# Poster Session

## P-01 Screening and activity of small molecule inhibitors targeting HGF-Met protein-protein interaction

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Activation of HGF (hepatocyte growth factor)-Met receptor pathway closely participates in cancer invasion-metastasis and drug resistance. HGF-Met pathway has become a hot target in anticancer drug discovery and development, particularly to overcome cancer metastasis and drug resistance. To obtain small molecule inhibitors that inhibit HGF-Met protein-protein interaction (PPI), the method to analyze HGF-Met PPI with flow cytometer was newly established, and the flow cytometry-based high-throughput screen (HTS) was applied for public chemical library. Hit compounds obtained by the first HTS were subjected to the second and third screenings. We confirmed inhibitory action of selected hit compounds for HGF-Met PPI and HGF-dependent Met activation. Selective inhibition of PPI by small molecules is current approach in anti-cancer drug discovery. Our goal is to obtain drug candidate that inhibit HGF-Met PPI.

## P-02 A novel approach for identification of hematopoietic stem cell population by nucleostemin promoter activity

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Hematopoietic stem cells (HSCs) maintain homeostasis by both self-renewing and differentiating into mature cells. Although recent improvements in cell purification techniques have contributed to efficient enrichment of HSCs, studies with single cell analysis suggested that such purified HSC population is still heterogeneous. In this study, we attempted to identify a novel subpopulation as HSCs by nucleostemin (NS) promoter activity. NS, nucleolar GTP binding protein, maintains self-renewal of embryonic stem cells and promotes reprogramming of somatic cells. We previously generated a transgenic mouse in which green florescence protein (GFP) is expressed under the control of NS promoter (NSP-GFP). We found that the highest NSP-GFP intensity was detected in HSCs (CD150+CD34-c-Kit+Sca-1+Lin-), whereas its down-regulation was associated with hematopoietic differentiation. In addition, we successfully enriched HSCs in immature cells (c-Kit+Sca-1+Lin-) by NSP-GFP, without other HSC markers, indicating that NS promoter activity may be a unique indicator for functional HSC properties. Thus, the NSP-GFP may contribute to further dissection of HSC population and deep understanding of regulation of HSC fate.

## P-03 Inflammatory chemokine, CCL3-mediated maintenance of leukemia initiating cells in the initiation process of chronic myeloid leukemia

**Tomohisa Baba and Naofumi Mukaida**
(Cancer Research Institute, Kanazawa University.)

In the initiation process of chronic myeloid leukemia (CML), a small number of transformed leukemia initiating cells (LICs) co-exist with a large number of normal hematopoietic cells, gradually increasing thereafter and eventually predominate in the hematopoietic space. However, the interaction between LICs and normal hematopoietic cells at the early phase has not been clearly delineated due to the lack of a suitable experimental model. In this study, we succeeded in causing a marked leukocytosis resembling CML from restricted foci of LICs in the normal hematopoietic system by direct transplantation of *BCR-ABL* gene-transduced LICs into the BM cavity of non-irradiated mice. Herein, we observed that BCR-ABL$^+$lineage$^-$ckit$^-$ immature leukemia cells produced high levels of an inflammatory chemokine, MIP-1 $\alpha$/CCL3, which promoted the development of CML. Conversely, the ablation of *CCL3* gene in LICs dramatically inhibited the development of CML. Furthermore, normal hematopoietic stem/progenitor cells (HSPCs) can directly impede the maintenance of LICs in BM in the absence of CCL3 signal.

## P-04 Inhibition of GSK3$\beta$ rectifies aberrant glucose metabolism in colon cancer cells

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Recently we discovered the pathological roles for glycogen synthase kinase-3$\beta$ (GSK3$\beta$) in promoting cancer cell survival, proliferation and invasion via the modulation of cell cycle, tumor suppressor and cell immortality pathways and cell motility machinery and in rendering cancer cells insusceptible to chemotherapy and radiation. This has led us to propose GSK3$\beta$ as a potential target for cancer treatment. A primary role for GSK3$\beta$ is the control of glycogen synthase activity that switches glycogenesis and glycolysis, the two major pathways of glucose metabolism. In this study, we investigated whether GSK3$\beta$ influences the aberrant glucose metabolism that preferentially fuels energy in cancer cells. Using the capillary electrophoresis-mass spectrometry (CE-MS)-based metabolome analysis, we found the distinct metabolic trait in colon cancer cells and their xenografts that suggest that inhibition of GSK3$\beta$ attenuates glycolysis (Warburg effect) and restores mitochondrial function. This trait provides an important molecular basis for cancer treatment targeting aberrant GSK3b.

