores. Furthermore, our electron microscopy studies ndicated a significant reduction in the number of inner nuclear filaments. In addition, Tpr siRNA treatment imp ired cell growth and proliferation compared to control siRNA-treated cells. In Tpr-depleted cells, the levels of p53 nd p21 proteins were enhanced. Surprisingly, Tpr depletion increased p53 nuclear accumulation and facilitated utophagy. Our study demonstrates for the first time that Tpr plays a role in autophagy through controlling HSP70 an(HSF1 mRNA export, p53 trafficking, and potentially through direct transcriptional regulation of autophagy factors.

P-24 Roles of mTORC1 signaling in the regulation of glioma ma lignancy

Daisuke Yamada[1], Takayuki Hoshii[1], Shingo Tanaka[1], Atsushi Hirao[1]
([1]Div. of Mol.Gene., Cancer Res. Inst., Kanazawa Univ.)

Glioblastoma (GBM) is the most common and malignant form of brain tumors. GBM h urbors genetic lesions affecting mTORC1 signaling. mTORC1 activity has been reported to increase with tumor gradir ; and to correlate with poor prognosis of GBM patients, suggesting that regulation of mTORC1 activity is involved in malignant progression of gliomas. However, it remains to be investigated whether mTORC1 activity directly c)ntrols the malignant properties. In this study, we investigated the roles of mTORC1 in mouse glioma model. M(use malignant glioma model was generated by the overexpression of mutant EGFR gene, EGFRvIII, in combinati(n with p16Ink4a/Arf deficiency. Using this glioma model, we established tamoxifen-inducible deletion of Tsc1 o1 Raptor to investigate effects of activation or inactivation of mTORC1. When mTORC1 was constitutively activate(by Tsc1-deletion, the survival of tumor-bearing mice was shortened. Histological analysis showed that Tsc1 defici nt tumors were more aggressive than control, and showed increased microvasculature. In contrast, Raptor-deletion emarkably suppressed the progression of glioma. Consistently, Raptor-deletion remarkably inhibited the growth of glio1 1a cells in vitro. These data demonstrate that mTORC1 plays a critical role in controlling the malignant properties of g oma.

P-25 The novel PI3K-mTOR inhibitor, BEZ235, circumvents erlotinib-resistance of *EGFR* mutant lung cancer cells triggered by HGF

Takako Sano[1], Shinji Takeuchi[1], Takayuki Nakagawa[1], Daisuke Ishikawa[1], Shigeki Nanjo[1], Tadaaki Yamada[1], Takahiro Nakamura[2], Kunio Matsumoto[2], Seiji Yano[1].
([1]Division of Medical Oncology, [2]Division of Tumor Dynamics and Regulation, Cancer Research Institute, Kanazawa University)

Acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs), such as gefitinib and erlotinib, is a critical problem in the management of patients with *EGFR* mutant lung cancer. Several mechanisms have been reported involved in this acquired resistance, including hepatocyte growth factor (HGF)/Met pathway. PI3K and mTOR are downstream molecules of receptor tyrosine kinases, such as EGFR and Met, and are thought to be ideal targets for controlling various tumor types. We assessed whether BEZ235, a dual inhibitor of PI3K and mTOR, could overcome the EGFR-TKI resistance induced by HGF in an *EGFR* mutant lung cancer. Exogenous and endogenous HGF triggered resistance to erlotinib in *EGFR* mutant lung cancer PC-9 and HCC827 cell lines. BEZ235 alone inhibited the viability of these cell lines *in vitro*, irrespective of the presence of HGF. Using a xenograft model of SCID mice with HGF-gene transfected PC-9 cells (PC-9/HGF), BEZ235 inhibited tumor growth, whereas erlotinib did not. BEZ235 monotherapy also inhibited the phosphorylation of Akt and p70S6K/S6RP, downstream molecules of PI3K and mTOR, respectively, as well as suppressing tumor-cell proliferation and angiogenesis of PC-9/HGF tumors. These results suggest that BEZ235 may be useful in managing HGF-induced EGFR-TKI resistance in *EGFR* mutant lung cancer.

P-26 Activation of phosphoinositide 3-kinase is required for the infection of human astrovirus type1

Shoichiro Tange[1,2], Yan Zhou[1], Akira Nakanishi[1]
([1]Section of Gene Therapy, Department of Aging Intervention, National Center for Geriatrics and Gerontology. [2]Division of Functional Genomics, Cancer Research Institute, Kanazawa University.)

A series of cellular signaling cascades are activated to help facilitate viral entry and viral propagation in the cell. Very little is known about how the human astrovirus type 1 (HAstV1) exploits signaling cascades for establishing infection to host cells. Recent finding showed that activation of extracellular signal-regulated kinase (ERK) 1/2 is important for the HAstV1 infection, though involvement of other signaling cascades is unclear. We used a panel of kinase blockers to search for cellular signaling pathways important for the infection of HAstV1 by examining the effect on the viral life cycles. Inhibitors that block phosphoinositide 3-kinase (PI3K) activation interfered with the infection, independently of an effect on ERK 1/2 activation. The activation of the PI3K signaling cascade occurred at an early phase of the infection, judged from the time frame of the phosphorylation of Akt, and inhibition of PI3K at early times, but not at later times, blocked viral gene expression. However, inhibiting the downstream targets of PI3K activation, Akt and Rac1, did not block the infection. Our results reveal a previously unknown essential role of PI3K in the life cycle of HAstV1 at an early stage of infection, likely in the viral entry process.

