Research Institute

Preface

The National Cancer Center Research Institute (NCCRI) was established in 1962 as one of the main parts of the National Cancer Center (NCC), and has been driving cancer research in Japan ever since. The NCC was designated a National Research and Development Agency in April 2015. Since then, there has been more demand than ever to promote research and development and to maximize results. To meet such demand, the NCCRI has been collaborating closely with the NCC Hospitals, the Exploratory Oncology Research & Clinical Trial Center, the Research Center for Cancer Prevention and Screening (the Center for Public Health Sciences from January 2016), and the Center for Cancer Control and Information Services, and thereby has tried to maximize the transition "from bench to bedside".

In addition to 19 divisions, the NCCRI also contains the Fundamental Innovative Oncology Center (FIOC), which is a core facility for the entire NCC. The FIOC consists of 15 departments, and it runs the NCC BioBank, provides specialized techniques, and also facilitates collaborative work with various private sectors outside the NCC. As of March 2016, the NCCRI has 83 research staff, 79 postdoctoral fellows and 131 graduate students/supporting staff, all of whom are dedicated to a wide range of cancer research including prevention of cancer, elucidating inter- or intra-tumor heterogeneity, identification of therapeutic targets and preclinical studies for novel anti-cancer reagents.

Outstanding achievements in 2015 in the NCCRI include the following:

1) Large-scale genomic analyses on biliary tract cancer
2) Discovery of the relationship between the expression level of a microRNA and resistance against anti-cancer drugs
3) Identification of stem cells in Barrett's esophagus lesions.
4) Development of a novel modality by the use of synthetic lethality for tumors with *CBP* mutations.
5) Large-scale genomic analyses on ampullary carcinoma.

The NCCI also actively participates in, and leads worldwide cancer research collaborations including the International Cancer Genome Consortium (ICGC) and the International Human Epigenome Consortium (IHEC). We are also collaborating with the Early Detection Research Network (EDRN) of the National Cancer Institute (NCI) of the United States.

As described above, through enhancing high-quality research and interaction with other institutes, the NCCRI is eagerly generating novel modalities to prevent and conquer cancer.

Hiroyuki Mano, M.D., Ph.D.
Director, National Cancer Center Research Institute

Organization

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Tomomitsu Hotta

Director:
Hitoshi Nakagama

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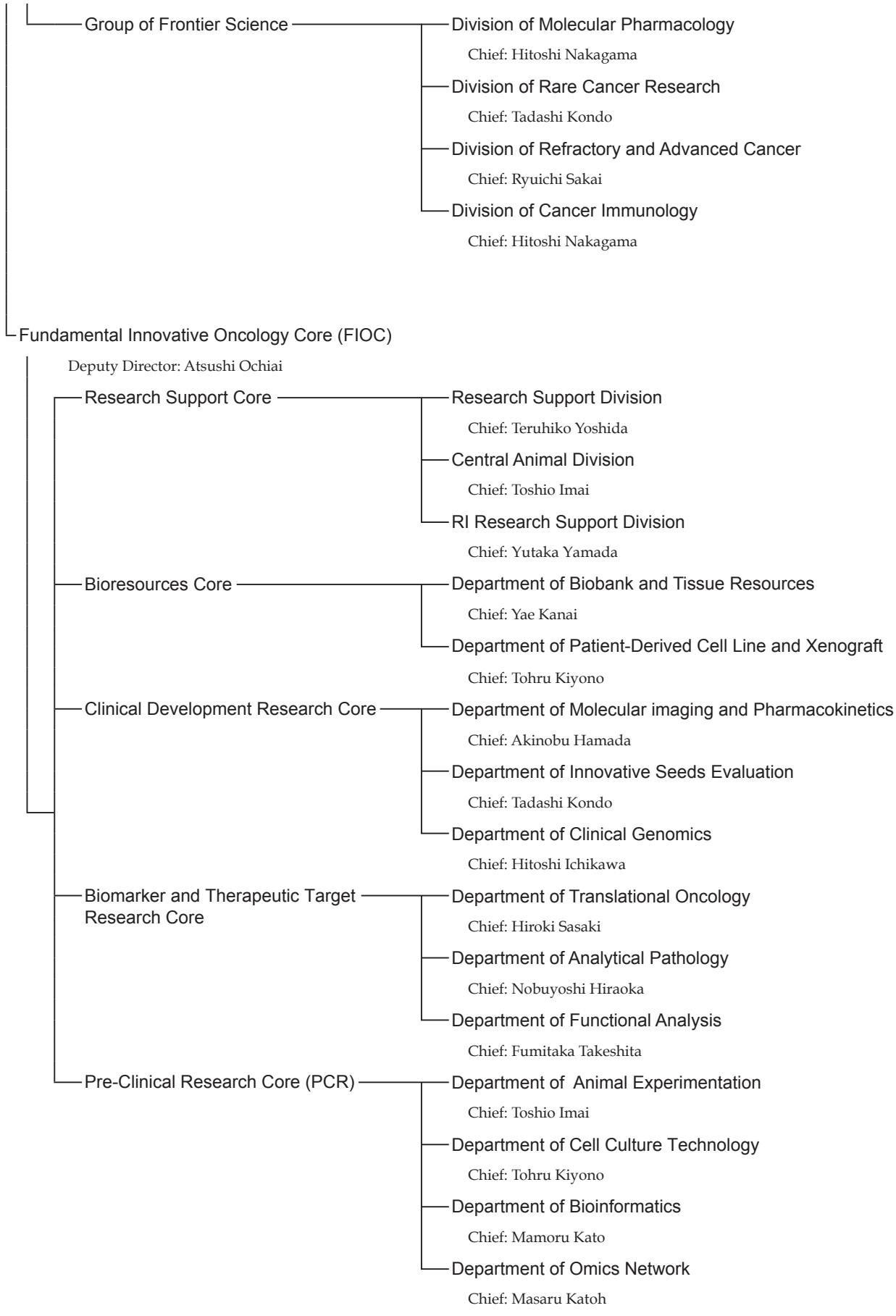
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Division of Rare Cancer Research
Chief: Tadashi Kondo
Division of Refractory and Advanced Cancer
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Division of Cancer Immunology
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Department of Cell Culture Technology
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Department of Bioinformatics
Chief: Mamoru Kato
Department of Omics Network
Chief: Masaru Katoh

Activities of the Divisions

Division of Molecular Pathology

Yae Kanai, Eri Arai, Masahiro Gotoh, Ying Tian, Yoshimasa Saito, Takuya Yotani, Yuriko Yamada, Koji Tsumura, Ayako Shibuya, Nanako Itoh, Toshihide Muramatsu, Toshiya Nakaoka, Michiko Suzuki

Introduction

On the basis of findings from routine diagnostic pathology work, we develop scientific ideas and follow them up using a molecular pathological approach to understand the molecular basis of diseases and mechanisms determining clinicopathological heterogeneity of cancers. Our Division mainly consists of researchers who also belong to academia and industry. Therefore, we focus on industrial‐academic‐government cooperation to yield potential benefits for cancer patients.

Research activities

Activities in the International Human Epigenome Consortium (IHEC)

We have participated in IHEC as a principal investigator supported by the Core Research for Evolutional Science and Technology (CREST) project by the Japan Agency for Medical Research and Development (AMED). We have worked in collaboration with research groups in Kyushu University and the University of Tokyo to reveal the epigenome landscape, whole-genome bisulfite sequencing using post-bisulfite adaptor tagging, chromatin immunoprecipitation sequencing, RNA sequencing and whole-genome sequencing using normal hepatocytes purified from partial hepatectomy specimens of six Japanese patients. CpG methylation levels were generally low in the region 200 bp upstream from the transcription start site (TSS200), the first coding exon and the CpG island. Considerable CHG and CHH methylation was observed. Personal differentially methylated regions (pDMRs) were observed less frequently in TSS200, the first coding exon and the CpG island. Histone modification profiles of pDMRs differed considerably among samples. pDMRs were observed around the TSSs of genes whose expression levels are reportedly regulated by CpG methylation. pDMRs were frequently observed in the vicinity of single-nucleotide variations and insertions/deletions, suggesting the possibility of cis-acting genome-epigenome interaction. Genetic variations may induce epigenetic variations and generate individual differences in the phenotypes of normal hepatocytes through variations in expression.

Epigenome maps are now being obtained from hepatocytes purified from diseased liver tissue with hepatitis C virus or hepatitis B virus-infection and epithelial cells purified from the stomach, colon and urogenital organs for IHEC activities. Such data we obtain will be made available by the National Bioscience Database Center (http://humandbs.biosciencedbc.jp/en/) and the IHEC database (http://epigenomesportal.ca/ihec/). Accurate epigenome profiling of normal cells will allow the identification of disease-specific epigenome profiles, thus facilitating a potential breakthrough in the prevention, diagnosis and therapy of diseases.

From November 16-18, 2015, AMED and our CREST team had the pleasure to host this year's annual meeting of IHEC and to welcome IHEC members from around the world to Tokyo, Japan. The three-day meeting offered various sessions including country updates, workgroup meetings and talks by both IHEC members and invited scientists on their latest findings in epigenomics. This year's meeting marked the highest number of attendees yet in the series of IHEC conferences.

Multilayer omics analysis in human cancers for personalized medicine

CpG-island methylator phenotype (CIMP)-positive clear cell renal cell carcinomas (RCCs), which we have originally identified, are characterized by

accumulation of DNA hypermethylation of CpG islands, clinicopathological aggressiveness and poor patient outcome. To clarify the molecular pathways participating in CIMP-positive renal carcinogenesis, genome (whole-exome and copy number), transcriptome and proteome (two-dimensional image converted analysis of liquid chromatography-mass spectrometry) analyses were performed using tissue specimens of CIMP-negative and CIMP-positive clear cell RCCs and corresponding specimens of non-cancerous renal cortex in a collaborative joint research project. Genes encoding microtubule-associated proteins, such as *DNAH2, DNAH5, DNAH10, RP1* and

Figure 1. Participants (upper) and discussion (lower) in IHEC Science Day and Annual Meeting in Tokyo (November 16-18, 2015).

HAUS8, showed a high incidence of mutations in CIMP-positive RCCs, whereas CIMP-negative RCCs lacked distinct genetic characteristics. Alterations of mRNA or protein expression were significantly accumulated in signaling pathways participating in the spindle checkpoint. All CIMP-positive RCCs showed overexpression of Aurora kinases, *AURKA* and *AURKB*, which are key players in the spindle checkpoint and this overexpression was mainly attributable to the increased copy number. These data suggest that abnormalities of the spindle checkpoint pathway participate in CIMP-positive renal carcinogenesis, and that *AURKA* and *AURKB* may be potential therapeutic targets in more aggressive CIMP-positive RCCs.

In order to make DNA methylation diagnosis applicable to clinical use, a high performance liquid chromatography (HPLEC)-based and scaled-down methylated DNA detection device, which can be introduced into clinical laboratories of each hospital and even into small clinics, is now being developed by a joint research program with Sekisui Medical Co., Ltd. This joint R&D was introduced in several newspapers and other media in 2015. We are now attempting to use this device for carcinogenetic risk estimation, liquid biopsy diagnosis, prognostication and companion diagnosis for molecular targeted therapy in cancers derived from various organs.

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 joint research through providing clinicopathological information.

Future prospects

We will continuously perform joint research using tissue specimens pathologically examined by ourselves with both academia and industry to develop new strategies for cancer prevention, diagnosis and therapy.

List of papers published in 2015

Journal

1. Arai E, Gotoh M, Tian Y, Sakamoto H, Ono M, Matsuda A, Takahashi Y, Miyata S, Totsuka H, Chiku S, Komiyama M, Fujimoto H, Matsumoto K, Yamada T, Yoshida T, Kanai Y. Alterations of the spindle checkpoint pathway in clinicopathologically aggressive CpG island methylator phenotype clear cell renal cell carcinomas. Int J Cancer, 137:2589-2606, 2015

2. Yamanoi K, Arai E, Tian Y, Takahashi Y, Miyata S, Sasaki H, Chiwaki F, Ichikawa H, Sakamoto H, Kushima R, Katai H, Yoshida T, Sakamoto M, Kanai Y. Epigenetic clustering of gastric carcinomas based on DNA methylation profiles at the precancerous stage: its correlation with tumor aggressiveness and patient outcome. Carcinogenesis, 36:509-520, 2015

3. Sato T, Soejima K, Arai E, Hamamoto J, Yasuda H, Arai D, Ishioka K, Ohgino K, Naoki K, Kohno T, Tsuta K, Watanabe S, Kanai Y, Betsuyaku T. Prognostic implication of PTPRH hypomethylation in non-small cell lung cancer. Oncol Rep, 34:1137-1145, 2015

4. Robles AI, Arai E, Mathé EA, Okayama H, Schetter AJ, Brown D, Petersen D, Bowman ED, Noro R, Welsh JA, Edelman DC, Stevenson HS, Wang Y, Tsuchiya N, Kohno T, Skaug V, Mollerup S, Haugen A, Meltzer PS, Yokota J, Kanai Y, Harris CC. An Integrated Prognostic Classifier for Stage I Lung Adenocarcinoma Based on mRNA, microRNA, and DNA Methylation Biomarkers. J Thorac Oncol, 10:1037-1048, 2015

5. Sakamoto A, Hino S, Nagaoka K, Anan K, Takase R, Matsumori H, Ojima H, Kanai Y, Arita K, Nakao M. Lysine Demethylase LSD1 Coordinates Glycolytic and Mitochondrial Metabolism in Hepatocellular Carcinoma Cells. Cancer Res, 75:1445-1456, 2015

6. Narukawa T, Hara T, Arai E, Komiyama M, Kawahara T, Kanai Y, Fujimoto H. Tumour multifocality and grade predict intravesical recurrence after nephroureterectomy in patients with upper urinary tract urothelial carcinoma without a history of bladder cancer. Jpn J Clin Oncol, 45:488-493, 2015

7. Hashimoto T, Ogawa R, Matsubara A, Taniguchi H, Sugano K, Ushiama M, Yoshida T, Kanai Y, Sekine S. Familial adenomatous polyposis-associated and sporadic pyloric gland adenomas of the upper gastrointestinal tract share common genetic features. Histopathology, 67:689-698, 2015

8. Matsubara A, Ogawa R, Suzuki H, Oda I, Taniguchi H, Kanai Y, Kushima R, Sekine S. Activating GNAS and KRAS mutations in gastric foveolar metaplasia, gastric heterotopia, and adenocarcinoma of the duodenum. Br J Cancer, 112:1398-1404, 2015

9. Yoshida M, Sekine S, Ogawa R, Yoshida H, Maeshima A, Kanai Y, Kinoshita T, Ochiai A. Frequent MED12 mutations in phyllodes tumours of the breast. Br J Cancer, 112:1703-1708, 2015

10. Terai H, Soejima K, Yasuda H, Sato T, Naoki K, Ikemura S, Arai D, Ohgino K, Ishioka K, Hamamoto J, Kanai Y, Betsuyaku T. Long-term exposure to gefitinib induces acquired resistance through DNA methylation changes in the EGFR-mutant PC9 lung cancer cell line. Int J Oncol, 46:430-436, 2015

11. Hiraoka N, Ino Y, Yamazaki-Itoh R, Kanai Y, Kosuge T, Shimada K. Intratumoral tertiary lymphoid organ is a favourable prognosticator in patients with pancreatic cancer. Br J Cancer, 112:1782-1790, 2015

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16. Nakamura H, Arai Y, Totoki Y, Shirota T, Elzawahry A, Kato M, Hama N, Hosoda F, Urushidate T, Ohashi S, Hiraoka N, Ojima H, Shimada K, Okusaka T, Kosuge T, Miyagawa S, Shibata T. Genomic spectra of biliary tract cancer. Nat Genet, 47:1003-1010, 2015

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18. Sekiguchi M, Kushima R, Oda I, Suzuki H, Taniguchi H, Sekine S, Fukagawa T, Katai H. Clinical significance of a papillary adenocarcinoma component in early gastric cancer: a single-center retrospective analysis of 628 surgically resected early gastric cancers. J Gastroenterol, 50:424-434, 2015

19. Yamada M, Sakamoto T, Otake Y, Nakajima T, Kuchiba A, Taniguchi H, Sekine S, Kushima R, Ramberan H, Parra-Blanco A, Fujii T, Matsuda T, Saito Y. Investigating endoscopic features of sessile serrated adenomas/polyps by using narrow-band imaging with optical magnification. Gastrointest Endosc, 82:108-117, 2015

20. Oguro S, Esaki M, Kishi Y, Nara S, Shimada K, Ojima H, Kosuge T. Optimal indications for additional resection of the invasive cancer-positive proximal bile duct margin in cases of advanced perihilar cholangiocarcinoma. Ann Surg Oncol, 22:1915-1924, 2015

21. Fujimoto A, Furuta M, Shiraishi Y, Gotoh K, Kawakami Y, Arihiro K, Nakamura T, Ueno M, Ariizumi S, Nguyen HH, Shigemizu D, Abe T, Boroevich KA, Nakano K, Sasaki A, Kitada R, Maejima K, Yamamoto Y, Tanaka H, Shibuya T, Shibata T, Ojima H, Shimada K, Hayami S, Shigekawa Y, Aikata H, Ohdan H, Marubashi S, Yamada T, Kubo M, Hirano S, Ishikawa O, Yamamoto M, Yamaue H, Chayama K, Miyano S, Tsunoda T, Nakagawa H. Whole-genome mutational landscape of liver cancers displaying biliary phenotype reveals hepatitis impact and molecular diversity. Nat Commun, 6:6120, 2015

22. Watanabe T, Ueno H, Watabe Y, Hiraoka N, Morizane C, Itami J,Okusaka T, Miura N, Kakizaki T, Kakuya T, Kamita M, TsuchidaA, Nagakawa Y, Wilber H, Yamada T, Honda K. ACTN4 copy number increase as a predictive biomarker for chemoradiotherapy of locally advanced pancreatic cancer. Br J Cancer,112:704-713, 2015

Division of Genetics

Teruhiko Yoshida, Hiromi Sakamoto, Hitoshi Zembutsu, Bunsyo Shiotani, Norihisa Saeki, Chihiro Udagawa, Marianne Hanae Mazevet, Mineko Ushiama, Yoko Odaka, Misuzu Tsukamoto, Sachiyo Mitani, Fumiko Chiwaki, Rie Komatsuzaki, Masumi Shimizu, Noriko Abe, Sayaka Mito, Shizuka Shinohara, Hitomi Gunji, Tomoko Ikegami, Akiko Sakamoto, Aya Imai, Naoya Hayashida

Introduction

In 2015, the major research themes of the Division were 1) molecular understanding of cancer susceptibility; 2) pharmacogenomics research on cancer treatment and 3) molecular understanding of DNA damage response. Dr. Shiotani joined the Division as a new Senior Staff Scientist in April 2015 to lead theme 3) above.

Research activities

1) LMO1 was identified as a neuroblastoma (NB)-susceptibility gene by a genome-wide association study in our previous study. Based on microarray expression analyses on LMO1-knockdown NB cells, several microRNAs were found suppressed and some of them, including the let-4 family miRNAs, showed cell-growth inhibition activities when introduced into NB cells. The results suggest that LMO1 contributes to tumorigenesis through down-regulation of tumor-suppressing microRNAs.

2) In the pharmacogenomics research, the prospective multi-center study of CYP2D6-Tamoxifen has completed its patient recruitment. The manuscript is in preparation. Other on-going projects have shown solid progress in the validation study for genetic markers of gemcitabine-induced neutropenia; some of the candidate SNPs were replicated successfully in the samples of the National Cancer Center biobank. The biomarker for Herceptin-induced cardiotoxicity has been searched by whole-exome sequencing analysis. Targeted sequencing has been performed on cancer-related genes to identify biomarkers for sensitivity to cytotoxic anticancer drugs using a PDX model. Serum biomarker screening for BRCTF1-targeted therapy has identified three candidate biomarkers.

3) ATR is a master checkpoint kinase orchestrating DNA damage response to maintain genome integrity and is a promising target in cancer chemotherapy. An analog sensitive (AS-ATR) was identified out of 60 candidates, which showed their kinase activity in the presence of both natural ATP and an analog ATP. A system is being developed utilizing AS-ATR and analog ATP for comprehensive identification of direct ATR substrates.

4) The Integrated Disease Omics Project supported by the National Institute of Biomedical Innovation (NiBio) finished in March 2015. Two staff of the Division are expected to continue to play a leading role in the Project for the development and public release of the Disease Omics database. Data cleaning and registration in the database have been the major focus of the painstaking efforts.

Education

A postdoctoral fellow was employed and an undergraduate student has been trained since 2015.

Future prospects

In addition to the research projects described above, the staff of the Division have engaged in the services of the genome core facility and biobank. In particular, the duties of the genome core facility have included the genetic test of hereditary cancer syndromes for the outpatient genetic counseling clinic in the National Cancer Center Hospital. On

the other hand, original research activities are being centered in the research themes 2) and 3). Because the staff leading the themes have stayed in the Division only from 1 to 2 years, further development is expected in the coming few years.

List of papers published in 2015

Journal

1. Zembutsu H. Pharmacogenomics toward personalized tamoxifen therapy for breast cancer. Pharmacogenomics, 16:287-296, 2015

2. Hashimoto T, Ogawa R, Matsubara A, Taniguchi H, Sugano K, Ushiama M, Yoshida T, Kanai Y, Sekine S. Familial adenomatous polyposis-associated and sporadic pyloric gland adenomas of the upper gastrointestinal tract share common genetic features. Histopathology, 67:689-698, 2015

3. Kumamoto K, Ishida H, Ohsawa T, Ishibashi K, Ushiama M, Yoshida T, Iwama T. Germline and somatic mutations of the APC gene in papillary thyroid carcinoma associated with familial adenomatous polyposis: Analysis of three cases and a review of the literature. Oncol Lett, 10:2239-2243, 2015

4. Saeki N, Ono H, Sakamoto H, Yoshida T. Down-regulation of Immune-related Genes by PSCA in Gallbladder Cancer Cells Implanted into Mice. Anticancer Res, 35:2619-2625, 2015

5. Matsunuma R, Niida H, Ohhata T, Kitagawa K, Sakai S, Uchida C, Shiotani B, Matsumoto M, Nakayama KI, Ogura H, Shiiya N, Kitagawa M. UV Damage-Induced Phosphorylation of HBO1 Triggers CRL4DDB2-Mediated Degradation To Regulate Cell Proliferation. Mol Cell Biol, 36:394-406, 2015

6. Tanaka Y, Aoyagi K, Minashi K, Komatsuzaki R, Komatsu M, Chiwaki F, Tamaoki M, Nishimura T, Takahashi N, Oda I, Tachimori Y, Arao T, Nishio K, Kitano S, Narumi K, Aoki K, Fujii S, Ochiai A, Yoshida T, Muto M, Yamada Y, Sasaki H. Discovery of a Good Responder Subtype of Esophageal Squamous Cell Carcinoma with Cytotoxic T-Lymphocyte Signatures Activated by Chemoradiotherapy. PLoS One, 10:e0143804, 2015

7. Saeki N, Ono H, Yanagihara K, Aoyagi K, Sasaki H, Sakamoto H, Yoshida T. rs2294008T, a risk allele for gastric and gallbladder cancers, suppresses the PSCA promoter by recruiting the transcription factor YY1. Genes Cells, 20:382-391, 2015

8. Yamanoi K, Arai E, Tian Y, Takahashi Y, Miyata S, Sasaki H, Chiwaki F, Ichikawa H, Sakamoto H, Kushima R, Katai H, Yoshida T, Sakamoto M, Kanai Y. Epigenetic clustering of gastric carcinomas based on DNA methylation profiles at the precancerous stage: its correlation with tumor aggressiveness and patient outcome. Carcinogenesis, 36:509-520, 2015

9. Saeki N, Komatsuzaki R, Chiwaki F, Yanagihara K, Sasaki H. A GSDMB enhancer-driven HSV thymidine kinase-expressing vector for controlling occult peritoneal dissemination of gastric cancer cells. BMC Cancer, 15:439, 2015

10. Suzuki M, Chiwaki F, Sawada Y, Ashikawa M, Aoyagi K, Fujita T, Yanagihara K, Komatsu M, Narita M, Suzuki T, Nagase H, Kushima R, Sakamoto H, Fukagawa T, Katai H, Nakagama H, Yoshida T, Uezono Y, Sasaki H. Peripheral opioid antagonist enhances the effect of anti-tumor drug by blocking a cell growth-suppressive pathway *in vivo*. PLoS One, 10:e0123407, 2015

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12. Fujita T, Chiwaki F, Takahashi RU, Aoyagi K, Yanagihara K, Nishimura T, Tamaoki M, Komatsu M, Komatsuzaki R, Matsusaki K, Ichikawa H, Sakamoto H, Yamada Y, Fukagawa T, Katai H, Konno H, Ochiya T, Yoshida T, Sasaki H. Identification and Characterization of CXCR4-Positive Gastric Cancer Stem Cells. PLoS One, 10:e0130808, 2015

13. Iwakawa R, Kohno T, Totoki Y, Shibata T, Tsuchihara K, Mimaki S, Tsuta K, Narita Y, Nishikawa R, Noguchi M, Harris CC, Robles AI, Yamaguchi R, Imoto S, Miyano S, Totsuka H, Yoshida T, Yokota J. Expression and clinical significance of genes frequently mutated in small cell lung cancers defined by whole exome/RNA sequencing. Carcinogenesis, 36:616-621, 2015

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

Tohru Kiyono, Takashi Yugawa, Tomomi Nakahara, Kenji Yamada, Ghani Farhana Ishrat, Takako Ishiyama, Katsuyuki Tanaka, Chiho Kohno, Shin-ichi Ohno, Yuki Inagawa, Kasumi Ohtsubo, 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 persistent infection and oncogenesis of 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). Persistent infection of the HR-HPVs is a major cause of cervical cancer and a subset of head and neck cancer. Our goal is to develop non-invasive therapies to prevent and cure HPV-associated cancers. We have developed a tissue culture model recapitulating the viral persistence as well as viral oncogene, E6 and E7, -induced cancer development. By using these tissue culture models, we are currently studying (1) mechanism of the viral genome replication and (2) the roles of E6, E7 and cellular oncogenes in multistep carcinogenesis.

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. To develop an anti-HPV drug, we are studying molecular mechanisms of HPV genome replication, a key aspect of the viral persistence.

Research activities

1) Molecular mechanism of HPV genome maintenance and anti-HPV drug development.