## P-05 A GO-based gene expression data analysis method of searching biomarker genes for pseudomyxoma peritonei

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In order to search genes which are specifically expressed in pseudomyxoma peritonei (PMP), namely, "biomarker genes" for PMP, we construct a new gene expression data analysis method for efficiently specifying a set of highly-selected genes which are promising candidates for biomarker, using Gene Ontology (GO) terms attached to each gene together with the information derived from several large web databases. We developed an efficient algorithm that can search GO terms in a practically short time. Experiments using real data of PMP and other types of cancers show that the proposed system is remarkably useful for discovering biomarker genes for PMP as well as for identifying the primary site of PMP.

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## P-06 Role of the scaffolding protein JLP in UVB-induced apoptosis

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Ultra-violet B (UVB) component of sunlight causes many adverse biological effects, including apoptosis, and eventually can lead to skin cancer. However, molecular mechanisms of which are not fully understood. Here, we examined a role of the scaffolding protein JLP (c-Jun $NH_2$-terminal kinase-associated leucine zipper protein) against UVB-induced apoptosis in mice skin. Upon UVB exposure, the number of apoptotic cells was significantly decreased in epidermis of *Jlp* KO mice compared with control mice. Furthermore, *Jlp* cKO mice specifically in epidermal stem cell (*Jlp*$^{flox/flox}$;*K5-Cre*) exhibited reduced number of apoptotic cells, which is consistent with the results obtained by using JLP-null mice. Moreover, we found a significant inhibition of UVB-induced p38 mitogen-activated protein kinase (MAPK) activation in skin of both *Jlp* KO and *Jlp* cKO mice. Taken together, these data indicate that the scaffolding protein JLP may play a key role in UVB-induced apoptosis by modulating p38 MAPK signaling pathways.

## P-07 Role of Gli1 transcription activator in the metastasis of melanoma

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Several lines of evidence suggest that the Hedgehog (Hh) pathway is important in metastasis of melanoma, although no genetic mutations were found in the Hh signaling component. The expression of Gli1 transcription activator might have a correlation with tumor progression and metastasis, but its mechanism remains elusive. We generated B16-F0 mouse melanoma cell lines overexpressing various levels of Gli1 transcription activator, and performed metastasis assay in chick embryo. The migration activity of these cell lines also seems to correlate with the expression level of Gli1. To gain insight into the molecular mechanisms, we searched for Gli1 target genes by gene expression profile analysis. We found that several pro-metastasis genes were highly up-regulated, although further analysis is required to confirm their correlation with Gli1 activity. Eventually, our finding might have implications that Gli1 transcription activator enhances metastasis of melanoma by up-regulating these pro-metastasis genes.

## P-08 Identification and Functional Study of Tumor Epithelial Cell-Specific MicroRNAs in Gastrointestinal Tumor

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*Helicobacter pylori* infection is one of the risk factors for gastric cancer. Deregulation of microRNAs (miRNAs) is important for several cancers. In this study, we aimed to identify tumor cell-specific miRNAs, and to find whether these miRNAs are related with inflammatory response and promotion of gastrointestinal tumor. To compare expression patterns of miRNAs between tumor cells and normal epithelial cells, only epithelial cells were isolated, and analyzed by microRNA microarray. Finally, we found that 18 miRNAs were commonly up-regulated, while 12 miRNAs were down-regulated in tumor epithelial cells. Among these miRNAs, miR-135b expression, which is potential oncogene induced by inflammatory response, was significantly increased in gastritis and gastric tumors. MiR-135b expression was also increased in human gastric cancer tissues and cell lines. Moreover, miR-135b expression was correlated with IL-6 and TNF- $\alpha$ expression level, suggesting inflammation-dependent upregulation of miR-135b. Notably, the miR-135b expression was also overexpressed in $Apc^{\Delta 716}$ mouse intestinal tumor epithelial cells. Knocking down of miR-135b expression suppressed proliferation of AGS, KATOIII and MKN74 gastric cancer cells, while overexpression of miR-135b led to increasing the cell growth. Taken together, these results show that miR-135b expression is upregulated in tumor epthielial cells in an inflammation-dependent manner and promotes gastrointestinal tumorigenesis.

