Advanced Clinical Research Center

Division of Clinical Genome Research
臨床ゲノム腫瘍学分野

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Research Projects

The aim of our research is the application of findings in basic cancer research to clinics. Currently, we are working on the following six projects: 1) identification of novel molecular targets for the treatment of colorectal cancer, 2) understanding the role of Wnt/β-catenin signaling pathway in human carcinogenesis, 3) discovery of Wnt inhibitors through a screening of large-scale chemical libraries, 4) development of therapeutic strategies for intrahepatic cholangiocarcinoma using syngeneic mouse tumor cells, 5) elucidation of molecular mechanisms underlying pseudomyxoma peritonei, and 6) clinical sequencing for the implementation of genomic medicine.

1. Identification of novel molecular targets for the treatment of colorectal cancer

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Epigenetic modifications such as DNA methylation and histone modification alter gene expression by the change of chromatin structure. Accumulated evidence has demonstrated that aberrant epigenetic modifications are involved in carcinogenesis. Bromodomains have been known as a protein-interaction module that recognizes acetylated lysine residues. Through this interaction, protein containing a bromodomain(s) directs the assembly of nuclear factor complexes to their target sites on chromatin, resulting in the transcriptional activation. Recently, we found that bromodomain containing 8 (BRD8) was frequently accumulated in colorectal cancer. Transcriptome analysis coupled with genome-wide mapping of BRD8-binding sites disclosed that BRD8 regulates the expression of multiple subunits of the pre-replicative complex in concert with the activator protein-1. Depletion of BRD8 induced cell-cycle arrest at the G1 phase, and reduced cell proliferation in vitro and in vivo. Importantly, we found that the bromodomain of BRD8 is indispensable for not only the interaction with histone H4 or transcriptional regulation but also its own protein stability. These findings suggested that the bromodomain will serve as a therapeutic target for colorectal cancer.

Since Brd8 is abundantly expressed in the testis, we additionally generated testis-specific *Brd8* knockout mice to investigate its roles in male fertility. Interestingly, the *Brd8* knockout mice exhibited reduced testis size and decreased sperm counts. Consistent with these features, immunohistochemical analyses using stage-specific markers of testicular cell differentiation suggested that Brd8 plays an important role in the regulation of spermatogenic differentiation. A comprehensive phenotypic and molecular characterization of these mice will help us understand how Brd8 is involved in male fertility.

2. Understanding the role of Wnt/β-catenin signaling pathway in human carcinogenesis

Saya Nakagawa, Kiyoshi Yamaguchi, Yoichi Furukawa

Key components of the Wnt/β-catenin pathway, such as APC, β-catenin, and AXIN1, are frequently mutated in human tumors particularly in colorectal and liver cancers. These mutations result in the stabilization of β-catenin, leading to its accumulation in the cytoplasm and nucleus. This activates the TCF/LEF transcription complex, resulting in the enhanced expression of target genes. Although a number of target genes of the β-catenin/TCF/Lef complex have been identified, comprehensive understanding of the downstream molecules with altered expression by this signal pathway is necessary to clarify the molecular basis of colorectal and liver cancer cells. In our earlier study, we demonstrated that the expression of histidine ammonia-lyase (*HAL*) and arginase1 (*ARG1*), two genes associated with catabolism of histidine and arginine, respectively, was suppressed by the Wnt/β-catenin signaling in liver cancer cells. Consistent with the findings, suppression of the signaling induced the expression of HAL and ARG1, and decreased the intracellular levels of histidine and arginine, corroborating that the Wnt/β-catenin signaling plays a role in regulating amino acid metabolism. Further studies will provide a deeper understanding of the molecular mechanisms involved in colorectal and liver cancer.

3. Discovery of Wnt inhibitors through a screening of large-scale chemical libraries

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Although a number of research groups and companies have tried to develop Wnt inhibitors, successful development of the inhibitors has not been achieved. We previously developed a robust cell-based reporter assay for the detection of the Wnt/β-catenin signaling activity, and established a high-throughput screening system. A screening of libraries containing more than twenty-thousand small molecules and natural products identified several hit compounds that inhibited the Wnt/β-catenin signaling activity. Characterization of the compounds and their mode of action are currently under investigation.