The HPV genome undergoes three phases of replication: initial amplification, maintenance replication and productive amplification in the viral life cycle. Upon infection, HPV establishes its genome as a nuclear episome through initial amplification and about 50 to 100 copies of the viral episomes are stably maintained in basal cells of the infected lesions such as cervical intraepithelial neoplasm (CIN). In terminally differentiating compartments of the lesions, HPV genome are drastically increased through productive amplification and incorporated in progeny virions. In a previous study, we demonstrated that the viral helicase E1 is dispensable for maintenance replication but indispensable for the initial and productive amplification of the HPV16 genome. In a recent study, we found that expression of HPV16 E1 results in activation of DNA damage response (DDR) which leads to activation of NF-κB pathway in human cervical keratinocytes (HCKs) stably maintaining HPV16 genome. The activation of NF-κB pathway suppressed the E1-dependent replication of HPV genome by promoting its proteosomal degradation. Furthermore, NF-κB activities are constitutively higher in HPV containing-cell lines derived from CIN biopsies than in normal HCKs and inhibition or activation of NF-κB resulted in an increase or decrease of HPV copy numbers, respectively. Thus, E1 and NF-κB may constitute a negative feedback that mediates transition from initial amplification to maintenance replication and also sustains an E1-independent replication during the viral persistence. We also developed HCKs maintaining recombinant "reporter" HPV16 genomes expressing a secreted luciferase and verified that luciferase activities in cultural medium collates with the viral copy numbers. By using this reporter system, we are

working on identifying NF-κB target genes that enables intervention in viral persistence as well as developing drug screening to eradicate HPV genomes. Once an 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 clustered regularly interspaced short palindromic repeats (CRISPR) / CRISPR-associated (Cas) made it possible to directly target an 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) *In vitro* human carcinogenesis model

p63, a member of the p53 family, is frequently overexpressed in squamous cell carcinomas (SCCs). Paradoxically, growing evidence points out the association of p63 loss with metastasis appearance and poor prognosis in SCCs, although the underlying molecular mechanism and its functional relevance to carcinogenesis remains largely unclear. We have previously demonstrated that p63 represses NOTCH1 gene expression to support the self-renewing capacity of normal human keratinocytes, and its overexpression enhances tumorigenicity. We also identified the novel NOTCH-ROCK pathway as a critical regulator for keratinocyte differentiation downstream of p63. Recently, we revealed that MYC overexpression rescues the proliferative ability of p63-deficient cells. Using our *in vitro* human multistep carcinogenesis model, we found that knock-down of p63 increased invasiveness through the NOTCH-ROCK pathway in a three-dimensional culture system. We aim to develop a novel therapeutic strategy to target poorly differentiated SCCs based on their cancer biology.

Using a novel culture method based on the finding of the NOTCH-ROCK pathway, normal bile duct epithelial cells and duodenal epithelial cells were isolated and immortalized. These cells have been used to model ampullary cancer.

Education

One Ph.D. student and one MD/Ph.D. student in local universities worked as trainees in our lab and had cancer research training. One post-doctoral researcher has been currently training in a cancer research field with us since April.

Future prospects

The current HPV vaccines have no therapeutic effect upon pre-existing CIN lesions. To clear HPV infection from CIN lesions, a possible strategy is eradication of HPV genomes with a specific inhibitor of HPV replication or elimination of HPV-infected cells with surgery or by induction of cell death with a drug or therapeutic vaccine. Further understanding of molecular mechanisms underlying the viral persistence through an E1-NFκB negative feedback will promote development of anti-HPV drug and possible immuno-therapies by enhancing presentation of the viral specific antigens in persistently infected basal cells.

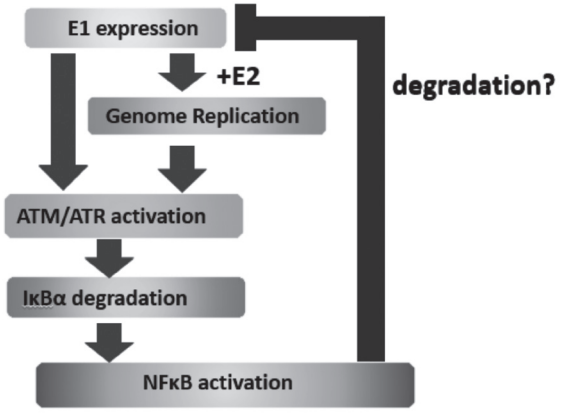

Figure 1. Regulation of HPV genome replication

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Division of Carcinogenesis and Prevention (Environmental Carcinogenesis and Prevention Group)

Yukari Totsuka, Michihiro Mutoh, Ken-ichi Yoshioka, Gen Fujii, Sachiko Dobashi, Masanori Goto, Haruna Sato, Nozomi Akiba, Wakana Onuma, Takahiro Hamoya, Yuri Fukushi, Shuuya Tamura, Yuko Atsumi, Yusuke Minakawa, Atsuhiro Shimizu, Tetsuya Mukasa

Introduction

Cancer is a disease associated with aging and environmental risk factors. It is well known that chemical substances form DNA adducts, which are considered to be a 'trigger' of mutagenesis. As cancer risk elevates in association with aging, genomic destabilization frequently arises in the cells of the elderly, which is associated with the impairment of DNA repair functions. The aims of our research projects are exploration of novel cancer etiology via identification of DNA adducts that are important for human cancer development, and clarification of the mechanisms for genomic instability associated with aging. On the other hand, cancer chemoprevention is one of the preemptive approaches that is strongly expected to reduce cancer morbidity and mortality. We are working to develop novel candidates for cancer chemopreventive agents and aim for their practical application using the concept of drug repositioning.

Research activities

1) Exploration of cancer etiology using comprehensive DNA adduct analysis

Nanosized-magnetite (MGT) showed genotoxicity in both *in vitro* and *in vivo* assay systems. Based on the mutational spectrum observed in the lungs of mice exposed to MGT, it was suggested that inflammatory responses exist behind the genotoxicity. To further clarify mechanisms underlying the genotoxicity, a comprehensive DNA adduct (DNA adductome) analysis was conducted using DNA samples derived from the lungs of mice exposed to MGT. Principal component analysis (PCA) against a subset of DNA adducts was applied and several adducts, which are deduced to be formed by inflammation or oxidative stress, as is the case of etheno-deoxycytidine (ε dC), revealed higher contributions to MGT exposure. From these observations, it is suggested that inflammatory responses might be involved in the genotoxicity induced by MGT in the lungs of mice.

2) Regulations for genome stability and the associated anti-cancer and aging effects

Senescent cells are usually defective in DNA damage repair, and hence widely accumulate irreparable DNA double strand breaks (DSBs). Such repair deficiency is associated with the decrease of histone H2AX that is required for efficient DSB repair and hence for genome stability. We showed that cells with largely down-regulated H2AX were defective in repairing DNA-replication stress-associated DSBs but still could repair directly caused DSBs through transient H2AX stabilization. H2AX stabilization upon DSB formation was mediated by the ATM kinase, sirtuin protein SIRT6, and ISWI family chromatin remodeler SNF2H.

3) Prevention of colorectal cancer

Familial adenomatous polyposis (FAP) patients are a well-known high-risk group with 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 a laboratory study. Moreover, we are searching for novel chemopreventive agents against CRC using animal models of FAP.

Education

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

Future prospects

- Establish a novel cancer prevention strategy based on the exploration of cancer etiology and mechanisms
- Develop novel candidates for cancer chemopreventive agents and aim for their practical application

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Division of Cancer Biology

Hirofumi Arakawa, Yasuyuki Nakamura, Masayuki Tsuneki, Hiroki Kamino, Yoko Sagami, Ruri Nakanishi, Natsuki Kinoshita, Chieko Haga, Ayami Kawashima, Katsuko Honjo, Tomonori Aikawa

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 roles of the Mieap-regulated mitochondrial quality control and cancer-specific unhealthy mitochondria in tumorigenesis have been studied to uncover the mechanisms of cancer initiation, growth, invasion and metastasis, based on which new cancer preventive, diagnostic, and therapeutic strategies could be developed.

Research activities

Mieap-regulated mitochondrial quality control

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 Adenosine TriPhosphate (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). 14-3-3 γ mediates the degradation of oxidized mitochondrial proteins within mitochondria during MALM (*Scientific Reports* 2: 379, 2012).

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).

Mieap-regulated mitochondrial quality control is frequently inactivated in human cancer

The accumulation of unhealthy mitochondria results in mitochondrial dysfunction, which has been implicated in aging, degenerative diseases and cancer. The Mieap-regulated mitochondrial quality control (MQC) was found to be frequently inactivated by p53 mutations or Mieap/BNIP3 promoter methylation in more than 70% of primary cancer tissues of colorectal cancer patients, leading to accumulation of unhealthy mitochondria and a high level of mitochondria reactive oxygen species generation (ROS).

The elevated mitochondrial ROS causes oxidative damage to DNA, RNA, protein and lipid and so on. This induces genomic instability. The mitochondrial ROS contribute to tumor growth, epithelial-to-mesenchymal transition, cancer invasion, cancer metastasis, tumor angiogenesis through the activation of HIF-1, NF-kB, MMPs, AKT, Erk1/2, JNK and so on. Therefore, the Mieap-regulated mitochondrial quality control is a tumor suppressor for colorectal cancer (Figure 2).

In order to further evaluate the clinical significance of the Mieap-regulated MQC in human cancer, the status of p53, Mieap, and BNIP3 are

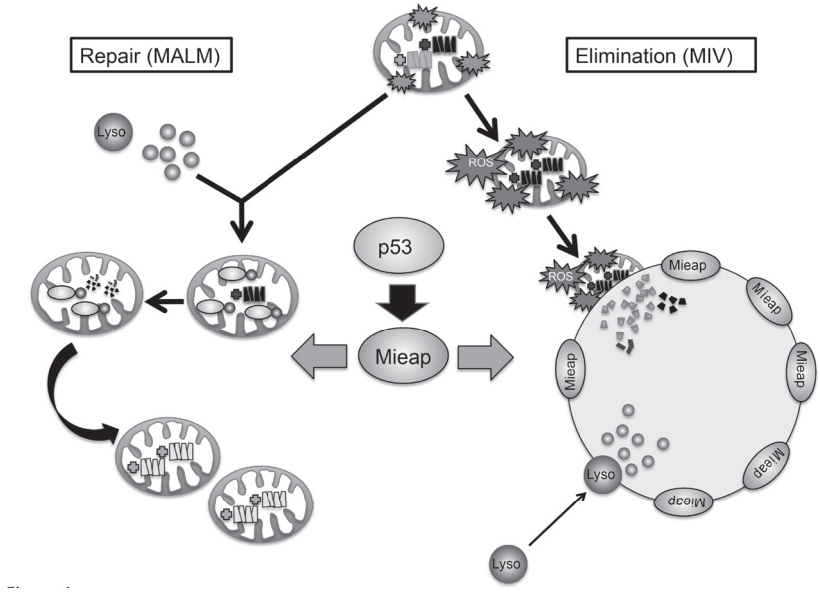

Figure 1. Mieap-regulated mitochondrial quality control

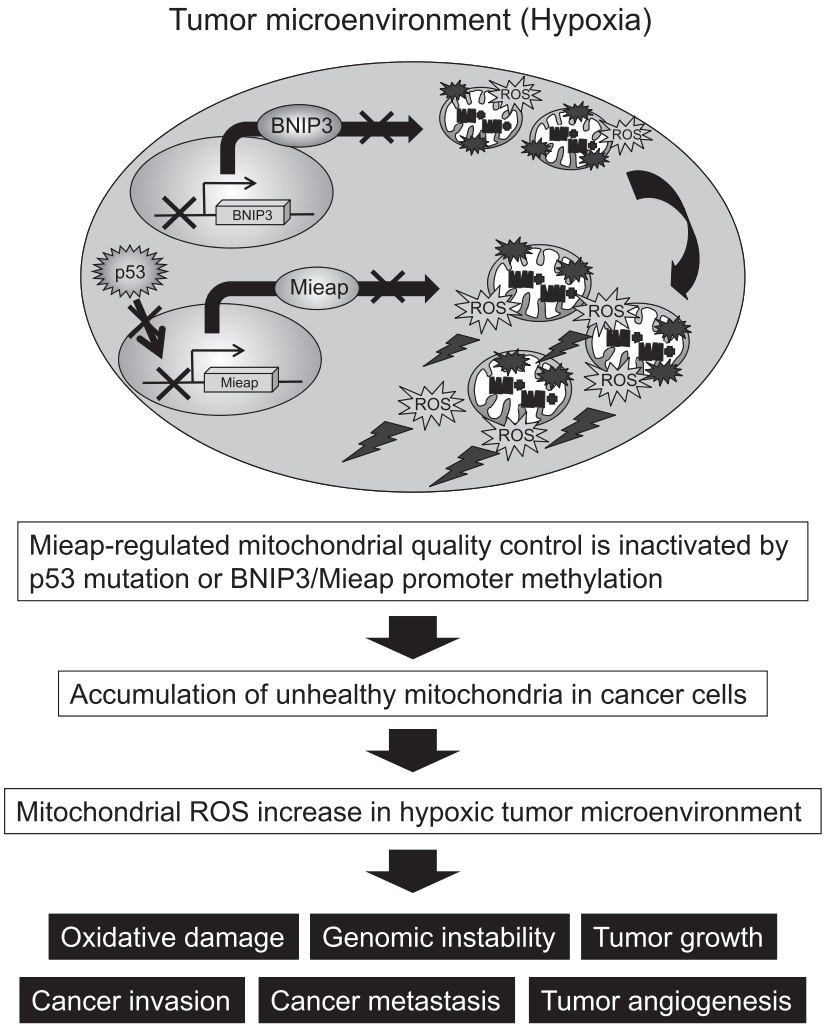

Figure 2. Alteration of Mieap-regulated mitochondrial quality control in cancer

being examined in primary cancer tissues of breast, gastric, pancreatic, lung and liver cancer patients.

Mieap-deficient cancer animal model

To clarify the in vivo role of the Mieap-regulated MQC in tumorignenesis, Mieap knockout mice were generated in the Division. Using the Mieap knockout mice, the Mieap-deficient $Apc^{Min/+}$ mice were also generated and analyzed in order to elucidate the role of Mieap in colorectal cancer tumorigenesis.

Interestingly, the Mieap-deficient $Apc^{Min/+}$ mice exhibited remarkably reduced lifespans compared with those of $Apc^{Min/+}$ mice. Furthermore, a substantial increase in the number and size of intestinal polyps was found in the Mieap-deficient $Apc^{Min/+}$ mice. Histopathologically, intestinal tumors in the Mieap-deficient $Apc^{Min/+}$ mice clearly exhibited advanced grades of adenoma and adenocarcinomas. Unhealthy mitochondria dramatically accumulated in the tumor cells and generated a high level of ROS in the Mieap-deficient $Apc^{Min/+}$ mice. These results suggest that the Mieap-regulated mitochondrial quality control pathway has a critical role in the suppression of intestinal tumor in vivo.

In addition to the colorectal cancer model, the Mieap-deficient pancreatic and gastric cancer models are being prepared at the Division.

Development of new cancer preventive/diagnostic/therapeutic methods through targeting cancer-specific unhealthy mitochondria

Unhealthy mitochondria are dramatically and specifically accumulated in cancer cells due to inactivation of the Mieap-regulated MQC. Therefore, the Division's working hypothesis proposes that cancer-specific unhealthy mitochondria owing to inactivation of the Mieap-regulated MQC may be very attractive and are promising targets for development of new cancer preventive/diagnostic/therapeutic methods. Now, in order to identify new cancer preventive/diagnostic/therapeutic targets, the nature and characteristics of these cancer-specific unhealthy mitochondria are being explored by metabolome, transcriptome, proteome analyses in the Division.

Education

To gain understanding and skill for cancer research, students attend lectures and seminars, and attend and/or practice research meetings, journal clubs, scientific meetings, and so forth. These practices will enable students to develop the ability to conduct their studies as an independent cancer researcher in the future. To obtain the high-level 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, and so forth.

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.

List of papers published in 2015

Journal

1. Tsuneki M, Nakamura Y, Kinjo T, Nakanishi R, Arakawa H. Mieap suppresses murine intestinal tumor via its mitochondrial quality control. Sci Rep, 5:12472, 2015

Division of Hematological Malignancy

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

Introduction

Acute myeloid leukemia (AML) is the most common leukemia in Japan and the 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 AML patients have a relapse and only 25-30% of young adults and fewer than 10% of older patients survive longer than 5 years, suggesting the presence of AML stem cells that are resistant to chemotherapy. Thus, AML stem cell eradication is thought to be crucial for the cure to AML. Chromosome abnormalities, which results in the 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 are essential for the maintenance of AML cells, especially AML stem cells.

Research activities

AML is a clonal malignant disorder derived from a small number of leukemic stem cells (LSCs). *MLL* gene rearrangements are found in AML associated with poor prognosis. The upregulation of *Hox* genes is critical for LSC induction and maintenance, but is unlikely to support malignancy and the high LSC frequency observed in MLL leukemias. The present study shows that MLL fusion proteins interact with the transcription factor PU.1 to activate the transcription of *CSF-1R*, which is critical for LSC activity. AML is cured by either deletion of *PU.1*, or ablation of cells expressing CSF-1R. Kinase inhibitors specific for CSF-1R prolong survival time. These findings indicate that PU.1-mediated upregulation of CSF-1R is a critical effector of *MLL* leukemogenesis.

IDH1 and IDH2 mutations occur frequently in AML and other cancers. The mutant IDH enzymes convert α-ketoglutarate (α-KG) to the oncometabolite 2-hydroxyglutarate (2-HG), which dysregulates a set of α-KG-dependent dioxygenases. To determine whether mutant IDH enzymes are valid targets for cancer therapy, we created a mouse model of AML in which mice were transplanted with *nucleophosmin1 (NPM1)*+/- hematopoietic stem/progenitor cells co-transduced with four mutant genes (*NPMc, IDH2/R140Q, DNMT3A/R882H,* and *FLT3/ITD*) which often occur simultaneously in human AML patients. Conditional deletion of *IDH2/R140Q* blocked 2-HG production and maintenance of leukemia stem cells, resulting in survival of the AML mice. IDH2/R140Q was necessary for the engraftment or survival of $NPMc^+$ cells in vivo. Gene expression analysis indicated that NPMc increased expression of Hoxa9. IDH2/R140Q also increased the level of Meis1 and activated the hypoxia pathway in AML cells. IDH2/R140Q decreased the 5hmC modification and expression of some differentiation-inducing genes (*Ebf1* and *Spib*). Taken together, our results indicated that IDH2 mutation is critical for the development and maintenance of AML stem-like cells, and they provided a preclinical justification for targeting mutant IDH enzymes as a strategy for anticancer therapy.

List of papers published in 2015

Journal

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Division of Cancer Stem Cell

Kenkichi Masutomi, Yoshiko Maida, Mami Yasukawa, Marco Ghilotti, Satoko Yamaguchi, Mihoko Tsurumaki, Akiya Tatsumi

Introduction

Research in the Division of Cancer Stem Cell is focused on deciphering the mechanisms that establish and maintain cancer stem cells and to develop 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). The RdRP enzyme complex is distinct from telomerase; TERT assembles with BRG1 and nucleostemin (NS), and the TERT-BRG1-NS complex (TBN complex) exerts RdRP activity[2]. We found that the TBN complex regulates miRNA expression, presumably at the transcriptional level[3] (Figure 2). 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)[2]. We confirmed that TERT protein levels and TERT-associated RdRP activity are positively correlated in human cancer cell lines.

RdRPs in yeast and worms 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. 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.

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. We found that the TBN complex participates in the regulation of tumor initiating cells (TICs) phenotypes through telomere-independent mechanisms[4] (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 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[5]. 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[5]. We found that eribulin mesylate (eribulin) effectively inhibits growth of platinum-resistant ovarian cancer cells. 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 eribulin. 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 might be a promising therapeutic agent for platinum-resistant ovarian cancer.

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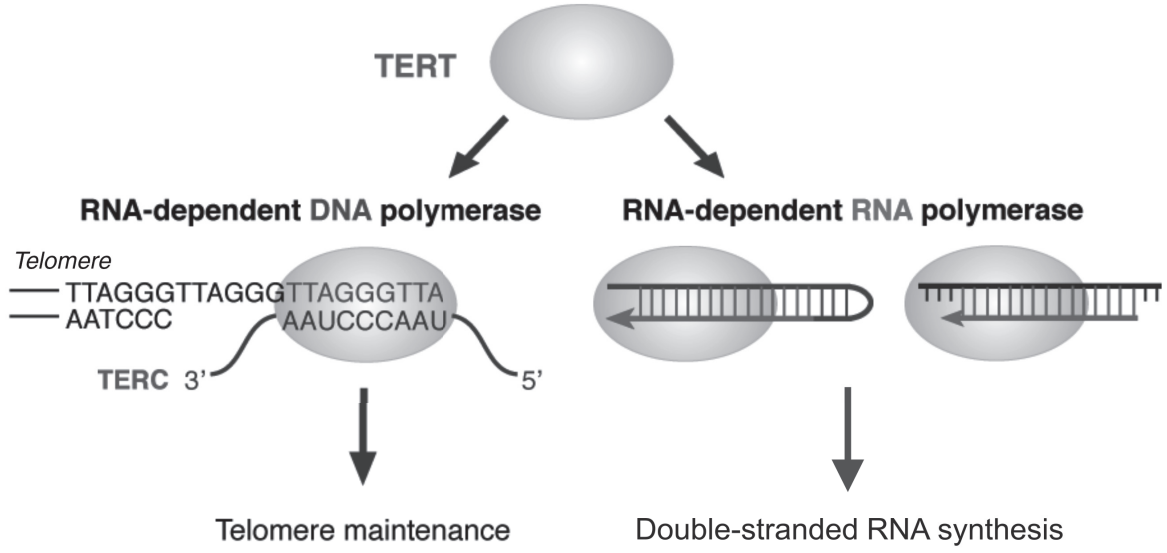

Figure 1. TERT exerts RdRP activity

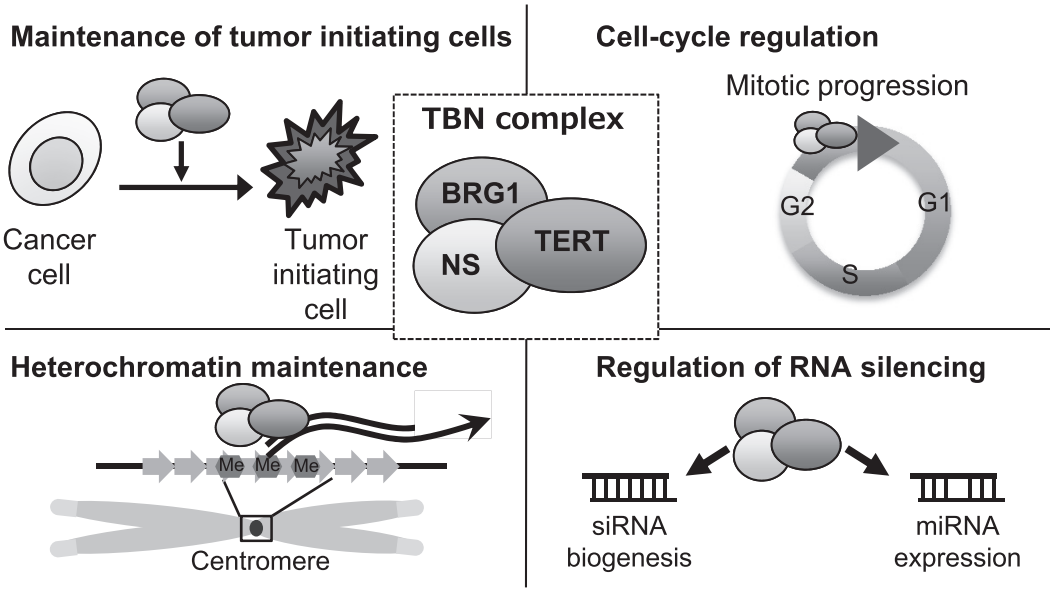

Figure 2. Various functions of the TBN complex

List of papers published in 2015

Journal

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Division of Cancer Differentiation

Koji Okamoto, Daisuke Shiokawa, Hirokazu Ohata, Toshiaki Miyazaki, Manami Miura, Naoko Osada, Kenta Takahashi, Rie Uchino, Wakako Hara, Ai Sato, Hiroaki Sakai, Seiko Ogawa

Introduction

It is proposed that cancer stem cells (CSCs) are responsible for the malignant traits of refractory cancer, that is, the ability to generate metastatic foci and chemoresistance. Our group mainly focuses on studying CSCs from colon cancer and ovarian cancer. We aim to find out the weaknesses of CSCs that are cultivated in vitro from various clinical specimens, and to exploit them for clinical purposes. In addition, we use the established CSCs to generate patient-derived xenograft tumors in order to understand the mechanisms of chemoresistance (Figure 1).

Routine activities

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

Research activities

1) Biological characterization of cancer stem cells in vitro from human refractory cancer

Recently, we isolated and expanded CSCs in vitro from human colon cancer and serous ovarian cancer. Using the cultivated CSCs, we demonstrated that activation of mTORC1 is responsible for proliferation and maintenance of stemness of colon CSCs. Furthermore, we revealed that reactive oxygen species (ROS) produced by NADPH oxidase contribute to the activation of mTORC1. In addition, we compared the metastatic and non-metastatic CSCs through microarray and metabolome analyses, and identified 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 addition to colon CSCs, we also investigated regulatory pathways of ovarian CSCs. We showed that ALDH1 is specifically expressed in ovarian CSCs. Further, we demonstrated the functional importance of ALDH for their proliferation.

2) Establishment of PDX models of refractory cancer through transplantation of CSCs into mice

Using xenograft tumors through transplantation of colon and ovarian CSCs into immuno-compromised MOG mice, we established chemoresistance models in vivo, in which mice carrying the tumors were treated with chemotherapeutic agents. By applying single-cell gene expression analyses, the cellular heterogeneity of xenograft tumors and identity of chemo-resistant cells were examined.

Education

Teaching students (one undergraduate student, four graduate students)

Future prospects

We will pursue biological characterization of CSCs derived from refractory cancer. We will aim to translate the acquired knowledge for CSCs into clinical practices.