## P-09 DNA methylation and heterochromatin formation at the *survivin* promoter responding to DNA damage is dependent on NBS1.

**Naoyuki Hayashi, Awad Shamma, Masahiko Kobayashi, Chiaki Takahashi, Ken-ichi Yamamoto**
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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. We identified DNMT1, which encodes DNA methyltransferase 1, as a binding factor of NBS1 via 2-hybrid screening. The binding between NBS1 and DNMT1 in vivo was observed in the condition of either HU or mitomycin C treatment, but not aphidicolin treatment. NBS1 binding region of DNMT1 was mapped at target recognition domain in its catalytic region, and DNMT1 binding region of NBS1 was in FHA domain. On the other hands, we examined DNA methylation in NBS patient cell line to examine epigenetic control function via DNMT1 of NBS1. The chromatin structure at the *survivin* promoter is regulated by p53 responding to DNA damage for repression of its expression to allow apoptosis. In NBS patient cells, neither DNA methylation nor heterochromatin formation was observed at this promoter, and consistently DNMT1 was not recruited there. These observations indicated that NBS1 function is required for DNA methylation and subsequent heterochromatin formation mediated by DNMT1.

## P-10 Therapeutic effect of the cocktail of GSK3$\beta$-inhibiting drugs against colon cancer

**Mayumi Hirose, Takahiro Domoto, Toshinari Minamoto**
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Glycogen synthase kinase 3$\beta$ (GSK3$\beta$) is a serine-threonine protein kinase initially identified in the insulin signaling pathway and later implicated in chronic, progressive diseases including diabetes mellitus, neurodegenerative brain disorders and cancer. Thus, GSK3$\beta$ has recently emerged as one of the most attractive therapeutic targets for these diseases. To date, there are no clinical trial reports describing the use of specific GSK3$\beta$ inhibitors for cancer treatment, although the laboratory research identified GSK3$\beta$ that is responsible for tumor progression and an attractive candidate for cancer molecular targets. A number of drugs prescribed for diseases other than cancer were shown to inhibit GSK3$\beta$ activity. Here we show that inhibition of GSK3$\beta$ activity by using these drugs compromises survival, proliferation, migration and invasion of human colon cancer cells. The cocktail of GSK3$\beta$-inhibiting drugs more efficiently inhibits GSK3$\beta$ activity in cancer cells and their proliferation, migration and invasion than single drug. Our findings warrant further investigation of the optimal combination of GSK3$\beta$-inhibiting drugs and chemotherapeutic agents for treatment of refractory colon cancer.

## P-11 Physiological role of Jmjd5, a novel p53 signal regulator, in embryonic development

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We have performed the retroviral insertional mutagenesis in mice, resulting in identification of *Jmjd5*, a member of the JmjC family. In this study, we have generated *Jmjd5*-deficient mice ($Jmjd5^{\Delta/\Delta}$ ) to investigate the physiological role of *Jmjd5*. The $Jmjd5^{\Delta/\Delta}$ mice showed severe growth retardation and abnormal vascular development, resulting in the embryonic lethality around E 11.0. Genetic studies using vascular-specific *Jmjd5*-deficient mice suggested that the embryonic lethality might be derived from the growth retardation. Thus, we prepared *Jmjd5* hypomorphic MEFs ($Jmjd5^{neo/neo}$), and growth defect in the mutant MEFs was observed as well as $Jmjd5^{\Delta/\Delta}$ embryos. We found that the expression of several p53-regulated genes including *p21* was elevated in $Jmjd5^{neo/neo}$ MEFs. The expression of *p53* RNA/protein was not altered, and immunoprecipitation studies showed no direct association between Jmjd5 and p53. Nevertheless, the growth defect of $Jmjd5^{neo/neo}$ MEFs was significantly recovered under $p53^{\Delta/\Delta}$ genetic background, suggesting that Jmjd5 may work as a novel p53 signal regulator involved in normal embryogenesis.

