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 Sciences (Division of Molecular Cell Biology) was organized 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 institute. This system also provides technical advice and guidance on specific fine-morphological or biochemical approaches to a registrant's experiment, if necessary. In 2017, 158 researchers registered at our annual registration system and we provided 189 research supports for electron microscopy and 2 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, or perform high-performance liquid chromatography and mass spectrometry. The service fee is minimal because services are limited to the university.

Research Activities

Activation of transcription factors by adjuvant therapy in brain tumor cells and the effect on expressions of metal proteinases

Malignant brain tumors, especially malignant gliomas and glioblastomas are poor prognosis and refractory, and therefore radiation therapy and chemotherapy are used with surgery. Previously we demonstrated that irradiation and anti-malignant drug temozolomide, which are standard adjuvant therapies for the diseases, does not increase activities of metal proteinases that affects invasiveness of tumors. To investigate the further mechanisms, the effects of these therapies on transcriptions of genes affect expression of metalloproteinases were investigated this year.

As a result, it was shown that irradiation and administration of alkylating agent activated a series of transcription factors that promote cell repair. In addition, they resulted in enhancement of the transcription factors itself. Among the metalloproteinases family, MMP 2 and MMP 9 are known to have the potency to degrade basement membrane.

From the sequences of the promoter, MMP 2 is less sensitive to transcription factors. But MMP 9 is highly vulnerable, and so we are trying to elucidate which factor is more important for invasiveness of brain tumors.

Hippo abnormality in thyroid carcinoma cell lines
Currently we are conducting clinical research of monoclonal antibody against thyroid papillary carcinoma established by Professor Hiroshi Takeyama of Surgical Department in our University. When cells proliferate, various growth factors dissociate Hippo complex via PI3 kinase and PD kinase 1, and this dephosphorylates the effector YAP and causes nuclear translocation. During the investigation of the relationship between the antigen recognized by the antibody and cell proliferation, we found that YAP protein which is normally phosphorylated and tethered in the cytoplasm, exists in the nucleus even in contact inhibited status or on serum depraved condition in some thyroid malignant cell lines producing the antigen. We are investigating the pathway.

Human hepatocyte chimeric mice and hepatitis virus 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 mechanism of progression to chronic infection, and ultrastructural alterations of intrahepatocellular organelle after viral eradication.

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

The association between serum microRNA expression levels and treatment outcome/prognosis in HCC
We measure serum microRNA expression levels in an intrahepatic feeding artery, proper hepatic artery, and peripheral vein when we perform TACE for patients with HCC, and are investigating the association between serum microRNA expression levels and treatment outcome/prognosis in HCC patients who were treated with TACE/RFA.

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) and have achieved successful outcomes of our research.

Comprehensive gene expression profiling analysis of microRNA/messenger RNA
We are profiling and analyzing the expression of microRNA/messenger RNA (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 investigating the association between serum microRNA expression level and treatment outcome/prognosis in HCC patients who were treated with TACE/RFA.

Examination of a direct analysis in real time (DART)-MS utility in everyday clinical practice
The DART-MS method is simple and easy technique to be able to do the MS measurement quickly just to expose a solid, a liquid, a gas sample to direct ionization gas without the sample preparation. It is important to get reliable diagnostic information timely and without taking time in actual clinical practice such as outpatient department or the operating room. From such a point of view, it was measured using dried blood spot (DBS) which was really used in a screening diagnosis whether DART-MS was useful in the clinical practice. As a result, 7-ketocholesterol of a family of oxysterol compounds which was a diagnostic marker for Fabry's disease was identified in the second from the DBS of Fabry's disease patient who was one of the rare diseases. 7-Ketocholesterol showed increase in comparison with the DBS of the healthy person conspicuously, and it is clear the DART-MS will become a powerful tool in rare disease screening examination on everyday clinical practice. Examination of the utility of the DART-MS method is going to be repeated using clinical various non-invasive samples which can be gathered in actual clinical practice in future.

Establishment and characterization of a squamous cell carcinoma cell line, designated hZK-1, derived from a metastatic lymph node tumor of the tongue
The hZK-1 cell line was successfully established from the metastatic foci of a lymph node of an 82-year-old Japanese woman with squamous cell carcinoma of the tongue. The pathological diagnosis of the tumor was moderately to well-differentiated squamous cell carcinoma. The hZK-1 cells were angular in shape, and had neoplastic and pleomorphic features. Adjacent hZK-1 cells were joined by desmosomes and well-developed microvilli, and many free ribosomes were observed in the cytoplasm. The doubling time of the hZK-1 cells was approximately 36, 33, and 29 h at the 10th, 20th, and 30th passages, respectively. The cell line was shown to be triploid, with a chromosomal distribution of 75-80. Immunocytochemical staining of the hZK-1 cells revealed cytokeratin (CK) 17-, Ki67-, and p53-positive staining, and negative staining for CK13. The hZK-1 cells were negative for human papillomavirus (HPV)-16 or-18 infection. Grafting was not successful when the hZK-1 cells were transplanted into the subcutis of SCID mice. The hZK-1 cells (2×10^6 cells/3 ml of growth medium) secreted vascular endothelial growth factor (VEGF) that reached a concentration of 2.6 ng/ml media after 3 days of culture. Hypoxia enhanced cellular HIF-1α expression and VEGF secretion in hZK-1 cells. The HIF-1α inhibitor YC-1 partially inhibited hypoxia-induced VEGF secretion in ZK-1 cells. The reverse transcription-polymerase chain reaction (RT-PCR) results revealed that the expression of CK17, Ki67, and p53 was elevated in the hZK-1 cells.

hZK-1 cells were not sensitive to CDDP, TXT, 5-FU, or a mixture of these three anti-tumor agents.

Effects of urocortins (Ucns) on insulin secretion from MIN6 mouse pancreatic β-cells in high glucose culture medium

We have been investigating the protective effects of urocortins, especially, Ucn II and Ucn III, which are expressing in MIN6 mouse pancreatic β-cells and family peptides of corticotropin-releasing hormone (CRH). Based on the previous results that Ucn III, a specific ligand of CRH type 2 receptor, modulates insulin secretion in various glucose concentration, we are trying to investigate MIN6 cells knocked out the peptide. And we are now constructing knock-in plasmids to insert after genomic edited site of Ucn II or III and seeking suitable conditions for introducing knock-in plasmids and agent for CRISPR treatment.

Discrimination of volatile components using sensors

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 systems are mainly used for analysis of volatile components. For example, numerous volatile components in foods, drinks, environments, and biomarker components have been identified by their systems. On the other hand, when there are many types of components in the samples, not only the component concentrations but also the component ratios may be important for characterization of the smell. Therefore, sensors can analyze from the viewpoint different from the current GC and GC/MS systems, because it can capture the characteristics of the whole scent at once. This viewpoint are possible to bring new knowledge.

In this fiscal year, in order to expand the diversity of the odor which can be discriminated by the sensor systems, we proceeded machine learning using the aroma kit, Le Nez du Café which composed of 36 kinds of scents. As a result, 36 types of scent axes could be added, leading to an increase in the type of expression about the fruity aroma that was difficult before. This result will be possible to improve the representation accuracy of sensors. This work was supported by JSPS KAKENHI Grant Number JP 16K 12709.

Publications

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