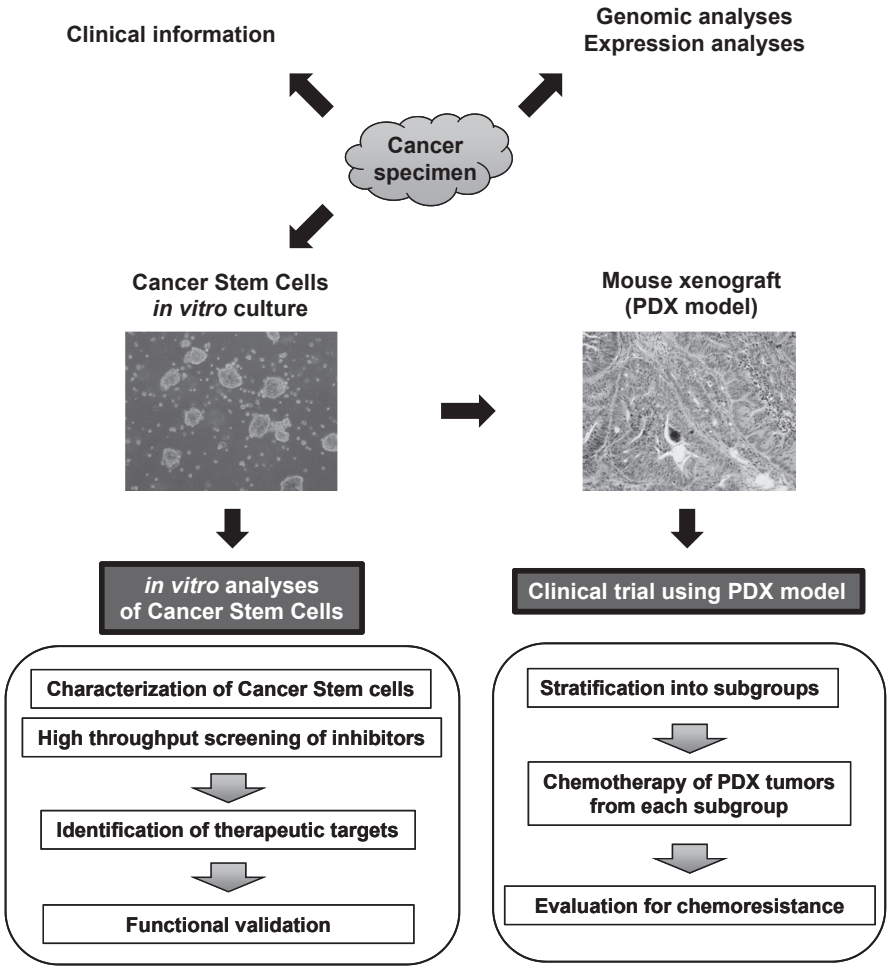

Figure 1. In vitro cultivation of Cancer Stem Cells and PDX models

List of papers published in 2015

Journal

1. Ujihira T, Ikeda K, Suzuki T, Yamaga R, Sato W, Horie-Inoue K, Shigekawa T, Osaki A, Saeki T, Okamoto K, Takeda S, Inoue S. MicroRNA-574-3p, identified by microRNA library-based functional screening, modulates tamoxifen response in breast cancer. Sci Rep, 5:7641, 2015
2. Miyazaki T, Ikeda K, Sato W, Horie-Inoue K, Okamoto K, Inoue S. MicroRNA library-based functional screening identified androgen-sensitive miR-216a as a player in bicalutamide resistance in prostate cancer. J Clin Med, 4:1853-1865, 2015

Division of Epigenomics

Toshikazu Ushijima, Satoshi Yamashita, Hideyuki Takeshima, Naoko Hattori, Masahiro Maeda, Emi Kubo, Naoko Iida, Akiko Mori, Kana Kimura, Kanako Sakashita, Naoko Kobayashi, Yuko Miyaji, Aya Nakajima, Mika Wakabayashi

Introduction

This Division has been focusing on the epigenetic mechanisms of carcinogenesis, and has identified many aberrantly methylated genes 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, 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 activities in 1) developing clinically useful biomarkers, a novel approach to cancer prevention, and epigenetic therapy, and 2) 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, *SMARCA1*, encoding an ISWI-type chromatin remodeling factor, was identified as a tumor-suppressor gene inactivated by either aberrant DNA methylation or somatic mutation in gastric cancer. It was also revealed that genetic and epigenetic alterations of *SMARCA1* were induced at an early stage of carcinogenesis and were frequently involved in the formation of a field defect.

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. It was revealed that about 40% of gastric cancer cases had no genetic alterations of known cancer-related genes, but frequently had epigenetic alterations of various genes involved in cancer-related pathways, such as WNT signaling and p53 signaling pathways.

2) Development of Biomarkers

This Division previously revealed that the degree of accumulated aberrant DNA methylation in normal-appearing gastric mucosae is expected to be a useful diagnostic marker to predict 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 revealed that cases with higher DNA methylation levels of *miR-124a-3* had a higher risk of developing metachronous gastric cancers (a multivariate-adjusted HR = 2.30 (95% CI = 1.03 to 5.10), $p = 0.042$) (Figure 1). Based on these results, we started a multicenter prospective cohort study for the prediction of gastric cancer risk in healthy volunteers who underwent eradication of *Helicobacter pylori (H. pylori)*, the almost exclusive cause of gastric cancers.

To establish clinically useful biomarkers to predict the response to cancer therapy is very important. This year, it was revealed that esophageal squamous cell carcinoma cases with DNA methylation of *ZNF695* benefitted from chemoradiotherapy treatment.

3) Development of epigenetic therapy

A combination of epigenetic modifications specifically present in cancer cells is a possible target in developing cancer cell-specific epigenetic therapy. This year, it was revealed that the combination of DNA methylation and trimethylation of histone H3 lysine 27 (H3K27me3) existed specifically in cancer

cells, and it was suggested that this combination is a possible target for cancer cell-specific epigenetic therapy.

Future prospects

Based on these results, this Division will 1) continue multicenter prospective cohort studies for the prediction of gastric cancer risk, 2) conduct the development of epigenetic therapy in gastric cancers and neuroblastomas, and 3) reveal the molecular mechanisms of how aberrant DNA methylation is induced by chronic inflammation.

Other activities

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

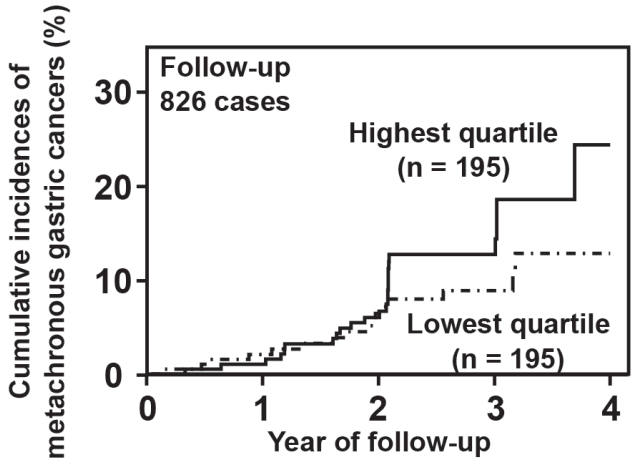

Figure 1. Prediction of gastric cancer risk by DNA methylation.

List of papers published in 2015

Journal

1. Wei FZ, Cao Z, Wang X, Wang H, Cai MY, Li T, Hattori N, Wang D, Du Y, Song B, Cao LL, Shen C, Wang L, Wang H, Yang Y, Xie D, Wang F, Ushijima T, Zhao Y, Zhu WG. Epigenetic regulation of autophagy by the methyltransferase EZH2 through an MTOR-dependent pathway. Autophagy, 11:2309-2322, 2015

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3. Takeshima H, Niwa T, Takahashi T, Wakabayashi M, Yamashita S, Ando T, Inagawa Y, Taniguchi H, Katai H, Sugiyama T, Kiyono T, Ushijima T. Frequent involvement of chromatin remodeler alterations in gastric field cancerization. Cancer Lett, 357:328-338, 2015

4. Asada K, Nakajima T, Shimazu T, Yamamichi N, Maekita T, Yokoi C, Oda I, Ando T, Yoshida T, Nanjo S, Fujishiro M, Gotoda T, Ichinose M, Ushijima T. Demonstration of the usefulness of epigenetic cancer risk prediction by a multicentre prospective cohort study. Gut, 64:388-396, 2015

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6. Takeshima, Hideyuki. Identification of coexistence of DNA methylation and H3K27me3 specifically in cancer cells as a promising target for epigenetic therapy. Carcinogenesis, 36:192-201, 2015

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8. Yamaguchi T, Mukai H, Yamashita S, Fujii S, Ushijima T. Comprehensive DNA methylation and extensive mutation analyses of HER2-positive breast cancer. Oncology, 88:377-384, 2015

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Division of Cancer Genomics

Tatsuhiro Shibata, Fumie Hosoda, Yasushi Totoki, Shinichi Yachida, Yasuhito Arai, Natsuko Hama, Hiromi Nakamura, Hirofumi Rokutan, Erina Takai, Koichi Ogura, Akihiro Oomoto, Mihoko Adachi, Masami Suzuki, Hiroko Shimizu, Shoko Ohashi, Wataru Omata, Wakako Mukai, Erika Arakawa, Keiko Igarashi, Risa Usui, Hiroki Sato, 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), contributing to the core facility of the center, and developing new informatics tools for data analysis from various types of next-generation high-performance sequencers (NGS).

Research activities

1) Comprehensive molecular genetic characterization of Asian cancer genomes

Biliary tract cancer (BTC) is an intractable cancer, with limited therapeutic options, in which the molecular mechanisms underlying tumor development remain poorly understood. We performed whole-exome and transcriptome analysis of 260 biliary tract cancers, including 145 intrahepatic and 86 extrahepatic cholangiocarcinoma and 29 gallbladder cancers as a Japan ICGC project. We uncovered spectra of molecular alterations that included new potential therapeutic targets and unique immune-signatures, confirmed genetic differences in distinct subtypes and demonstrated that approximately 40% of cases harbored potentially targetable genetic aberrations (Figure 1). Notably, FGFR2 kinase fusion genes are identified as one of the high-potential therapeutic targets in BTC, and we have started-up BT-SCRUM (genomic screening consortium for biliary tract cancer) for the prospective study of genotype-based molecular therapy through collaboration with the Department of Hepatobiliary and Pancreatic Oncology.

We performed whole genome sequencing of adult T cell leukemia/lymphoma cases and identified somatic mutations, genomic rearrangements including fusion genes and human T cell leukemia virus type-1 (HTLV-1) genome integrations. We found many gene loci where genome deletions occurred frequently, including NRXN3, IMMP2L, DPYD and IKZF2, and in-frame fusion genes including CTLA4-CD28 and ICOS-CD28. We also found that all cases have one or more (up to three) HTLV-1 genome integration sites and some integrated HTLV-1 genomes had large deletions.

We have performed large-scale whole transcriptome and whole exome sequencing of gastric cancer as a Japan ICGC project. The whole transcriptome analysis identified multiple novel fusion genes including protein kinases. The whole exome analysis of 68 mucinous gastric carcinomas identified a characteristic mutational profile and driver mutations including potential therapeutic target genes.

We established international multicenter collaboration and conducted in-depth analysis of the genomic abnormalities of ampullary carcinomas. Whole exome sequencing and subsequent targeted deep sequencing led to the identification of a tumor suppressor gene, ELF3, characteristic of ampullary carcinomas.

2) New technological developments for next-generation genome medicine

Liquid biopsy

We developed a new mutation call program that can detect somatic mutations in samples with low (≧ 2%) tumor content such as circulating cell-free DNA (cfDNA) using deep sequencing. We performed targeted deep sequencing of 60 genes of cfDNA in 48 patients of pancreatic ductal adenocarcinoma (PDAC) and identified potentially targetable somatic mutations in 14 of 48 patients (29.2%) (Figure 2). We also analyzed somatic copy number alterations using our in-house algorithm and detected potentially targetable amplifications. Assessment of mutations and copy number alterations in plasma cfDNA may provide a prognostic and diagnostic tool to assist decisions regarding optimal therapeutic strategies for PDAC patients.

Microbiome

We are clarifying the relationships between the luminal microbiota and colorectal cancers and the mechanisms of potential contribution of the microbiome in colorectal cancer development.

Germline evaluation

We have examined the genetic polymorphisms of 147 drug metabolism-related genes in 75 ALK-positive lung cancer patients treated with crizotinib. We found possible functional SNPs in two genes, showing statistically significant differences between the patients with or without severe adverse effects. By whole exome sequencing of germline DNA, pancreatic cancer susceptibility genes in Japanese familial pancreatic cancer patients have been identified.

Education

Six young researchers, one cancer specialist training doctor, and one visiting researcher have been trained in this Division. Three young bioinformaticians have been trained and two of them prepared for papers as the first author.

Future prospects

By utilizing current and cutting-edge sequencing technologies (for example, single cell sequencing), this Division will actively investigate cancer genomics from both basic (new biomarkers including therapeutic targets, epigenomics, metagenomics and immune-genomics) and translational research (preclinical research, liquid clinical sequencing, PGx and germline evaluation) viewpoints. Especially tighter collaboration with cancer-immunology groups by applying single cell immune-profiling and TCR repertoire sequencing will be achieved. This Division will also contribute to the development of bioinformatics tools and human resources for analyzing large cancer genomics data.

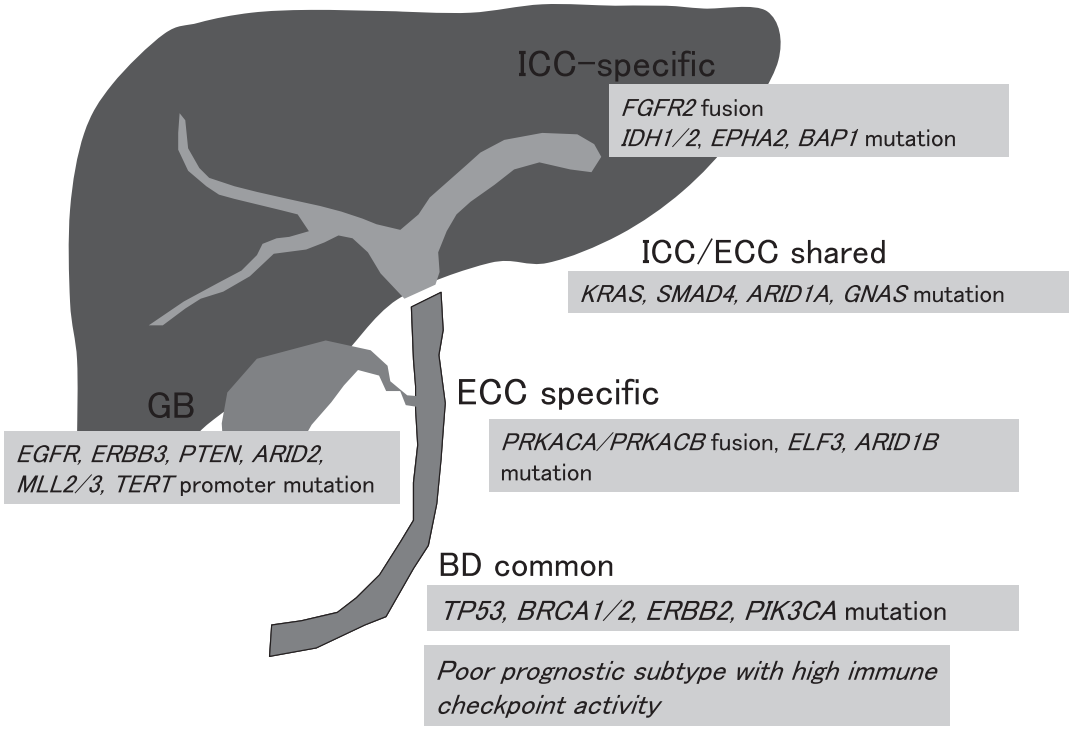

Figure 1. Subtype-specific omics (genome + RNA) signatures in BTC

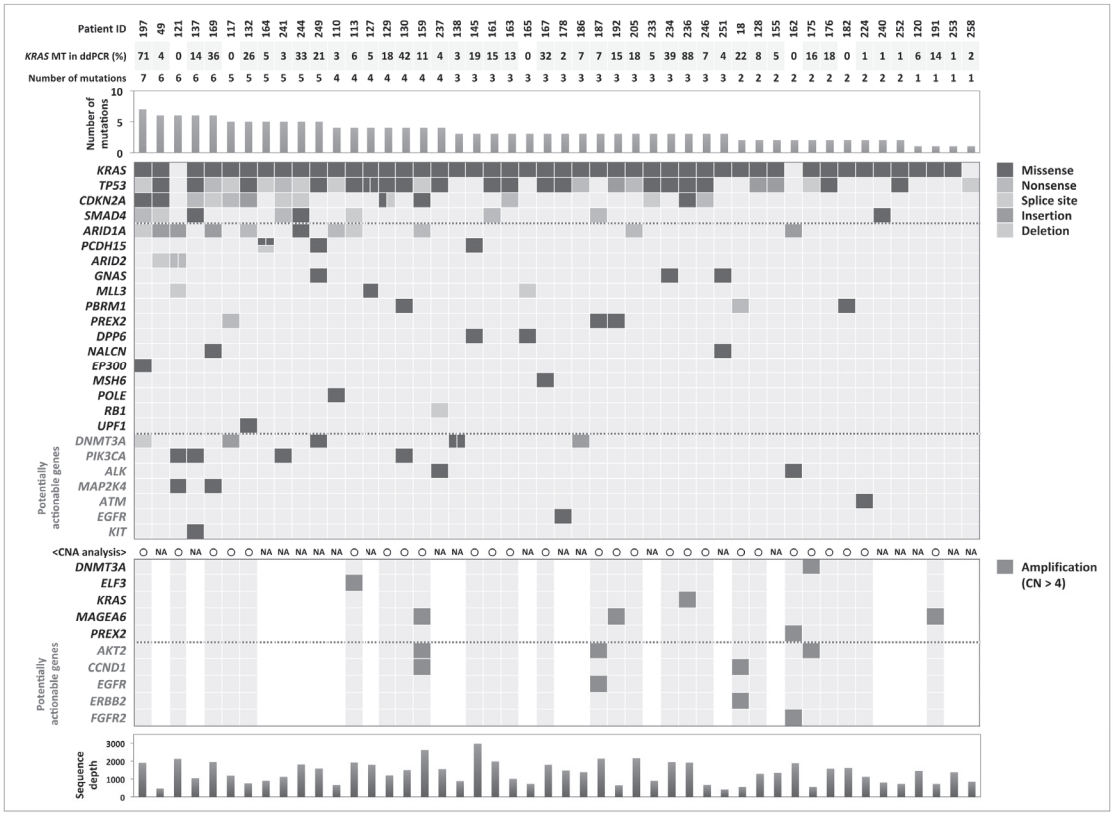

Figure 2. Somatic mutations and amplification detected by targeted sequencing of plasma cell-free DNA in 48 patients with pancreatic cancer

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Journal

1. Wild CP, Bucher JR, de Jong BWD, Dillner J, von Gertten C, Groopman JD, Herceg Z, Holmes E, Holmila R, Olsen JH, Ringborg U, Scalbert A, Shibata T, Smith MT, Ulrich C, Vineis P, McLaughlin J. Translational cancer research: balancing prevention and treatment to combat cancer globally. J Natl Cancer Inst, 107:353, 2015

2. Fujimoto A, Furuta M, Shiraishi Y, Gotoh K, Kawakami Y, Arihiro K, Nakamura T, Ueno M, Ariizumi S, Nguyen HH, Shigemizu D, Abe T, Boroevich KA, Nakano K, Sasaki A, Kitada R, Maejima K, Yamamoto Y, Tanaka H, Shibuya T, Shibata T, Ojima H, Shimada K, Hayami S, Shigekawa Y, Aikata H, Ohdan H, Marubashi S, Yamada T, Kubo M, Hirano S, Ishikawa O, Yamamoto M, Yamaue H, Chayama K, Miyano S, Tsunoda T, Nakagawa H. Whole-genome mutational landscape of liver cancers displaying biliary phenotype reveals hepatitis impact and molecular diversity. Nat Commun, 6:6120, 2015

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7. Kataoka K, Nagata Y, Kitanaka A, Shiraishi Y, Shimamura T, Yasunaga J, Totoki Y, Chiba K, Sato-Otsubo A, Nagae G, Ishii R, Muto S, Kotani S, Watatani Y, Takeda J, Sanada M, Tanaka H, Suzuki H, Sato Y, Shiozawa Y, Yoshizato T, Yoshida K, Makishima H, Iwanaga M, Ma G, Nosaka K, Hishizawa M, Itonaga H, Imaizumi Y, Munakata W, Ogasawara H, Sato T, Sasai K, Muramoto K, Penova M, Kawaguchi T, Nakamura H, Hama N, Shide K, Kubuki Y, Hidaka T, Kameda T, Nakamaki T, Ishiyama K, Miyawaki S, Yoon SS, Tobinai K, Miyazaki Y, Takaori-Kondo A, Matsuda F, Takeuchi K, Nureki O, Aburatani H, Watanabe T, Shibata T, Matsuoka M, Miyano S, Shimoda K, Ogawa S. Integrated molecular analysis of adult T cell leukemia/lymphoma. Nat Genet, 47:1304-1315, 2015

8. Shibata T. Current and future molecular profiling of cancer by next-generation sequencing. Jpn J Clin Oncol, 45:895-899, 2015

9. Mutation Consequences and Pathway Analysis working group of the International Cancer Genome Consortium. Pathway and network analysis of cancer genomes. Nat Methods, 12:615-621, 2015

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17. Nakamura H, Arai Y, Totoki Y, Shirota T, Elzawahry A, Kato M, Hama N, Hosoda F, Urushidate T, Ohashi S, Hiraoka N, Ojima H, Shimada K, Okusaka T, Kosuge T, Miyagawa S, Shibata T. Genomic spectra of biliary tract cancer. Nat Genet, 47:1003-1010, 2015

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19. Iwakawa R, Kohno T, Totoki Y, Shibata T, Tsuchihara K, Mimaki S, Tsuta K, Narita Y, Nishikawa R, Noguchi M, Harris CC, Robles AI, Yamaguchi R, Imoto S, Miyano S, Totsuka H, Yoshida T, Yokota J. Expression and clinical significance of genes frequently mutated in small cell lung cancers defined by whole exome/RNA sequencing. Carcinogenesis, 36:616-621, 2015

Division of Genome Biology

Takashi Kohno, Naoto Tsuchiya, Hideaki Ogiwara, Motonobu Saito, Kouya Shiraishi, Kuniko Sunami, Ken Ishida, Mariko Sasaki, Yoko Shimada, Yoshitaka Seki, Kazuaki Takahashi, Yuko Fujiwara, Takayuki Honda, Yoshie Iga, Ayaka Otsuka, Takashi Nakaoku, Yusuke Sugiyama, Yujin Ishihara, Mei Tanabe, Mokuri Masuda, Takashi Mitachi, Tomoaki Yoshizawa, Jun Yokota

Introduction

Somatic mutations in the cancer genome and inter-individual variations in the human genome are critical to improving cancer medicine. Our Division aims to find "seeds" that are applicable for development of novel strategies for the treatment and prevention of cancer through identifying and understanding the biological relationship of their seeds with cancer pathogenesis caused by somatic mutations and/or genetic polymorphisms of the patients. In order to attain our goal, we are working together with the National Cancer Center (NCC) staff from the hospital, evaluation system of postgraduate clinical training (EPOC) and the Research Center for Cancer Prevention and Screening to fight lung cancer, the most common cause of cancer-related death in the world.

Routine activities

A weekly research seminar and journal club are held with all the members of the division.

Research activities

1) Genes for personalized cancer medicine

Whole exome sequencing using 200 lung adenocarcinomas (LADC) demonstrated that cases with driver fusion genes showed a distinct profile with a smaller number of somatic mutations in cancer-related genes than those in the others. It was also found that genes encoding chromatin-remodeling factors are frequently mutated in the LADCs, which are negative for driver oncogene mutations, providing a novel concept that regulation of epigenetic status has an important role in lung carcinogenesis. In relation to this, a synthetic lethal screen against histone modification enzyme CBP, which are highly mutated in a certain kind of cancers, identified a novel molecular target, whose inhibition will be expected to eliminate CBP-deficient cancers. Therefore, development of compounds that specifically inhibit the function of a target molecule is now being conducted in collaboration with a pharmaceutical company. Whole RNA sequencing of 32 invasive mucinous adenocarcinomas (IMAs) identified the NRG fusion genes as a novel oncogenic fusion in IMA. It was clarified that a gene product of NER fusion is involved in the augmentation of stemness in lung cancer cells. A genome-wide association study (GWAS) led us to identify a novel LADC susceptibility locus. An international and pan-Japan collaborative GWAS study uncovered the relationship between telomere length and lung cancer risk. International collaboration is still under way to further identify genetic factors involved not only in susceptibility but also in prognosis of lung cancer.

2) Research for the development of nucleic acid drugs based on the properties of miRNA

NEK9 was previously identified by an miR-22 target screen as a regulator for cell cycle progression in p53-deficient cancer cells. Depletion of NEK9 selectively repressed the proliferation on p53 mutant cancer cells both in vivo and in vitro. Screening of molecules that are critical regulators in the NEK9 network is being conducted to establish the strategy for selective elimination of p53 deficient cancer cells. It was uncovered that tumor-suppressive miR-101 is a critical factor for precise activation of an intrinsic p53 tumor-suppressor network. Reduced-expression of miR-101 in p53 wild-type LADCs exhibited significant poor prognosis, suggesting that administration of miR-101 into cancer tissues is

a potential approach for the development of nucleic acid drug.

Clinical trials

A phase II clinical trial, which investigates the therapeutic effect of a RET-tyrosine kinase inhibitor, vandetanib, was conducted by identifying >20 RET-fusion positive lung cancers among >1,500 non-small cell lung carcinoma cases in 190 hospitals. The genetic screening was done by the LC-SCRUM-Japan (Lung Cancer Genomic Screening Project for Individualized Medicine in Japan) consortium.

Education

Supervising research and presentation skills for students and young researchers

Future prospects

Our Division aims to establish novel strategies for personalized cancer medicine, including prevention, diagnosis and therapy, through the finding of unique "seeds", which are identified by genetic and biological analyses using cancer cells and clinical samples. A nationwide clinical trial of RET-tyrosine kinase inhibitor will confirm the efficacy of RET-tyrosine kinase inhibitor for the treatment RET-fusion LADC. Furthermore, understanding biological roles of novel molecular targets, including chromatin-remodeling factors, histone modifiers and cell cycle regulators, which are identified by synthetic lethal screen and comprehensive genome analyses, provide unique and/or novel concepts for the development of cancer therapy. In addition, practical application of miRNAs as diagnostic biomarkers will be expected in the near future.