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## P-12 Wnt activation by RUNX3 in Kato-III gastric cancer cells

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It has been demonstrated that RUNX3 functions as a tumor suppressor in various cancers including gastric cancer. RUNX3 forms a ternary complex with β-catenin/TCFs and attenuates transactivation potential of β-catenin/TCFs in colon cancer cell lines. Based on these results, we further examined the role of RUNX3 in Wnt/β-catenin signaling activity in various types of gastric cancer cells by TOPFLASH analysis. When RUNX3 expression was suppressed by siRNA in AZ521 cells that have endogenous RUNX3 expression, Wnt activity level was significantly increased, which was consistent with the previous reports. We next transfected RUNX3 expression vector in Kato III gastric cancer cells that lack RUNX3 expression by gene methylation. Unexpectedly, RUNX3 transfection significantly increased Wnt signaling activity in Kato-III cells, which was suppressed by RUNX3 siRNA. Notably, in the RUNX3-transfected Kato-III cells, β-catenin level was not increased, which ruled out the possibility that RUNX3 enhances β-catenin stabilization. By ChIP analysis, we found that TCF4, β-catenin, and RUNX3 bind c-myc gene promoter. Accordingly, it is possible that RUNX3 activates Wnt signaling in Kato III cells by direct binding to promoter of Wnt target genes. Although Wnt is activated, RUNX3 suppressed tumorigenicity of Kato-III cells possibly through other mechanism(s) than Wnt inhibition.

## P-13 Critical role of Rheb in immediate response of myeloid progenitor cells to proliferative stimuli.

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Fine-tuning of mammalian target of rapamycin complex 1 (mTORC1) controls hematopoietic stem cell homeostasis. mTORC1 activity is strictly regulated by multiple upstream molecules, but little is known about the physiological role of mTORC1 activator in hematopoiesis. In this study, we investigated roles of Rheb (Ras homolog enriched in brain), a well-known activator of mTORC1, in adult hematopoiesis, by using $Rheb^{flox}$/Rosa-$CreER^{T2}$ mice, in which Rheb is deleted in all tissues by tamoxifen treatment. Rheb deficiency in vivo exhibited myeloid lineage expansion in peripheral blood, bone marrow and spleen. Rheb deficiency showed extramedullary hematopoiesis characterized by hematopoietic stem and progenitor cells (HSPCs) expansion in spleen. However, when Rheb-deficient HSPCs were transplanted, the hematopoietic reconstitution was significantly defective. Furthermore, Rheb deficiency resulted in impaired recovery and expansion of myeloid cells after 5-FU treatment. These data demonstrate that Rheb plays crucial roles in immediate response of myeloid progenitor cells to proliferative stimuli after tissue damage. We expect that these results provide new insight into the role of Rheb-dependent mTORC1 regulation in hematopoiesis.

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## P-14 Undifferentiated state induced by Rb inactivation associated with enhanced inflammatory signaling

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Inactivation of Rb protein is frequently found during tumor progression. To address the role of Rb in tumor progression, we established a mouse model in which Rb-deficiency in varied genetic backgrounds generates calcitonin-producing cell-derived tumors, and only in p53 null background, C-cell adenocarcinoma lost the expression of neuroendocrine lineage markers and exhibited high sphere-forming activity. These findings indicate that Rb inactivation in p53 null background may contribute to the acquisition of undifferentiated state. We succeeded in the enrichment of cell populations with high sphere-forming activity from Rb-depleted p53 null mouse-derived sarcoma cells. RNA sequence analysis revealed that these cells exhibited signatures of inflammatory conditions. Knockdown experiments demonstrated that sphere-forming activity induced by Rb depletion depend on activation of IL6 -STAT3 axis. Our model may contribute to elucidating the mechanisms that underlie the stem cell-like behaviors seen in cancer cells.

