Research Institute

Preface

The National Cancer Center Research Institute (NCCRI) was established in 1962 as a department of the National Cancer Center (NCC), and has been the nation's leading cancer research institute for nearly 50 years. It is now internationally recognized for major contributions to various aspects of cancer research worldwide. The mission of the NCCRI is to advance our knowledge of cancer prevention, diagnosis and therapy, toward the ultimate goal of cancer control. Collaborative research integration between other departments of the NCC, including NCC Hospitals, and the Research Institute is highly encouraged. The NCCRI is now composed of 25 divisions, and they are sub-grouped into four major Research Groups and one Project Group; namely, the Group for Cancer Development and Progression, Group for Research into Molecular Functions and Targets, Group for Development of Molecular Diagnostics and Individualized Therapy and Group for Translational Research and Project Group. Core Facilities for Research and Innovative Medicine, which consist of the Central Animal Division, Central Radioisotope Division and Core Facility Division, provide several kinds of technical support in molecular biology, high-throughput omics-type analyses, biological analysis and animal experiments for researchers in both the Research Institute and Hospital to further encourage and facilitate the development of translational-type studies in our Institute. The NCCRI currently has approximately 90 research staff, around 50 postdoctoral fellows, and more than 100 supporting staff. Foreign scientists and research fellows are also welcomed on a regular basis. The "Annual Report 2011" of the NCCRI summarizes the recent research activities of each division, which cover the following areas: (i) environmental human carcinogens and cancer chemoprevention, including the use of animal models; (ii) clarification of molecular mechanisms underlying cancer development, invasion and metastasis; (iii) investigation of genetic and epigenetic alterations in a variety of cancers; (iv) clarification of the molecular bases underlying the susceptibility to cancer development; (v) exploration of novel biomarkers with diagnostic, therapeutic and prognostic value; and (vi) functional analyses of various cancer-related genes. We have also been participating in worldwide research interactions, such as the International Cancer Genome Consortium (ICGC), International Cancer Biomarker Consortium (ICBC), and International Human Epigenome Consortium (IHEC). We further encourage our members to develop international collaborations in various other areas. The activities of the research institute can also be viewed on the home page: http://www.ncc.go.jp/en/nccri/index.html.

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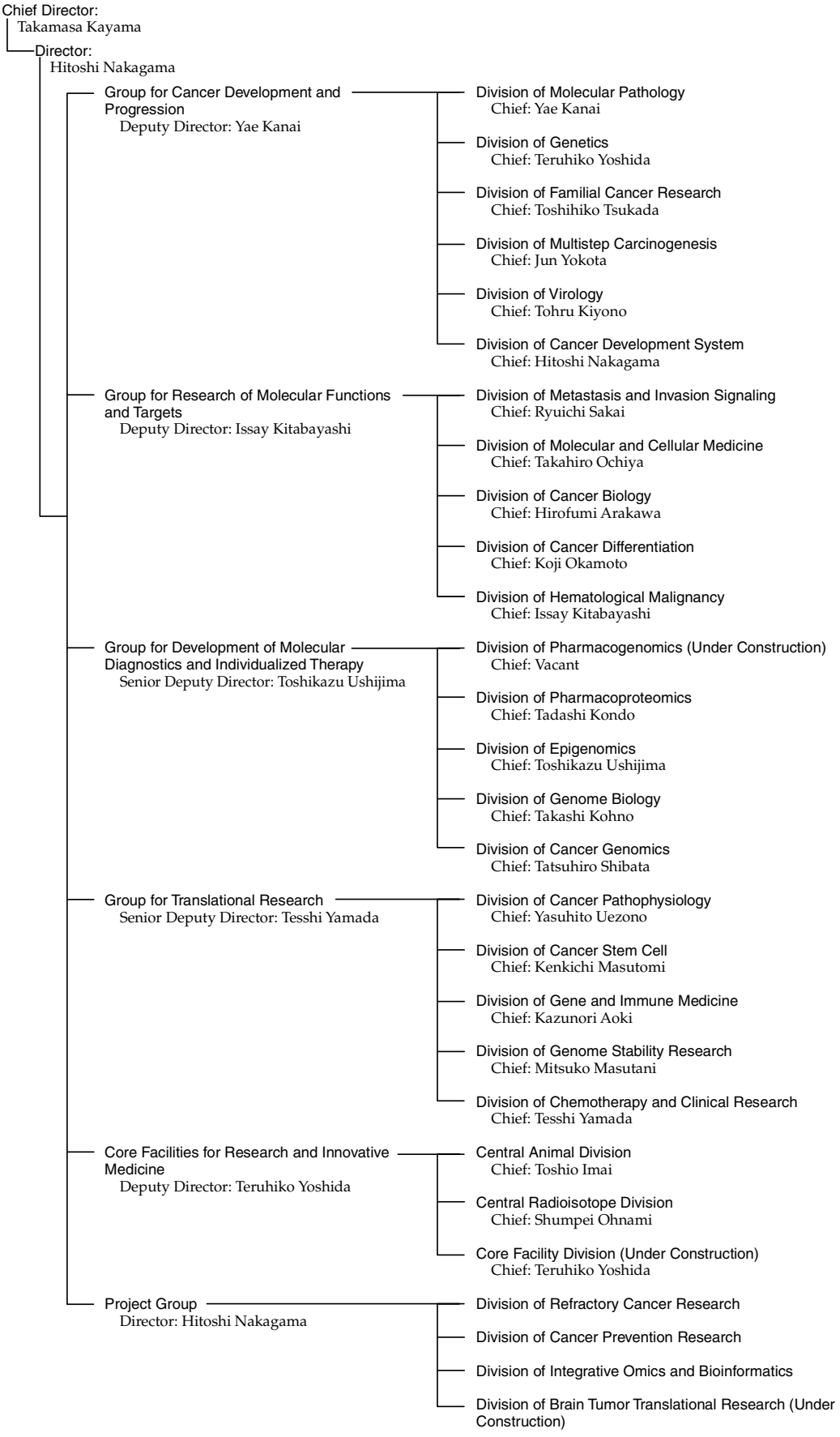
Organization



Activities of the Divisions

DIVISION OF MOLECULAR PATHOLOGY

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Research in the Division of Molecular Pathology is based on a combination of clinicopathological observations and molecular pathological analyses.

Epigenetic and Genetic Alterations During Multistage Carcinogenesis

To understand the significance of DNA methylation alterations underlying the clinicopathological diversity of human cancers, a genome-wide DNA methylation analysis has been performed. Since renal carcinogenesis is associated with neither chronic inflammation nor persistent viral infection, and hardly any histological change is evident in corresponding non-tumorous renal tissue from patients with renal tumors, precancerous conditions in the kidney have been rarely described. However, the bacterial artificial chromosome (BAC) array-based methylated CpG island amplification method, which may be suitable for overviewing the DNA methylation tendency of individual large regions among all chromosomes, has revealed that non-tumorous renal tissue from patients with renal tumors are already at the precancerous stage. DNA methylation profiles determining the histological subtypes of renal tumors developing in individual patients may be already established in non-tumorous renal tissue at the precancerous stage (1). DNA methylation profiles at the precancerous stage may be basically inherited by the cancers developing in individual patients (2). DNA methylation alterations at the precancerous stage may confer vulnerability to further epigenetic and genetic alterations and determine both the malignant potential of the corresponding cancer and the outcome of patients (2).

Even non-cancerous urothelia showing no remarkable histological features obtained from patients with urothelial carcinomas can be considered to be at the precancerous stage, because they may be exposed to carcinogens in the urine. Actually, genome-wide DNA methylation alterations accumulated in both non-cancerous urothelia obtained from patients with urothelial carcinomas and urothelial carcinomas themselves. An array comparative genomic hybridization analysis revealed that losses of 5q14.1-q23.1, 6q14.1-q27, 8p22-p21.3, 11q13.5-q14.1 and 15q11.2-q22.2 and gains of 7p11.2-q11.22 and 19q13.12-q13.2 were correlated with the development of aggressive non-papillary urothelial carcinomas (3). Losses of 1p32.2-p31.3, 10q11.23-q21.1 and 15q21.3 were correlated with tumor recurrence (3). An unsupervised hierarchical clustering analysis based on copy number alterations clustered urothelial carcinomas into three subclasses: copy number alterations associated with genome-wide DNA hypomethylation, regional DNA hypermethylation on C-type CpG islands associated with frequent chromosomal losses, and genome-wide DNA hypo- and hypermethylation, were accumulated in clusters A, B_1 and B_2, respectively (3). Both epigenetic and genetic events appear to accumulate during urothelial carcinogenesis, reflecting the clinicopathological diversity of urothelial carcinomas.

DNA methylation alterations are stably preserved on DNA double strands by covalent bonds, and they can be detected using highly sensitive methodology. Therefore, genome-wide DNA methylation profiling may be advantageous to establish optimal diagnostic indicators for cancer practice. For example, the diagnostic criteria of ductal adenocarcinomas of the pancreas based on DNA methylation profiling may be advantageous for supporting the histological and cytological assessment of pancreatic biopsy and juice specimens, respectively (4). In order to decide the indications of adjuvant chemotherapy for patients with pancreatic cancers, which should be carried out carefully, paying close attention to adverse reactions, the criteria for prognosis using the 11 BAC clones were established. The DNA methylation status on the 11 BAC clones significantly correlated with both cancer-free and overall survival rates of patients with pancreatic cancers. A multivariate analysis revealed that our criteria were an independent predictor of poor outcome (4).

For appropriate surveillance of patients at the precancerous stage for hepatocellular carcinomas (HCCs), the 45 CpG sites, whose DNA methylation levels differed significantly between normal liver

tissues and non-cancerous liver tissues obtained from HCC patients in the learning cohort, were identified using a highly quantitative pyrosequencing method (5). The criteria combining the DNA methylation status for the 45 CpG sites were able to diagnose non-cancerous liver tissues obtained from HCC patients as being at high risk of carcinogenesis with 100% sensitivity and specificity in both the learning and validation cohorts (5). Pyrosequencing can be performed using a very small amount of degraded DNA extracted from formalin-fixed and paraffin-embedded liver biopsy specimens. These criteria may be applicable for liver biopsy specimens obtained prior to interferon therapy from patients who are followed up because of chronic liver diseases.

Recently, a high-throughput platform using the BeadChip microarray, which is able to interrogate the human DNA methylome at single CpG resolution, has been employed. Such ongoing projects may provide more accurate classifications of human cancers. Such subclassification may yield clues for clarification of distinct mechanisms of carcinogenesis in various organs, and identify possible target molecules for therapy in patients belonging to specific clusters.

Antitumor Immune Responses

The anti-tumor immune reaction changed drastically during carcinogenesis (6, 7). Gene expression analyses using microarray and RT-PCR revealed that CXCL17 and ICAM2 were involved in the immune surveillance during the development of pancreatic cancers (8). CXCL17 and ICAM2 induced infiltration and accumulation of immature myeloid dendritic cells in the tumor epithelial layer. This was followed by an active cellular immune reaction. ICAM2 simultaneously promoted the susceptibility of the tumor cells to cytotoxic T cell-mediated cytolysis. Immune surveillance occurs during the early intraepithelial stages of human pancreatic carcinogenesis, which is mediated by the expression of CXCL17 and ICAM2.

Arginine is essential for T cell activity and survival. Arginase II, an arginine-catabolizing enzyme, in pancreatic ductal carcinoma tissue was clinicopathologically studied. Arginase II was characteristically expressed in cancer-associated fibroblasts under hypoxia. The presence of arginase II-expressing cancer-associated fibroblasts is an independent factor for a worse prognosis, and it is a new hypoxic indicator in pancreatic ductal carcinoma tissue.

Role of β-catenin in Hepatocarcinogenesis

CTNNB1, encoding β-catenin, is one of the most frequently mutated oncogenes in HCCs. However, it has been unclear if active β-catenin signaling confers growth advantage to hepatocytes under physiological conditions. Our analysis of hepatocyte-specific β-catenin-deficient mice showed that hepatocytes with wild-type β-catenin exhibited survival advantage over β-catenin-deficient hepatocytes *in vivo* (9). Furthermore, paradoxically, β-catenin-mutated hepatomas were frequently developed in β-catenin-deficient livers. These suggest that a non-cell autonomous mechanism is involved in the hepatocarcinogenesis mediated by active β-catenin signaling. Our analyses also showed overexpression of AMACR and SLCO1B3, both of which are involved in bile acid metabolism, in *CTNNB1*-mutated human HCCs (10, 11).

Clinicopathological Studies

Breast carcinomas sometimes metastasize to the stomach, and the histopathologic distinction of such metastases from primary gastric adenocarcinomas is often difficult: most metastatic breast and primary gastric carcinomas have contained signet ring cell components. Immunohistochemical examination using a panel of antibodies identified hepatocyte nuclear factor 4A as an excellent marker for differentiating between the 2 lesions (12). Other clinicopathological studies were also conducted to further the understanding of the pathogenesis and promote the diagnosis and treatment of various tumors (13-15).

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DIVISION OF GENETICS

Teruhiko Yoshida, Hiromi Sakamoto, Fumiaki Koizumi, Hiroki Sasaki, Hitoshi Ichikawa, Norihisa Saeki, Kazuhiko Aoyagi, Takao Nishimura, Hiroo Takahashi, Aya Kuchiba, Low Siew Kee, Yusuke Nakadate, Akio Ashida, Sumiko Ohnami, Mineko Ushiama, Yoko Odaka, Misuzu Okuyama, Akiko Takahashi, Masumi Shimizu, He Haiping, Mika Shioya, Sayaka Mito, Mayumi Akitaya, Yuka Kitamura, Yuri Uehara, Rumi Koyama, Rie Komatsuzaki, Fumiko Chiwaki, Sachiyo Mitani, Hiroe Ono, Asami Kikawada

Introduction

In 2011, the three major research areas of the Division of Genetics were 1) molecular understanding of cancer susceptibility for the application to cancer diagnosis and prevention, 2) basic research for the development of molecular targeting and personalized cancer chemotherapy, and 3) transcriptome analyses of solid tumors and leukemia. In addition, the Division has also continued its participation in the biobanking project of the Tsukiji campus of the NCC, particularly in the DNA, RNA and plasma banking of the peripheral blood samples. The Division has been also involved in the construction of the biobank network among the 6 National Centers.

Genetic Susceptibility to Cancers

The Division continued an investigation of the genetic susceptibility of diffuse type gastric cancer by focusing on the second candidate locus at 1q22, which was identified by the previous genome-wide association study (GWAS) by the Division. An LD (linkage disequilibrium) block in the region contained the *MUC1* (mucin 1) gene which was identified as the most likely susceptibility factor based on the annotation and the results of previous candidate gene analyses. Functional studies demonstrated that the rs4072037 SNP (P=1.43x10^{-11}; odds ratio=1.66 by meta-analysis) in *MUC1* affects promoter activity and determines the major splicing variants of *MUC1* in the gastric epithelium. The result suggests that the polymorphism modulates the protective function of the protein against a variety of external insults to the gastric mucosa and thus influences the individual susceptibility to gastric carcinogenesis (1). The researchers of the Division also participated in the international collaboration to identify the *LMO1* (LIM domain only 1) gene as a susceptibility gene of neuroblastoma (2, 3).

Clinical genetic testing on hereditary cancer syndromes has been continued as a long-standing collaboration with the outpatient clinic in the National Cancer Center Hospital to support its genetic diagnosis. Collaboration with other institutions evaluated a role of family history and other general cancer risk factors in the PET cancer screening, but there was no significant association between the cancer risks and detection rate (4).

Basic Research for Molecular Targeting and Personalized Cancer Chemotherapy

Lymphangiogenesis has been considered an important element in the development of metastasis. Sunitinib, a multi-kinase inhibitor and clinically available as an angiogenesis inhibitor, is a promising candidate to suppress the lymphangiogenesis. *In vitro*, sunitinib blocked both VEGFR-2 and VEGFR-3 phosphorylation and downstream signaling induced by VEGF-C or VEGF-D in human lymphatic endothelial cells (LECs). Sunitinib also prevented VEGF-C-induced proliferation, migration and tube formation of the LECs. An *In vivo* breast cancer xenograft model showed that sunitinib significantly reduced the number of blood and lymphatic vessels and suppressed axillary lymph node metastasis, suggesting the usefulness of sunitinib in the treatment of breast cancer (5).

The Division has continued multiple collaborations in the field of pharmacogenetic and pharmacodynamics biomarker development research (pharmacogenomics in a broad sense) with the researchers at the National Cancer Center Hospital and other institutions on cytotoxic or molecular target agents. A GWAS was conducted on 105 chemotherapy-naïve stage IIIB or IV non-small cell lung cancer patients treated with carboplatin and paclitaxel, and 3 SNPs were identified as new prognostic biomarker candidates (6).

Collaborative research found a strong correlation between a high-serum heparan sulfate concentration and a poor treatment outcome of

EGFR-tyrosine kinase inhibitors (TKIs) suggesting the possibility to develop a promising noninvasive and repeatable glycobiological biomarker (7). Acquired resistance to an anti-angiogenic VEGFR2-TKI was analyzed on HUVEC clones. Expression analysis including microarray experiments revealed that an escape from VEGFR2 signaling-dependency is one of the cellular mechanisms of resistance to VEGFR2-TKI in vascular endothelial cells (8).

Gene Expression Profiling Analyses

Surgical specimens have long been used as important subjects for cancer research; however, it is still unclear how concordant the expression profiles of the surgical specimens are with those obtained from biopsy samples. Gene expression profiles were compared between 77 biopsy and 89 surgical samples. Artificially induced epithelial-mesenchymal transition (aiEMT) was found in the surgical specimens, possibly leading to various fundamental misinterpretations in previous cancer research, which relied on surgical archives to establish predictive/ prognostic markers based on biopsy samples (9). As a part of a collaboration on gene expression profiling of esophageal cancers, Reg4 (regenerating islet-derived family, member 4) protein expression was found to be highly specific for adenocarcinoma, but not squamous cell carcinoma, of the esophagus. The diagnostic utility of serum Reg4 concentration will be examined (10).

The researchers in the Division have been involved in gene expression profiling analyses of leukemic and normal hematopoietic cells to understand the molecular pathways leading to leukemia and to develop their clinical applications. This year, the roles of Fbxl10 and Bmi1 in hematopoietic stem cell self-renewal and leukemogenesis were investigated as collaborative works with the researchers in Chiba University (11, 12). Another collaboration also addressed identification and characterization of cancer stem cells in the spheres derived from a canine mammary gland adenocarcinoma model (13).

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DIVISION OF FAMILIAL CANCER RESEARCH

Toshihiko Tsukada, Yuko Nagamura, Satoko Shimazu

Introduction

The Division of Familial Cancer Research is focusing research activities on the development of new methods for diagnosis and treatment of familial cancer syndromes. The genotype-phenotype correlation in multiple endocrine neoplasia type 1 (MEN1) has been investigated. Drug resistance of pituitary tumors and pharmacological actions of rikkunshito, a traditional Japanese herbal medicine, are also being currently investigated.