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Journal

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Division of Brain Tumor Translational Research

Koichi Ichimura, Shintaro Fukushima, Kai Yamasaki, Kohei Fukuoka, Hirokazu Takami, Taishi Nakamura, Shunichiro Miki, Yuko Matsushita, Hideyuki Arita, Yuki Yomoda, Yumiko Miyamoto, Emiko Yamamoto

Introduction

Our laboratory focuses on translational research on various types of malignant brain tumors. Extensive genomic studies in recent years dramatically improved our understanding of the molecular mechanisms of brain tumors. It is highly anticipated that the new WHO Classification for central nervous system tumors (revised 4th edition) will incorporate molecular diagnostics as a part of criteria in some tumors. Through a nationwide multi-center collaboration including the Japan Clinical Oncology Group (JCOG), the Japan Children's Cancer Group (JCCG), the Japan Pediatric Molecular Neuro-oncology Group (JPMNG) and the Intracranial Germ Cell Tumor Consortium (iGCT), we are setting up and standardizing a robust molecular classification and a diagnostic system. This will enable more accurate and objective diagnosis of brain tumors and increase the efficacy of clinical trials. We are also developing a novel molecular-targeted therapy against TERT, which is very frequently mutated in the promoter region in adult gliomas. Through our research, we aim to establish a molecular diagnostic system for adult and pediatric brain tumors in Japan and utilize it for better stratification and assessment, as well as to develop a novel targeted therapy for one of the most therapy-resistant tumors of humans.

Routine activities

In collaboration with the Department of Neurosurgery and Neuro-oncology in the National Cancer Center Hospital, we routinely perform molecular diagnosis for all gliomas operated on in the National Cancer Center. Tumor tissues are snap-frozen immediately after removal at the operation theater and the samples are subjected to DNA extraction and analysis for mutations of IDH1, IDH2, TERT promoter, H3F3A and BRAF, as well as methylation of MGMT by using pyrosequencing in a single experiment. The results are reported back to clinics within two weeks of the operation to aid treatment decisions for the patient. Co-deletion of 1p/19q, which is a hallmark of oligodendroglioma, is examined using a customized FISH protocol or MLPA.

Research activities

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

We have previously discovered that the three major classes of adult glioma have distinct molecular profiles: oligodendroglioma, a chemo-sensitive glioma with relatively long survival, is characterized by combined IDH mutations, TERT promoter mutations and 1p/19q co-deletion; astrocytoma, less chemo-sensitive with significantly shorter survival than oligodendroglioma, harbors IDH mutation but not TERT mutation nor 1p/19q co-deletion; glioblastoma, the most therapy-resistant tumors with the poorest prognosis, often have TERT promoter mutations but seldom IDH mutations or 1p/19q co-deletion. We have performed a multicenter study to develop a novel molecular classification system utilizing the statuses of these molecular markers. We have studied more than 1,000 adult gliomas collected from 18 centers and showed that the combination of these markers defines 4 molecular subgroups with significantly different overall survival. In order to meet the requirement in the revised WHO Classification for central nervous system tumors, in which molecular tests will be mandatory to make diagnosis of astrocytomas and oligodendrogliomas, we are currently setting up robust and practicable molecular tests for clinical use in collaboration with industry.

2) Development of a novel targeted therapy for glioblastoma

A novel targeted therapy against TERT is being developed for glioblastoma in collaboration with the Division of Cancer Stem Cell at the National Cancer Center Research Institute. We have performed a series of pre-clinical experiments to investigate the efficacy of anti-TERT therapy. The results showed that glioblastoma cell lines that have TERT mutations are highly sensitive to the TERT inhibitor, and the survival of mice transplanted with TERT-mutated glioblastoma cell lines in the brain was significantly prolonged.

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 (iGCT Consortium), a nationwide collaborative network to study germ cell tumors, through which tumor samples of more than 240 cases from 22 centers have been collected. We have previously performed a whole exome and targeted sequencing in 197 germ cell tumors of CNS or testicular origin, the results of which showed a high prevalence of mutations affecting the MAPK and/or PI3K pathway. We have now analyzed the genome-wide DNA methylation status as well as transcriptome and are investigating the potential cell of origin and mechanism of GCT development.

4) Molecular diagnosis of pediatric brain tumors

In order to build a central molecular diagnosis service for pediatric brain tumors nationwide, we have established JPMNG. More than 100 ependymomas collected through JPMNG have so far been analyzed and molecularly diagnosed using an Illumina HumanMethylation 450 BeadChip and a custom FISH protocol. Based on these results, we are now in collaboration with the German Cancer Research Center (DKFZ) as a part of an international effort to build up a consensus on molecular classification of ependymomas. A practical molecular diagnostic scheme for pediatric gliomas is also being set up. We also act as one of the central molecular diagnostic laboratories to perform molecular testing and classification for clinical trials and other clinical research conducted under JCCG.

Clinical trials

We continue to offer an 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 only be given TMZ chemotherapy while those with unmethylated MGMT will only receive radiation therapy. In this trial, tumor specimens are sent to our laboratory from the participating centers immediately after the operation. For them, we perform an MGMT methylation test using our custom-designed pyrosequencing assay. Up to the end of 2015, 68 patients were tested for MGMT methylation. Registration continues in 2016. We are also preparing a clinical trial to test the efficacy of the TERT-targeted drug for recurrent glioblastomas, for which full support from the Center for Research Administration and Support at the National Cancer Center for the clinical trial has been approved.

Education

Three postgraduate students, three Research Residents, one Clinical Resident did research work during 2015 at the Division of Brain Tumor Translational Research.

Future prospects

As one of the leading translational research centers on malignant brain tumors in Japan, we continue to organize nationwide collaboration and perform research. We offer a central molecular diagnostic service in a number of clinical trials and clinical research. We develop novel targeted therapies, rigorously validated in pre-clinical settings and plan clinical trials. We also support young dedicated clinician investigators and help them with their PhD projects. Our goal is to be able to offer better patient care and treatment for brain tumor sufferers and help develop world-class neuro-oncology research in Japan.

List of papers published in 2015

Journal

1. Arita H, Narita Y, Matsushita Y, Fukushima S, Yoshida A, Takami H, Miyakita Y, Ohno M, Shibui S, Ichimura K. Development of a robust and sensitive pyrosequencing assay for the detection of *IDH1/2* mutations in gliomas. Brain Tumor Pathol, 32:22-30, 2015

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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 Sasaki, Hiroko Ito, Junhui Wang, Haruyo Tozaki, Yuko Miyamoto, Naoko Goto, Takako Sakamoto, Keiko Takeuchi, Nobuhiko Nishijima, Takanori Kakuya, Makoto Kobayashi, Hirokazu Shoji, Teppei Sugano, Takahisa Hirai, Tasuku Itoh, Miyuki Hozumi, Sota Kikuhara, Kumiko Kinoshita, Noriko Shibata, Akira Sato, Gui Zhen Chen

Introduction

The Division has been devoted to the clinical application/translation of basic research findings obtained through the comprehensive genomics and proteomics approaches.

Therapeutic targets in the Wnt signaling pathway: feasibility of targeting TNIK in colorectal cancer

Wnt signaling is a major force driving colorectal carcinogenesis, but no molecular targeting therapy has yet been established. In the majority of colorectal cancers, the β-catenin destruction complex is not properly formed due to premature termination of the APC (adenomatous polyposis coli) protein (Figure 1), and only the molecules downstream of APC can be considered as targets for Wnt signal blockage. TRAF2 and NCK-interacting protein kinase (TNIK) is a regulator of the β-catenin and TCF4 (T-cell factor-4) transcription complex, the most downstream component of Wnt signaling. TNIK is essential for the full activation of Wnt signal, and colorectal cancer cells are highly dependent on TNIK expression. We have reported some of our research data as evidence for the legitimacy of targeting TNIK in our recent review articles.

Plasma biomarker for detection of early stage pancreatic cancer and risk factors for pancreatic malignancy using antibodies for apolipoprotein-AII isoforms

We previously reported that circulating apolipoprotein AII (apoAII) isoforms apoAII-ATQ/AT (C-terminal truncations of the apoAII homo-dimer) decline significantly in pancreatic cancer and thus might serve as plasma biomarkers for the early detection of this disease. We report here the development of novel enzyme-linked immunosorbent assays (ELISAs) for measurement of apoAII-ATQ/AT and their clinical applicability for early detection of pancreatic cancer.

Plasma and serum concentrations of apoAII-ATQ/AT were measured in three independent cohorts, which comprised healthy control subjects and patients with pancreatic cancer and gastroenterologic diseases (n = 1,156, two Japanese cohorts and one US cohort). These cohorts included 151 cases of stage I/II pancreatic cancer. Significant reductions in apoA2-ATQ/AT levels were recognized in patients with pancreatic cancer in comparison with healthy controls in both independent Japanese cohorts ($P = 1.34 \times 10^{-18}$ and 5.09×10^{-39}). Areas under the receiver operating characteristic curve (AUCs) were > 0.92 for distinguishing patients with stage-I/II pancreatic cancer from healthy controls in the Japanese cohorts. The AUCs of apoA2-ATQ/AT to distinguish patients with pancreatic cancer from healthy controls were higher than those of CA19-9 in both Japanese cohorts. Better discrimination of pancreatic cancer was also observed with apoA2-ATQ/AT than with CA19-9 in the blind test using the pancreatic cancer reference set of NCI EDRN (US National Cancer Institute's Early Detection Research Network) data; combining apoA2-ATQ/AT with CA19-9 led to significantly improved AUC compared to CA19-9 alone. ApoAII-ATQ/AT is a potential biomarker for screening patients for the early stage of pancreatic cancer and identifying patients at risk for pancreatic malignancy.

ACTN4 copy number as a predictive biomarker for chemoradiotherapy of locally advanced pancreatic cancer

The copy number increase (CNI) of *ACTN4* is well known to be a good prognostic biomarker

for some cancers. We evaluated the copy number of *ACTN4* in 91 biopsy specimens of LAPC before treatment using fluorescence in situ hybridisation (FISH) to determine if it could be used as a predictive biomarker for selection of the therapeutic strategy of LAPC. 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 CNI of *ACTN4* had a worse prognosis of OS than patients 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 an NCN of *ACTN4*. We concluded that the copy number of *ACTN4* is a predictive biomarker for the decision of a personalized therapeutic strategy for LAPC.

The alternatively spliced actinin-4 variant as a prognostic marker for metastasis in small-cell lung cancer

The alternatively spliced actinin-4 variant (ACTN4va) is expressed in small-cell lung cancer (SCLC) and is thought to be a potential diagnostic marker. However, ACTN4va expression has not been examined in transbronchial biopsy specimens. We retrospectively examined the relationship between ACTN4va expression, clinical factors and survival in 104 consecutive newly diagnosed SCLC patients. Of the 104 screened cases, 83 (median age = 69 years; transbronchial biopsy, 71) were included in our study. Survival was significantly different in the group with no distant metastasis (1996 vs. 422 days, respectively; $P = 0.000115$) but was not significantly different with regard to ACTN4va expression in the group with distant metastasis (293 vs. 254 days, respectively; $P = 0.678$). ACTN4va expression was identifiable in small biopsy samples. ACTN4va expression was also significantly related to distant metastasis and could stratify SCLC patients according to prognosis.

Proteomic analysis of ligamentum flavum from patients with lumbar spinal stenosis

We report a first proteomic analysis of ligamentum flavum from patients with lumbar spinal stenosis. We analyzed 73 ligamentum flavum tissues from patients with lumbar spinal stenosis and lumbar disc herniation, and detected 316 peptides differentially expressed between them using our originally developed proteomic analyzing system 2DICAL (2-dimensional image converted analysis of LC/MS). From the differentially expressed peptides, we found several proteins important for the pathogenesis of ligamentum flavum and confirmed the expression difference by SRM (selected reaction monitoring)/MRM (Multiple Reaction Monitoring) and immunohistochemical study.

ATM and SIRT6/SNF2H Mediate Transient H2AX Stabilization When DSBs Form by Blocking HUWE1 to Allow Efficient gammaH2AX Foci Formation

H2AX was rapidly induced in response to DNA double-strand breaks (DSBs), thereby enabling γ H2AX foci formation and DSB repair. Such rapid H2AX induction resulted from continuous protein production. Synthesized H2AX was ordinarily degraded through the proteasome pathway mediated by the E3 ubiquitin ligase HUWE1. However, this process was transiently halted in response to DSBs, which was mediated by ATM and involved the dissociation of HUWE1 from poly-ubiquitinated H2AX. The serine-139 residue of H2AX was required for such transient H2AX expression and γ H2AX foci formation. Intriguingly, such H2AX expression was required for proper DSB repair even in H2AX-expressing cancer cells, as well as in H2AX-diminished quiescent cells. We contributed the paper for finding HUWE1 by 2DICAL.

Boron-captured neutron therapy

The development of accelerator-based BNCT (boron neutron-capture therapy) system is an ongoing collaborative project of the NCC (National Cancer Center) and industries. For biological evaluation of the safety and effectiveness of this BNCT system, the experimental systems are being set up using mouse models. Melanoma cell lines were evaluated as grafted tumor models in nude mice and further optimized for validation of BNCT effectiveness. The ICP-AES (Inductively

Coupled Plasma Atomic Emission Spectroscopy) procedure to measure the dynamic changes of ^{10}B-para-boronophenylalamine (BPA) in the blood and tumor tissues has been optimized. Biology of boron neutron-capture reaction (BNCR) has been also studied as a collaborative experimental research project of Kyoto University Reactor (KUR). Using SAS (human squamous cell carcinomas derived) cell line, cellular responses, DNA damage induction including γ H2AX foci induction and apoptosis were characterized after BNCR using comprehensive molecular approaches.

A comprehensive analysis of radiosensitization targets

A comprehensive genome-wide screening of targets using lenti virus shRNA library for radiosensitization was conducted with a functional cluster analysis. From the validated targets, we have focused on the DNA methyltransferase 3B (*DNMT3B*) gene, because expression of this gene often showed aberrant overexpression in various types of cancers. The radiosensitization by *DNMT3B* RNAi was caused through impairment of ionizing radiation (IR)-induced HP1 β foci formation, defective γ H2AX signaling and consequent attenuated DNA damage responses after IR. DNMT3B was found to interact with HP1 β in an untreated condition; however, after IR, DNMT3B no longer interacts with HP1 β and is associated with H2AX, suggesting that DNMT3B/H2AX interaction is required for an efficient H2AX accumulation after IR. This study suggests that comprehensive screening with cluster analysis is useful to identify the radiosensitization targets.

The studies on PARP and PARG inhibitors as anti-cancer drug

Poly(ADP-ribose) polymerase (PARP) is frequently upregulated in cancers and is involved in DNA repair. The action mechanism of PARP inhibitors, now approved as an anti-cancer drug, has been further investigated. Dysfunction of poly(ADP-ribose) glycohydrolase (PARG), a main enzyme for poly(ADP-ribose) degradation, suppresses DNA repair and poly(ADP-ribose) accumulation within the cells induces cell death. Development of PARG inhibitors as a potential anti-cancer target has been conducted as a collaboration study. Through comprehensive screening approaches, the genes that affect lethality under PARG functional inhibition have been identified and the mechanism of the synthetic lethality has been investigated.

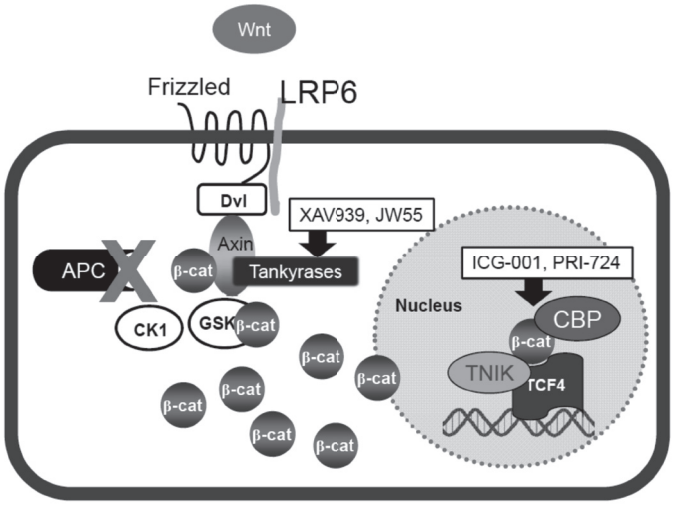

Figure 1. Pharmacological blocking of Wnt signaling has been considered an attractive therapeutic approach for colorectal cancer. TRAF2 and NCK-interacting protein kinase (TNIK) has been identified as a regulatory component of the T-cell factor-4 (TCF4) and β-catenin (β-cat) transcriptional complex. TNIK regulates Wnt signaling in the most downstream part of the pathway, and its inhibition is expected to block the signal even in colorectal cancer cells with *APC* gene mutation.

List of papers published in 2015

Journal

1. Okamoto N, Suzuki H, Kawahara K, Honda K, Miura N, Hirashima T, Tamiya M, Morishita N, Shiroyama T, Tanaka A, Tani E, Hamaguchi M, Kitani M, Yamada T, Kawase I. The alternatively spliced actinin-4 variant as a prognostic marker for metastasis in small-cell lung cancer. Anticancer Res, 35:1663-1667, 2015

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4. Arai E, Gotoh M, Tian Y, Sakamoto H, Ono M, Matsuda A, Takahashi Y, Miyata S, Totsuka H, Chiku S, Komiyama M, Fujimoto H, Matsumoto K, Yamada T, Yoshida T, Kanai Y. Alterations of the spindle checkpoint pathway in clinicopathologically aggressive CpG island methylator phenotype clear cell renal cell carcinomas. Int J Cancer, 137:2589-2606, 2015

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9. Fujimori H, Sato A, Kikuhara S, Wang J, Hirai T, Sasaki Y, Murakami Y, Okayasu R, Masutani M. A comprehensive analysis of radiosensitization targets; functional inhibition of DNA methyltransferase 3B radiosensitizes by disrupting DNA damage regulation. Sci Rep, 5:18231, 2015

10. Kishi Y, Fujihara H, Kawaguchi K, Yamada H, Nakayama R, Yamamoto N, Fujihara Y, Hamada Y, Satomura K, Masutani M. PARP Inhibitor PJ34 Suppresses Osteogenic Differentiation in Mouse Mesenchymal Stem Cells by Modulating BMP-2 Signaling Pathway. Int J Mol Sci, 16:24820-24838, 2015

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12. Kamita M, Mori T, Sakai Y, Ito S, Gomi M, Miyamoto Y, Harada A, Niida S, Yamada T, Watanabe K, Ono M. Proteomic analysis of ligamentum flavum from patients with lumbar spinal stenosis. Proteomics, 15:1622-1630, 2015

13. Watanabe T, Ueno H, Watabe Y, Hiraoka N, Morizane C, Itami J, Okusaka T, Miura N, Kakizaki T, Kakuya T, Kamita M, Tsuchida A, Nagakawa Y, Wilber H, Yamada T, Honda K. ACTN4 copy number increase as a predictive biomarker for chemoradiotherapy of locally advanced pancreatic cancer. Br J Cancer, 112:704-713, 2015

14. Masuda M, Yamada T. Signaling pathway profiling by reverse-phase protein array for personalized cancer medicine. Biochim Biophys Acta, 1854:651-657, 2015

15. Shiba S, Morizane C, Hiraoka N, Sasaki M, Koga F, Sakamoto Y, Kondo S, Ueno H, Ikeda M, Yamada T, Shimada K, Kosuge T, Okusaka T. Pancreatic neuroendocrine tumors: A single-center 20-year experience with 100 patients. Pancreatology, 16:99-105, 2015

Book

1. Wang J, Sato A, Fujimori H, Miki Y, Masutani M. PARP and carcinogenesis. In: Curtin N, Sharma R (eds), PARP inhibitors for cancer therapy, Switzerland, Humana Press, pp 99-124, 2015

Division of Cancer Pathophysiology

Yasuhito Uezono, Kanako Miyano, Seiji Shiraishi, Sadamoto Zenda, Junko Ezuka, Yukiko Araki, Kiyoshi Terawaki, Katsuya Ohbuchi, Chika Miyagi, Koichiro Minami, Tohru Yokoyama, Satoshi Murakami, Hideya Kokubun, Yoshiyuki Meguro, Akinobu Yokoyama, Hitomi Nishimura, Moeko Eto, Megumi Kawaida, Shiori Sato, Etsuko Nemoto, Hoko Ohguri, Tomoko Matsude, Takamichi Arima, Hirotsugu Kuwata, Yusuke Hamada, Tomoyuki Takahashi, Mio Sekiguchi, Airi Mizukami

Introduction

Since its establishment in January 2009, the Division of Cancer Pathophysiology has focused on two major research issues regarding 1) the improvement of the quality of life (QOL) of patients with cancer suffering from severe or intolerable pain, and 2) the 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. Since 2015, the Chief of this Division holds both the posts in Exploratory Oncology Research and Clinical Trial Center (for phase I clinical study) and Innovation Center for Supportive, Palliative and Psychosocial Care (for phase II and III clinical studies), to accelerate the development of novel drugs for cancer patients.

Routine activities

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

Research activities

1) 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 QOL 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. On the clinical side, the local anesthetic lidocaine is normally used for the relief of pain in cancer patients with stomatitis. However, lidocaine removes not only pain but also the ability to discriminate taste and texture, since it non-selectively 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. Since last year, we have been focusing on a "compound X" as a novel analgesic drug for stomatitis, and evaluated the intensity of oral pain using newly established stomatitis model animals. With the model, as expected, 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 without effects on normal tissues. Further, the analgesic effect of the compound X persisted longer than that of lidocaine. Based on our basic research results, we have been developing "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, by intellectually and financially supporting the Project Promoting Support for Drug Discovery, Japan Agency for Medical Research and Development.

The second target is severe pain such as one with bone-metastasized patients. We showed that a platelet-activating factor (PAF) receptor antagonist produced profound and long lasting anti-allodynia effects in several different neuropathic pain models in mice. We have demonstrated that

the PAF antagonist showed extremely excellent analgesic effects on both the bone-metastasized cancer pain model and also the chemotherapy-induced peripheral neuropathy model. Further, we discovered that knocking out of inducible PAF synthase type 2 inhibited pain with siRNA technology in mouse models and also the type 2 PAF synthase knockout mice model. These results demonstrated that PAF seems to produce and maintain persistent pain. We now are collaborating with the members of the Department of Lipid Signaling, National Center for Global Health and Medicine to find novel PAF receptor and PAF synthase antagonists.

2) Prevention and decrease of the cachexic symptoms or chemotherapy-induced side effects and also prolonging survival in a mice model of human aging by Japanese traditional KAMPO medicines

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 QOL of patients suffering from cancer cachexia. We found that a Japanese Kampo (traditional Oriental) medicine "rikkunshito" usually administered for the prevention of gastritis, nausea and vomiting, improved the symptoms of cancer cachexia. In addition to rikkunshito, we analyzed and summarized the action mechanisms of other traditional Japanese Kampo medicines to improve chemotherapy-induced side effects such as pain and allodynia.

In addition, we demonstrated that rikkunshito prolonged survival in mouse models of human aging by activation of ghrelin signaling, suggesting that potentiation of this signal with rikkunshito may be useful to extend health and lifespan.

Education

We have three graduate students and 11 students.

Future prospects

The goal of the Division of Cancer Pathophysiology is to improve the QOL of cancer patients.

List of papers published in 2015

Journal

1. Okura D, Horishita T, Ueno S, Yanagihara N, Sudo Y, Uezono Y, Minami T, Kawasaki T, Sata T. Lidocaine preferentially inhibits the function of purinergic P2X7 receptors expressed in *Xenopus* oocytes. Anesth Analg, 120:597-605, 2015

2. Miyano K, Minami K, Yokoyama T, Ohbuchi K, Yamaguchi T, Murakami S, Shiraishi S, Yamamoto M, Matoba M, Uezono Y. Tramadol and its metabolite M1 selectively suppress transient receptor potential ankyrin 1 activity, but not transient receptor potential vanilloid 1 activity. Anesth Analg, 120:790-798, 2015

3. Kubota K, Ohtake N, Ohbuchi K, Mase A, Imamura S, Sudo Y, Miyano K, Yamamoto M, Kono T, Uezono Y. Hydroxy-α sanshool induces colonic motor activity in rat proximal colon: a possible involvement of KCNK9. Am J Physiol Gastrointest Liver Physiol, 308:G579-G590, 2015

4. Suzuki M, Chiwaki F, Sawada Y, Ashikawa M, Aoyagi K, Fujita T, Yanagihara K, Komatsu M, Narita M, Suzuki T, Nagase H, Kushima R, Sakamoto H, Fukagawa T, Katai H, Nakagama H, Yoshida T, Uezono Y, Sasaki H. Peripheral opioid antagonist enhances the effect of anti-tumor drug by blocking a cell growth-suppressive pathway *in vivo*. PLoS One, 10:e0123407, 2015

5. Hitomi S, Ono K, Miyano K, Ota Y, Uezono Y, Matoba M, Kuramitsu S, Yamaguchi K, Matsuo K, Seta Y, Harano N, Inenaga K. Novel methods of applying direct chemical and mechanical stimulation to the oral mucosa for traditional behavioral pain assays in conscious rats. J Neurosci Methods, 239:162-169, 2015

6. Hisaoka-Nakashima K, Miyano K, Matsumoto C, Kajitani N, Abe H, Okada-Tsuchioka M, Yokoyama A, Uezono Y, Morioka N, Nakata Y, Takebayashi M. Tricyclic antidepressant amitriptyline-induced glial cell line-derived neurotrophic factor production involves pertussis toxin-sensitive $G\alpha_{i/o}$ activation in astroglial cells. J Biol Chem, 290:13678-13691, 2015

7. Minami K, Sudo Y, Miyano K, Murphy RS, Uezono Y. μ-Opioid receptor activation by tramadol and O-desmethyltramadol (M1).