## P-15 Role of RB tumor suppressor gene in central carbon metabolism

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The retinoblastoma protein has been shown to regulate cell cycle and terminal differentiation in various contexts. It is thought that RB inactivation is required for tumor to overcome the RB mediated cell cycle progression in tumor initiation. However, RB inactivation is frequently observed in tumor progression. To investigate physiological role of RB inactivation in tumor progression, we established a model cells which is derived from soft-tissue sarcoma in p53 KO mouse. p53 null tumor cells derived from soft-tissue sarcoma formed tumor sphere by RB depletion under suspension culture condition. Although the cells derived from tumor sphere showed high sphere forming activity and tumor forming activity in nude mice, however the cells incorporated a small amount of BrdU. Metabolomic and transcriptomic analysis revealed that "glycolytic to glutaminolytic" switch (metabolic rewiring) governed by the RB status. Remarkably, glutamine addition to media did not stimulate oxygen consumption in Rb depleted cells derived from tumor spheres. These data suggested that some glutamine entering the citric acid cycle is metabolized to citrate via reductive carboxylation in Rb depleted cells derived tumor spheres. We will further argue that this specific metabolism would be required for malignant tumor progression and stem cell-like features appearing in cancer cells.

## P-16 Trichostatin A induces macrophage IL-1β production by activating NLRP3 inflammasome

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Macrophages produce IL-1β in response to not only infectious agents but also endogenous and environmental inflammatory substances. Recently, it was discovered that cytosolic pattern recognition receptors including NLRP3 form an inflammasome, a caspase-1-activating protein complex, in response to these stimuli, and thereby promote caspase-1-mediated maturation of IL-1β. In this study, we discovered that trichostatin A (TSA) induces IL-1β secretion from phorbol 12-myristate 13-acetate (PMA)-activated human monocytic THP-1 cells and peripheral blood monocytes. Gene knockdown experiments using siRNAs indicated that NLRP3 and its signal transducing partner, ASC, are essential for this response. TSA induced the expression of NLRP3 but not ASC or caspase-1. In addition, trichostain A promoted conversion of pro-caspase-1 into mature caspase-1 in PMA-activated THP-1 cells. PKC inhibitors, a potassium channel blocker, glibenclamide and an NADPH oxidase inhibitor, diphenyliodonium prevented TSA-induced IL-1β secretion. These results suggest that TSA promotes macrophage IL-1β production in cooperation with PKC by activating NLRP3 through the modulation of cytoplasmic potassium concentration and the ROS generation.

## P-17 Co-ordinate action of MT1-MMP with serine protease is essential for tumor invasive growth

**Zichen Li, Takahiro Domoto, Takahisa Takino, Hiroshi Sato**
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Hepatocyte growth factor activator inhibitor-1 (HAI-1) is a membrane-associated Kunitz-type serine protease inhibitor. HAI-1 is recognized as a cognate inhibitor of matriptase, a multi domain, transmembrane serine protease of the S1 trypsin like family. Matriptase was detected in a variety of human tumors of epithelial origin or phenotype and has been implicated in the initiation and progression of human carcinomas. However, matriptase is always inactivated by HAI-1, and the activation mechanism has been unknown. We found that membrane-type-1 matrix metalloproteinase (MT1-MMP) activate matriptase by cleaving HAI-1, which subsequently accelerated invasive growth of epithelial tumor cells in collagen gel (Cancer Sci., 2013). Recently, we designed HAI-1 mutant, which is resistant to cleavage by MT1-MMP, and introduced it to fibrosarcoma derived HT1080 cells. HT1080 cells express serine proteases other than matriptase. HT1080 cells expressing wild-type HAI-1, mutant HAI-1 lacking protease inhibitory function showed invasive growth as effective as control cells, however, cells expressing HAI-1 mutant, which was resistant to cleavage by MT1-MMP did not grew in collagen gel. These results suggest that co-ordinate action of serine protease with MT1-MMP is essential for tumor invasive growth.

