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

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General Summary

Core Research Facilities for Basic Science (Division of Molecular Cell Biology) was organized on April 1, 2014. The mission of the 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 equipments to researchers of the university to perform experiments. Once registered, researchers can freely use the various devices in our institute. This system also provides technical advice and guidance on specific fine-morphological or biochemical approaches to a registrant's experiment, if necessary. In 2016, 160 researchers registered at our annual registration system and we provided 197 research supports for electron microscopy and 3 for laboratory experiments.

2. Research Support System

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, or perform high-performance liquid chromatography and mass spectrometry. The service fee is minimal because services are limited to the university.

Research Activities

Change of MMPs expression of malignant brain tumor cells after exposure to anti-malignancy agent, temozolomide

Malignant brain tumors, especially malignant glioma and glioblastoma, are poor prognosis. Therefore, adjuvant therapies such as radiation and chemotherapy are required for the combination to surgical therapy. In this case, temozolomide is the first line drug used as an anti-neoplastic agent, but the influence on the invasivness of the tumor is major concern. While temozolomide suppresses proliferation of cell growth through methylation of DNA, it transiently activates transcription factors such as EGR-1 and NF-κB. Enzymes such as MMP9 chop off the basement membrane, and expression of the enzyme increases by transcriptional factors and enhance of the expression might affect invasion. In the study, gene transcription, protein amount, and activity of MMP2 and MMP9, were quantified after exposure to the therapeutic concentrations of temozolomide. As a result, the enzymatic activity of the MMP9 was not influenced by the agent. The therapeutic dose of

temozolomide was concluded less likely to have an effect on invasion via MMP activity. Also, feasibility of combination of temozolomide and MMP inhibitor was proposed.

Utilization of thermoresponsive magnetic nanoparticles with lower critical solutim temperature (LCST) in aqueous solution for detection of thyroid papillary carcinoma antigen
Thyroid carcinoma is one of the favorable prognostic malignancies in human body. For the reason, early diagnosis and treatment are important. While thyroid is easily reached by physical examination, the disease is generally diagnosed by ultrasound and fine needle aspiration. However, both methods have limitations. Previously, professor Takeyama Hiroshi, Department of Surgery in the university, established a monoclonal antibody for thyroid carcinoma and presented the effectiveness by immunostaining for clinical diagnosis. Whereas the result was obtained mainly by histological examination, the antigen was also present in blood. In current study, thermoresponsive magnetic nanoparticles with LCST in aqueous solution, was conjugated to the antibody for the application of the antibody for blood screening. Nanoparticles have larger surface and capacity for holding the larger amounts of antibody on the surface. The particle has thermoresponsive activity as well as magnetic properties, existence of antigen was detected by the method. Conjugation to the nanoparticle, the antibody may contribute to handy and more reliable screening of thyroid papillary carcinoma patients.

Human hepatocyte chimeric mice and hepatitis infection animal model
We have established human hepatocyte chimeric mice by an efficient method that we had developed and an animal model infected with hepatitis B or C virus by using the chimeric mice. Currently, we are intensely researching the efficacy of novel anti-viral agents, the infection mechanism, and ultrastructural alterations of intrahepatocellular organelle after viral eradication.

Intrahepatic cellular localization of ATP7B
ATP7B protein, also known as Wilson disease protein, is a copper-trans-porting P-type ATPase which is encoded by the *ATP7B* gene, locates in trans-Golgi network of liver, and balances the copper level by excreting excess copper into bile and plasma. However, the exact localization of ATP7B in the hepatocyte is controversial and remains to be determined. We have been cooperating with the seminal research in The University of Barcelona (Spain).

SNPs, and RAVs in the treatment of chronic HCV infection
DAAs are the first-line treatment for chronic HCV infection. We are investigating the association of SNPs of the genes with the blood drug concentration, treatment response, and DAA-induced liver damage. RAVs are also being investigated in detail.

Comprehensive gene expression profiling analysis of microRNA/mRNA
We are profiling and analyzing the expression of microRNA/mRNA in the liver tissue of HBV-infected human hepatocyte chimeric mice. We have found the novel interaction between microRNA and mRNA in HBV replication and lifecycle. We are also investigat-

ing the association between serum microRNA expression level and treatment outcome/prognosis in HCC patients who were treated with TACE/RFA.