J Anesth, 29:475-479, 2015

8. Kitagawa H, Munekage M, Matsumoto T, Sadakane C, Fukutake M, Aoki K, Watanabe J, Maemura K, Hattori T, Kase Y, Uezono Y, Inui A, Hanazaki K. Pharmacokinetic profiles of active ingredients and its metabolites derived from rikkunshito, a ghrelin enhancer, in healthy Japanese volunteers: a crossover, randomized study. PLoS One, 10:e0133159, 2015

9. Yoshimura M, Uezono Y, Ueta Y. Anorexia in human and experimental animal models: physiological aspects related to neuropeptides. J Physiol Sci, 65:385-395, 2015

10. Kono T, Shimada M, Yamamoto M, Kaneko A, Oomiya Y, Kubota K, Kase Y, Lee K, Uezono Y. Complementary and synergistic therapeutic effects of compounds found in Kampo medicine: analysis of daikenchuto. Front Pharmacol, 6:159, 2015

11. Minami K, Ogata J, Uezono Y. What is the main mechanism of tramadol? Naunyn Schmiedebergs Arch Pharmacol, 388:999-1007, 2015

12. Kono T, Suzuki Y, Mizuno K, Miyagi C, Omiya Y, Sekine H, Mizuhara Y, Miyano K, Kase Y, Uezono Y. Preventive effect of oral goshajinkigan on chronic oxaliplatin-induced hypoesthesia in rats. Sci Rep, 5:16078, 2015

Division of Molecular and Cellular Medicine (Ochiya Group)

Takahiro Ochiya, Yusuke Yamamoto, Ryou-u Takahashi, Takeshi Katsuda, Yusuke Yoshioka, Ayako Inoue, Kana Kurosaki, Mayuko Yamamura, Luc Nicolas Gailhouste, Ai Hironaka, Satomi Fukuda, Takumi Sonoda, Hiroko Tadokoro, Juntaro Matsuzaki, Akira Yokoi, Yutaka Nezu, Mizuyo Arashi, Naomi Nomura, Teruko Yamaguchi, Kazumi Nagao, Satoko Takizawa, Yutaka Naito, Maki Abe, Kurataka Otsuka, Nao Nishida, Tsukasa Kadota, Makiko Ichikawa, Naoomi Tominaga, Liew Lee Chuen, Hayato Kurata, Yumi Kawamura

Introduction

The focus of the Division of Molecular and Cellular Medicine lies in the development of novel treatments and diagnosis against cancer. The specific activities are as follows: 1) studies on microRNA (miRNA) regulation in cancer cells and development of RNA interference (RNAi)-based therapeutics; 2) an exosome as a novel diagnosis and therapeutic tool against cancer; 3) the study of stem cells and their therapeutic applications.

Research activities

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

RNAi-based therapeutics is a promising approach as a novel and potentially more effective treatment for cancer, and miRNA is one of the targets involved in the regulation of tumor-related genes（Urata, Sci Rep, Osaki Ther Deliv）.

By screening with the natural substance library, three compounds were identified as inducers of miR-200c in breast cancer cells (Hagiwara, Sci Rep).

The up-regulation of miR-200c suppressed the invasiveness of cancer cells mediated by ZEB1 inhibition and E-cadherin induction. We identified an miR-197/CKS1B/STAT3-mediated PD-L1 network in chemoresistant NSCLC. miR-197 is downregulated in platinum-resistant NSCLC specimens, resulting in the promotion of chemoresistance, tumorigenicity, and pulmonary metastasis in vitro and in vivo (Fujita, Mol Ther).

We previously demonstrated that silencing of RPN2 efficiently reduced resistance to docetaxel in human breast cancer cells. Recently, we also reported the clinical and functional correlations of RPN2 expression in lung cancer (Fujita, Int J Mol Sci, Fujita, Oncotarget). Higher RPN2 expression was significantly correlated with poor prognosis (Ono, Pathol Int). In July 2015, an investigator-initiated clinical trial (first-in-human phase I study) with intratumoral administration of RPN2-siRNA for treatment-resistant breast cancer was started at the National Cancer Center (NCC) Hospital.

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

The circulating exosomes could be found in a variety of body fluids including serum, plasma, urine, saliva, and breast milk (Nishida-Aoki, CMLS). The existence of circulating exosomes in the blood of cancer patients has raised the possibility that exosomes may serve as a novel diagnostic marker.

To more precisely understand the functions of circulating miRNAs and extracellular vesicles in cancer biology, we developed a mouse model for brain metastasis using breast cancer cells and identified cancer-derived extracellular vesicles containing mir-181c, which trigger the breakdown of the blood-brain barrier (BBB). Importantly, miR-181c promotes the destruction of BBB through the abnormal localization of actin via the downregulation of its target gene, PDPK1, whose degradation leads to the downregulation of phosphorylated cofilin and the resultant activated cofilin-induced modulation of actin dynamics (Tominaga, Nat. Commun.).

Also, we have conducted a proteomics approach to reveal a molecular mechanism in which miRNA is transferred into extracelluar vesicles and identified Annexin A2 as a key player in this approach (Hagiwara, FEBS lett.).

We showed that suppression of autophagy by extracellular vesicles promotes myofibroblast

differentiation in chronic obstructive pulmonary disease (COPD) pathogenesis (Fujita, J Extracell Vesicles). These findings prompted us to consider the application of exosomes in diagnosis and therapy against cancer development (Tominaga, Adv Drug Deliv Rev; Fujita, Trends Mol Med; Lener, J Extracell Vesicles).

3) Molecular analysis of cancer stem cells governing breast cancer generation and related miRNAs

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. MicroRNA (miRNA) is identified as the targets involved in the regulation of CSC properties (Osaki, Ther Deliv; Takahashi Cancers (Basel)). We identified microRNA-27b (miR-27b) as a key regulator for the generation of a side-population in breast cancer cells that showed CSC properties, and also found that the anti-type II diabetes (T2D) drug metformin reduced this side-population via miR-27b-mediated repression of ENPP1, which is involved in T2D development (Takahashi, Nat. Commun.). We found that some specific miRNAs played an important role in the acquisition of CSC properties. Therefore, these results suggest that conventional cancer therapy with modulation of the expression of miRNA may eradicate CSC fraction and improve the treatment of cancer patients.

4) Chemical reprogramming of adult hepatocytes modelling for hepatocellular carcinomas

We are interested in modelling hepatocellular carcinomas utilizing chemically-induced liver progenitor cells. Recently, we have developed a highly efficient strategy for small molecular cocktail-enabled mature hepatocytes to reprogram bi-potential liver progenitor cells. Also, we have established an orthotopic transplantation method for reprogrammed liver progenitor cells that showed remarkably high repopulation rates. Combining these techniques, our main focus is on the elucidation of the multi-step carcinogenesis process of normal liver cells to hepatocellular carcinomas.

List of papers published in 2015

Journal

1. Hagiwara K, Katsuda T, Gailhouste L, Kosaka N, Ochiya T. Commitment of Annexin A2 in recruitment of microRNAs into extracellular vesicles. FEBS Lett, 589:4071-4078, 2015

2. Naito Y, Tanaka Y, Ochiya T. microRNAs and Hepatitis B. Adv Exp Med Biol, 888:389-399, 2015

3. Lener T, Gimona M, Aigner L, Börger V, Buzas E, Camussi G, Chaput N, Chatterjee D, Court FA, Del Portillo HA, O'Driscoll L, Fais S, Falcon-Perez JM, Felderhoff-Mueser U, Fraile L, Gho YS, Görgens A, Gupta RC, Hendrix A, Hermann DM, Hill AF, Hochberg F, Horn PA, de Kleijn D, Kordelas L, Kramer BW, Krämer-Albers EM, Laner-Plamberger S, Laitinen S, Leonardi T, Lorenowicz MJ, Lim SK, Lötvall J, Maguire CA, Marcilla A, Nazarenko I, Ochiya T, Patel T, Pedersen S, Pocsfalvi G, Pluchino S, Quesenberry P, Reischl IG, Rivera FJ, Sanzenbacher R, Schallmoser K, Slaper-Cortenbach I, Strunk D, Tonn T, Vader P, van Balkom BW, Wauben M, Andaloussi SE, Théry C, Rohde E, Giebel B. Applying extracellular vesicles based therapeutics in clinical trials - an ISEV position paper. J Extracell Vesicles, 4:30087, 2015

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5. Fujita Y, Kuwano K, Ochiya T. Development of small RNA delivery systems for lung cancer therapy. Int J Mol Sci, 16:5254-5270, 2015

6. Fujita Y, Yagishita S, Takeshita F, Yamamoto Y, Kuwano K, Ochiya T. Prognostic and therapeutic impact of RPN2-mediated tumor malignancy in non-small-cell lung cancer. Oncotarget, 6:3335-3345, 2015

7. Nishida-Aoki N, Ochiya T. Interactions between cancer cells and normal cells via miRNAs in extracellular vesicles. Cell Mol Life Sci, 72:1849-1861, 2015

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9. Tominaga N, Kosaka N, Ono M, Katsuda T, Yoshioka Y, Tamura K, Lötvall J, Nakagama H, Ochiya T. Brain metastatic cancer cells release microRNA-181c-containing extracellular vesicles capable of destructing blood-brain barrier. Nat Commun, 6:6716, 2015

10. Takahashi RU, Miyazaki H, Ochiya T. The roles of microRNAs in breast cancer. Cancers (Basel), 7:598-616, 2015

11. Sugimachi K, Matsumura T, Hirata H, Uchi R, Ueda M, Ueo H, Shinden Y, Iguchi T, Eguchi H, Shirabe K, Ochiya T, Maehara Y, Mimori K. Identification of a bona fide microRNA biomarker in serum exosomes that predicts hepatocellular carcinoma recurrence after liver transplantation. Br J Cancer, 112:532-538, 2015

12. Fujita Y, Yagishita S, Hagiwara K, Yoshioka Y, Kosaka N, Takeshita F, Fujiwara T, Tsuta K, Nokihara H, Tamura T, Asamura H, Kawaishi M, Kuwano K, Ochiya T. The clinical relevance of the miR-197/CKS1B/STAT3-mediated PD-L1 network in chemoresistant non-small-cell lung cancer. Mol Ther, 23:717-727, 2015

13. Osaki M, Okada F, Ochiya T. miRNA therapy targeting cancer stem cells: a new paradigm for cancer treatment and prevention of tumor recurrence. Ther Deliv, 6:323-337, 2015

14. Akagi T, Kato K, Kobayashi M, Kosaka N, Ochiya T, Ichiki T. On-chip immunoelectrophoresis of extracellular vesicles released from human breast cancer cells. PLoS One, 10:e0123603, 2015

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16. Fujita T, Chiwaki F, Takahashi RU, Aoyagi K, Yanagihara K, Nishimura T, Tamaoki M, Komatsu M, Komatsuzaki R, Matsusaki K, Ichikawa H, Sakamoto H, Yamada Y, Fukagawa T, Katai H, Konno H, Ochiya T, Yoshida T, Sasaki H. Identification and Characterization of CXCR4-Positive Gastric Cancer Stem Cells. PLoS One, 10:e0130808, 2015

17. Matsumura T, Sugimachi K, Iinuma H, Takahashi Y, Kurashige J, Sawada G, Ueda M, Uchi R, Ueo H, Takano Y, Shinden Y, Eguchi H, Yamamoto H, Doki Y, Mori M, Ochiya T, Mimori K. Exosomal microRNA in serum is a novel biomarker of recurrence in human colorectal cancer. Br J Cancer, 113:275-281, 2015

18. Ochiya T, Takenaga K, Asagiri M, Nakano K, Satoh H, Watanabe T, Imajoh-Ohmi S, Endo H. Efficient inhibition of tumor angiogenesis and growth by a synthetic peptide blocking S100A4-methionine aminopeptidase 2 interaction. Mol Ther Methods Clin Dev, 2:15008, 2015

19. Ono M, Tsuda H, Kobayashi T, Takeshita F, Takahashi RU, Tamura K, Akashi-Tanaka S, Moriya T, Yamasaki T, Kinoshita T, Yamamoto J, Fujiwara Y, Ochiya T. The expression and clinical significance of ribophorin II (RPN2) in human breast cancer. Pathol Int, 65:301-308, 2015

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23. Izumi H, Tsuda M, Sato Y, Kosaka N, Ochiya T, Iwamoto H, Namba K, Takeda Y. Bovine milk exosomes contain microRNA and mRNA and are taken up by human macrophages. J Dairy Sci, 98:2920-2933, 2015

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25. Kawaharada K, Kawamata M, Ochiya T. Rat embryonic stem cells create new era in development of genetically manipulated rat models. World J Stem Cells, 7:1054-1063, 2015

26. Urata YN, Takeshita F, Tanaka H, Ochiya T, Takimoto M. Targeted knockdown of the kinetochore protein D40/Knl-1 inhibits human cancer in a p53 status-independent manner. Sci Rep, 5:13676, 2015

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28. Hagiwara K, Gailhouste L, Yasukawa K, Kosaka N, Ochiya T. A robust screening method for dietary agents that activate tumour-suppressor microRNAs. Sci Rep, 5:14697, 2015

29. Murakami Y, Kubo S, Tamori A, Itami S, Kawamura E, Iwaisako K, Ikeda K, Kawada N, Ochiya T, Taguchi YH. Comprehensive analysis of transcriptome and metabolome analysis in Intrahepatic Cholangiocarcinoma and Hepatocellular Carcinoma. Sci Rep, 5:16294, 2015

30. Yokoi A, Yoshioka Y, Ochiya T. Towards the realization of clinical extracellular vesicle diagnostics: challenges and opportunities. Expert Rev Mol Diagn, 15:1555-1566, 2015

31. Tominaga N, Yoshioka Y, Ochiya T. A novel platform for cancer therapy using extracellular vesicles. Adv Drug Deliv Rev, 95:50-55, 2015

32. Fujita Y, Araya J, Ito S, Kobayashi K, Kosaka N, Yoshioka Y, Kadota T, Hara H, Kuwano K, Ochiya T. Suppression of autophagy by extracellular vesicles promotes myofibroblast differentiation in COPD pathogenesis. J Extracell Vesicles, 4:28388, 2015

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Book

1. Yoshioka Y, Katsuda T, Ochiya T. Circulating microRNAs as hormones: intercellular and inter-organ conveyors of epigenetic information? In: Igaz P (ed), Circulating microRNAs in disease diagnostics and their potential biological relevance, Switzerland, Springer Basel, pp 255-267, 2015

2. Fujiwara T, Fujita Y, Nezu Y, Kawai A, Ozaki T, Ochiya T. MicroRNAs in boen and soft tissue sarcoma and their values as biomarkers. In: García-Giménez JL (ed), Epigenetic Biomarkers and Diagnostics, 1st Edition, UK, USA, Academic Press, pp 613-635, 2015

Division of Molecular and Cellular Medicine (Aoki Group)

Kazunori Aoki, Chie Kudo, Kenta Narumi, Yoko Kobayashi, Yukihiro Mizoguchi , Ryousuke Ueda, Hisayoshi Hashimoto, Yuki Yamamoto, Yosei Rin, Masaki Nagasato, Chihiro Shibasaki, Marina Henmi

Introduction

Research programs in the Division of Molecular and Cellular Medicine (Aoki group) consist of development of novel therapeutic strategies for solid cancers based on the analysis of host-immune response against cancer cells and exploitation of cancer-targeting technologies. The specific activities in 2015 were as follows: 1) Clarification of immunological effects of myeloid-derived suppressor cells (MDSC) and type I neutrophils on antitumor immune reaction induced by immune therapies; and 2) Identification of cancer-targeting peptides using the peptide-display adenovirus library.

Research activities

Immunological effects of MDSC and neutrophils on antitumor immune reaction

We investigated the immunological effect of myeloid lineage cells such as MDSC and neutrophils in the tumor microenvironment on antitumor immune reaction induced by chemotherapy and hematopoietic stem cell transplantation.

1) Some anti-cancer drugs have been found to induce positive immune reactions against cancers, leading to the new concept of "chemo-immunotherapy". We found that the high frequency of MDSC in peripheral blood and tumor tissue was strongly associated with poor survival in patients with colorectal cancer who received the standard chemotherapy. Then, we examined whether the chemotherapy-induced tumor immunity is enhanced by the depletion of MDSC in murine colon cancer models. An Ly6G antibody was used to deplete MDSC. The tumor growth was retarded in treatment of 5-FU and Ly6G antibody alone, while a combination more strongly suppressed the growth (Figure. 1). In the spleens of 5-FU-treated mice, the number of AH-1 tetramer$^+$ cells was increased. The combination increased more the number of AH-1$^+$ cells in spleens and IFN-γ^+ CD8$^+$ T cells in tumors. In addition, the frequency of CD107a$^+$ NK cells/total NK cells was also increased by the combination. The results suggested that a combination of chemotherapy and inhibition of immune suppressive cells is a promising strategy in inducing strong tumor immunity.
2) It was reported that autologous hematopoietic stem cell transplantation (HSCT) also can induce a strong antitumor immunity following preconditioning-induced lymphopenia. However, the underlying mechanisms were fully understood. First, we showed that syngeneic HSCT-activated NK cells contributed to an antitumor effect using mouse colon cancer models. Then, we examined what factor influenced the activation of NK cells in tumors. We found that a large number of neutrophils accumulated in tumors especially in the early period after HSCT. The depletion of neutrophils significantly decreased the antitumor effect of HSCT. The fraction of IFN-γ^+ NK cells was clearly elevated in HSCT tumors compared with non-HSCT tumors, and the neutrophil depletion decreased the IFN-γ^+ fraction. The fraction of dead NK cells in the tumor was significantly increased by the neutrophil depletion (Figure. 2). The results indicated that neutrophils in tumors prevented NK cells from cell-death induction during homeostatic proliferation. This relationship between neutrophils and NK cells may reveal an important aspect of antitumor immunity.

Identification of cancer-targeting ligands using the peptide-display adenovirus library

The cancer-targeting ligands are useful to deliver

therapeutic reagents such as chemotherapeutic drugs, molecular targeted drugs, gene therapy vectors and oncolytic viruses to tumor regions in vivo. To identify the cancer-targeting ligands, we have constructed an adenovirus library displaying random peptides on the fiber, and have developed the screening procedures on cancer cell lines and murine cancer models. This year, we successfully isolated pancreatic and prostate cancer-targeting peptides and confirmed the specificity and effectiveness of targeting ligands on several cancer and normal cell lines. Furthermore, we began to develop the system to comprehensively explore the cognate receptors of identified targeting-ligands using the Human Proteome Expression Resource in collaboration with the National Institute of Advanced Industrial Science and Technology. The identification of cancer-targeting ligands and their cellular receptors are useful to develop novel diagnostic and therapeutic strategies for cancer.

Education

Two graduate students (doctoral course) linked with Keio University, three graduate students (doctoral course: one, master's course: two) linked with Tokyo Medical and Dental University and the undergraduate students in Tokyo University of Pharmacy and Life Sciences studied cancer immunology and cancer-targeted therapy in our laboratory.

Future prospects

We are investigating the molecular basis of an immune-suppressive microenvironment and the interaction between cancer cells, stromal cells and immune cells in a tumor microenvironment, which may open a new perspective on immune therapy for cancer. In addition, tumor-targeting ligands are also promising as a next-generation of molecular targeting therapy.

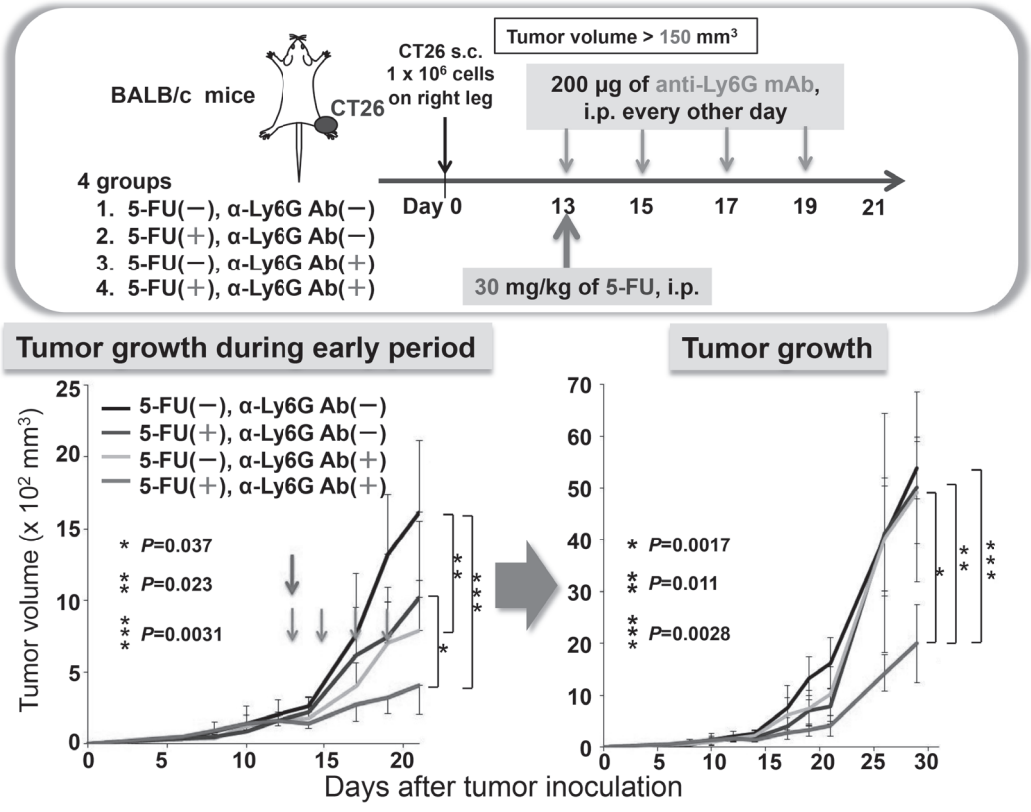


Figure 1. MDSC Depletion Enhanced 5-FU-mediated Antitumor Effect

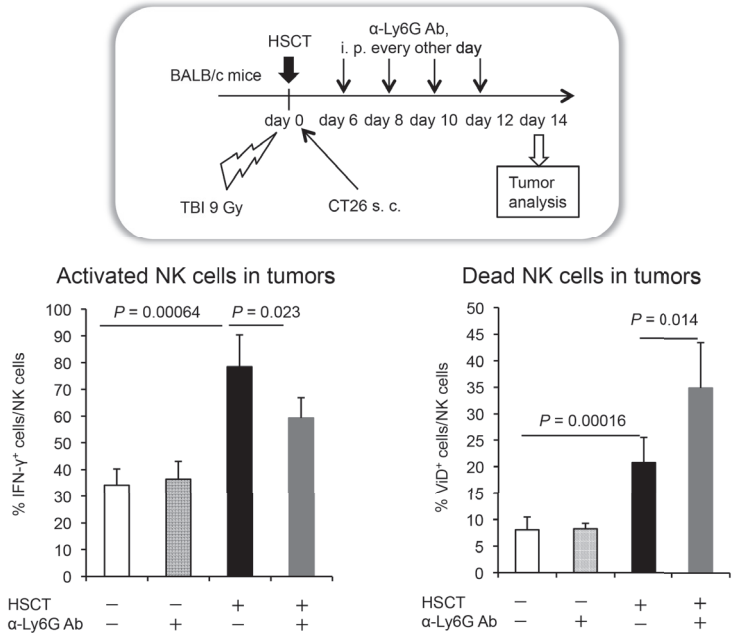


Figure 2. Neutrophils in tumors prevent NK cells from activation-induced cell death during HP

List of papers published in 2015

Journal

1. Narumi K, Miyakawa R, Ueda R, Hashimoto H, Yamamoto Y, Yoshida T, Aoki K. Proinflammatory Proteins S100A8/S100A9 Activate NK Cells via Interaction with RAGE. J Immunol, 194:5539-5548, 2015

2. Terracina KP, Aoyagi T, Huang WC, Nagahashi M, Yamada A, Aoki K, Takabe K. Development of a metastatic murine colon cancer model. J Surg Res, 199:106-114, 2015

3. Kudo-Saito C. Cancer-associated mesenchymal stem cells aggravate tumor progression. Front Cell Dev Biol, 3:23, 2015

4. Tanaka Y, Aoyagi K, Minashi K, Komatsuzaki R, Komatsu M, Chiwaki F, Tamaoki M, Nishimura T, Takahashi N, Oda I, Tachimori Y, Arao T, Nishio K, Kitano S, Narumi K, Aoki K, Fujii S, Ochiai A, Yoshida T, Muto M, Yamada Y, Sasaki H. Discovery of a Good Responder Subtype of Esophageal Squamous Cell Carcinoma with Cytotoxic T-Lymphocyte Signatures Activated by Chemoradiotherapy. PLoS One, 10:e0143804, 2015

5. Kosaka T, Davydova J, Ono HA, Akiyama H, Hirai S, Ohno S, Takeshita F, Aoki K, Ochiya T, Yamamoto M, Kunisaki C, Endo I. Imaging and antitumoral effect of a cyclo-oxygenase 2-specific replicative adenovirus for small metastatic gastric cancer lesions. Anticancer Res, 35:5201-5210, 2015

Book

1. Kawakami Y, Qian L, Kawamura N, Miyazaki J, Tsubota K, Kinoshita T, Nakaura K, Ohmura G, Satomi R, Sugiyama J, Nishio H, Hayakawa T, Popivanova B, Nuchsupha S, Liu TH, Kamijuku H, Kudo-Saito C, Tsukamoto N, Sakurai T, Fujita T, Yaguchi T. Cancer induced immunosuppression and its modulation by signal inhibitors. In: Bonavida B, Chouaib S (eds), Resistance of Cancer Cells to CTL-Mediated Immunotherapy, Switzerland, Springer International Publishing, pp 287-301, 2015

2. Kawakami Y, Qian L, Kawamura N, Miyazaki J, Nagumo H, Tsubota K, Kinoshita T, Nakamura K, Ohmura G, Satomi R, Sugiyama J, Nishio H, Hayakawa T, Popivanova B, Nuchsupha S, Liu TH, Kamijuku H, Kudo-Saito C, Tsukamoto N, Sakurai T, Fujita T, Yaguchi T. Development of personalized combination cancer immunotherapy based on the patients' immune status. In: Seya T, Matsumoto M, Udaka K, Sato N (eds), Inflammation and Immunity in Cancer, Japan, Springer Japan, pp 255-266, 2015

Division of Rare Cancer Research

Tadashi Kondo, Xiaoqing Pan, Rieko Ohki

Introduction

The research goal of the Division of Rare Cancer Research is to create the innovative seeds for better clinical outcomes for rare cancer patients. A rare cancer is defined as a cancer with prevalence of less than six. Rare cancer includes about 200 cancer types, and despite the rarity of each rare cancer, the rare cancers represent in total about 20% of all cancer cases in Japan. Thus, the rare cancer research deals with a wide-ranging subject. Although the research themes of rare cancer research are quite general ones such as those for prevention, diagnosis and treatment, as the clinical materials of rare cancers are limited, we need to make a special effort for rare cancer research. With this notion, the fundamental tools were created for rare cancer research. The establishment of the patient-derived cancer model, and the database for meta-analysis of genes are the efforts to solve the problems of the limited amount of clinical material. Re-localization of cancer drugs is a practical approach to rare cancer, and the experimental systems for re-localization of cancer drugs were created in our laboratory. Those include the high-throughput screening system and the application of Connectivity Map. Sarcomas are the major subjects of our rare cancer research, and the identification of candidates of therapeutic targets and biomarkers were undertaken. The predictive and prognostic biomarkers are the subjects of our research. Moreover, through the above-mentioned approach, we are discovering the novel utility of existing cancer drugs for rare cancers. Our experience and fundamental systems for rare cancer research will be applicable for major cancer research.

