Core Research Facilities

Yoshinobu Manome, *Professor and Director* Hiroyuki Sasaki, *Associate Professor*
Takeo Iwamoto, *Associate Professor*

General Summary

Core Research facilities were reorganized on April 1, 2009, in the Research Center for Medical Sciences and consist of the Division of Fine Morphology, the Division of Biochemistry, and the Division of Advanced-Research Laboratory. The mission of the facilities is the facilitation of research in the university. Two systems are constituted for the use of the facilities.

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-pressure 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 registrant's experiment, if necessary.

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 high technology and various new 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 experimental. 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

Biotracing using fluorescent nanoparticles
The monoclonal antibody JT95 was developed at this university. It specifically recognizes an antigen expressed in differentiated thyroid carcinomas. For the use of serum in the diagnosis of thyroid cancer, the antibody was conjugated to fluorescent nanoparticles. The localization of the antigen was visualized with fluorescence microscopy after

reactions with thyroid carcinoma cells. In addition, the conjugate demonstrated greater sensitivity than the enzyme-linked immunosorbent assay for quantitative analysis of the antigen in human serum.

Functional analysis of tight junctions

Tight junctions (TJs) in epithelia and endothelia restrict the paracellular flux of water and solutes. Epidermal TJs are thought to restrict molecular movement and to help the stratum corneum serve as a secondary barrier in the skin. Calcium ion (Ca^{2+}), a well-known differentiation inducer for keratinocytes, distributes to form a vertical gradient peaking in the stratum granulosum. In this study, we applied sodium caprate, which elicits dilations of TJs on human reconstructed epidermis, and investigated Ca^{2+} distribution in the epidermis. Ion-capture cytochemistry and electron energy-loss spectroscopy revealed that treatment with sodium caprate markedly altered Ca^{2+} localization in the epidermis. Additionally, abnormal differentiation (e.g., parakeratosis) was observed in the stratum granulosum. To confirm that these changes were caused by TJ disruption, we observed the structure of TJ strands with the freeze-fracture replica method and measured transepidermal Ca^{2+} permeability by quantifying diffused Ca^{2+} through the epidermis. We found that the TJ strands had fragmented and that Ca^{2+} permeability had increased. These data suggest that epidermal TJs maintain Ca^{2+} under the stratum corneum and regulate epidermal differentiation.

A channel-forming peptide that modulates drug delivery across in vitro corneal epithelium

The goal of this study was to determine whether a synthetic peptide, NC-1059, can modulate the corneal epithelium to increase the permeation of therapeutic agents across this barrier. An *in vitro* system employing transformed human corneal epithelial cells was optimized for this study. Culture conditions were identified to promote the formation of a confluent monolayer that rapidly develops a substantial transepithelial electrical resistance. Electrical parameters were measured with a modified Ussing flux chamber, and solute flux was quantified with fluorescently labeled compounds. The peptide NC-1059 causes a concentration-dependent increase in short-circuit current and an increase in transepithelial electrical conductance when assessed in a modified Ussing chamber. The effect of NC-1059 on transepithelial electrical resistance was reversible. To test for paracellular permeability and size exclusion, fluorescein isothiocyanate-labeled dextran ranging in size from 10 to 70 kDa was used. Dextran permeated the corneal cell monolayer in the presence, but not the absence, of NC-1059.

When fluorescein sodium and carboxyfluorescein were used as low molecular weight markers, similar NC-1059-modulated kinetics were observed.

Maximum permeation for the fluorescein derivatives occurred 30 to 90 minutes after exposure to NC-1059 for 5 minutes.

A prototypical drug, methotrexate, also exhibited increased permeation in the presence of NC-1059. NC-1059 enhances drug permeation across cultured corneal epithelial cell monolayers by transiently affecting the paracellular pathway. Thus, NC-1059 is a lead compound for the development of cotherapeutic agents to enhance the access and

effectiveness of ophthalmic compounds.

Publications

Funamizu N, Okamoto A, Kamata Y, Misawa T, Uwagata T, Gocho T, Yanaga K, Manome Y. Is the resistance of gamecitabine for pancreatic cancer settled only by overexpression of deoxycytidine kinase? *Oncol Rep* 2010; **23:** 471–5.

Manome Y, Mizuno S, Akiyama N, Fujioka K, Saito H, Hataba Y, Kobayashi T, Watanabe M. Three-dimensional cell culture of glioma and morphological comparison of four different human cell lines. *Anticancer Res* 2010; **30:** 383–90.

Fujioka K, Futamura Y, Shiohara T, Hoshino A, Kanaya F, Manome Y, Yamamoto K. Amino acid synthesis in a supercritical carbon dioxide-water system. Inte J Mol Sci 2009; **10:** 2722–32.

Fujioka K, Arakawa E, Kita J, Aoyama Y, Okuda T, Manome Y, Yamamoto K. Combination of real-value smell and metaphor expression aids yeast detection. *PLoS One* 2009; **4(11):** e7939.

Manome Y, Furuhata H, Hashimoto A, Funamizu N, Suzuki R, Ishizawa S, Akiyama N, Kobayashi T, Watanabe M. Application of therapeutic insonation to malignant glioma cells and facilitation by echo-contrast microbubbles of levovist. *Anticancer Res* 2009; **29:** 235–42

Kubo A[1], Nagao K[1], Yokouchi M[1], Sasaki H, Amagai M[1] ([1]Keio Univ Sch Med). External antigen uptake by Langerhans cells with recognition of epidermal tight junction barriers. *J Exp Med* 2009; **206:** 2937–46.

Morimoto S, O-Uchi J, Kawai M, Hoshina T, Kusakari Y, Komukai K, Sasaki H, Hongo K, Kurihara S. Protein kinase A-dependent phosphorylation of ryanodine receptors increases Ca^{2+} leak in mouse heart. *Biochem Biophys Res Commun* 2009; **390:** 87–92.

Konishi H[1], Kikuchi S[1], Ochiai T[1], Ikoma H[1], Kubota T[1], Ichikawa D[1], Fujiwara H[1], Okamoto K[1], Sakakura C[1], Sonoyama T[1], Kokuba Y[1], Sasaki H, Matsui T[1], Otsuji E[1] ([1]Kyoto Pref Univ Med). Latrunculin A has a string anticancer effect in a peritoneal dissemination model of human gastric cancer in mice. *Anticancer Res* 2009; **29:** 2091–8.

Kuroda S[1], Kurasawa M[1], Mizukoshi K[1], Maeda T[1], Yamamoto T[1], Oba A[1], Sasaki H ([1]Pola Chem Ind). Epidermal tight junction: the master skin barrier regulator. *IFSCC Magazine* 2009; **12:** 2–6.

Kurasawa M[1], Kuroda S[1], Kida N[1], Murata M, Oba A[1], Yamamoto T[1], Sasaki H ([1]Pola Chem Ind). Regulation of tight junction permeability by sodium caprate in human keratinocytes and reconstructed Epidermis. *Biochem Biophys Res Commun* 2009; **381:** 171–5.

Martin J, Malreddy P, Iwamoto T, Freeman L, Davidson H, Tomich J, Schultz B. NC-1059: a channel-forming peptide that modulates drug delivery across in vitro corneal epithelium. *Invest Ophthalmol Vis Sci* 2009; **50:** 3337–45.

Tomich J, Iwamoto T. Peptide-enhanced corneal drug delivery. *US Patent* 2009: US 7592341B2.