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Preface

It is a great pleasure for me to share with you the 24th Scientific Report (2016-2017) of the Aichi Cancer Center Research Institute. Since its establishment in 1964, reports have been published biannually to detail our major research activities and highlight recent progress.

As illustrated on the following pages, the Research Institute consists of 8 Divisions, along with a Central Service Unit and an Animal Facility, which in total include permanent positions for 42 staff researchers and 11 technicians, as well as support for 17 research residents and 9 temporary research assistants. Totals of 6 graduate students and 65 visiting research fellows are also conducting studies under supervision of staff researchers. Our overall research emphasis is on three areas: cancer prevention/epidemiology, preclinical/experimental therapy, and carcinogenesis/molecular biology. The institute is also affiliated with the Nagoya University Graduate School of Medicine and the Nagoya City University Graduate School of Pharmaceutical Sciences, with 7 professors and 6 associated professors nominated from our division chiefs and section heads.

The major areas being pursued are as follows:
- descriptive and analytical epidemiology of cancers
- molecular epidemiology and its application in clinical practice/prevention
- molecular pathogenesis and pathophysiology of lung, colorectal and breast cancers as well as various other malignancies such as mesotheliomas
- molecular mechanisms underlying cancer cell proliferation, motility and metastasis
- basic and applied studies on cancer immunology

More comprehensive descriptions of the individual research topics of each Division appear in the contents of this report.

We are committed to obtaining mechanistic insights into how various human cancers develop, with a clear focus on our major objective "from bench to bedside", and translating our findings into development of novel strategies for better diagnosis, treatment, and prevention.

Finally, we would like to express our deep appreciation to the Aichi Prefectural Government for its continued support since foundation of our institution in 1964. Grants from the Ministry of Education, Science, Sports, Culture and Technology (MEXT), and the Ministry of Health, Labor, and Welfare (MHLW), of Japan, as well as the Japan Agency for Medical Research and Development (AMED), and the Japan Science and Technology Agency (JST), are also gratefully acknowledged.

January, 2018

Takashi Takahashi, M.D., Ph.D.
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- Acting Director: T. Takahashi (as of April 2017)
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Division of Epidemiology and Prevention
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- (To be appointed)

Division of Molecular Oncology
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Division of Molecular and Clinical Epidemiology
- (K. Matsuo)

Division of Immunology
- (K. Kuzushima)

Division of Microbiology and Oncology
- (C. Onyama)

Division of Molecular Pathology
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Division of Biochemistry
- (M. Inagaki, until June 2016)

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- (M. Inagaki, until June 2016; M Aoki, as of July 2016)

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- (C. Onyama)

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SCIENTIFIC REPORTS
From left to right

First row: Dr. H. Nakagawa, Dr. I. Oze, Dr. S. H. Ito, Dr. H. Tanaka, Dr. S. Hosono, Ms. M. Watanabe

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Isao Oze, M.D. Senior Researcher (until March 2017)
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General Summary
The current research activities of the Division of Epidemiology and Prevention cover the following: (1) descriptive epidemiology of cancer incidence, mortality and survival using data from the Aichi Prefectural Cancer Registry and other population-based registries in collaborative studies; (2) analytical epidemiology based on the hospital-based epidemiologic research program at Aichi Cancer Center (HERPACC) to determine risk and protective factors for cancer.

Our main research achievements in 2016 and 2017 were: 1) Determination of trends in incidence of colorectal cancer by anatomical subsites, using data from population-based cancer registries in Japan between 1978 and 2004; 2) Estimation of cancer prevalence in 2012 based on cancer incidence and 5-year survival rate in Aichi Prefecture using data from our population-based cancer registry, the Aichi Cancer Registry, which covers 7.4 million people; and 3) Two case-control studies examining the association between coffee consumption and CRC risk by anatomic subsite among Japanese using data from HERPACC-I and II.

1. Changes in trends in colorectal cancer incidence rate by anatomic site between 1978 and 2004 in Japan
Although colorectal cancer (CRC), a major type of cancer worldwide, has shown a proximal or right-sided shift in subsite distribution in western countries, trends in subsite incidence in Asian countries remain unclear. Here, we evaluated subsite-specific trends in CRC incidence rate between 1978 and 2004 in Japan using a large body of data from 10 population-based cancer registries. The colorectal sites (C18-C20) were categorized into three groups: proximal colon...
(C18.0-C18.5), distal colon (C18.6-C18.7), and rectum (C19.9 and C20.9). Trends in age-standardized incidence rates (ASRs) were characterized by joinpoint regression analysis. A total of 303,802 CRC cases were analyzed. Overall, ASRs increased remarkably until 1993, with an annual percentage change (APC) of 4.9%, and then stabilized thereafter. By subsite, however, ASRs of proximal colon significantly increased, with APCs of 7.1% (1978-1991), 3.8% (1991-1996), and 0.9% (1996-2004); distal colon showed an initial significant increase, with an APC of 7.6%, but stabilized from 1991 until the end of the observation period; and rectal cancer showed an initial significant increase, with an APC of -1.0%. Thus, we revealed that changes in incidence trends for the three anatomic sites apparently began to differ in the 1990s. Careful monitoring is necessary to confirm whether these trends are typical of the Japanese population.

*1Chiba Cancer Center
*2Department of Cancer Therapy Center, Fukui Prefectural Hospital
*3Department of Epidemiology and Public Health, Kanazawa Medical University
*4Department of Epidemiology, Radiation Effects Research Foundation, Hiroshima and Nagasaki
*5Center for Cancer Control and Statistics, Osaka Medical Center for Cancer and Cardiovascular Diseases

2. Cancer Prevalence in Aichi, Japan for 2012: Estimates Based on Incidence and Survival Data from the Population-based Cancer Registry
Nakagawa-Senda, H., Yamaguchi, M., Matsuda, T.,*1, Koide, K.*2, Kondo, Y.*2, Tanaka, H., and Ito, H.
Cancer is the leading cause of death among both men and women in Japan. Monitoring cancer prevalence is important because the relevant data play a critical role in the development and implementation of health policy. We estimated cancer prevalence in 2012 based on cancer incidence and 5-year survival rate in Aichi Prefecture using data from our population-based cancer registry, the Aichi Cancer Registry, which covers 7.4 million people. The annual numbers of incident cases between 2008 and 2012 were used. Data for patients diagnosed in 2006-2008 and followed up until the end of 2012 were selected for survival analysis. Cancer prevalence was estimated from incidence and year-specific survival probabilities and stratified by sex, organ site (25 major cancers), and age group at diagnosis. The estimated total prevalence for all cancers in 2012 was 68,013 cases among men and 52,490 cases among women, with 120,503 cases for both sexes. Colorectal cancer was the most common cancer with 6,654 cases, accounting for 16.0% of overall incident cases, followed by stomach cancer with 5,749 cases (13.8%) and lung cancer with 5,593 cases (13.4%). Prostate cancer was the most prevalent among men, accounting for 21.5%, followed by colorectal and stomach cancers. Breast cancer was the most prevalent among women, accounting for 28.6%, followed by colorectal, stomach, and uterine cancers. This study provided cancer prevalence data that could serve as useful essential information for local governments in cancer management, to carry out more practical and rational countermeasures against cancer.

