Research Institute

Preface

For more than 50 years since its establishment in 1962 as a department of the National Cancer Center (NCC), the National Cancer Center Research Institute (NCCRI) has been the leading cancer research institute. The NCCRI is now internationally recognized for its major contributions to various aspects of cancer research. Its mission 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 the NCC Hospitals, the Exploratory Oncology Research & Clinical Trial Center (EPOC), the Research Center for Cancer Prevention and Screening and the Research Institute, is highly encouraged.

The NCCRI consists of the Advanced Biomedical Research Faculty and the Fundamental Innovative Oncology Core (FIOC). The former body now comprises 18 divisions which are sub-grouped into four major Research Groups and one Project Group, that is, the Group for Cancer Development and Progression, the Group for Research into Molecular Functions and Targets, the Group for Development of Molecular Diagnostics and Individualized Therapy and the Group for Translational Research and Project Group. On the other hand, the FIOC is established in 2014 as a core facility to bridge the gap between preclinical and clinical studies for efficient drug development. It consists of 5 cores comprising 15 departments and provides several kinds of technical support for molecular biology, high-throughput omics-type analyses, biological analysis and animal experiments to researchers in both the Research Institute and Hospitals in order to further encourage and facilitate the development of translational-type studies in the Institute.

Currently, there are approximately 80 research staff and around 30 postdoctoral fellows in the NCCRI with over 230 supporting staff members. Foreign scientists and research fellows also visit the NCCRI on a regular basis.

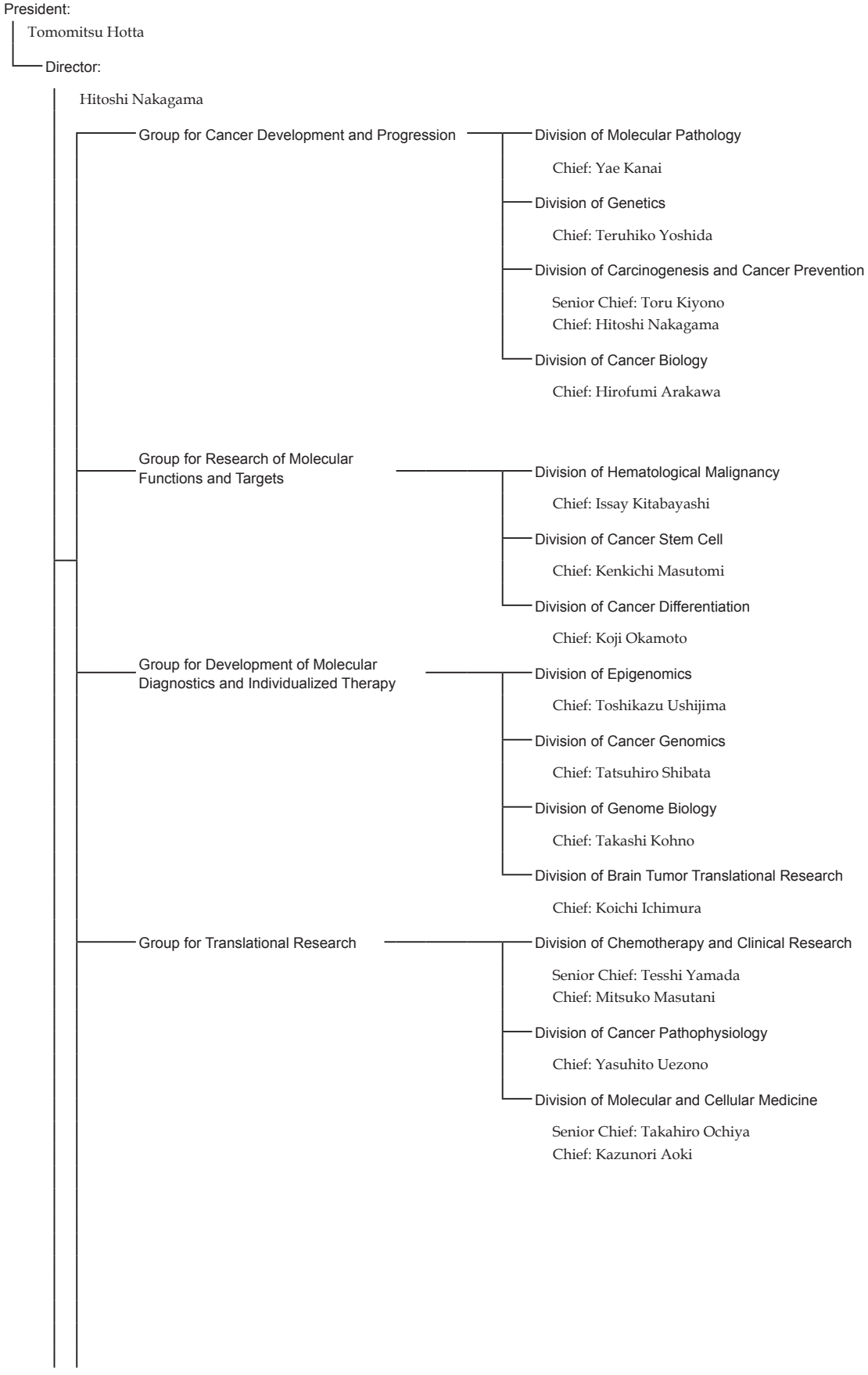
The "Annual Report" of the NCCRI summarizes the recent research activities of each division. Our recent major accomplishments are as follows:

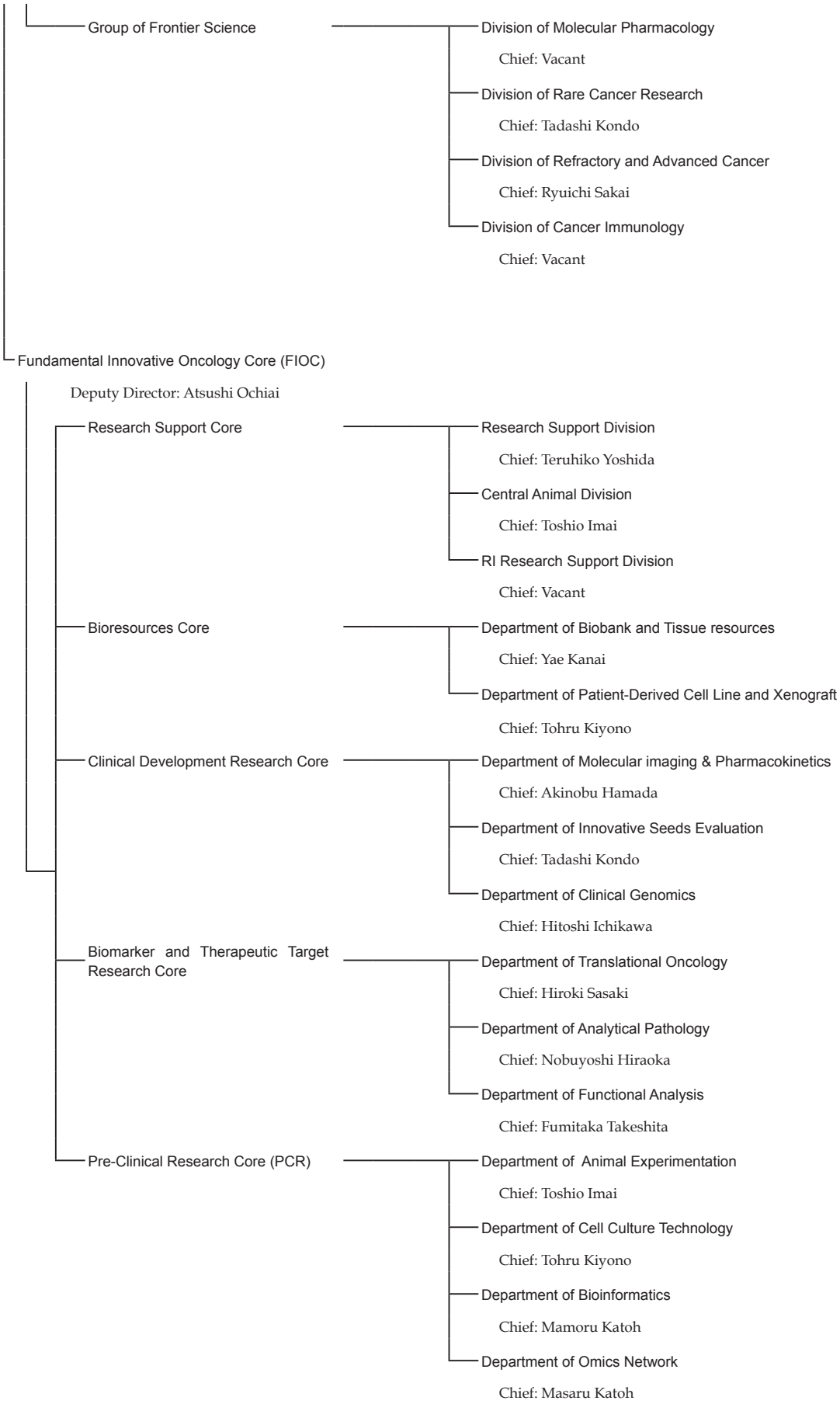
(i) Identification of novel fusion genes in lung cancers, and characterized mutations in hepatitis C-associated liver cancers;
(ii) Identification of novel druggable targets, such as TNIK, RPN2, and GLIS2;
(iii) Establishment of the concept of epigenetic field for cancerization; and
(iv) Development of markers for patient stratification in multiple types of cancers.

We have participated in worldwide research consortia, such as the International Cancer Genome Consortium (ICGC) and International Human Epigenome Consortium (IHEC), and joined the Early Detection Research Network (EDRN) of the National Cancer Institute (NCI), the National Institutes of Health (NIH). In addition to further encouraging collaborative research with NIH, we are now developing international collaborative research projects in other various areas. Information on research activities of NCCRI is also available on the website: http://www.ncc.go.jp/en/nccri/index.html.

Hitoshi Nakagama, M.D., D.M.Sc.
Director, National Cancer Center Research Institute

Organization




Group of Frontier Science
Division of Molecular Pharmacology
Chief: Vacant
Division of Rare Cancer Research
Chief: Tadashi Kondo
Division of Refractory and Advanced Cancer
Chief: Ryuichi Sakai
Division of Cancer Immunology
Chief: Vacant
Fundamental Innovative Oncology Core (FIOC)
Deputy Director: Atsushi Ochiai
Research Support Core
Research Support Division
Chief: Teruhiko Yoshida
Central Animal Division
Chief: Toshio Imai
RI Research Support Division
Chief: Vacant
Bioresources Core
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Chief: Yae Kanai
Department of Patient-Derived Cell Line and Xenograft
Chief: Tohru Kiyono
Clinical Development Research Core
Department of Molecular imaging & Pharmacokinetics
Chief: Akinobu Hamada
Department of Innovative Seeds Evaluation
Chief: Tadashi Kondo
Department of Clinical Genomics
Chief: Hitoshi Ichikawa
Biomarker and Therapeutic Target Research Core
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Department of Analytical Pathology
Chief: Nobuyoshi Hiraoka
Department of Functional Analysis
Chief: Fumitaka Takeshita
Pre-Clinical Research Core (PCR)
Department of Animal Experimentation
Chief: Toshio Imai
Department of Cell Culture Technology
Chief: Tohru Kiyono
Department of Bioinformatics
Chief: Mamoru Katoh
Department of Omics Network
Chief: Masaru Katoh

Activities of the Divisions

Division of Molecular Pathology

Yae Kanai, Eri Arai, Masahiro Gotoh, Ying Tian, Hidenori Ojima, Takuya Yotani, Yuriko Yamada, Ayako Shibuya, Nanako Itoh, Michiko Suzuki

Introduction

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

Routine activities

Staff scientists of the Division of Molecular Pathology are also Japanese Board-certified pathologists, who are engaged in routine pathology work involving diagnosis of biopsy and surgically resected materials at the National Cancer Center Hospital. On the basis of findings from our routine diagnostic work, we develop scientific ideas and follow them up using a molecular pathological approach, which can yield potential benefits for patients with cancer.

Research activities

Multilayer omics analysis in human cancers for personalized medicine

We have participated in the Research Project, "Comprehensive Exploration of Drug Targets Based on Multilayer/Integrative Disease Omics Analyses," as a PI supported by the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation. In March 2015, "Multilayer/Integrative Disease Omics Database" will be launched to disclose data obtained from this project.

To reveal the molecular pathways significantly participating in CpG-island methylator phenotype (CIMP)-positive renal carcinogenesis, genome (whole-exome), transcriptome and proteome analyses were performed in the collaborative project study. A signaling pathway most frequently affected by multilayer omics abnormalities in CIMP-positive clear cell renal cell carcinomas (RCCs), which are characterized by accumulation of DNA hypermethylation of CpG islands, clinicopathological aggressiveness and poor patient outcome, was identified as the potential therapeutic target. The effectiveness of the inhibitor of the identified pathway has been revealed in CIMP-positive RCC cell lines.

In order to make DNA methylation diagnosis, such as prognostication of patients with RCCs using RCC-specific CIMP marker genes, widely applicable for clinical use in each hospital and clinic, we are now innovating a Scaled-Down, Methylated DNA Detection Device in collaboration with a medical device company. We have made patent publications (JP2014-514703, US14/399591, EP13787593.6, CN201380036415.8, KR10-2014-7032254) and put out a media release.

The whole transcriptome analysis (RNA sequencing) and exploration of candidate chimeric transcripts using the deFuse program were performed on cancerous tissue specimens obtained from patients with clear cell RCCs. After verification by reverse transcription-PCR and Sanger sequencing, 26 novel chimeric transcripts were identified in 25% of the examined clear cell RCCs. Genomic breakpoints were determined in the chimeric transcripts. Quantitative RT-PCR analysis revealed that the mRNA expression levels for the MMACHC, PTER, EPC2, ATXN7, FHIT, KIFAP3, CPEB1, MINPP1, TEX264, FAM 107A, UPF3A, CDC16, MCCC1, CPSF3 and ASAP2 genes, being partner genes involved in the chimeric transcripts in the initial cohort, were significantly reduced in cancerous tissue samples relative to the corresponding non-cancerous renal cortex tissue samples in the second cohort. Moreover, the mRNA expression levels for the above partner genes in cancerous tissue samples were significantly correlated with tumor aggressiveness and poorer patient outcome, indicating that reduced expression

of these genes may participate in malignant progression of RCCs. As is the case when their levels of expression are reduced, these partner genes also may not fully function when involved in chimeric transcripts. These data suggest that generation of chimeric transcripts may participate in renal carcinogenesis by inducing dysfunction of tumor-related genes.

Activities in the International Human Epigenome Consortium (IHEC)

We have participated in the IHEC as a PI supported by the Core Research for Evolutional Science and Technology (CREST) project by the Japan Science and Technology Agency (JST). In collaboration with research groups in Kyushyu University and the University of Tokyo, we perform whole-genome bisulfite sequencing using the post-bisulfite adaptor-tagging method, chromatin immunoprecipitation-sequencing and RNA-sequencing of various cell lineages of the gastrointestinal and urinary systems. Epigenome maps of hepatocytes purified from normal liver tissue and diseased liver tissue with hepatitis C virus (HCV) or hepatitis B virus (HBV) infection have been deposited in the National Bioscience Database Center supported by the JST. In addition to standard protocols recommended by the IHEC, the whole genome sequencing was performed in purified hepatocytes and genome-epigenome crosstalk was examined. Our data indicated that genomic variations, such as single-nucleotide polymorphisms, insertions and deletions, may induce personal variations of DNA methylation status in cis-acting manner.

Clinicopathological studies of human cancers based on the practice of diagnostic pathology

Using morphological, histological, immunohistochemical and molecular pathological approaches, diagnostic and prognostic criteria which are applicable to histological specimens were explored. We collect tissue samples for the National Cancer Center Biobank and contribute to collaboration researches through providing clinicopathological information.

Future prospects

We will perform joint researches with users of "Multilayer/Integrative Disease Omics Database" among government, industry and academia. We will hold the sixth annual meeting of the IHEC in next November in Tokyo. Accurate standard epigenome profiles of digestive and urogenital organ epithelilal cells obtained through IHEC activities will be used to explore more useful biomarkers and drug targets of cancers.

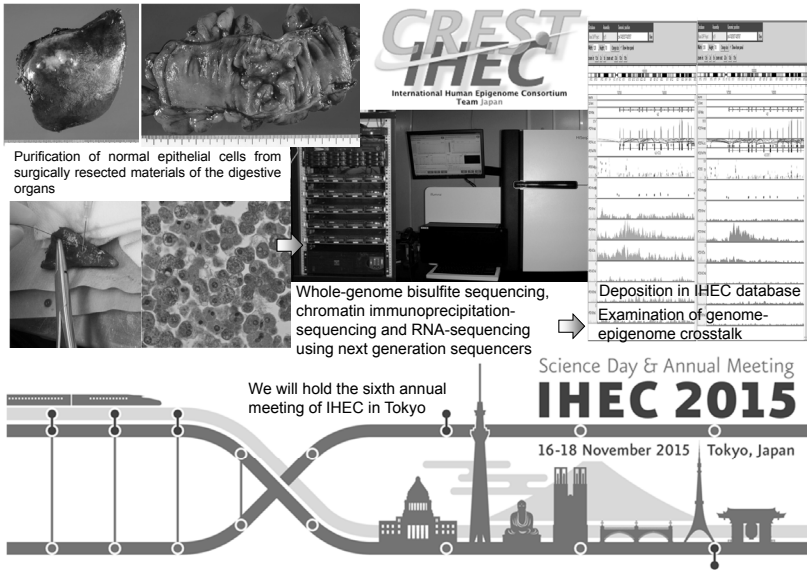

Figure 1. Activities in The International Human Epigenome Consortium

List of papers published in 2014

Journal

1. Arai E, Sakamoto H, Ichikawa H, Totsuka, H Chiku S, Gotoh M, Mori T, Nakatani T, Ohnami S, Nakagawa T, Fujimoto H, Wang L, Aburatani H, Yoshida T, Kanai Y. Multilayer-omics analysis of renal cell carcinoma, including the whole exome, methylome and transcriptome. Int J Cancer, 135:1330-1342, 2014

2. Sato T, Arai E, Kohno T, Takahashi Y, Miyata S, Tsuta K, Watanabe S, Soejima K, Betsuyaku T, Kanai Y. Epigenetic clustering of lung adenocarcinomas based on DNA methylation profiles in adjacent lung tissue: Its correlation with smoking history and chronic obstructive pulmonary disease. Int J Cancer, 135:319-334, 2014

3. Gotoh M, Ichikawa H, Arai E, Chiku S, Sakamoto H, Fujimoto H, Hiramoto M, Nammo T, Yasuda K, Yoshida T, Kanai Y. Comprehensive exploration of novel chimeric transcripts in clear cell renal cell carcinomas using whole transcriptome analysis. Genes Chromosomes Cancer, 53:1018-1032, 2014

4. Tian Y, Arai E, Gotoh M, Komiyama M, Fujimoto H, Kanai Y. Prognostication of patients with clear cell renal cell carcinomas based on quantification of DNA methylation levels of CpG island methylator phenotype marker genes. BMC Cancer, 14:772, 2014

5. Kanai Y, Arai E. Multilayer-omics analyses of human cancers: exploration of biomarkers and drug targets based on the activities of the International Human Epigenome Consortium. Front Genet 5:24, 2014

6. Sato Y, Ojima H, Onaya H, Mori T, Hiraoka N, Kishi Y, Nara S, Esaki M, Shimada K, Kosuge T, Sugihara K, Kanai Y. Histopathological characteristics of hypervascular cholangiocellular carcinoma as an early stage of cholangiocellular carcinoma. Hepatol Res, 44:1119-1129, 2014

7. Iwao Y, Ojima H, Onaya H, Sakamoto Y, Kishi Y, Nara S, Esaki M, Mizuguchi Y, Ushigome M, Asahina D, Hiraoka N, Shimada K, Kosuge T, Kanai Y. Early venous return in hepatic angiomyolipoma due to an intratumoral structure resembling an arteriovenous fistula. Hepatol Res, 44:700-706, 2014

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9. Sekine S, Ogawa R, Oshiro T, Kanemitsu Y, Taniguchi H, Kushima R, Kanai Y. Frequent lack of GNAS mutations in colorectal adenocarcinoma associated with GNASmutated villous adenoma. Genes Chromosomes Cancer, 53:366-372, 2014

10. Matsubara A, Nara S, Sekine S, Ojima H,Kosuge T, Shimada K, Kushima R, Kanai Y, Hiraoka N. Intraductal dissemination of papillary adenocarcinoma of the ampulla of Vater in the pancreatic duct. Pathol Int, 64:39-44, 2014

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12. Hori M, Takahashi M, Hiraoka N, Yamaji T, Mutoh M, Ishigamori R, Furuta K, Okusaka T, Shimada K, Kosuge T, Kanai Y, Nakagama H. Association of pancreatic fatty infiltration with pancreatic ductal adenocarcinoma. Clin Transl Gastroenterol, 5:e53, 2014

13. Nakahata S, Ichikawa T, Maneesaay P, Saito Y, Nagai K, Tamura T, Manachai N, Yamakawa N, Hamasaki M, Kitabayashi I, Arai Y, Kanai Y, Taki T, Abe T, Kiyonari H, Shimoda K, Ohshima K, Horii A, Shima H, Taniwaki M, Yamaguchi R, Morishita K. Loss of NDRG2 expression activates PI3K-AKT signalling via PTEN phosphorylation in ATLL and other cancers. Nat Commun, 5: 3393, 2014.