MEN1

MEN1 is a familial cancer syndrome caused by heterozygous germline mutation of the *MEN1* gene, which encodes a tumor suppressor protein named menin, and is characterized by the multiple occurrences of endocrine tumors in the pituitary, parathyroid, pancreas, gastrointestinal tracts, adrenal cortex and thymus. Because the optimal therapies for MEN1-associated tumors, especially for multicentric parathyroid and pancreatic tumors, are different from those for sporadic endocrine tumors, accurate differential diagnoses between these hereditary and non-hereditary diseases is mandatory before planning treatment. A DNA test for *MEN1* germline mutations is a powerful tool for the differential diagnosis of patients with endocrinopathy suggestive of MEN1. However, it is often difficult to distinguish a disease-causing mutation from a rare benign polymorphism especially when a novel missense mutation is identified in a patient with incomplete forms of MEN1. We previously demonstrated that menin missense mutants associated with typical MEN1 are degraded rapidly by the ubiquitin-proteasome pathway when expressed in culture cells. To examine whether the intracellular stability of mutant menin is correlated with clinical phenotypes, we developed a method of evaluating menin stability, and examined missense mutants associated with typical MEN1 and those associated with incomplete phenotypes such as familial isolated hyperparathyroidism (FIHP) and apparently sporadic parathyroid tumors (ASPTs) (1). All tested mutants associated with typical MEN1 showed reduced stability. Some missense and in-frame deletion mutants associated with FIHP or ASPT were almost as stable as, or only slightly less stable than, wild type menin, while others were as unstable as those associated with typical MEN1. Some stable mutants exhibited substantial biological activities when tested with a JunD-dependent transactivation assay. These findings suggest that certain missense and in-frame mutations are fairly stable and retain intrinsic biological activity, and may be specifically associated with incomplete clinical phenotypes. This menin stability test will provide useful information for the management of patients carrying germline *MEN1* mutations especially when they have missense or in-frame variants of ambiguous clinical significance.

Resistance to Dopamine Agonists in Prolactinoma

The first line treatment of prolactinoma is dopamine (DA) agonists, bromocriptine or cabergoline, which normalize prolactin levels and reduce tumor size of DA-sensitive prolactinoma. However, some tumors (10-15% of cases) are resistant to DA agonists from the beginning of the treatment (primary resistance) and are treated surgically. A few prolactinomas initially respond to DA agonists but become resistant after prolonged treatment with DA (secondary resistance). Although the reduction of the dopamine D2 receptor (DRD2) expression in tumor cells may explain the resistance, the exact mechanism is not fully understood. We examined 13 cases of surgically resected prolactinomas, which were divided into three groups according to the responsiveness to DA agonists: the sensitive, the primary resistant and the secondary resistant tumors. DRD2 expression was investigated by measuring mRNAs of the short isoform (D2S) and the long isoform (D2L) of DRD2. DNA methylation patterns in the promoter region of the *DRD2* gene were also analyzed. The D2L expression was much lower in the secondary resistant tumors than in sensitive or primary resistant tumors. Primary resistant tumors showed lower D2L expression than sensitive tumors. The D2S / D2L receptor mRNA ratio was not correlated with tumor response to DA

agonists. The DNA methylation patterns in the *DRD2* gene promoter region were not different between sensitive and resistant tumors. These findings suggest that the silencing of the *DRD2* gene expression, though not related to promoter methylation, is a possible mechanism for DA resistance in prolactinoma.

Effects of Rikkunshito on Adrenal Chromaffin Cells

Rikkunshito is widely used to treat appetite loss associated with various disorders, and may be a useful regimen for cancer cachexia. Because β-adrenergic agonists have been shown to prevent muscle wasting in experimental cancer cachexia, we investigated the effects of rikkunshito on cAMP-dependent gene expression in PC12 cells, a catecholamine-producing adrenal chromaffin cell line. We previously established modified PC12 cells (PCVG cells) by stable transfection of the bacterial *lacZ* gene fused to the cAMP-dependent vasoactive intestinal peptide gene promoter. β-galactosidase activity is increased in PCVG cells in response to stimuli that increase intracellular cAMP. In PCVG cells, rikkunshito alone increased β-galactosidase expression and also enhanced forskolin-induced expression by two-fold at the forskolin concentration of 0.1 μM. A concomitant increase of cAMP was demonstrated in PCVG cells stimulated with rikkunshito. These findings suggest that rikkunshito increases cAMP levels in adrenal chromaffin cells and may enhance the biosynthesis of epinephrine, which is known to be stimulated by cAMP.

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DIVISION OF MULTISTEP CARCINOGENESIS

Jun Yokota, Naoto Tsuchiya, Reika Iwakawa-Kawabata, Hiroko Ogata-Kawata, Mariko Sasaki, Yuko Fujiwara, Takahiro Oike, Masataka Takenaka, Ryo Nagahiro, Kouhei Katoh, Hisanori Isomura, Daisuke Kurioka, Yusuke Kimura, Momoyo Nishida, Tomoyo Kobayashi

Lung cancer is the leading cause of cancer death worldwide. To develop novel ways of lung cancer prevention, diagnosis and treatment, it is important to elucidate the molecular processes of multistep lung carcinogenesis and genetic/environmental factors involved in the development of lung cancer. For this reason, genetic alterations in lung cancer cells and genetic polymorphisms in lung cancer patients have been studied over the long term in the Division of Multistep Carcinogenesis. In 2011, the following results were obtained.

Even in small-sized (≤2 cm in greatest dimension) and/or stage I lung adenocarcinoma (ADC), a considerable proportion of the patients will show a poor prognosis. Therefore, we conducted a study to identify genetic alterations that define the prognosis of patients with early-stage lung ADC (1). Regions of copy number alterations in 65 small-sized lung ADCs and 40 ADC cell lines were determined with GeneChip Human Mapping 10-K and 250-K single-nucleotide polymorphism (SNP) arrays, respectively. Several regions on chromosomes 5p, 7p, 8q, and 14q were frequently (>10%) amplified in both small-sized ADCs and lung ADC cell lines. In particular, the MYC gene was mapped in the minimum common region at chromosome 8q24.21, and therefore was indicated to be a target of gene amplification in lung ADCs. MYC amplification correlated with a poor prognosis (P=0.031) in patients with small-sized ADCs. MYC amplification detected by SNP array analysis was well reproduced by real-time genomic PCR analysis. Therefore, to investigate the utility of MYC amplification as a prognostic marker for early-stage lung ADCs, 162 stage I lung ADCs were subjected to the analysis. MYC amplification was associated with relapse-free survival in these patients (P=0.013). Thus, it was strongly indicated that MYC amplification is a prognostic marker for patients with early-stage lung ADCs.

Lung cancer cells often show invasive phenotypes; however, causative genetic alterations for the acquisition of invasive phenotypes remain unclear. PTEN is inactivated in a subset of lung cancer; therefore, we investigated the possible involvement of PTEN inactivation in the invasiveness of lung cancer cells (2). AKT at Ser473 was phosphorylated in several lung cancer cell lines with loss of PTEN expression. Therefore, we created a tetracycline inducible expression system of wild-type PTEN (PTEN-WT) as well as catalytically (PTEN-G129R) and lipid phosphatase (PTEN-G129E) inactive PTEN mutants using the PC14, PC9 and PC3 lung ADC cell lines, in which endogenous PTEN expression was not detected and AKT at Ser473 was phosphorylated by Western blot analysis. Induction of PTEN-WT reduced phosphorylation of AKT and inhibited the transcriptional activity of NFkB, whereas PTEN mutants did not, suggesting that PTEN inactivation results in the activation of the AKT/NFkB pathway in ADC cells. Furthermore, overexpression of PTEN-WT suppressed anchorage independent growth and reduced invasiveness in PC14 cells. Neither PTEN-G129R nor PTEN-G129E had suppressive effects on anchorage independent growth and invasiveness. Therefore, it was indicated that activation of the PI3K/AKT/NFkB pathway by PTEN inactivation results in augmented invasiveness in lung ADC cells and lipid phosphatase activity of PTEN plays a key role in this process.

There is increasing evidence that altered microRNA expression is associated with tumor progression and survival in cancer patients. We tested if the expression of specific microRNAs was associated with prognosis and disease progression in early-stage lung ADC (3). Expression of miR-21, miR-17, and miR-155 was measured by quantitative RT-PCR in tissues from 317 non-small cell lung cancer (NSCLC) patients that originated from the U.S., Norway, and Japan. Elevated miR-21 (HR=2.06, 95%CI=1.13-3.75), miR-17 (HR=2.00, 95%CI=1.10-3.61), and miR-155 (HR=2.37, 95%CI=1.27-4.42) was associated with worse cancer-specific mortality in the U.S. cohort. Among three microRNAs, only miR-21 was associated with worse cancer-specific mortality in the Norwegian cohort (HR=2.78, 95%CI=1.22-6.31) and worse relapse-free survival in the Japanese cohort (HR=2.82, 95%CI=1.57-5.07). More advanced stage tumors expressed significantly higher levels of miR-21 compared with stage I tumors. In stage I patients, high levels of miR-21 were associated with worse cancer-specific mortality (HR=2.16,

95%CI=1.11-4.21) and relapse-free survival (HR=3.40, 95%CI=1.57-7.36) independent of other clinical factors. This suggests that the expression of miR-21 may contribute to lung carcinogenesis and could serve as a therapeutic target or early-stage prognostic biomarker for lung ADC.

Recent genome-wide association studies (GWASs) have identified polymorphisms in several genes associated with lung cancer risk. Nevertheless, functional polymorphisms in DNA repair and metabolic genes that had been reported as being associated with risk for lung cancer, particularly for lung squamous cell carcinoma (SQC), were not examined in those studies. Therefore, the significance of these functional polymorphisms was evaluated in a population in which polymorphisms in the GWAS genes showed associations with lung SQC risk. Polymorphisms in three DNA repair genes, TP53, MDM2, and OGG1, and two metabolic genes, CYP1A1 and GSTM1, were examined for associations with lung SQC risk in a hospital-based case-control study consisting of 377 cases and 325 controls, which had been previously subjected to association studies on GWAS genes, CHRNA3, TERT, and HLA-DQA1. Genotypes for two DNA repair genes, TP53 and OGG1, showed significant associations with SQC risk ($P<0.05$), and those for two GWAS genes, CHRNA3 and HLA-DQA1, showed significant associations with SQC risk ($P<0.05$) with odds ratios between 1.65 (95%CI=1.06-2.57 for OGG1) and 2.57 (95%CI=1.03-6.87 for CHRNA3). Marginally significant associations were also observed for the MDM2 and CYP1A1 genes. This result indicates the necessity of reevaluation for the significance of functional polymorphisms in DNA repair and metabolic genes for lung cancer risk in other populations subjected to GWASs.

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DIVISION OF VIROLOGY

Tohru Kiyono, Takashi Yugawa, Nagayasu Egawa, Shin-ichi Ohno

Introduction

Approximately 15% of human cancers have a viral etiology, and seven viruses have been elucidated as being associated with human cancers (Table 1). Among these recognized viruses, research in the Division of Virology is mainly focused on the molecular mechanisms of oncogenesis by the human papillomavirus (HPV). A subset of HPVs including types 16 and 18 are closely associated with human cancers and have thus been called high-risk HPVs (HR-HPVs). E6 and E7 proteins of HR-HPVs are known to inactivate the major tumour suppressors, p53 and retinoblastoma protein (pRB), respectively. We have developed an *in vitro* multistep carcinogenesis model for both HPV-positive and –negative human oral squamous carcinomas (1), which could be a good model for analyzing the multistep carcinogenesis of other cancers (Figure 1).

HPV and Oral Cancer

Oral squamous cell carcinomas (OSCCs) are considered to arise from human oral keratinocytes. DNAs of human papillomaviruses (HPVs), predominantly types 16 and 18, etiological agents of cervical cancer, have been detected in approximately 25% of OSCCs. In addition to inactivation of the p53 and pRB pathways, other alterations such as overexpression of epidermal growth factor receptor (EGFR) are often observed in both HPV-positive and -negative OSCCs. However, causal-relationships between accumulation of these abnormalities and multi-step carcinogenesis are not fully understood. To elucidate underlying processes, we transduced either HPV16 E6/E7 or mutant CDK4 ($CDK4^{R24C}$), cyclin D1 and human telomerase reverse transcriptase (TERT) into primary human tongue keratinocytes (HTK), and obtained immortal cell populations, HTK-16E6E7 and HTK-K4DT. Additional transduction of oncogenic HRAS or EGFR together with MYC into the HTK-16E6E7 and dominant-negative p53 expressing HTK-K4DT resulted in anchorage-independent growth and subcutaneous tumor formation in nude mice. This *in vitro* model system recapitulating the development of OSCCs should facilitate further studies of mechanisms of carcinogenesis in the oral cavity (Figure 1) (1). Since E6 and E7 are ideal molecular targets for HPV-positive cancers, synthetic small interfering RNA(siRNAs) against E6 and E7 are potent drugs for these cancers. To minimize undesirable effects, including silencing of unintended genes (off-target effect) and nonspecific cytotoxicity, we have developed a new double-strand RNA-DNA chimera (dsRDC) targeting human papillomavirus 16 (HPV16) E6 and E7 oncogenes. The dsRDC modification reduced nonspecific cytotoxicity in two of three siRNAs, while their silencing activity was marginally impaired. Finally, one of the dsRDC induced E6E7-specific growth suppression of cervical cancer cells as well as E6E7-immortalized human keratinocytes (2). By using an *in vitro* multistep carcinogenesis model for cervical cancer, we found that the expression of disintegrin-metalloproteases and their endogenous regulators was dysregulated during cervical carcinogenesis. The aberrant expression of A Disintegrin And Metalloproteases (ADAMs) might contribute to the pathogenesis of cervical cancer formation and progression (3). Matrix metalloproteinase (MMP) production from stroma cells could be stimulated by emmprin expressed in adjacent carcinoma cells. Synthetic peptides carrying a part of the extra-cellular domain of emmprin inhibited emmprin-stimulated production of MMP-2 in co-cultures of fibroblasts and several different human tumor cells types (4).

NF-κB Activation and Progestin-induced FOXO1 Expression in Endometrial Cancer

We have successfully transformed primary endometrial epithelial cells by transduction of several oncogenes, and revealed the importance of the RAS-MAPK and the PI3K-AKT pathways in transformation. In this *in vitro* carcinogenesis model for endometrial cancer, we demonstrated that NF-κB activation is a novel target of oncogenic KRAS in endometrial carcinogenesis, implying the potential utility of NF-κB inhibitors for endometrial cancer chemoprevention, especially with KRAS mutation (5). Despite the therapeutic utility of progestin in invasive and preinvasive endometrial

neoplasias, the molecular mechanisms through which it exerts inhibitory effects on endometrial epithelial growth are largely unknown. We demonstrated that progestin markedly induced FOXO1 gene expression to inhibit cell growth, implicating novel molecular mechanisms of progestin to eradicate endometrial neoplasias (6).

Immortalization of Normal Human cells and Its Application for Cancer Therapy

We have immortalized various types of normal human cells (7-9). Among them, immortalized myoblasts showed normal diploid and conserved differentiation potential (7). Similarly immortalized human peritoneal mesothelial cells (MCs) were used as carrier cells of the oncolytic HSV-1 mutant viruses, HF10 and Hh101, for intraperitoneal therapy against ovarian cancer. In a mouse xenograft model of ovarian cancer, the injection of infected carrier cells led to a significant reduction of tumor volume and prolonged survival in comparison with the injection of the virus alone (8). Several immortalized human epithelial cells were also used for analyzing novel functions of trichoplein and Chk1 (10, 11).

Cell Cycle Regulation of DNA Replication in Mammalian Cells and Its Implication in Oncology

Genomic DNA has to be replicated only once during the cell cycle. During late mitosis through the G1 phase, the MCM complex, a central component of replicative helicase, is loaded onto chromatin by the ORC, CDC6 and Cdt1 proteins. SNF2H, a member of the ATP-dependent chromatin-remodeling complex, was recruited onto DNA replication origins in human cells in a Cdt1-dependent manner and positively regulated MCM loading. Thus SNF2H may promote MCM loading at DNA replication origins via interaction with Cdt1 in human cells (12).

Table 1

Tumor Virus	Malignancy
Human Papillomavirus (HPV)	Cervical Cancer, Anal Cancer, Head and Neck Cancer etc.
Epstein-Barr virus (EBV)	Burkitt Lymphoma, Nasopharyngeal Carcinoma, Gastric Cancer etc.
Hepatitis Virus B (HBV)	Hepatocellular Carcinoma
Hepatitis Virus C (HCV)	Hepatocellular Carcinoma
Human Adult T Cell Leukemia Virus-1 (HTLV-1)	Adult T Cell Leukemia (ATL)
Human Herpes Virus 8 (HHV-8)	Kaposi Sarcoma
*Merkel Cell Polyomavirus (MCPyV)	Merkel Cell Carcinoma

*isolated in 2008, and closely associated with Merkel cell carcinoma, though as yet not defined as a group 1 carcinogen by WHO

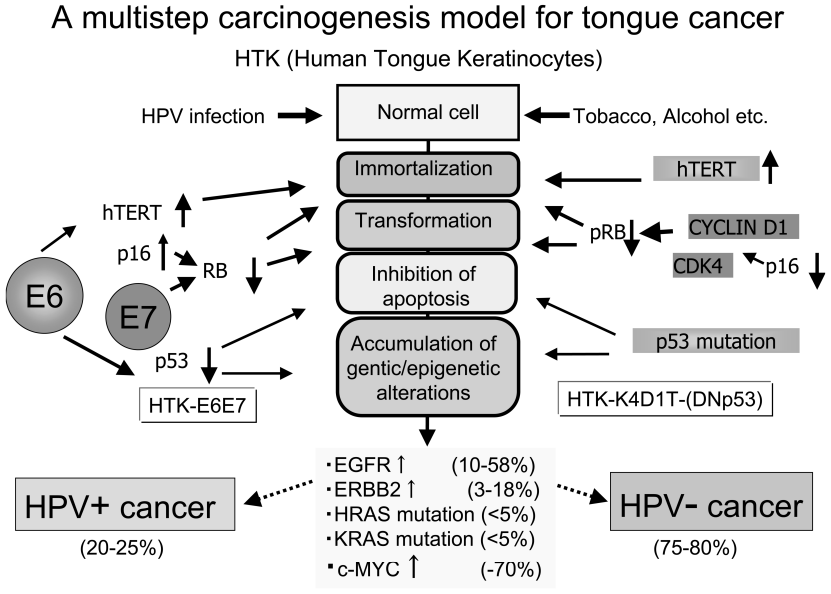

Figure 1

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DIVISION OF CANCER DEVELOPMENT SYSTEM

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Introduction

Research in the Division of Cancer Development System is focused on elucidation of the mechanisms underlying cancer development, which are initiated and promoted by the combination of environmental and genetic factors. Our goal is to dissect the multi-stage carcinogenesis in humans into its component stages, using animal models and *in vitro* systems, for the future development of novel and precise modalities in the field of prevention and early diagnosis of cancer.

