Matching event●

Establishment of 137 patient-derived cancer cell lines from the ascites with omics information and their high utility in translational research

Hiroki Sasaki

Chief, Division of Biomarker Discovery

Exploratory Oncology Research & Clinical Trial Center

Chief, Department of Translational Oncology

Fundamental Innovative Oncology Core Center

National Cancer Center

Genome-wide genetic information in about 1,000 cancer cell lines is available on COSMIC DB (Sanger Center, UK); however, among them, only 28 cell lines are derived from gastric cancer (GC). Since driver gene mutation frequency in a certain cancer is often less than 5%, the establishment of cell lines from each patient to be analyzed is desired for functional selection of driver gene mutations. Furthermore, almost all of the 28 GC cell lines were established many years ago, thereby, the clinical and pathological information is insufficient. 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.

In 2010-2018, from the ascites, we successfully established 90 diffuse-type GC cell lines (National Cancer Center Stomach Cancer (NSC) series) from 53 patients, and also established 34 pancreatic and 9 ovarian cancer cell lines, and more. We are conducting omics analyses for gene expression and copy number variation, and hot spot- and genome wide-gene alteration in t hese cell lines. Moreover, for in vivo preclinical study, their tumorigenicity and histopathological c haracteristics in the xenograft, such as fibroblast rich-, hypovascular-, and dormant-state, were e valuated. Through collaboration with some pharmaceutical industries, in vitro and in vivo preclinical studies have been conducted to derivate clinical trials in our hospital.

Here I will present the characteristics of the patient’s derived cancer cell lines and will underline their utility in translational research.

Matching event●

Establishment of gastric cancer Patient-Derived-Xenograft (PDX) models and cell lines for new drug development

Takeshi Kuwata
Chief
Department of Pathology and Clinical Laboratories
National Cancer Center Hospital East

[Background] It is important to have an appropriate pre-clinical model for developing new drugs. From May 2015, we have been conducting patient derived xenograft models as well as cell lines establishment from gastric cancer (GC) patients in NCC.
[Result] Total 250 GC patients, including 233 patients underwent surgery and 17 patients underwent Cell-free and Concentrated Ascites Reinfusion Therapy (CART), were enrolled. From 233 surgically resected GC specimen, 36 PDXs and 23 cell lines have been established. From 17 CART specimen, 1 PDXs and 8 cell lines have been established. Totally we have 37 PDX GC models and 24 GC cell lines, and among these 20 cases have both PDX and cell lines. All the GC PDX tumors have been confirmed pathologically and more than 80% of PDX tumors showed an intestinal phenotype. Immunohistochemical examination identified HER2 positive GC tumors as well. NGS-based gene mutation analysis revealed repetitive so-called driver mutations (SNV/Indel) in several genes including KRAS, PIK3CA and PTEN. In some cases, gene mutation profiles were compared between primary tumor, PDX and cell lines, and most of drive mutations were observed in all specimens.
[Conclusion and future perspective] We have established new GC PDXs and/or cell lines, supplemented with clinicopathological information, for accelerating new drug development. This bio-resource may help pre-clinical studies as well as finding new biomarkers for treating GC patients.

Matching event●

Chemotherapy payload of anti-insoluble fibrin antibody drug conjugate is released exclusively on the fibrin clot within aggressive solid tumors.

Yasuhiro Matsumura
Director
Division of Developmental Therapeutics
Exploratory Oncology Research & Clinical Trial Center
National Cancer Center

The first study of the enhanced permeability and retention (EPR) effect was published in 1986. Since then, various formulations of drug delivery system (DDS) based on the EPR effect have been developed and applied in clinical settings. Meanwhile, we reported that tissue factor (TF) on cancer cell surfaces and hemorrhage caused by cancer erosion can induce fibrin clot formation, as well as tumor vascular permeability. Accordingly, common cancers possess a fibrin-rich stroma that hinders the distribution of macromolecular DDS, including monoclonal antibodies (mAbs) and antibody–drug conjugates (ADCs). Recently, we developed an anti-insoluble fibrin (IF) mAb that does not react with fibrinogen or soluble fibrin. We subsequently created an ADC consisting of the anti-IF mAb conjugated with an ACA via a Val-Leu-Lys linker, which is severed specifically by the fibrinolytic protease plasmin. The anti-IF mAb ADC (IF-ADC) can selectively extravasate through leaky tumor vessels, bind to specific pits in fibrin clots that are uncovered only when a fibrin clot forms, and create a scaffold from which effective sustained release of the free ACA occurs. Because plasmin is only active on insoluble fibrin and is neutralized by the innate α2-plasmin inhibitor circulating in the blood, free ACA is only released when the IF-ADC is bound to the previously hidden epitopes in the pits on insoluble fibrin. This free ACA can easily reach cancer cells by diffusion through the stromal barrier. Another benefit of this approach is that ACA released from IF-ADC can also attack vascular endothelial cells. In this project, we will develop ADCs using mAbs recognizing IF produced in tumor tissues, and optimize these ADCs during future clinical development with the goal of achieving cancer stromal targeting (CAST) therapy.