14. Takaki Y, Saito Y, Takasugi A, Toshimitsu K, Yamada S, Muramatsu T, Kimura M, Sugiyama K, Suzuki H, Arai E, Ojima H, Kanai Y, Saito H. Silencing of microRNA-122 is an early event during hepatocarcinogenesis from non-alcoholic steatohepatitis. Cancer Sci, 105:1254-1260, 2014

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16. Sato Y, Sasaki M, Harada K, Aishima S, Fukusato T, Ojima H, Kanai Y, Kage M, Nakanuma Y, Tsubouchi H, Hepatolithiasis Subdivision of Intractable Hepatobiliary Diseases Study Group of Japan (Chairman, Hirohito Tsubouchi). Pathological diagnosis of flat epithelial lesions of the biliary tract with emphasis on biliary intraepithelial neoplasia. J Gastroenterol, 49:64-72, 2014

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19. Taoka M, Morofuji N, Yamauchi Y, Ojima H, Kubota D, Terukina G, Nobe Y, Nakayama H, Takahashi N, Kosuge T, Isobe T, Kondo T. Global PROTOMAP profiling to search for biomarkers of early-recurrent hepatocellular carcinoma. J Proteome Res, 3:4847-4858, 2014

20. Takahashi H, Ojima H, Shimizu H, Furuse J, Furukawa H, Shibata T. Axitinib (AG-013736), an oral specific VEGFR TKI, shows potential therapeutic utility against cholangiocarcinoma. Jpn J Clin Oncol, 44:570-578, 2014

21. Okusaka T, Ojima H, Morizane C, Ikeda M, Shibata T. Emerging drugs for biliary cancer. Expert Opin Emerg Drugs, 19:11-24, 2014

22. Arai Y, Totoki Y, Hosoda F, Shirota T, Hama N, Nakamura H, Ojima H, Furuta K, Shimada K, Okusaka T, Kosuge T, Shibata T. Fibroblast growth factor receptor 2 tyrosine kinase fusions define a unique molecular subtype of cholangiocarcinoma. Hepatology, 59:1427-1434, 2014

Division of Genetics

Teruhiko Yoshida, Hiromi Sakamoto, Hitoshi Zenbutsu, Norihisa Saeki, Fumiaki Koizumi, Misuzu Okuyama, Yoko Odaka, Mineko Ushiama, Masumi Shimizu, Sayaka Mito, Hitomi Gunji, Tomoko Ikegami, Hiroe Ishii, Norie Kowatari, Misaki Ono, Sumiko Ohnami

Introduction

In 2014, the Division continued to pursue its ongoing research agenda except the departure of a laboratory head, Dr. Koizumi in March 2014. Dr. Zenbutsu has joined the Division as a new laboratory head. The 3 major research themes of the Division were #1) molecular understanding of cancer susceptibility; #2) development of personalized cancer diagnosis and treatment and #3) pharmacogenomics research on cancer treatment.

Research activities

#1) The Division has been engaged in the studies on prostate stem cell antigen (PSCA) gene related to cancer susceptibility identified by a genome-wide association studies. The Division demonstrated previously that the T allele of a single nucleotide polymorphism (SNP) rs2294008 (T/C) in the gene is associated with gastric cancer, and that the T allele significantly suppresses the transcriptional activity of the PSCA promoter. Replacing the C allele of rs2294008 to the T generates a binding consensus for Yin Yang 1 (YY1), a multifunctional zinc-finger transcription factor member of the Polycomb Group protein family, suggesting that YY1 contributes to PSCA promoter suppression. The PSCA protein is thought to be involved in some form of intracellular signaling. Gene-expression profiling on tumors with and without expression of PSCA unveiled several immune-related genes down-regulated by PSCA, including IL1RN and S100A9.

#2) The Division has continued the Integrated Disease Omics Project to explore target candidates for drug and biomarker development on 13 diseases supported by NiBio (National Institute of Biomedical Innovation). Dr. Sakamoto is in charge of the genomics core facility of the project and contributed to the construction of the Integrated Omics Database. Moreover, as a collaborative study with Drs. Hiroki Sasaki and Hitoshi Ichikawa, who were laboratory heads of the Division by 2013, Dr. Sakamoto performed SNP array and whole exome sequence analyses on diffuse-type gastric cancer and identified several new therapeutic target candidates (Figure 1).

#3) In 2014, Dr. Zenbutsu published in the following research on pharmacogenomics of breast cancer: CYP2D6 genotype and adjuvant tamoxifen (Figure 2): meta-analysis of heterogeneous study populations, Polygenic inheritance of paclitaxel-induced sensory peripheral neuropathy driven by axon outgrowth gene sets in CALGB 40101, VAV3 mediates resistance to breast cancer endocrine therapy.

Clinical trials

#3) CYP2D6 is well-known to be a key enzyme in the generation of the endoxifen which is a principal active metabolite of tamoxifen, and the genetic polymorphisms of CYP2D6 have been extensively investigated in association with the plasma endoxifen concentrations and clinical outcome of tamoxifen therapy. To prospectively investigate the effects of CYP2D6 genotype on the response of patients with hormone receptor-positive breast cancer, prospective clinical trial is ongoing using the patients receiving the preoperative tamoxifen therapy.

Education

Dr. Zenbutsu contributed to the pharmacy residency program in the NCC.

Future prospects

#1) Elucidation of the mechanism of carcinogenic effect of the genes identified by a hypothesis-free observational study on human clinical cancers is critically important for the translation of the scientific findings to the medical and health care of the society. It could be, however, a high-risk research, and PSCA seems to be a difficult case to dissolve its mystery. The Division highly appreciates Dr. Saeki for his remarkable perseverance and effort even in the limited research resource allocation.

#2) As in the case with #1), observational study should be followed by a functional exploration to identify an actionable drug target. The gastric cancer genome project by the Division has an advantage in that context, because it has entailed the establishment of primary culture cell lines, which have turned out to be precious resource for drug screening.

#3) The genome information-based companion diagnostics (CDx) could be one of the most important devices for precision medicine. The Division keeps working on the establishment of prediction system for the efficacy and adverse events of cancer chemotherapy through pharmacogenomics study.

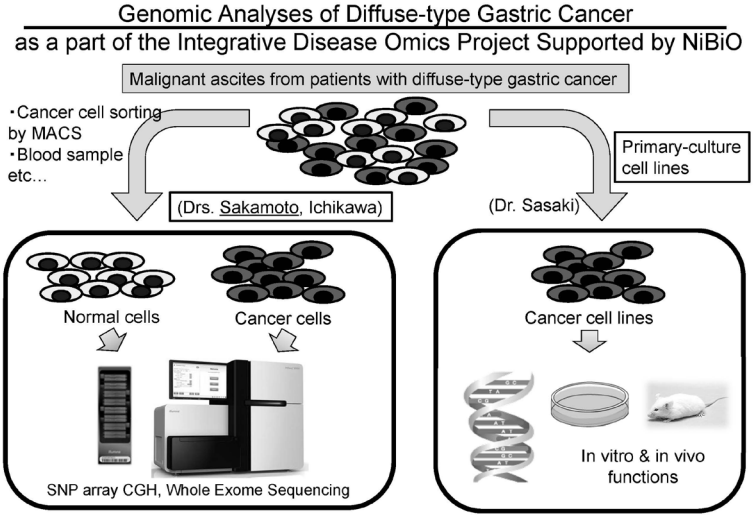

Figure 1. Genomic analyses of diffuse-type gastric cancer

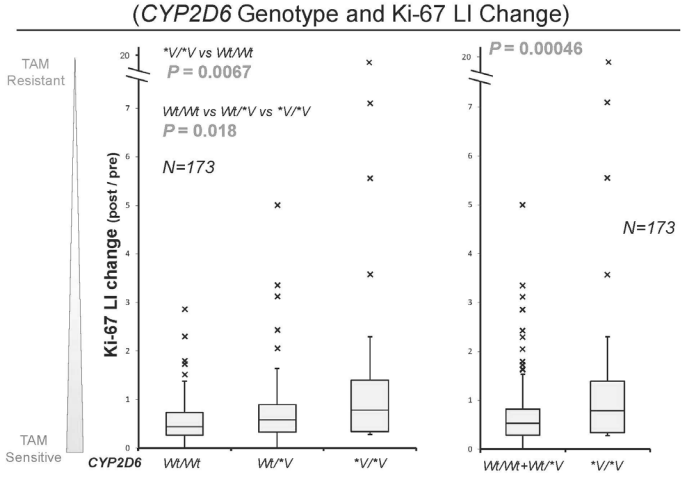

Figure 2. Clinical research on CYP2D6 polymorphism and personalized tamoxifen treatment

List of papers published in 2014

Journal

1. Nakaoku T, Tsuta K, Ichikawa H, Shiraishi K, Sakamoto H, Enari M, Furuta K, Shimada Y, Ogiwara H, Watanabe S, Nokihara H, Yasuda K, Hiramoto M, Nammo T, Ishigame T, Schetter AJ, Okayama H, Harris CC, Kim YH, Mishima M, Yokota J, Yoshida T, Kohno T. Druggable oncogene fusions in invasive mucinous lung adenocarcinoma. Clin Cancer Res, 20:3087-3093, 2014
2. Takahashi H, Sai K, Saito Y, Kaniwa N, Matsumura Y, Hamaguchi T, Shimada Y, Ohtsu A, Yoshino T, Doi T, Okuda H, Ichinohe R, Takahashi A, Doi A, Odaka Y, Okuyama M, Saijo N, Sawada J, Sakamoto H, Yoshida T. Application of a combination of a knowledge-based algorithm and 2-stage screening to hypothesis-free genomic data on irinotecan-treated patients for identification of a candidate single nucleotide polymorphism related to an adverse effect. PLoS One, 9:e105160, 2014
3. Arai E, Sakamoto H, Ichikawa H, Totsuka H, Chiku S, Gotoh M, Mori T, Nakatani T, Ohnami S, Nakagawa T, Fujimoto H, Wang L, Aburatani H, Yoshida T, Kanai Y. Multilayer-omics analysis of renal cell carcinoma, including the whole exome, methylome and transcriptome. Int J Cancer, 135:1330-1342, 2014
4. Mizukami T, Shiraishi K, Shimada Y, Ogiwara H, Tsuta K, Ichikawa H, Sakamoto H, Kato M, Shibata T, Nakano T, Kohno T. Molecular mechanisms underlying oncogenic RET fusion in lung adenocarcinoma. J Thorac Oncol, 9:622-630, 2014
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11. Takahashi A, Nakayama R, Ishibashi N, Doi A, Ichinohe R, Ikuyo Y, Takahashi T, Marui S, Yasuhara K, Nakamura T, Sugita S, Sakamoto H, Yoshida T, Hasegawa T, Takahashi H. Analysis of gene expression profiles of soft tissue sarcoma using a combination of knowledge-based filtering with integration of multiple statistics. PLoS One, 9:e106801, 2014
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14. Chhibber A, Mefford J, Stahl EA, Pendergrass SA, Baldwin RM, Owzar K, Li M, Winer EP, Hudis CA, Zembutsu H, Kubo M, Nakamura Y, McLeod HL, Ratain MJ, Shulman LN, Ritchie MD, Plenge RM, Witte JS, Kroetz DL. Polygenic inheritance of paclitaxel-induced sensory peripheral neuropathy driven by axon outgrowth gene sets in CALGB 40101 (Alliance). Pharmacogenomics J, 14:336-342, 2014
15. Province MA, Goetz MP, Brauch H, Flockhart DA, Hebert JM, Whaley R, Suman VJ, Schroth W, Winter S, Zembutsu H, Mushiroda T, Newman WG, Lee MTM, Ambrosone CB, Beckmann MW, Choi JY, Dieudonne AS, Fasching PA, Ferraldeschi R, Gong L, Haschke-Becher E, Howell A, Jordan LB, Hamann U, Kiyotani K, Krippl P, Lambrechts D, Latif A, Langsenlehner U, Lorizio W, Neven P, Nguyen AT, Park BW, Purdie CA, Quinlan P, Renner W, Schmidt M, Schwab M, Shin JG, Stingl JC, Wegman P, Wingren S, Wu AHB, Ziv E, Zirpoli G, Thompson AM, Jordan VC, Nakamura Y, Altman RB, Ames MM, Weinshilboum RM, Eichelbaum M, Ingle JN, Klein TE. CYP2D6 genotype and adjuvant tamoxifen: meta-analysis of heterogeneous study populations. Clin Pharmacol Ther, 95:216-227, 2014
16. Oue N, Anami K, Schetter AJ, Moehler M, Okayama H, Khan MA, Bowman ED, Mueller A, Schad A, Shimomura M, Hinoi T, Aoyagi K, Sasaki H, Okajima M, Ohdan H, Galle PR, Yasui W, Harris CC. High miR-21 expression from FFPE tissues is associated with poor survival and response to adjuvant chemotherapy in colon cancer. Int J Cancer, 134:1926-1934, 2014

Book

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Division of Carcinogenesis and Cancer Prevention (Viral Carcinogenesis and Prevention Group)

Tohru Kiyono, Takashi Yugawa, Tomomi Nakahara, Kenji Yamada, Satomi Kikawa, Yuki Inagawa, Takako Ishiyama, Katsuyuki Tanaka, Shin-ichi Ohno, Kasumi Ohtsubo, Kazuki Shimomura, Shotaro Tsunoda, Akiko Noguchi, Etsuko Kabasawa

Introduction

Approximately 15% of human cancers have a viral etiology, and seven viruses have been elucidated as being associated with human cancers. Among these recognized viruses, research in the Division of Carcinogenesis and Cancer Prevention is mainly focused on the molecular mechanisms of oncogenesis by human papillomaviruses (HPVs). 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). The E6 and E7 proteins of HR-HPVs are known to inactivate the major tumor suppressors, p53 and retinoblastoma protein (pRB), respectively. By using an in vitro multistep carcinogenesis model for cervical cancer, we are elucidating the roles of E6, E7 and cellular oncogenes in multistep carcinogenesis (Figure 1).

Routine activities

To clarify molecular mechanisms of oncogenesis by viral and cellular oncogenes and inactivation of tumor suppressors, we are establishing ex vivo carcinogenesis models for cervical cancer and other cancers by transducing abnormalities of genes found in cancer into normal cells-of-origin of each cancer.

Research activities

1. HPV-induced carcinogenesis and its prevention

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). Persistent infection of the HR-HPVs is a major cause of cervical cancer. About 50 to 100 copies of HPV genome are maintained in basal cells of the infected lesions such as cervical intraepithelial neoplasm (CIN). We demonstrated previously that the viral helicase E1 is dispensable for maintenance replication of the HPV genome in basal cell layer but indispensable for the initial amplification of the genome soon after primary infection and productive amplification in suprabasal layer. We previously established a human cervical keratinocyte cell line that harbors about 50 copies of episomal HPV16 genome. By using the tetON system, we transduced E1 and E2 in the cells where these two genes were induced upon doxycycline (DOX) treatment. These cell lines showed robust amplification of the HPV genome upon induction of E1 and E2 by DOX treatment. With this newly developed cell lines, we are analyzing the mechanism of transition from initial amplification to maintenance replication. We also study what facilitates loss of episomal HPV and emergence of viral genome integration which potentiates cancer progression. Once HPV genome is integrated in the form that E6 and E7 genes can be highly expressed in the basal cells, these oncogenes cooperatively immortalize and transform cells so as to induce CIN2/3 lesions. Recent genome editing technology with nucleases such as Zinc finger nuclease and CRISPR/Cas made it possible to directly target HPV genome whether or not it is integrated. With the CRISPR/Cas system, we are developing targeting vector to knock down E6/E7 regions of HPV16 and 18.

2. Human cancer xenograft model utilizing normal pancreatic duct epithelial cells

Pancreatic ductal adenocarcinomas (PDACs) are considered to arise through neoplastic transformation of human pancreatic duct epithelial cells (HPDECs). In order to evaluate the biological significance of genetic and epigenetic alterations in PDACs, we isolated primary HPDECs and established an in vitro carcinogenesis model.

Firstly, lentivirus-mediated transduction of $KRAS^{G12V}$, MYC and human papillomavirus 16 (HPV16) E6/E7 under the control of a tetracyclin-inducible promoter efficiently immortalized and transformed primary HPDECs, which gave rise to adenocarcinomas subcutaneously in an immune-deficient mouse xenograft model, depending on expression of the four genes. The tumors regressed promptly upon shutting-off the oncogenes, and the remaining tissues showed histological features corresponding to normal ductal structures with simple columnar epithelium. Re-expression of the oncogenes resulted in development of multiple PDACs through pancreatic intraepithelial neoplasia-like structures (Fig 1). We also succeeded in efficient immortalization of primary HPDECs with transduction of mutant CDK4, cyclin D1 and TERT. In combination with p53 silencing, $KRAS^{G12V}$ alone was sufficient to fully transform the immortalized HPDECs, and MYC markedly accelerated the development of tumors.

Education

Five undergraduate and graduate students in local universities worked as trainees in our lab and had cancer research training.

Future prospects

The current HPV vaccines have no therapeutic effect upon pre-existing CIN lesions. To clear HPV infection from CIN lesions, possible strategy is eradication of HPV genome with specific inhibitor of HPV replication or elimination of HPV-infected cells with surgery or by induction of cell death with drug or therapeutic vaccine. Elucidation of the transition mechanism from initial amplification to maintenance replication will facilitate development of such drugs. If such drugs are developed, prevention of cervical cancer will be much easier.

The in vitro carcinogenesis model with reversible control of oncogene expression enabled de novo development of PDAC from quasi-normal human tissues pre-formed subcutaneously in mice and might be applicable to carcinogenesis models in many organ sites. These models will be useful for preclinical assessment of new cancer therapies.

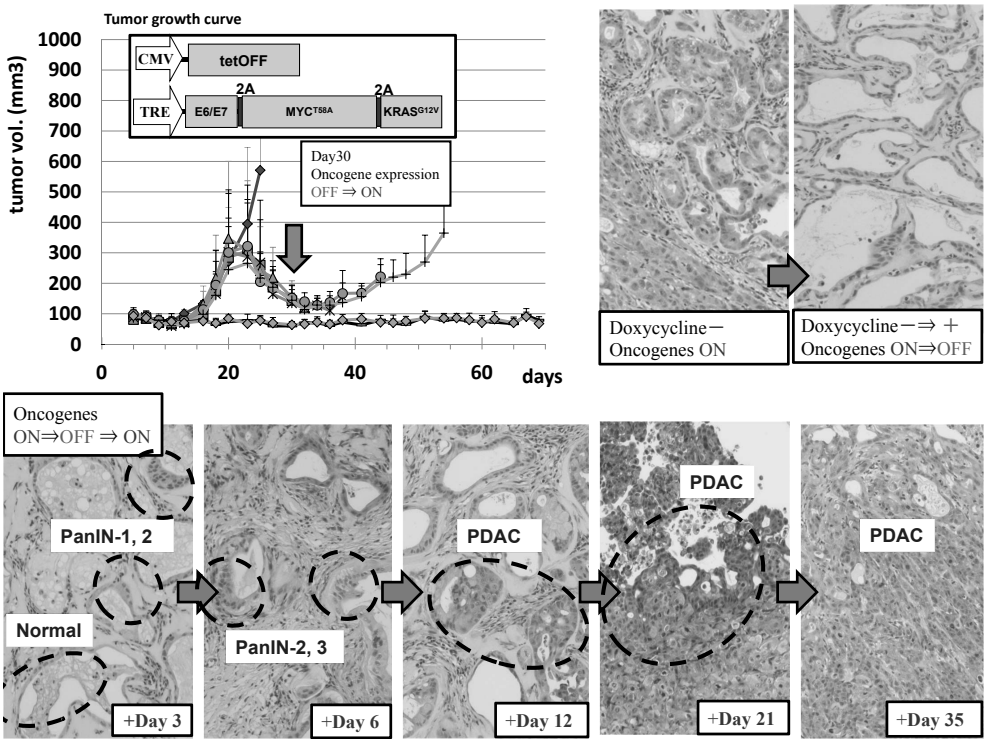

Figure 1. Ex vivo Pancreatic Carcinogenesis Model

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Journal

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Division of Carcinogenesis and Cancer Prevention (Chemical Carcinogenesis and Prevention Group)

Hitoshi Nakagama, Yukari Totsuka, Ken-ichi Yoshioka, Sachiko Dobashi, Kazuhiro Shiizaki, Masanori Goto, Michihiro Mutoh, Gen Fujii, Satomi Shimizu, Wakana Onuma, Takahiro Hamoya, Masahiro Ishikawa

Introduction

Cancer is a disease associated with environmental factors and aging. Most of them are developed without particular backgrounds that risk genomic destabilization, such as repair deficiency. This poses multiple questions, including how genomic instability is developed in association with aging and how genomic instability drives cancer development. To address these questions, we are studying environmental and aging-associated risk factors of genomic instability and cancer. We are also evaluating the usefulness and safety of thorough endoscopic polypectomy and of cancer chemopreventive agents in familial adenomatous polyposis (FAP) patients.

Routine activities

To clarify cancer risks, we are studying DNA adducts and damages and genomic instabilities that are triggered by those DNA lesions and cause cellular transformation. In addition, we are also studying DNA damage response and repair mechanisms, which can neutralize the cancer risks.

Research activities

1. DNA damage repair study in senescent cells

Senescent cells are defective in DNA damage repair, hence usually accumulating unrepairable DNA lesions. Here we showed such repair deficiency was partly because of the decrease of histone H2AX that was required for DNA repair. Intriguingly, such cells still can repair DNA double strand breaks (DSBs) because H2AX transiently expresses in response to DSBs.

2. Study of genomic instability development

Mismatch repair deficient cancer cells generally show microsatellite instability (MSI). Here we have observed that such MSI induction was triggered by DNA replication stress.

3. Identification of novel mutagens/carcinogens

A new mutagenic Maillard reaction product formed from glucose and L-tryptophan, an aminobenzoazepinoquinolinone derivative (ABAQ) was identified. To investigate *in vivo* mutagenicity of ABAQ, *gpt* delta transgenic mice were treated with five consecutive administrations of ABAQ by gavage at doses of 25 or 50 mg/kg per week for 3 weeks. *gpt* mutation frequencies (MF) in the liver of mice treated with ABAQ significantly increased in a dose-dependent manner. Mutation spectra analysis showed that G:C to A:T transition and A:T to C:G transversion were the most significant. Moreover, we determined the tumor-initiating potency of ABAQ using an inflammation-related, two-stage mouse colon carcinogenesis model. Male Crj: CD-1 (ICR) mice were treated with the single intragastric administration (100 or 200 mg/kg body weight) of ABAQ followed by subsequent 1-week oral exposure to 2% dextran sodium sulfate (DSS) in drinking water. The ABAQ treatment alone resulted in high-grade dysplasia, which is a precursor to colorectal cancer, in the colon. Following the administration of DSS after ABAQ treatment, the incidence and frequency of high-grade dysplastic lesions increased. These findings indicate that ABAQ is mutagenic, and might contribute to cancer development in animal models.