In Vitro Reconstitution of Intestinal Tumorigenesis by Genetic and Environmental Factors

We recently developed a cell-based assay that recapitulates intestinal tumorigenesis by genetic reconstitution. Primary murine intestinal cells were transduced with lentivirus vectors for knockdown of tumor suppressor genes, or activation of oncogenes. Tumor formation could be observed within 2 months of the lentiviral infection by the right combinations of genetic alterations, such as inactivation of APC and PTEN. Basically, tumor formation potential observed in this system was concordant with the results from earlier studies using genetically modified mice. Moreover, the generated tumors closely resembled human colorectal adenocarcinoma in their histology. These results suggest that this approach might be indeed mimicking carcinogenesis *in vivo*. Accordingly, this model might allow us to investigate the precise mechanism in the early stage of intestinal carcinogenesis or validate the functional roles of novel cancer-related genes. We further integrated inflammation, as an environmental factor, into this system. Intestinal cells transduced with shAPC were briefly co-cultured with activated inflammatory cells, which resulted in the development of more aggressive tumors, raising the possibility that this model might be useful in dissecting the complex mechanism in inflammation-related carcinogenesis. Reports related to colon carcinogenesis can be found in the attached list of references (1-3).

PhIP-dependent Rat Colon Carcinogenesis

The F344 rat is a sensitive strain for carcinogenesis chemically induced by PhIP, a dietary colon carcinogen. The mechanisms by which PhIP exerts oncogenic effects still remain largely elusive. We previously identified a candidate modifier gene for tumor susceptibility by genetic linkage analysis. Overexpression of the gene in transgenic rats resulted in resistance to PhIP-induced colon carcinogenesis, confirming the tumor suppressive role of the gene. To gain further insight into the mechanism, we asked if miRNA could be involved in mediating the oncogenic properties of PhIP. Six heterocyclic amines (HCAs) with differential carcinogenic potentials have been administered to F344 rats, and the expression profiles of miRNA were obtained. Strikingly, HCAs were separated by hierarchical clustering analysis, and even three miRNAs were sufficient to discriminate carcinogens from non-carcinogens. The *in vitro* responses to PhIP-exposure, including DNA-damage checkpoint, oxidative stress and epigenetic changes, are currently being investigated as well. Reports related to colon carcinogenesis can be found in the attached list of references (4, 5).

Roles of Tumor-suppressor miRNAs in Colon Cancer Development

It has been shown that aberrant expression of miRNA genes, observed in almost all types of human cancers, contributes to cancer development and metastasis. Through functional and comprehensive genomic screens, we recently identified miR-22 as a candidate for a tumor-suppressor gene in human colon cancer (6). The miR-22 gene showed highly frequent hemizygous loss and decreased expression level in human colon cancers. We found that p53 directly regulated the transcription of miR-22, which in turn directly suppressed p21. Indeed, introduction of miR-22 robustly inhibited the accumulation of p21, after activation of p53 by DNA damage, and sensitized cells to p53-dependent apoptosis. The p53-dependent activation of miR-22 was achieved only when the genotoxic stress was so severe that the damaged cells were destined to undergo

apoptosis. These findings indicate that miR-22 is an intrinsic and critical molecular determinant of the p53-dependent response to various oncogenic stresses, partly through p21 repression at a post-transcriptional level (7).

Molecular Targets and Agents for Colon Cancer Prevention

Obesity, consumption of a high-fat diet and hyperlipidemia are epidemiologically associated with the risk of colon cancer. *Apc*-deficient Min mice show age-dependent intestinal polyp development and a hyperlipidemic state, along with suppression of lipoprotein lipase (LPL), which catalyzes the hydrolysis of TG. Statins, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitors, are clinically used for reducing serum lipid levels. The effects of a novel statin, pitavastatin, on intestinal polyp formation in Min mice were examined. Treatment with pitavastatin decreased the total number of polyps in a dose-dependent manner (8), and reduced serum adipocytokine levels and pro-inflammatory gene levels in the intestinal mucosa. Moreover, lipid oxidation observed in the serum and the small intestinal epithelial cells were reduced (9). These results indicate that statins could thus be a good candidate for colorectal chemopreventive agents.

Identification of Novel Mutagens and Carcinogens

Nanomaterials are commonly used in various fields due to their characteristic properties. Accordingly, risk assessment of their use, especially on genotoxicity, is an issue of serious concern. We examined various nanomaterials, including fullerenes and kaolins, and observed a significant induction of micronuclei, and sister chromatid exchange at a high frequency in cultured cells. Moreover, DNA damage of the lungs, from ICR mice intratracheally instilled with a single dose of these nanomaterials, was about 2-3 fold more intense than vehicle control, as revealed by a comet assay. DNA adducts in the lungs were analyzed in parallel, with stable isotope dilution LC-MS/MS, which identified 2 to 5-fold more 8-oxodeoxyguanosine and other lipid peroxide-related adducts in the nanomaterial-exposed mice. Multiple instillations of C60 or kaolin resulted in higher mutant frequencies in the lungs of gpt delta transgenic mice, involving the increase of G:C to C:G transversions for both C60 and kaolin, and the increase of G:C to A:T specifically for kaolin. Immunohistochemical analysis demonstrated many regions in the lungs that were positively stained for nitrotyrosine. These observations suggest that oxidative stress and inflammatory responses might account for the genotoxicity associated with these nanomaterials (10, 11). We characterized an ADP-ribosyltransferase from *Streptomyces coelicolor* that targets guanine mononucleic acids, including guanosine, deoxyguanosine, cyclic GMP, GTP, and dGTP. ADP-riboyslation of the nucleotide pool was indeed confirmed in HeLa cells constitutively expressing the enzyme.

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DIVISION OF METASTASIS AND INVASION SIGNALING

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Introduction

The malignant characteristics of cancers causing invasion into surrounding tissue and metastasis to distant organs are serious threats to the clinical treatment of cancer. It is suggested that numbers of receptor and non-receptor tyrosine kinases are involved in the multiple steps of cancer progression. Signals from activated tyrosine kinases are mediated through phosphorylation of substrate molecules to modulate cell characteristics during tumor proliferation and metastasis. The main object of our Division is to elucidate the roles of tyrosine phosphorylation of signaling molecules during cancer metastasis and invasion. One of the goals of our research is to establish models of the novel therapy for progressed cancer by regulating phosphotyrosine-dependent interactions between signaling molecules.

Molecules Modulating Cancer Invasion and Metastasis

During the analysis of phosphotyrosine-containing proteins in scirrhous gastric carcinoma cell lines, we observed unusual phosphorylation of ARAP3, a multi-modular signaling protein that is a substrate of the Src family kinases. Unlike other phosphotyrosine proteins such as p130Cas, CDCP1 or C9orf10/Ossa that are overexpressed and hyperphosphorylated in scirrhous gastric carcinomas, ARAP3 was underexpressed in cancerous human gastric tissues. Overexpression of ARAP3 in the scirrhous gastric carcinoma cell lines caused marked reduction of peritoneal dissemination without significantly affecting their proliferation. *In vitro* studies also showed that ARAP3 suppressed cell attachment to the extracellular matrix (ECM) as well as invasive activities. These effects were partially lost by mutations in the Rho-GAP domain or in the two tyrosine residues at the C-terminus that are phosphorylated by Src. Our results suggest that ARAP3 is a unique Src substrate that suppresses peritoneal dissemination of scirrhous gastric carcinoma cells (1).

Invadopodia are extracellular matrix-degrading protrusions formed by invasive cancer cells that function in cancer metastasis. We found that PI3-kinase p110α, a frequently mutated gene product in human cancers, and its downstream molecules are essential regulators of invadopodia formation. This study provides evidence that oncogenic activation of p110α promotes invadopodia formation, which contributes to cancer invasion and metastasis (2). We are currently establishing the co-culture system of scirrhous gastric carcinoma cells with fibroblasts for analyzing the cancer-stromal interactions which are characteristic to this type of invasive gastric cancers.

We have reported that CUB-domain-containing protein 1 (CDCP1) regulates anoikis resistance as well as metastatic and invasive properties of cancer cells. It was revealed that CDCP1 phosphorylated at tyrosines by Src family kinases (SFKs) mediates cell migration, invasion and ECM degradation in a tyrosine phosphorylation-dependent manner (3). Cortactin, which was detected as a CDCP1 dependent-binding partner of PKCδ, showed a significant role in migration and invasion but not in the ECM degradation of pancreatic cells. In the invasion model of breast cancer cells, CDCP1 was found to be localized near the invadopodia, which are actin-rich subcellular protrusions with associated proteases used by cancer cells to degrade extracellular matrix cells. On the other hand, human lung cancer tissues or cell lines with Ras mutations show significantly higher expression of CDCP1 than those without Ras mutations. Expression of activated Ras clearly induced CDCP1 expression, while knockdown of CDCP1 abrogated Ras-induced anoikis resistance and enhanced migration/invasion. The cancer progression model of human cervical keratinocytes also validated the involvement of CDCP1 and its phosphorylation by SFKs in the multistep carcinogenesis by Ras and Myc. We demonstrated the CDCP1 protein is required for the functional link between Ras and SFK signaling during the multistage progression of human malignant tumors, highlighting CDCP1 as a potent target for treatment in the broad spectrum of human cancers associated with activating Ras mutations (Figure 1).

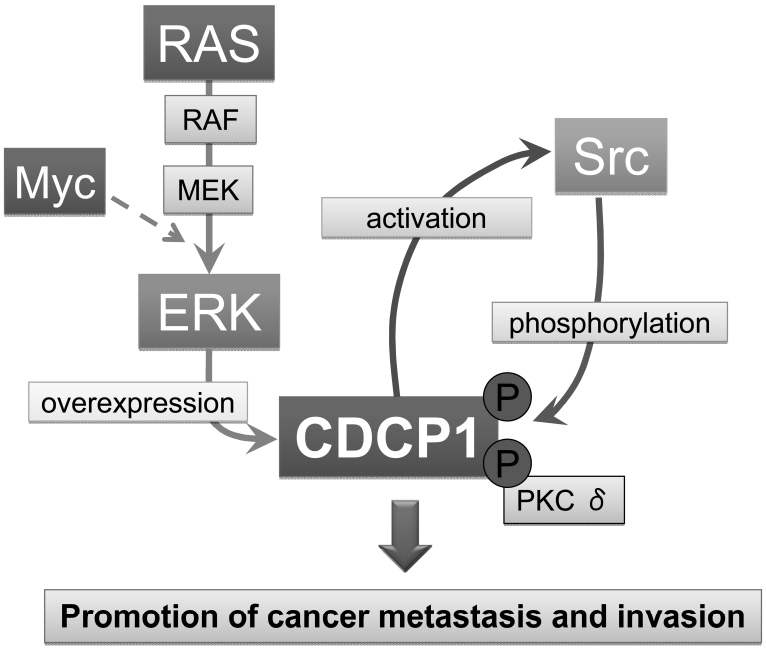

Figure 1

Oncogenic Signals in Neuroblastomas

Neuroblastomas, one of the most common pediatric solid tumors, arise from the immature sympathetic nervous system. Recently, activation of anaplastic lymphoma kinase (ALK) either by mutation or overexpression, has been indicated as a significant oncogenic factor in neuroblastoma formation. During a search of the proteins associating with oncogenic ALK in neuroblastomas, p130Cas (Cas) was detected as forming a complex with activated ALK, and ALK regulates Cas phosphorylation in neuroblastoma cell lines. We previously reported that activated ALK physically binds to the PTB domain of ShcC, while Cas appears to be associated with the SH2 domain of ShcC. Several other phosphortyrosine-containing proteins associated with ALK were identified by mass spectrometry and the oncogenic roles of these molecules in neuroblastoma are being investigated.

On the other hand, it was observed that expression of a receptor tyrosine kinase Ret which is highly expressed in some of the neuroblastoma cell lines was suppressed by knockdown of ALK or by the ALK inhibitor in the neuroblastoma cell lines. Since activation of Ret kinase by its ligands such as GDNF was shown to contribute to the anchorage independent growth of neuroblastoma cells, an indirect effect of ALK activation through Ret kinase might affect the oncogenic aspects of neuroblastomas. The combinatory effect of inhibiting both ALK and Ret kinases are being analyzed for evaluation of its clinical significance.

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DIVISION OF MOLECULAR AND CELLULAR MEDICINE

Takahiro Ochiya, Fumitaka Takeshita, Masaki Kawamata, Nobuyoshi Kosaka, Ryou-U Takahashi, Ayako Inoue, Wakako Kobayashi, Tomohiro Fujiwara, Makiko Ono, Luc Gailhouste, Thirion Muriel, Satoshi Seino, Yusuke Yoshioka, Keitaro Hagiwara, Takeshi Katsuda, Keita Uchino

Introduction

The main focus of the Division of Molecular and Cellular Medicine is the development of novel strategies to study tumorigenesis, cancer metastasis, and drug resistance. The specific activities in 2011 were as follows: 1) Preclinical studies on RNAi-mediated cancer therapy; 2) An exosome-carrying microRNA as a novel communication tool for cancer development; and 3) The generation of novel animal models for cancer study.

RNAi-mediated Cancer Therapy

Since small interfering RNAs (siRNAs) and microRNAs (miRNAs) are two main types of silencing small RNAs that modulate tumor-related genes and multiple oncogenic pathways, extensive investigation efforts have been directed to siRNAs and miRNAs into clinical therapeutic applications. In our previous study, we identified Ribophorin-2 (RPN2) as a novel regulator of drug resistance in breast cancer and found that RPN2 affects docetaxel resistance through the regulation of *N*-linked glycosylation of P-glycoprotein (1). Further analysis showed that RPN2 is highly expressed in cancer stem cells (CSCs) and regulates tumourigenic and metastatic activities in breast cancer by stabilizing mutant p53. Therefore, RPN2 knockdown profoundly suppressed CSC phenotypes *in vitro* and *in vivo*. Moreover, we demonstrated that therapeutic silencing of RPN2 induced highly significant tumor growth inhibition and regression in a dog with naturally occurring breast cancer. These findings revealed a previously undescribed molecular mechanism for mtp53 stabilization in breast cancer and suggested that the RPN2/mtp53 regulatory network could be a promising target for anti-CSC therapy.

Pulmonary metastases are the main cause of death in patients with osteosarcoma. We identified microRNA-143 (miR-143) as a lung metastasis-related miRNA and found that the treatment with synthetic miR-143 molecules inhibited lung metastasis of mouse model for osteosarcoma (2). Furthermore our data showed that the expression of miR-143 is inversely correlated with its predicted target MMP-13 level in osteosarcoma patients.

Our group also showed that synthetic miR-22 delivery suppresses tumor growth and metastasis by inducing cellular senescence in a mouse model of breast carcinoma (3). Our study suggested that miR-22 restored the cellular senescence program in cancer cells and has a therapeutic potency for cancer treatment. Our *in vivo* studies, tumor growth and metastasis in a mouse model of human cancer have been evaluated *in vivo* by bioluminescence-based imaging analysis (4).

Chronic hepatitis C (CH) can develop into liver cirrhosis (LC) and hepatocellular carcinoma (HCC). We detected several human miRNAs whose expression levels were correlated with the degree of progression of liver fibrosis. In both the mouse and human studies, the expression levels of miR-199 and 200 families were positively correlated to the progression of liver fibrosis (5).

An integrated genomic analysis combined with array-based comparative hybridization, miRNAs, and a gene expression microarray elucidated the mechanism of drug-resistance in a drug-resistant breast cancer cell line (6). One of the down-regulated miRNAs, miR-505, whose genome regions were deleted in a drug-resistant breast cancer cell line, is a novel tumor suppressive miRNA and inhibits cell proliferation by inducing apoptosis. In addition, Akt3, correlated inversely with miR-505, modulated drug sensitivity in a drug-resistant breast cancer cell line. These results showed that various genes and miRNAs can be orchestrated to temper the drug-resistance in cancer cells, and thus acquisition of drug-resistance is intricately controlled by genomic status, gene and miRNA expression changes.

Intercellular Transfer of microRNAs in Living Cells

Evidence is presently increasing to show that miRNAs contained in exosomes are released from mammalian cells and act as a signal transducer. It has already been demonstrated that normal

epithelial cells regulate the secretion of autocrine and paracrine factors that prevent aberrant growth of neighboring cells, leading to healthy development and normal metabolism, indicating that one reason for tumor initiation is considered to be a failure of this homeostatic cell competitive system. Taken together, these findings suggest that secretory miRNAs may have favorable aspects for antiproliferative signals mediating the interaction between cancer cells and non-cancer cells. Through the extension of this concept, it was first found that exosomal tumor-suppressive miRNAs secreted by non-cancerous cells inhibited the proliferation of cancerous cells.

Generation of Genetically Modified Rats from Embryonic Stem Cells

Rats have important advantages as an experimental system for physiological and pharmacological investigations (7). In extensive chemical carcinogenesis studies, rats have been used for a long period. Despite this history, functional genetic studies and generation of human disease models are poor in rats. At present, only a few groups have generated genetically modified rats from embryonic stem (ES) cells because stable ES cells are not available. We have recently established authentic rat ES cells developing a new culture medium composed of 20% fetal bovine serum and cell signaling inhibitors for ROCK, MEK, TGF beta and GSK3 (8). The rat ES cells expressed ES cell markers such as *Oct4*, *Nanog*, *Sox2* and *Rex1* and retained a normal karyotype. Embryoid bodies and teratomas were also produced from the rat ES cells. All six ES cell lines derived from 3 different rat strains successfully achieved germline transmission, which strongly depended on the presence of the inhibitors during the injection process. Most importantly, *Oct4*-Venus transgenic (Tg) rats were successfully generated via the selection of gene-manipulated ES cell clones through germline transmission. In the Tg rats, Venus fluorescence can be detected in cells expressing *Oct4* (9). Since somatic and cancer stem cells expressthe *Oct4* gene, we anticipate that the mechanisms of tumor initiation could be solved using the Tg rats. Our rat ES cell technology has the potential to generate extensive numbers of knockout as well as Tg rats, which will contribute to research for cancer therapy and regenerative medicine for humans (10).

