Research Center for Medical Sciences Core Research Facilities for Basic Science (Division of Molecular Cell Biology)

Yoshinobu Manome, *Professor and Director*
Takeo Iwanoto, *Professor*
Keiichi Ikeda, *Assistant Professor*
Akihito Tsubota, *Professor*
Toshiaki Tachibana, *Professor*
Kouki Fujioka, *Assistant Professor*

General Summary

Core Research Facilities for Basic Sciences (Division of Molecular Cell Biology) was established on April 1, 2014. The mission of our facilities is the facilitation of research in the university. Two systems are constituted for the use of our facilities.

1. Annual Registration System

This system is intended to supply research benches and other equipment to researchers of the university to perform experiments. Once registered, researchers can freely use the various devices in our institution. This system also provides, if necessary, technical advice and guidance on specific fine-morphological or biochemical approaches to a registrant's experiment. In 2018, 160 researchers registered at our annual registration system, and we provided research support 214 times for electron microscopy and 1 time for laboratory experiments.

2. System for Providing Research Services

Advances in research technologies and equipment enable us to perform more precise and accurate observations of specimens in medical sciences. For researchers who cannot perform experiments owing to limits of time and funds, our staff can prepare samples for scanning electron microscopy and transmission electron microscopy, record images, and perform high-performance liquid chromatography and mass spectrometry. The service fee is minimal because services are limited to the university.

Research Activities

Investigation of the nicotinamide phosphoribosyltransferase as a potential target for treatment of malignant brain tumors

Mutations in isocitrate dehydrogenases 1/2 are involved in the development of brain tumors, and research on treatment by intervention of this pathway is actively in progress. However, such proteins as epithelial cell growth factor receptor and nicotinamide phosphoribosyltransferase (NAMPT) are overexpressed in brain tumors and are considered to be performant and useful targets of treatment. In particular, NAMPT is a rate-limiting step enzyme of mammalian nicotinamide adenine dinucleotide synthesis, and the amounts of messenger RNA of the enzyme are directly correlated with the prognosis of patients. Therefore, modulation of the NAMPT transcripts is useful as an adjunct to radiation therapy and chemotherapy. This year, vectors transcribing short hairpin interference RNA to NAMPT were constructed and cell lines of stably transcribed short hairpin interference

RNA were established. We are using these cell lines to investigate the effects of NAMPT suppression on cell cycles, growth, drug and radio resistance, and colony-formation of malignant brain tumor.

Establishment of diagnostic method for papillary thyroid carcinoma by measuring tumor-associated antigen

We are currently investigating a clinical application of a monoclonal antibody to papillary thyroid carcinoma established by Professor Takeyama of the Department of Surgery of The Jikei University. In thyroid cells, when quiescent, phosphorylated Yes-associated protein (YAP) is anchored in the cytoplasm, and cells do not proliferate because the activities of phosphoinositide 3 kinase and pyruvate dehydrogenase kinase 1 are not sufficient to activate the Hippo pathway. However, malignant thyroid carcinoma SW1,736 cells have been shown to dephosphorylate YAP and translocate to the nucleus, even in the cells are confluent or in a serum-deprived condition or both. Because the antigen recognized by the monoclonal antibody is glycosylated and localize on the cell surface, the relationship of the antigen with the turbulence of the Hippo pathway is being investigated with cell fractionations and measurements of the quantities of total YAP and phosphorylated YAP.

Characteristics of palmitoyl protein thioesterase 1 and tripeptidyl peptidase 1 enzymes in dried blood spots and leukocytes from patients with neuronal ceroid lipofuscinosis 1 or 2 and their application to newborn screening

We first characterized the enzymes palmitoyl protein thioesterase 1 (PPT1) and tripeptidyl peptidase 1 (TPP1) in dried blood spots (DBSs), plasma/serum, and leukocytes/lymphocytes from patients with neuronal ceroid lipofuscinosis (NCL) 1 or 2 and from control subjects. The enzyme PPT1 had only 1 acid form in control DBSs, plasma/serum, and leukocytes/lymphocytes and showed deficient activities in these samples from patients with NCL 1. In contrast, TPP1 in control DBSs and leukocytes/lymphocytes consisted of 2 forms, an acidic form and a neutral form, whereas serum TPP1 had only a neutral form. In control subjects, the optimal pH of PPT1 in DBSs, plasma/serum, and leukocytes/lymphocytes was 4.5 to 5.0 in the acidic form, whereas the optimal pH of TPP1 was 4.5 in control DBSs and 6.5 in leukocytes/lymphocytes. Regarding samples from patients with NCL 1 or 2, both PPT1 and TPP1 activities in DBSs, plasma, and leukocytes/lymphocytes were markedly reduced in acidic pH, whereas those from heterozygotes with NCL 1 or 2 in the acidic form showed activities intermediate between those of patients and control subjects. In neutral conditions, pH 6.0, the PPT1 activities in patients with NCL 1 showed higher residual activities and intermediate activities in heterozygotes in NCL 1, which was probably caused by mutated proteins in 3 patients with NCL 1. The activities of TPP1 at neutral pH 6.5 to 7.0 in DBSs and leukocytes/lymphocytes were higher in patients with NCL 2 and in heterozygotes. The reason for the increases of neutral TPP1 activities at pH 6.5 to 7.0 in NCL 2 DBSs and leukocytes/lymphocytes is obscure, but the increases might have been caused by secondary activation of neutral TPP1 due to the absence of the acidic form. Interestingly, TPP1 activity in serum consisted of only a neutral form, no an acidic form, and was not deficient in any patient with NCL 2. Therefore,

we can diagnose NCL 1 in patients via a plasma/serum enzyme assay for PPT1, but we cannot diagnose NCL 2 via a serum TPP1 enzyme assay. A pilot study of neonatal screening of NCL 1 and 2 has been established by assays of DBSs from more than 1,000 neonates. Using this assay system, we will be able to perform neonatal screening for NCL 1 and 2 via DBS assays.