*1Division of Surveillance, Center for Cancer Control and Information Service, National Cancer Center, Japan
*2Health and Welfare Department, Aichi Prefectural Government, Japan

3. Coffee consumption and risk of colorectal cancer by anatomical subsite in Japan: Results from the HERPACC studies
Nakagawa-Senda, H., Ito, H., Hosono, S., Oze, I., Tanaka, H., Matsuo, K.
Consumption of coffee, a popular beverage worldwide, has been associated with lower colorectal cancer (CRC) risk. Although it is clear that CRC exhibits different biological characteristics by anatomical subsite, the possibly heterogeneous impact of coffee on CRC by anatomical subsite has remained unclear. Here, we conducted two case-control
studies to examine the association between coffee consumption and CRC risk by anatomic subsite among Japanese using data from the Hospital-based Epidemiological Research Program at Aichi Cancer Center I and II (HERPACC-I and II). Subjects were enrolled in HERPACC-I between 1988 and 2000 and in HERPACC-II between 2001 and 2005. Coffee consumption was measured with a self-administered questionnaire. A conditional logistic regression model was used to calculate odds ratios (ORs) for CRC with coffee consumption, adjusted for the potential confounders age, smoking, alcohol drinking, red meat intake, BMI, exercise, family history of CRC, and diabetes mellitus history. We estimated summary ORs by pooling study-specific ORs with a fixed effects model. In total, 2,696 CRC cases and 13,480 non-cancer outpatients as controls were included. Overall, compared to non-drinkers, ORs for less than 1 cup/day, 1-2 cups/day and 3 or more cups/day for CRC were 0.88 (95%CI:0.77-1.00), 0.90 (95%CI:0.80-1.01) and 0.78 (95%CI:0.65-0.92), respectively (trend-P=0.009). Subsite-specific analysis revealed a significant inverse linear trend between coffee consumption and distal colon cancer (P-trend=0.048), and a tendency toward a lower risk of rectal cancer (P-trend=0.068). These findings suggest that coffee consumption might impact the prevention of CRC, especially distal colon cancer.
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First row: Dr. Maho Okuda, Ms. Ikue Hasegawa, Dr. Yoshitaka Sekido, Dr. Yuko Murakami-Tonami, Dr. Satomi Mukai
Second row: Dr. Tatsuhiro Sato, Dr. Ryota Yamagishi, Ms. Miwako Nishizawa, Ms. Haruna Ikeda
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Akihiro Matsushita, M.D., Research Resident (until March 2017)
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Kosuke Tanaka, M.D., Nagoya University Graduate School of Medicine (until January 2017)
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Anna Ogiso, Meijo University (until September 2016)
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Yusa Takabayashi, Kinjo Gakuin University (December 2016–March 2017)
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General Summary
Our goal is to identify genetic lesions and epigenetic alterations associated with the development of human solid cancers and to use this information to develop approaches for the prevention, diagnosis, and treatment of these diseases. Currently, we are focusing on malignant mesothelioma (MM), which develops following asbestos exposure and has a long latent period. MM is very aggressive and highly refractory to conventional therapeutic modalities such as chemotherapy, surgery, and radiation therapy. We are studying the genetics and biology of MM cells using various approaches, including the establishment of cell lines from patient tissues, comprehensive analysis of genetic and epigenetic alterations using next-generation sequencing, and in vitro analyses of cell signaling pathways in MM cells and immortalized mesothelial cells.

We have already identified key tumor suppressor genes in MM cells, including \( NF2 \) and \( LATS2 \). Alterations in these genes cause dysregulation of the tumor-suppressive Hippo signaling pathway, known to play a critical role in various biological functions such as cell proliferation, apoptosis, and organ size control. Inactivation of the Hippo signaling pathway induces constitutive activation of \( YAP/TAZ \) transcriptional coactivators in MM cells and enhances the transcription of their downstream genes, including cyclin D1 (\( CCND1 \)) and connective tissue growth factor (\( CTGF \)). Upregulation of these prooncogenic genes confers a more aggressive malignant phenotype on MM cells by promoting cell cycle progression, extracellular matrix
formation, epithelial-mesenchymal transition, and cytokine production. We are attempting to develop new therapeutic modalities based on NF2-Hippo pathway inactivation in MM cells using several approaches aimed at regulating constitutively activated YAP/TAZ coactivators. Successful regulation of these coactivators may allow us to inhibit MM cell proliferation and progression. We are also trying to target key genes of epithelial-mesenchymal transition, identify genes that result in a synthetic lethal phenotype with an NF2 or LATS2 mutation, and identify vulnerabilities in metabolomic changes caused by the inactivation of these genes. Finally, we are also performing drug screening using our MM cell line panel. We hope that our studies will help develop a promising therapeutic modality against this formidable disease.

1. Statin suppresses proliferation of Hippo pathway-inactivated malignant mesothelioma cells and blocks the YAP/CD44 growth stimulatory axis

Tanaka, K., Osada, H., Murakami-Tonami, Y., Horio, Y.*, Hida, T.*1 and Sekido Y.

Malignant mesothelioma frequently features Hippo signaling pathway inactivation. This is mainly due to NF2 and/or LATS2 mutations, which lead to activation of YAP transcriptional co-activator. In this study, we assessed antitumor effects of statin on MM cells with Hippo pathway inactivation, through the interplay of the mevalonate and Hippo signaling pathways. Statin attenuated proliferation and migration of MM cells harboring an NF2 mutation by accelerating YAP phosphorylation/inactivation (Fig. 1). CD44 expression was decreased by statin, in parallel with YAP phosphorylation/inactivation. Importantly, we discovered that YAP/TEAD activated CD44 transcription by binding to the CD44 promoter at TEAD-binding sites. On the other hand, CD44 regulated Merlin phosphorylation according to cell density and sequentially promoted the YAP transcriptional co-activator, suggesting that CD44 plays two pivotal functional roles as an upstream suppressor of the Hippo pathway and one of the downstream targets regulated by YAP/TEAD. Moreover, the YAP/CD44 axis conferred cancer stem cell (CSC)-like properties on MM cells leading to chemoresistance, which was blocked by statin. Together, our findings suggest that YAP mediates CD44 up-regulation at the transcriptional level, conferring CSC-like properties in MM cells, and statin offers a potential therapeutic option against MM by inactivating YAP.

1Department of Thoracic Oncology, Aichi Cancer Center Hospital

Figure 1. A. Cell proliferation assay. Fluvastatin (FLV), simvastatin and zoledronic acid inhibition of cell proliferation of NCI-H290 in a dose dependent manner. B. Wound healing assay. Treatment with FLV significantly inhibited the cell migration of NCI-H290, whereas geranylgeranyl-pyrophosphate (GGPP) had a moderately accelerating effect.

2. E-cadherin expression correlates with resistance to focal adhesion kinase inhibitor in Merlin-negative malignant mesothelioma cells

Kato, T., Sato, T., Yokoi, K.*1, and Sekido, Y.

Malignant mesothelioma is an aggressive tumor commonly caused by asbestos exposure after a long latent period. Focal adhesion kinase (FAK) inhibitors inhibit the cell growth of Merlin-deficient MM cells, but their clinical efficacy has not been determined.
clearly. The aim of this study was to evaluate the growth inhibitory effect of an FAK inhibitor, VS-4718, on MM cell lines and find biomarkers for its efficacy. Although most Merlin-deficient cell lines were more sensitive to VS-4718 compared with control MeT-5A cells, a subset of the cell lines exhibited resistance to the drug. Microarray and qRT-PCR analyses using RNA isolated from Merlin-deficient MM cell lines revealed a significant correlation between E-cadherin mRNA levels and VS-4718 resistance (Fig. 2). Merlin- and E-cadherin-negative Y-MESO-22 cells underwent apoptosis upon treatment with a low concentration of VS-4718, whereas Merlin-negative E-cadherin-positive Y-MESO-9 cells did not. Furthermore, E-cadherin knockdown in Merlin-negative MM cells significantly sensitized cells to VS-4718 with induction of apoptotic cell death upon VS-4718 treatment. Together, our results suggest that E-cadherin serves as a predictive biomarker for molecular target therapy with FAK inhibitors for mesothelioma patients and that it confers MM cells with resistance to FAK inhibitors.