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DIVISION OF CANCER BIOLOGY

Hirofumi Arakawa, Yasuyuki Nakamura, Noriaki Kitamura, Hiroki Kamino, Masaki Yoshida, Ryuya Murai, Izumi Hyo

The scope of the research at the Division of Cancer Biology is broad, covering numerous areas including the cloning of genes involved in carcinogenesis, biological and structural analyses of proteins, analyses of animal models, and the development of new strategies for cancer therapy. In particular, the tumor suppressor p53 and the genes that are directly regulated by p53 have been studied to uncover the mechanism of p53-mediated tumor suppression, based on which new cancer therapies could be developed.

Identification and Characterization of p53-target Genes

Using a combination of a microarray analysis and a chromatin immunoprecipitation assay, identification of p53-target genes in the human genome has been conducted at the Division. In 2011, *Mieap* (1) was identified and analyzed as new p53-target genes. The results of these studies enabled us to further understand the physiological functions of p53.

There are a number of p53-target genes, and the function of the p53-target varies from gene to gene. The important question is how p53 regulates a number of target genes and/or functions. Recently, modifications of p53, such as phosphorylation, acetylation and sumoylation have been suggested to play an important role in this process. To clarify the mechanisms, classification of the p53-target genes is being conducted according to their individual functions.

Mieap-induced Accumulation of Lysosome-like Organella within Mitochonria

Mieap controls mitochondrial quality via two distinct novel mechanisms. One of the mechanisms has been designated MALM for Mieap-induced accumulation of lysosome-like organelles within mitochondria (1). In this mechanism, Mieap induces the accumulation of intramitochondrial lysosome-like organelles to eliminate oxidized mitochondrial proteins in response to mitochondrial damage. This leads to a decrease in reactive oxygen species generation and an increase in mitochondrial ATP synthesis activity, implying MALM plays a role in repairing unhealthy mitochondria.

Mieap-inudced Vacuole

Alternatively, another mechanism has been designated MIV, standing for Mieap-induced vacuole (2). When MALM is inhibited, Mieap induces a vacuole-like structure known as the MIV. The MIV engulfs the damaged mitochondria and accumulates lysosomes, leading to the degradation of unhealthy mitochondria. MIV likely represents a novel mechanism for mitochondrial autophagy, also called "mitophagy". Therefore, Mieap controls mitochondrial quality by repairing or eliminating unhealthy mitochondria via MALM or MIV generation, respectively (Figure 1) (1, 2).

Mitochondrial Quality Control and Cancer

Mitochondria play a critical role in a number of cellular functions, being involved in aging, degenerative diseases and cancer. However, the mechanisms involved in maintenance of the quality of healthy mitochondria still remain unclear. A new protein, Mieap, was discovered, which plays a critical role in mitochondrial quality control (1, 2).

Aerobic glycolysis is a common feature of human cancers, which is also known as the Warburg effect. Although the nature of cancer cells has been applied to the development of positron emission tomography (PET) for the whole body screening of human cancers, the mechanism for the phenomenon remains to be elucidated. The p53-Mieap pathway is frequently inactivated in human cancers because of p53 mutations and/or Mieap methylation. This leads to accumulation of unhealthy mitochondria and consequently the Warburg effect (Figure 2). This finding could explain the reason why cancer cells preferentially utilize aerobic glycolysis, as observed by Warburg. Therefore, the mechanisms of maintenance of healthy mitochondria are currently being investigated at this Division.

New Therapeutic Strategies for Cancer Therapy

Several p53-mutants are known to show enhanced apoptosis inducing activity, and are believed to be some kind of activated forms of p53. On the other hand, several p53-target genes have also been reported to induce marked apoptosis in cancer cells. Therefore, to improve p53 gene therapy, adenovirus-mediated gene transfer of the active forms of p53 or apoptotic p53-target genes may well become a new therapeutic strategy for the treatment of p53-resistant cancers. In addition, adenovirus-mediated gene transfer of *Mieap* has been found to strongly suppress the tumor growth, suggesting that normalization of unhealthy mitochondria could be a novel strategy to suppress cancers *in vivo*. Toward the development of new strategies for cancer therapy, the *in vitro* and *in vivo* antitumor effects of these genes are being examined at this Division.

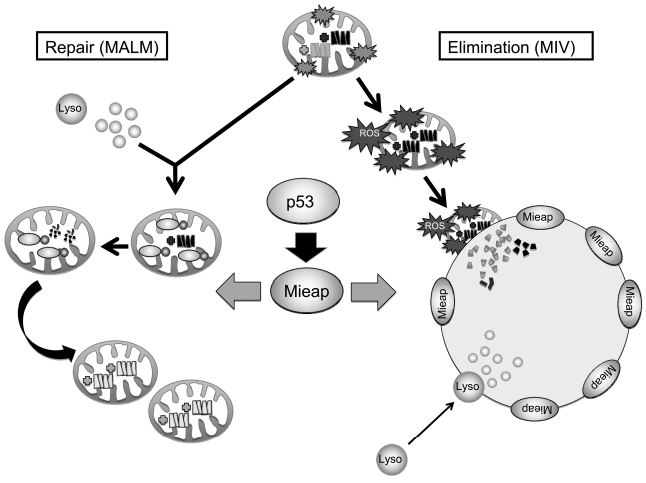

Figure 1

Inactivation of the p53-Mieap pathway in cancer

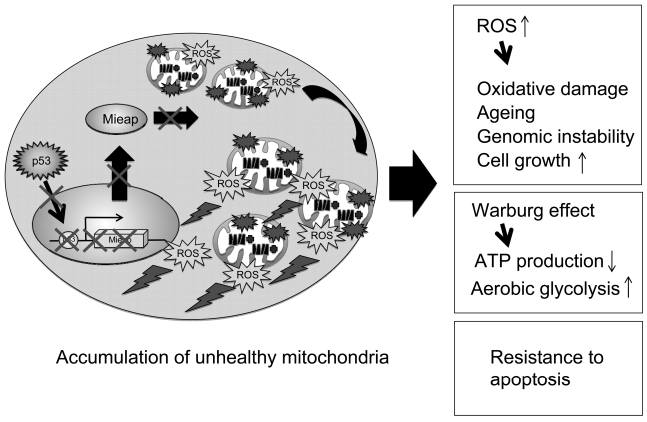

Figure 2

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DIVISION OF CANCER DIFFERENTIATION

Koji Okamoto, Hirokazu Ohata, Tatsuya Ishiguro, Yuki Aihara, Ai Sato, Hiroaki Sakai

Introduction

The main goals of our division center around the elucidation of the biological mechanism behind the metastasis of cancer cells that are refractory to conventional therapy. In general, we took two approaches to understand the mechanism of metastasis. In the first approach, we are attempting to identify crucial regulators of metastasis, especially liver metastasis from colon cancer. In order to identify such regulators, we performed a functional screening in a mouse model of liver metastasis. Once such key regulators are identified we will attempt to understand the mechanism of metastasis via elucidation of their biological roles. In the second approach, we are analyzing human cancer-initiating cells that are derived from refractory cancer of colon or ovary origin. Through analyses of these cancer-initiating cells, we hope to get biological insight into the mechanism of cancer metastasis as it has been reported that metastatic cancer cells share a variety of phenotypic resemblances to cancer stem cells.

Identification of microRNAs (miRNAs) that Inhibit Colon Cancer Metastasis

In order to recapitulate liver metastasis from colon cancer, we developed an experimental model in which cancer metastasis was generated with high efficiency in highly immunocompromised NOG mice. Colon cancer cells were injected into the spleen or tail vein of NOG mice to generate liver or lung metastasis, and generated metastatic foci were visualized with bioimaging or quantified by counting the number of metastatic cells with flow cytometry after dissociation of targeted organs. To determine the role of the regulatory factors of metastasis, lentiviruses that express such factors were introduced in HCT116/GFP, and the effects of the regulatory factors were determined by examining the effects of expression of the factor on generating metastasis.

This metastasis model mentioned above was used to functionally isolate regulatory factors involved in liver metastasis of colon cancer cells. It has been reported that a subset of miRNAs is involved in suppression of metastasis of cancers. Therefore, we looked for miRNAs that can inhibit colon cancer cell metastasis to the liver by applying a systematic screening approach (dropout screening). Through the dropout screening of a miRNA library after the introduction of HCT116 colon cancer cells, one miRNA was isolated that reproducibly inhibited the liver metastasis of colon cancer cells.

The impact of expression of this miRNA on the metastasis and prognosis of colon cancer has been investigated. A functional assay showed that one of the microRNAs identified via the functional screening, miR-493 was capable of inhibiting liver metastasis. miR-493 inhibited retention of metastasized cells in the liver parenchyma and induced cell death. IGF1R was identified as a direct target of miR-493, and its inhibition partially phenocopied the anti-metastatic effects. High levels of miR-493 in primary colon cancer were inversely related to the presence of liver metastasis, and attributed to an increase of miR-493 expression during carcinogenesis. Therefore, in a subset of colon cancer, up-regulation of miR-493 during carcinogenesis may prevent liver metastasis via the induction of cell death in metastasized cells.

Identification of shRNAs that Inhibit Colon Cancer Metastasis

We attempted to isolate genes that regulate liver metastasis of colon cancer by using a functional screening method that is conceptually similar to the dropout screening of miRNA. Screening of the shRNA library for human annotated genes revealed that 8 candidate genes were targeted by two independent shRNAs that inhibit liver metastasis. Individual evaluation of these clones is in progress.

Establishment and Characterization of *in vitro* Culture System of Cancer Stem Cells

Metastatic cells share phenotypic resemblances to cancer stem cells, and acquisition of “stemness” may confer metastatic function to cancer cells. Accumulating reports indicate that “cancer stem cells” exist in various types of cancer, and they are responsible for metastatic processes as well as the tumorigenicity and chemoresistance of cancer. In

order to examine the role of cancer stem cells in metastasis, we isolated cancer stem cells from human colon cancer, and established the condition that allows stable *in vitro* propagation of colon cancer stem cells in a spheroid form (colonosphere).

The difficulty in expanding cancer-initiating cells *in vitro* is one of major obstacles for their biochemical characterization. We found that inhibitors of some kinases greatly facilitated the establishment of spheroids from primary colon cancer. Under such conditions, the spheroid cells expressed cancer stem cell markers, showed the ability to differentiate, and induced tumors in mice. The spheroids were composed of cells that express various levels of CD44, and while $CD44^{high}$ cells exhibited increased sphere-forming ability, $CD44^{-/low}$ cells showed increased levels of differentiation markers and apoptotic cells. As expected from the predicted hierarchy, $CD44^{high}$ cells differentiated into $CD44^{-/low}$ cells. Unexpectedly, a fraction of $CD44^{-/low}$ cells generated $CD44^{high}$ cells, and the kinase inhibitor primed the transition by inducing CD44 expression. In accordance, $CD44^{-/low}$ cells resumed CD44 expression and formed tumors in mice. Therefore, the transition from $CD44^{-/low}$ to $CD44^{high}$ state may enhance tumorigenicity by maintaining a $CD44^{high}$ fraction in colon cancer.

In addition to colon cancer, the attempt to establish similar *in vitro* cultivation system of cancer-initiating cells from ovarian cancer is in progress.

DIVISION OF HEMATOLOGICAL MALIGNANCY

Issay Kitabayashi, Akihiko Yokoyama, Kimiko Shimizu, Kazutsune Yamagata, Takuo Katsumoto, Yutaka Shima

Leukemia is a heterogeneous disease with distinctive biological and clinical properties that are conferred by a variety of acquired genetic mutations. Specific chromosomal translocations and other mutations associated with acute myeloblastic leukemia (AML) often involve transcription factors and transcriptional coactivators. Such target genes include AML1, C/EBPα, RARα, MOZ, p300/CBP, and MLL, all of which are important in the regulation of hematopoiesis. The resultant fusion or mutant proteins deregulate the transcription of the affected genes and disrupt their essential role in hematopoiesis, causing differentiation block and abnormal proliferation and/or survival. Our research focuses on such transcription factors and coactivators, and describes their roles in leukemogenesis and hematopoiesis (1).

Chromosomal translocations of the mixed lineage leukemia (*MLL*) gene account for 5%–10% of acute leukemias and are generally associated with a poor prognosis. The *MLL* gene rearrangements create fusion genes that contain the 5′ portion of *MLL* and the 3′ portion of its fusion partner, whose products cause sustained expression of MLL target genes and consequent enhanced proliferation of hematopoietic progenitors. The MLL proto-oncogenic protein is a histone-lysine N-methyltransferase that is produced by proteolytic cleavage and self-association of the respective functionally distinct subunits (MLL(N) and MLL(C)) to form a holocomplex involved in epigenetic transcriptional regulation. On the basis of studies in Drosophila it has been suggested that the separated subunits might also have distinct functions. In this study, we used a genetically engineered mouse line that lacked MLL(C) to show that the MLL(N)-MLL(C) holocomplex is responsible for MLL functions in various developmental processes. The stability of MLL(N) is dependent on its intramolecular interaction with MLL(C), which is mediated through the first and fourth plant homeodomain (PHD) fingers (PHD1 and PHD4) and the phenylalanine/tyrosine-rich (FYRN) domain of MLL(N). Free MLL(N) is destroyed by a mechanism that targets the FYRN domain, whereas free MLL(C) is exported to the cytoplasm and degraded by the proteasome. PHD1 is encoded by an alternatively spliced exon that is occasionally deleted in T-cell leukemia, and its absence produces an MLL mutant protein that is deficient for holocomplex formation. Therefore, this should be a loss-of-function mutant allele, suggesting that the known tumor suppression role of MLL may also apply to the T-cell lineage. Our data demonstrate that the dissociated MLL subunits are subjected to distinct degradation pathways and thus not likely to have separate functions unless the degradation mechanisms are inhibited (2).

The histone acetyltransferases (HATs) of the MYST family include TIP60, HBO1, MOZ/MORF, and MOF and function in multisubunit protein complexes. Bromodomain-containing protein 1 (BRD1), also known as BRPF2, has been considered as a subunit of the MOZ/MORF H3 HAT complex based on its analogy with BRPF1 and BRPF3. However, its physiologic function remains obscure. Here we show that BRD1 forms a novel HAT complex with HBO1 and regulates erythropoiesis. Brd1-deficient embryos showed severe anemia because of impaired fetal liver erythropoiesis. Biochemical analyses revealed that BRD1 bridges HBO1 and its activator protein, ING4. Genome-wide mapping in erythroblasts demonstrated that BRD1 and HBO1 largely colocalize in the genome and target key developmental regulator genes. Of note, levels of global acetylation of histone H3 at lysine 14 (H3K14) were profoundly decreased in Brd1-deficient erythroblasts and depletion of Hbo1 similarly affected H3K14 acetylation. Impaired erythropoiesis in the absence of Brd1 accompanied reduced expression of key erythroid regulator genes, including Gata1, and was partially restored by forced expression of Gata1. Our findings suggest that the Hbo1-Brd1 complex is the major H3K14 HAT required for transcriptional activation of erythroid developmental regulator genes (3).

In acute promyelocytic leukemia (APL), the promyelocytic leukemia (PML) gene is frequently fused to the retinoic acid receptor α (RARα) gene, generating the PML-RARα fusion gene. While PML normally forms discrete nuclear speckles called nuclear bodies (NBs), PML-RARα disrupts NBs via an unknown mechanism. It is also unclear whether this disruption is related to leukemia pathogenesis. In the present study, PML-RARα was found to mediate NB disruption by inhibiting PML oligomerization while cAMP/PKA-dependent PML-RARα phosphorylation restored NBs and

promoted APL cell differentiation. These results suggest that NB restoration might be an appropriate therapeutic strategy for APL. Recently, cAMP/PKA pathway activators have shown efficacy in t(15;17) APL. The disruption of PML NBs, a defining cellular feature of t(15;17) APL, has been regarded as unimportant in the pathogenesis of the disease because it is not observed in other forms of APL. Therefore, this disruption has also been considered as an inappropriate target for therapy. The present study refutes this view and demonstrates that PML-RARα disrupts NBs by blocking PML oligomerization. Results further show that cAMP/PKA-dependent phosphorylation of PML-RARα restores NBs and that forskolin, a cAMP/PKA pathway activator, restores NBs and promotes ATRA-dependent APL cell differentiation. Thus, the restoration of PML NBs may be a suitable therapeutic approach for t(15;17) APL.

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DIVISION OF PHARMACOPROTEOMICS

Tadashi Kondo, Daisuke Kubota, Hiroshi Ichikawa, Noriyuki Hosoya, Kazutaka Kikuta, Yukiko Nakamura, Youko Takai, Ruriko Sakamoto, Kazuya Arai, Satomi Ikeda, Kano Sakamoto, Fusako Kito, Marimu Sakumoto, Ryousuke Yamaka, Jyunya Otake, Yutaka Sugihara, Hirotaka Yonemori, Ayako Haga, Kazuya Kimura, Chen Chen, Yuka Takaku, Reina Tamura

The Division of Pharmacoproteomics focuses on the identification of novel therapeutic targets, and biomarkers for personalized medicine. The major strategies of the Division are comprehensive protein expression studies using original technologies and clinical samples as well as clinico-pathological information. In collaboration with clinicians, pathologists and researchers inside and outside the Center, and commercial companies, the Division aims to realize the practical use of research outcomes in the clinical field.

Bone and Soft-tissue Sarcoma Study

Response to adjuvant chemotherapy is a major prognostic factor in osteosarcomas. To develop predictive modalities for the response to chemotherapy, the protein and microRNA contents in the incisional biopsy samples before treatments were compared between the responder- and non-responder-groups. A global expression study identified the proteins and microRNA corresponding to the response to the treatments. An *in vitro* function study revealed the molecular backgrounds of these biomarkers.

Prognostic proteins were investigated in synovial sarcomas. The proteomic contents of surgically resected tissues were compared with two-dimensional difference gel electrophoresis (2D-DIGE) and mass spectrometry among patients with different clinical outcomes. Prognostic proteins were identified among more than 3,000 protein species, and the association of sesernin-1 with the prognosis was confirmed in an independent sample set using our original antibody (1). A patent was applied for regarding the utility of the identified protein.

A global expression study on proteins and microRNA for lung-metastasis associated genes in osteosarcomas and a multi-institutional validation study for the prognostic value of nucleophosmin in Ewing sarcoma are on-going with the aims of discovering novel therapeutic targets and biomarkers.

Liver Cancer Study

To develop the prognostic modalities and identify the therapeutic targets in hepatocellular carcinoma (HCC), the expression of 580 unique nuclear factors were examined with an antibody-based proteomics approach. This study included 100 surgical specimens from the patients with HCC in collaboration with Zhongshan Hospital (Shanghai, China). A monoclonal antibody library identified approximately 200 nuclear factors associated with carcinogenesis and early recurrence. A subsequent tissue microarray study revealed the localization of the identified nuclear factors in tumor cells and tumor vascular structures. Among them, function assays identified the nuclear factor with a regulatory function on malignant features of HCC. The proteomic approach using 2D-DIGE also identified the proteins with predictive value for early intrahepatic recurrence in the same sample set. A patent application for the clinical utility of the identified proteins is currently pending.