Reports related to other environmental mutagens/carcinogens can be found in the attached list of references.

4. Prevention of colorectal cancer

FAP patients are a well-known high risk group of colorectal cancer (CRC). We are evaluating the usefulness and safety of thorough endoscopic polypectomy and of cancer chemopreventive

agents in FAP patients. Based on these findings, we are trying to clarify the underlying mechanism of colorectal carcinogenesis in laboratory study. Moreover, we are searching for novel chemopreventive agents against CRC using animal models of FAP.

Education

Six undergraduate and graduate students in local universities worked as trainees in our lab and had cancer research training.

List of papers published in 2014

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Future Prospects

Our challenge is to characterize the risk factors of cancer and the regulation to control those risks. As a future direction, we are trying to establish the strategies to prevent cancer.

Book

1. Mutoh M, Takahashi M and Wakabayashi K. Chapter 20 ”Chemoprevention of colorectal cancer by anti-inflammatory agents”. In: Hiraku Y, Kawanishi S, Ohshima H (eds), Cancer and Inflammation Mechanisms: Chemical, Biological, and Clinical Aspects, First edition, USA, John Wiley & Sons, Inc., 2014

Division of Cancer Biology

Hirofumi Arakawa, Yasuyuki Nakamura, Noriaki Kitamura, Masayuki Tsuneki, Sayaka Yasuda, Saori Morota, Hiroki Kamino, Yoko Sagami, Ruri Nakanishi, Yoko Takahashi

Introduction

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 preventive, diagnostic, and therapeutic strategies could be developed.

Research activities

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. Thus far, a number of p53-target genes including *DFNA5*, *SEMA3F*, *BLNK*, *UNC5A*, *NEEP21*, and *TMPS* have been identified and characterized at the Division. Along the line, a new p53-target gene was identified, and designated Mieap for mitochondria-eating protein, reflecting its unusual function of the protein. Surprisingly, the function of Mieap is involved in mitochondrial quality control (MQC).

Mieap-induced accumulation of lysosome-like organelles 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 (*PLoS ONE* 6: e16054, 2011). In this mechanism, Mieap induces the accumulation of intramitochondrial lysosomal proteins in order 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.

BNIP3 and NIX, mitochondrial outer membrane proteins, two Mieap-interacting proteins mediate the translocation of lysosomal proteins from cytosol into mitochondria during MALM by forming an unknown pore in the mitochondrial double membrane (*PLoS ONE* 7: e30767, 2012). 14-3-3γ mediates the degradation of oxidized mitochondrial proteins within mitochondria during MALM (*Scientific Reports* 2: 379, 2012).

Mieap-inudced vacuole

Alternatively, the other mechanism has been designated MIV for Mieap-induced vacuole (*PLoS ONE* 6: e16060, 2011). When MALM is inhibited, Mieap induces a vacuole-like structure, 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).

Mitochondrial quality control and cancer

The accumulation of unhealthy mitochondria results in mitochondrial dysfunction, which has been implicated in aging, degenerative diseases and cancer. The Mieap-regulated MQC is frequently inactivated by p53 mutations or Mieap-methylation or BNIP3 methylation in more than 80% primary colorectal and pancreatic cancer tissues. In order to further evaluate the clinical significance of the Mieap-regulated MQC, the status of p53 (gene mutation), Mieap (methylation), and BNIP3/NIX (methylation) are being examined in many primary

cancer tissues including breast and gastric cancer patients.

To clarify the in vivo role of Mieap in tumorignenesis, the Mieap knockout mice were generated in the Division. Using the Mieap knockout mice, the Mieap-deficient $Apc^{MIN/+}$ mice were also generated and being analyzed in order to elucidate the role of Mieap in colorectal cancer tumorigenesis. In addition, the Mieap-deficient pancreatic and gastric cancer models are being prepared at the Division.

Aerobic glycolysis is a common feature of human cancers, which is also known as the Warburg effect. 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). The accumulated unhealthy mitochondria in cancer cells also produce high level of reactive oxygen species (ROS). The increased mitochondrial ROS dramatically enhance cancer migration and invasion (Figure 2).

Education

To acquire knowledge and skills for cancer research, students attend lectures and seminars, and attend and/or practice research meeting, journal club, scientific meeting, etc. These practices will enable students to develop an ability to conduct their studies as an independent cancer researcher in the future. To obtain good skills to carry out experiments that are required for cancer research, students belong to one of our research groups, and conduct their own studies under the guidance of the instructor and/or staff. Students perform various experiments involved in genetics, gene technology, biochemistry, cellular biology, molecular biology, physiology, experimental animal, pathology, genomic/epigenomic/proteomic analysis, imaging, next generation sequencing, etc.

Future prospects

Analyses of clinical cancer tissues and various cancer-mouse models enable us to understand the actual role of the Mieap-regulated mitochondrial quality control in human cancer formation, progression, invasion and metastasis. Finally, we will be able to establish a solid foundation for development of new strategies for cancer prevention, diagnosis, and therapy in the future.

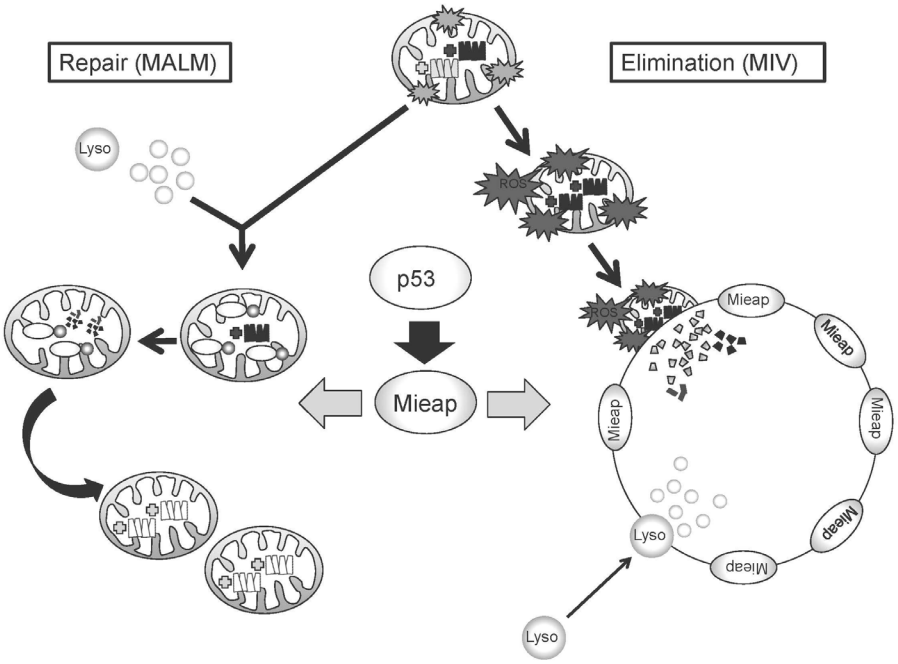

Figure 1.

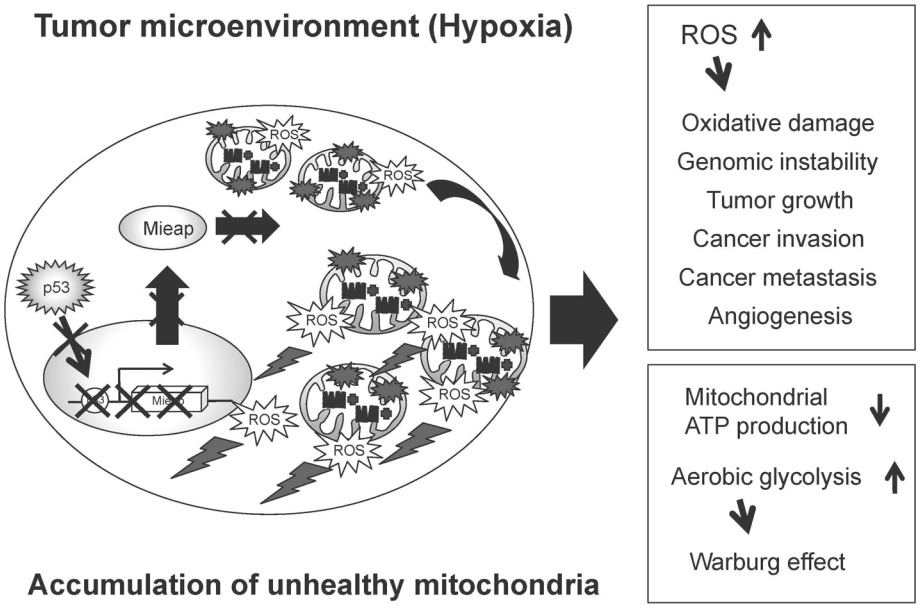
Tumor microenvironment (Hypoxia)
ROS
Mieap
p53
Mieap
Accumulation of unhealthy mitochondria
ROS
Oxidative damage
Genomic instability
Tumor growth
Cancer invasion
Cancer metastasis
Angiogenesis
Mitochondrial ATP production
Aerobic glycolysis
Warburg effect

Figure 2.

Division of Hematological Malignancy

Issay Kitabayashi, Kazutsune Yamagata, Takuo Katsumoto, Yutaka Shima, Yoko Ogawara, Emi Takamatsu, Yuuki Kagiyama, Mai Suzuki, Shuhei Fujita, Yukiko Aikawa, Mika Shino, Rieko Furuya

Introduction

AML is the most common leukemia in Japan and U.S. With current standard chemotherapy, approximately 70% of adults with AML can be expected to attain complete remission status following appropriate induction therapy. However, many of the patients relapse AML and only 25-30% of young adults and fewer than 10% of older patients survive longer than 5 years, suggesting presence of AML stem cells that are resistant to chemotheraphy. Thus, AML stem cell eradication is thought to be crucial for cure of AML. Chromosome abnormalities, which result in generation of specific fusion genes, are observed in ~50% of AML patients. AML associated with fusion genes involving MLL, MOZ, CALM or NUP98 have an extremely poor outcome. Normal cytogenetics portend average-risk AML. Recent genome analysis revealed that mutations in NPM, IDH1/IDH2/TET2, DNMT3a and FLT3 genes are often simultaneously observed in patients with normal cytogenetics. Our research purpose is to establish new therapeutic methods by identifying molecular targets that is essential for maintenance of AML cells, especially AML stem cells.

Research activities

Chromosomal translocations that involve the monocytic leukemia zinc finger (MOZ) gene are typically associated with human acute myeloid leukemia (AML) and often predict a poor prognosis. Overexpression of HOXA9, HOXA10, and MEIS1 was observed in AML patients with MOZ fusions. To assess the functional role of HOX upregulation in leukemogenesis by MOZ-TIF2, we focused on bromodomain-PHD finger protein 1 (BRPF1), a component of the MOZ complex that carries out histone acetylation for generating and maintaining proper epigenetic programs in hematopoietic cells. Immunoprecipitation analysis showed that MOZ-TIF2 formed a stable complex with BRPF1, and chromatin immunoprecipitation analysis showed that MOZ-TIF2 and BRPF1 interacted with HOX genes in MOZ-TIF2-induced AML cells. Depletion of BRPF1 decreased the MOZ localization on HOX genes, resulting in loss of transformation ability induced by MOZ-TIF2. Furthermore, mutant MOZ-TIF2 engineered to lack histone acetyltransferase activity was incapable of deregulating HOX genes as well as initiating leukemia. These data indicate that MOZ-TIF2/BRPF1 complex upregulates HOX genes mediated by MOZ-dependent histone acetylation, leading to the development of leukemia. We suggest that activation of BRPF1/HOX pathway through MOZ HAT activity is critical for MOZ-TIF2 to induce AML.

The CALM-AF10 fusion gene, which results from a t(10;11) translocation, is found in a variety of hematopoietic malignancies. Certain HOXA cluster genes and MEIS1 genes are upregulated in patients and mouse models that express CALM-AF10. Wild-type clathrin assembly lymphoid myeloid leukemia protein (CALM) primarily localizes in a diffuse pattern within the cytoplasm, whereas AF10 localizes in the nucleus; however, it is not clear where CALM-AF10 acts to induce leukemia. To investigate the influence of localization on leukemogenesis involving CALM-AF10, we determined the nuclear export signal (NES) within CALM that is necessary and sufficient for cytoplasmic localization of CALM-AF10. Mutations in the NES eliminated the capacity of CALM-AF10 to immortalize murine bone-marrow cells in vitro and to promote development of acute myeloid leukemia in mouse models. Furthermore, a fusion of AF10 with the minimal NES can immortalize bone-marrow cells and induce leukemia in mice.

These results suggest that during leukemogenesis, CALM-AF10 plays its critical roles in the cytoplasm.

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Division of Cancer Stem Cell

Kenkichi Masutomi, Yoshiko Maida, Satoko Yamaguchi, Mami Yasukawa, Marco Ghilotti, Yosuke Satomura

Introduction

Research in the Division of Cancer Stem Cell is focused on deciphering the mechanisms that establish and maintain cancer stem cells and developing novel therapeutic approaches to treat cancer stem cells. In particular, the Division studies the molecular links between a) telomerase and RNA-dependent RNA polymerase (RdRP); b) telomerase and cancer stem cells; and c) RdRP and anticancer drugs.

Telomerase and RNA-dependent RNA polymerase

Telomerase is a ribonucleoprotein complex that elongates telomeres. Human TERT is known as the catalytic subunit of the enzyme. TERT acts as an RNA-dependent DNA polymerase (RdDP) and synthesizes telomere DNA from a non-coding RNA template human TERC. Although the major function of TERT is believed to be telomere elongation, emerging evidence indicates that TERT exhibits various functions beyond telomere maintenance. We reported that TERT has an RdRP activity and mediates post-transcriptional gene silencing through the production of endogenous siRNAs[1] (Figure 1). To further investigate biological functions of TERT-RdRP, we generated a new anti-TERT monoclonal antibody and established an RdRP assay using TERT immune complexes isolated from cell lysate (IP-RdRP assay). We confirmed that both TERT levels and TERT-associated RdRP activity are increased during mitosis while telomerase activity is upregulated in S phase[2]. These observations indicate a non-telomere directed function of TERT during mitosis.

RdRPs in yeast and worm regulate centromeric heterochromatin formation, and RdRPs are required for proper chromosome segregation during mitosis in these organisms. The RNA-directed RNA polymerase complex (RDRC) contributes to the regulation, and the complex contains RdRP and RNA helicase. In our study, TERT assembles with BRG1 and nucleostemin (NS) in mitotic cells, and the TERT/BRG1/NS complex (TBN complex) exerts RdRP activity. Because TERT has RdRP activity, and BRG1 has helicase activity, we speculated that the TBN complex might have similar functions with the RDRC. We confirmed that TERT-RdRP suppresses transcription from heterochromatic regions at centromeres and transposons, and suppression of TERT-RdRP complex results in the increase of the cells arrested in mitosis, binucleate cells and the heterochromatic transcription[2]. These observations indicate that TERT-RdRP contributes to mitotic progression through the regulation of heterochromatin maintenance (Figure 2). Our findings suggest that inhibitors for the novel functions of TERT may prove useful in targeting cancer cells.

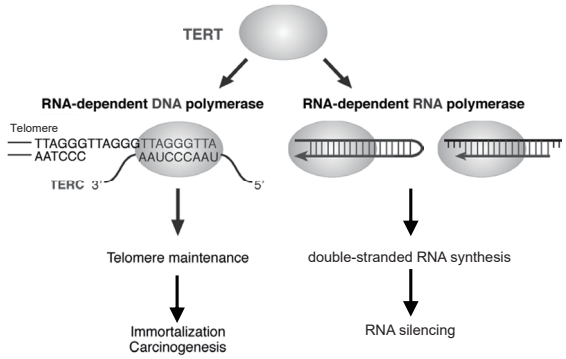

Figure 1. TERT exerts RdRP activity

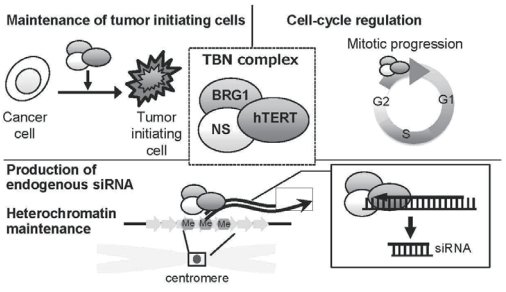

Figure 2. Various functions of the TBN complex

Telomerase and cancer stem cells

Previous studies indicated that TERT has activities beyond telomere maintenance, and it is speculated that the constitutive expression of TERT not only stabilizes telomere length and facilitates cell immortalization but also contributes to tumor susceptibility and alters stem cell cycling in vivo even when telomere lengths are not limited. As mentioned above, we found that TERT forms a protein complex with the SWI/SNF component (BRG1) and the nucleolar GTP-binding protein (NS); the TBN complex participates in the regulation of tumor initiating cells (TICs) phenotypes through telomere-independent mechanisms[3] (Figure 2). We also confirmed that the cells that constitutively express NS exhibited increased beta-catenin signaling and elevated MYC, OCT3/4, KLF4 and TWIST (master regulator of epithelial mesenchymal transition [EMT]) expression. Moreover, cells that constitutively express elevated levels of TERT, BRG1 and NS exhibit increased CD133 and CD44 expression and enhanced tumorigenicity at limiting cell numbers. These observations indicate that the TBN complex is essential for the maintenance of TICs.

RdRP and anticancer drugs

Ovarian cancer is the most lethal of all gynecological malignancies in Japan. The majority of ovarian cancers are diagnosed at an advanced stage. Currently, platinum-based chemotherapy is the standard first-line treatment for advanced ovarian cancer patients; however, chemoresistance is a major obstacle for long-term survival after initial treatment. Using platinum-sensitive and platinum-resistant ovarian cancer cell lines, we screened a series of anti-cancer compounds for growth suppression of platinum-resistant ovarian cancer cell lines[4]. We found that eribulin mesylate (eribulin) effectively inhibits growth of platinum-resistant ovarian cancer cells. Eribulin is available for the treatment of breast cancer in Japan. Although, it has been confirmed that eribulin exerts its anticancer effect by blocking the elongation of microtubules, we found that eribulin specifically inhibits the RdRP activity of TERT in vitro, suggesting TERT-RdRP as a novel molecular target of the drug beyond tubulin. This hypothesis was further supported by the results showing that 1) eribulin-sensitive ovarian cancer cell lines express high levels of TERT, and 2) suppression of TERT expression reduced sensitivity to erilubin. The eribulin-sensitive cell lines have enhanced cancer stem cell (CSC)-like traits, the characteristics related to TERT, as well. Our study demonstrated that eribulin may be a promising therapeutic agent for platinum-resistant ovarian cancer.

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Division of Cancer Differentiation

Koji Okamoto, Daisuke Shiokawa, Hirokazu Ohata, Su Youn Chung, Noriyuki Yokomichi, Hirokazu Takahashi, Waka Kato, Naoko Osada, Kenta Takahashi, Rie Uchino, Ai Sato, Hiroaki Sakai

Introduction

Cancer stem cells (CSCs) are likely to be responsible of malignant traits of refractory cancer, i.e. ability to generate metastatic foci and chemoresistance. Our group mainly focuses on studying CSCs from colon cancer and serous ovarian cancer. We aim to investigate biological feature of CSCs by cultivating them *in vitro* from various clinical specimens. In addition, we use the established CSCs to generate patient-derived xenograft tumor by injecting them into immuno-compromized mice. As an alternative approach to understand cancer metastasis, we have performed functional screening using lentivirus libraries of shRNAs and miRNAs.

Routine activities

A weekly conference is held with members of the Division of Cancer Differentiation.

Research activities

Biological studies of cancer stem cells *in vitro* from human refractory cancer

Recently, by performing spheroid culture in the presence of an inhibitor of Rho-associated protein kinase (ROCK), we isolated and expanded cancer stem cells *in vitro* from human colon cancer and serous ovarian cancer. In addition, we successfully cultivated CSCs from metastatic foci in liver. We are comparing the metastatic and non-metastatic CSCs through microarray analyses and metabolome analyses. These investigations revealed several specific genes and metabolites that are specifically expressed at high levels in metastatic liver. We are now examining if they are linked to any functional roles in liver metastasis of colon cancer.

In our previous studies, we demonstrated that, in colon CSCs, CD44 induction after suppression of ROCK is associated with the maintenance of stemness of colon CSCs. We demonstrated that activation of mTORC1 by reactive oxygen species (ROS) is responsible for the induction of CD44 and the stemness of colon CSCs. Furthermore, we revealed that NADPH oxidase is responsible for ROS production in colon CSCs.

In addition to colon CSCs, we also looked for the crucial regulatory pathways for proliferation of ovarian CSCs. We showed that ALDH, one of the established CSC markers for various types of CSCs, is specifically expressed in ovarian CSCs. Further, ALDH activity is functionally important for their proliferation.

Functional identification and characterization of regulatory factor of cancer metastasis

Through functional screening of miRNA lentivirus library, we previously identified miR-493 as metastasis-inhibitory miRNA, and demonstrated that up-regulation of miR-493 during carcinogenesis may prevent liver metastasis via the induction of cell death of metastasized cells, through inhibition of its targets, i.e. IGF-1R and MKK7. We also performed the functional screening of shRNA lentivirus library and identified several metastasis-suppressive shRNA. Characterization of the corresponding genes is under way.

Education

Teaching students (2 undergraduate students, 2 graduate students)

Future prospects

We will pursue the basic research on CSCs derived from refractory cancer. In future, we aim to translate the acquired knowledge for CSCs into clinical purposes.