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Fig. 2. Dot plots showing a significant correlation between the levels of E-cadherin mRNA and IC\textsubscript{50}\textsuperscript{VS-4718} in Merlin-negative MM cells.
From left to right
- First row: Ms. Y. Kasugai, Dr. I. Oze, Dr. K. Matsuo, Dr. H. Ito, Ms. M. Nakata
- Second row: Ms. S. Inui, Ms. K. Hirano, Ms. T. Nishiwaki, Ms. T. Ito, Ms. S. Sato, Ms. I. Hanaoka
- Third row: Dr. S. Inoue, Dr. M. Sawabe, Dr. A. Kropp, Dr. J. Ishiguro, Dr. T. Ugai, Ms. I. Morikawa, Ms. K. Yoshida, Ms. M. Kawaguchi
- Inset: Dr. H. Masaoka, Dr. M. Katayama
Division of Molecular and Clinical Epidemiology

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General Summary
Research in this laboratory is focused on elucidating genetic and molecular bases of human cancer in conjunction with environmental exposures, with a view to applying the obtained knowledge in the areas of clinical oncology and prevention. Currently we are working on molecular epidemiology of cancer and its practical application, with physicians/researchers within and outside Aichi Cancer Center.

Sporadic cancers are consequences of molecular/genetic events after environmental exposure in combination with the genetic background. Elucidating optimal combination of environmental and genetic factors including treatment is essential part of targeted treatment and prevention. In collaboration with the Division of Epidemiology and Prevention, we are trying to: (1) elucidate
new gene-environment interactions between genetic background and environmental factors; (2) develop risk prediction models integrating genetic and environment factors; and (3) apply the developed models in pre- and post-clinical setting.

1. Establishing prediction models for breast cancer using molecular and environmental information.
   Ito, H., Ugai, T., Oze, I., Kasugai, Y., Ishiguro J., Iwata, H., Matsuo, K.

2. Randomized-controlled trial for risk feedback with or without genetic information.
   Ugai, T., Kasugai, Y., Ishiguro J., Oze, I., Iwata, H., Ito, H., Matsuo, K.

   Masaoka, H., Ito, H., Soga, N., Matsuo, K.

4. Heterogeneous impact of alcohol consumption according to treatment method on survival in head and neck cancer: A prospective study.
   Sawabe, M., Ito, H., Oze, I., Kawakita, D., Hasegawa, Y., Matsuo, K.

5. Smoking and subsequent risk of acute myeloid leukemia in Japan.
   Ugai, T., Matsuo, K., Swada, N., Iwasaki, M., Yamaji, T., Shimazu, T., Sasazuki, S, Inoue, M., Tsugane, S.

1. Establishing prediction models for breast cancer using molecular and environmental information.

More than 100 common breast cancer susceptibility loci have been identified in genome-wide association studies (GWAS) world-wide. The utility of these variants in breast cancer risk prediction models has not been thoroughly evaluated in Japanese women. In 3 case-control studies with 1,319 cases and 2,094 controls we evaluated 116 breast cancer risk loci reported in previous GWASs. We selected 22 risk loci out of 116 with P<0.05 for summary estimates in meta-analyses of 3 studies. Compared to women with 6-14 risk alleles in 22 SNPs, the odds ratio for women with 25-33 risk alleles was 3.4 (95% CI, 1.8-6.4). Next, we constructed a genetic risk prediction model using only SNP information, a conventional risk model using 13 environmental risk factors and an inclusive model with 22 SNPs and environmental risk factors. Performance of each model was evaluated by summary c-statistics in the meta-analysis. The results were 0.63, 0.68 and 0.72, respectively. In conclusion, addition of common SNPs to conventional risk factors improves breast cancer risk prediction. It can help in identifying women with high risk of breast cancer for targeted prevention.

2. Randomized-controlled trial for risk feedback with or without genetic information.
   Ugai, T., Kasugai, Y., Ishiguro J., Oze, I., Iwata, H., Ito, H., Matsuo, K.

Genome-wide association studies (GWASs) have been conducted to identify genetic susceptibility loci for various kinds of diseases including malignancies worldwide. Regarding breast cancer, following initial reports about associations with FGFR2 loci, many risk-linked loci have been identified. Despite accumulation of findings about such risk loci, evidence is still scarce about effective applications in cancer prevention. We have developed a risk prediction model for breast cancer among Japanese using a pooled analysis of three case-control studies conducted in Nagano, Kagoshima and Nagoya using 22 GWAS-identified loci. We are now conducting an intervention study to evaluate the impact of feedback of predicted risk with our without 22 SNPs information. The study
is designed as a randomized controlled trial and the primary endpoint is frequency of breast self-examination, a proxy for breast cancer awareness. This presentation will cover progress of the study and perspectives for future individualized cancer prevention. Acknowledgement: The study is supported by a grant from Japan Agency for Medical Research and Development (17ck0106177h0003).

Masaoka, H., Ito, H., Soga, N*1, Oze, I., Matsuo, K.
Although a range of exposures (chemicals in cigarette smoke and occupational agents) are recognized risk factors for the development of bladder cancer (BCa), many epidemiological studies have demonstrated that alcohol drinking is not associated with BCa risk. However, it is known that aldehyde dehydrogenase 2 (ALDH2; rs671, Glu504Lys) and alcohol dehydrogenase 1B (ADH1B; rs1229984, His47Arg) polymorphisms impact on accumulation of acetaldehyde, resulting in an increased risk of various cancers. Hitherto, however, no studies evaluating the association between BCa risk and alcohol drinking have considered such polymorphisms. Here, we conducted a matched case-control study to investigate whether ALDH2 and ADH1B polymorphisms influence BCa risk associated with alcohol drinking. Cases were 74 BCa patients and controls were 740 first-visit outpatients without cancer at Aichi Cancer Center Hospital between January 2001 and December 2005. Odds ratios (ORs), 95% confidence intervals (CIs) and gene-environment interactions were assessed by conditional logistic regression analysis with adjustment for potential confounders. ALDH2 Glu/Lys was associated with a significantly increased risk of BCa compared with Glu/Glu (OR 2.03, 95% CI 1.14-3.62, P = 0.017). In contrast, ALDH2 Glu/Lys showed no increase in risk among the stratum of never drinkers compared with Glu/Glu, indicating a gene-environment interaction. ADH1B His/Arg had an OR of 1.98 (1.20-3.24, P = 0.007) compared with His/His. ADH1B Arg+ showed a similar OR and 95% CI. Individuals with ALDH2 Glu/Lys and ADH1B Arg+ had the highest risk of BCa compared with ALDH2 Glu/Glu and ADH1B His/His [OR 4.00 (1.81-8.87), P = 0.001].