Research activities

1) Establishment of fundamental research system
   - Patient-derived cancer models were created from the clinical materials of sarcoma patients.
   - Screening system for the study of re-localization of cancer drugs was established and used for the cell panel.
   - Platform of bioinformatics such as the original Connectivity Map was created and applied to the study of re-localization of cancer drugs.
   - Database of gene status of rare cancer was created using bioinformatics approach.

2) Study of individual rare cancer

Sarcomas are presently major subjects of our rare cancer research. The identification of therapeutic targets and biomarkers was undertaken using clinical materials. The biomarker candidates to predict the resistance of molecular targeting drugs such as those used for sarcomas were identified by a multi-omics approach. Their molecular backgrounds and validation using additional cases are under consideration.

3) Reverse innovation

The research platforms were developed with the idea that they will be applicable to other malignancies.

Education

One PhD student and two post-doctorate researchers were educated.

Future prospects

Our research activities will benefit patients with rare cancers. The fundamental system for rare cancer research will be applicable to the research of all cancers.

List of papers published in 2015

Journal

1. Tajima T, Kito F, Ohta T, Kawai A, Kondo T. Interactome analysis reveals molecular mechanisms underlying the association between selenium binding protein 1 expression and the malignant features of tumor cells. J Electrophoresis, 59:1-6, 2015

2. Uemura N, Kondo T. Current advances in esophageal cancer proteomics. Biochim Biophys Acta, 1854:687-695, 2015

3. Fujita T, Yuno M, Okuzaki D, Ohki R, Fujii H. Identification of non-coding RNAs associated with telomeres using a combination of enChIP and RNA sequencing. PLoS One, 10:e0123387, 2015

4. Kikuta K, Morioka H, Kawai A, Kondo T. Global protein-expression profiling for reclassification of malignant fibrous histiocytoma. Biochim Biophys Acta, 1854:696-701, 2015

5. Ichikawa H, Yoshida A, Kanda T, Kosugi S, Ishikawa T, Hanyu T, Taguchi T, Sakumoto M, Katai H, Kawai A, Wakai T, Kondo T. Prognostic significance of promyelocytic leukemia expression in gastrointestinal stromal tumor; integrated proteomic and transcriptomic analysis. Cancer Sci, 106:115-124, 2015

Book

1. Kondo T. Novel prognostic biomarker, pfetin, in gastrointestinal stromal tumors: proteomics study. In: Victor RP, Vinood BP (eds), General methods in biomarker research and their applications 1st Edition, Netherlands, Springer Netherlands, pp 251-266, 2015

Division of Refractory and Advanced Cancer

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

Introduction

The malignant characteristics of cancers causing invasion into surrounding tissue, metastasis to distant organs, and acquired resistance to therapeutics are serious threats to the clinical treatment of cancer. A number of receptor and non-receptor tyrosine kinases are involved in the acquisition of such malignant characteristics. 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 novel therapy to overcome these malignant characteristics of progressed cancers by targeting critical proteins and signals involved in these procedures.

Routine activities

A weekly conference is held with the members of the Division of Refractory and Advanced Cancer. In addition, a monthly progress report is held with the members of the research institute.

Research activities

Molecules and Microenvironments regulating Metastasis and Invasion of Cancers

Scirrhous gastric carcinoma (SGC) show rapid expansion through progressive invasion, peritoneal dissemination and frequent metastasis to lymph nodes. Receptor tyrosine kinases such as fibroblast growth factor receptors (FGFRs) and Met are frequently activated in SGC and the contribution of signaling from these kinases to unique clinical aspects of SGC are suggested. We have recently identified numbers of phosphotyrosine-containing molecules under the regulation of these receptor tyrosine kinases by mass-spectrometry analysis. The function of these potent signal mediators involved in progression of SGC are being investigated.

It was revealed that the interaction of lung cancer with fibroblasts participates in the malignancy of lung cancer and that some factors secreted from cancer cells inactivate the p53 pathway in fibroblasts. Furthermore, we identified TSPAN12 as a factor that induced p53 inactivation in fibroblasts to promote cancer progression.

CDCP1 is a critical regulator of anoikis resistance, distant metastasis, and peritoneal dissemination of cancer cells. It was also shown that CDCP1 is required for the functional link between Ras and Src signaling during the multistage progression of human malignant tumors. Therapeutic antibodies and chemicals that block CDCP1-mediated signaling are being screened and several candidates were obtained.

In collaboration with Rome University, we found that clathrin heavy chain, which plays a role in endocytosis and p53 transactivation, interacts with the estrogen receptor, which leads to sustained signals from estrogen.

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

ALK fusion-positive lung adenocarcinoma cell lines were successfully established from patients and it was revealed by utilizing these cells that the combination of the ALK inhibitor with the p53 activator can reduce the resistance of ALK fusion-positive lung cancer cells to the ALK inhibitor. In addition, the combination treatment of the ALK inhibitor with the p53 activator was also effective in the ALK-positive neuroblastoma.

Flotillin-1 (FLOT1), a plasma-membrane-localizing protein, and SHP2/PTPN11, a tyrosine

phosphatase, were identified as the ALK-binding tyrosine-phosphorylated proteins in neuroblastoma. It was revealed that FLOT1 negatively regulates ALK expression and signaling via endocytosis. It was shown that SHP2 is tyrosine-phosphorylated by ALK and appears to mediate the ALK-dependent oncogenic property of NB-39-nu cells, while SHP2 induces dephosphorylation of ALK protein. Our findings suggest that the loss of FLOT1-mediated regulation of ALK or enhanced expression of SHP2 contributes to malignancy of clinical neuroblastoma cases and those cases might be sensitive to ALK inhibitors even without the genetic alteration of ALK.

Education

We accept students or graduate students as trainees from various institutes including the University of Tokyo and educate future basic cancer researchers. We also make efforts in the education of young post-doctoral researchers.

Future prospects

In patients with advanced stages of cancers, the control of metastasis, invasion and drug resistance is crucial for maintaining quality of life (QOL) in addition to prolonged survival. Our approach to elucidate the underlying mechanism of these malignant characteristics of cancers will give us ways to develop novel therapeutic strategies for advanced cancers. Especially, identification of molecules and signals involved in drug resistance and cancer-stromal interaction will be intensively studied to find novel approaches to overcome refractory cancers.

List of papers published in 2015

Journal

1. Totta P, Pesiri V, Enari M, Marino M, Acconcia F. Clathrin heavy chain interacts with estrogen receptor α and modulates 17β-estradiol signaling. Mol Endocrinol, 29:739-755, 2015
2. Ueno H, Tomiyama A, Yamaguchi H, Uekita T, Shirakihara T, Nakashima K, Otani N, Wada K, Sakai R, Arai H, Mori K. Augmentation of invadopodia formation in temozolomide-resistant or adopted glioma is regulated by c-Jun terminal kinase-paxillin axis. Biochem Biophys Res Commun, 468:240-247, 2015
3. Yamaguchi H, Sakai R. Direct interaction between carcinoma cells and cancer associated fibroblasts for the regulation of cancer invasion. Cancers (Basel), 7:2054-2062, 2015

Division of Cancer Immunology

Hitoshi Nakagama, Yuka Maeda

Introduction

Our Division was established in April 2015. The recent success of cancer immunotherapy makes it an important strategy for cancer treatment, and a lot of clinical trials are ongoing to develop new reagents. However, not all subjects expect to derive therapeutic benefits; in some patients it does not work. Based on our previous work, we found that regulatory T cells rendered self-tumor-antigen-specific $CD8^+$ T cells anergic (that is, hypo-proliferative and cytokine hypo-producing upon antigen re-stimulation) to maintain self-tolerance (Maeda Y et al. *Science*, 346:1536-40, 2014).

Research activities

1) Clarify immune suppressive mechanism of Tregs at tumor sites with melanoma samples.
2) Analyze immunologically and pathologically BRAF mutated melanoma pre-and post-treated with a BRAF inhibitor.

Future prospects

As most tumor antigens are derived from self-antigens, immunological self-tolerance induced by natural occurring Tregs may hamper the induction of effective anti-tumor T-cell responses. When responder patients with non-small cell lung cancers treated with anti-PD-1 mAb were examined by whole-exome sequencing, $CD8^+$ T-cells mainly recognized antigens generated by tumor-specific mutations (neo-antigens) (Rizvi NA…Maeda Y.. et al. Science, 348:124-128, 2015). Therefore, Tregs-mediated anergy induction in tumor (self) antigen-specific $CD8^+$ T cells may inhibit their activation, and neo-antigens stemmed from tumor-specific gene mutations could become the main target of $CD8^+$ T cells that actually induce tumor regression. We reveal Treg suppressive mechanisms against anti-tumor responses in tumor local sites and offer new combination immunotherapy.

List of papers published in 2015

Journal

1. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, Lee W, Yuan J, Wong P, Ho TS, Miller ML, Rekhtman N, Moreira AL, Ibrahim F, Bruggeman C, Gasmi B, Zappasodi R, Maeda Y, Sander C, Garon EB, Merghoub T, Wolchok JD, Schumacher TN, Chan TA. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science, 348:124-128, 2015

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

Introduction

The concept of the Research Core Facility (CF) originated from the first 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. 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, its maintenance and human expertise, especially in the field of bioinformatics, which are increasingly difficult if not impossible to afford by 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. The current NCC CF is a virtual organization based on mutual support among research scientists and laboratories, each engaging in their own competitive research.

Figure 1 shows the original CF framework officially started on September 5, 2011 with four 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.

In August 2014, the original CF system was incorporated into the newly established Fundamental Innovative Oncology Core Center (FIOC). The Research Support Division of the FIOC corresponds to the Genome, Epigenome and Proteome CF and is reported here. The biology CF function is being offered by the Central Animal Division and reported in its pages.

Research activities

The mission of the CF is not limited to mutual support and collaboration inside the NCCRI, but extends to other sectors of the NCC. For instance, the CF offers a 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 (Figure 2). One important mission of the CF is its contribution to the genetic diagnosis of hereditary cancer syndromes at the outpatient genetic counseling clinic in the NCC hospital. Such services, however, are undoubtedly in the transitional zone between research and clinical practice and need special consideration and practice to assure its analytical validity.

Because the CF covers such diverse activities, its performance is difficult to quantify, but just as a simplified example, the numbers of individual research projects and samples submitted to the CF are summarized in Table 1.

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

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

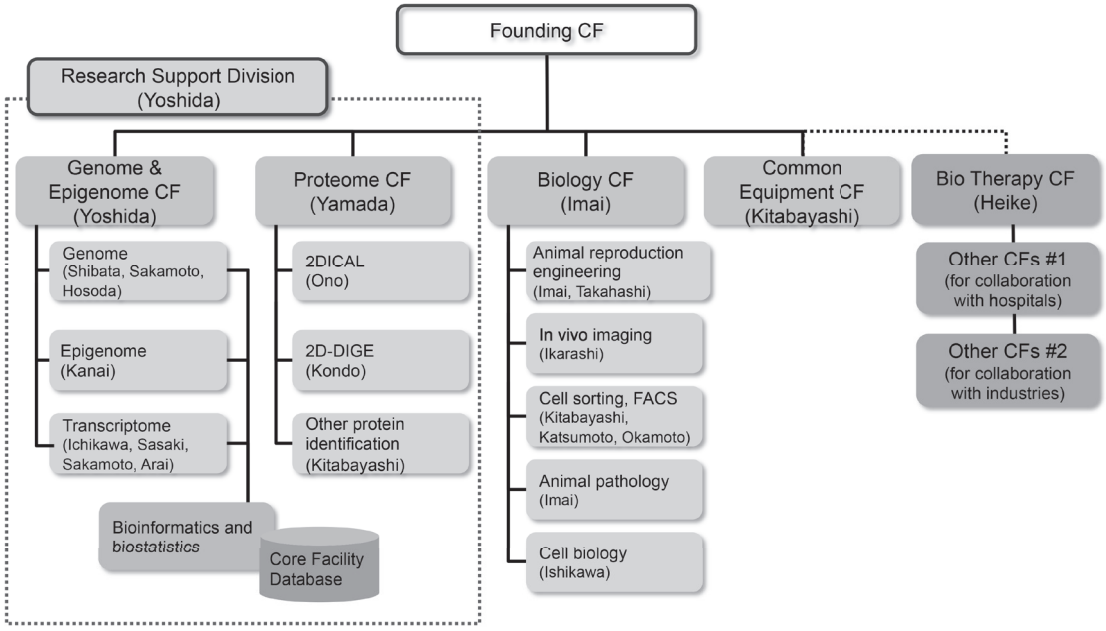


Figure 1. CF Organization (as of 2014)

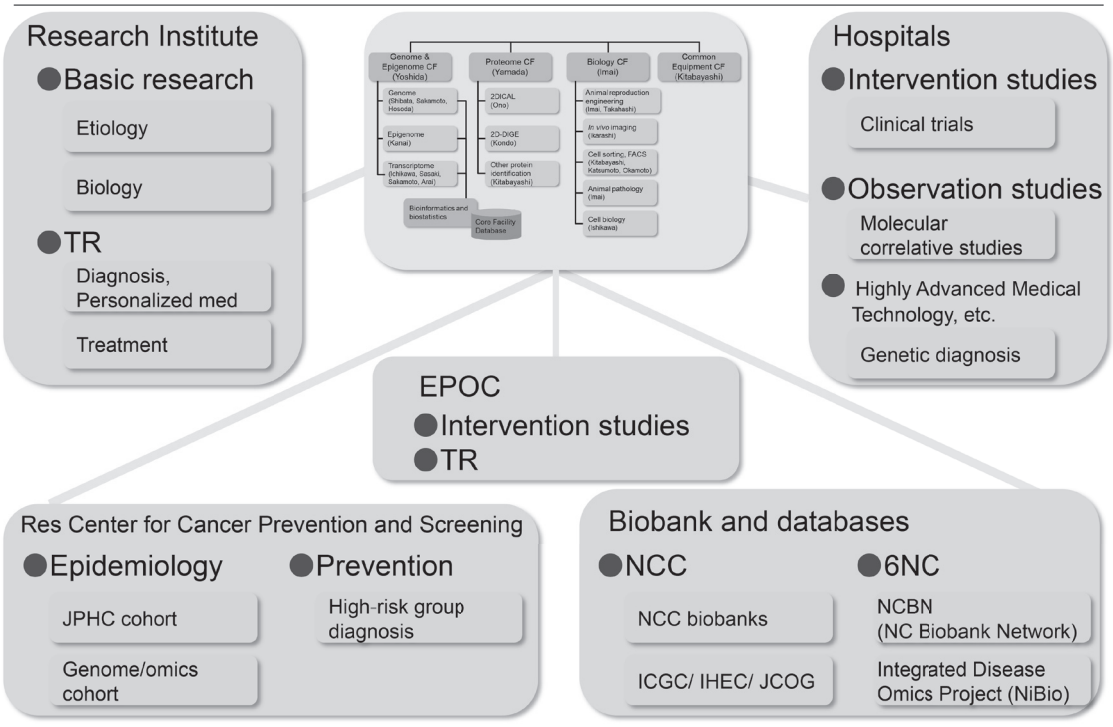


Figure 2. CF Interactions and Participations

Table 1. CF Activities in FY 2011-2015 (excluding the self-service type)

Omics	Applications	# samples				
		FY 2011	FY 2012	FY2013	FY2014	FY2015
Genome	Next Generation Sequencer	248	180	160	1,203	1,063
	SNP array/CGH array analyses	2,359	2,226	1,885	529	7,309
Epigenome	NGS	102	14	8	30	697
	Infinium array	1,646	569	801	705	63
Transcriptome	NGS	44	157	0	243	15
	Oligonucleotide microarray	155	132	178	232	403
Proteome	2DICAL	524	112	54	126	42
	2D-DIGE	0	308	83	199	24
	Protein identification	0	483	612	1,573	1,555
Total		5,078	4,181	3,781	4,840	11,171

List of papers published in 2015

Journal

1. Hashimoto T, Ogawa R, Matsubara A, Taniguchi H, Sugano K, Ushiama M, Yoshida T, Kanai Y, Sekine S. Familial adenomatous polyposis-associated and sporadic pyloric gland adenomas of the upper gastrointestinal tract share common genetic features. Histopathology, 67:689-698, 2015

2. Kumamoto K, Ishida H, Ohsawa T, Ishibashi K, Ushiama M, Yoshida T, Iwama T. Germline and somatic mutations of the APC gene in papillary thyroid carcinoma associated with familial adenomatous polyposis: Analysis of three cases and a review of the literature. Oncol Lett, 10:2239-2243, 2015

3. Saeki N, Ono H, Sakamoto H, Yoshida T. Down-regulation of Immune-related Genes by PSCA in Gallbladder Cancer Cells Implanted into Mice. Anticancer Res, 35:2619-2625, 2015

4. Tanaka Y, Aoyagi K, Minashi K, Komatsuzaki R, Komatsu M, Chiwaki F, Tamaoki M, Nishimura T, Takahashi N, Oda I, Tachimori Y, Arao T, Nishio K, Kitano S, Narumi K, Aoki K, Fujii S, Ochiai A, Yoshida T, Muto M, Yamada Y, Sasaki H. Discovery of a Good Responder Subtype of Esophageal Squamous Cell Carcinoma with Cytotoxic T-Lymphocyte Signatures Activated by Chemoradiotherapy. PLoS One, 10:e0143804, 2015

5. Saeki N, Ono H, Yanagihara K, Aoyagi K, Sasaki H, Sakamoto H, Yoshida T. rs2294008T, a risk allele for gastric and gallbladder cancers, suppresses the PSCA promoter by recruiting the transcription factor YY1. Genes Cells, 20:382-391, 2015

6. Yamanoi K, Arai E, Tian Y, Takahashi Y, Miyata S, Sasaki H, Chiwaki F, Ichikawa H, Sakamoto H, Kushima R, Katai H, Yoshida T, Sakamoto M, Kanai Y. Epigenetic clustering of gastric carcinomas based on DNA methylation profiles at the precancerous stage: its correlation with tumor aggressiveness and patient outcome. Carcinogenesis, 36:509-520, 2015

7. Suzuki M, Chiwaki F, Sawada Y, Ashikawa M, Aoyagi K, Fujita T, Yanagihara K, Komatsu M, Narita M, Suzuki T, Nagase H, Kushima R, Sakamoto H, Fukagawa T, Katai H, Nakagama H, Yoshida T, Uezono Y, Sasaki H. Peripheral opioid antagonist enhances the effect of anti-tumor drug by blocking a cell growth-suppressive pathway in vivo. PLoS One, 10:e0123407, 2015

8. Budhathoki S, Iwasaki M, Yamaji T, Sasazuki S, Takachi R, Sakamoto H, Yoshida T, Tsugane S. Dietary heterocyclic amine intake, NAT2 genetic polymorphism, and colorectal adenoma risk: the colorectal adenoma study in Tokyo. Cancer Epidemiol Biomarkers Prev, 24:613-620, 2015

9. Fujita T, Chiwaki F, Takahashi RU, Aoyagi K, Yanagihara K, Nishimura T, Tamaoki M, Komatsu M, Komatsuzaki R, Matsusaki K, Ichikawa H, Sakamoto H, Yamada Y, Fukagawa T, Katai H, Konno H, Ochiya T, Yoshida T, Sasaki H. Identification and Characterization of CXCR4-Positive Gastric Cancer Stem Cells. PLoS One, 10:e0130808, 2015

10. Iwakawa R, Kohno T, Totoki Y, Shibata T, Tsuchihara K, Mimaki S, Tsuta K, Narita Y, Nishikawa R, Noguchi M, Harris CC, Robles AI, Yamaguchi R, Imoto S, Miyano S, Totsuka H, Yoshida T, Yokota J. Expression and clinical significance of genes frequently mutated in small cell lung cancers defined by whole exome/RNA sequencing. Carcinogenesis, 36:616-621, 2015

Central Animal Division

Toshio Imai, Mami Takahashi, Tetsuya Ishikawa, Teruo Komatsu, Kotomi Otsubo, Yoshinori Ikarashi, Naoaki Uchiya, Rikako Ishigamori, Yukiko Nakamura, Masashi Yasuda, Manabu Tsuchida, Ayami Kawashima, Satoshi Ikeda, Junichi Zukeyama, Shiho Ozawa, Yudai Seki, Karin Miura, Junya Asahira

Routine activities

The important role of the Central Animal Division is health management of experimental animals and maintenance of the animal experimentation facility. Some researchers and technical staff also act 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

The 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) Involvement of obesity/pancreatic fatty infiltration (FI) in pancreatic carcinogenesis

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 the degree of FI in the pancreas. Our recent clinical studies have showed that there is a positive correlation between FI of the pancreas and pancreatic ductal adenocarcinomas, suggesting severe pancreatic FI could be a risk factor of pancreatic cancer. To clarify the role of obesity/pancreatic fatty infiltration (FI) in pancreatic carcinogenesis, we crossed pancreas-specific K-ras mutant mice with obesity model mice. A significant increase of tumor development was observed and the enhancing mechanisms are being investigated.

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. Recently, a BALB/c strain background heterozygous p53 deficient mouse model is also used. The results obtained indicated that HFD promoted carcinogenesis. Molecular mechanisms of the promotion as assessed with DNA microarray analysis for non-cancerous mammary tissues were suggested to be associated with the altered expression of a planar cell polarity-related gene, which was at least partly affected by DNA methylation.

3) Human-induced hepatic lineage-oriented stem cells (hiHSCs)

hiHSCs were generated and expanded as a new type of hiPSC under non-typical coculture with feeder cells in a chemically defined hiPSC medium at a very high density. Self-renewing hiHSCs expressed markers of both human embryonic stem cells and hepatocytes. Those cells were highly expandable, markedly enhancing gene expression of serum hepatic proteins and cytochrome P450 enzymes with the omission of FGF-2 from an undefined hiPSC medium. Approximately 90% of hiHSCs autonomously differentiated to hepatocyte-like cells, even in a defined minimum medium without any of the exogenous growth factors necessary for hepatic specification.

Future prospects

Research approaches using immune deficient/severely immune-deficient mice have become increasingly important over the past few 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 2015

Journal

1. Kitahashi T, Takahashi M, Imai T. Biphasic alterations in expression and subcellular localization of MUC1 in pancreatic ductal carcinogenesis in Syrian hamsters. Pancreas, 44:76-86, 2015

2. Ishikawa T, Kobayashi M, Yanagi S, Kato C, Takashima R, Kobayashi E, Hagiwara K, Ochiya T. Human Induced Hepatic Lineage-Oriented Stem Cells: Autonomous Specification of Human iPS Cells toward Hepatocyte-Like Cells without Any Exogenous Differentiation Factors. PLoS One, 10:e0123193, 2015

RI Research Support Division

Yutaka Yamada, Mitsuko Masutani, Gen Fujii

Introduction

The RI Research Support 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 roles of the RI Research Support Division are exposure control of radiation workers and management of the radiation controlled area.

Research activities

The research activities of the RI Research Support Division have focused on studies of radiation effect. The mechanism of cell death induced by boron neutron capture reaction (BNCR) were investigated using human oral squamous carcinoma cell line, SAS cells. The cells were irradiated with a thermal neutron beam after incubation with or without boronophenylalanine (BPA). BNCR (irradiation with BPA) induced typical apoptosis in the cells 24 hours after irradiation. Proteins functioning in endoplasmic reticulum, DNA repair, and RNA processing showed dynamic changes at the early phase after BNCR, suggesting that the proteins could be involved in the regulation of cellular response to BNCR. In addition, BNCR induces fragments of endoplasmic reticulum-localized lymphoid-restricted protein (LRMP). The fragmentation of LRMP was also observed in the rat tumor graft model 20 hours after boron neutron capture therapy. These data suggest that dynamic changes of LRMP could be involved during cellular response to BNCR.

Future prospects

These changes will be available as biomarkers for evaluating the effects of boron neutron capture therapy (BNCT). For clinical application of BNCT, the identification of appropriate biomarkers is needed. Further analysis of different types of cancer cells will facilitate the identification of useful biomarkers for BNCT.

List of papers published in 2015

Journal

1. Sato A, Itoh T, Imamichi S, Kikuhara S, Fujimori H, Hirai T, Saito S, Sakurai Y, Tanaka H, Nakamura H, Suzuki M, Murakami Y, Baiseitov D, Berikkhanova K, Zhumadilov Z, Imahori Y, Itami J, Ono K, Masunaga S, Masutani M. Proteomic analysis of cellular response induced by boron neutron capture reaction in human squamous cell carcinoma SAS cells. Appl Radiat Isot, 106:213-219, 2015

Department of Biobank and Tissue Resources

Yae Kanai, Masumi Tanaka, Teiko Yamane, Nobuko Nangi

Activities of National Cancer Center Biobank

The Department of Biobank Tissue Resources operates National Cancer Center Biobank under the supervision of the National Cancer Center Biobank Administration Committee (Figure 1).

In 2015, 9,652 vials of tissue specimens obtained from surgically resected materials of 1,796 patients were newly deposited at the National Cancer Center Biobank and 1,786 vials of tissue specimens obtained from surgically resected materials of 1,370 patients were provided for research approved by the National Cancer Center Ethics Committee. The ratio of the number of patients from whom samples were provided for research to those of whom samples were newly deposited at the Biobank was about 76%. At the end of 2015, we reposited 81,314 vials of tissue specimens of 19,355 patients.

In 2015, 43,902 vials of plasma samples drawn from 9,831 patients were newly deposited at the National Cancer Center Biobank and 3,482 vials of plasma samples drawn from 3,340 patients were provided for research approved by the National Cancer Center Ethics Committee. At the end of 2015, we reposited 151,796 vials of blood samples of 37,570 patients who consented to give blood samples for research purposes.

We have built up the catalog database, HosCanR Biobank Edition, by extracting appropriate information from the Interview Sheet Database in a common form among six National Centers in Japan and HosCanR, an application specialized for the National Program of Cancer Registries. Researchers and biobank users can find samples suitable for their own research plans using the search commands of this catalog database. In 2015, we adjusted the HosCanR Biobank Edition to correspond to the central database platform of the National Center Biobank Network (NCBN) and prepared a wide distribution of samples from NCBN, which are not based on collaborative research, for outside researchers including researchers employed by industry.