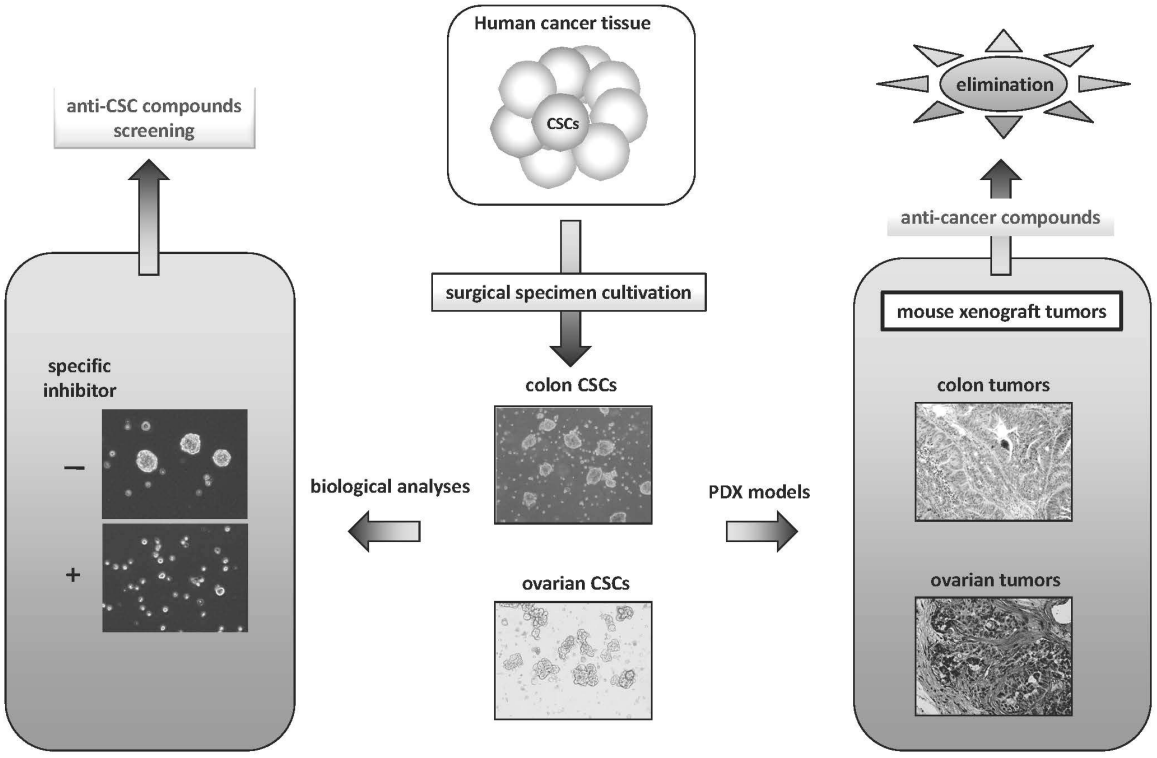

Figure 1. Experimental platforms for identification of novel anti-cancer compounds

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Division of Epigenomics

Toshikazu Ushijima, Satoshi Yamashita, Kiyoshi Asada, Hideyuki Takeshima, Naoko Hattori, Takayoshi Kishino, Yuka Takeuchi, Yukie Yoda, Takamasa Takahashi, Satoshi Yoshida, Masahiro Maeda, Zong Liang, Akiko Mori, Kana Kimura, Naoko Kobayashi, Yuko Miyaji, Aya Nakajima

Introduction

This Division has been focusing on the epigenetic mechanisms of carcinogenesis, and has identified many aberrantly methylated CpG islands (CGIs) in various cancers, including gastric cancers, esophageal squamous cell carcinomas (ESCCs), neuroblastomas, breast cancers, pancreatic cancers, lung cancers, ovarian cancers, and melanomas. This has led to identification of novel tumor-suppressor genes in various cancers, development of a powerful prognostic marker in neuroblastomas, and establishment of the concept of an "epigenetic field for cancerization (field defect)." This Division continues its activity in 1) developing clinically useful biomarkers, a novel approach of cancer prevention, and epigenetic therapy, and 2) in revealing molecular mechanisms of aberrant DNA methylation induction.

Research activities

1. Identification of Novel Epigenetic Alterations

Identification of tumor-suppressor genes silenced by aberrant DNA methylation is important. This year, *ANGPTL4* was identified as a tumor-suppressor gene inactivated by either aberrant DNA methylation or genetic alteration, namely a somatic 21-bp deletion. It was also revealed that ANGPTL4, which is a secreted protein, functions as a tumor-suppressor by suppressing the proliferation of human vascular endothelial cells and vascular tube formation. This showed that ANGPTL4 is a secreted tumor-suppressor that inhibits tumor angiogenesis inactivated by both genetic and epigenetic alterations.

The recent development of personal sequencers and bead array technology has made it possible to conduct integrated analysis of genetic and epigenetic alterations in multiple cancer samples. This year, integrated analysis was conducted in 50 primary gastric cancers, and it was revealed that epigenetic alterations were more frequently observed than genetic alterations in gastric cancers.

2. Development of Biomarkers

This Division previously revealed that neuroblastomas with the CpG island methylator phenotype (CIMP) have a worse prognosis than those without. This year, a diagnostic assay based on pyrosequencing technology was developed for clinical practice in collaboration with a diagnostic company, SRL, Inc (Figure 1). Now, the clinical usefulness of CIMP in neuroblastomas is being analyzed using primary neuroblastoma samples collected in a prospective manner. A pre-clinical study using a combination of a DNA demethylating drug, 5-aza-2'-deoxycytidine (decitabine), and a differentiation-inducing drug, tamibarotene, is also being conducted for the development of epigenetic therapy in neuroblastomas.

In gastric cancers, the degree of accumulated aberrant DNA methylation in normal-appearing gastric mucosae is expected to be a useful diagnostic marker to predict a gastric cancer risk. To bring this concept into clinical practice, a multicenter prospective cohort study has been conducted for the prediction of metachronous gastric cancer risk after endoscopic resection. This year, an intermediate analysis was conducted, and it was suggested that cases with higher DNA methylation levels of *miR-124a-3* had a higher risk of metachronous gastric cancers.

Future Prospects

Based on these results, this Division will conduct 1) a multicenter prospective cohort study for the prediction of gastric cancer risk in

healthy volunteers who underwent eradication of *Helicobacter pylori*, the almost exclusive cause of gastric cancers, and 2) the development of epigenetic therapy in gastric cancers and neuroblastomas.

Other Activities

This Division assisted with 1) epigenetic and genetic analyses of primary cancer samples in several translational researches that are being conducted in the National Cancer Center and other institutions, and 2) epigenetic analysis in various animal models.

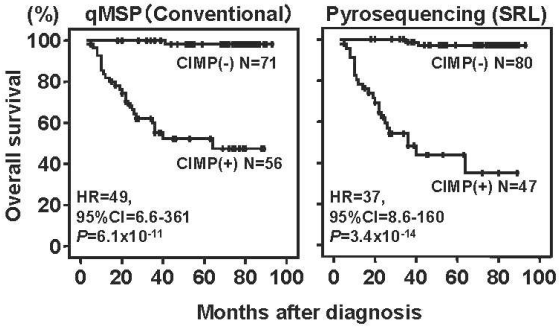

Figure 1. Diagnosis of prognosis in neuroblastoma by pyrosequencing

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Division of Cancer Genomics

Tatsuhiro Shibata, Fumie Hosoda, Yasushi Totoki, Mamoru Kato, Shinichi Yachida, Yasuhito Arai, Natsuko Hama, Hiromi Nakamura, Isao Kurosaka, Masami Suzuki, Hirofumi Rokutan, Erina Takai, Ogura Koichi, Tomoko Urushidate, Akiko Kokubu, Hiroko Shimizu, Shoko Ohashi, Wakako Mukai, Momoko Nagai, Erika Arakawa, Chika Shima, Keiko Igarashi, Hiroki Sato, Asmaa Elzawahry, Machiko Watanabe

Introduction

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 heterogeneous but *intervention-able* "biological systems" that contribute to the pathogenesis of cancer. This Division has also been participating in the international consortium (International Cancer Genome Consortium; ICGC), contributed to the core facility of the center, and developing new informatics tools for the data analysis from various types of next-generation high-performance sequencers (NGS).

Research activities

To elucidate genetic diversities in liver cancer with regards to ethnic and epidemiological differences, we have conducted the trans-ethnic cancer genome research under the umbrella of ICGC and US-based The Cancer Genome Atlas. We performed whole exome sequencing and copy number analysis of 619 pairs of liver cancers, which overrides in several ethnic populations (424 cases from the Japanese cohort and 195 from the US cohort) with various etiological backgrounds. Principal component analysis of six substitution patterns demonstrated that diversity of somatic substitution patterns existed among ethnic groups, which was not associated with known etiological backgrounds. Non-negative matrix factorization analysis extracted unique combinations of mutation signatures in each ethnic group. Trans-ethnic cancer genome sequencing first uncovered the existence of ethnicity-related mutagenesis processes in common human cancer (published in *Nature genetics*).

Whole genome sequencing of chondrosarcoma, rare bone cancer subtype, identified somatic alterations of the COL2A1 gene, which encodes an essential extracellular matrix protein in chondroskeletal development, in 19.3% of chondrosarcoma and 31.7% of enchondroma cases. Furthermore, a novel FN1-ACVR2A fusion transcript was observed in both chondrosarcoma and osteochondromatosis cases. We also found that mutational signature of chondrosarcoma shares significant commonalities with that of prostate cancer (published in *Genome Research*).

Biliary tract cancer (BTC) is an intractable cancer, with limited therapeutic options, in which the molecular mechanisms underlying tumor development remain poorly understood. To find out cancer driver alterations and biomarkers for personalized therapy, we performed whole exome and transcriptome sequencing analyses of BTCs (manuscript in revision). FGFR2 fusion kinase genes we identified are one of the high-potential therapeutic targets of BTC (published in *Hepatology*).

Whole exon sequencing and copy number analysis of rare hepato-biliary pancreatic tumors were conducted. "Liquid Clinical Sequencing" project in which digital PCR or NGS analysis of cell-free DNA from blood samples is used for molecular diagnosis, especially identification of actionable mutations, has been actively conducted in our group. We also launched the cancer-metagenomics project: establishment of the Japanese gut metagenomics database and exploring roles of gut microbiome in human carcinogenesis.

To understand the genetic basis of developing gastric cancer and to identify new drug targets for malignant gastric cancer, transcriptome sequence analysis of 200 gastric cancers has been performed.

Paired-end RNA sequence analysis detected thousands of abnormal structural variations and a verification experiment identified a hundred of fusion genes including protein kinase fusions. Further molecular and functional studies on oncogenic properties of these fusions promise to identify novel therapeutic targets.

Education

Three young research residents have been trained in this Division.

Future prospects

By utilizing current and cutting-edged sequencing technologies (e.g. single cell sequencing), this Division will actively investigate the cancer genomics from both basic (new biomarkers including therapeutic targets, epigenomics, metagenomics and immune-genomics) and translational research (preclinical research and liquid clinical sequencing) viewpoints. This Division will also contribute to the development of bioinformatics tools and human resources for analyzing the large cancer genomics data.

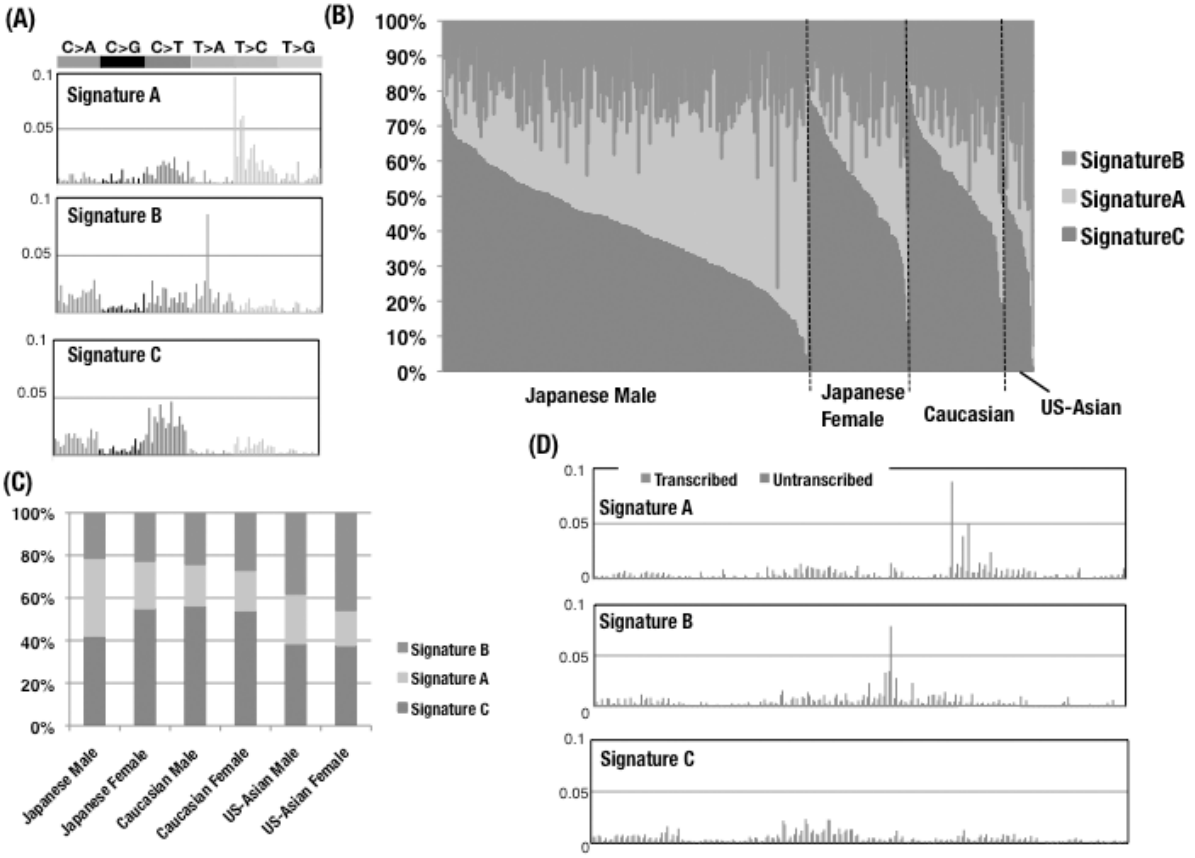

Figure 1.

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Division of Genome Biology

Takashi Kohno, Naoto Tsuchiya, Hideaki Ogiwara, Kouya Shiraishi, Motonobu Saito, Yoko Shimada, Mariko Sasaki, Ayaka Ohtsuka, Yuko Fujiwara, Keiko Igarashi, Reika Iwakawa-Kawabata, Takashi Nakaoku, Yoshitaka Seki, Kazuaki Takahashi, Kuniko Sunami, Takashi Mitachi, Yujin Ishihara, Keisuke Sugiyama, Daisuke Kurioka, Yoshiaki Onozato, Mei Tanabe, Ryo Okada

Introduction

Somatic mutation in the cancer genome and inter-individual variations in the human genome are crucial keys to improve cancer clinics. The aim of our Division is to find "seeds" that are expected to drastically improve treatment and prevention of cancer through identifying and understanding the biological significances of somatic mutations of their seeds in cancer genomes and genetic polymorphisms of cancer patients. To this end, we are working together with NCC staff from hospital, 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 worldwide.

Routine activities

Weekly research seminar and journal club are held with all members of the Division.

Research activities

1. Genes for personalized cancer medicine

Oncogenic fusion of the RET gene was recently identified as a novel driver gene aberration in lung adenocarcinoma (LADC) by us. Mouse transgenic model demonstrated that expression of *RET* fusion gene promotes tumor development in the lung. Treatment of the transgenic mice with RET inhibitor, vandetanib, showed marked reduction in the number of lung tumors. The results indicate that *RET* fusion functions as a driver for the onset of LADC. Furthermore, molecular mechanism for rearrangement of RET locus was addressed through the structural analysis of breakpoints and it was revealed that oncogenic RET fusion in LADC occurs through multiple pathways of illegitimate repair of DNA strand breaks. Whole RNA sequencing of 32 invasive mucinous adenocarcinomas (IMAs), including 27 cases without KRAS mutations, led us to identify the NRG1/neuregulin fusion gene as a novel druggable oncogenic fusion in IMA (Figure 1A). We proposed a synthetic lethality therapy approach using synthetic lethal relationship between two paralogous genes, based on our finding that BRG1-deficient cancer cells are susceptible to depletion of its paralogue, BRM. Microarray-based screening of microRNAs (miRNAs) was performed to identify miRNA species that are differentially expressed in chemotherapy responders and non-responders, and demonstrated that the three-miRNA signature in surgically resected primary LADC tissues could be clinically useful for predicting responsiveness to platinium-based doublet chemotherapy in recurrent LADC patients. Genome-wide association study (GWAS) led us to identify a novel LADC susceptibility locus. International and pan-Japan collaborative GWAS are underway to further identify genetic factors involved not only in susceptibility but also in prognosis of lung cancer.

2. Basic research for the development of miRNA drugs

Tumor-suppressive miRNA, miR-22, is a regulator for p53 tumor-suppressor network. By the miR-22 target screening, we identified NIMA-related kinase 9, NEK9, as a novel factor required for cell cycle progression in p53-inactivated cancer cells. NEK9 repression inhibited cell proliferation selectively in p53-deficient cancer cells in vitro and in vivo. Lung adenocarcinoma patients with positive staining for NEK9 and mutant p53 proteins exhibited significantly poorer prognosis, suggesting

that expression of both proteins promotes tumor growth. Collectively, these results provided possibility that NEK9 inhibition could be a novel strategy for development of cancer therapy (Figure 1B). In addition, we have successfully identified 6 circulating serum exosomal miRNAs as promising diagnostic biomarkers for the detection of colon cancer patients.

Clinical trials

A phase II clinical trial, which investigates the therapeutic effect of a RET-tyrosine kinase inhibitor, vandetanib, has been started by us in Japan in Q1 of 2013. For the purpose, >1500 non-small cell lung carcinoma cases have been screened by an all-Japan consortium consisting of 190 hospitals, LC-SCRUM-Japan (Lung Cancer Genomic Screening Project for Individualized Medicine in Japan), using RT-PCR and FISH assays developed by us. More than 10 RET fusion-positive cases are being treated with vandetanib.

Education

Supervising for the investigation and presentation skills of students and young researchers

Future prospects

Our Division aims to contribute for establishment of novel strategies of personalized cancer medicine, including prevention, diagnosis and therapy, through the finding of unique "seeds". A clinical trial, which investigates the therapeutic effect of a RET-tyrosine kinase inhibitor, will be expected to be a good response. Furthermore, identification and understanding biological roles of novel molecular targets, which are screened by synthetic lethality, provide unique and/or novel concepts for development cancer therapy. Furthermore, practical applications of miRNAs as a diagnostic biomarker for cancer detection will be expected in the near future.

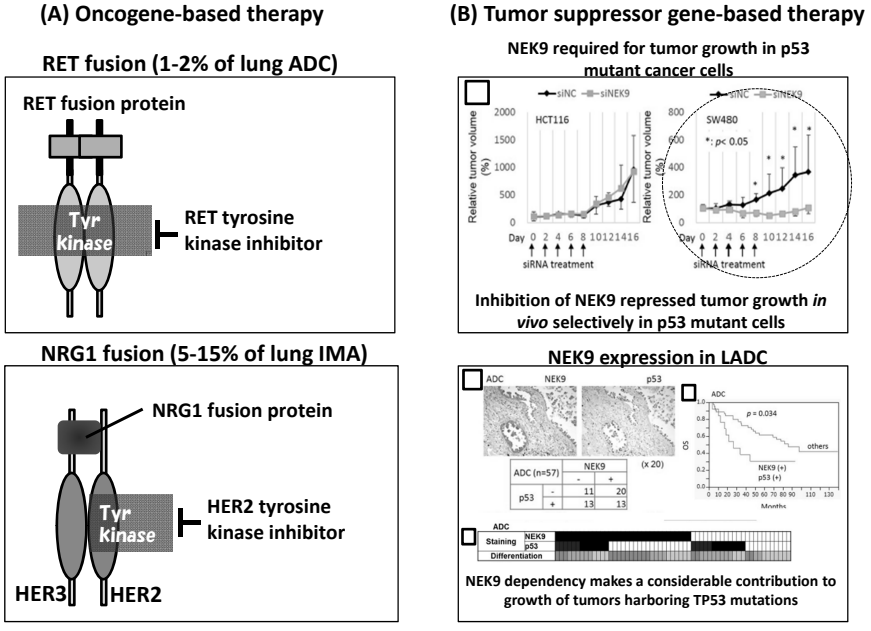

Figure 1. Div. Genome Biol.

List of papers published in 2014

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1. Saito M, Shiraishi K, Matsumoto K, Schetter AJ, Ogata-Kawata H, Tsuchiya N, Kunitoh H, Nokihara H, Watanabe S, Tsuta K, Kumamoto K, Takenoshita S, Yokota J, Harris CC, Kohno T. A three-microRNA signature predicts responses to platinum-based doublet chemotherapy in patients with lung adenocarcinoma. Clin Cancer Res, 20:4784-4793, 2014
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Division of Brain Tumor Translational Research

Koichi Ichimura, Shintaro Fukushima, Kai Yamasaki, Taishi Nakamura, Hirokazu Takami, Emiko Yamamoto, Kohei Fukuoka, Yuko Matsushita, Hideyuki Arita, Motoki Yonezawa

Introduction

Our laboratory focuses on translational research on various types of malignant brain tumor, which remains one of the most difficult cancer to cure in humans. There are more than 130 different types of brain tumors, each developing through distinct molecular pathogenesis. Morphological ambiguity across some tumor types makes accurate diagnosis sometimes challenging. To develop a novel molecular diagnostics and an effective personalized therapy, we set our aim to investigate the molecular pathogenesis of key malignant brain tumors including adult gliomas, primary central nervous system lymphomas (PCNSL) and pediatric brain tumors, in particular intracranial germ cell tumors (iGCT), to identify novel tumor markers to aid making diagnosis, to establish a novel molecular classification and an optimal assay for the molecular tests, and to more accurately predict outcome of the patients as well as novel therapeutic targets. For this purpose, we have organized nationwide multicenter collaborations to collect a large number of cases. The results will be applied to clinical trials and a routine clinical practice. The details of selected projects are described below.