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4. Heterogeneous impact of alcohol consumption according to treatment method on survival in head and neck cancer: A prospective study.
Sawabe, M., Ito, H., Oze, I., Kawakita, D., Hasegawa, Y*1, Matsuo, K.
Alcohol consumption is an established risk factor, and also a potential prognostic factor, for squamous cell carcinoma of the head and neck (HNSCC). However, little is known about whether the prognostic impact of alcohol consumption differs by treatment method. We evaluated the association between alcohol drinking and survival by different therapeutic approaches and primary sites in 427 patients with HNSCCs treated between 2005 and 2013 at Aichi Cancer Center Central Hospital (Nagoya, Japan). The impact of alcohol on prognosis was measured by multivariable Cox regression analysis adjusted for established prognostic factors. Among all HNSCC patients, the overall survival rate was significantly poorer with increased levels of alcohol consumption in multivariable analysis (trend P = 0.038). Stratification by treatment method and primary site revealed that the impact of drinking was heterogeneous. Among laryngopharyngeal cancer (laryngeal, oropharyngeal, and hypopharyngeal cancer) patients receiving radiotherapy (n = 141), a significant dose-response relationship was observed (trend P = 0.034). In contrast, among laryngopharyngeal cancer patients treated with surgery (n = 80), no obvious impact of alcohol was observed. This heterogeneity in the impact of alcohol between surgery and...
radiotherapy was significant (for interaction, \( P = 0.048 \)). Furthermore, among patients with oral cavity cancer treated by surgery, a significant impact of drinking on survival was seen with tongue cancer, but not with non-tongue oral cancer. We observed a significant inverse association between alcohol drinking and prognosis among HNSCC patients, and its impact was heterogeneous by treatment method and primary site.

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5. Smoking and subsequent risk of acute myeloid leukemia in Japan.

Ugai, T., Matsumi, K., Sawada, N*1, Iwasaki*1, M., Yamaji, T*1, Shimazu, T*1, Sasazuki, S*1, Inoue, M*1, Tsugane, S*1.

Cigarette smoking has been reported to be associated with an increased risk of leukemia. Most epidemiological evidence on the association between cigarette smoking and leukemia risk is from studies conducted in Western populations, however, and evidence from Asian populations is limited. Therefore, we conducted a large-scale population-based cohort study of 96,992 Japanese subjects (46,493 men and 50,499 women; age 40-69 years at baseline) with an average 18.3 years of follow-up, during which we identified 90 cases of acute myeloid leukemia (AML), 19 of acute lymphoblastic leukemia (ALL), and 28 of chronic myeloid leukemia (CML). Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated using a Cox regression model adjusted for potential confounders. When we adjusted for age, sex, and study area, our findings showed no significant association or dose-response relationship between risk of AML and cigarette smoking overall. However, after further adjustment for body mass index and occupation, current smokers with more than 30 pack-years of cigarette smoking had a significantly increased risk of AML compared to never smokers among men (HR 2.21; 95% CI, 1.01-4.83). This increased risk was not clear among women. In conclusion, our results suggest that cigarette smoking increases the risk of AML in Japanese men. Associations of smoking with AML among women, and with CML and ALL among men and women, should be assessed in future studies.

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General Summary
Cytotoxic T lymphocytes (CTLs) play a central role in cancer immunity, recognizing target antigens expressed on cancer cell surfaces via T cell receptors (TCRs). TCRs bind to cognate peptide antigens presented on major histocompatibility complex (MHC) molecules on the target surface. Though administration of immune checkpoint inhibitors has brought one of the most remarkable advances in cancer therapy history, substantial numbers of patients still await additional or alternative treatments. The object of our research is to establish molecular and cellular bases for novel cancer therapy taking advantage of updated knowledge of immune responses. The achievements during past two years are as detailed below.

Firstly, we have found an interesting phenomenon in antigen presentation to CTLs in association with levels of transporters associated with antigen processing (TAP) molecules. The CTL clone 32D12 appears to be cancer-specific since it does not kill TAP-proficient normal cells. It only kills cells with impaired TAP function, such as some cancerous or TAP gene-edited cells. The obtained data point to an importance of TAP expression and cancer immune-surveillance.

Secondly, we explored a new strategy to obtain cancer-specific TCRs with high affinity to improve efficacy of adoptive administration of TCR gene transferred T cells. To enhance the affinity of TCR against HLA-A*24:02/hTETRT peptide complexes, we generated libraries with PCR-mediated saturation mutagenesis in complementarity-determining regions (CDRs). Transient transfection of the library into CD3-introduced 293T cells enabled us to isolate many high affinity TCR clones. We believe our novel strategy may circumvent technical obstacles in phage- or yeast-mediated TCR display systems.

1. **In vitro affinity maturation of human T cell receptors on 293T cells**

The efficacy of cytotoxic T lymphocytes (CTLs) against cancer is often suppressed by
regulatory T cell and immune checkpoint mechanisms such as PD-1-PD-L1 ligation. Administration of immune checkpoint inhibitors has thus brought one of the most remarkable advances in recent cancer therapy history. However, objective responses are only achieved in around 10-20% of patients. Alternative treatments that could benefit other cancer cases are still needed.

Adoptive therapy with tumor-specific T cells may be a promising therapy in patients who do not benefit from immune checkpoint inhibitors. CTLs recognize target antigens via T cell receptors (TCRs) on their surfaces. A TCR binds to its cognate peptide antigen presented on major histocompatibility complex (MHC) molecules on the target surface. However, because most tumor antigens are derived from self-proteins, cancer-specific TCR typically have weaker affinity for MHC/cognate peptide complexes compared to those specific to foreign antigens. The most effective technique to enhance the affinity of human TCRs in vitro is phage display. As evidenced by the limited number of groups have been successful with this technique, there are substantial difficulties. Therefore, it is imperative to develop an alternative method to obtain high affinity TCRs against tumor-related self-proteins.

We explored a new strategy to establish in vitro affinity maturation of TCRs expressed on mammalian cells. First, we focused on a low affinity TCR against the hTERT tumor antigen expressed on various tumor cells. To enhance the affinity of TCR against the HLA-A*24:02/hTETRT peptide complex, we generated TCR libraries featuring PCR-mediated saturation mutagenesis in their complementarity-determining regions (CDRs). We transiently transfected each library into 293T-CD3 cells and examples binding to PE-labelled tetramers were sorted with the aid of FACSArea III. By cloning, we established 34 independent TCRs which showed higher binding affinity to the HLA-A*24:02/hTETRT peptide complex than the wild type. Now we are planning to quantify affinity improvement of these clones with a Biacore surface plasmon resonance system.

2. Aberrant TAP creates a cancer-specific epitope

Demachi-Okamura, A., Akatsuka, Y., Kuzushima, K.

Cytotoxic T lymphocytes (CTLs) exert cytotoxicity against tumors specifically through T-cell receptors (TCRs) that recognize peptides presented in the context of the major histocompatibility complex (MHC). However, cancer cells may escape from the immune system owing to loss or downregulation of MHC, which blinds T cells so that immune surveillance is avoided. The MHC defects are caused by genetic, transcriptional and epigenetic alterations, including losses and aberrant gene regulation, and change in beta 2 microglobulin and antigen-processing machinery genes. Especially, impairment of TAP is one contributor observed in cancer immunoediting.

We here report a CTL clone 32D12 isolated after two rounds of stimulation with artificial antigen-presenting cells responding to HLA-A24-expressing K562 and colon cancer cells, but not to HLA-A24-expressing normal fibroblasts, normal bronchial epithelial cells or EBV-transformed B lymphoblastoid cells. The clone recognizes heat shock protein (HSP) 90β that is ubiquitously expressed in both normal and malignant cells. To examine direct associations of antigen processing machinery with this epitope generation that mimics a tumor antigen, we applied chemical inhibitors and siRNA inhibition. Treatment of target cells with siRNAs to TAP1 or viral TAP inhibitors such as HSV ICP47 and CMV US6 genes resulted in an augmented 32D12 response. To study roles of TAP in this epitope generation more precisely, we employed CRISPR/Cas9 system that allows introduction of the TAP gene mutation into KP-3 cells, a pancreatic cancer cell line. The result was CTL clone 32D12 production of IFN-γ against KP-3 having aberrant TAP expression. These data imply TAP-proficient normal cells could not generate this epitope because it might bind with MHC weakly. When TAP expression is reduced in cancer cells, the epitope repertoire on MHC is changed. These data suggest that a detailed evaluation of antigen processing machinery in tumors may lead to improved
cancer immunotherapy.