## P-18 MTHFD2 is a key molecule in EGF receptor tyrosine kinase and regulates lung cancer cell growth

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Non-small cell lung cancer is a major subtype of lung cancer and is the most common and fatal cancer worldwide. We have previously identified 139 genes as EGF receptor tyrosine kinase (RTK) key molecules. In this study, we examined the role of MTHFD2 (methylenetetrahydrofolate dehydrogenase (NADP+dependent)2, methenyltetrahydrofolate cyclohydrolase), one of the EGF RTK key molecules, for lung tumorigenesis. MTHFD2 is localized in mitochondria, and is an enzyme in the folate metabolism. We found that MTHFD2 is expressed at low levels in normal lung epithelial cells, while it is abundantly expressed in many lung cancer cell lines. We knocked down the expression of MTHFD2, and found that the adhesion-dependent and independent lung cancer cell growth was inhibited with the MTHFD2 knock-down. These results suggest that MTHFD2 regulates lung cancer cell growth.

## P-19 Effects of FOXO inactivation on leukemia differentiation

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Myeloid leukemias are essentially hematopoiesis gone awry at the primitive levels, including hematopoietic stem cells (HSCs) / progenitor cells. Forkhead members of the class O transcription factor (FOXO), plays a critical role of maintenance of HSCs, leukemia initiating cells. Thus, FOXO are key regulators for the maintenance of leukemia stem cells, but these functions remain unclear. In this study, we attempted to establish a system for identification of molecules regulating differentiation blockade of leukemia stem cells by monitoring FOXO activity. We established high-throughput screening system and explored chemical compounds that inhibit FOXO transcriptional activity. We found that chemical compounds that inhibit FOXO transcriptional activity efficiently cause leukemia differentiation. Furthermore, analysis of effects of the pharmaceutical inactivation of FOXO on leukemia differentiation revealed unique FOXO function in maintaining leukemia stem cells.

## P-20 Post-tralslational regulation of polycomb family protein involved in invasive- and inflammatory- phenotypes

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Based on the growing evidences demonstrating epigenetic pathways play a significant role in oncogenesis and malignant progression, several epigenetic drugs are under clinical trials or available for cancer treatment. Nevertheless, most of the drugs are relatively non-specific, it is necessary to understand the mechanisms of epigenetic regulation relatively selective in terms of target genes, cellular context, and pathologies. Here we show that a post-translational regulation of a polycomb family protein regulates invasive- and inflammatory- phenotypes of human mesothelioma cells. Our results provide the evidence that targeting post-translational regulation of epigenetics possibly lead to new strategies of epigenetic therapy.

## P-21 The metabolic function of RB in controlling mevalonate (MVA) pathway and cancer cells

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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 function in CSCs.

## P-22 MIP-1α regulates progression of fibrosis occurring as a result of chronic colitis in mice

**Soichiro Sasaki, Tomohisa Baba and Naofumi Mukaida**
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Patients with inflammatory bowel diseases often develop colon carcinoma. Combined treatment of azoxymethane (AOM) and dextran sulfate sodium (DSS) recapitulates colitis-associated cancer in mice. AOM/DSS-induced tumor formation was reduced in *CCL3*- or its specific receptor, *CCR5*-deficient mice despite the presence of a massive infiltration of inflammatory cells. However, AOM/DSS-induced type I collagen-positive fibroblast accumulation in the colon was reduced in *CCL3*- or *CCR5*-deficient mice. This was associated with depressed expression of heparin-binding epidermal growth factor (HB-EGF), which is expressed mainly by fibroblasts. Moreover, CCL3 induced *in vitro* fibroblasts to proliferate and to express HB-EGF. Furthermore, CCR5 blockade reduced tumor formation together with reduced fibroblast accumulation and HB-EGF expression, even when administered after the development of multiple colon tumors. Taken together, CCL3-CCR5-mediated fibroblast accumulation may be required, in addition to leukocyte infiltration to induce full-blown colitis-associated carcinogenesis. Thus, blockade of the CCL3-CCR5 axis can be a potential therapeutic target against colon cancer, particularly one that is associated with fibrosis.