Colorectal Cancer Study

To develop the biomarkers for the early diagnosis of colorectal cancer, proteomic contents were compared between normal and tumor tissues (2). A 2D-DIGE study of 5000 protein species and an antibody-based proteomics approach for 400 proteins resulted in the identification of the proteins with significant levels of overexpression in the tumor tissues. Further studies revealed that the product of the oncogene was detected in the conditioned medium of colorectal cancer cell lines, and in plasma samples of the patients. A patent application for the clinical utility of the identified proteins is currently pending.

Gastrointestinal Stromal Tumor Study

Our previous proteomic studies using 2D-DIGE identified a novel prognostic biomarker, pfetin, in gastrointestinal stromal tumors. After the prognostic

value of pfetin was validated in 210 cases in the National Cancer Center, a monoclonal antibody was generated for clinical application, and the prognostic value of pfetin was confirmed in 100 cases in Niigata University Hospital. Following this, we further validated the clinical utilities of pfetin in an additional 40 cases in Juntendo University Shizuoka Hospital (3). A multivariate study demonstrated the independent prognostic values of pfetin among the other clinico-pathological parameters in all validation studies. A patent examination for the clinical utility of pfetin is currently pending.

Novel Plasma Proteomics Approach

A novel proteomics protocol for plasma samples was developed using a solid-phase hexapeptide ligand library in combination with a multi-dimensional chromatography system, 2D-DGIE and mass spectrometry. Extensive fractionation resulted in the observation of up-to 8000 intact proteins (4). The application of this protocol to plasma biomarker studies has been undertaken.

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DIVISION OF EPIGENOMICS

Toshikazu Ushijima, Eriko Okochi-Takada, Satoshi Yamashita, Kiyoshi Asada, Tohru Niwa, Hideyuki Takeshima, Naoko Hattori

This Division has been focusing on the epigenetic mechanisms of carcinogenesis, mainly DNA methylation. Using our original genome-wide screening technique for differences in DNA methylation, methylation-sensitive-representational difference analysis, tumor suppressor genes and many aberrantly methylated CpG islands (CGIs) have been identified in various cancers, i.e., gastric cancers, breast cancers, pancreatic cancers, lung cancers, ovarian cancers, neuroblastomas, and melanomas. This led to identification of a novel tumor-suppressor gene in gastric cancers, development of a novel and powerful prognostic marker in neuroblastomas, and revelation of an "epigenetic field for cancerization".

This Division continues its activity in identifying novel epigenetic alterations in various cancers and normal tissues, and is applying its past discoveries to the development of clinically useful biomarkers. It is also interested in the development of epigenetic therapy and clarification of mechanisms of how epigenetic alterations are induced.

Identification of Novel Epigenetic Alterations

Detection of lymph node metastasis is critically important for deciding on the treatment strategy for esophageal squamous cell carcinomas, and this Division identified CGIs whose methylation levels are associated with the presence of lymph node metastasis in human esophageal cancers (1). Aberrant DNA methylation in animal models is useful to analyze modifiers of methylation induction, and such genes were isolated in rat mammary carcinomas (2). The presence of aberrant hypermethylation in predisposed epithelial cells is now known, and hypomethylation of repetitive elements was shown to be present in gastric mucosae with *Helicobacter pylori* (*H. pylori*) infection (3). State-of-the-art technologies are constantly employed for genome-wide methylation analyses, and bead array technology and high-throughput sequencing technologies are now being adopted.

Development of Biomarkers

This Division previously revealed that accumulation of aberrant methylation of multiple genes in normal-appearing gastric mucosae is associated with gastric cancer risk (epigenetic field for cancerization) (4). Levels of such accumulated methylation are expected to be useful as a gastric cancer risk marker, and a prospective study is being conducted to bring this into clinical practice. The presence of an epigenetic field for cancerization is now recognized for various other cancers. In the esophagus, in collaboration with the National Taiwan University Hospital, methylation levels in esophageal mucosae were shown to increase following exposure to carcinogens, such as alcohol, betel quid, and cigarettes, and to be higher in cancer patients ($P<0.05$) (5). The clinical usefulness of prognostic markers in neuroblastomas is being analyzed using materials collected in a prospective manner.

Development of Epigenetic Therapy

Epigenetic therapy is expected as a next-generation strategy in cancer chemotherapy. Since many genes are now known to be silenced in a single cancer, simultaneous reversal of silencing of multiple genes could be an effective treatment. This Division is working on this strategy as a novel therapeutic concept using neuroblastomas as a model. Assay systems for novel epigenetic reagents are also being developed.

Induction Mechanisms of Epigenetic Alterations

Clarification of induction mechanisms of epigenetic alterations is critically important for public health, including cancer prevention. This Division showed that chronic inflammation is critically important for induction of aberrant methylation (Figure 1). The effects of various kinds of inflammation due to different inducers, such as high concentrations of ethanol (EtOH) and saturated sodium chloride (NaCl), were examined. Methylation was induced only in gerbils infected with *H. pylori*. Histologically,

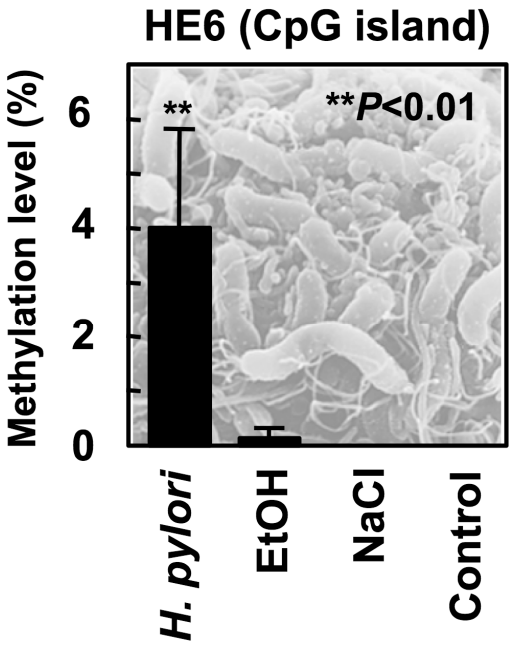

Figure 1. Specific inflammation is critical for aberrant DNA methylation induction

chronic inflammation with lymphocyte and macrophage infiltration was prominent in *H. pylori* infection, whereas neutrophil infiltration was mainly observed in the EtOH and NaCl treatment. Cell proliferation was most strongly induced by the NaCl treatment. Therefore, it was considered that specific types of inflammation are necessary for methylation induction (6).

Aberrant DNA methylation is known to be induced in specific genes, and this Division demonstrated that the absence of RNA polymerase II and the presence of H3K27me3 predispose genes to become methylated. In addition to these epigenetic factors, it was demonstrated that genome architecture, namely a remote location from SINE and from LINE, was associated with increased susceptibility (7).

Other Activities

This Division assisted in a genome-wide screen for aberrant methylation in uterine leiomyoma (8), analysis of histone modifications of the *Bdnf* gene in the brain (9), and analysis of SNPs in the MFSD2A gene in Asian lung cancer patients (10). This Division also contributed to communicating recent technologies in DNA methylation analysis in toxicology (11).

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DIVISION OF GENOME BIOLOGY

Takashi Kohno, Hideaki Ogiwara, Kouya Shiraishi, Yoko Shimada

Introduction

Biological research using genome information is changing medical treatments of cancer. In particular, interindividual variations in the human genome and somatic mutations in the cancer genome have become critical keys to improving the diagnosis and treatment of cancer. The aim of our division is to find "seeds" that improve the prevention, diagnosis and treatment of cancer, by identifying and elucidating the biological significance of somatic mutations in cancer genomes, and genetic polymorphisms of cancer patients. We are working together with NCC staff from hospitals, the Research Center for Cancer Prevention and Screening, and the Center for Cancer Control and Information Service to fight lung cancer, the most common cause of cancer-related deaths in Japan and worldwide.

Research Projects

1. Genes for cancer treatment

Genes involved in DNA repair and/or chromatin remodeling are being analyzed to improve the efficiency of existing therapeutic methods. Non-homologous end joining (NHEJ) is a major repair pathway for DNA double strand breaks (DSBs) generated by ionizing radiation and anti-cancer drugs. Therefore, inhibiting the activity of proteins involved in this pathway is a promising way of sensitizing cancer cells to radio- and chemotherapy. We established an assay for evaluating NHEJ activity against DSBs in chromosomal DNA in human cells. Using this assay, we revealed that CBP and p300, histone acetyltransferases (HATs), promote repair by facilitating accumulation of NHEJ proteins (1). The utility of CBP/p300 as a target for sensitization of tumors to radio- and chemotherapies was indicated. To translate the results into a clinical setting, radiosensitizing effects of natural compounds with an inhibitory activity against HATs involved in NHEJ are being searched for. Up to the present, curcumin (contained in the spice turmeric) and anacardic acid (contained in the shell of the cashew nut), have been identified as promising radiosensitizers (1).

NHEJ is responsible for the repair of DSBs with incompatible DNA ends, which are often generated by ionizing irradiation. Therefore, we defined essential factors involved in incompatible DNA end joining using the *in vivo* assay described above. The results indicate that DNA end resection (Artemis) and ligation (LIG4) factors are critical for the efficient joining of incompatible ends *in vivo*, further emphasizing the importance of synapsis and gap-filling factors (POLλ and POLμ) in preventing illegitimate joining (2). In addition, PALF (Polynucleotide kinase and aprataxin-like forkhead-associated protein) was identified as a novel DNA end resection factor involved in incompatible DNA end joining (3). These essential factors will also be a target for radiosensitization.

Genes that can be used as targets for therapy are also being investigated by analyzing genomic DNA and RNA from lung cancer tissues supplied from the National Cancer Center Biobank. Intragenic mutations in the EGFR gene and rearrangements of the ALK gene are critical information to select patients with lung adenocarcinoma (LADC), which responds to molecular targeting therapy using specific tyrosine kinase inhibitors. In collaboration with the Department of Pathology and Clinical Laboratories, methods to accurately detect EGFR mutations and ALK rearrangements are being investigated. We also demonstrated that N-cadherin signaling contributes to the survival mechanisms of gefitinib-resistant lung cancer cells and N-cadherin is a potential molecular target to cure gefitinib-resistant lung cancer cells (4). Novel genes mutated and/or rearranged in lung cancer are being intensively searched for by conducting whole RNA/exome sequencing using high-speed DNA sequencers.

2. Genes for cancer prevention

By analyzing genetic polymorphisms of cancer patients, we aim to improve cancer prevention through identification of high-risk individuals for cancer development. Recent genome-wide association studies (GWASs) by us and others have identified genes whose polymorphisms are associated with lung cancer risk. However, the significance of functional polymorphisms in DNA repair and metabolic genes that had been reported as being associated with risk for tobacco-related

cancers, particularly for lung squamous cell carcinoma (LSQC), remains unclear, since those polymorphisms were not examined in the GWASs. The significance of such polymorphisms was evaluated here in a cohort of LSQC patients, in which polymorphisms in the GWAS genes showed associations with risk. The association of polymorphisms in TP53 and OGG1 involved in DNA damage response/repair and that in CYP1A1 involved in bio-activation of polycyclic aromatic hydrocarbon, a major tobacco procarcinogen, was validated (5). This result indicates that multiple genes contribute to inter-individual susceptibility to LSQC. GWASs of LADC patients are being conducted to identify genes that enable identification of high risk individuals for targeted screening and/or prevention. LADC, particularly bronchioloalveolar carcinoma, is believed to develop from a benign adenomatous lesion, atypical adenomatous hyperplasia (AAH). AAH is detected as ground-glass opacity (GGO) by computed tomography (CT) examination, therefore, association studies on genetic polymorphisms of CT-based cancer screening examinees with and without GGO are also underway to establish methods to select individuals at high risk for the development of AAH/LADC.

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DIVISION OF CANCER GENOMICS

Tatsuhiro Shibata, Fumie Hosoda, Yasushi Totoki, Yasuhito Arai, Takuya Shirakihara, Hiromi Nakamura, Natsuko Hama, Hiroyuki Takahashi, Wataru Munakata, Naoko Okada, Akiko Kokubu, Tomoko Urushidate, Hiroko Shimizu, Shoko Ohashi

The Division of Cancer Genomics focuses on comprehensive characterization of the cancer genome on the basis of tumor pathology and aims to make a "breakthrough" by identifying novel cancer-related genes, including potential therapeutic targets and biomarkers, and to understand the cancer genome as global and interconnected "biological systems" that contribute to the pathogenesis of cancer. This Division has also organized the facility and developed new informatics methodologies for the analysis of a next-generation high-performance sequencer.

Whole Genome Sequencing Analysis of Liver Cancer and the International Cancer Genome Project

Thirteen countries including Japan participated in the International Cancer Genome Consortium to generate a comprehensive, high-resolution catalog of genomic changes for major cancer types world-wide. The National Cancer Center has joined this consortium and the Division of Cancer Genomics has taken the initiative in the execution of this international project as a representative research group to analyze virus-associated liver cancer (Figure 1).

Massively parallel sequencing of short-insert genomic libraries of a primary hepatitis C virus-positive hepatocellular carcinoma and matched lymphocytes identified a characteristic mutation signature and potential driver gene candidates (1) (Figure 2). Whole genome sequencing of 10 additional hepatitis B and C virus-associated HCC cases revealed the significant influences of diverse environmental and genetic backgrounds on the somatic mutation patterns and an important role of epigenetic remodeling by genetic alterations in liver carcinogenesis.

Whole Exon Sequencing Analysis of Metastatic Breast Cancer and Other Tumors

Metastasis is the major cause of therapeutic failure and death in cancer patients. The status of metastasis to the axillary lymph nodes is an important prognostic factor in patients with breast cancer. A whole exome sequencing (WES) of three trios of primary breast cancers, their matched noncancerous tissues and lymph node metastatic tumors identified 7 nonsynonymous mutations specific to primary tumors, 4 mutations specific to metastatic tumors and 4 mutations observed in both. This suggests that heterogeneous genetic alterations may occur during the metastatic process in individual breast cancers. WES analysis of hematological cancers and other solid tumors are in progress.

Analysis of Fusion Genes in Lung Cancer

EML4-ALK is a novel transforming fusion product in lung cancer and recent clinical trials for ALK inhibitors reported promising results. The characteristic histopathological features of ALK-positive lung cancer were identified.

To explore molecular genetics and identify new molecular targets in lung cancer, combined sequencing analysis of whole-genome and transcriptome was performed. We identified a novel tyrosine kinase fusion gene which showed oncogenic activity *in vitro* and *in vivo*.

Genome-wide Search for EZH2 Targets in Triple Negative Breast Cancer (TNBC)

Polycomb group proteins, including EZH2, play a master regulatory role in maintaining cancer stem cell population, cell proliferation and metastasis. The primary activity of the EZH2 protein complex is to trimethylate histone H3 lysine 27 at target gene promoters. EZH2 is up-regulated in a broad range of solid human malignancies including TNBC, whose effective therapy remains to be determined. Genome-wide analysis by chromatin immunoprecipitation coupled with sequencing (ChIP-Seq) identified novel EZH2 targets in TNBC cells, which may include potential therapeutic targets.

New Bioinformatics Analysis Pipelines for Cancer Genomics

We developed new algorithms to classify small RNA from RNA sequencing data and applied them to uncover the epigenetic roles of piRNA (2, 3). To support analysis of the huge amount of data generated by a next-generation sequencer, we developed original quality check programs and pipeline programs to efficiently detect somatic mutation, rearrangement, fusion gene, copy number alteration and substitution pattern in cancer genome and transcriptome sequencing. We also estimated the optimum sequence depth for detecting mutations by WES.

Identification of Novel Cancer-related Genes and Their Biology for Translational Research

A resequencing analysis of primary melanoma samples identified somatic *IDH1/2* mutations, which co-exist with *BRAF* and *KIT* mutations (4). Recurrent mutations of *NRF2*, a novel oncogene, in esophageal cancers were associated with therapeutic resistance (5). MET overexpression was a prognostic factor in cholangiocarcinoma (6).

During the EMT process, TGF-beta induced isoform switching of FGF receptors, causing the cells to become sensitive to FGF-2 and combined TGF-beta and FGF-2 stimulation promoted the invasion of cancer cells. TGF-beta and FGF-2 may cooperatively regulate EMT in the cancer microenvironment (7).

Predisposition to cancer is a primary feature of Li-Fraumeni syndrome (LFS). We identified oncogene amplification including BIRC2/3 and TRIB1 along with TP53 missense mutation in LFS-associated tumor (8).

Diagnostic Pathology Research at the Center Hospital

Collaborative clinico-pathological researches have been conducted as a pathologist concurrent in the Division of Clinical Laboratory in the Center Hospital (9).

Figure 1

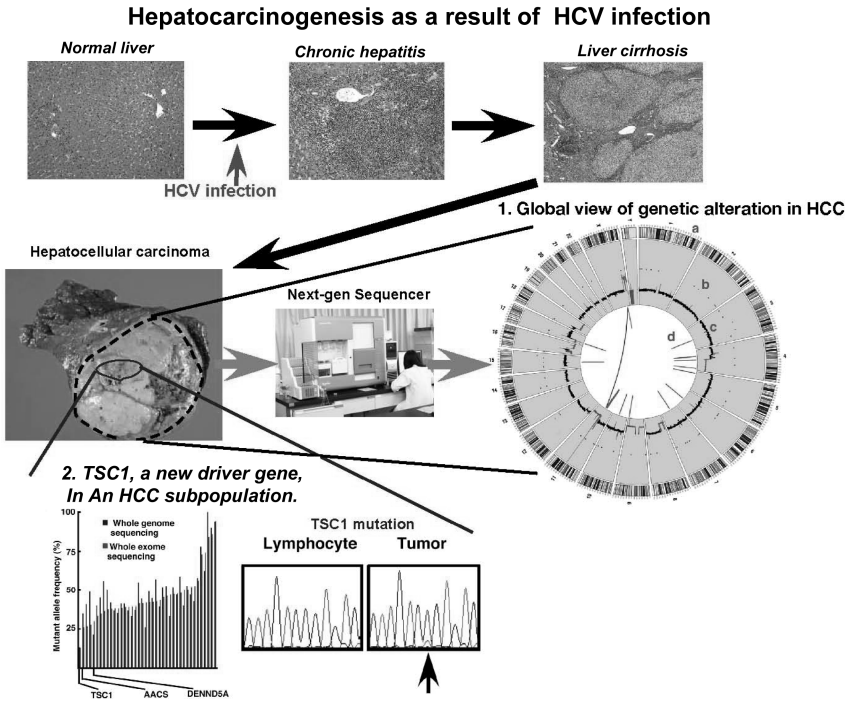

Figure 2

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DIVISION OF CANCER PATHOPHYSIOLOGY

Yasuhito Uezono, Seiji Shiraishi, Kiyoshi Terawaki, Masami Suzuki, Kanako Miyano, Junko Ezuka, Mari Sakamaki, Yuka Sudo, Katsuya Morita, Koichiro Minami, Shun Muramatsu, Tohru Yokoyama, Junichi Ogata, Shiro Tomiyasu, Masato Fukutake, Katsuya Ohbuchi, Naoyo Motoyama, Yohei Kashiwase, Naofumi Oyanagi, Maho Ashikawa, Tempei Miyaji, Miki Inoue, Atsumi Nagasawa

Introduction

Since its establishment in January 2009, the Division of Cancer Pathophysiology has focused on two major research issues regarding 1) improvement of the quality of life of patients with cancer suffering from severe or intolerable pain, and 2) studies on the prevention and development of novel treatment for cancer cachexia. In particular, basic to clinical, and clinical to basic translational interactive collaboration with the Palliative Care and Psychooncology Division in the National Cancer Center Hospital comprises our main research protocols and is now ongoing.