Optimization of proteomics analysis by LC-MS bottom-up and top-down method
Currently, the analysis of the proteomics measured it by bottom up method or top-down method, and it was performed in combination with a powerful bioinformatics. Digested intact proteins in a restriction enzyme were measured by the bottom-up LC-MS/MS, and measurements ware provided peptide exact masses and fragment patterns. The protein database retrieval was performed based on these fragments pattern information and exact precursor molecular ion. These assigned proteins results were significantly useful for differential expression analyzes between a cancer tissue and the penumbra organization for a dynamic state profiling. To improve a detection limit, ionization efficiency was increased by using of matrix effected nanobooster and optimized LC separation condition. Now it was capable to identify 100 amol proteins routinely using nanoLC-MS/MS analysis. It was still difficult to obtain information about the PTM (phosphorylation, carbohydrate, drug conjugates) data using the conventional bottom-up method approach, but this information could be got by the LC-MS top-down method. In late years it was assumed that high molecular compounds detection including intact proteins ware difficult in spite of progress of the mass spectroscopy. Most of PTM studies were performed by immunoassays such as the Western blots and infusions MS method using highly pre-purified protein. Analysis of modification of carbohydrates and/or drug conjugates with intact protein were enabled at a routine level using optimized LC-ultra high resolution exact mass spectrometry device (Maxis3G) and Maximum Entropy Deconvoluted algorithm.

Isolation and characterization of embryonic ameloblast lineage cells derived from tooth buds of fetal miniature swine
Dental enamel formation, known as "amelogenesis," is initiated by cytodifferentiation of the ectodermally derived dental epithelium. Enamel cannot regenerate itself because once it is completely formed, ameloblasts are lost as the tooth erupts. Rodent teeth have been useful for studying the mechanisms of amelogenesis because ameloblast cell lines can be derived from the ever-growing incisors. However, higher mammals such as humans have no growing teeth, and cell lines derived from larger animals that are more similar to humans are required for higher fidelity studies. Here, we isolated embryonic enamel epithelium-derived epithelial cells from fetal swine. The explant culture of the developing deciduous molars that had been removed from the dental papilla-derived mesenchymal tissue and cells inside the tooth buds provided the epithelial cell population for the primary culture. To isolate the cell population, we performed a unique cell isolation technique called cell fishing. The isolated cells showed clear embryonic-stage ameloblast characteristics with appropriate gene/protein expressions of enamel matrix and proteinases, abundant glycogen pools, and secretory granular materials. They could be continuously subcultured several times and are presently being maintained. This cell population will facilitate the establishment of a stable cell line and allow us to characterize the definitive phenotype and functional behavior of porcine ameloblasts, which, in turn, promises to yield useful and practical findings that are more relevant than those provided by rodent

studies. Finally, analysis of in vitro enamel formation will be important for engineering "bio-enamel" as a new dental therapy to restore enamel defects.

Effects of urocortin III on insulin secretion from MIN6 mouse pancreatic β-cells in high glucose culture medium

We have been investigating the protective effects of urocortins, family peptides of CRH. We already reported that urocortin III stimulated insulin secretion from pancreatic β-cells in low to moderate high glucose (glucose concentration 1-4.5 g/L), but decreased in extremely high glucose condition (glucose concentration 9 g/L). Then, we also investigated the effects of urocortin III from the view point of histology. As the results, we confirmed that urocortin III increased dense-core granule at 1 g/L glucose, but decreased at 9 g/L glucose. These results indicate that urocortin III may differentially regulate insulin secretion as plasma glucose concentration in pancreatic β-cells.

Discrimination of volatile components using sensor description system

In this research, we aim to develop a system to objectively quantify fragrance by learning scent expressions using standards for sensor devices. Currently, GC and GC/MS are utilized for analysis of volatile components, and numerous components that are volatile components and biomarkers that characterize scents have been identified. On the other hand, when there are many types of volatile components, not only the component concentration but also the component ratio contained may be important for discrimination. Since the sensor can capture the characteristics of the whole scent, it can analyze from a different viewpoint from the current GC and GC/MS analyses, and it can analyze the flavor expression of food and the pattern discrimination of volatile biomarkers in the medical field. In order to achieve these objectives, we conducted training using flavor standards on sensor devices, and we developed an algorithm that expresses the characteristics of time-dependent changes during incubation of coffee which containing more than 600 chemical components as test samples. As a result, we found that fruity aroma increased with time. It is known that organic acids are produced by long-time keeping of coffee, and it is considered that these ingredients may have influences on the fruit scents. Thus, it was suggested that even with samples containing many kinds of volatile components, it is possible to express and discriminate change in scent easily and objectively by using our sensor description system. This research was supported by JSPS Grants-in-Aid for Science and Technology JP 16K12709.

Publications

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