Researchers who received samples from the Biobank have published 354 scientific papers (total impact factor: 1,831.289, total citation index; 5,630). A total of 64% of the published papers were based on collaborative research between researchers of the National Cancer Center and outside researchers; in particular, 21% were based on collaborative research with industry.

Many founders and/or contact persons of bioresource repositories of other universities and hospitals and television crews visited the National Cancer Center Biobank to learn about the management knowhow of the biobank. We have been consulted by contact persons of bioresource repositories of other universities about the storage system for specimens.

Staff of the National Cancer Center Biobank participate in the General Ethics Support Sector, Sample Utilization Review Working Group, Sample Handling System Review Working Group and Medical Information System Review Working Group of NCBN.

Future prospects

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

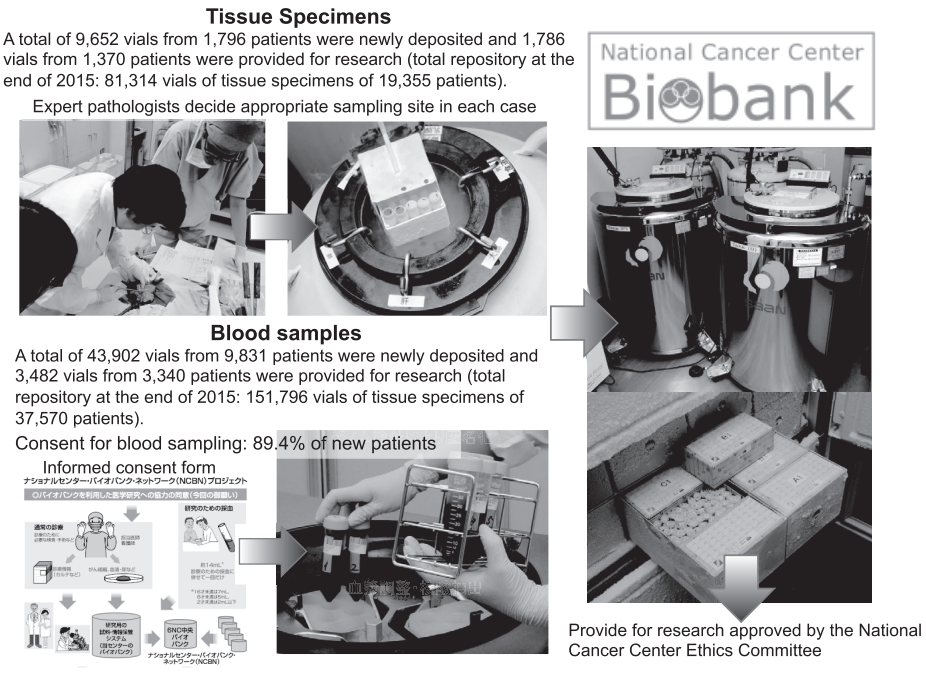


Figure 1. National Cancer Center Biobank

Department of Patient-Derived Cell Line and Xenograft

Tohru Kiyono, Farhana Ishrat Ghani, Chiho Kohno

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. Recently, both PDX models and cell line-derived xenograft (CDX) models are considered to be important preclinical tools. However, the success rate to establish new cell lines or PDX lines is not satisfactory.

Routine activities

This Department was founded in 2014 for the establishment of new cancer cell lines and PDX lines. With the help of the pathology division, we have systemically started to store valuable cancer specimens so that cancer tissues or cancer cells can be transplanted into immune-deficient mice or cultivated *in vitro* in the future.

Research activities

So far we have tried to cultivate ovarian cancer cells from more than 30 operative specimens. This year, we have succeeded to establish 14 novel ovarian cancer cell lines out of 15 operative specimens, including three clear cell carcinomas.

Future prospects

The number of cell lines derived from Asian patients is limited. Cell lines from some of the rare cancers remain to be established. Since we have developed a method to efficiently establish ovarian cancer cell lines, we will expand our method to other cancers. These cell lines are valuable resources with detailed clinical information. For example, the correlation between genomic mutations and drug sensitivity can be evaluated.

Department of Molecular imaging and Pharmacokinetics

Akinobu Hamada, Mitsuhiro Hayashi, Makiko Yamashita, Shoraku Ryu, Tomomi Nishijo, Mayu Ohuchi, Mariko Mizui

Introduction

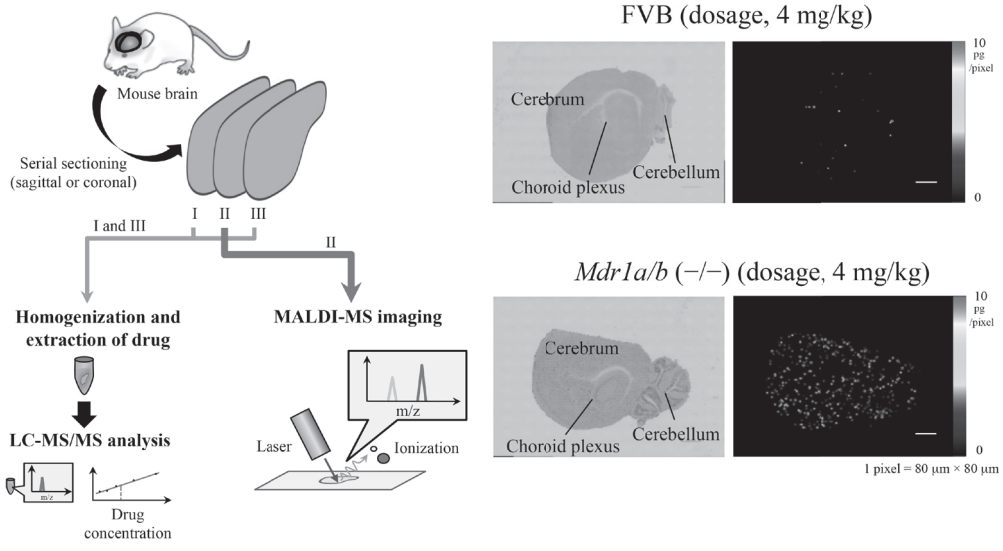
The Department of Clinical Pharmacology is focused on the development of a pharmacokinetics/pharmacodynamics (PK/PD) analyzing system. The system provides drug exposure in blood and tissues by using a high-sensitivity liquid chromatography tandem mass spectrometry (LC-MS/MS) and spatial drug distribution on tissue by using mass spectrometry imaging without labeling reagents. We are also focused on the development of an immunomonitoring system detecting patient's antibody-dependent cellular cytotoxicity (ADCC) activity.

Research activities

We established a quantitative mass spectrometry imaging system that provides both quantitative information and spatial distribution of target drugs. We used this system to visualize the amount and the distribution of anti-cancer drugs in several mouse models. To assess the efficacy and the behavior of target drugs in tumor tissues, we are now establishing not only a cell-derived xenotraft mouse model but also a patient-derived xenograft model. Moreover, we established an immunomonitoring system that can detect ADCC activity from patient's peripheral blood mononuclear cell (PBMC). Both research projects were submitted as papers to scientific journals.

Future prospects

The combination of PK/PD analysis, mass spectrometry imaging, measurement of ADCC activity, and establishment of a patient-derived xenograft (PDX) model can provide us with more accurate information about patients. These systems will help to actualize personalized medicine in the future.



P糖タンパク質(Mdr1)の基質薬剤の脳内組織移行性は、ノックアウトマウスで上昇

Figure 1. MALDI-MSI imaging analysis showed that the intra-brain transitivity of the drug was elevated on Mdr1(-/-) mouse brain.

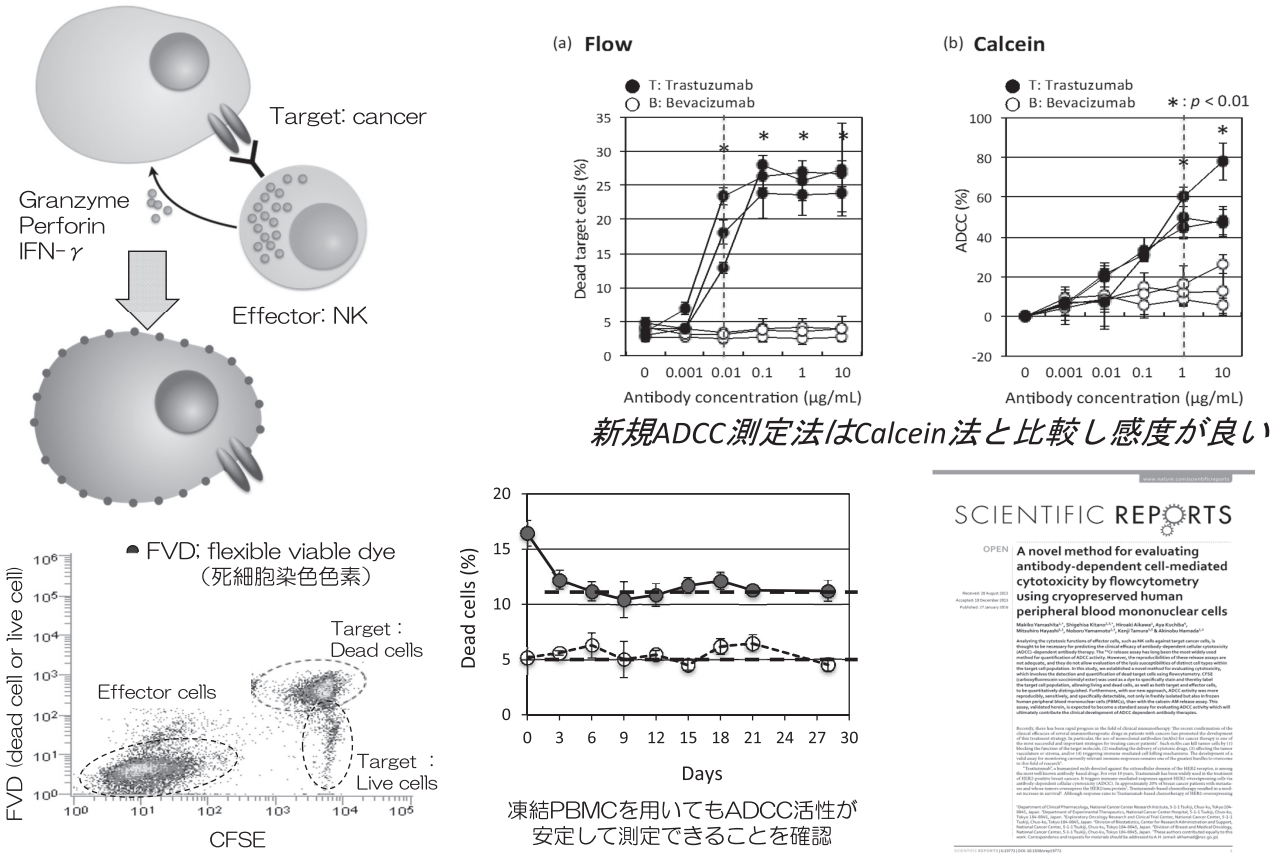


Figure 2. We developed a highly sensitive method for quantifying the ADCC activity of patient's blood.

List of papers published in 2015

Journal

1. Fujiwara Y, Kobayashi S, Nagano H, Kanai M, Hatano E, Toyoda M, Ajiki T, Takashima Y, Yoshimura K, Hamada A, Minami H, Ioka T. Pharmacokinetic Study of Adjuvant Gemcitabine Therapy for Biliary Tract Cancer following Major Hepatectomy (KHBO1101). PLoS One, 10:e0143072, 2015

2. Katsuya Y, Fujiwara Y, Sunami K, Utsumi H, Goto Y, Kanda S, Horinouchi H, Nokihara H, Yamamoto N, Takashima Y, Osawa S, Ohe Y, Tamura T, Hamada A. Comparison of the pharmacokinetics of erlotinib administered in complete fasting and 2 h after a meal in patients with lung cancer. Cancer Chemother Pharmacol, 76:125-132, 2015

3. Kurihara H, Hamada A, Yoshida M, Shimma S, Hashimoto J, Yonemori K, Tani H, Miyakita Y, Kanayama Y, Wada Y, Kodaira M, Yunokawa M, Yamamoto H, Shimizu C, Takahashi K, Watanabe Y, Fujiwara Y, Tamura K. ^{64}Cu-DOTA-trastuzumab PET imaging and HER2 specificity of brain metastases in HER2-positive breast cancer patients. EJNMMI Res, 5:8, 2015

4. Otani S, Hamada A, Sasaki J, Wada M, Yamamoto M, Ryuge S, Takakura A, Fukui T, Yokoba M, Mitsufuji H, Toyooka I, Maki S, Kimura M, Hayashi N, Ishihara M, Kasajima M, Hiyoshi Y, Katono K, Asakuma M, Igawa S, Kubota M, Katagiri M, Saito H, Masuda N. Phase I and pharmacokinetic study of erlotinib administered in combination with amrubicin in patients with previously treated, advanced non-small cell lung cancer. Am J Clin Oncol, 38:405-410, 2015

5. Sakata S, Sasaki J, Saeki S, Hamada A, Kishi H, Nakamura K, Tanaka H, Notsute D, Sato R, Saruwatari K, Iriki T, Akaike K, Fujii S, Hirosako S, Kohrogi H. Dose Escalation and Pharmacokinetic Study of Carboplatin plus Pemetrexed for Elderly Patients with Advanced Nonsquamous Non-Small-Cell Lung Cancer: Kumamoto Thoracic Oncology Study Group Trial 1002. Oncology, 88:201-207, 2015

6. Hayashi M, Yamamoto Y, Sueta A, Tomiguchi M, Yamamoto-Ibusuki M, Kawasoe T, Hamada A, Iwase H. Associations Between Elastography Findings and Clinicopathological Factors in Breast Cancer. Medicine (Baltimore), 94:e2290, 2015

7. Iwamoto N, Umino Y, Yamane N, Hamada A, Shimada T. The development of the validated LCMS bioanalysis of trastuzumab in human plasma using a selective detection method for complementarity-determining regions of monoclonal antibodies: nano-surface and molecular-orientation limited (nSMOL) proteolysis. Anal Methods, 7:9177-9183, 2015

8. Oguri T, Shimokata T, Ito I, Yasuda Y, Sassa N, Nishiyama M, Hamada A, Hasegawa Y, Ando Y. Extension of the Calvert formula to patients with severe renal insufficiency. Cancer Chemother Pharmacol, 76:53-59, 2015

9. Yoshitake Y, Fukuma D, Yuno A, Hirayama M, Nakayama H, Tanaka T, Nagata M, Takamune Y, Kawahara K, Nakagawa Y, Yoshida R, Hirosue A, Ogi H, Hiraki A, Jono H, Hamada A, Yoshida K, Nishimura Y, Nakamura Y, Shinohara M. Phase II clinical trial of multiple peptide vaccination for advanced head and neck cancer patients revealed induction of immune responses and improved OS. Clin Cancer Res, 21:312-321, 2015

Department of Innovative Seeds Evaluation

Tadashi Kondo, Tsutomu Ohta, Rieko Oyama, Yoko Takai, Fusako Kito, Marimu Sakumoto, Shoko Tanahashi

Introduction

The establishment of a patient-derived cancer model is our research activity. Although there are many patient-derived cancer models, those for rare cancers are rarely available. A rare cancer is defined as a cancer with prevalence of less than six. Rare cancer includes about 200 cancer types, and despite the rarity of each rare cancer, the rare cancers represent in total about 20% of all cancer cases in Japan. Thus, the rare cancers are quite important subjects. With this notion, the fundamental tools were created for rare cancer research. The establishment of patient-derived cancer models, especially those for sarcomas, has been conducted in our laboratory. The established models are currently used to evaluate the efficacy of novel cancer drugs.

Research activities

The clinical materials obtained in the National Cancer Center Hospital are used to develop the patient-derived cancer models. The development of methods for establishment of cancer models and the characterization of established cancer models are the major activities of our laboratory. The clinical materials are quite diverse depending on the original tissue samples, and the methods for individual histology are required to establish the cancer models. The molecular characterization is also important to use the cancer models in the research. It is quite important to know how the developed cancer models retain the original molecular backgrounds. For this sake, we employ the multi-omics approach. The DNA, RNA and proteins are comprehensively and intensively examined, comparing the original tissue samples and the established models. Our cancer models are included in the collaborative study with pharmaceutical companies. The research activity of our Department is linked to that of the Division of Rare Cancer Research. The ideas and the fundamental research tools are shared between two laboratories for novel innovative seeds development.

Future prospects

Our research activities will benefit the patients with rare cancers by contributing to the development of novel cancer drugs. We will establish more collaborative studies with pharmaceutical companies as well as academic research groups.

List of papers published in 2015

Journal

1. Tajima T, Kito F, Ohta T, Kawai A, Kondo T. Interactome analysis reveals molecular mechanisms underlying the association between selenium binding protein 1 expression and the malignant features of tumor cells. J Electrophoresis, 59:1-6, 2015

2. Uemura N, Kondo T. Current advances in esophageal cancer proteomics. Biochim Biophys Acta, 1854:687-695, 2015

3. Kikuta K, Morioka H, Kawai A, Kondo T. Global protein-expression profiling for reclassification of malignant fibrous histiocytoma. Biochim Biophys Acta, 1854:696-701, 2015

4. Ichikawa H, Yoshida A, Kanda T, Kosugi S, Ishikawa T, Hanyu T, Taguchi T, Sakumoto M, Katai H, Kawai A, Wakai T, Kondo T. Prognostic significance of promyelocytic leukemia expression in gastrointestinal stromal tumor; integrated proteomic and transcriptomic analysis. Cancer Sci, 106:115-124, 2015

Book

1. Kondo T. Novel prognostic biomarker, pfetin, in gastrointestinal stromal tumors: proteomics study. In: Victor RP, Vinood BP (eds), General methods in biomarker research and their applications 1st Edition, Netherlands, Springer Netherlands, pp 251-266, 2015

Department of Clinical Genomics

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

Introduction

The aim of our Department is to contribute to realize precision medicine for cancer patients based on the molecular profiles of their malignancies. To this end, we have developed a next-generation sequencing (NGS)-based genomic testing system using original gene panels. In addition, we are working on identification of novel diagnostic and therapeutic biomarkers for several types of malignancies by the use of NGS and microarray technologies.

Research activities

1) Development of an original NGS-based genomic testing system

We developed an NGS-based in-house genomic testing system, and have been continuously improving this system to become an accurate and clinically useful in vitro diagnostics (IVD) system. In this system, an original cancer gene panel (NCC oncopanel) was designed and used. With this gene panel, mutations and amplifications of ~100 genes and fusions of ~10 genes can be accurately identified from FFPE tumor tissue samples. In 2015, we renewed our gene panel (NCC oncopanel v3), and confirmed its performance in a prospective feasibility study to examine patients considering entry into an early-phase clinical trial. In addition, we supported the setting-up of SCI-Lab in the Hospital, in which our system was used as a clinical test with quality assurance to detect actionable genetic alterations for cancer patients.

2) Development of novel biomarkers

Through the use of NGS and microarray technologies, we are searching novel diagnostic and therapeutic biomarkers for sarcoma, gastric cancer and pediatric acute myeloid leukemia (AML). In 2015, from microarray-based gene expression profiling analysis of 130 Japanese pediatric AML patients, we found that the *EVI1* and *MEL1* genes were overexpressed in approximately 30% of patients, and that their high expression was significantly associated with inferior survival. High *EVI1* expression was detected mainly in myelomonocytic-lineage leukemia with *MLL* rearrangements and in megakaryocytic-lineage leukemia. On the other hand, high *MEL1* expression was detected in myelocytic-lineage and myelomonocytic-lineage leukemia without *MLL* rearrangements. Because of their subtype-dependent and mutually exclusive expression, a combined evaluation of their high expression enabled a clear distinction of patients with inferior survival. This association was confirmed by quantitative RT-PCR analysis of an independent cohort of 81 patients. We propose that the combined estimation of *EVI1* and *MEL1* expression will be an effective method to predict the prognosis of pediatric AML.

3) Target sequencing services

We provided target sequencing services using our original genomic testing system and commercially available cancer panel systems, upon requests from researchers in the Research Institute and Hospital. In 2015, about 600 samples from various types of cancers were analyzed.

List of papers published in 2015

Journal

1. Nakamura H, Arai Y, Totoki Y, Shirota T, Elzawahry A, Kato M, Hama N, Hosoda F, Urushidate T, Ohashi S, Hiraoka N, Ojima H, Shimada K, Okusaka T, Kosuge T, Miyagawa S, Shibata T. Genomic spectra of biliary tract cancer. Nat Genet, 47:1003-1010, 2015

2. Gocho Y, Kiyokawa N, Ichikawa H, Nakabayashi K, Osumi T, Ishibashi T, Ueno H, Terada K, Oboki K, Sakamoto H, Shioda Y, Imai M, Noguchi Y, Arakawa Y, Kojima Y, Toyama D, Hata K, Yoshida T, Matsumoto K, Kato M, Fukushima T, Koh K, Manabe A, Ohara A, Tokyo Children's Cancer Study Group. A novel recurrent EP300-ZNF384 gene fusion in B-cell precursor acute lymphoblastic leukemia. Leukemia, 29:2445-2448, 2015

3. Jo A, Mitani S, Shiba N, Hayashi Y, Hara Y, Takahashi H, Tsukimoto I, Tawa A, Horibe K, Tomizawa D, Taga T, Adachi S, Yoshida T, Ichikawa H. High expression of EVI1 and MEL1 is a compelling poor prognostic marker of pediatric AML. Leukemia, 29:1076-1083, 2015

4. Nakaguro M, Kiyonari S, Kishida S, Cao D, Murakami-Tonami Y, Ichikawa H, Takeuchi I, Nakamura S, Kadomatsu K. Nucleolar protein PES1 is a marker of neuroblastoma outcome and is associated with neuroblastoma differentiation. Cancer Sci, 106:237-243, 2015

5. Yamanoi K, Arai E, Tian Y, Takahashi Y, Miyata S, Sasaki H, Chiwaki F, Ichikawa H, Sakamoto H, Kushima R, Katai H, Yoshida T, Sakamoto M, Kanai Y. Epigenetic clustering of gastric carcinomas based on DNA methylation profiles at the precancerous stage: its correlation with tumor aggressiveness and patient outcome. Carcinogenesis, 36:509-520, 2015

6. Takenaka M, Saito M, Iwakawa R, Yanaihara N, Saito M, Kato M, Ichikawa H, Shibata T, Yokota J, Okamoto A, Kohno T. Profiling of actionable gene alterations in ovarian cancer by targeted deep sequencing. Int J Oncol, 46:2389-2398, 2015

7. Fujita T, Chiwaki F, Takahashi RU, Aoyagi K, Yanagihara K, Nishimura T, Tamaoki M, Komatsu M, Komatsuzaki R, Matsusaki K, Ichikawa H, Sakamoto H, Yamada Y, Fukagawa T, Katai H, Konno H, Ochiya T, Yoshida T, Sasaki H. Identification and Characterization of CXCR4-Positive Gastric Cancer Stem Cells. PLoS One, 10:e0130808, 2015

8. Saito M, Shimada Y, Shiraishi K, Sakamoto H, Tsuta K, Totsuka H, Chiku S, Ichikawa H, Kato M, Watanabe S, Yoshida T, Yokota J, Kohno T. Development of lung adenocarcinomas with exclusive dependence on oncogene fusions. Cancer Res, 75:2264-2271, 2015

Department of Translational Oncology

Hiroki Sasaki, Masayuki Komatsu, Rie Komatsuzaki, Fumiko Chiwaki, Tomoko Hiroki, Akio Ashida, Kanako Nakamura, Daichi Inami

Introduction

In 2015, the two major research areas of the Department of Translational Oncology were 1) preclinical studies using newly established gastric, esophageal, pancreatic, and ovarian cancer cell lines for derivation of industrial and academia seeds/drugs to the Exploratory Oncology Research & Clinical Trial Center (EPOC), and 2) basic research and development of personalized cancer diagnosis and treatment for gastric and esophageal cancers.

Preclinical Studies Using Newly Established Cell Lines from Common Cancers in Asia

Genome-wide genetic information in about 1,000 cancer cell lines is available on COSMIC DB (Sanger Center, UK) and on recent NGS analyses (Klijin C et al, Nat Biotech 2015); however, among them, only 28 cell lines are derived from gastric cancer (GC). Almost all of the 28 GC cell lines were established many years ago, thereby, insufficient clinical and pathological information is attached. The wait is on for the establishment of new GC cell lines, especially from metastatic sites after therapy. 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 the Division of Genetics, we have newly established 59 diffuse-type GC-derived cell lines (NSC-1~49 series) from the cancer ascites of 34 patients. Now, we have 94 GC cell lines including 80 diffuse-type (new 59 and existing 21) and 14 intestinal-type, and also have 52 esophageal squamous cell carcinoma (ESCC) cell lines. In 2015, we successfully established six pancreatic and one ovarian cancer cell lines. 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, for in vivo preclinical study, their tumorigenicity and histopathological characteristics of PDXs, such as fibroblast rich-, hypovascular-, and dormant-state, were evaluated. Through collaboration with five pharmaceutical industries, in vitro and in vivo preclinical studies were conducted to derivate them to EPOC.

Basic Research and Development of Personalized Diagnosis and Treatment for GC and ESCC

Two major research projects, basic research and personalized medicine for GC and ESCC, are under way.

The study for GC: Diffuse-type solid tumors are often composed of a high proportion of rarely proliferating (that is, dormant) cancer cells, strongly indicating the involvement of cancer stem cells (CSCs). Although diffuse-type GC patients have a poor prognosis due to high-frequency development of peritoneal dissemination (PD), knowledge is limited about the PD-associated CSCs and efficacy of CSC-targeting therapy in diffuse-type GC. We established highly metastatic GC cell lines by in vivo selection designed for the enrichment of PD-associated GC cells. By microarray analysis, we found that C-X-C chemokine receptor type 4 (CXCR4) can be a novel marker for highly metastatic CSCs, since CXCR4-positive cells can grow anchorage-independently, initiate tumors in mice, be resistant to cytotoxic drugs, and produce differentiated daughter cells. In clinical samples, these CXCR4-positive cells were found from not only in the late metastasis stage (accumulated ascites) but also in the earlier stage (peritoneal washings). Moreover, treatment with transforming growth factor-β enhanced the anti-cancer effect of docetaxel via induction of cell differentiation/asymmetric cell division of the CXCR4-positive gastric CSCs even in a dormant state. Therefore,

differentiation inducers hold promise for obtaining the maximum therapeutic outcome from currently available anti-cancer drugs through re-cycling of CSCs (Fujita T et al, PLoS One 2015). Thus, the dormancy of tumor cells is a major problem in chemotherapy. One potential way to overcome chemo-resistance is to "wake up" these dormant cells. We showed that the opioid antagonist methylnaltrexone (MNTX) enhances the effect of docetaxel (Doc) by blocking a cell growth-suppressive pathway (Suzuki M et al, PLoS One 2015). PENK, which encodes opioid growth factor (OGF) and suppresses cell growth, was predominantly expressed in diffuse-type GCs. The blockade of OGF signaling by MNTX released cells from their arrest and boosted the effect of Doc. In comparison with the use of Doc alone, the combined use of Doc and MNTX significantly prolonged survival, alleviated abdominal pain, and diminished Doc-resistant spheroids on the peritoneal membrane in model mice. These results suggest that blockade of the pathways that suppress cell growth may enhance the effects of anti-tumor drugs.