Improvement of Pain Treatment for Patients with Severe and Intolerable Cancer Pain

In the treatment of pain in cancer patients, opioids and related analgesics are mainly and routinely used. However, opioids can prove ineffective in not a few patients. For such patients, several adjuvant analgesics such as anti-convulsants, anti-depressants, anesthetics and anti-arrhythmias are used; they are chosen based mainly on their history of clinical experience. In order to clarify the mechanisms by which adjuvant analgesics have analgesic effects in some particular types of pain, basic research analyses with molecular and cellular biological approaches are conducted in this Division (1, 2, 3, 4).

For instance, voltage-dependent Na^+ channels (Nav) in the peripheral neurons could be involved in certain types of intolerable pain. Accordingly, one of our ongoing studies involves elucidating the mechanisms as to how Nav is modulated by several drugs or endogenous active agents (3, 4). In addition, the transient receptor potential (TRP) channel family, especially the TRP Vanilloid channels 1 (TRPV1) and TRP ankyrin 1 (TRPA1) are reported to transduce a large group of of signal such as pain. We also are trying to investigate the mechanisms of the TRP family functions (2). In addition, analysis of the functions of another analgesic, tramadol, is our current research project (1).

Development of Novel Analgesics with Less Tolerance

Although μ-opioid receptors (μOR) are targets for opioid analgesics, they have been modulated by numerous numbers of drugs and anesthetics. We currently examine whether adjuvant analgesics or anesthetics have a direct or indirect effect on μOR-mediated signaling in cell-based studies and models (5, 6).

γ-Aminobutyric acid receptor type B ($GABA_BR$) is expressed in the central and peripheral neurons, and the $GABA_BR$ agonist baclofen has been used as an anti-spasticity agent. Intrathecal baclofen (ITB) therapy is an established treatment for severe spasticity. Recently, ITB therapy has also been recognized as a powerful antinociceptive tool in patients suffering from chronic pain including cancer pain in whom opioids were ineffective. However, long-term ITB therapy results in the development of tolerance, which makes pain control difficult. Such decreased responsiveness to baclofen is due to $GABA_BR$ desensitization, recognized as occurring due to the formation of complexes of $GABA_BR$ and either G protein-coupled receptor kinase 4 (GRK4) or GRK5.

Some reports have shown that the intrathecal administration of the NMDA receptor antagonist ketamine prevents the development of tolerance against morphine. μ-Opioid receptors are associated with GRK2 or GRK3 and the GRKs are involved in desensitization of μ-opioid receptors. In case of $GABA_BR$, desensitization of $GABA_BR$ would be suppressed by the modification of the properties of GRK4 or GRK5. We showed that ketamine was proved to suppress desensitization of $GABA_BR$ signaling by inhibition of a complex formation between $GABA_BR$ and GRK4 and GRK5 (7). Another project on ketamine concerned its suggested efficacy in the treatment of pain for cancer patients with spinal bone metastasis. We established a mouse spinal bone metastasis model and examined whether ketamine was effective for the pain control in this model. Our data suggested that ketamine was in fact effective for the pain control in the mouse spinal bone metastasis model.

Collectively, ketamine could be a candidate to prevent the development of tolerance against ITB therapy (7) as well as for pain control of bone metastasis from cancers.

Study on the Prevention and Effective Treatment of Cancer Cachexia

Cancer cachexia is often observed in patients with advanced cancer, and is characterized by anorexia and weight loss associated with reduced muscle mass and adipose tissue. The prevention and effective treatment of cachexia are important in the management of patients with cancer because cachexia induces increased morbidity and mortality, and impinges on the patients' quality of life. There is also a trend towards lower response rates with the use of chemotherapy in patients with cancer cachexia. The study of cancer cachexia is indispensable to improve the quality of life in cancer patients and is being conducted in this Division. With support from a Grant-in-Aid for the Third-Term Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health, Labour and Welfare, Japan, we demonstrated that a Japanese *kampo* (traditional Oriental medicine) medication, *rikkunshito*, which is well known to improve gastrointestinal motility, potentiated orexigenic peptide ghrelin signaling attenuated cancer anorexia-cachexia and prolonged survival in an animal model of cancer cachexia (8).

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DIVISION OF CANCER STEM CELL

Kenkichi Masutomi, Seii Ohka, Mami Yasukawa, Keita Kinoshita, Yuko Jincho, Naoko Okamoto

Introduction

Research in the Division of Cancer Stem Cells is focused on deciphering the mechanisms that establish and maintain cancer stem cells and to develop a novel approach targeting cancer stem cells. In particular, the Division studies the molecular links between a) telomerase and RNA dependent RNA polymerase b) telomerase and cancer stem cells and c) telomerase and epigenetics.

Telomerase and RNA Dependent RNA Polymerase

Telomerase is a ribonucleoprotein complex that elongates telomeres. Human telomerase (hTERT) acts as an RNA dependent DNA polymerase (RdDP) and synthesizes telomere DNA from a non-coding RNA (ncRNA) template *human TERC* (*hTERC*). We found that in addition to *hTERC*, hTERT binds a second non-coding RNA, *RMRP*, the RNA component of RNase MRP (1), and TERT and *RMRP* form an RNA dependent RNA polymerase (RdRP) and produce double-stranded *RMRP* that can be processed into an endogenous small interfering RNA (siRNA) to regulate *RMRP* expression levels. To have a deeper understanding of the biological functional role of RdRP (TERT), we generated *RMRP* knockout mice. We have confirmed that the phenotypes of *RMRP* null mice are embryonic lethal (2). These observations indicate the possibilities that the hTERT-*RMRP* complex may essential for ontogeny and biological functions.

Telomerase and Cancer Stem Cells

Accumulating evidence suggests that hTERT has activities beyond telomere maintenance, and it is speculated that the constitutive expression of hTERT not only stabilizes telomere length and facilitates cell immortalization but also increases tumor susceptibility and alters stem cell cycling *in vivo* even when telomere lengths are not limiting. We showed that hTERT forms a protein complex with the SWI/SNF component BRG1 and the nucleolar GTP-binding proteins, nucleostemin (NS) or GNL3L, and the complex composed of hTERT, BRG1 and NS or GNL3L participates in the regulation of tumor initiating cells (TICs) phenotypes (Figure 1) (3). We confirmed that the cells that constitutively express NS/GNL3L exhibited increased beta-catenin signaling and elevated MYC, OCT3/4, KLF4, hTERT and TWIST (master regulator of epithelial mesenchymal transition (EMT)) expression. Moreover, cells that constitutively express elevated levels of hTERT, BRG1 and NS/GNL3L exhibit increased CD133 and CD44 expression and enhanced tumorigenicity at limiting cell numbers. These observations indicate that the TERT–BRG1–NS/GNL3L complex is essential for the maintenance of TICs. Since NS contributes to the maintenance of TICs, we hypothesized that NS may act as a predictive marker for recurrence after neoadjuvant chemotherapy. We examined the expression of CD133, CD44, NS, GNL3L, and TWIST with immunohistochemistry in a series of 54 surgically-resected specimens of esophageal squamous cell carcinomas after neoadjuvant chemotherapy. We identified that that high NS proportion, TWIST intensity, and advanced pathological N (lymph nodes) stage significantly correlated with poor relapse-free survival. Moreover, we confirmed that a high NS proportion, strong TWIST intensity, and advanced pathological N stage were significantly correlated with poor recurrence-free survival in a multivariate analysis adjusted for pathological T (tumor) and N stages. In addition, we examined the correlation between NS and TWIST using several human esophageal cancer cell lines. We confirmed that the ectopic expression of NS induced the upregulation of TWIST expression, and we also found that the endogenous NS expression level correlated with the TWIST expression. These observations implicate NS and TWIST as the predictive markers for postoperative recurrence. Our data suggest that the expression level of NS is correlated with clinical prognosis in esophageal cancer patients. It is noteworthy that this is the first clinical attempt to examine the clinical impact of the cancer stem cell factor(s) of NS in esophageal cancer.

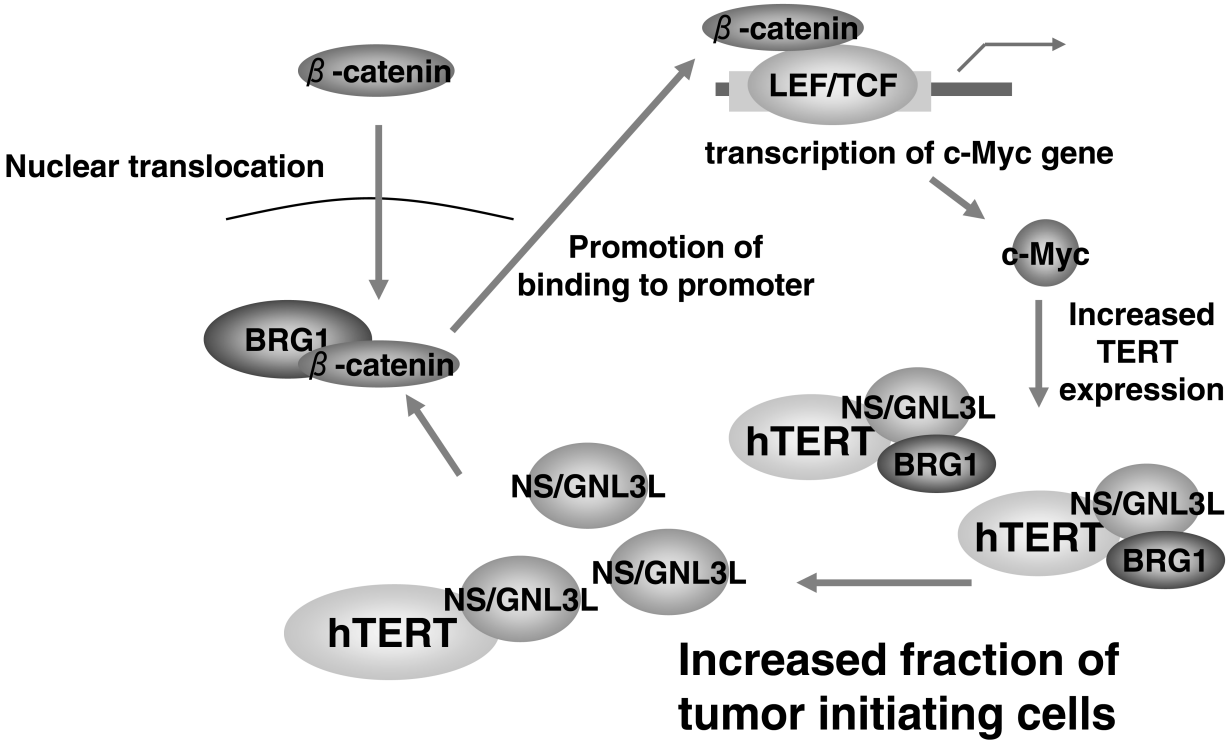

Figure 1

Telomerase and Epigenetics

Functional non-coding RNA is widely involved in the physiology of organisms through its epigenetic regulation. We focus on studying the molecular basis of maintenance of the heterochromatin formation by RNAs, especially by non-coding RNAs such as siRNAs, miRNAs and snoRNAs. It is widely known that epigenetic abnormalities contribute to tumor progression, but the detailed mechanisms are unclear. Since previous studies have shown that hTERT expression reduces the frequency of dicentric chromosomes and suppresses aneuploidy, we speculated that hTERT monitors the genome stability during centromeric heterochromatin maintenance as well as telomere maintenance. Moreover, we have identified that hTERT acts as RdRP, and produces a double-stranded, endogenous siRNA (1). These observations suggest that the mammalian homologue of RdRP (TERT) may regulate heterochromatin formation through its epigenetic regulation, and we are analyzing the link between telomerase and epigenetics.

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DIVISION OF GENE AND IMMUNE MEDICINE

Kazunori Aoki, Naoko Goto, Kouichirou Aida, Koji Suzuki, Kenta Narumi, Takeshi Udagawa, Jun Kimura, Reina Miyakawa, Yuki Yamamoto

Introduction

Research programs in the Division of Gene and Immune Medicine consist of the development of gene and cell therapies for solid cancers based on the analysis of host-immune response against cancer and the development of novel cancer-targeting vectors. In particular, the Division investigates therapeutic methods to inhibit the immune-tolerant environment developed by cancer.

Research Activities

1) Type I IFN Gene Therapy against Solid Cancers

Sarcoma at advanced stages remains a clinically challenging disease. Interferons (IFN) can target cancer cells via multiple antitumor activities including the induction of cancer cell death and enhancement of the immune response. Although the delivery of IFN protein is insufficient and/or results in an unsustainable level in the tumor site in conventional regimens with recombinant IFN proteins, the gene transfer approach allows an increased and sustained local concentration of IFN in the target sites with minimal leakage of the cytokine into the systemic blood circulation. The Division demonstrated that a type I IFN gene transfer significantly suppressed the cell growth of various sarcoma cell lines, and that IFN-β gene transfer was more effective in inducing cell death than was IFN-α in sarcoma cells. Then, to examine the antitumor effect *in vivo*, human sarcoma cells were inoculated in immune-deficient mice, and a lipofection of an IFN-β-expressing plasmid was found to suppress the growth of subcutaneous tumors significantly (Fig. 1). The treated mice showed no significant adverse events. An intratumoral IFN gene transfer could be a promising therapeutic strategy for sarcoma. Based on these basic data, the Division is planning a Phase I clinical trial on intratumoral injection of IFN-β plasmid/liposome complex in patients with sarcoma at advanced stages in collaboration with the Central Hospital.

2) Combination of Hematopoietic Stem Cell Transplantation and Immune Gene Therapy

Allogeneic hematopoietic stem cell transplantation (HSCT) has proved to be an effective therapeutic approach for several types of leukemia and, recently, has also been applied for solid cancers such as renal and breast cancers. The benefit of the graft-versus-tumor (GVT) effect is, however, often offset by the development of graft-versus-host disease (GVHD). Enhancement of the tumor-specific response of allogeneic HSCT against solid cancers is a major issue in clinical oncology. It is commonly believed that the target antigens for a GVT effect include tumor-associated antigens (TAAs) and minor histocompatibility antigens (mHAs), whereas the targets for GVHD are thought to be mHAs. Therefore, efforts to selectively enhance a donor T cell response to TAAs may provide a means to augment antitumor activity without a concomitant increase in toxicity. The Division showed that a combination of immune gene therapy (intratumoral allogeneic major histocompatibility complex gene transfer) can augment the systemic antitumor activity of allogeneic HSCT without exacerbating GVHD (1). The Division also recognizes the utility of autologous HSCT due to the lack of GVHD and independence of donor availability. It is reported that lymphopenia-induced homeostatic proliferation (HP) of T cells after autologous HSCT is driven by the recognition of self antigens, and there is an opportunity to skew the T-cell repertoire during the T-cell recovery by engaging TAAs, leading to an induction of tumor immunity. However, HP-driven antitumor responses gradually decay in association with tumor growth. Type I IFN has important roles in regulating the innate and adaptive immune system. The Division showed that an intratumoral IFN-α gene transfer resulted in marked tumor suppression when administered in the early period of syngeneic HSCT, and was evident even in distant tumors that were not transduced with the IFN-α vector. The intratumoral IFN-α gene transfer creates an environment strongly supporting the enhancement of antitumor immunity in reconstituted lymphopenic recipients.

3) Development of cancer-targeting vectors using the peptide-display adenovirus library

Attempts to redirect adenovirus vectors to alternative receptors by engineering the capsid-coding region have shown limited success because proper targeting ligand-receptor systems on the cells of interest are generally unknown. To overcome the limitation, the Division has developed a direct selection method of the targeted vector from a random peptide library displayed on the adenoviral fiber knob. However the library constructed in the primary method contains residual adenovirus vectors displaying no peptide, which may disturb the extensive exploration of cancer-targeting vectors. To establish more efficient screening methods, the Division is constructing novel adenovirus libraries, which eliminate unnecessary expansion of adenovirus vectors displaying no peptide.