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General Summary
In the Division of Microbiology and Oncology we seek to understand the mechanisms maintaining cellular homeostasis and their dysfunction in cancer. Normal cellular homeostasis requires the coordinated regulation of signaling molecules in space and time. Accumulation of genetic and epigenetic alterations or oncogenic viral infections disrupts the stringent regulation of signaling networks and can lead to cellular transformation and tumor progression. Our studies involve a focus on genes, proteins, and signaling mechanisms directly responsible for oncogenic phenotypes and identifying novel therapeutic targets. Currently, the primary goal of our research is to elucidate the molecular mechanisms underlying aberrant activation of Src pathways in a wide variety of human cancer cells. During the period 2016-2017, our research interest was concentrated on the following issues: 1) Mechanisms underlying the regulation of exosome biogenesis; 2) Spatial regulation of Src via lipid rafts in control of cancer progression; 3) Regulation of cancer progression by microRNA-mediated Src oncogenic signaling; and 4) Preclinical development of small molecule drug candidates which mimic the function of tumor suppressor 4E-BP1.

1. Mechanisms underlying the regulation of exosome biogenesis.
Hikita, T., Naito, Y., Kuwahara, A., Miyata, M., Oneyama, C.
Exosomes are small membrane vesicles that are believed to play important roles in intercellular communication. The amounts and composition of exosomes vary depending on the physiological state of cells in normal tissue and cancers, but the mechanisms underlying their control have yet to be established in detail. In this study, we found that exosome secretion is enhanced by kinase activity of c-Src, a non-receptor tyrosine kinase which is frequently overexpressed or hyper-activated in human cancers. To elucidate the molecular mechanisms underlying c-Src-mediated exosome secretion, we explored c-Src-interacting proteins in exosomes by mass-spectrometry. Among several candidates, Alix, an ESCRT-associated protein, was found to interact with c-Src via SH3 domain-mediated binding. Inhibition of Src activity with dasatinib treatment suppressed exosome secretion as well as shRNA-mediated Alix knockdown. These data indicate that Alix regulates c-Src-mediated exosome production. Further, we are currently trying to develop a new analytical method for exosomes and clarify the mechanisms underlying the regulation of exosome biogenesis by identifying and analyzing molecules such as Alix responsible for exosome formation and encapsulation.

2. Spatial regulation of Src via lipid rafts controls cancer progression.
Oneyama, C., Yamauchi T.
c-Src is upregulated in various human cancers, suggesting roles in malignant progression. However, the molecular circuits of c-Src oncogenic signaling remain elusive. We have
shown that Fer tyrosine kinase oligomer mediates and amplifies Src-induced tumor progression. Previously, we established that transformation of fibroblasts is promoted by relocation of c-Src to non-raft membranes. Under these conditions, we identified Fer and ezrin as non-raft c-Src targets. c-Src directly activated Fer by initiating its autophosphorylation, which was further amplified by Fer oligomerization. Fer was also crucial for cell transformation induced by v-Src or epidermal growth-factor receptor activation. Furthermore, Fer activation was required for tumorigenesis and invasiveness in some cancer cells in which c-Src is upregulated. We propose that the Src–Fer axis represents a new therapeutic target for treatment of a subset of human cancers.

Oneyama, C., Yagi, R., Watanabe, R.
The tyrosine kinase c-Src is frequently overexpressed and activated in a wide variety of human cancers. However, the underlying molecular mechanisms remain largely unknown. To examine whether microRNA-mediated c-Src upregulation promotes cancer progression, we screened miRNAs with complementarity to the 3’-UTR of c-Src mRNA. Downregulation of miR-137 was tightly associated with c-Src-mediated tumor progression of human colon cancer cells/tissues and its re-expression in human colon cancer cells suppressed tumor growth and caused disruption of focal contacts, and suppression of cell adhesion and invasion, although restoration of c-Src in miR-137-treated cells could not fully rescue the tumor-suppressive effects. We found that miR-137 targets AKT2 and also paxillin and miR-137-mediated regulation of c-Src /AKT2 is crucial for controlling tumor growth, while that of c-Src/paxillin contributes to malignancy. miR-137 suppressed Src-related oncogenic signaling and changed the expression of miRNAs that are regulated by Src activation. In addition, miR-137 could be shown to control the expression of c-Src/AKT2/paxillin and synergistically suppress Src oncogenic signaling evoked from focal adhesions. In various human cancers that harbor c-Src upregulation, dysfunction of this novel mechanism could serve as a critical trigger for tumor progression.

4. Preclinical development of small molecule drug candidates which mimic the function of tumor suppressor 4E-BP1.
Oneyama, C., Watanabe, R.
4E-BP1 (eIF4E-binding protein 1) plays a critical role in the control of protein synthesis by eIF4E (eukaryotic translation initiation factor 4E), related to survival and cell growth
in cancer cells. Since 4E-BP1 expression is extinguished in more than half of human pancreatic ductal adenocarcinomas, restoration of the function of 4E-BP1 is a conceivable therapeutic approach. MO30003 is a lead compound which mimics the function of tumor suppressor 4E-BP1. With the aim of improving efficacy, we evaluated the effects of a number of MO30003-derived compounds in pancreatic cancer cells (PANC-1) and normal cells (MEFs and HaCaT). Under the conditions previously optimized, we found that several compounds suppressed growth of PANC-1 cells as well or better than MO30003. Importantly, these compounds had no cytotoxic effects in normal cells. Further, to confirm the mechanism of action of MO compounds as 4E-BP1 mimics, we demonstrated influence on protease susceptibility of eIF4E. We currently are investigating in vivo anti-oncogenic activity and side effects of MO compounds in a mouse orthotopic pancreatic cancer model as a preclinical test of small molecule drug candidates which mimic the function of tumor suppressor 4E-BP1.
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General Summary
Cancer is a systemic disease. Heterotypic interactions between cancer cells and non-cancer stromal cells in the tumor microenvironment play essential roles in cancer progression. Cancer cells are not limited to the immediate environment, however, but can also interact with more distant tissues or organs. Metastasis, a spreading of cancer cells from the primary site to different parts of the body, and cachexia, a wasting syndrome characterized by the loss of skeletal muscle mass that leads to progressive weight loss, represent systemic changes which are major causes of cancer mortality.

Using genetically-engineered and other mouse models of colorectal cancer, we are currently focusing on the following: (1) Clarifying roles of the tumor microenvironment in cancer progression; (2) Elucidating the molecular mechanisms of metastasis; and (3) Unraveling the pathophysiology of cancer cachexia.

1. Tumor microenvironment events confer mTOR inhibitor resistance in invasive intestinal adenocarcinomas
Fujishita, T., Kojima, Y., Kajino, R., Taketo, MM.*1, and Aoki, M.