Research activities

1. Development of a novel molecular classification and optimal molecular tests for adult gliomas

In 2013, we discovered that hotspot mutations in the promoter region of TERT, the reverse transcriptase subunit of human telomerase, were very common in oligodendrogliomas and glioblastomas however rare in astrocytomas. We are now conducting a multicenter study to develop a novel molecular classification scheme utilizing the statuses of the TERT promoter, IDH1/2, chromosomal arms 1p/19q and MGMT methylation. More than 800 adult glioma cases have been collected from 13 centers and a central pathology diagnosis is being carried out. They will be classified according to the molecular profiles and compared with the survival data to validate the efficacy of the system.

2. Development of a novel targeted therapy for glioblastoma

A novel therapy for glioblastoma targeting TERT is being developed in collaboration with the Division of Cancer Stem Cell. Pre-clinical experiments are currently being carried out. The initial results showed that the compound significantly suppressed growth of cultured or transplanted glioblastoma cell lines, indicating a strong anti-tumor activity. A successful result will lead to a clinical trial.

3. Genomic analysis of intracranial germ cell tumors

Intracranial germ cell tumors are the second most common pediatric brain tumors in Japan. We have established the Intracranial Germ Cell Tumor Genome Analysis Consortium, a nationwide collaborative network to study germ cell tumors, through which tumor samples of more than 170 cases from 22 centers have been so far collected. A whole exome sequencing was performed for 41 tumors and a targeted sequencing for further 91 intracranial and 65 testicular germ cell tumors. The results showed a high prevalence of mutations affecting the MAPK pathway, most notably in KIT, which appear to be one of the main driving forces of germ cell tumorigenesis. A genome-wide DNA methylation analysis, hCG expression in the tumor tissues, tumor-infiltrating lymphocytes in germinomas are also being investigated to elucidate the pathogenesis of these tumors with the aim for better prognostication and treatment.

4. Molecular diagnosis of pediatric brain tumors

In order to establish a central molecular diagnostic system, we have established the Japan Pediatric Molecular Neuro-oncology Group (JPMNG) to collect pediatric brain tumor samples nationwide and offer various molecular tests according to the internationally accepted procedures. More than 100 ependymomas have been collected through JPMNG, 70 of which have been subjected to a genome-wide methylation analysis using an Illumina HumanMethylation 450 BeadChip. The results were comparable to those published, indicating the accuracy of the method. An integrated protocol to genotype pediatric low-grade gliomas is also being developed.

Clinical trials

We continue to offer a MGMT methylation test for the patients enrolled in the EGGTRIAL, a clinical trial to evaluate the feasibility of the treatment strategy for elderly (70 or older) glioblastoma patients based on the MGMT status, in which those with methylated MGMT will be given TMZ chemotherapy alone while those with unmethylated MGMT will receive radiation alone. During 2014, 30 tumors from 28 patients were tested for MGMT methylation, 9 of which were judged methylated. The trial continues in 2015.

Education

Two postgraduate students, 3 Research Residents, 2 Clinical Residents were conducting research during 2014.

Future prospects

We aim to establish ourselves as a translational research center on malignant brain tumors in Japan through setting up efficient molecular tests and a molecular classification system, applying them for clinical trials and other clinical studies, developing novel therapeutic strategy for glioblastomas, educating young researchers and organizing a multicenter collaboration. Our goal is to contribute to facilitate brain tumor research and to provide better clinical management of malignant brain tumors in Japan.

List of papers published in 2014

Journal

1. Fukushima S, Otsuka A, Suzuki T, Yanagisawa T, Mishima K, Mukasa A, Saito N, Kumabe T, Kanamori M, Tominaga T, Narita Y, Shibui S, Kato M, Shibata T, Matsutani M, Nishikawa R, Ichimura K. Mutually exclusive mutations of KIT and RAS are associated with KIT mRNA expression and chromosomal instability in primary intracranial pure germinomas. Acta Neuropathol, 127:911-925, 2014
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Division of Chemotherapy and Clinical Research

Tesshi Yamada, Mitsuko Masutani, Masaya Ono, Kazufumi Honda, Mari Masuda, Hiroaki Fujimori-Sakuma, Nami Miura, Ayako Mimata, Masahiro Kamita, Shoji Imamichi, Yuka Sakaki, Hiromi Harada, Naoko Goto, Hiroko Ito, Junhui Wang, Haruyo Tozaki, Yuko Miyamoto, Nobuhiko Nishijima, Takanori Kakuya, Makoto Kobayashi, Kengo Inoue, Takahisa Hirai, Tasuku Itoh, Miyuki Hozumi, Sota Kikuhara, Gui Zhen Chen

Introduction

Advances in so-called "-omics" technologies have contributed tremendously to the discovery of therapeutic target molecules and diagnostic biomarkers. The Division has been devoted to the clinical application/translation of basic research findings obtained through the comprehensive genomics and proteomics approaches.

Signaling pathway profiling by reverse-phase protein arrays

Sorafenib monotherapy is the current standard treatment for unresectable hepatocellular carcinoma (HCC). However, not all HCC patients receive therapeutic benefits. Some tumors show marked shrinkage after short-term administration of sorafenib, but many others show no response. Sorafenib is a multi-kinase inhibitor, and its precise target molecule/pathway has not been established. We newly developed fluorescence reverse-phase protein arrays and profiled the phosphorylation status of signaling molecules in 23 HCC cell lines with different sensitivities to sorafenib. We found that sorafenib-resistant HCC cells showed constitutive activation of mammalian target of rapamycin (mTOR) signaling, and that increased expression of ribosomal protein S6 phosphorylated at serine residues 235/236 (p-rpS6 S235/236) was able to serve as a predictive biomarker of HCC unresponsiveness to sorafenib (5,7).

Proteomic analysis of ligamentum flavum from patients with lumbar spinal stenosis

Lumbar spinal stenosis (LSS) is a syndromic degenerative spinal disease and is characterized by spinal canal narrowing with subsequent neural compression causing gait disturbances. Although LSS is a major age-related musculoskeletal disease that causes large decreases in the daily living activities of the elderly, its molecular pathology has not been investigated using proteomics. We used 2-dimensional image converted analysis of LC/MS (2DICAL) to compare LF obtained from individuals with LSS to that obtained from individuals with disc herniation (non-degenerative control). We detected 64,781 MS peaks and identified 1675 differentially expressed peptides derived from 286 proteins. We verified four differentially expressed proteins (fibronectin, serine protease HTRA1, tenascin, and asporin) by quantitative proteomics using SRM/MRM. The present proteomic study is the first to identify proteins from degenerated and hypertrophied LF in LSS, which will help in studying LSS.

Hippo pathway gene mutations in malignant mesothelioma

Malignant mesothelioma (MM) is often unresectable and rarely responds to conventional cytotoxic drugs. It is therefore necessary to develop new therapeutics specifically targeting molecules essential for the development and/or progression of MM. In this study we first adopted an unbiased approach to examining the MM genome using whole-exon (exome) and RNA (transcriptome) sequencing. We found a novel gene fusion between the large tumor suppressor-1 and presenilin-1 genes (LATS1-PSEN1) (Fig. 1). The fusion gene product lacked the kinase activity of LATS1, and the LATS1 locus was inactivated by a two-hit genetic event. This initial discovery prompted us to sequence all exons of the 40 known Hippo pathway genes in 23 patients with MM. We found that the NF2, LATS2, RASSF1, and SAV1 genes were mutated with a frequency of 35% (8/23).

Prognostic and predictive significance of ACTN4 in locally advanced pancreatic cancer

Several clinical trials have compared

chemotherapy alone and chemoradiotherapy (CRT) for locally advanced pancreatic cancer (LAPC) treatment. However predictive biomarkers for optimal therapy of LAPC remain to be identified. We retrospectively estimated amplification of the ACTN4 gene to determine its usefulness as a predictive biomarker for LAPC. The copy number of ACTN4 in 91 biopsy specimens of LAPC before treatment was evaluated using fluorescence in situ hybridisation (FISH). There were no statistically significant differences in overall survival (OS) or progression free survival (PFS) of LAPC between patients treated with chemotherapy alone or with CRT. In a subgroup analysis of patients treated with CRT, patients with a copy number increase (CNI) of ACTN4 had a worse prognosis of OS than those with a normal copy number (NCN) of ACTN4 (P = 0.0005 log-rank test). However, OS in the subgroup treated with chemotherapy alone was not significantly different between patients with a CNI and a NCN of ACTN4. In the patients with a NCN of ACTN4, the median survival time (MST) of PFS in CRT-treated patients was longer than that of patients treated with chemotherapy alone (P = 0.049) (Fig. 2). The copy number of ACTN4 is a predictive biomarker for CRT of LAPC.

Research of PARP and PARG inhibitors for cancer treatment

Poly(ADP-ribose) polymerase (PARP) inhibitors are now in clinical trials and act by blocking DNA repair. On the other hand, PARP inhibitor olaparib was demonstrated to affect epigenetic regulation in cancer cells through DNA methyltransferase 3b downregulation. PARP inhibitor can target various PARP family proteins for epigenetic regulation. A collaborative study further showed the involvement of PARP7 as well as PARP1 in the maintenance of epigenetic regulation in ES cell models (11). Inhibition of poly(ADP-ribose) glycohydrolase (PARG) leads to block of DNA repair and causes cell death through poly(ADP-ribose) accumulation. Therefore, PARG inhibitors have been recently considered as a potential anti-cancer target (13). To develop specific and potent PARG inhibitors, a collaborative study with other institutions was initiated and novel PARG inhibitors have been identified and their structures are being optimized. Phenolic hydrazide hydrazines are one group of these compounds that show inhibition of PARG catalytic activity (12). Through genome-wide analysis, the genes that affect lethality by PARG inhibition have been identified and validated using cancer cell lines.

Studies for biological radiosensitization

To achieve biological radiosensitization in tumor radiation therapy, a comprehensive screening of the genes, which cause radiosensitization were performed. Besides known radiosensitizing genes when knocked down, including *PARP1*, *PARP-2* and *Rad51* genes, genes of various functions, such as DNA repair and chromatin regulation were picked up as candidate genes. The generation of gene clusters based on the networks of functional interactions was found to be a useful method for identifying 'radiosensitizing gene clusters'. A mechanistic study of a focused candidate gene that could effectively radiosensitize the particular cancer cells has been carried out.

Basic studies on boron neutron-captured therapy

The project of developing accelerator-based BNCT (boron neutron-capture therapy) system in NCC is ongoing. To support the biological evaluation of the accelerator-based BNCT, a collaborative study of BNCT with other institutes has been undertaken from 2012. The experimental systems to evaluate the radiation protection, safety and effectiveness of BNCT have been established using mouse models. The basic studies to optimize therapeutic effects of BNCT and search for the biomarkers for tumor cell death are also ongoing (14). Using tumor graft models, dynamics of DNA damage response markers after BNCT irradiation were studied. The early upregulation of HMGB1 and later augmentation pattern of poly(ADP-ribose) and γH2AX were found as the characteristic changes after BNCT (3). Comprehensive analysis of protein and gene expression was also performed after BNCT condition.

Future Prospects

With the increase of the cancer patients of advanced age, noninvasive cancer treatments with lower side-effects are currently being required. Through the further accumulation of the knowledge of cancer therapeutic targets and cancer heterogeniety, cancer treatment strategies including chemotherapy and radiation therapy are expected to become patient-friendly and effectively optimized for individual patients.

Figure 1. The novel gene fusion between the large tumor suppressor-1 and presenilin-1 genes (LATS1-PSEN1)

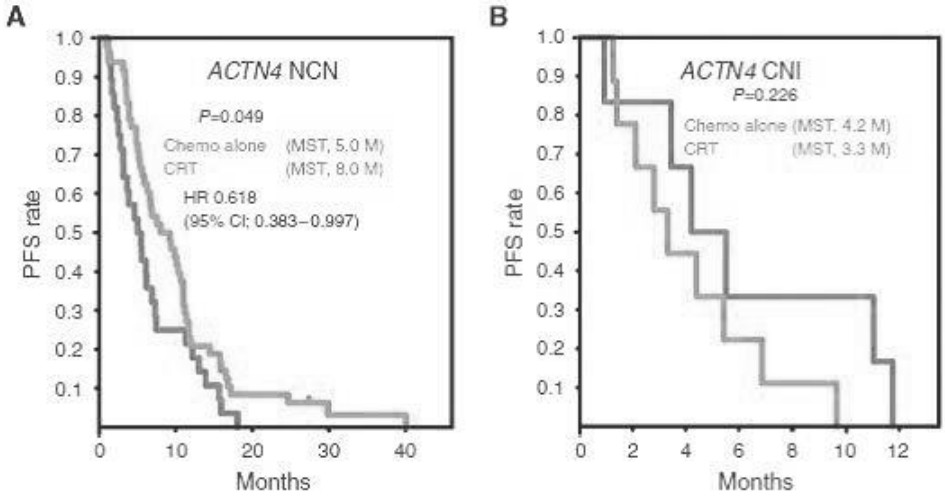

Figure 2. Progression-free survival of LAPC patients with NCN (A) and CNI (B) of ACTN4

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Journal

1. Fukushima S, Yoshida A, Honda K, Maeshima AM, Narita Y, Yamada T, Shibui S, Tsuda H. Immunohistochemical actinin-4 expression in infiltrating gliomas: association with WHO grade and differentiation. Brain Tumor Pathol, 31:11-16, 2014
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9. Taira N, Yamaguchi T, Kimura J, Lu Z-G, Fukuda S, Higashiyama S, Ono M, Yoshida K. Induction of amphiregulin by p53 promotes apoptosis via control of microRNA biogenesis in response to DNA damage. Proc Natl Acad Sci U S A, 111:717-722, 2014
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Division of Cancer Pathophysiology

Yasuhito Uezono, Seiji Shiraishi, Masami Suzuki, Kanako Miyano, Junko Ezuka, Yukiko Araki, Kiyoshi Terawaki, Katsuya Ohbuchi, Chika Miyagi, Koichiro Minami, Tohru Yokoyama, Satoshi Murakami, Hideya Kokubun, Akinobu Yokoyama, Hitomi Nishimura, Megumi Kawaida, Shiori Sato, Etsuko Nemoto, Takamichi Arima, Hirotsugu Kuwata, Chihiro Kojima, Yusuke Hamada

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) prevention and development of novel treatments for cancer cachexia symptoms. Based on the 2nd Basic Plan to Promote Cancer Control Programs established in Japan in 2012, basic to clinical, and also clinical to basic translational collaborative research with the clinical laboratory groups comprises our main research protocols and has been ongoing.

Routine activities

A weekly conference/research seminar is held with all members including students at the Division of Cancer Pathophysiology.

Research activities

Translational research to innovate new strategies to improve pain analgesia in cancer patients

The aim of our studies is to develop new therapies for chemotherapy-induced peripheral neuropathy, and refractory cancer pain, both of which make the quality of life of cancer patients even worse. One of the targets is oral stomatitis induced by chemotherapy and/or radiotherapy.

The cancer patients who undergo chemotherapy, radiotherapy and terminal palliative care often have a wide range of stomatitis, which induces severe pain and limits the fundamental basics of life such as eating, drinking and talking. In clinical sides, lidocaine is normally used for cancer patients with stomatitis to relieve oral pain. However, lidocaine removes not only the pain but also the ability to discriminate taste and texture, since it nonselectively suppresses the activation of all neurons by blocking the voltage-gated Na^+ channels. Therefore, a novel analgesic drug, which selectively blocks the pain-related neuron alone, is required to allow patients to eat without losing or changing the taste and texture. We have focused on a "compound X" as the novel analgesic drug for stomatitis, and established the method to evaluate the intensity of oral pain using stomatitis model animals. With the model, lidocaine not only inhibited pain but also caused numbness in normal oral mucosa. On the contrary, the compound X suppressed the pain in the ulcer, but had no effects on normal tissues. Further, the analgesic effect of the compound X was longer than that of lidocaine, indicating that the compound X is expected to be a more superior analgesic drug than lidocaine. Further, we have been elucidating the pharmacological actions of the compound X (e.g., how does it block only the pain-related neurons?) with cultured cell models. By connecting such a basic study to a clinical study, we want to develop "the new pain-killer compound X, which can remove the oral pain without changing the texture and taste of food" for cancer patients with severe painful stomatitis. This research project now has been intellectually and financially supported as "an innovative seed" by the Drug Discovery Support Network, which was newly established at the National Institute of Biomedical Innovation.

Second target is severe pain such as one with bone-metastasized patients. We previously showed that a platelet-activating factor (PAF) antagonist produced profound and long lasting anti-allodynia effects in several different neuropathic pain models in mice including a partial sciatic nerve ligation

injury model. Also we have found that the PAF antagonist showed extremely excellent analgesic effects on both the bone-metastasized cancer pain model mice and the chemotherapy-induced peripheral neuropathy model mice. In addition, we discovered that knocking out PAF synthase by siRNA technology in mouse model with severe pain by nerve ligation injury model, significantly reduced pain, demonstrating that existence of PAF seems to produce pain. We currently are developing both novel PAF receptor and PAF synthase antagonists. The pain-relieving action of PAF-signal antagonists are found to be effective for the treatment of pain.

Prevention, and decrease the cachexic symptoms or chemotherapy-induced side effects by Japanese traditional KAMPO medicines and opioid-related compounds

We established novel cancer cachexia animal models and then undertook molecular and cellular analyses to identify the mechanisms of action of the expected compounds to improve the quality of life of patients suffering from cancer cachexia with biological, biochemical and electrophysiological approaches. We found that a Japanese Kampo (traditional Oriental) medicine "rikkunshito" usually administered for the prevention of gastritis, nausea and vomiting since the 17th century in Japan, improved the symptoms of cancer cachexia. In addition to rikkunshito, we summarize the mechanisms of action of other traditional Japanese Kampo medicines to improve chemotherapy-induced side effects, and their potential use for improvement of the symptoms of cancer cachexic patients and the side effects in cancer patients who take anticancer agents.

In addition, the dormancy of tumor cells is a major problem in chemotherapy, since it limits the therapeutic efficacy of anti-tumor drugs that only target dividing cells. One potential way to overcome chemo-resistance is to “wake up” these dormant cells. Here we show that the opioid antagonist methylnaltrexone (MNTX) enhances the effect of docetaxel (Doc) by blocking a cell growth-suppressive pathway. We found that PENK, which encodes opioid growth factor (OGF) and suppresses cell growth, is predominantly expressed in diffuse-type gastric cancers (GCs). Blockade of OGF signaling by MNTX released cancer cells from their arrest and enhanced the effect of Doc. The combined use of Doc and MNTX significantly prolongs survival, alleviates abdominal pain, and diminishes Doc-resistant spheroids on the peritoneal membrane in model mice in comparison with the use of Doc alone. We showed that blockade of these pathways by MNTX may enhance the effects of anti-tumor drugs.

Education

We have two graduate students and 6 students.

List of papers published in 2014

Journal

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Division of Molecular and Cellular Medicine

Kazunori Aoki, Kenta Narumi, Yoko Kobayashi, Tsukasa Shinohara, Ryousuke Ueda, Hisayoshi Hashimoto, Yosei Rin, Masaki Nagasato, Yuki Yamamoto

Introduction

Research programs in the Division of Molecular and Cellular Medicine (Aoki group) 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. The specific activities in 2014 were as follows: 1) Investigation of molecular basis of immune microenvironment in pancreatic and colon cancer; 2) Development of cancer-targeting vectors using the peptide-display adenovirus library.

Research activities

Investigation of molecular basis of immune microenvironment in pancreatic and colon cancer

We investigated the immunological function of inflammaoty protein S100A8/A9 and immunostimulatory cytokine type I interferon (IFN) in tumor microenvironment.

1) Whether the expression of S100A8/A9 in tumors predicts a good or poor prognosis is controversial in the clinical setting. To clarify the in vivo role of S100A8/A9 in the tumor microenvironment, we subcutaneously inoculated Pan02 (pancreatic cancer cell line) stably expressing S100A8 and S100A9 proteins (Pan02-S100A8/A9) in syngeneic mice. Unexpectedly, after small tumor nodules were once established, they rapidly disappeared. Flow-cytometry showed that the number of NK cells in the tumors was increased, and a depletion of NK cells promoted the growth of Pan02-S100A8/A9 subcutaneous tumors. Although the S100A8/A9 proteins alone did not change the IFN-γ expression of NK cells in vitro, a co-culture with Pan02 cells, which express Rae-1, induced IFN-γ production, and Pan02-S100A8/A9 cells further increased the number of IFN-γ^+ NK cells, suggesting that S100A8/A9 enhanced the NKG2D ligand-mediated intracellular activation pathway in NK cells. We then examined whether NK cell activation by S100A8/A9 was via their binding to RAGE by using the inhibitors. RAGE antagonistic peptide and anti-RAGE antibody inhibited the IFN-γ production of NK cells induced by S100A8/A9 proteins. Since S100A8/A9 strongly enhances the activity of NK cells, the S100A8/A9-NK cells axis may be useful for cancer immunotherapy.
2) IFN-α can effectively induce an antitumor immunity by the activation of tumor-specific T cells and maturation of dendritic cells. Unknown, however, is how the type I IFN alters the immunotolerant microenvironment in the tumors. Here, we found that intratumoral IFN-α gene transfer significantly decreased the frequency of regulatory T cells (Tregs) per $CD4^+$ T cells in tumors. The concentration of a Treg-inhibitory cytokine, IL-6, was correlated with the IFN-α expression level in tumors, and intratumoral $CD11c^+$ cells produced IL-6 in response to IFN-α stimulation. To confirm the role of IL-6 in the suppression of Tregs in tumors, an anti-IL-6 receptor antibody was administered in IFN-α-treated mice. The antibody increased the frequency of Tregs in the tumors, and attenuated systemic tumor-specific immunity induced by IFN-α. Furthermore, the IFN-α-mediated IL-6 production increased the frequency of Th17 cells in the tumors, which may be one of the mechanisms for the reduction of Tregs. The study demonstrated that IFN-α creates an environment strongly supporting the enhancement of antitumor immunity through the suppression of Tregs.