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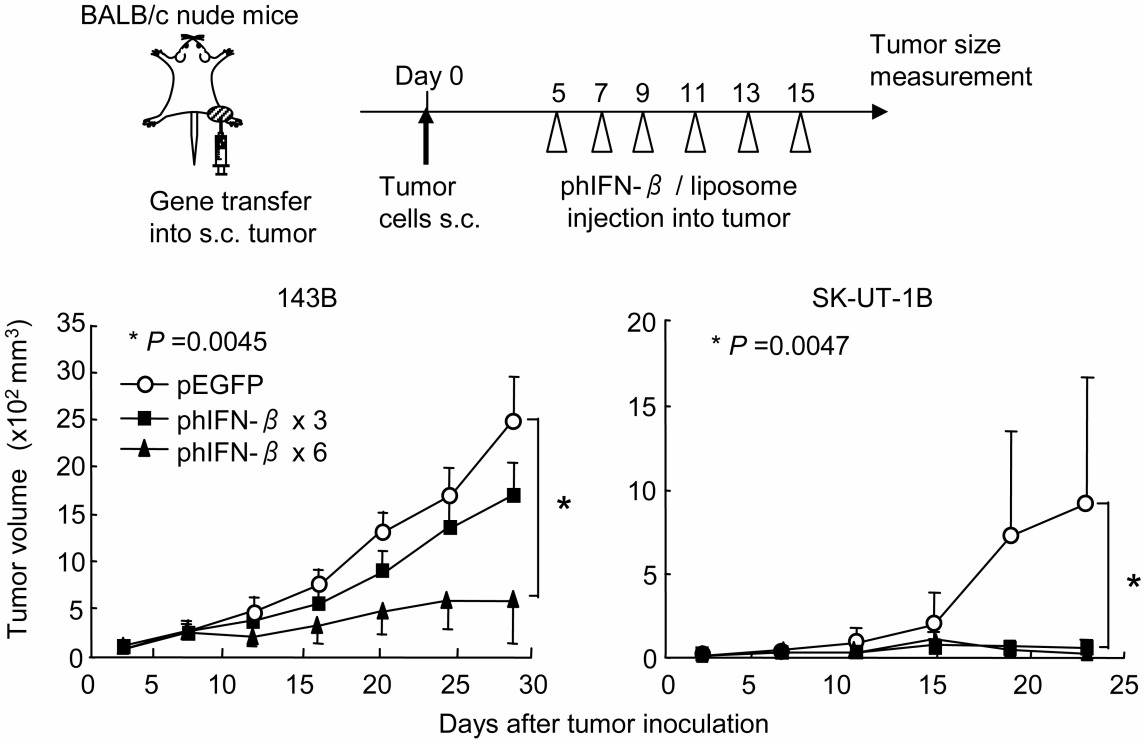

Figure 1

DIVISION OF GENOME STABILITY RESEARCH

Mitsuko Masutani, Ken-ichi Yoshioka, Hiroaki Fujimori-Sakuma, Takahisa Hirai, Anna-Margareta Rydén, Tsubasa Sekiguchi, Hiromi Harada, Kumiko Kinoshita, Junhui Wang, Soichiro Saito, Yuko Atsumi, Yuko Kudo, Tomoyuki Osawa, Hiroaki Mukai

Introduction

The multiple mechanisms for the maintenance of genomic stability contribute to cancer suppression. DNA repair pathways and cell cycle checkpoint systems are the main body of the DNA damage response (DDR) system. Functional studies of the mechanisms for the maintenance of genomic stability are conducted focusing on the poly(ADP-ribosylation) reaction, one of the dynamic post-translational modifications, and key proteins involved in DDRs (1, 2). Elucidation of the DDR is also important in developing novel strategies in chemotherapy and radiation therapy for cancer. The Division started collaborative research with clinical researchers inside and outside of the NCC to develop innovative strategies for chemotherapy and radiation sensitization.

Involvement of PARP-1 in Epigenetic Regulation and Trophoblast Differentiation

Parp-1 deficient embryonic stem cells (ESCs) showed induction and acceleration of trophoblast differentiation of ESCs. *Parp-1* deficient ESCs showed alteration in methylation profiles of the particular CpG islands, including hypomethylation in *H19/Igf2* ICR. Dnmt1 dysregulation and histone H3 acetylation was suggested to be involved in the epigenetic conversion and *H19* transcriptional activation of the ICR in *Parp-1* deficient ESCs. This work, presented by one member of the Division staff (H. F.), was awarded the Koichi Suzuki Memorial Award from the Japanese Biochemical Society.

Cellular DDR Mechanism Suppressing Mutations

Poly(ADP-ribose) polymerase (PARP)-1 is a nuclear enzyme that promotes base excision repair and DNA strand break repair. *Parp-1*$^{-/-}$ mice showed increased frequencies of deletion mutations after treatment with alkylating agents or aging. This could contribute to the augmented susceptibility to carcinogenesis. Using a reconstituted repair assay system, it was shown that processing from 5′-blocked double-strand breaks caused by dephosphorylation was enhanced in the absence of Parp-1, which possibly leads to deletion-type mutations (Fig. 1). Characterization of tumorigenic mutations in a representative tumor suppressor gene, p53 gene, was also analyzed (2).

Radiosensitization by a PARP Inhibitor to Low and High LET Radiation

There are a limited number of factors known to induce sensitization to charged particle radiation. The radiosensitization effect of PARP on low and high linear-energy-transfer (LET) radiation was studied. Treatment of cells with a PARP inhibitor enhanced the effect of γ-, LET 13 and LET 70 carbon-ion irradiation. The mechanism underlying the sensitization effect of PARP inhibitors on γ- and carbon-ion irradiation was a local delay in DNA double-strand break processing. These results suggested that PARP inhibitors might be applicable to a wide therapeutic range of LET radiation through their effects on the DNA damage response. This work, presented by a Division Research Resident (T. H.), was awarded the Radiation and Cancer Biology 1st place Poster Viewing Recognition Award at the American Society for Radiation Oncology in Miami, Florida.

Function of PARG in DNA Damage Response and Cell Death Regulation

Poly(ADP-ribose) glycohydrolase (PARG) is the main enzyme involved in poly(ADP-ribose) degradation. *Parg* and poly(ADP-ribose) polymerase-1 (*Parp-1)* deficiency on ES cell sensitivity to low and high LET radiation was assessed. *Parg*$^{-/-}$ ES cells were more sensitive to γ-irradiation compared to *Parp-1*$^{-/-}$ cells. *Parg*$^{-/-}$ cells also exhibited sensitization to carbon-ion irradiation, whereas *Parp-1*$^{-/-}$ ES cells did not. A further study suggested that *Parg* deficiency sensitizes mouse ES cells to low and high LET radiation through effects on the DNA damage

response and enhanced cell death.

PARG deficiency also causes enhanced cytotoxicity to methylmethanesulfonate (MMS) in mouse ES cells and human cancer cell lines. The mechanism of cell death elevation in these different cell types was characterized. *Parg*$^{-/-}$ ES cells mainly underwent caspase-dependent apoptosis. In *PARG* knocked-down MIAPaCa2, a human pancreatic cancer cell line, enhanced necrotic cell death with augmented HMGB-1 secretion was observed, indicating that different cell death pathways were augmented. This study implies that the functional inhibition of *PARG* may be useful for sensitization of particular cancer cells to alkylating agents.

Arf/p53 Dependent H2AX Diminution and Quiescent Cellular State

Normal cells, both *in vivo* and *in vitro*, attain a growth-arrested state after serial cell proliferation, during which cells are led to a quiescent state but are simultaneously subjected to the risk of developing immortality with genomic instability and mutations, such as in the Arf/p53 module. However, it is still unclear how the cells are regulated for the preservation and the abrogation of cellular homeostasis and how the Arf/p53 module acts in these steps. Addressing these issues, our study showed that a growth-arrested cellular status is produced with histone H2AX diminution in normal cells under the regulation of the Arf/p53 module (Fig. 2) (1). Normal mouse embryonic fibroblast cells undergo growth-arrested status with diminished H2AX only through p53 regulation. Such quiescent status is preserved with genome stability, but is abrogated under continuous growth stimulation because of the induction of DNA replication stress-associated lesions with the resulting genomic destabilization. Although the cells with genomic instability initially remain growth-arrested, immortalized cells eventually appear and become predominant because the Arf/p53 module is mutated with the consequence of genomic instability, resulting in the recovery of H2AX and growth activity. Thus, although cellular homeostasis is preserved under quiescence with genome stability, genomic destabilization induced under growth stimulation disrupts the homeostasis and triggers immortality acquisition.

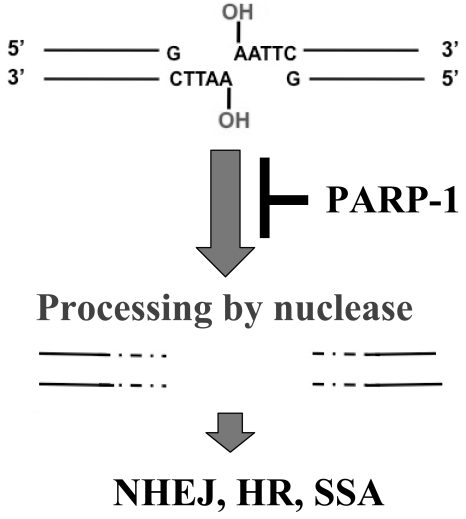

Fig. 1. PARP-1 has been suggested to suppress excessive processing from 5'-blocked termini during double-strand break repair.

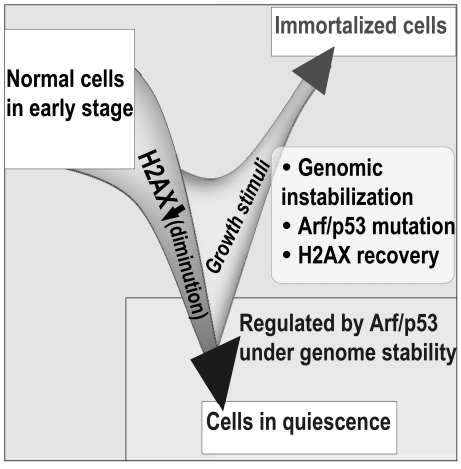

Fig. 2. Life cycle of normal cells, undergoing a growth-arrested phase and developing immortality.

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DIVISION OF CHEMOTHERAPY AND CLINICAL RESEARCH

Tesshi Yamada, Masaya Ono, Kazufumi Honda, Miki Shitashige, Mari Masuda, Nami Miura, Ayako Mimata, Masahiro Kamita, Tomoko Umaki, Ayako Ikarashi, Yuka Nakamura, Miwako Matsuda, Hiroko Ito, Haruyo Tozaki, Akihiko Miyanaga, Takafumi Watanabe

Introduction

Cancer is a genetic disease, but the biological behaviour of cancer is directly regulated by protein quantity, protein post-translational modifications, and protein-protein interactions. These alterations can serve as direct targets for diagnosis and therapy (1, 2). With the aim of discovering molecular biomarkers for early diagnosis and therapy personalization, comprehensive genomic and proteomic analyses of cancer cell lines and clinical samples have been undertaken at the Division of Chemotherapy and Clinical Research.

Combined Functional Genome Survey of Therapeutic Targets for Clear Cell Carcinoma of the Kidney

We adopted a combined functional genomic approach to catalogue potential therapeutic target molecules for clear cell (CC) renal cell carcinoma (RCC). We first selected genes up-regulated in CCRCC relative to surrounding normal kidney tissues in 10 patients using Exon Arrays that detect all potential transcripts predicted in the human genome. The selected genes were subjected to functional screening using small interfering RNA (siRNA) in six CCRCC cell lines. We finally extracted 33 genes over-expressed in CCRCC and required for maintaining cell proliferation in RCC cell lines (3).

Revision of 2-dimensional Image Converted Analysis of Liquid Chromatography and Mass Spectrometry (2DICAL)

Shotgun proteomics has recently attracted considerable attention because of its comprehensive protein identification capacity: protein samples are enzymatically digested into a large array of peptides with uniform physical characteristics, and every peptide is analyzed with low-speed liquid-chromatography (LC) and high-speed scan mass spectrometry (MS). However, LC/MS have been considered unsuitable for quantitative proteomics because of their relatively poor reproducibility. We reviewed various aspects of LC/MS and established a new quantitative proteome platform, namely 2DICAL (Ono et al., Mol Cell Proteomics, 2006). We further refined the algorithm for peak picking and alignment and made 2DICAL ready for large-scale comparative plasma proteomics studies.

We compared the relative quantity of a total of 94,803 peptide peaks between 31 colorectal cancer patients and 59 age/sex-matched healthy controls using the new version of 2DICAL and identified the ninth component of complement (C9) as a novel diagnostic biomarker for colorectal cancer (4).

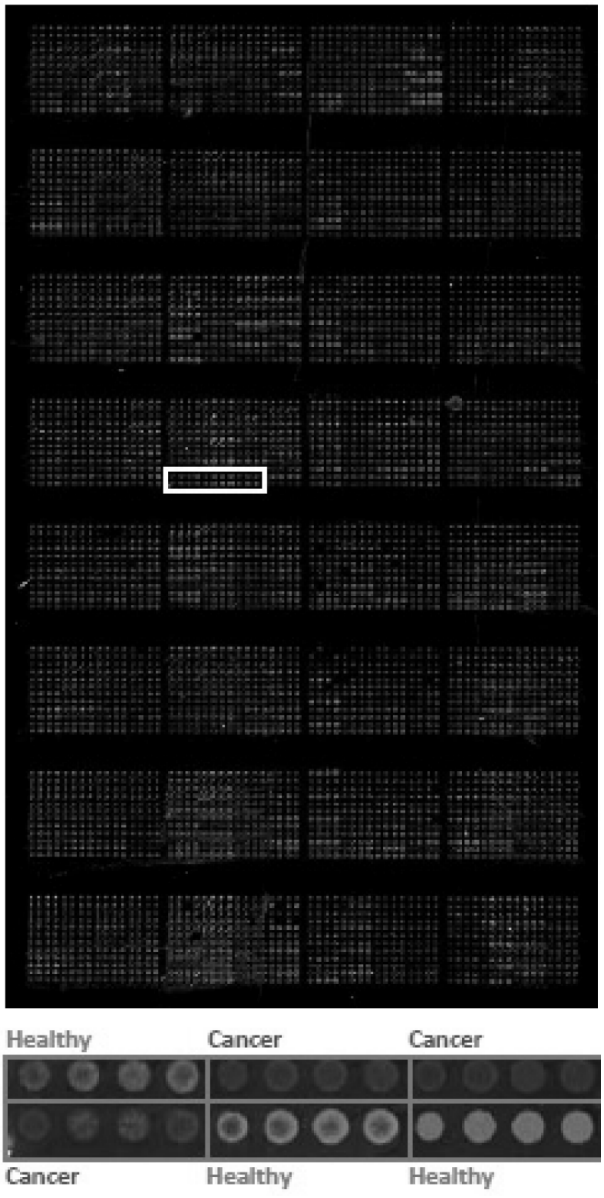
Hollow-fiber-membrane (HFM)-based Low-molecular-weight Protein Enrichment

The proteomics analysis of plasma/serum samples has been hampered by the prominence of a handful of particularly abundant proteins such as albumin and immunoglobulin. The efficient depletion of these proteins is essential for the detection of low-abundant biomarker proteins. We combined a method for the pretreatment of serum/plasma using the HFM filtration technique with 2DICAL. HFM filtration employs multistage filtration and cascaded cross-flow processes, enabling fully automated separation of proteins below a predetermined molecular weight. As the more abundant plasma proteins generally have relatively large molecular weights, they can be efficiently eliminated. Using the combination, we identified a significant decrease in the plasma level of CXC chemokine ligand 7 (CXCL7) in patients with pancreatic cancer. (5).

Biomarker Validation by Protein Microarray

Any biomarker candidates identified using proteomic approaches must be validated in a statistically sufficient number of cases and controls using a different quantitative method before they can be considered for clinical application. We developed a new method of large-scale validation

of proteomics data called high-density reverse-phase protein microarrays (RPPM) and validated the selected biomarker candidates in hundreds of subjects (6).



Dual-color scanning image of a reverse-phase protein microarray (RPPM) on which serially diluted plasma samples of cancer patients and healthy controls were randomly spotted in quadruplicate.

The standard sandwich enzyme-linked immunosorbent assay requires two antibodies, which do not interfere with each other, and more importantly requires a relative large volume of samples. Because the supply of clinical materials is often limited, it may not be possible to use hundreds of microliters of precise samples for preliminary experiments. Our high-density RPPM require a minimal sample volume in the order of the nanoliters and only one antibody. RPPM is considered to be an alternative validation method that can determine rapidly the clinical utility of candidate biomarker protein.

Participation in the International Cancer Biomarker Consortium (ICBC)

Plasma and serum samples were collected prospectively from 7 medical institutions in Japan for biomarker discovery and validation. The multi-institutional collaborative study group was organized by the Third-Term Comprehensive Control Research for Cancer and affiliated to the ICBC (http://www.fhcrc.org/science/international_biomarker/).

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CENTRAL ANIMAL / RADIOISOTOPE DIVISIONS

Toshio Imai, Mami Takahashi, Tetsuya Ishikawa, Yoshinori Ikarashi, Masafumi Yamamoto, Kotomi Otsubo, Naoaki Uchiya, Teruo Komatsu, Masashi Yasuda, Manabu Tsuchida, Masahiro Nakashima, Ayami Kawashima, Daiju Mutoh, Susumu Tezuka, Ikuo Onodera, Daisuke Akagi, Shumpei Ohnami

Introduction

The important role of the Central Animal Division is health management of the experimental animals and maintenance of the animal experimentation facility in the National Cancer Center Research Institute. Some researchers and technical staff act also in the Core Facilities for Research and Innovative Medicine, and several support services are provided based on their biological skills, such as reproductive technologies for animal cleaning/embryo-sperm preservation and histopathological techniques for animal tissues. Research activities have focused on studies of chemical carcinogenesis using laboratory animals, the process of graft-versus-host disease using *in vivo* imaging technologies and human induced hepatic stem cells for anti-cancer drug screening.

The Central Radioisotope Division provides advanced technical training and education for researchers in the fields of molecular genetics and radiology. This division is equipped with separate laboratories where registered users can conduct experiments safely with various types of radioisotopes. Research activities have been performed in collaboration with the Division of Genetics and the Division of Gene and Immune Medicine.

Fatty Infiltration in the Pancreas in Association with Invasive Ductal Carcinogenesis in Hamsters and Man

Obesity is associated with pancreatic cancer risk, but the mechanisms of obesity-associated carcinogenesis have not yet been clearly understood. Syrian golden hamsters, which are susceptible to chemical carcinogenesis in the pancreatic ducts, are in a hyperlipidemic state even under normal diet condition. In the BOP-treated hamster model, a high fat diet increased the levels of serum lipids and leptin, and induced severe fatty infiltration in the pancreas with abnormal adipokine production, which may enhance cell proliferation, and promoted pancreatic cancer development (1). The role of fatty infiltration in pancreatic carcinogenesis is being further investigated in human and animal models.

Pancreatic Ductal Carcinogenesis and Epithelial Mesenchymal Transision in Hamsters

The poor prognosis of pancreatic cancer has been attributed to the difficulty in detection of this cancer in its early operable stages, resulting from its aggressive invasive and distant metastatic activities. To clarify the mechanisms of increased motility and invasiveness of pancreatic carcinoma cells, in the context of epithelial to mesenchymal transition (EMT), the expression of Slug was evaluated in early and advanced stage lesions in a BOP-treated hamster model. Immunohistochemical analysis revealed increased Slug expression not only in invasive carcinomas but also in the early stages of carcinogenesis, suggesting an important role of EMT in the aggressiveness of pancreatic carcinomas. In addition, several invasion-associated proteins, such as kallikrein 7, were found to be over-expressed in the early lesions and carcinomas (2). Investigations for therapeutic target molecules relating to EMT are now ongoing.

Mechanisms of Carcinogenesis by Chemicals in Food

Acrylamide (AA) has been reported to be formed via the baking and frying processes and to show genotoxicities and carcinogenicities in rats and mice (3). Some hormone-related organs, such as the thyroid and mammary gland were revealed as the target organs in the rat model. Therefore, carcinogenic mechanisms associated with systemic hormonal dysregulation have been considered. In a recent study, the effects of 78-week AA exposure on carcinogenic target organs were evaluated in hamsters. Benign and malignant tumor incidences were increased in the forestomach but not in hormone-related organs. Further studies are needed to clarify the cause of species difference in the target organs.