The mechanistic target of rapamycin (mTOR) complex 1 (mTORC1) is frequently activated in cancers but can be controlled with the clinically available mTORC1 inhibitors, everolimus and temsirolimus. Although mTORC1 and dual mTORC1/2 inhibitors are currently under development for treatment of various cancers, the emergence of drug resistance represents a major complication. Administration of everolimus or the dual mTORC1/2 inhibitor AZD8055 significantly reduced the growth of intestinal tumors in cis-Apc/Smad4 mice, a model of locally invasive intestinal adenocarcinoma. In contrast, neither inhibitor exhibited major blocking effects on invasion of the tumors. Biochemical and immunohistochemical analyses revealed that treatment of cis-Apc/Smad4 mice with everolimus or AZD8055 induces marked increases in EGFR and MEK/ERK signaling in tumor epithelial and stromal cells, respectively. Co-administration of AZD8055 and the EGFR inhibitor erlotinib or the MEK
inhibitor trametinib suppressed tumor invasion in cis-Apc/Smad4 mice. These data indicate that mTOR inhibitor resistance in invasive intestinal adenocarcinomas involves feedback signaling from both cancer epithelial and stromal cells, and underscores the role of the tumor microenvironment in drug resistance. Our findings also suggest that simultaneous inhibition of mTOR and EGFR or MEK may be more effective in treating colon cancer.

Fig.1. A schematic model for the mechanism of mTOR inhibitor resistance in invasive intestinal adenocarcinomas, showing EGFR and MEK/ERK activation in tumor epithelia and stroma, respectively.

2. Roles of intestinal epithelial MyD88 in tumor formation in Apc mutant mice.
Kajino, R., Fujishita, T., Taketo, MM.*1, and Aoki, M.

The APC tumor suppressor gene is commonly mutated in colorectal adenomas and adenocarcinomas, and Wnt signaling activation due to LOH of the Apc locus in intestinal epithelial cells of Apc+/−Δ716 (Apc) mice initiates formation of adenomatous polyps. However, the development of large polyps requires JNK-mediated activation of mTORC1 in tumor epithelial cells. To identify molecules involved in this pathway, we generated organoids derived from intestinal polyps in Apc mice. We found that IL-1β, an inflammatory cytokine upregulated in intestinal polyps, caused activation of the JNK-mTORC1 pathway. We then investigated the role of MyD88, a critical adaptor protein in the IL-1 signaling pathway, in intestinal tumorigenesis. Conditional knockout of MyD88 in intestinal epithelial cells reduced the number of intestinal tumors in Apc mice, accompanied by attenuation of JNK signaling in the tumors. Consistently, tamoxifen-induced MyD88 deletion in intestinal polyp-derived organoids resulted in apoptotic cell death. These data suggest that intestinal epithelial MyD88 functions in the maintenance and growth of intestinal tumors.

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3. HNRNPLL promotes cell cycle progression in colorectal cancer cells by stabilizing mRNAs for DNA replication proteins
Sakuma, K., Sasaki, E., Kimura, K., Komori, K., Shimizu, Y., Yatabe, Y., and Aoki, M.

HNRNPLL (heterogeneous nuclear ribonucleoprotein L-like) is an RNA-binding protein that has been shown to regulate alternative splicing of pre-mRNAs and thereby control differentiation of lymphocytes, as well as metastasis of colorectal cancers. We have found that HNRNPLL promotes cell cycle progression and hence proliferation of colorectal cancer cells. Functional annotation analysis of those genes whose expression levels were changed by three-fold or more in RNA sequencing analysis between SW480 cells overexpressing or knocked down for HNRNPLL revealed enrichment of DNA replication-related genes by HNRNPLL overexpression. Among 13 genes detected in the DNA replication pathway, PCNA, RFC3, and FEN1 showed reproducible upregulation with HNRNPLL overexpression at both mRNA and protein levels in SW480 and HT29 cells. Importantly, knockdown of any of these genes alone suppressed the proliferation promoting effect of HNRNPLL overexpression. RNA-immunoprecipitation assays and experiments using actinomycin D revealed that HNRNPLL could bind to and stabilize mRNAs of PCNA, RFC3, and FEN1. Moreover, analysis of a public RNA sequencing dataset of clinical samples suggested a correlation between overexpression of HNRNPLL and levels of PCNA, RFC3, and FEN1. This correlation was further evidenced by immunohistochemistry of colorectal cancer clinical samples. These results indicate that HNRNPLL promotes colorectal cancer cell proliferation through stabilization of mRNAs encoding regulators of DNA replication.

4. Systemic metabolic changes in mouse models of cancer cachexia.
Kojima, Y., Fujishita, T., Soga, T. *1, Taketo, MM.*2, and Aoki, M.

Cachexia is a wasting syndrome characterized by the loss of skeletal muscle mass that leads to a progressive drop in body weight. Cancer cachexia occurs in up to 80% of advanced cancer patients and is a direct cause of as many as 20% of cancer deaths. Although increasing knowledge indicates that cancer cachexia is a complex whole-body metabolic disorder, systemic studies on associated changes have not been reported. We employed two
independent mouse models of cancer cachexia; *cis-Apc/Smad4 mice*, genetically-engineered animals that develop intestinal adenocarcinomas, and a xenograft model of human melanoma (SEKI cells) in nude mice. Exploratory analysis of metabolome data obtained by capillary electrophoresis coupled to mass spectrometry (CE-MS) and subsequent mathematical analysis revealed characteristic alteration in essential metabolic pathways in the liver and skeletal muscle of the cachectic mice in both models, as compared with appropriate non-cachectic controls. The roles of these metabolic parameters in the development and progression of cancer cachexia are currently under investigation.

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General Summary
How cells coordinate proliferation and differentiation is a fundamental question in cell and developmental biology, and alterations in these processes are of prime importance to cancer development. As cells progress toward cell division, a number of cell-cycle checkpoints ensure that they do not prematurely undergo cell-cycle transitions such as exit from G0 and entry into S phase or mitosis. In the normal development of multi-cellular organisms, signals controlling these cell-cycle checkpoints are thought to come from both intrinsic (e.g. centrosomes or cell polarity signaling) and extrinsic (e.g. cell-cell contacts or planar cell polarity signaling) sources. We are seeking to define the mechanisms by which cells link polarity signaling (tissue architecture) with cell-cycle control. Identification of regulatory mechanisms and their failure to operate in cancer cells should contribute to novel strategies for cancer therapy.

Our attention is focused on 2 specific areas: (1) Identification of novel molecular targets downstream of protein kinases involved in cell cycle checkpoints; (2) Regulation of cytoskeletal proteins and associated elements active in cell adhesion and determination of cell polarity. Specific projects/results are described in detail below.

1. Identification of novel molecular targets downstream of checkpoint kinase 1 (Chk1)
Goto, H., Tanigawa, N. and Kanemaki, M *1.

Normal cells have two major DNA damage response pathways, involving ATM-Chk2-p53 and ATR-Chk1-Cdc25A. Hereditary mutations have been reported in ATM, CHEK2, and TP53 gene loci in patients with cancer preposition syndromes. The fact that homozygous impairment of these genes is frequently observed in a variety of sporadic human cancers is further evidence of the importance of the ATM-Chk2-p53 pathway in tumor suppression. On the other hand, no homozygous loss-of-function mutations have been detected in CHEK1 gene locus, although heterozygous deletion was reported in a few types of cancers. Rather, Chk1 had been reported to be upregulated in a variety of neoplasms. Impairment of the ATM-Chk2-p53 pathway likely leads to upregulation of ATR-Chk1-Cdc25A in a majority of cancer cells.