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

The addition of a targeting strategy is necessary to enhance oncolysis and secure safety of a conditionally replicative adenovirus (CRAd). We have constructed an adenovirus library displaying random peptides on the fiber, and have successfully identified a pancreatic cancer-targeting ligand (SYENFSA). The usefulness of cancer-targeted CRAd for pancreatic cancer was examined as a preclinical study. First, we constructed a survivin promoter-regulated CRAd expressing enhanced green fluorescent protein gene (EGFP), which displayed the identified targeting ligand (AdSur-SYE). The AdSur-SYE resulted in higher gene transduction efficiency and oncolytic potency than the untargeted CRAd (AdSur) in several pancreatic cancer cell lines. An intratumoral injection of AdSur-SYE significantly suppressed the growth of subcutaneous tumors, in which AdSur-SYE effectively proliferated and spread. An ectopic infection in adjacent tissues and organs of intratumorally injected AdSur-SYE was decreased compared with AdSur. Then, to examine whether the targeting ligand actually enhanced the infectivity of CRAd in human pancreatic cancer tissues, tumor cells prepared from surgical specimens were infected with viruses. The AdSur-SYE increased gene transduction efficiency 6.4-fold higher than did AdSur in single cells derived from human pancreatic cancer, whereas the infectivity of both vectors was almost the same in the pancreas and other cancers. AdSur-SYE resulted in a stronger oncolysis in the primary pancreatic cancer cells co-cultured with mouse embryonic fibroblasts than AdSur did. We showed the usefulness of enhanced tumor targetability in oncolytic therapy.

Education

2 graduate students (doctoral course) linking with Keio University, 1 graduate student (master course) and 1 student linking with Tokyo Medical Dental University, studied about cancer immunology and virus therapy in our laboratory.

Future prospects

We are investigating the relationship between cancer gene expression profile and immune micoenvironment in pancreatic and colon cancer, which may open a novel perspective on immune therapy for cancer. In addition, CRAd in combination with a tumor-targeting ligand is promising as a next-generation of oncolytic virotherapy for pancreatic cancer.

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Journal

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Division of Molecular and Cellular Medicine

Takahiro Ochiya, Fumitaka Takeshita, Ryou-u Takahashi, Takeshi Katsuda, Yusuke Yoshioka, Yu Fujita, Hiroaki Miyazaki, Yutaka Nezu, Akira Yokoi, Ayako Inoue, Maki Abe, Mizuyo Arashi, Satoko Takizawa, Makiko Ichikawa, Kana Kurosaki, Mayuko Yamamura, Satomi Fukuda, Kazumi Nagao, Luc Gailhouste, Kurataka Ootsuka, Hayato Kurata, Rie Tamai, Hiroshi Naito, Nao Nishida, Keitaro Hagiwara, Naoomi Tominaga, Ken Yasukawa, Liew Lee Chuen

Introduction

The focus of the Division of Molecular and Cellular Medicine lies in the development of novel diagnosis and treatments for cancer patients. The specific activities were as follows: 1) Studies on microRNA (miRNA) regulation in cancer cells and development of RNA interference (RNAi) -based therapeutics; 2) Exosomes as a novel diagnosis and therapeutic tool against cancer; 3) Study of stem cells and its therapeutic applications.

Research activities

1) Studies on miRNA regulation in cancer cells and development of RNAi-based therapeutics.

RNAi-based therapeutics is promising approach as novel and potentially more effective treatments for cancer and miRNAs are identified as important modulators of tumor-related genes (4,5,17, 27,30).

We identified miRNAs which show abnormal expression in a highly malignant osteosarcoma (1,21-23). Silencing of miR-133a with locked nucleic acid (LNA) reduced cell invasion and systemic administration of LNA along with chemotherapy suppressed lung metastasis and prolonged the survival of osteosarcoma-bearing mice. Furthermore, in a clinical study, high expression levels of CD133 and miR-133a were significantly correlated with poor prognosis, whereas high expression levels of the four miR-133a target genes were correlated with good prognosis. We previously demonstrated that silencing of ribophorin II (RPN2) efficiently induced apoptosis and reduced resistance to docetaxel in human breast cancer cells (9,24). Recently, we also reported the clinical relevance of RPN2 expression in osteosarcoma (3). Higher RPN2 expression was significantly correlated with poor prognosis. The RNAi-induced RPN2 knockdown showed reduced tumor growth and lung metastasis in mice model of osteosarcoma. Now we proceed with the preparation of clinical trials of RPN2-siRNA treatment for drug-resistant breast cancer. This will be the first clinical trial of siRNA on human performed in Japan.

2) Exosomes as a novel diagnosis and therapeutic tool against cancer

The circulating exosomes could be found in variety of body fluids including serum, plasma, urine, saliva, and breast milk (18). The existence of circulating exosomes in the blood of cancer patients has raised the possibility that exosomes may serve as a novel diagnostic marker (7,13,16,19,20,26). For this reason, a new high sensitive method of circulating exosomes has been developed (2). Moreover, we found that CD147 and CD9 double-positive exosomes were significantly higher in serum from colorectal cancer patients than in serum from healthy donors (2). Cell-cell communication of cancer cells and surrounding noncancerous cells are critical for the acquisition of malignancy in human cancer. We discovered that the effects of bone marrow mesenchymal stem cells (BM-MSCs) on breast CSCs were attributable to the transfer of miRNAs from BM-MSCs to breast CSCs through exosomes. We revealed that exosomal miR-23b promoted dormancy and decreased CD44 surface abundance in breast cancer cells (28). These findings prompted us into applying exosomes in diagnosis and therapy against cancer development (11,12,14).

3) Study of stem cells and its therapeutic

applications

While cancer stem cell (CSC) properties such as tumorigenicity and drug resistance are a major focus in current cancer research, the molecular mechanisms for the regulation of CSC properties are not fully understood. MiRNA is also identified as the target involved in the regulation of CSC properties (10, 15). We found that some specific miRNAs played an important role in the acquisition of CSC properties (10, 15). We also investigated the functions of these miRNAs in vivo using animal model and found that modulation of miRNAs efficiently suppressed the tumor seeding ability and drug resistance of CSCs (29). Therefore, these results suggest that conventional cancer therapy with modulating the expression of miRNA improves the treatment of cancer patients.

4) Mesenchymal Stem Cell (MSC) Therapy

We are interested in the therapeutic potential of MSCs. Especially, our main focus is on the realization of the clinical application of adipose tissue derived-mesenchymal stem cells (ADSC) in liver diseases (8,25). Recently, MSCs attract much attention not only for their own potential as cells, but also for their secretory capacity of extracellular vesicles (EVs) that can have therapeutic benefits (6).

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Division of Rare Cancer Research

Tadashi Kondo, Rieko Ohki, Bunsho Shiotani, Xiaoqing Pan, Parlayan Cuneyd, Yukiko Nakamura, Yohko Yamaguchi, Yoko Takai, Yukiko Araki, Fusako Kito, Marimu Sakumoto, Aska Matsuo, Naofumi Asano, Kosuke Hirota, Takashi Tajima, Kazutaka Kikuta, Yuki Tani, Junko Otsuka, Yoshinori Asano, Issei Ezawa, Yu Chen, Miku Shimizu, Shiori Suzuki, Yuhei Takano, Raira Saigawa, Shu Matsushita, Maiko Minegishi

Introduction

The Division of Rare Cancer Research aims the innovative seeds for novel therapy for rare cancers. The rare cancer research has unique difficulty due to small number of patients and clinical samples. We challenge the fundamental problems in rare cancer research, discover innovative seeds for novel therapy, and establish unique approach to cancers with low incidence.

Research activities

Our research activities focus on three subjects. [Establishment of fundamental research system of rare cancers] Patient Derived Xenograft models and primary cell lines were established from surgical specimens of sarcoma patients. Omics database was constructed by integrating our original data and publically available data. [Study on rare cancers] <Biomarker developement> Omics study for biomarker discovery was performed using the clinical samples (tumor tissues and blood samples) of sarcoma patients. Evaluation and validation studies were done for biomarker candidates. Study for assay system development was done for predictive diagnosis. <Discovery of therapeutic target> By comparative proteomic study, tyrosine kinases (TKs) with aberrant auto-phosphorylation were identified, and the effects of TK inhibitors were examined using *in vitro* cells. <Molecular backgrounds of rare cancers> Associations of ATR and clinical and pathological features of sarcomas were examined at molecular level. Aberrant regulation of novel p53 target gene, PHLDA3, was discovered in pancreatic neuroendocrine tumor. Its significances in clinical features and carcinogenesis were examined at molecular level. {Reverse innovation] <Systematic survey of effects of cancer drugs> To identify cancer drugs applicable for sarcomas, the inhibitor library was screened in the sarcoma cell line panel. Molecular backgrounds of sensitivity and resistance were examined by multi-OMICS study. <Development of novel methods for scare amount samples> Novel proteomics application based on unique separation modes was developed for scare amount samples.

Education

Young doctors, PhD students, and students joined our Division from Keio University, Kyorin University, Chiba University, Waseda University, and Tokyo College of Biotechnology.

Future prospects

Fundamental research system to approach to the essential problem in rare cancer research, such as “small number of cases and samples for research” will be established. Novel innovative seeds for rare cancer therapies will be discovered and applied to clinical applications. Methods for cancers with small number of cases and samples will be established, and applied to cancer research in future.

List of papers published in 2014

Journal

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9. Kubota D, Yoshida A, Kikuta K, Saito T, Suehara Y, Gotoh M, Kawai A, Kondo T. Proteomic Approach to Gastrointestinal Stromal Tumor Identified Prognostic Biomarkers. J Proteomics Bioinform, 7:010-016, 2014
10. Ichikawa H, Kanda T, Kosugi S, Kawachi Y, Wakai T, Kondo T. Proteomic and metatranscriptomic study on lymph node metastasis in gastric cancer. EuPA Open Proteomics, 3:183?194, 2014
11. Kondo T, Kawai A. A proteomics approach for the development of sarcoma biomarkers. EuPA Open Proteomics, 4:121-128, 2014
12. Uemura N, Kondo T. Current status of predictive biomarkers for neoadjuvant therapy in esophageal cancer. World J Gastrointest Pathophysiol, 5:322-334, 2014
13. Kondo T. Inconvenient truth: cancer biomarker development by using proteomics. Biochim Biophys Acta, 1844:861-865, 2014
14. Kondo T. Casting doubt on the traditional approach of cancer biomarker discovery through proteomics. Expert Rev Proteomics, 11:9-12, 2014
15. Suehara Y, Kohsaka S, Kubota D, Mukaihara K, Akaike K, Mineki R, Fujimura T, Kaneko K, Ladanyi M, Saito T, Kondo T. Proteomic technologies to develop biomarkers and functional analyses for bone and soft tissue tumors. J Proteomics Bioinform, S3:1-13, 2014
16. Tani Y, Tajima T, Kawai A, Unuma Y, Kinoshita H, Kondo T. Evaluation of a novel automated machine, the auto2D, for two-dimensional gel electrophoresis. J Proteomics Bioinform, 7:108-111, 2014
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Division of Refractory and Advanced Cancer

Ryuichi Sakai, Hideki Yamaguchi, Masato Enari, Takuya Shirakihara, Katsuhiko Nakashima, Yumi Hasegawa, Emi Saito, Yuko Hibiya

Introduction

The malignant characteristics of cancers causing the invasion into surrounding tissue, metastasis to distant organs, and acquirement of resistance to therapeutics are serious threats to the clinical treatment of cancer. Interaction of cancer cells with neighboring cells such as cancer associated fibroblasts (CAFs) has recently been shown to have critical roles in this procedure. It is also 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 signaling molecules during cancer metastasis, invasion and drug resistance. One of the goals of our research is to establish models of the novel therapy to overcome these malignant characteristics of progressed cancers by targeting critical proteins and signals involved in these procedures.

Research activities

Regulation of Anaplastic Lymphoma Kinase (ALK) activity and drug resistance in cancers

Although the ALK-fusion gene is detected in approximate 5% of pulmonary cancer and the ALK inhibitor is used as therapeutic drug for this type of lung cancer, the problem of recurrence elicited by tiny fraction of cancer cells resistant to the ALK inhibitor remains unresolved. We recently found that ALK-fusion protein inhibited the p53 pathway via the unique mechanism and that activation of the p53 pathway reduced the drug resistance to the ALK inhibitor. We are now pushing forward further conclusive evidence using clinical specimens.

Activation of ALK either by mutation or overexpression, has been indicated as a significant oncogenic factor in neuroblastoma. Flotillin-1 (FLOT1), a plasma membrane protein known to be involved in endocytosis, was found among binding partners of ALK. It was suggested that FLOT1 controls the amount of ALK protein at the cell surface through the regulation of receptor endocytosis. RNAi-mediated attenuation of FLOT1 expression in neuroblastoma cells caused ALK dissociation from endosomes along with membrane accumulation of ALK, thereby triggering activation of ALK and downstream effector signals. These features enhanced the malignant properties of neuroblastoma cells in vitro and in vivo. Our findings suggest that the loss of FLOT1-mediated regulation of ALK contributes to malignancy of clinical neuroblastoma cases and those cases might be sensitive to ALK inhibitors even without the genetic alteration of ALK.

Interaction between Cancers and Stromal Cells

Scirrhous gastric carcinoma (SGC) shows rapid expansion through progressive invasion, peritoneal dissemination and frequent metastasis to lymph nodes. We investigated the role of stromal fibroblasts (SFs) in invasion and extracellular matrix (ECM) remodeling by SGC cells. When SGC cells were cocultured with SFs on three-dimensional (3D) Matrigel, they were attracted together to form large cellular aggregates that invaded into the Matrigel. By utilizing this assay system for inhibitor library screening, we have identified several inhibitors that potently suppress the cooperation between SGC cells and SFs to form the invasive structures. Among them, a Src inhibitor dasatinib impaired the interaction between SGC cells and SFs both in vitro and in vivo and effectively blocked peritoneal dissemination of SGC cells. These results indicate that SFs mediate mechanical remodeling of the ECM by SGC cells, thereby promoting invasion and peritoneal dissemination of SGC.

It was revealed that cancer cells transact the surrounding fibroblasts to cause decreased expression of p53, which results in CAF-like transition of these cells and enhanced expression of a four transmembrane-spanning protein, TSPAN12. The fibroblasts expressing TSPAN12 could trigger the invasiveness of the cancer cell through direct attachment between these cells.

Molecules regulating Metastasis and Invasion of Cancers

We have demonstrated that a membrane protein CDCP1 is the critical regulator of anoikis resistance, distant metastasis, and peritoneal dissemination of cancer cells. It was shown that CDCP1 is required for the functional link between Ras and Src 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 activation of Ras pathway. Therapeutic antibodies and chemicals which block the CDCP1-mediated signaling are being screened.

TSPAN2, another four transmembrane-spanning protein, was found among the proteins induced by p53-inactivation. It was demonstrated that TSPAN2 interacts with CD44 and enhances invasion and metastasis of lung adenocarcinoma cells through efficient removal of reactive oxygen species (ROS) in cancer cells.

Future prospects

In patients with advanced stages of cancers, the control of metastasis, invasion and drug resistance is crucial for maintaining the quality of life (QOL) in addition to the prolonged survival. Our approach to elucidate the underling mechanism to these malignant characteristics of cancers will give way to develop novel therapeutic strategies for advanced cancers.

List of papers published in 2014

Journal

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2. Uekita T, Fujii S, Miyazawa Y, Iwakawa R, Narisawa-Saito M, Nakashima K, Tsuta K, Tsuda H, Kiyono T, Yokota J, Sakai R. Oncogenic Ras/ERK signaling activates CDCP1 to promote tumor invasion and metastasis. Mol Cancer Res, 12:1449-1459, 2014
3. Otsubo C, Otomo R, Miyazaki M, Matsushima- Hibiya Y, Kohno T, Iwakawa R, Takeshita F, Okayama H, Ichikawa H, Saya H, Kiyono T, Ochiya T, Tashiro F, Nakagama H, Yokota J, Enari M. TSPAN2 is involved in cell invasion and motility during lung cancer progression. Cell Rep, 7:527-538, 2014
4. Otomo R, Otsubo C, Matsushima-Hibiya Y, Miyazaki M, Tashiro F, Ichikawa H, Kohno T, Ochiya T, Yokota J, Nakagama H, Taya Y, Enari M. TSPAN12 is a critical factor for cancer-fibroblast cell contact-mediated cancer invasion. Proc Natl Acad Sci U S A, 111:18691-18696, 2014
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Research Support Division

Teruhiko Yoshida, Tesshi Yamada, Toshio Imai, Issay Kitabayashi, Tatsuhiro Shibata, Hiromi Sakamoto, Fumie Hosoda, Yae Kanai, Hitoshi Ichikawa, Hiroki Sasaki, Yasuhito Arai, Masaya Ono, Tadashi Kondo, Mami Takahashi, Yoshinori Ikarashi, Takuo Katsumoto, Koji Okamoto, Tetsuya Ishikawa (including collaborating staff).

Introduction

The concept of the Research Core Facility (CF) has originated from the 1st lecture given by Dr. Hitoshi Nakagama on May 9, 2011 after his appointment as the Director of the National Cancer Center (NCC) Research Institute (RI). Along with the biobank, the CF has been positioned between the NCCRI and the NCC hospital to establish a bidirectional translational bridge (Fig. 1). The combination of the rich collection of high quality clinical samples and advanced, reliable analytical power should be a crucial asset of our Institute. However, the latest genome and other omics technologies demand heavy and stable investments both in hardware and its maintenance and human expertise, especially in the field of bioinformatics, which are increasingly difficult if not impossible to afford for individual laboratories, such as those led by young PIs and physician scientists. As a consequence, the CF has become an essential component integrated in many leading biomedical research institutes in the world. Characteristically, our CF is a virtual organization based on the mutual help among the research scientists and laboratories, each engaging in their own competitive research.

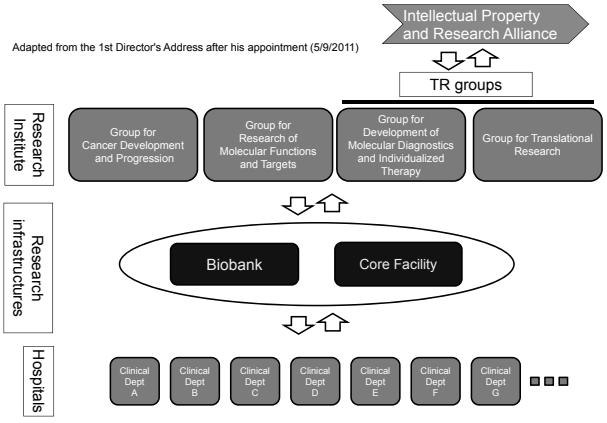

Figure1. Concept of CF: Director's Initiative

Figure 2 shows the original CF system officially started on September 5, 2011 with 4 major arms: Genome & Epigenome, Proteome, Biology, and Common Equipment for self-service use of shared resource-demanding machines in terms of cost, space and other installation specifications. The Research Support Division corresponds to the Genome & Epigenome CF and Proteome CF. The Biology CF function is being offered by the Central Animal Division and reported in its pages.

In August 2014, the Fundamental Innovative Oncology Core (FIOC) has been established, and the Research Support Division now belongs to the new FIOC system.

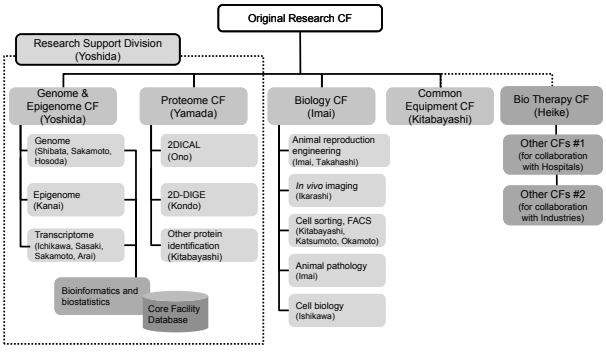

Figure 2. CF Organization

Research activities

The mission of the CF is not limited to the mutual support and collaboration inside the NCCRI, but extends to other sectors of NCC as a whole. For instance, the CF offers genotyping service for population-based cohort studies in the Research Center for Cancer Prevention and Screening (RCCPS), and helping observation studies in the framework of clinical trials in the hospital. The CF is also supporting a transitional

zone between research and clinical practice, such as genetic diagnosis of hereditary cancer syndromes (Fig. 3).

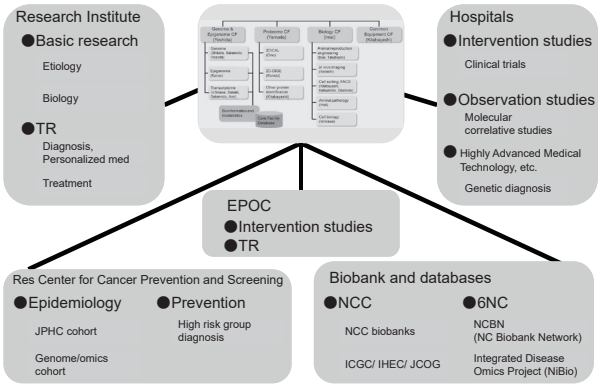

Figure 3. CF Interactions and Participations

Because the CF covers such diverse activities, its performance is difficult to quantify, but just as a simplified example, the numbers of the individual research projects and samples submitted to the CF are summarized in Figure 4.

Education

Although not always apparent, one of the most important contributions of the CF may be the discussion and consultation BEFORE offering the actual CF service.