In vivo Fluorescence Imaging of Donor Cells after Allogeneic Hematopoietic Stem Cell Transplantation (HSCT)

Visualizing the *in vivo* dynamics of donor cells after allogeneic HSCT could be useful for an understanding of the process of graft-versus-host disease (GVHD). *In vivo* fluorescence imaging using green fluorescent protein (GFP)-transgenic mice allowed visualization of GFP donor cells at the single cell level in the tissues after transplantation. Furthermore, *in vivo* cellular fluorescence imaging is a very useful tool for monitoring individual donor cells and for exploring immunomodulatory reagents for allogeneic HSCT as well as understanding the mechanism of GVHD.

Human Induced Hepatic Stem Cells for Anti-cancer Drug Screening

Gene transfer of OCT3/4, SOX2, and KLF4 could induce human hepatic stem (iHS) cells from the skin or gastric tissues. Expandable iHS cells would have an advantage in practical use. They are similar to human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells in morphology and cell surface antigens. Human iHS cells markedly expressed many hepatic genes; in addition, these cells expressed ES cell-specific genes at an equivalent level. Both hepatic and stem cell marker expressions have been confirmed by immunocytochemistry. Human hepatocytes derived from such stem cells would be useful for anti-cancer drug screening.

Possible Role of Genes Related to Folate Metabolism and Global DNA Methylation in Pancreatic Carcinogenesis

Global DNA methylation is known to be involved in the process of carcinogenesis. We have previously shown that a common missense SNP of methionine synthasereductase (MTRR) was a novel pancreatic cancer susceptibility factor. MTRR is an enzyme involved in folate metabolism, and serves as a molecular chaperone for MTR. We thereafter identified a thymidilate synthase (TYMS), a folate dependent enzyme, using a gene-gene interactions analysis based on a data mining method (multifactor-dimensionality reduction) for SNPs in the same study population. Knockdown of MTRR or TYMS expression by siRNAs reduced the global DNA methylation level measured by a methyl-CpG content assay in MIAPaCa-2 cells, in which both genes are highly expressed, as compared to the negative control. It is plausible that the polymorphisms and expression of the genes related to the folate metabolism are involved in pancreatic carcinogenesis through modulation of the methylation status.

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DIVISION OF REFRACTORY CANCER RESEARCH

Hitoshi Nakagama, Masato Enari, Rieko Ohki, Yuko Hibiya, Yukie Aita, Yosuke Ohsawa, Chihiro Otsubo, Ryo Otomo, Makoto Miyazaki, Yoshinori Asano, Issei Ezawa, Kozue Saito, Mayuko Matsumoto

Introduction

Our main focus is to clarify the molecular mechanisms of tumor progression in refractory cancers including lung cancers, pancreatic cancers and brain tumors, and to develop various novel therapeutic strategies for cancer prevention. In particular, the Division studies how cancer cells acquire invasiveness, metastatic activity and drug resistance, which are characteristics of refractory cancers. For this purpose, the functional analyses of a tumor suppressor gene p53, which plays a central role in regulating tumor progression, have been studied using cellular and molecular biological techniques. The specific activities in 2011 were as follows: 1) p53 inactivation and cell-surface proteins during tumor progression in lung cancer; 2) The inhibitory mechanism of p53 function by anaplastic lymphoma kinase (ALK); 3) Involvement of cancer susceptibility polymorphism of p53 at codon 72 in phosphorylation and degradation of the p53 protein.

p53 Inactivation and Cell-surface Proteins During Tumor Progression in Lung Cancer

In lung cancer progression, p53 mutations are often observed more in invasive tumors than in non-invasive tumors, suggesting that p53 is involved in tumor invasion and metastasis. For the understanding of the nature of the function of p53 as a tumor suppressor, it is crucial to elucidate the detailed mechanisms of the alteration in epithelial cells, the main origin of solid tumors, following p53 inactivation. Using immortalized small airway epithelial cells (SAEC) from human lung, many genes altered by p53 inactivation were identified. Among them, two up-regulated genes, *epithelial membrane protein 2* (*emp2*) and *tetraspanin 2* (*tspan2*), encoding a cell-surface protein, were selected because such proteins have emerged as key factors in invasion and cell motility, and may be utilized as targets for cancer therapy such as antibody therapeutics and nucleic acid drugs. Various functional analyses reveal that these cell-surface proteins are involved in cell motility and invasion elicited by p53 inactivation and highly expressed in lung cancer cells with the p53 mutation. Down-regulation of these cell-surface proteins by RNAi suppresses metastasis to the lung organs in immuno-deficient mice, causing prolonged survival. Furthermore, these cell-surface proteins bind to CD44, which is known to be a cancer-initiating cell marker, and regulate the production of reactive oxygen species (ROS) through the modulation of CD44 function. These data suggest that cell-surface proteins induced by p53 inactivation enhance cell motility and invasiveness through the sustained anti-oxidant system to scavenge intracellular ROS (Figure 1).

The Inhibitory Mechanism of p53 Function by Anaplastic Lymphoma Kinase (ALK)

In anaplastic large cell lymphoma, p53 mutations are rarely detected, suggesting that the p53 pathway is inactivated by negative regulators. Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase involved in tumorigenesis and the translocations of the ALK gene locus to various gene loci have recently been identified in many cancers including lung and renal carcinomas. Although ALK-fusion proteins are important for oncogenesis, the relationship between ALK and p53 is poorly understood. Here, we found that oncogenic ALK-fusion proteins inhibit p53 transactivation and that the inhibition of the p53 pathway by ALK is required for its kinase activity. Moreover, three tyrosine residues on p53 are phosphorylated by ALK to inactivate p53 function (Figure 2). This is the first finding of tyrosine phosphorylation on p53 in the world.

Involvement of Cancer Susceptibility Polymorphism of p53 at Codon 72 in Phosphorylation and Degradation of p53 Protein

The common polymorphism of p53 at codon 72, either encoding proline or arginine, has drawn attention for the last 2 decades as a genetic factor associated with clinical outcome or cancer risk. We now show that these two polymorphic variants differ in protein structure, especially within the

N-terminal region and, as a consequence, differ in post-translational modification at the N terminus. The arginine form (p53-72R) shows significantly enhanced phosphorylation at Ser-6 and Ser-20 compared with the proline form (p53-72P). We also show diminished Mdm2-mediated degradation of p53-72R compared with p53-72P, which is at least partly brought about by higher levels of phosphorylation at Ser-20 in p53-72R. Furthermore, enhanced p21 expression in p53-72R-expressing cells, which is dependent on phosphorylation at Ser-6, was demonstrated. Differential p21 expression between the variants was also observed upon activation of TGF-β signaling (1). Collectively, we demonstrate a novel molecular difference and simultaneously suggest a difference in the tumor-suppressing function of the variants.

Published Papers

1. Ozeki C, Sawai Y, Shibata T, Kohno T, Okamoto K, Yokota J, Tashiro F, Tanuma S, Sakai R, Kawase T, Kitabayashi I, Taya Y, Ohki R. Cancer susceptibility polymorphism of p53 at codon 72 affects phosphorylation and degradation of p53 protein. J Biol Chem, 286:18251-18260, 2011

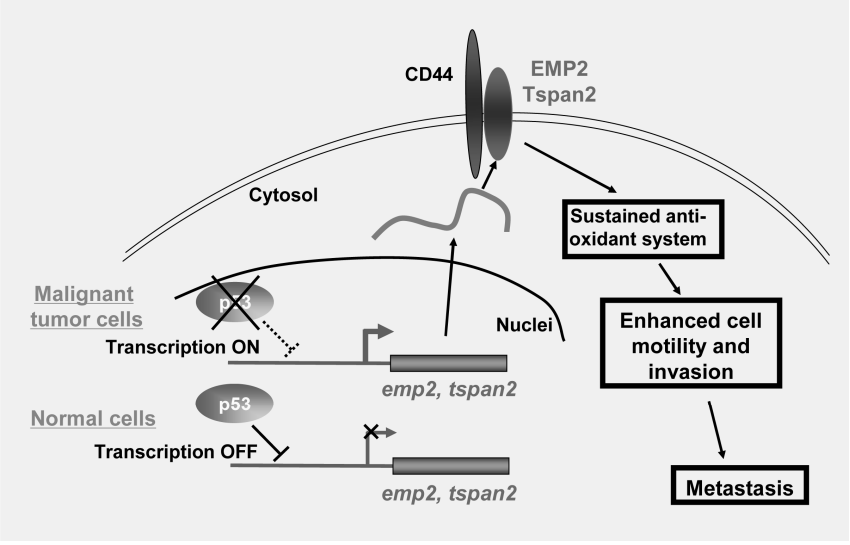

Figure 1. A model of malignant transformation by p53 inactivation

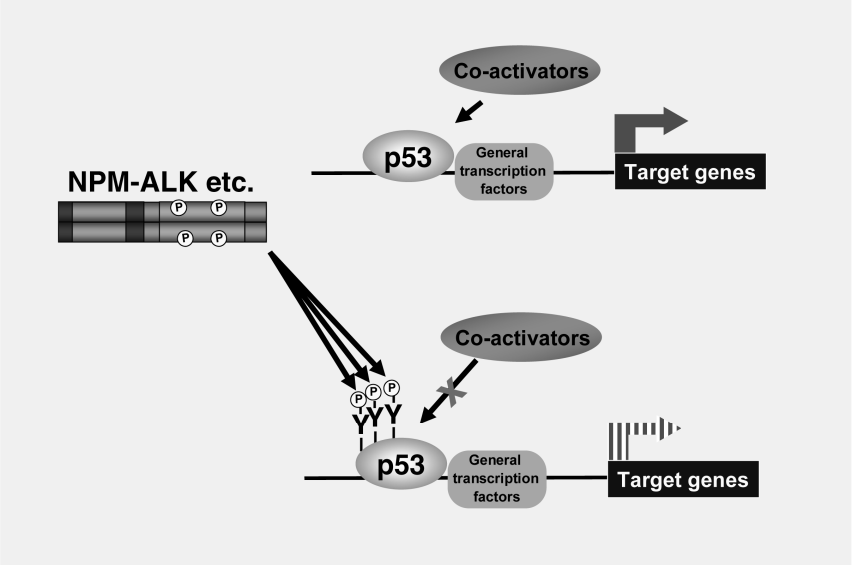

Figure 2. A model of the inhibitory mechanism of p53-mediated transcription by ALK-fusion proteins

DIVISION OF CANCER PREVENTION RESEARCH

Hitoshi Nakagama, Michihiro Mutoh, Gen Fujii, Masafumi Yamamoto

Introduction

Obesity and abnormal lipid metabolism are associated with development of many cancers, including colon and pancreas cancer. Dyslipidemia, alterations of adipocytokine balance and pro-inflammatory status were suggested to be involved in the development of colon and pancreatic cancer. In animal studies, improvement of dyslipidemia and an abnormal adipocytokine balance suppressed both colon and pancreas carcinogenesis. However, underlying suppressive mechanisms are not known in detail, such as lipid metabolism changes in the cancer cells and cross-talk changes between the epithelial cells, adipocytes and macrophages. Thus, we are investigating the mechanisms of obesity- and dyslipidemia-related carcinogenesis in the colon and pancreas to develop effective approaches for human cancer prevention.

Molecular Targets for Cancer Prevention and the Search for a Chemopreventive Agent against Colon Cancer

Obesity is a risk factor for human colorectal cancer development. A colorectal carcinogenesis study using obese KK-A^y mice revealed that the KK-A^y mice are highly susceptible to azoxymethane (AOM)-induced colorectal aberrant crypt foci (ACF) and tumor development. KK-A^y mice showed high serum Pai-1, leptin, IL-6 levels and low adiponectin levels (1). Visceral fat accumulation and low plasma adiponectin levels are reported to be associated with development of human colorectal tumors. Thus, we introduced an adiponectin-knockout mutation into *Min* mice which resulted in an increased total number of intestinal polyps compared with those of adiponectin-wild *Min* mice (2). Moreover, the incidences of AOM-induced tumors in C57BL/6J mice with each genotype, adiponectin (+/-) and (-/-), increased the incidences of colon tumors. Among serum adipocytokines, the levels of serum Pai-1 increased with adiponectin-deficiency. AOM-induced colorectal ACF in adiponectin-deficient mice decreased with administration of a Pai-1 blocker suggesting that adiponectin and its receptors might be good targets for colon cancer chemopreventive agents (3).

Published Papers

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2. Mutoh M, Teraoka N, Takasu S, Takahashi M, Onuma K, Yamamoto M, Kubota N, Iseki T, Kadowaki T, Sugimura T, Wakabayashi K. Loss of adiponectin promotes intestinal carcinogenesis in *Min* and wild-type mice. Gastroenterology, 140:2000-2008.e2, 2011

3. Fujii G, Yamamoto M, Takahashi M, Mutoh M. Role of adpicytokines in colorectal carcinogenesis. Curr Res in Cancer, 5:39-48, 2011

DIVISION OF INTEGRATIVE OMICS AND BIOINFORMATICS

Hitoshi Nakagama, Tsutomu Ohta, Masaru Katoh, Mamiko Miyamoto, Kohtaro Oda, Yuuki Yamamoto, Teruaki Tsuji, Saho Kawamoto

Our Division, consisting of Ohta's and Katoh's Units, contributes to the development of innovative cancer diagnosis and treatment based on an integrative omics approach.

Ohta's Unit

Roles of Nrf2 in Lung Cancer

Oxidative and electrophilic stresses are sensed by Keap1, which activates Nrf2 to achieve cytoprotection by regulating the expression of drug-metabolizing and anti-oxidative stress enzymes/proteins. Since oxidative and electrophilic stresses cause many diseases including cancer, an abnormality in the Nrf2-Keap1 system may provide advantages for the growth of cancer cells. Many synonymous somatic *KEAP1* gene mutations and lower expression of *KEAP1* were identified in lung cancer. In cancer cells, enfeebled Keap1 activity due to the mutations or low-level expression led to nuclear localization and constitutive activation of Nrf2, which resulted in constitutive expression of cytoprotective genes encoding multi-drug resistance pumps, phase II detoxifying enzymes and anti-oxidative stress enzymes/proteins. Up-regulation of these target genes in lung cancer cells led to resistance to anti-cancer drugs. Nrf2 activation also provided growth stimulation in lung cancer-derived *KEAP1*-lower expression and -mutant cell lines and in *Keap1*-null mouse embryonic fibroblasts under homeostatic conditions. These results suggest that inhibition of *NRF2* may provide a new direction for therapeutic approaches in lung cancers with activation of Nrf2. The search system for Nrf2 inhibitors was developed.

Roles of SYT-SSX in Synovial Sarcoma

Chromosomal translocations are frequently associated with soft tissue sarcomas. Fusion proteins generated by such translocations often play critical roles in tumorigenesis. Therefore, it is important to understand the function of the fusion protein to develop therapeutic interventions. The t(X;18)(p11.2;q11.2) translocation found in synovial sarcomas results in a fusion between the SYT gene on chromosome 18 and an SSX gene on the X chromosome. Although SYT-SSX fusion proteins appear to trigger synovial sarcoma development, little is known about the downstream targets of SYT-SSX. The SYT-SSX fusion protein produces a dominant-negative function for the SYT, which is a transcriptional co-activator. To search for the downstream targets of SYT-SSX, the gene expression profiles in SYT-SSX-knockdown SYO-1 cells with a microarray were analyzed. The expression levels of about three hundred genes were increased in the SYT-SSX2-knockdown SYO-1 cells.

Roles of hTERT in Cancer

Telomerase, a ribonucleoprotein enzyme that maintains telomere length, is crucial for cellular immortalization and cancer progression. Telomerase activity is attributed primarily to the expression of telomerase reverse transcriptase (TERT). The gene responsible for the regulation of hTERT transcription was identified with a microarray (1).

Katoh's Unit

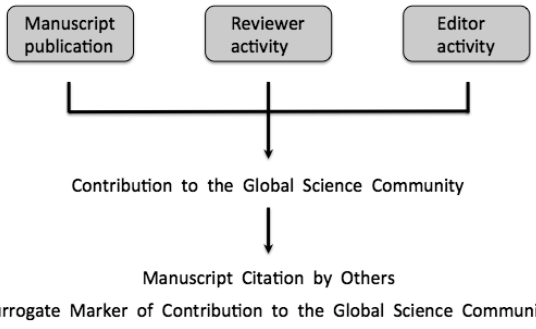
Stem Cell-signaling Network Project

Canonical WNT signaling activation leads to transcriptional up-regulation of the FGF, Notch and non-canonical WNT ligands, the WNT and TGFβ antagonists, and MYC (2). Hedgehog up-regulates the Notch ligand, WNT antagonist, BMP antagonists, and MYCN. TGFβ up-regulates the non-canonical WNT ligand, CDK inhibitors, and NANOG, while BMP up-regulates the Hedgehog ligand. Based on these mutual regulations, WNT, FGF, Notch, Hedgehog, and TGFβ/BMP signaling cascades constitute the stem-cell signaling network, which plays a key role in the maintenance or homeostasis of pluripotent stem cells and cancer stem cells. Human embryonic stem cells (ESCs) are supported by FGF and TGFβ/Nodal/Activin signals, whereas mouse ESCs by LIF and canonical

WNT signals. The combination of a TGFβ inhibitor and a canonical WNT activator alter the character of human induced pluripotent stem cells (iPSCs) from human ESC-like to mouse ESC-like. Because FGF, Hedgehog, TGFβ, and non-canonical WNT signals synergistically induce EMT regulators, such as Snail (SNAI1), Slug (SNAI2), TWIST, and ZEB2 (SIP1), tumor-stromal interaction at the invasion front aids cancer stem cells to acquire a more malignant phenotype (2). Cancer stem cells occur as mimetics of normal tissue stem cells based on germ-line variation, epigenetic change, and somatic mutation of stem-cell signaling components, and then acquire a more malignant phenotype based on accumulation of additional epigenetic and genetic alterations, and tumor-stromal interaction at the invasion front (2).

Contribution to the Global Science Community

Katoh is contributing to the global science community based on manuscript publication, reviewer activity, and editor activity. Katoh carried out peer review of grant proposals or journal manuscripts written in English 73 times/year. Katoh is an editorial board member of several scientific journals, such as PLoS ONE, the Asia-Pacific Journal of Clinical Oncology, and the International Journal of Oncology. PLoS ONE is an open access journal contributing to "Creative Commons." Katoh made an editorial decision on 100 manuscripts submitted to PLoS ONE in 2011.



Manuscript citation count is a surrogate marker of contribution to the global science community. Katoh's citation count by others was 495 in 2011 (Web of Science Database, Thomson Reuters).

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2. Katoh M. Network of WNT and other regulatory signaling cascades in pluripotent stem cells and cancer stem cells. Curr Pharm Biotechnol, 12:160-170, 2011