Since Chk1 upregulation is well correlated with tumor grade and recurrence in some types of cancers, it may be associated with the resistance to DNA-damaging therapies, such as radiotherapy or conventional chemotherapy. Thus, Chk1 inhibitors were initially expected to sensitize cancer cells to conventional treatment approaches including radiation. However, the use of several drugs likely increases the risk of undesirable effects, such as off-target toxicity. Therefore, more attention has been paid to use of single Chk1 inhibitors. In preclinical models, cancer cells are more sensitive to Chk1 inhibitors than normal cells. However, the precise mechanism by which Chk1 inhibitors preferentially kill cancer cells is largely unknown.
In order to elucidate signaling pathways downstream of Chk1 without exogenous DNA-damaging reagents, we have employed a strategy to induce rapid degradation of endogenous Chk1 protein in cells. For this purpose, we established a colorectal carcinoma cell line HCT116 in which a minimum auxin-inducible degron (mAID) sequence is inserted prior to stop codons on both \( \text{CHEK1} \) loci (\( \text{CHEK1}^{\text{mAID/mAID}} \)). After UV irradiation, Chk1-mAID was phosphorylated in \( \text{CHEK1}^{\text{mAID/mAID}} \) cells, like Chk1 in parent HCT116 cells (\( \text{CHEK1}^{\text{WT/WT}} \)). Chk1-mAID was degraded rapidly after the addition of auxin to the growth medium. In \( \text{CHEK1}^{\text{mAID/mAID}} \) cells, incubation with auxin resulted in aberrant accumulation of gamma-H2AX and p53, and elevated apoptosis together with reduced cell proliferation. Now, using these established cell lines, we are trying to identify putative substrates downstream of Chk1 involved in cancer cell viability.

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2. Analysis of centrosomes for protein functions and label-free visualization
Hayashi, Y., Kiyono, T.*, Goshima, N.*, Inagaki, M., Kano H.*, and Inoko, A.

The centrosome is a small but important organelle that functions in centriole duplication, spindle formation, and ciliogenesis. Each event is regulated by key enzymatic reactions, but how these processes are integrated is still unknown. Recent studies reported that ciliogenesis is controlled by distal appendage proteins such as FBF1, also known as Albatross, but its precise role in centrosome dynamics remains to be determined. Here, we found a novel function for Albatross at the proximal ends of centrioles. Using Albatross monospecific antibodies, full-length constructs, and siRNAs for rescue experiments, we found that Albatross mediates centriole duplication by recruiting HsSAS-6, a cartwheel protein of centrioles. Moreover, Albatross participates in centrosome separation during mitosis by recruiting Plk1 to residue S348 of Albatross after its phosphorylation, most probably by Cdk1. Taken together, our results show that Albatross is a novel protein that spatiotemporally integrates different aspects of centrosome function, namely, ciliogenesis, centriole duplication, and centrosome separation.

In addition, despite growing demand for truly naïve imaging, label-free observation of cilium-related structures remains challenging, and validation of pertinent molecules is correspondingly difficult. Recently, in retinas and cultured cells, we distinctively visualized Rootletin filaments in rootlets in the second harmonic generation (SHG) channel, integrated in custom coherent nonlinear optical microscopy (CNOM) with a simple, compact, and ultra-broadband supercontinuum light source. This SHG signal was primarily detected on rootlets of connecting cilia in retinal photoreceptors and was validated by colocalization with anti-Rootletin staining. Transfection of cells with Rootletin fragments revealed that the SHG signal can be ascribed to filaments assembled from the R234 domain, but not to cross-striations assembled from the R123 domain. Consistent with this, Rootletin-depleted cells lacked the SHG signals expected of a centrosome linker. As a proof of concept, we confirmed that similar fibrous SHG was observed even in unicellular ciliates. These findings have potential for broad applications in clinical diagnosis and biophysical experiments in various organisms.

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First row: Ms. Y. Nakai, Dr. M. Aoki, and Ms. R. Nishida
Second row: Ms. M. Nishizawa, Mr. Y. Minoura, Dr. H. Kumimoto, Ms. Y. Shinohara and Mr. Y. Okumoto
Central Service Unit

Masaki Inagaki, M.D.  Chief (until June 2016)
Masahiro Aoki, M.D.  Chief (as of July 2016)
Hiroshi Kumimoto, Ph.D.  Senior Researcher
Yukiko Nakai, B.P.,  Research Assistant
Reina Nishida, B.P.,  Research Assistant (as of April 2017)
Yasushi Minoura, B.P.,  Semi-regular Employee
Miwako Nishizawa, B.P.,  Semi-regular Employee
Yoshimi Shinohara,  Semi-regular Employee
Naomi Tanigawa,  Semi-regular Employee (until September 2016)
Yasuhide Okumoto,  Semi-regular Employee (as of March 2017)

General summary

Our main research project is molecular epidemiologic analysis of human esophageal cancer. Especially, we have focused on the relationship between numbers of polymorphisms in the D-loop region of mitochondrial DNA (mtDNA) and risk of esophageal cancer development.

1. Relationship between risk of esophageal cancer and the number of polymorphisms in mitochondrial DNA

Kumimoto, H.

Mitochondria are well known as the organelles in eucaryote for energy production for cells as ATPs and also they have roles in apoptosis. Recently, frequent mutations in mitochondrial DNA (mtDNA) have been found in various types of cancer, such as breast cancer and stomach cancer. Our previous analysis of mutations in the D-loop region of mtDNA in esophageal tumor demonstrated frequent somatic mutations (in 34 % of cases). We also determined nuclear genomic instability in same subjects, but did not find any correlation with somatic mtDNA mutations, suggesting that instability of mtDNA in esophageal cancer might be an independent of nuclear genomic instability.

Energy as ATP is produced in mitochondria with reactive oxygen species (ROS) as byproducts. Polymorphisms in the genes related to proteins of oxidative phosphorylation may elevate ROS production by leaking electrons. Therefore the number of polymorphisms in mtDNA may influence ROS levels in cells, which would be expected to increase the risk of introducing mutations into mtDNA and nuclear genomic DNA. We therefore analyzed the number of polymorphisms in mtDNA as a surrogate marker for ROS generating level, then evaluating the relationship with risk of esophageal cancer.

We performed sequencing analysis of D-loop region in mtDNA using DNA samples from esophageal cancer subjects and non-cancer controls collected in the HERPACC study. At first, we used re-sequencing primers sets made by ABI, mitoSEQr. We found that the whole D-loop region could be sequenced with 4 of 8 primer sets of mitoSEQr. After sequencing the whole D-loop region, we identified polymorphisms by comparing these sequences with the common mtDNA sequence.

So far, we have completed analyses of polymorphisms in 88 subjects with esophageal cancer and 67 non-cancer controls (see Table 1). After analyses of polymorphisms in 185 esophageal cancer patients and 185 non-cancer controls, we will evaluate the relationship between esophageal cancer risk and number of mitochondrial polymorphisms.
<table>
<thead>
<tr>
<th>Type of polymorphism</th>
<th>total</th>
<th>Insertion</th>
<th>deletion</th>
<th>SNP</th>
<th>heteroplasmy</th>
<th>Number of subjects analyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esphaegal cancer</td>
<td>6.6</td>
<td>0.2</td>
<td>0.9</td>
<td>5.2</td>
<td>0.3</td>
<td>88</td>
</tr>
<tr>
<td>Non-cancer</td>
<td>6.7</td>
<td>0.2</td>
<td>0.9</td>
<td>5.2</td>
<td>0.4</td>
<td>67</td>
</tr>
</tbody>
</table>

Data are shown as average numbers / subject except 'number of subjects analyzed'.
Librarians

From left to right
Ms. Y. Naruse, Ms. A. Kakizoe, Ms. N. Jinbo, Mr. T. Matsunaga
Publications

Journals


J048. Kar, S.P., Beesley, J., Amin, A.I., Olama, A.,


(PMID: 26980212)


J134. Takahama, H., Araki, R., Nishimura, R., Yachie, A., Espinoza, J.L., Okumura, H., Yoshida,


J147. Ugai, T., Kanda, Y., Morishima, Y., Matsuo,


Reviews and Books


Abstracts for international conferences


A008. Sekido, Y.: Constitutive YAP activation induces malignant phenotypes of immortalized mesothelial cells. iMig, Birmingham, 2016.