## P-23 Regulation of Gli1 transcriptional activity by FGF2-JNK signaling in the differentiation of cerebellar granule cell precursors

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Abnormal activation of the Sonic hedgehog (Shh) signaling pathway has been described in a wide variety of human cancers. Shh signaling through Gli1 transcription factors regulates cell proliferation. We previously found that FGF2-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 FGF2-JNK signaling regulates Shh-Gli1 signaling pathway. We found that FGF2 signaling decreased the Gli1 transcriptional activity in primary cultured GCPs and HEK293T cells. This decrement of Gli1 transcriptional activity was partially rescued by addition of JNK inhibitor. We also found that Gli1 subcellular localization was changed by reatment with FGF2. Furthermore, we found that JNK binds to Gli1 through N-terminal region of Gli1. Taken together, these results suggest that FGF2-JNK signaling plays a key role in the Gli1 transcriptional activity, and that this regulation would be a critical step of suppression Shh signaling pathway during the differentiation of GCPs.

## P-24 Chemokines in gemcitabine-induced cell senescence in Human Pancreatic Cancer Cell Lines

**Yao Song, Tomohisa Baba, and Naofumi Mukaida**
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Gemcitabine, a widely-used drug for the treatment of pancreatic cancer, cannot eradicate pancreatic cancer completely in most cases. Hence, we explored the mechanism responsible for gemcitabine-induced incomplete eradication to improve its efficacy. Gemcitabine efficiently induced senescence (60 to 80%) but not apoptosis (6 to 12%) in Miapaca-2 and Panc-1 human pancreatic cancer cells. Gemcitabine could not completely kill both cell lines in culture, even at its highest concentrations that we examined. Among the cytokines (IL-1 $\alpha$, $\beta$, IL-6) and chemokines (CXCL1, CXCL8/IL-8, CCL2, CCL3, CCL4, CX3CL1) that we determined, gemcitabine induced selectively the secretion of CXCL8/IL-8 and its related chemokines, GRO, by Miapaca-2 and Panc-1 cells. However, CXCR1 and CXCR2, specific receptors for IL-8/CXCL8, were not detected at both mRNA and protein levels in these cells. Moreover, the IL-8/IL-8 receptor axis blockade had few effects on gemcitabine-induced senescence of human pancreatic cancer cells. Hence, gemcitabine-induced IL-8 secretion may not act on tumor cells in an autocrine manner but may act on stroma cells present in tumor tissues.

## P-25 Spred-1 regulates the competitive advantage of hematopoietic stem cells in the bone marrow microenvironment

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Hematopoietic stem cells (HSCs) are maintained within bone marrow niches for a long-term. However, little is yet known about the quality control of HSC population. To understand this paradigm, we examined the role of Spred-1, a negative regulator of cytokine signals, in adult HSCs. In competitive reconstitution assays or serial transplantations, the chimerism of Spred-$1^{-/-}$ cells, including HSC population, gradually increased. Colony-forming ability of Spred-$1^{-/-}$ HSCs was enhanced by SCF stimulation on feeder cells, but not in feeder-free culture. Although Spred-1 deficiency enhanced occupancy by competitive assay *in vivo*, the absolute number of HSCs was not increased. These data suggest that Spred-$1^{-/-}$ HSCs don't expand in a cell-intrinsic manner and might have influence toward neighbor cells by cell competition. Further analyses of HSC regulation by Spred-1 may provide competitive fitness and clonal advantage of HSCs.

## P-26 mTORC1 activation accelerates malignant gliomagenesis and expands glioma-initiating cells

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Malignant gliomas (GBM) frequently harbor genetic lesions stimulating the activity of mammalian target of rapamycin complex 1 (mTORC1). Loss of heterozygosity of TSC1 or TSC2 gene, which forms a critical negative regulator protein complex for mTORC1, is also found in GBM; however, it has not been elucidated how mTORC1 activation affect the development of malignant gliomas. In this study, we investigated the roles of mTORC1 in mouse glioma model. Mouse malignant glioma model was generated by the overexpression of mutant EGFR gene, EGFRvIII, in combination with p16Ink4a/Arf deficiency. Using this glioma model, we established tamoxifen-inducible deletion of Tsc1 or Raptor, an essential component of mTORC1, to investigate effects of activation or inactivation of mTORC1. Tsc1 deletion accelerated malignant phenotypes, including increased tumor mass and intra-cranial hemorrhage with enhanced micro-vasculature. Tsc1 deficiency increased frequency of glioma-initiating cells (GICs) *in vivo* and induced cytokine-independent growth of GICs *in vitro*. In contrast, Raptor-deletion remarkably suppressed the progression of glioma. These data demonstrate that mTORC1 plays a critical role in controlling the malignant properties of glioma.