In personalized medicine, we have developed mini DNA chips containing six marker and three control genes for predicting GC recurrence from peritoneal washings. Peritoneal cytology (CY) offers important prognostic information for GC after surgery; however, CY provides only a limited sensitivity and the task requires great skill. Our collaborating company continues to prepare a lot of supporting data for submitting the mini DNA chip to the Pharmaceuticals and Medical Devices Agency (PMDA) for marketing approval as an in vitro diagnostic (IVD).

The study for ESCC: Definitive chemoradiotherapy (CRT) is a less invasive therapy for esophageal squamous cell carcinoma (ESCC); however, the five-year survival rate of locally advanced ESCC patients was only 37%. Therefore, a prediction of CRT-responder is awaited. We have successfully identified 5 intrinsic subtypes of ESCCs by 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 that received CRT or surgery, respectively) and a validation set (167 cases containing 90 and 77 cases, respectively). Five intrinsic subtypes (1a/M1, 2a/I, 3b, 5/M2, 7/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-year 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. We previously reported that tumor-specific cytotoxic T-lymphocyte (CTL) activation signatures were preferentially found in long-term survivors. However, it is unknown whether the tumor-specific cytotoxic T-lymphocyte (CTL) activation is actually driven by CRT. We compared gene expression profiles among pre- and post-treatment biopsy specimens of 30 ESCC patients and 121 pre-treatment ESCC biopsy specimens. In the complete response (CR) cases, 999 overexpressed genes including at least 234 tumor-specific CTL-activation associated genes such as IFNG, PRF1, and GZMB, were found in post-treatment biopsy specimens. Clustering analysis using expression profiles of these 234 genes allowed us to distinguish the immune-activated cases, designating them as I-type, from other cases. However, despite the better CR rate in the I-type, overall survival was not significantly better in both these 30 cases and another 121 cases. Further comparative study identified a series of epithelial to mesenchymal transition-related genes overexpressed in the early relapse cases. Importantly, the clinical outcome of CDH2-negative cases in the I-type was significantly better than that of the CDH2-positive cases in the I-type. Furthermore, NK cells, which were activated by neutrophils-producing S100A8/S100A9, and CTLs were suggested to cooperatively enhance the effect of CRT in the CDH2-negative I-type. These results suggested that CTL gene activation may provide a prognostic advantage in ESCCs with epithelial characteristics (Tanaka Y et al, PLoS One 2015). Our findings may contribute not only to the elucidation of CRT responsiveness but also for future therapeutic development. To develop an IVD, we are collaborating with a pharmaceutical company.

List of papers published in 2015

Journal

1. Tanaka Y, Aoyagi K, Minashi K, Komatsuzaki R, Komatsu M, Chiwaki F, Tamaoki M, Nishimura T, Takahashi N, Oda I, Tachimori Y, Arao T, Nishio K, Kitano S, Narumi K, Aoki K, Fujii S, Ochiai A, Yoshida T, Muto M, Yamada Y, Sasaki H. Discovery of a Good Responder Subtype of Esophageal Squamous Cell Carcinoma with Cytotoxic T-Lymphocyte Signatures Activated by Chemoradiotherapy. PLoS One, 10:e0143804, 2015

2. Tanabe S, Aoyagi K, Yokozaki H, Sasaki H. Regulated genes in mesenchymal stem cells and gastric cancer. World J Stem Cells, 7:208-222, 2015

3. Saeki N, Ono H, Yanagihara K, Aoyagi K, Sasaki H, Sakamoto H, Yoshida T. rs2294008T, a risk allele for gastric and gallbladder cancers, suppresses the PSCA promoter by recruiting the transcription factor YY1. Genes Cells, 20:382-391, 2015

4. Yamanoi K, Arai E, Tian Y, Takahashi Y, Miyata S, Sasaki H, Chiwaki F, Ichikawa H, Sakamoto H, Kushima R, Katai H, Yoshida T, Sakamoto M, Kanai Y. Epigenetic clustering of gastric carcinomas based on DNA methylation profiles at the precancerous stage: its correlation with tumor aggressiveness and patient outcome. Carcinogenesis, 36:509-520, 2015

5. Saeki N, Komatsuzaki R, Chiwaki F, Yanagihara K, Sasaki H. A GSDMB enhancer-driven HSV thymidine kinase-expressing vector for controlling occult peritoneal dissemination of gastric cancer cells. BMC Cancer, 15:439, 2015

6. Higuchi Y, Kojima M, Ishii G, Aoyagi K, Sasaki H, Ochiai A. Gastrointestinal Fibroblasts Have Specialized, Diverse Transcriptional Phenotypes: A Comprehensive Gene Expression Analysis of Human Fibroblasts. PLoS One, 10:e0129241, 2015

7. Tanabe S, Komatsu M, Aoyagi K, Yokozaki H, Sasaki H. Implications of epithelial-mesenchymal transition in gastric cancer. Transl Gastrointest Cancer, 4:258-264, 2015

8. Naito Y, Oue N, Pham TT, Yamamoto M, Fujihara M, Ishida T, Mukai S, Sentani K, Sakamoto N, Hida E, Sasaki H, Yasui W. Characteristic miR-24 Expression in Gastric Cancers among Atomic Bomb Survivors. Pathobiology, 82:68-75, 2015

9. Suzuki M, Chiwaki F, Sawada Y, Ashikawa M, Aoyagi K, Fujita T, Yanagihara K, Komatsu M, Narita M, Suzuki T, Nagase H, Kushima R, Sakamoto H, Fukagawa T, Katai H, Nakagama H, Yoshida T, Uezono Y, Sasaki H. Peripheral opioid antagonist enhances the effect of anti-tumor drug by blocking a cell growth-suppressive pathway *in vivo*. PLoS One, 10:e0123407, 2015

10. Fujita T, Chiwaki F, Takahashi RU, Aoyagi K, Yanagihara K, Nishimura T, Tamaoki M, Komatsu M, Komatsuzaki R, Matsusaki K, Ichikawa H, Sakamoto H, Yamada Y, Fukagawa T, Katai H, Konno H, Ochiya T, Yoshida T, Sasaki H. Identification and Characterization of CXCR4-Positive Gastric Cancer Stem Cells. PLoS One, 10:e0130808, 2015

Department of Analytical Pathology

Nobuyoshi Hiraoka, Yoshinori Ino-Ishikawa

Introduction

In the Department of Analytical Pathology, the pathobiological and clinicopathological characteristics of the target molecules are analyzed for evaluating their potential significance in applying diagnostic or treatment use in the future. Expression of the molecules or genes in human tissues is assessed by morphological techniques, immunohistochemistry, RT-PCR, in situ hybridization, and so forth, 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

Tertiary lymphoid organs (TLOs) are induced in various inflamed tissues. There were two different localizations of pancreatic ductal carcinoma (PDC)-associated TLOs: intratumoral and peritumoral. The presence of intratumoral TLOs represents a microenvironment that has an active immune reaction, and shows a relatively intact vascular network being retained.

Education

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

Future prospects

We will answer requests from a selected project in various types of studies 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 2015

Journal

1. Hiraoka N, Ino Y, Yamazaki-Itoh R, Kanai Y, Kosuge T, Shimada K. Intratumoral tertiary lymphoid organ is a favourable prognosticator in patients with pancreatic cancer. Br J Cancer, 112:1782-1790, 2015

2. Yoshida A, Yoshida H, Yoshida M, Mori T, Kobayashi E, Tanzawa Y, Yasugi T, Kawana K, Ishikawa M, Sugiura H, Maeda D, Fukayama M, Kawai A, Hiraoka N, Motoi T. Myoepithelioma-like Tumors of the Vulvar Region: A Distinctive Group of SMARCB1-deficient Neoplasms. Am J Surg Pathol, 39:1102-1113, 2015

3. Yoshida M, Ogawa R, Yoshida H, Maeshima A, Kanai Y, Kinoshita T, Hiraoka N, Sekine S. TERT promoter mutations are frequent and show association with MED12 mutations in phyllodes tumors of the breast. Br J Cancer, 113:1244-1248, 2015

4. Oguro S, Ino Y, Shimada K, Hatanaka Y, Matsuno Y, Esaki M, Nara S, Kishi Y, Kosuge T, Hiraoka N. Clinical significance of tumor-infiltrating immune cells focusing on BTLA and Cbl-b in patients with gallbladder cancer. Cancer Sci, 106:1750-1760, 2015

Department of Functional Analysis

Fumitaka Takeshita, Megumi Miyagi

Introduction

The Department of Functional Analysis carries out functional analysis for the development of scientific-based diagnosis and pre-clinical studies in corporation with other departments in the Fundamental Innovative Oncology Core Center (FIOC).

In 2015, we supported the following projects:

1) The project in FIOC for the establishment of xenografts and cell lines derived from cancer patients.
2) Investigator-initiated clinical trial for treatment-resistant breast cancer patients with novel siRNA against ribophorin II (RPN2) gene.
3) Development of Diagnostic Technology for Detection of miRNA in Body Fluids, grant from AMED.
4) Training and counseling for techniques for developing cancer models through experiments on animals and in vivo imaging for cancer model animals.

Research activities

In our laboratory, evaluation of treatments with cancer model studies and imaging for gene medicine molecules such as microRNA are undertaken by making good use of imaging devices that detect luminescence and fluorescence from living animals (Kosaka, Anticancer Res, Urata, Sci Rep, Takahashi, Nat Commun, Ono, Pathol Int, Fujita, Oncotarget, Fujita, Mol Ther).

Clinical trials

An investigator-initiated clinical trial (first-in-human phase I study) for treatment-resistant breast cancer patients with novel siRNA against ribophorin II (RPN2) gene was started from July 2015. This clinical trial is conducted by the Department of Breast and Medical Oncology in the National Cancer Center (NCC) Hospital. We have evaluated the POC of RPN2-siRNA using clinical samples.

List of papers published in 2015

Journal

1. Fujita Y, Yagishita S, Takeshita F, Yamamoto Y, Kuwano K, Ochiya T. Prognostic and therapeutic impact of RPN2-mediated tumor malignancy in non-small-cell lung cancer. Oncotarget, 6:3335-3345, 2015

2. Fujita Y, Yagishita S, Hagiwara K, Yoshioka Y, Kosaka N, Takeshita F, Fujiwara T, Tsuta K, Nokihara H, Tamura T, Asamura H, Kawaishi M, Kuwano K, Ochiya T. The clinical relevance of the miR-197/CKS1B/STAT3-mediated PD-L1 network in chemoresistant non-small-cell lung cancer. Mol Ther, 23:717-727, 2015

3. Takahashi RU, Miyazaki H, Takeshita F, Yamamoto Y, Minoura K, Ono M, Kodaira M, Tamura K, Mori M, Ochiya T. Loss of microRNA-27b contributes to breast cancer stem cell generation by activating ENPP1. Nat Commun, 6:7318, 2015

4. Ono M, Tsuda H, Kobayashi T, Takeshita F, Takahashi RU, Tamura K, Akashi-Tanaka S, Moriya T, Yamasaki T, Kinoshita T, Yamamoto J, Fujiwara Y, Ochiya T. The expression and clinical significance of ribophorin II (RPN2) in human breast cancer. Pathol Int, 65:301-308, 2015

5. Urata YN, Takeshita F, Tanaka H, Ochiya T, Takimoto M. Targeted knockdown of the kinetochore protein D40/Knl-1 inhibits human cancer in a p53 status-independent manner. Sci Rep, 5:13676, 2015

6. Kosaka T, Davydova J, Ono HA, Akiyama H, Hirai S, Ohno S, Takeshita F, Aoki K, Ochiya T, Yamamoto M, Kunisaki C, Endo I. Imaging and antitumoral effect of a cyclo-oxygenase 2-specific replicative adenovirus for small metastatic gastric cancer lesions. Anticancer Res, 35:5201-5210, 2015

Department of Animal Experimentation

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

Introduction

A pivotal role of this Department is the establishment of cancer animal models (human cancer tissue/cell-transplanted immune-deficient mice) used for exploration/screening of molecular target drugs. The goal is to set up flexible models that have greater accuracy than previous ones using cancer cell lines. In experiments using immune-deficient/severely immune-deficient mice, the microbiological environment of the animal experimentation facility should be strictly controlled and technical staff take great care of the facility.

Research activities

The 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 cultured murine primary epithelial cells, so called organoids, could lead to the development of adenocarcinoma in the dorsal skin of immunedeficient mice. Notably, tumor initiation and subsequent stepwise progression from normal cells via precancerous 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. Large intestinal and pulmonary tissue-originated organoids have been newly established this year, and they are confirmed to lead to the development of adenocarcinoma by introduction of cancer-related genetic alterations.

Future prospects

The staff of the Department of Animal Experimentation are united in our resolve to establish a wide-ranging cancer animal model panel, which can be selected depending on the intended use.

List of papers published in 2015

Journal

1. Sakamaki A, Katsuragi Y, Otsuka K, Tomita M, Obata M, Iwasaki T, Abe M, Sato T, Ochiai M, Sakuraba Y, Aoyagi Y, Gondo Y, Sakimura K, Nakagama H, Mishima Y, Kominami R. Bcl11b SWI/SNF-complex subunit modulates intestinal adenoma and regeneration after γ-irradiation through Wnt/β-catenin pathway. Carcinogenesis, 36:622-631, 2015

Department of Cell Culture Technology

Tohru Kiyono, Farhana Ishrat Ghani, Chiho Kohno

Introduction

Human cells in culture have a limited lifespan and undergo a 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 a 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 that 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 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. A method for a long-term culture of these cell types has been established, and also can be immortalized by transduction of CDK4, cyclin D1 and TERT so as to be cultivated in more general culture conditions.

Future prospects

Our goal is to establish a cell culture method that can easily amplify every cell type including normal, pre-neoplastic and cancer cells. Our trials include organoid culture as well as conventional two-dimensional culture. Once cells-of-origin of every cancer can be easily amplified *in vitro*, they can be used for normal control cells for each cancer. The causal relationship of a gene mutation found in cancer and a certain phenotype such as drug resistance can be directly evaluated by transducing the mutant gene into them. They might also be applied to development of cell transplantation therapy.

Department of Bioinformatics

Mamoru Kato, Asmaa Elzawahry, Eisaku Furukawa, Joe Miyamoto, Momoko Nagai, Daichi Narushima

Introduction

The missions of our Department are 1) to develop new bioinformatics and data-analysis methods necessary for cancer studies, 2) to build new theories for understanding cancer through data analysis and computational approaches, and 3) bioinformatics analysis support for experimental groups in the Center as well as other research institutions.

Research activities

- We took charge of the bioinformatics part in the clinical sequencing project in the National Cancer Center (NCC). The bioinformatics part consists of 1) development of DNA-alteration calling programs, 2) development of medical information systems for clinical sequencing, and 3) construction of the computer network for clinical sequencing.

1) We improved our computer programs optimized for FFPE samples used in clinical sequencing. These programs detect SNV, indels, gene fusions, and copy number alterations from a large amount of data produced by the next generation sequencer (NGS). We compared our programs with other well-known programs (originally developed for cell-line and frozen samples for research purposes). We confirmed that our programs greatly outperformed the other programs. We also developed a special program that detects known alterations that were in principle hard to detect previously.
2) We developed a pipeline system for detection of somatic and germline alterations based on tumors and two types of normal tissue samples (matched and un-matched mixed normal). We also developed a program to output an Expert Panel report that integrates detected alterations with clinical information.
3) We set up a cluster machine and constructed a computer network for clinical sequencing in the NCC Central Hospital. We implemented the calling programs into the network to automatically run procedures from alteration detection to making a report.

- We performed big data analysis for trans-omics data of lung adenocarcinoma and hepatocellular carcinoma as a part of the Medical Big Data project, and found new prognosis markers.
- We provided bioinformatics support for studies such as those on liver cancer and bile duct cancer as a part of ICGC, pancreas cancer, tumor immunity, and cell-free DNA in the Division of Cancer Genomics; on DNA adductome, gene expression of cancer stem cells, and carcinogen DNA variants in the Division of Cancer Development System; and on germinoma in the Division of Brain Tumor Translational Research.
- We performed single-cell sequencing to reveal intra-tumor heterogeneity and cancer-cell evolution, collaborating with the Division of Cancer Genomics and Chiba Cancer Center. Using a mouse model for cancer development, we performed single-cell exome and transcriptome sequencing and developed pipeline programs to analyze the dynamics of cancer-cell subpopulations along the time-course.

Education

Our Department employed three technical staff members this year (two of them were staff-transferred) and educated them through on-the-job training. Our Department gave advice to bioinformatics technical staff in the Division of Cancer Genomics.

Future prospects

We will develop information technologies for clinical sequencing and promote technology transfer to implement precision 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 promote single-cell sequencing studies to reveal the dynamics of intra-tumor heterogeneity. We will keep continue to provide bioinformatics support for other groups in the Center.

List of papers published in 2015

Journal

1. Takai E, Totoki Y, Nakamura H, Morizane C, Nara S, Hama N, Suzuki M, Furukawa E, Kato M, Hayashi H, Kohno T, Ueno H, Shimada K, Okusaka T, Nakagama H, Shibata T, Yachida S. Clinical utility of circulating tumor DNA for molecular assessment in pancreatic cancer. Sci Rep, 5:18425, 2015

2. Ishino K, Kato T, Kato M, Shibata T, Watanabe M, Wakabayashi K, Nakagama H, Totsuka Y. Comprehensive DNA adduct analysis reveals pulmonary inflammatory response contributes to genotoxic action of magnetite nanoparticles. Int J Mol Sci, 16:3474-3492, 2015

3. Nakamura H, Arai Y, Totoki Y, Shirota T, Elzawahry A, Kato M, Hama N, Hosoda F, Urushidate T, Ohashi S, Hiraoka N, Ojima H, Shimada K, Okusaka T, Kosuge T, Miyagawa S, Shibata T. Genomic spectra of biliary tract cancer. Nat Genet, 47:1003-1010, 2015

4. Takenaka M, Saito M, Iwakawa R, Yanaihara N, Saito M, Kato M, Ichikawa H, Shibata T, Yokota J, Okamoto A, Kohno T. Profiling of actionable gene alterations in ovarian cancer by targeted deep sequencing. Int J Oncol, 46:2389-2398, 2015

5. Saito M, Shimada Y, Shiraishi K, Sakamoto H, Tsuta K, Totsuka H, Chiku S, Ichikawa H, Kato M, Watanabe S, Yoshida T, Yokota J, Kohno T. Development of lung adenocarcinomas with exclusive dependence on oncogene fusions. Cancer Res, 75:2264-2271, 2015

Department of Omics Network

Masaru Katoh

Introduction

The Department of Omics Network, established in August 2014, is derived from Masaru Katoh's laboratory, which was also known as the Genetics and Cell Biology Section (from 1998 to 2009) and the Katoh's Unit (from 2009 to 2014). The Department is involved in innovation based on the balance between its main world-class projects and cutting-edge new projects. Masaru Katoh has been changing his field in medical and life sciences: he was engaged in clinical medicine from 1986 to 1990, basic medicine from 1990 to 2002 and information science from 2003 to 2011. Since 2012, Masaru Katoh has been engaged in the Knowledgebase Project with the slogan "Back to the Medicine".

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) are the main (fundamental) projects of the Department. Cell adhesion (PMID 25865774), epigenetics (PMID 26411517) and microRNA (miRNA) (PMID 25364765) are new (cutting-edge) projects of the Department. The fundamental projects and cutting-edge projects are the essential constituent parts of the Department (Figure 1).

Research activities

Genomics, transcriptomics, proteomics and metabolomics are representative "omics" disciplines of life science that deal with the entirety of genes, transcripts, proteins, and metabolites, respectively. Omics medicine is an emerging discipline of medical science that produces large amounts of omics data on genetics, genomics, epigenetics, transcriptomics, proteomics, and metabolomics. Omics medicine consists of three layers: the first layer corresponds to clinical medicine that involves patients' care and clinical sampling of blood and tissues (bio-banks); the second layer corresponds to basic medicine that produces high-throughput omics data using microarrays and next-generation sequencing technologies (databases); and the third layer corresponds to translational medicine that generates knowledge on mechanisms of pathogenesis, diagnostics, therapeutics, and so forth (knowledge base). The goal of the Department is the establishment of a knowledge base focused on the regulatory signaling network for the development of novel diagnostics and therapeutics. Integrative clinicopathological analyses have been carried out in the Department using the solid data on genomics, epigenomics, proteomics, exosome, and so forth.

ASXL1, ASXL2 and ASXL3 are epigenetic regulators associated with BAP1, EZH2 and nuclear hormone receptors. Germline mutations in the *ASXL* family genes cause Bohring-Opitz and related syndromes presented with intellectual disability, cranioskeletal features and feeding problems. Somatic alterations in the *ASXL* family genes occur in hematological malignancies, such as myelodysplastic syndrome (MDS), myeloproliferative neoplasms (MPN), acute myeloid leukaemia (AML) and chronic lymphocytic leukaemia (CLL), as well as solid tumors, such as colorectal cancer with microsatellite instability (MSI), breast cancer, prostate cancer and pancreatic cancer. ASXLs themselves are not appropriate drug targets, because ASXLs are adaptor molecules without their intrinsic enzymatic function. At present, there are no therapeutics for cases with ASXL mutations.

ALK, CSF1R (FMS), DDR2, EGFR, ERBB2, EFBB3, ERBB4, FGFR1, FGFR2, FGFR3, FGFR4, FLT3, IGF1R, KIT, MET, PDGFRA, RET and

VEGFR2 (KDR) are receptor tyrosine kinases (RTKs) on the Oncomine Cancer Research Panel (OCP) for clinical sequencing. FGFR1, FGFR2, FGFR3 and FGFR4 are activated in breast cancer, gastric cancer, glioblastma, leukemia, lung cancer, prostate cancer, soft-tissue sarcomas, and so forth, owing to gene amplification, gene fusion or gain-of-function mutation. AZD4547, BGJ398 and dovitinib are representative FGFR inhibitors that are in clinical trials for cancer patients with FGFR alterations. The tumor microenvironment consists of cancer cells and stromal/immune cells, such as cancer-associated fibroblasts (CAFs), endothelial cells, myeloid-derived suppressor cells (MDSCs), tumor-associating macrophages of M2 type (M2-TAMs) and regulatory T (Treg) cells. Therapeutics targeting cancer cells, tumor angiogenesis and immune evasion are ongoing themes of the in-house study in the Department.

CD44 is a cell-adhesion molecule that functions as a hyaluronan receptor as well as a co-receptor of growth factors and is utilized as a functional biomarker of cancer stem cells. Exploration of drug targets and development of disease biomarkers related to CD44 and other cell-adhesion molecules are ongoing themes of the international collaboration study in the Department.

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 reviews of grant proposals or journal manuscripts written in English 64 times in 2015. Katoh is an Academic Editor of *PLoS ONE*, and carried out editorial decisions 102 times in 2015. 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 113 editorial board members.

The manuscript citation count in the Web of Science Database (Thomson Reuters) is a surrogate marker of the contribution to the global science community. Katoh's manuscripts were cited at least 640 times by others in 2015.

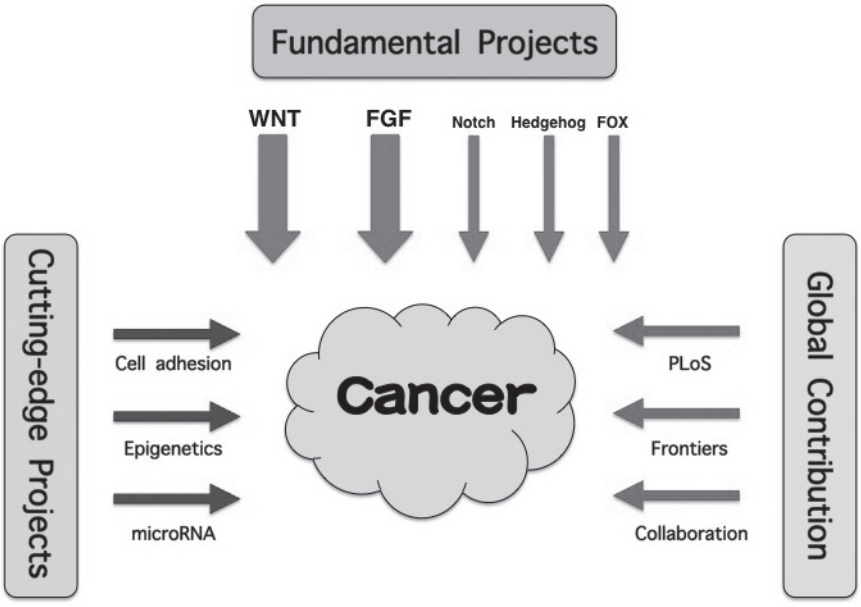


Figure 1. Fundamental Projects, Cutting-edge Projects and International Contribution in the Department of Omics Network

List of papers published in 2015

Journal

1. Jiang WG, Sanders AJ, Katoh M, Ungefroren H, Gieseler F, Prince M, Thompson SK, Zollo M, Spano D, Dhawan P, Sliva D, Subbarayan PR, Sarkar M, Honoki K, Fujii H, Georgakilas AG, Amedei A, Niccolai E, Amin A, Ashraf SS, Ye L, Helferich WG, Yang X, Boosani CS, Guha G, Ciriolo MR, Aquilano K, Chen S, Azmi AS, Keith WN, Bilsland A, Bhakta D, Halicka D, Nowsheen S, Pantano F, Santini D. Tissue invasion and metastasis: Molecular, biological and clinical perspectives. Semin Cancer Biol, 35:S244-S275, 2015
2. Katoh M. Functional proteomics of the epigenetic regulators ASXL1, ASXL2 and ASXL3: a convergence of proteomics and epigenetics for translational medicine. Expert Rev Proteomics, 12:317-328, 2015