(References)

1. Prince R, Matsumura Y, Angelillo-Scherrer A. et al Targeting anticoagulant protein S to improve hemostasis in hemophilia. Blood 131, 1360-1371 (2018).
2. Fujiwara Y, Furuta M, Manabe S, Koga Y, Yasunaga M, Matsumura Y. Imaging mass spectrometry for the exquisite design of antibody-drug conjugates. Sci. Rep. 6, 24954 (2016).
3. Obonai T, Fuchigami H, Furuya F, Kozuka N, Yasunaga M, Matsumura Y. Tumour imaging by detecting fibrin clots in tumour stroma with anti-fibrin Fab fragment. Sci. Rep. 6, 23613 (2016).
4. Hisada Y, Yasunaga M, Hanaoka S, Saijou S, Sugino T, Tsuji A, Saga T, Tsumoto K, Manabe S, Kuroda J, Kuratsu J, Matsumura Y. Discovery of an uncovered region in fibrin clots and its clinical significance. Sci. Rep. 3, 2604 (2013)
5. Matsumura Y. Cancer stromal targeting (CAST) therapy Adv. Drug Deliv. Rev. 64, 710-719 (2012) .
6. Matsumura Y, Maeda H. A new concept for macromolecular therapeutics in cancer chemotherapy: Mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. Cancer Res. 46, 6387-6392 (1986).

Matching event●

Development of novel transcription-based cancer therapies

Susumu S. Kobayashi
Chief
Division of Translational Genomics
Exploratory Oncology Research & Clinical Trial Center
National Cancer Center

Recent progress of genetic studies has dramatically unveiled pathogenesis of cancer. In addition to conventional cytotoxic agents, targeted therapies and immunotherapies have revolutionized the way to treat cancers such as chronic myeloid leukemia, melanoma, and non small cell lung cancer. However, overall survival of cancer, particularly advanced cancer, remain unsatisfactory due to primary and acquired resistance to current therapies. Therefore, development of novel therapeutics is required. It has been shown that CCAAT/Enhancer Binding Protein α (C/EBPα) is one of crucial transcription factors that induce differentiation and growth arrest in many types of cells. Mice deficient in C/EBPα demonstrate a block of differentiation in myeloid cells, hepatocytes, and alveolar cell. Furthermore, C/EBPα are perturbed in many cancers through multiple mechanisms, such as transcriptional silencing, translational inhibition, posttranslational modification, decrease in DNA binding, or point mutations. We have previously demonstrated that transfection of C/EBPα can induce differentiation and growth arrest of leukemia cells and lung cancer cells *in vitro*. Moreover, C/EBPα is expressed in many tissues such as skin, pancreas, adipocytes, prostate, and liver. Therefore, we hypothesize that an increase in expression/activity of C/EBPα may induce differentiation and subsequent apoptosis of cancer cells, leading to elimination of tumors. To answer this question, we have established a cell-based high-throughput screening using cell lines in which C/EBPα activity is detected by luciferase assay. Among several hits identified, 2-[(*E*)-2-(3,4-dihydroxyphenyl)vinyl]-3-(2-methoxyphenyl)-4(3H)-quinazolinone (ICCB280), induced myeloid differentiation of leukemia cell lines accompanied by increased expression of C/EBPα and its downstream target genes. Primary blast cells isolated from human AML patients treated with ICCB280 demonstrated evidence of differentiation and massive apoptosis. To further characterize chemical and biological nature of ICCB280, we are aiming to: (1) ***Explore molecular mechanisms and functions of ICCB280;*** and (2) ***Conduct structure-activity-relationship studies to improve the potency of ICCB280***. We believe that the approach described here will lead to development of novel differentiation therapies and significantly impact care of patients with various cancers with unfavorable prognosis in the near future.

Matching event●

A Novel method for screening peptide-hormone receptors

Shosei Kishida
Professor, Kagoshima University
Akihiro Asakawa
Professor, Kagoshima University
Yasuhito Uezono
Chief, Department of Supportive Care Research
Exploratory Oncology Research & Clinical Trial Center
National Cancer Center

After identification of human whole genome was completed in 2003, the clarification about the function of each gene have been become more important in the post genome era. Taken that membrane proteins consists more than 30 % of human 25000 genes and 60 % of the commercially available drugs target membrane/membrane-associated proteins (ligands, receptors, RTK, etc), analyses of interaction of membrane proteins are still considered to be an important method for the drug discovery.

However, it is not so easy to handle with membrane proteins. They are assumed to have physiological structures embedded in the lipid-layers. Expression and analyses of recombinant membrane proteins are often difficult due to their hydro-phobic properties.

We developed a novel modified assay system using yeasts for intermolecular interactions of membrane-associated proteins (ligands, receptors, transmembrane proteins, etc), whereas original two-hybrid method could not handle with membranous proteins or ligands. It enables rapid and efficient screening of several interactions such as receptor-ligand interaction, dimerization of membrane proteins etc. Novel receptors for peptide hormones, neuropeptides could be identified theoretically by our new method, which might lead to the understanding of its signal transduction, the discovery of novel agonists and antagonists for interesting ligands. Actually, we found three novel interaction of ligand-membranous proteins which are not reported before.

Recently, we are proceeding the project for identifying new receptors for neuropeptides including ghrelin and trying to extend our method for detection of interaction of non-peptide ligands and their receptors.

We would like to find collaborators/companies which are interested in the molecular interactions we have found or interested in our method to seek out unknown counterparts of ligands or receptors or chemicals.