A021. **Masaoka, H., Ito, H., Soga, N., Yokomizo, A., Eto, M., Matsuo, K.** - Aldehyde dehydrogenase 2 (ALDH2) and alcohol dehydrogenese 1B (ALDH1B) polymorphisms exacerbate bladder cancer risk associated with alchol drinking : Gene-environment interaction. 24th Biennial Congress of the European Association for Cancer Reasearch, Manchester, 2016.


Record of Seminars

Invited Speakers

2016

Jan. 18 Harada, H. (Hakubi Center, Kyoto University and Department of Radiation Oncology and Image-applied Therapy, Kyoto University Graduate School of Medicine)
   Cancer progression and treatment resistance mediated by HIF-1

Feb. 4 Takeda, H. (Department of Pathology, Kanazawa Medical University)
   Identification of genes involved in colon cancer formation by Sleeping Beauty (SB) transposons

May. 17 Hoshino, S. (Department of Biological Chemistry, Nagoya City University Graduate School of Pharmaceutical Sciences)
   Molecular mechanisms of mRNA degradation

Jun. 10 Shirahige, K. (Laboratory of Genome Structure & Function, Research Center for Epigenetic Disease, IMCB, The University of Tokyo)
   The roles of the elongation factor cohesin learned from the common molecular mechanisms shared by rare diseases and cancer

Jun. 29 Kikuchi, A. (Department of Molecular Biology and Biochemistry, Graduate School of Medicine, Osaka University)
   Tubulogenesis and Tumorigenesis Regulated by Wnt Signaling

Dec. 14 Miyamoto, T. (Department of Genetics and Cell Biology, Research Institute for Radiation Biology and Medicine, Hiroshima University)
   Identification of mutations causing rare genetic diseases using genome editing

2017

Jan. 26 Suzuki, A. (Division of Molecular and Cellular Biology, Kobe University Graduate School of Medicine)
   Functional analysis of the Hippo signaling using genetically engineered animals

Feb. 9 Nojima, H. (Department of Molecular Genetics, Research Institute for Microbial Diseases, Osaka University)
   Diagnostic and therapeutic strategies for cancer through Cyclin G and Cyclin G-associated kinase (GAK)
February 16, Nakanishi, M. (Division of Cancer Cell Biology, Department of Cancer Biology, Institute of Medical Science, The University of Tokyo)

**Mechanisms for induction and maintenance of cellular senescence and protection of carcinogenesis**

February 17, Fujita, M. (Department of Cellular Biochemistry, Graduate School of Pharmaceutical Sciences, Kyushu University)

**Identification of a novel oncogene, GRWD1, and future development of the study**

March 17, Kondo, Y. (Department of Biomolecular Engineering, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology)

**Development of anticancer agents using in vivo optical imaging**

June 7, Nishikawa, H. (Exploratory Oncology Research and Clinical Trial Center, National Cancer Center Research Institute, Department of Immunology, Nagoya University Graduate School of Medicine)

**Challenges of current cancer immunotherapy and future prospects**

June 20, Enomoto, A. (Department of Pathology, Nagoya University Graduate School of Medicine)

**Understanding cancer based on diversity of its stroma and mechanobiology**

July 5, Ebi, H. (Institute for Frontier Science Initiative, Division of Medical Oncology, Cancer Research Institute, Kanazawa University)

**Development of therapeutics based on the organ specificity of tumors harboring driver gene mutations**

July 7, Sabe H. (Department of Molecular Biology, Hokkaido University Graduate School of Medicine)

**The Arf6 pathway: a central pathway driving mesenchymal malignancies and drug-resistance of refractory cancers**

July 28, Taguchi, A. (Department of Translational Molecular Pathology M.D. University of Texas MD Anderson Cancer Center)

**Molecular biomarkers: Towards an integrated and translational cancer research**

August 17, Matsuda, M. (Department of Pathology and Biology of Diseases, Graduate School of Medicine, Kyoto University)

**What would we learn from visualizing intracellular signaling pathways in live mice?**

August 22, Imoto, I. (Department of Human Genetics, Graduate School of Biomedical Sciences, Tokushima University)

**Cancer genetics in the era of genome medicine**
Sep, 21  Okada, Y. (Department of Statistical Genetics, Osaka University Graduate School of Medicine)  
Elucidation of pathophysiology of diseases and genome-based drug discovery approached by statistical genetics

Oct, 4  Curtis C. Harris. (Laboratory of Human Carcinogenesis, National Cancer Institute, NIH)  
Precision medicine: Lung biomarkers

Oct, 25.  Soga, T. (Institute for Advanced Biosciences, Keio University)  
Elucidating colorectal cancer metabolism using multiomics approach

Nov, 24.  Fujita, Y. (Division of Molecular Oncology, Institute for Genetic Medicine, Hokkaido University)  
Cell competition between normal and mutated epithelial cells

Dec, 13  Miyano, S. (Human Genome Center, The Institute of Medical Science, The University of Tokyo)  
Cancer genomics and clinical sequencing studies accelerated by supercomputers and artificial intelligence

Institute Speakers

2016
Jan, 21  Kojima, Y. (Molecular Pathology):  
Unraveling the pathophysiology of cancer cachexia

Feb, 18  Inaba, H. (Biochemistry):  
Regulation of primary cilia by Ndel1

Feb, 26  Kanda, T. (Microbiology and Oncology):  
Isolation and characterization of EB virus strains derived from stomach cancer using genome editing technology

Mar, 3  Murakami, Y. (Molecular Oncology):  
SGO1 is involved in DNA damage response in neuroblastoma cells with MYCN amplification

Nov, 24  Sato, T. (Molecular Oncology):  
Functional analyses of the mTOR activator Rheb

2017
Jan, 31  Sakuma, K. (Molecular Pathology):  
Toward the elucidation of molecular mechanism of cancer metastasis

Feb, 27  Hikita, T. (Microbiology and Oncology):  
Elucidating the mechanisms of exosome formation and cargo loading initiated by c-Src
Mar, 22  Kajino, R. (Molecular Pathology):
The roles of MyD88 in intestinal tumor formation in Apc mutant mice

Mar, 29  Ohta, R. (Immunology):
Attempts for obtaining high-affinity T-cell receptors specific for cancer antigens: Toward development of novel cancer immunotherapies

May, 19  Fujishita, T. (Molecular Pathology):
Studies on cancer microenvironment using mouse models for intestinal tumors

Jun, 8  Mukai, S. (Molecular Oncology):
Unexpected relationship between LATS1/2 and BAP1, tumor suppressors mutated in malignant mesotheliomas

Aug, 10  Ugai, T. (Molecular and Clinical Epidemiology):
Japanese lifestyle and risk for hematologic diseases: achievements so far and challenges ahead

Sep, 14  Inoko, A. (Biochemistry):
Individualization/stratification of prostate cancers using stem-cell differentiation technologies and cell biology

Oct, 12  Oze, I. (Molecular and Clinical Epidemiology):
Epidemiologic studies on risk for esophageal cancer

Nov, 16  Kojima Y. (Molecular Pathology):
Studies toward elucidation of the pathophysiology of cancer cachexia and identification of its preventive/therapeutic targets

Dec, 14  Sawabe, M. (Molecular and Clinical Epidemiology):
Effects of lifestyles on carcinogenesis and treatment of head and neck cancers