Platelets play an essential role in murine lung development through C-type lectin-like receptor 2/podoplanin interaction

Platelets participate in thrombosis, hemostasis, and other pathophysiological processes, including tumor metastasis and inflammation. However, the putative role of platelets in the development of solid organs has not yet been described. Here, we report that platelets regulate lung development through the interaction between the platelet-activation receptor, C-type lectin-like receptor-2 (Clec-2; encoded by the C-type lectin domain family 1, member b gene [*Clec1b*]) and its ligand, podoplanin, a membrane protein. Deletion of Clec-2 in mouse platelets led to lung malformation, which caused respiratory failure and neonatal lethality. In these embryos, alveolar duct myofibroblasts positive for α-smooth muscle actin were almost completely absent in the primary alveolar septa, which resulted in a loss of alveolar elastic fibers and in lung malformation. Our data suggest that the lack of alveolar duct myofibroblasts is due to the abnormal differentiation of lung mesothelial cells, the major progenitors of alveolar duct myofibroblasts. In the developing lung, podoplanin expression is detected in alveolar epithelial cells, lung mesothelial cells, and lymphatic endothelial cells. Lymphatic endothelial cell-specific podoplanin knockout mice showed neonatal lethality and Clec1b2/2-like lung developmental abnormalities. Notably, these Clec1b2/2-like lung abnormalities were also observed after thrombocytopenia or transforming growth factor β depletion in fetuses. We propose that the interaction of Clec-2 on platelets and podoplanin on lymphatic endothelial cells stimulates alveolar duct myofibroblast differentiation of lung mesothelial cells through transforming growth factor β signaling and, thus, regulates normal lung development.

Human hepatocyte chimeric mice and an animal model of hepatitis virus infection

We have established human hepatocyte chimeric mice with an efficient method that we had developed and then used the mice to establish an animal model of infection with hepatitis B virus (HBV) or hepatitis C virus (HCV). We are now investigating the efficacy of novel antiviral agents, the mechanism of progression to chronic infection, and ultrastructural alterations of intrahepatocellular organelles after viral eradication.

Single nucleotide polymorphisms, and resistant-associated variants in the treatment of chronic HCV infection

Direct-acting antiviral agents are the first-line treatment for chronic HCV infection. We are investigating the association of single nucleotide polymorphisms of genes with the blood drug concentration, treatment response, and direct-acting antiviral agent-induced liver damage. Resistant-associated variants are also being investigated in detail.

The association between serum microRNA expression levels and treatment outcome/prognosis in hepatocellular carcinoma
We measure serum microRNA expression levels in intrahepatic feeding arteries, proper hepatic arteries, and peripheral veins when we perform transarterial chemoembolization for patients with hepatocellular carcinoma (HCC) and are investigating the association between serum microRNA expression levels and prognosis/outcome of treatment in patients with HCC who have been treated with transarterial chemoembolization plus radiofrequency ablation.

Intrahepatic cellular localization of ATPase copper transporter beta
The protein ATPase copper transporter beta (ATP7B), also known as Wilson disease protein, is a copper-transporting P-type ATPase that is encoded by the ATPase copper transporting beta gene (*ATP7B*). This protein is located in the trans-Golgi network of the liver and balances the copper level by excreting excess copper into bile and plasma. However, the exact location of ATP7B in hepatocytes is controversial and remains to be determined. We have been cooperating with the seminal research of the University of Barcelona (Spain) and have achieved successful outcomes.

Comprehensive gene expression profiling analysis of microRNA/messenger RNA
We are profiling and analyzing the expression of microRNA/messenger (m) RNA in the liver tissue of HBV-infected human hepatocyte chimeric mice. We have found novel interactions between microRNA and messenger RNA in HBV replication and lifecycle. We are also investigating the association between the serum microRNA expression level and the prognosis/outcome of treatment in patients with HCC who have been treated with transarterial chemoembolization plus radiofrequency ablation.

Identification of cellular secretory pathway of urocortin 2 in HL-1 cardiomyocytes
Because the secretory pathway of urocortins (Ucns), which are members of the corticotropin-releasing factor family of peptides, is only partially understood, we attempted to identify the pathway(s) of Ucn. After the construction of plasmids expressing hybrid proteins of fluorescent protein and Ucn I or II and the instruction of these plasmids into HL-1 cardiomyocytes natively expressing Ucn I and II, the cells were subjected to live cell imaging to track fluorescent proteins with or without brefeldin A, which affects the intracellular dynamics of Ucn I in A172 human glioblastoma cells. We found that the cellular dynamics of Ucn II was not affected by brefeldin A, although that of Ucn I was affected, as previously described.

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