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

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General Summary

The Core Research Facilities were reorganized on April 1, 2014, and its name was changed to Core Research Facilities for Basic Sciences (Division of Molecular Cell Biology). The mission of the facilities is the facilitation of research in the university. Two systems are constituted for the use of the Facilities for Basic Sciences (Division of Molecular Cell Biology).
1. Annual Registration System
This system is intended to supply research space, benches, and other equipment to researchers of the university to perform experiments. Once registered, researchers can freely use the various devices, such as fluorescent microscopes, optical microscopes, and equipment for the preparation of samples for histological examinations, high-performance liquid chromatographs, and nucleic acid amplification systems (polymerase chain reaction). Because inspections and maintenance are regularly performed by the staff, the equipment is reliable and available at any time. This system also provides technical advice and guidance on specific fine morphological or biochemical approaches to a registrant's experiment, if necessary.
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. However, the various new high technologies and devices require specialized knowledge. These advances can cost the researchers both time and money. Also, all researchers are not necessarily familiar with all the equipment for medical experiments. 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. By using this system, researchers can proceed efficiently. The service fee is minimal because services are limited to the university.

Research Activities

Drug and molecular delivery by a frog into malignant brain tumor cells
The introduction of foreign materials into cell bodies is an important technique for investigating the functions of corresponding materials. We have previously used ultrasound to transduce such materials. A recently produced mass-producible nanoprinting frog has enabled high-performance drugs or nucleic acids to be transferred into cells. Because lit-

tle is known about the mechanism of action, we investigated its effects on a malignant glioma cell line. Local oxidation reaction during the nanoprinting by the frog caused reversible cell perforation. The perforation was clearly observed by morphology. The system might be useful for transferring foreign materials into malignant brain tumor cells.

Morphological observation and metabolism of gemcitabine in a cholangiocarcinoma cell line

Cholangiocarcinoma is one of the most intractable malignancies in the human body. The disease was recently highlighted in Japan when cholangiocarcinomas were found to have developed in 11 of 62 persons who had worked in an offset color proof-printing room or in the front room of a printing company for at least 1 year from 1991 through 2006. The incidence suggests that cholangiocarcinoma is a new occupation-related disease. A cholangiocarcinoma cell line, TK, was established at our university. For further research use, morphology and key enzymes, especially for the metabolism of gemcitabine, a drug against malignant chemotherapeutic, were investigated and reported. Since this cell line was well characterized, it may play a role in investigating carcinogenesis and chemotherapy for patients with cholangiocarcinoma.

Transporter genes, single nucleotide polymorphisms, and resistance-associated variants in the treatment of chronic hepatitis C virus infection

Direct-acting antiviral agents (DAAs) or ribavirin or both are the main components of combination treatment for chronic infection with hepatitis C virus (HCV). For the virus to be eradicated with ribavirin-combined treatment, the HCV in hepatocytes must be exposed to ribavirin. Ribavirin is transported into hepatocytes by cell membrane transporters. We have discovered and are investigating the novel function of transporters and the association of single nucleotide polymorphisms of the gene with treatment response. Currently, the role of DAA transporters, causing single nucleotide polymorphisms of DAA-induced liver hepatitis, and resistant-associated variants are being investigated in detail.

Comprehensive gene expression profiling analysis of microRNA/messenger RNA in liver tissue

We are profiling and analyzing the expression of microRNA (miRNA)/messenger RNA (mRNA) in the liver tissue of patients with chronic HCV infection who would undergo combination treatment with PEGylated interferon-α and ribavirin. We have analyzed whether the microRNA/mRNA candidates can be associated with treatment responses in chronic HCV infection. We have found the novel interaction between miRNA and mRNA in replication and lifecycle of HCV. Currently, the expression of miRNA/mRNA in mice that are infected with hepatitis B virus and have humanized liver is being investigated in detail.

Study of the biophysical properties and transfection efficiency for branched amphiphilic peptide capsules to form peptiplexes with DNA

We recently designed a new class of branched amphiphilic peptide capsules (BAPCs) that

self-assemble into extremely stable nanospheres. We attempted to use BAPCs as a carrier of vector plasmids for gene therapy. The BAPCs mixed with genes were delivered to cells without cytotoxicity. However, the transfection efficiency was not high enough to apply gene therapy to mice. We investigated the biophysical properties and transfection efficiency for BAPCs to form peptiplexes with DNA. We found that, in the presence of double-stranded plasmid DNA, BAPCs are unable to be formed. Instead, depending of the peptide/DNA ratios, the peptides either coat the plasmid surface forming nanofibers (high peptide to DNA charged ratio) or condense the plasmid into nanometer-sized compacted structures (at low peptide to DNA charged ratios). Different gene delivery efficiencies are observed for the 2 types of assemblies. The compacted nanometer-sized structures display much higher transfection efficiencies in HeLa cells. This level of transfection is greater than that observed for a lipid-based reagent when the total number of viable transfected cells is taken into account.

Cytopathological analysis of the spinal ganglion in the novel ataxia mouse
Many vacuolar degenerated perikarya were found in the spinal ganglion of an ataxic mouse. No nucleus and endoplasmic reticulum was found in the vacuole, which contained mitochondria, microtubules, and neurofilaments. Each vacuole was encompassed with satellite cells. The satellite cell of a phenotypically normal mouse extended many cellular processes, while that of the ataxic mouse had few processes. These results suggest that the satellite cell is involved in the vacuolar degeneration of the ganglion cell.

Intracellular dynamics of urocortin I in A172 human glioblastoma cells
We recently reported that urocortins (UCNs; UCN I-III) and corticortropin-releasing factor (CRF) receptors are expressed in human gliomas and gastric cancers. However, the secretory pathways of UCNs have not been clearly investigated. Therefore, we investigated the secretory pathway of UCN I in human glioblastoma cells, A172. After construction of a plasmid expressing the hybrid protein of UCN signal peptide and mCherry fluorescent protein (pUCNIS-mCherry plasmid), the plasmid was introduced into A172 human glioblastoma cells. The intracellular dynamics of pUCNIS-mCherry were tracked with a DeltaVision Core-SP fluorescent microscope (GE Healthcare Life Sciences, Marlborough, MA, USA) and several agents. This study suggests that UCN I is secreted through the constitutive pathway.

Development of an in-vitro brain model for nano-brain toxicology assay
Recent technical innovations has made it possible to mass produce various nano materials. Although nano materials have improved in quality and are being used to produce consumer products, such as foods and cosmetics, their safety is still being investigated. Recently, we have investigated the effect of nano particles on several brain cells. Our results showed < 100 nm nano particles affected the cellular viability and differentiation of neural stem cells. This year, we published these results in the *International Journal of Molecular Sciences* and started a new toxicological study of nano particles against cells related to the blood-brain barrier.

Publications

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