4. Development of therapeutic strategies for intrahepatic cholangiocarcinoma using syngeneic mouse tumor cells

Kiyoko Takane, Yoichi Furukawa

Genetically engineered mice are useful tools for studying human diseases including cancer. In this project, we previously generated a novel mouse model of intrahepatic cholangiocarcinoma (ICC) using liver-specific expression of oncogenic *Kras* and homozygous *Pten* deletion (AKPP: *Alb-Cre*$^{+}$; *LSL-Kras*$^{G12D/+}$; *Pten*$^{flox/flox}$). Subsequently, a cell line was established from the ICC of AKPP mice and named AKPP cells. We confirmed that AKPP cells showed high expression of ICC markers, including cytokeratin-19 and pan-cytokeratin, while expressing low levels of the hepatocellular carcinoma marker HNF4α. To investigate the downstream effects expected from the loss of Pten and the activation of Kras, the phosphorylation levels of Akt (Ser473) and Erk1/2 were analyzed by immunoblotting. As a result, the phosphorylation of these downstream effectors was increased in AKPP cells compared with the non-malignant cholangiocyte line 603B. Consistent with the observations, pharmacological inhibition of PI3K potently suppressed AKPP cell proliferation, and the combination of KRAS G12D-specific inhibition with PI3K inhibition exhibited a synergistic effect in reducing the cell viability. To evaluate the biological properties of AKPP cells in vivo, we transplanted the cells into syngeneic immunocompetent mice and confirmed that they retained the characteristics of ICC.

5. Elucidation of molecular mechanisms underlying pseudomyxoma peritonei

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Pseudomyxoma peritonei (PMP) is a rare disease and is characterized by an excessive accumulation of mucin in the abdominal cavity. This disease is caused by neoplasms that originate primarily from the appendix, and frequent mutations of the *KRAS* and *GNAS* genes have been found in the tumor. We previously performed RNA-seq analysis of ten PMPs and their matched non-tumorous colonic epithelium. As a result, we identified a total of 32 differentially expressed genes between the tumors and non-tumorous colonic epithelium. A cell-of-origin subtype analysis with the nearest template prediction algorithm cor-

roborated that PMP cells belonged to the goblet cell subtype, suggesting that the tumor cells appear to differentiate into goblet cells or originate from goblet cells.

In addition, we performed genome-wide DNA methylome analysis of appendiceal PMP samples, and consequently found that the PMPs were classified into at least two epigenotypes, unique methylation epigenotype and normal-like methylation epigenotype. We are currently investigating the molecular function of genes whose expression is decreased by the DNA methylation in PMP. Our efforts will contribute to the better understanding of molecular mechanisms underlying PMP, and help in the diagnosis, treatment, and prevention of this life-threatening disease.

6. Clinical sequencing for the implementation of genomic medicine

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The application of Next-Generation Sequencing (NGS) technology in clinical medicine has revolutionized molecular diagnostics by enabling multiple gene testing, or analysis of the entire exon or whole genome with a limited amount of DNA. In collaboration with Human Genome Center and Advanced Clinical Research Center, we have been working on three projects: 1) genetic diagnosis of patients with suspected hereditary cancer predisposition, 2) application of nanopore sequencing technology for the detection of complex structural rearrangements, and 3) implementation of precision medicine for patients with rare or intractable cancer.

In the first project, we performed whole genome sequencing for molecular diagnostics of hereditary colon cancer syndromes such as familial adenomatous polyposis (FAP), Lynch syndrome (LS), and polymerase proofreading-associated polyposis (PPAP). Through genetic counseling, we provided patients and/or their families with appropriate information about these hereditary diseases and collaborated with clinical psychologists to deliver psychological care. In the second project, we have been analyzing complex structural rearrangements and alterations of DNA methylation patterns in tumors using Nanopore PromethION platform. As a result, we could identify breakpoints of pathogenic structural variants that were difficult to detect using short-read sequencing technologies. In the third project, we have been working on the clinical implementation of genomic data. We offered consultation of genetic analysis to patients with rare or intractable cancer as a clinical research in IMSUT hospital. The study enrolled patients with various types of cancer who provided written informed consent for genetic analysis and treatment prediction using artificial intelligence. Genetic alterations in their tumors were determined by NGS, and the data were subsequently analyzed by QIAGEN Clinical Insights (QCI). Actionable variants were reviewed and discussed in a tumor board to determine recommended therapeutic options. This multidisciplinary board, comprised of physicians, medical oncologists, genetic counselors, geneticists, bioinformaticians, and ethics experts, convened online every two weeks.

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