Future prospects

CF should keep exploring the latest needs among the NCC researchers and revising its service menu accordingly. It is also crucial to evaluate the effort offered by the CF staff in an appropriate way and develop sound and effective incentive for an active commitment to the CF service. At least a part of the CF financial fundamentals need to be supported by the NCC in-house budget, such as machine maintenance and basic human resource cost.

As a member of the newly established FIOC, the Research Support Division will contribute to its mission in line with the grand strategy and directives of FIOC.

CF Area	Applications	# projects				# samples			
		FY 2011	FY 2012	FY2013	FY2014	FY 2011	FY 2012	FY2013	FY2014
Genome	Next Generation Sequencer	11	8	5	18	248	180	160	1,203
	SNP array/TagMan assay	10	9	8	8	1,993	1,574	1,762	529
	Agilent array and others	5	9	4	0	366	652	123	0
Epigenome	NGS	2	2	1	1	102	14	8	30
	Infinium array	7	6	9	11	1,646	569	801	705
Transcriptome	NGS	3	8	0	7	44	157	0	243
	Affymetrix GeneChip	5	4	2	2	97	76	110	208
	Agilent array	5	3	4	2	58	56	68	24
Proteome	2DICAL	7	2	2	3	524	112	54	126
	2D-DIGE	0	7	4	1	0	308	83	199
	Protein identification	0	1	2	6	0	483	612	1,573
Animal reproduction engineering	Embryo/ sperm freezing stock	2	5	5	4	9	36	17	6
	Microbiological cleaning	1	1	5	1	-	1	5	1
In vivo imaging	IVIS/ OV110	12	4	4	5	-	-	-	-
Animal histopathology	FFPE, frozen sections	12	12	11	14	1,743	2,974	1,778	1,721
	Examination and diagnosis	4	4	6	14	-	-	-	-
Cell biology	Cell line/xenograft establishment	1	1	0	3	5	2	0	56
Total		87	86	72	100	6,835	7,194	5,581	6,624

Figure 4. CF Activities in FY 2011-2014 (excluding the self-service type)

Central Animal Division

Toshio Imai, Mami Takahashi, Tetsuya Ishikawa, Yoshinori Ikarashi, Teruo Komatsu, Kotomi Otsubo, Naoaki Uchiya, Masashi Yasuda, Manabu Tsuchida, Ayami Kawashima, Satoshi Ikeda, Junichi Zukeyama, Shiho Ozawa, Yudai Seki, Takuya Matsuyama, Junya Asahira, Shumpei Ohnami, Hitoshi Nakagama, Mitsuko Masutani, Gen Fujii

Introduction

A pivotal role of the Central Animal Division is supportive actions for basic/clinical/public health researchers on the basis of biological resources in National Cancer Center.

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.

Routine activities

The important role of the Central Animal Division is health management of the experimental animals and maintenance of the animal experimentation facility. Some researchers and technical staff act also for several support services, which are provided based on their biological skills, such as reproductive technologies for animal cleaning/embryo-sperm preservation, histopathological techniques for animal tissues and establishment of expandable cells/xenograft transplantable models from clinical cancer tissues (PDX models).

Research activities

Research activities of the Central Animal Division have focused on studies of chemical carcinogenesis using laboratory animals, genetically modified cancer developing animal models and occasionally clinical samples.

1) Association of pancreatic fatty infiltration (FI) with pancreatic ductal adenocarcinoma

Epidemiologically, obesity and diabetes are risk factors for pancreatic cancer, but the underlying mechanisms are not clearly understood. Obesity and diabetes are also associated with degree of FI in the pancreas. We reported that the degree of FI in non-cancerous part of pancreatic sections was significantly higher in pancreatic cancer patient cases than in the controls and positively associated with pancreatic cancer development. Severe pancreatic FI could be a risk factor of pancreatic cancer. The role of obesity and pancreatic fatty infiltration (FI) in pancreatic carcinogenesis is being investigated in animal models.

2) Mechanisms of promotion of mammary carcinogenesis associated with a high-fat diet

The effects of a high-fat diet (HFD) during prepubertal and pubertal stages were investigated in 7,12-dimethylbenz(a)anthracene-induced mammary carcinogenesis in female F344 rats. The results obtained indicated that HFD promoted carcinogenesis. Molecular mechanisms of the promotion as assessed with DNA microarray analysis for the non-cancerous mammary tissues were suggested to be associated with altered expression of cell cycle/differentiation-related genes, which were at least partly affected by DNA methylation.

3) Mechanistic analysis of inflammation in tumorigenesis with an intestinal orgnoid 3D culture approach

Mechanisms of tumorigenesis by inflammation are not clearly understood. An inflammatory response *in vivo* is very complicated because it involves many kinds of cytokines and immune cells. In this approach, we focused on a pro-inflammatory cytokine interleukin-22 (IL-22) and analyze its roles with an intestinal organoid 3D cultural method. IL-22 was found to disrupt

intestinal barrier functions, and it was suggested that unregulated IL-22 signaling could enhance tumorigenic inflammatory responses.

4) Human induced hepatic lineage-oriented stem cells (hiHSCs); autonomous specification of human iPS cells toward hepatocyte-like cells without any exogenous differentiation factors

Self-renewing hiHSCs mixed with Matrigel were subcutaneously injected into immune-deficient mice and serum biochemical analysis revealed elevated human albumin levels to 0.6–1.6 μg/mL. In this study, a differentiation potential of hiHSCs, which were histopatholgically developed to teratomas *in vivo*, to hepatocytes was at least partly confirmed.

Future prospects

Research approaches using immune-deficient/severely immune-deficient mice have become increasingly important these several years, and microbiological controls of the animal experimentation facility should become more strictly controlled. For development of research fields to conquer rare cancers/refractory cancers, establishment of their PDX models should be systematically organized.

List of papers published in 2014

Journal

1. Hori M, Takahashi M, Hiraoka N, Yamaji T, Mutoh M, Ishigamori R, Furuta K, Okusaka T, Shimada K, Kosuge T, Kanai Y, Nakagama H. Association of pancreatic Fatty infiltration with pancreatic ductal adenocarcinoma. Clin Transl Gastroenterol, 5:e53, 2014
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4. Imai T, Kitahashi T. A 13-week toxicity study of acrylamide administered in drinking water to hamsters. J Appl Toxicol, 34:57-65, 2014

Department of Biobank and Tissue resources

Yae Kanai, Izumi Kobayashi, Teiko Yamane, Masumi Tanaka

Activities of the National Cancer Center Biobank

The National Cancer Center Biobank was conducted under the supervision by the National Cancer Center Biobank Administration Committee (Figure 1).

In 2014, 8,738 vials of tissue specimens obtained from surgically resected materials of 1,657 patients were newly deposited into the National Cancer Center Biobank and 2,316 vials of tissue specimens obtained from surgically resected materials of 1,344 patients were provided to researches approved by the National Cancer Center Ethics Committee. The ratio of the number of the patients of whom samples were provided to researches to that of whom samples were newly deposited into the Biobank was about 81%. At the end of 2014, we reposited 72,510 vials of tissue specimens of 17,577 patients.

In 2014, 27,133 vials of plasma samples drawn from 6,837 patients were newly deposited into the National Cancer Center Biobank and 1,688 vials of plasma samples drawn from 1,053 patients were provided to researches approved by the National Cancer Center Ethics Committee. At the end of 2014, we reposited 110,214 vials of blood samples of 27,743 patients who consented to blood sampling for research purposes.

We have built up the catalog database, named the HosCanR Biobank Edition, by extracting appropriate information from the Interview Sheet Database in the common form among six National Centers in Japan and HosCanR, an application specialized for the National program of Cancer Registry. Researchers, biobank users, can find out samples which are suitable for their own research plans using search commands of this catalog database. In 2014, we made it able to automatically extract appropriate information, which should be provided to the central database of the National Center Biobank Network (NCBN), from the

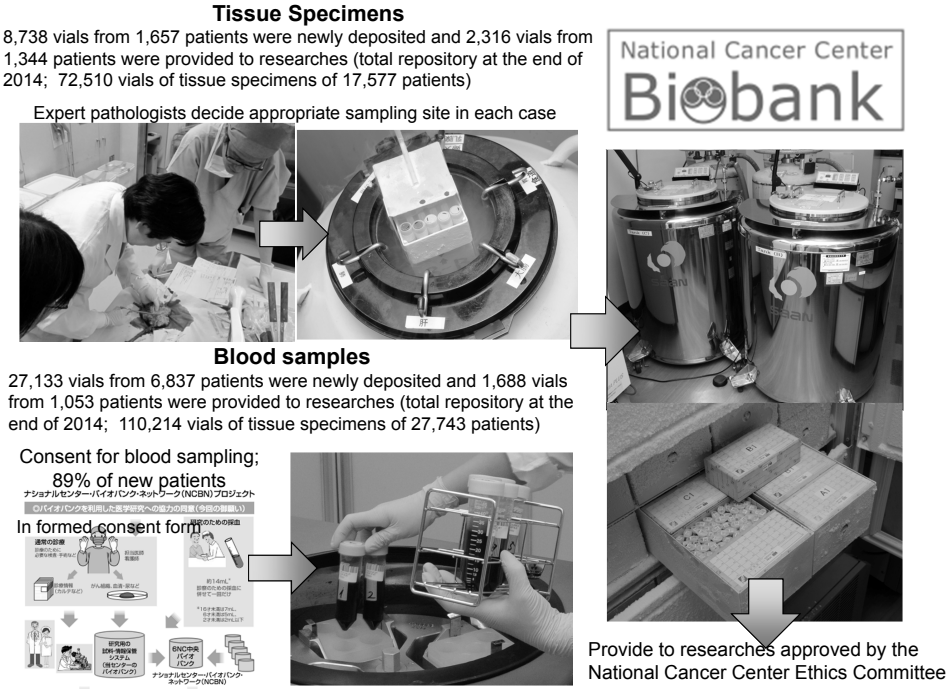

Figure 1. National Cancer Center Biobank

HosCanR Biobank Edition. In addition, we now able to confirm the informed consent status in the HosCanR Biobank Edition which has been connected to computerized medical records system.

Researchers who received samples from the Biobank have published 279 scientific papers (total impact factor; 1435.574, total citation index; 3,586). 63% of the published papers were based on collaborative researches between researchers of National Cancer Center and other institutes or universities.

Sixty-six founders and/or contact persons of 11 bioresource repositories of other universities and hospitals and two television crews visited the National Cancer Center Biobank to study knowhow of management of biobank in 2014. We have been consulted by contact persons of 15 bioresource repositories of other universities about storage system of specimens.

Staffs of the National Cancer Center Biobank participate in the General Ethics Support Sector, the Sample Utilization Review Working Group, the Sample Handling System Review Working Group and the Medical Information System Review Working Group of the NCBN. In 2014, the Central Database Management Sector of the NCBN collected individual data sheets of samples of each National Center. Ethical, legal and social issues about wide distribution of samples which are not based on collaborative researches have been discussed in the NCBN.

Future prospects

Consecutive collection of samples for various research needs and management of biobank including quality clinicopathological information database are considered as national mission. The National Cancer Center Biobank should be continued and become a more robust and permanent research base. The National Cancer Center Biobank should continuously support the NCBN and connect intention of voluntary donors to next generation personalized medicine.

Department of Patient-Derived Cell Line and Xenograft

Tohru Kiyono

Introduction

There are mainly two approaches to amplify cancer cells from patients, in vitro cell culture and patient-derived xenograft (PDX). Since HeLa cell line, the first human cancer cell line, has been established, human cancer cell lines have been essential for cancer research. Patient-derived xenografts (PDXs) generated from fresh tumor specimens generally reflect histopathology, tumor behavior, and the metastatic properties of the original tumor. Both PDX models and cell line-derived xenograft (CDX) models are considered to be important preclinical tools in recent years. However, the success rate to establish new cell lines or PDX lines is not satisfactory.

Routine activities

This Department was founded in 2014 for establishment of new cancer cell lines and PDX lines. We are preparing for the system that stores valuable cancer specimens so that cancer tissues or cancer cells can be transplanted into immune-deficient mice or cultivated in vitro.

Research activities

This year, a series of metastatic liver cancer specimens of colorectal carcinomas were selected for initial test for the storage since large amount of specimens can be generally obtained from each patient. Once the freezing condition has been fixed, other cancer specimens will be added for the storage.

Future prospects

The in vitro carcinogenesis model with reversible control of oncogene expression enabled de novo development of PDAC from quasi-normal human tissues pre-formed subcutaneously in mice and might be applicable to carcinogenesis models in many organ sites. These models will be useful for preclinical assessment of new cancer therapies.

Department of Molecular imaging & Pharmacokinetics

Akinobu Hamada, Shuichi Shimma, Makiko Yamashita, Hiroaki Aikawa, Shoraku Ryu

Introduction

Development of Pharmacokinetic analysis for a new anticancer agent using LC-MS/MS and MALDI imaging Mass Spectrometry.

Research activities

Drug exposure and distribution in tumor tissues impact pharmacology and efficacy in drug development. However, a conventional PK analysis, using HPLC and LC-MS/MS, has limitations in providing a comprehensive assessment of real tissue distribution. Now investigators in PK lab demonstrated a drug visualizing system using MALDI (matrix-assisted laser desorption ionization) Imaging Mass Spectrometry that provides the possibility to evaluate the concentration and spatial distribution in target tumor tissue.

List of papers published in 2014

Journal

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Department of Innovative Seeds Evaluation

Tadashi Kondo, Tsutomu Ohta

Introduction

The Department of Innovative Seeds Evaluation aims at the establishment of efficient methods to evaluate novel seeds, and evaluate research outcome for innovative therapies. Taking advantages of National Cancer Center such as easy access to clinical materials with high quality, we challenge the two following subjects. [In vitro evaluation system for novel innovative seeds] In vitro assay system is essential for preclinical study. Establishment of Patient Derived Xenograft (PDX) and primary cultured cell lines, and experiment system for evaluation of novel drug candidates is the first step for our goal. [Screening system of cancer drugs] Establishment of effective evaluation system using cell line panels is undertaken in our Department. Omics data are linked to the response to drug treatments, and integrated in the database to characterize the molecular backgrounds of response, and predict the effects of novel drugs.

Research activities

[In vitro evaluation system for novel innovative seeds] Protocol to establish PDX and primary cultured cell lines from surgical specimens were established, and applied to sarcomas. [Screening system of cancer drugs] Protocol to screen the effects of approximately 400 inhibitors in the cell line panel was established. Development of novel algorithm to examine and integrate the transcriptome data, which were obtained before and after the treatments with cancer drugs, was launched.

Future prospects

To facilitate the clinical applications of research outcome, methods to evaluate innovative seeds for novel therapy will be established, and applied to our findings.

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Journal

1. Katsui-Taniyama T, Nokihara H, Tsuta K, Horinouchi H, Kanda S, Fujiwara Y, Yamamoto N, Koizumi F, Yunokawa M, Tamura T. Clinicopathological features in young patients treated for small-cell lung cancer: significance of immunohistological and molecular analyses. Clin Lung Cancer, 15:244-247, 2014
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Department of Clinical Genomics

Hitoshi Ichikawa, Fumie Hosoda, Sachiyo Mitani, Shizuka Shinohara, Erika Arakawa

Introduction

The aim of our Department is to realize molecular profile-based personalized medicine for cancer patients by supporting genome and transcriptome analyses of clinical tumor samples. For personalized cancer medicine, next generation sequencer-based clinical sequencing is a promising molecular profiling method to efficiently detect genetic alterations from tumor tissues. We are developing and improving an original target sequencing system for clinical sequencing, and are providing sequencing services with small-scale next generation sequencers, Illumina MiSeq and Ion Proton.

Research activities

Development of a target sequencing system for FFPE tumor samples

We developed an original target sequencing system for clinical sequencing, which can identify gene amplifications and fusions as well as mutations from FFPE tumor tissue samples. In the present version of this system, 90 potentially targetable or actionable genes were selected as an in-house cancer panel (NCC oncopanel v2), and all exons of these 90 genes and introns of 10 genes among them are captured and sequenced for detection of mutations/gene amplifications and gene fusions, respectively. To ensure a stable operation on FFPE samples, we also developed a method to qualify FFPE tissue-derived DNA using the content of PCR-amplifiable DNA as a quality value. By changing the DNA quantity used for sequencing depending on their quality values, we were able to successfully analyze approximately 90% of the archived FFPE samples. For detection of genetic alterations, we adopted a novel algorithm which was developed by researchers of the Department of Bioinformatics. By the use of this algorithm, we achieved highly accurate mutation detection with more than 99% accuracy for base substitution mutations and more than 90% accuracy for insertion/deletion mutations.

Use for clinical sequencing

As a collaborative work with the Division of Translational Research and the Department of Experimental Therapeutics of EPOC, we designed and performed a feasibility study to use our target sequencing system for patient entry into early phase clinical trials of molecular target drugs, which was named TOPICS-1 (Trial of Onco-Panel for Introduction into Clinical Study-Phase 1). Totally 131 patients with many types of cancers were subjected to the sequencing analysis in this study. The results showed the feasibility of our system in a clinical setting. On average, 1.9 mutations and 0.3 amplifications were found in each patient, and nearly half of the patients had at least 1 actionable alteration, which is informative for entry into some clinical trials.

Other target sequencing services

We provided target sequencing services using our original target sequencing system and commercially available cancer panel systems, upon requests from researchers in Research Institute and Hospital. This year, more than 500 samples of various types of cancers were analyzed.

List of papers published in 2014

Journal

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6. Mizukami T, Shiraishi K, Shimada Y, Ogiwara H, Tsuta K, Ichikawa H, Sakamoto H, Kato M, Shibata T, Nakano T, Kohno T. Molecular mechanisms underlying oncogenic RET fusion in lung adenocarcinoma. J Thorac Oncol, 9:622- 630, 2014
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Department of Translational Oncology

Hiroki Sasaki, Kazuhiko Aoyagi, Masashi Tamaoki, Masayuki Komatsu, Rie Komatsuzaki, Fumiko Chiwaki, Akio Ashida, Kanako Nakamura, Risa Ichinohe

Introduction

In 2014, the three major research areas of the Department of Translational Oncology were 1) preclinical studies using newly established gastric cancer cell lines for derivation of industrial and academia seeds/drugs to EPOC, and 2) development of personalized cancer diagnosis and treatment.

Preclinical Studies Using Newly Established Gastric Cancer Cell Lines

Genome-wide genetic information in 963 cancer cell lines is available on COSMIC DB (Sanger Center, UK); however, among them, only 21 cell lines are derived from gastric cancer (GC). Almost all of the 21 GC cell lines have been established many years ago. Only insufficient clinical and pathological information is attached. Establishment of new GC cell lines, especially from metastatic sites after therapy, has been awaited. Peritoneal metastasis is most frequent in GCs, especially diffuse-type GCs. Furthermore, since driver gene mutation frequency in a certain cancer is often less than 5%, establishment of cell lines from each patient to be analyzed is desired for functional selection of driver gene mutations. In collaboration with Division of Genetics, we have newly established 44 diffuse-type GC derived cell lines (NSC-1~37 series) from the cancer ascites of 24 patients. Now we possess 77 GC cell lines including 65 diffuse-type (new 44 and existing 21) and 12 intestinal-type. We are conducting omics analyses for gene expression and copy number variation, and hot spot- and genome wide-gene alteration in these cell lines. Moreover, to establish the peritoneal metastasis model, their tumorigenicity and histopathological characteristics of PDXs, such as fibroblast rich-, hypovascular-, and dormant-state, were evaluated. By collaboration with three pharmaceutical industries, *in vitro* and *in vivo* preclinical studies were conducted to derivate them to the Exploratory Oncology Research & Clinical Trial Center (EPOC).

Development of Personalized Diagnosis and Treatment for Cancer

Two major research projects are underway. First, we developed mini DNA chips containing 6 marker and 3 control genes for predicting gastric cancer recurrence from peritoneal washings. Peritoneal cytology (CY) offers important prognostic information for gastric cancer after surgery; however, CY provides only a limited sensitivity and the task requires great skill. Our goal is to develop a sensitive tool that could be used in a clinical laboratory agency as a substitute for skilled cytology. Our collaborating company continues to prepare many supporting data for submitting the mini DNA chip to Pharmaceuticals and Medical Devices Agency (PMDA) for marketing approval as an in vitro diagnostic (IVD). Second, we successfully identified 5 intrinsic subtypes of esophageal squamous cell carcinomas by hundreds' gene expression profile-based unsupervised clustering of 274 biopsy samples obtained before treatment. The 274 profiles were divided into a test set (107 cases containing 35 and 72 cases received CRT or surgery) and a validation set (167 cases containing 90 and 77 cases, respectively). Five intrinsic subtypes (1a/F3/M1, 2a/I, 3b, 5/D/M2, 7/B/E) including 2 new subtypes (2a/I, 3b) were identified in the test set, and these were reproducibly found in the validation set. For the cases treated with CRT, the 5-y survival rate was 24% in subtype M2, whereas it was 74% in subtype E. Furthermore, we found transcriptional pathways activated characteristically in each subtype; the subtype E showed a differentiation phenotype, while the non-E subtypes including M1 and M2 showed an epithelial-mesenchymal transition phenotype. Our findings may contribute not only to the elucidation of CRT responsiveness but also for the future therapeutic development. To develop

an IVD, we successfully selected each 50 genes for predicting these two CRT-sensitive and –insensitive subtypes.