## P-27 MicroRNA targeted by *Rb1* in a *Trp53*-null background

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Multifaceted function of RB might be explained by its genetic interaction with microRNAs that simultaneously suppress varieties of target loci. We previously demonstrated that RB inactivation was allowed to induce highly undifferentiated phenotypes specifically in mice lacking *Trp53* loci. In this study, we determined microRNAs those expression levels were affected by *Rb1* depletion in soft tissue sarcoma cells derived from *Trp53-/-* mice. Agilent microRNA array system and following analysis by GeneSpring detected microRNAs regulated by *Rb1*. To select microRNAs that may mediate RB's effects on gene transcription, we employed GSEA method to compare gene lists to microRNA target gene sets provided by TargetScan. We are currently focusing on mmu-miR-140 and verifying its functional relationship to RB.

## P-28 Membrane-type 1 matrix metalloproteinase rescues from growth inhibition by three dimensional fibronectin matrix

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The extracellular microenvironment regulates cellular functions and growth. Here we demonstrate that Membrane-type 1 matrix metalloproteinase (MT1-MMP) rescues cells of confluent culture from growth suppression. When MT1-MMP was silenced in HT1080 fibrosarcoma cells, cells created three dimensional (3D) fibronectin matrix and were embedded within it. Formation of 3D fibronectin matrix was impeded by double knockdown with MT1-MMP and either FN or integrin $\beta$ 1, which resulted in restoration of cell growth. When pre-formed 3D fibronectin matrix was rapidly destructed by treatment with integrin $\beta$ 1 inhibitory antibody, cells underwent S phase entry. Ectopic expression of MT1-MMP in Rat1 fibroblasts exhibited destruction of 3D fibronectin matrix and growth induction. These results indicate that MT1-MMP induces cell growth by impeding 3D fibronectin matrix formation in confluent culture.

# P-29 Paracrine activation of MET promotes peritoneal carcinomatosis in scirrhous gastric cancer

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Scirrhous gastric cancer is associated with abundant stroma and frequently develops into peritoneal carcinomatosis with malignant ascites. Although malignant ascites is among the most deadly diseases worldwide, its molecular pathogenesis is poorly understood. We investigated the role of hepatocyte growth factor (HGF) in the production of peritoneal carcinomatosis with malignant ascites. We examined 3 scirrhous and 3 non-scirrhous human gastric cancer cell lines for the production of peritoneal carcinomatosis *in vivo* and responses to HGF *in vitro*. Furthermore, clinical scirrhous gastric cancer specimens were examined for HGF production. Among the 6 cell lines examined, only 2 scirrhous cell lines (NUGC4 and GCIY) produced peritoneal carcinomatosis with massive ascites after intraperitoneal injection in nude mice. Their proliferation was stimulated by exogenous HGF *in vitro*. On the other hand, a non-scirrhous cell line, MKN45, with *MET* amplification generated peritoneal tumors but not ascites. MET tyrosine kinase inhibitors, crizotinib and TAS-115, inhibited HGF-stimulated proliferation of NUGC4 and GCIY as well as constitutive proliferation of MKN45. Furthermore, crizotinib and TAS-115 prolonged the survival of mice bearing established tumors by NUGC4 or MKN45. In clinical specimens, HGF was markedly produced by stromal fibroblasts. Malignant ascitic fluids from patients with peritoneal carcinomatosis contained high levels of HGF. Our results strongly suggest that paracrine HGF-induced activation of MET-mediated signaling pathways plays an important role in the pathogenesis of peritoneal carcinomatosis in scirrhous gastric cancer. Thus, MET signaling pathway may be a potential therapeutic target for peritoneal carcinomatosis of gastric cancer, even without *MET* amplification.