P-27 Regulation of Shh-Gli signaling pathways by JNK signaling cascades during the differentiation of cerebellar granule cell precursors

Tokiharu Sato, Baljinnyam Tuvshintugs, Rong Li, Katsuji Yoshioka
(Div. Mol. Cell Signaling., Cancer Res. Inst., Kanazawa Univ)

Abnormal activation of the Sonic hedgehog (Shh) signaling pathway has been described in a wide variety of human cancers. Shh signaling through Gli transcription factors regulates cell proliferation. We previously found that bFGF-JNK signaling promotes cell-cycle exit and differentiation of cerebellar granule cell precursors (GCPs) in spite of the presence of Shh signaling, which is a potent mitogen of GCPs. Here we studied how bFGF-JNK signaling regulates Shh-Gli signaling pathway. We found that bFGF signaling decreased the Gli1 transcriptional activity and the expression level of Gli1 protein in primary cultured GCPs and HEK293T cells. This decrement of Gli1 transcriptional activity and expression level of Gli1 protein were rescued, in part, by addition of JNK inhibitor. We also found that MG132 proteasome inhibitor blocked the reduction of Gli1 protein level. Furthermore, we found that JNK binds to Gli1 through N-terminal region of Gli1. Taken together, these results suggest that bFGF-JNK signaling plays a key role in the degradation of Gli1 protein, and that this regulation would be a critical step of suppression Shh signaling pathway during the differentiation of GCPs.

P-28 The metabolic function of RB tumor suppressor gene

Susumu Kohno, Shunsuke Kitajima, Nobunari Sasaki, Hayato Muranaka, Chiaki Takahashi
(Division of Oncology and Molecular Biology, Cancer Research Institute, Kanazawa University)

The RB tumor suppressor gene has been implicated primarily in the control of cell cycle and terminal differentiation. However recent findings indicate that pRB may possess much more roles than previously thought beyond such well-appreciated ones.

We previously reported that pRB regulates mevalonate pathway through SREBPs thus affects isoprenylation of Ras proteins (Shamma et al., Cancer Cell 15:255, 2009). A recent report from another group demonstrated that the product of mutated p53 tumor suppressor gene also targets SREBP although in a different fashion. We therefore hypothesized that these two tumor suppressors are redundant in the control of lipid synthesis. Depending on the context, mouse cells deficient of both Rb and p53 genes showed metabolic reprogramming or some of stem cell-like features. In addition, mice deficient of both genes generated tumors with unique undifferentiated phenotypes. We further analyzed such cells or tumor cells by high-throughput drug screening. These experiences indicated that technically lack of p53 might facilitate us to unveil unique roles of pRB. In this presentation, we will shed light on previously unexpected function of pRB in controlling cell metabolism and its clinical significance.

P-29 The impact of RB status on the lipidogenic phenotype in cancer cells

Hayato Muranaka, Shunsuke Kitajima, Nobunari Sasaki, Susumu Kohno, Awad Shamma, Chiaki Takahashi
(Division of Oncology and Molecular Biology, Cancer Research Institute, Kanazawa University)

Tumor progression is prevalently associated with the rewiring of metabolic pathways; of these glycolytic and lipidogenic pathways have been mostly featured. The retinoblastoma tumor suppressor protein (pRB) is frequently inactivated during tumor progression. However, the exact impact of pRB inactivation on tumor progression is still unclear. Here, we propose a critical role for pRB in lipid metabolism that favors tumor progression. We previously reported that pRB regulates isoprenylation of Ras proteins through sterol regulatory element-binding proteins (SREBPs). Recent our efforts suggested that pRB also affects nuclear transport of SREBPs. Most probably in a SREBP-dependent manner, pRB regulates genes involved in lipid biosynthesis including fatty acid synthase (FASN) which has been shown to play pivotal roles in tumor progression. Furthermore, lipidomics analyses demonstrated that pRB depletion increased the abundance of several fatty acids in cancer cells. Since p53 has been suggested to be functionally redundant with pRB in SREBP regulation, we additionally deleted *RB* loci from *Trp53*-null tumor cells and we found that RB deletion dramatically enhanced their cancer stem cell ability. Inhibition of SREBPs significantly antagonized the effect of pRB inactivation to induce such alterations. These findings suggest that pRB-SREBPs-fatty acids pathway might be a plausible target of cancer therapy.

P-30 Therapeutic effect of GSK3β inhibition by drugs in clinical use against colon and pancreatic cancer

Mayumi Hirose, Takahiro Domoto, Yumi Ito, Kazuyuki Kawakami, Toshinari Minamoto
(Division of Translational and Clinical Oncology, Cancer Research Institute, Kanazawa University)

GSK3β is a serine/threonine protein kinase that regulates various cellular pathways and has been implicated in glucose intolerance, neurodegenerative disorders, inflammation and cancer. In the field of medicinal chemistry, GSK3β has recently emerged as one of the most attractive therapeutic targets for these chronic and progressive diseases. To date, there are no clinical trial reports describing the use of specific GSK3β inhibitors for cancer treatment, although the laboratory research identified GSK3β that is responsible for tumor progression and a promising candidate for cancer molecular targets. A number of drugs prescribed for diseases other than cancer were shown to inhibit GSK3β activity. Here we show that inhibition of GSK3b by using these drugs compromises survival, proliferation, migration and invasion of human colon cancer cells (HCT116, SW480, SW620, HT29, and RKO) and pancreatic cancer cells (PANC-1, BxPC-3, and MIA-PaCa-2). Combined treatment with the GSK3b-inhibiting drugs more efficiently inhibits cancer cell proliferation, migration and invasion than single drug. Our findings warrant further investigation to optimise combination of GSK3b-inhibiting drugs for cancer treatment and to understand the molecular basis of drug combination.