List of papers published in 2014

Journal

1. Oue N, Naito Y, Hayashi T, Takigahira M, Kawano-Nagatsuma A, Sentani K, Sakamoto N, Zarni Oo H, Uraoka N, Yanagihara K, Ochiai A, Sasaki H, Yasui W. Signal peptidase complex 18, encoded by SEC11A, contributes to progression via TGF-α secretion in gastric cancer. Oncogene, 33:3918-3926, 2014
2. Kojima M, Higuchi Y, Yokota M, Ishii G, Saito N, Aoyagi K, Sasaki H, Ochiai A. Human subperitoneal fibroblast and cancer cell interaction creates microenvironment that enhances tumor progression and metastasis. PLoS One, 9:e88018, 2014
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4. Oue N, Anami K, Schetter AJ, Moehler M, Okayama H, Khan MA, Bowman ED, Mueller A, Schad A, Shimomura M, Hinoi T, Aoyagi K, Sasaki H, Okajima M, Ohdan H, Galle PR, Yasui W, Harris CC. High miR-21 expression from FFPE tissues is associated with poor survival and response to adjuvant chemotherapy in colon cancer. Int J Cancer, 134:1926-1934, 2014
5. Sakamoto N, Naito Y, Oue N, Sentani K, Uraoka N, Zarni Oo H, Yanagihara K, Aoyagi K, Sasaki H, Yasui W. MicroRNA-148a is downregulated in gastric cancer, targets MMP7, and indicates tumor invasiveness and poor prognosis. Cancer Sci, 105:236-243, 2014
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8. Tanabe S, Aoyagi K, Yokozaki H, Sasaki H. Gastric-related markers and their significance in cancer. J Med Genom Biomark, 44:1955-1970, 2014

Department of Analytical Pathology

Nobuyoshi Hiraoka, Yoshinori Ino-Ishikawa

Introduction

In the Analytical Pathology Department the pathobiological and clinicopathological characteristics of the target molecules are analyzed for evaluating their potential significance in applying diagnostic or treatment use in future. Expression of the molecules or genes in human tissues is assessed by morphological techniques, immunohistochemistry, RT-PCR, i*n situ hybridization*, etc., and the results are compared to clinicopathological information. We also try to develop new, more reliable, or more effective analytical methods and tools.

Research activities

In 2014 two staff members made preparations for the Department start and studied tumor immune microenvironment clinicopathologically and pathobiologically.

Education

Teaching the analytical techniques to technicians and researchers in several departments of National Cancer Center was performed.

Future prospects

We will answer the requests from the selected project in various types of study containing basic, preclinical, and clinical studies, and assess the clinicopathological or pathobiological significance of the target molecules. We will develop methods of quantitative analysis to evaluate morphological findings that are currently analyzed qualitatively.

List of papers published in 2014

Journal

1. Furukawa D, Chijiwa T, Matsuyama M, Mukai M, Matsuo E, Nishimura O, Kawai K, Suemizu H, Hiraoka N, Nakagohri T, Yasuda S, Nakamura M. Zinc finger protein 185 is a liver metastasis-associated factor in colon cancer patients. Mol Clin Oncol, 2:709-713, 2014
2. Hayashi T, Horiuchi A, Sano K, Hiraoka N, Ichimura T, Sudo T, Ishiko O, Yaegashi N, Aburatani H, Konishi I. Potential diagnostic biomarkers: differential expression of LMP2/ β 1i and cyclin B1 in human uterine leiomyosarcoma. Tumori, 100:99e-106e, 2014
3. Hori M, Takahashi M, Hiraoka N, Yamaji T, Mutoh M, Ishigamori R, Furuta K, Okusaka T, Shimada K, Kosuge T, Kanai Y, Nakagama H. Association of pancreatic Fatty infiltration with pancreatic ductal adenocarcinoma. Clin Transl Gastroenterol, 5:e53, 2014
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12. Yamamoto Y, Hiraoka N, Goto N, Rin Y, Miura K, Narumi K, Uchida H, Tagawa M, Aoki K. A targeting ligand enhances infectivity and cytotoxicity of an oncolytic adenovirus in human pancreatic cancer tissues. J Control Release, 192:284-293, 2014
13. Yamazaki K, Masugi Y, Effendi K, Tsujikawa H, Hiraoka N, Kitago M, Shinoda M, Itano O, Tanabe M, Kitagawa Y, Sakamoto M. Upregulated SMAD3 promotes epithelialmesenchymal transition and predicts poor prognosis in pancreatic ductal adenocarcinoma. Lab Invest, 94:683-691, 2014

Department of Functional Analysis

Fumitaka Takeshita

Introduction

The Department of Functional Analysis carries out functional analysis to development of scientific basis diagnosis and pre-clinical studies in corporation with other department in Core Center.

Recently, optical imaging technologies for clinical use have been sized down to make them available for non-invasive in vivo imaging for small animals. The development of molecular imaging using luminescence or fluorescence in vitro have facilitated in vivo imaging to detect the molecular events in tumor cells on living animals. "In vivo imaging" and "In vivo molecular imaging" are now being established as a new field of research. Promptness and handiness of the in vivo imaging are rapidly changing the design of the animal experiments not only in cancer research but in the whole of biology.

Research activities

In our laboratory, evaluation of treatments with cancer model studies and imaging for the gene medicine molecule such as microRNA are done by making good use of this imaging device that detects luminescence and fluorescence from the living animals (Fujita, Biomed Res Int., Seino, J Food Sci., Ono, Sci Signal., Fujiwara, Stem Cells).

Clinical trials

The preparation of clinical trial of RPN2-siRNA treatment for chemo-resistant breast cancer has been preceded collaborated with the Division of Molecular and Cellular Medicine and the Department of Breast and Medical Oncology in the NCC Hospital.

Department of Animal Experimentation

Toshio Imai, Masako Ochiai, Yoshitaka Hippo, Tetsuya Matsuura, Takashi Nishizawa

Introduction

A pivotal role of this Department is establishment of cancer animal models (human cancer tissue/cell-transplanted immune-deficient mice). In experiments using immune-deficient/severely immune-deficient mice, microbiological environment of the animal experimentation facility should be strictly controlled and technical staffs take great care.

Routine activities

Establishment of xenograft transplantable models from clinical cancer tissues (patient-derived xenograft-PDX models) or cultured cancer cells (ectopic or orthotopic cancer cell implantation models) and evaluation of drug efficacy using PDX or cancel cell implantation models are performed.

Research activities

Research activities of the Department of Animal Experimentation are focused on studies of recapitulation of multi-step adenocarcinogenesis for diverse organs through an *in vitro* approach. Whereas both genetic and environmental factors cooperate for tumorigenesis *in vivo*, we demonstrated that the lentivirus-mediated introduction of genetic alterations in murine primary epithelial cells could lead to development of adenocarcinoma in the dorsal skin of immune-deficient mice. Notably, tumor initiation and subsequent step-wise progression from normal cells via pre-cancerous lesions to carcinoma could be accurately recapitulated for various vital organs in a cell-autonomous manner. By taking this approach, genetic and/or environmental interactions toward tumorigenesis could be conveniently investigated *in vitro*, which would likely accelerate elucidation of the molecular mechanisms underlying carcinogenesis.

List of papers published in 2014

Journal

1. Ochiai M, Hippo Y, Izumiya M, Watanabe M, Nakagama H. Newly defined aberrant crypt foci as a marker for dysplasia in the rat colon. Cancer Sci, 105:943-950, 2014

2. Igarashi M, Hippo Y, Ochiai M, Fukuda H, Nakagama H. AKT is critically involved in cooperation between obesity and the dietary carcinogen amino-1-methyl-6-phenylimidazo [4,5-b] (PhIP) toward colon carcinogenesis in rats. Biochem Biophys Res Commun, 443:852-857, 2014

Department of Cell Culture Technology

Tohru Kiyono

Introduction

Human cells in culture have limited life span and undergo non-dividing state named senescence. The replicative senescence is caused by telomere shortening since most human somatic cells do not express telomerase to the level sufficient for maintenance of telomere length. Human epithelial cells also undergo non-dividing state much earlier not because of telomere shortening but because of accumulation of $p16^{INK4A}$ and activation of pRB. Stem or progenitor cells of human epithelia often express higher levels of TERT so that telomere shortening is delayed. In a certain culture condition which induces higher levels of TERT expression and inhibits $p16^{INK4A}$ induction, they could proliferate infinitely without any transgenes.

Routine activities

This Department was founded in 2014 for developing better methods to cultivate normal human cells as well as cancer cells derived from clinical specimens obtained by operation, biopsy and therapy.

Research activities

Recently a culture condition with feeder layer cells and the ROCK inhibitor, Y-27632, has been developed for infinite proliferation of several epithelial cell types. Based on the improved method developed by the Division of Carcinogenesis and Cancer Prevention, we now can cultivate so far difficult-to-cultivate primary human cells, such as hepatocytes, pancreatic duct cells, gastric epithelial cells and colon epithelial cells without feeder cells. These cell types have been immortalized by transduction of CDK4, cyclin D1 and TERT so as to be cultivated in more general culture conditions. On the other hand, some cell types are still difficult to passage and quickly amplify in vitro without transgenes. Our goal is to establish the cell culture method that can easily amplify every cell type including normal, pre-neoplastic and cancer cells. These include organoid culture as well as conventional two-dimensional culture.

Future prospects

Once cells-of-origin of every cancer could be easily amplified in vitro, they can be used for normal control cells for each cancer. Causal relationship of a gene mutation found in cancer and a certain phenotype such as drug resistance could be directly evaluated by transducing the mutant gene into them. They might also be applied to development of cell transplantation therapy.

Department of Bioinformatics

Isao Kurosaka, Eisaku Furukawa, Joe Miyamoto

Introduction

Missions of our department are 1) bioinformatics analysis support for experimental groups in the Fundamental Innovative Oncology Core, 2) bioinformatics analysis support for other groups in NCC, and 3) to develop new bioinformatics and data-analysis methods necessary for emerging genomics technologies.

Research activities

- We took charge of the bioinformatics part in the clinical sequencing project in NCC. The bioinformatics part consists of 1) development of DNA-alteration calling program and 2) development of medical information system for clinical sequencing.
  1). We developed a new software system optimized for FFPE samples used in clinical sequencing. This system detects SNV, indels, gene fusions, and copy number alterations from a large amount of data produced by the next generation sequencer (NGS). We compared our program with other well-known programs (, which were originally developed for cell-line or frozen samples for research purposes). We confirmed that our program greatly outperformed the other programs.
  2) We developed the first version of a medical information system that integrates DNA alterations detected in clinical sequencing with clinical information taken from electronic medical records. By this system, medical doctors can view, search, and edit results of expert panel. This system can automatically calculate the statistics and can be used as a database.
- We developed a pipeline program to help discover new cancer subtypes, using trans-omics data in lung adenocarcinoma as a part of the Medical Big Data project.
- We provided bioinformatics support for studies on liver cancer and bile duct cancer as a part of ICGC, and on multi-regional liver cancer and on pancreas cancer in the Division of Cancer Genomics; for studies on DNA adductome, gene expression of cancer stem cells, miRNAs in the Division of Cancer Development System; and for a study on germinoma in the Division of Brain Tumor Translational Research.
- We conducted an experiment of a new technology – single-cell sequencing – to reveal intra-tumor heterogeneity and cancer-cell evolution, collaborating with the Division of Caner Genomics and the Division of Cancer Development System. We performed single-cell exome and transcriptome sequencing and informatics analysis, confirming the feasibility of this newly emerging technology.

Education

We educated two new technical staff members through on-the-job training, and advised bioinformatics technical staff in the Division of Cancer Genomics.

Future prospects

We will advance development and management of information system for clinical sequencing ultimately aiming at personalized medicine. We will also develop algorithms to find novel tumor molecular markers and cancer subtypes, using medical big data that will be accelerated by clinical sequencing. We will keep continue to provide bioinformatics support for other groups in the Center. We will develop new bioinformatics methodologies and theories for the

emerging technology, single-cell sequencing, to reveal tumor heterogeneity.

List of papers published in 2014

Journal

1. Fukushima S, Otsuka A, Suzuki T, Yanagisawa T, Mishima K, Mukasa A, Saito N, Kumabe T, Kanamori M, Tominaga T, Narita Y, Shibui S, Kato M, Shibata T, Matsutani M, Nishikawa R, Ichimura K. Mutually exclusive mutations of KIT and RAS are associated with KIT mRNA expression and chromosomal instability in primary intracranial pure germinomas. Acta Neuropathol, 127:911-925, 2014
2. Totoki Y, Tatsuno K, Covington KR, Ueda H, Creighton CJ, Kato M, Tsuji S, Donehower LA, Slagle BL, Nakamura H, Yamamoto S, Shinbrot E, Hama N, Lehmkuhl M, Hosoda F, Arai Y, Walker K, Dahdouli M, Gotoh K, Nagae G, Gingras M-C, Muzny DM, Ojima H, Shimada K, Midorikawa Y, Goss JA, Cotton R, Hayashi A, Shibahara J, Ishikawa S, Guiteau J, Tanaka M, Urushidate T, Ohashi S, Okada N, Doddapaneni H, Wang M, Zhu Y, Dinh H, Okusaka T, Kokudo N, Kosuge T, Takayama T, Fukayama M, Gibbs RA, Wheeler DA, Aburatani H, Shibata T. Trans-ancestry mutational landscape of hepatocellular carcinoma genomes. Nat Genet, 46:1267-1273, 2014
3. Mizukami T, Shiraishi K, Shimada Y, Ogiwara H, Tsuta K, Ichikawa H, Sakamoto H, Kato M, Shibata T, Nakano T, Kohno T. Molecular mechanisms underlying oncogenic RET fusion in lung adenocarcinoma. J Thorac Oncol, 9:622-630, 2014
4. Suenaga Y, Islam SMR, Alagu J, Kaneko Y, Kato M, Tanaka Y, Kawana H, Hossain S, Matsumoto D, Yamamoto M, Shoji W, Itami M, Shibata T, Nakamura Y, Ohira M, Haraguchi S, Takatori A, Nakagawara A. NCYM, a Cisantisense gene of MYCN, encodes a de novo evolved protein that inhibits GSK3 β resulting in the stabilization of MYCN in human neuroblastomas. PLoS Genet, 10:e1003996, 2014

Department of Omics Network

Masaru Katoh

Introduction

The Department of Omics Network, established in August 2014, is derived from Masaru Katoh's laboratory, which is also known as the Katoh's Unit (from 2009 to 2014) and the Genetics and Cell Biology Section (from 1998 to 2009). The Department has been researching the WNT (PMID: 17634527), FGF (PMID: 23696246), Notch (PMID: 17143535) and Hedgehog (PMID: 19860666) signaling cascades and the Forkhead-box (FOX) family of transcription factors (PMID: 23022474). The goal of the Department is the establishment of Knowledgebase focused on the regulatory signaling network for the development of novel diagnostics and therapeutics.

Fundamental and constitutive projects

WNT, FGF, Notch, Hedgehog and FOX are fundamental and constitutive projects of the Department. The fundamental theme in 2014 was therapeutics targeted to the FGF receptors (FGFRs).

FGFs are involved in a variety of cellular processes, such as stemness, proliferation, anti-apoptosis, drug resistance and angiogenesis. Gene amplification of *FGFR1* occurs in lung cancer and estrogen receptor (ER)-positive breast cancer, and that of *FGFR2* in diffuse-type gastric cancer and triple-negative breast cancer. Chromosomal translocation of *FGFR1* occurs in the 8p11 myeloproliferative syndrome and alveolar rhabdomyosarcoma, as with *FGFR3* in multiple myeloma and peripheral T-cell lymphoma. *FGFR1* and *FGFR3* genes are fused to neighboring *TACC1* and *TACC3* genes, respectively, due to interstitial deletions in glioblastoma multiforme. Missense mutations of *FGFR2* are found in endometrial uterine cancer and melanoma, and similar *FGFR3* mutations in invasive bladder tumors, and *FGFR4* mutations in rhabdomyosarcoma. Dovitinib, Ki23057, ponatinib and AZD4547 are orally bioavailable FGFR inhibitors, which have demonstrated striking effects in preclinical model experiments. Because there are multiple mechanisms of actions for FGFR inhibitors to overcome drug resistance, FGFR-targeted therapy is a promising strategy for the treatment of refractory cancer (Figure 1).

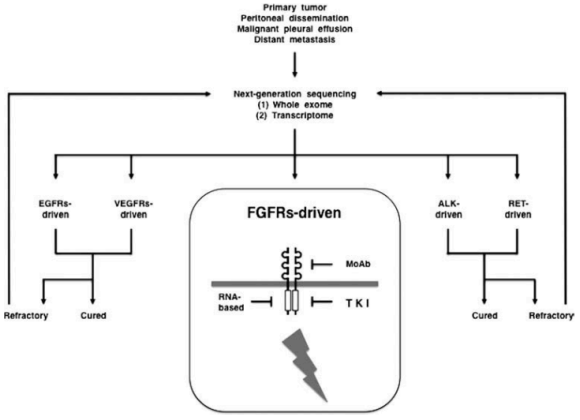

Figure 1. Perspectives on FGFR-targeted therapeutics

Whole-exome sequencing combined with transcriptome sequencing will be applied in clinical laboratory test to determine driver mutations in tumor samples. Primary and refractory tumors driven by aberrantly activated FGFRs will be treated with FGFR-targeted therapeutics, such as small-molecule FGFR inhibitor (TKI), human/humanized anti-FGFR monoclonal antibody (MoAb), and RNA-based drug.

Cutting-edge and mobile projects

Cellular adhesion, epigenetics and microRNA (miRNA) are cutting-edge and mobile projects of the Department. The cutting-edge theme in 2014 was miRNA-based diagnostics.

Cardiovascular diseases and cancers are the leading causes of morbidity and mortality in the world. miR-24, miR-125b, miR-195 and miR-214 were selected as representative cardio-miRs that are upregulated in human heart failure. ACVR1B, BCL2, BIM, eNOS, FGFR3, JPH2, MEN1, MYC,

p16, and ST7L are miR-24 targets that have been experimentally validated in human cells. ARID3B, BAK1, BCL2, BMPR1B, ERBB2, FGFR2, IL6R, MUC1, SITR7, Smoothened, STAT3, TET2, and TP53 are representative miR-125b targets. ACVR2A, BCL2, CCND1, E2F3, GLUT3, MYB, RAF1, VEGF, WEE1, and WNT7A are representative miR-195 targets. BCL2L2, ß-catenin, BIM, CADM1, EZH2, FGFR1, NRAS, PTEN, TP53, and TWIST1 are representative miR-214 targets. miR-125b is a good cardio-miR that protects cardiomyocytes; miR-195 is a bad cardio-miR that elicits cardiomyopathy and heart failure; miR-24 and miR-214 are bi-functional cardio-miRs. By contrast, miR-24, miR-125b, miR-195, and miR-214 function as oncogenic or tumor suppressor miRNAs in a cancer (sub) type-dependent manner. Circulating miR-24 is elevated in diabetes, breast cancer and lung cancer. Circulating miR-195 is elevated in acute myocardial infarction, breast cancer, prostate cancer and colorectal adenoma. Circulating miR-125b and miR-214 are elevated in some cancers. Cardio-miRs and onco-miRs bear some similarities in functions and circulation profiles. Because circulating miRNA profiles are modulated by genetic and environmental factors and are dysregulated by genetic and epigenetic alterations in somatic cells (Figure 2), circulating miRNA association studies (CMASs) within several thousands of cases each for common non-cancerous diseases and major cancers are necessary for miRNA-based diagnostics.

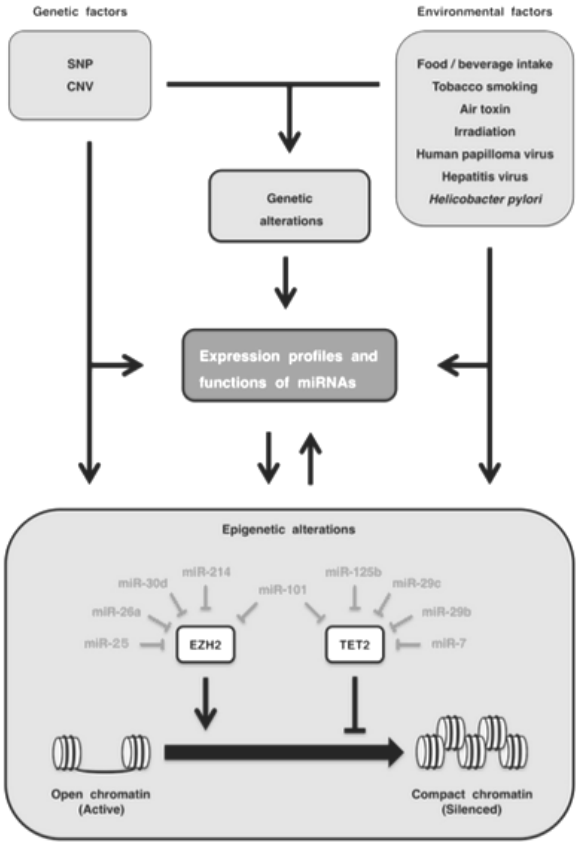

Figure 2. Regulation of circulating miRNAs

Genetic factors and environmental factors are involved in the regulation of expression profiles and functions of miRNAs (upper part). miRNA expression is downregulated by epigenetic silencing, while epigenetic regulators are repressed by multiple miRNAs. miRNAs and epigenetics are in the relationship of mutual regulation (lower part). Genetic and environmental factors regulate circulating miRNA profiles directly as well as indirectly through genetic and epigenetic alterations.

Contribution to the global scientific community

Masaru Katoh has been contributing to the global scientific community based on manuscript publication, reviewer activity and editor activity. Katoh carried out peer review of grant proposals or journal manuscripts written in English 69 times in 2014. Katoh is an Academic Editor of *PLoS ONE*, and has carried out editorial decision 177 times in 2014. Masaru Katoh is the Chief Editor of *Frontiers in Molecular Medicine* that aims to address the gap between cell and developmental biology and clinical medicine, together with 92 editorial board members.

Manuscript citation count in the Web of Science Database (Thomson Reuters) is a surrogate marker of contribution to the global science community. Katoh's manuscripts were cited 641 times by others in 2014.

List of papers published in 2014

Journal

1. Katoh M, Nakagama H. FGF receptors: cancer biology and therapeutics. Med Res Rev, 34:280-300, 2014
2. Katoh M. Cardio-miRNAs and onco-miRNAs: circulating miRNA-based diagnostics for noncancerous and cancerous diseases. Front Cell Dev Biol, 2:61, 2014

Book

1. Katoh M. WNT signaling in neoplasia. In: Gelmann EP, Sawyers CL, Rauscher FJ III (eds), Molecular Oncology, U.K., Cambridge University Press, pp 243-257, 2014