Core Research Facilities

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General Summary

The Core Research Facilities were reorganized on April 1, 2009, as the Research Center for Medical Sciences and consists 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-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 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 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

Application of a monoclonal antibody to thyroid papillary carcinoma for bioimaging and high-sensitive assay of tumor cells
A monoclonal antibody developed at our university specifically recognizes an antigen expressed in differentiated papillary thyroid carcinoma cells. Because the antibody is considered useful for detecting early lesions or metastases of thyroid carcinoma, a method

for determining trace amounts of antigen was developed. Although a method for labeling the antibody is being developed, the coupling efficiency remains unsatisfactory for immunoglobulin M molecules, such as our antibody, and, therefore, a simple and corroborative approach has been required. Previously, we coupled the antibody to quantum dots for immunnofluorescent assay or bioimaging. This year, we devised a sandwich assay for highly sensitive detection or quantification of the antigen. Because even a small amount of the antigen can be quantified with our method, we started a clinical study using the antibody.

Functional analysis of tight junctions in the epidermis

Tight junctions (TJs) among adjacent epithelial cells control the paracellular permeability of solutes. Epidermal TJs restricts molecular movement to assist the stratum corneum as a secondary barrier in the skin. However, the role of TJs in molecular distribution in the epidermis has not been studied in detail. Calcium ions (Ca^{2+}) induce keratinocyte differentiation and distribute to form a vertical gradient that peaks at the stratum granulosum. The stratum corneum forms the Ca^{2+} gradient because it is considered the only permeability barrier in the skin. However, the epidermal TJs in the stratum granulosum have recently been suggested to restrict molecular movement to assist the stratum corneum as a secondary barrier. The objective of this study was to clarify the contributions of TJs to the Ca^{2+} gradient and to epidermal differentiation in reconstructed human epidermis. When the epidermal TJ barrier function was disrupted by treatment with sodium caprate, Ca^{2+} flux increased and the gradient changed in ion-capture cytochemical images. Ultrastructural changes and alterations of proliferation/differentiation markers revealed that both hyperproliferation and precocious differentiation occurred regionally in the epidermis. These results suggest that TJs play a crucial role in maintaining epidermal homeostasis by controlling the Ca^{2+} gradient.

Study of interaction between staphylococcal exfoliative toxin A and ganglioside GM1 using surface plasmon resonance

Several molecular species of gangliosides have been shown to act as receptors for various bacterial toxins. To date, various analytical methods, such as inactivation of toxic activity, inhibition of toxin binding, and changes in mobility on polyacrylamide gel electrophoresis, have been reported. We now describe the formation of intermolecular complexes between ganglioside GM1 and staphylococcal exfoliative toxin A (sETA) using the surface plasmon resonance technique, native polyacrylamide gel mobility shift assay, and the sETA toxicity inactivation test in the epidermis of newborn mice. The electrophoretic pattern of sETA showed a single faster band than control sETA when 4.5 μg of sETA was mixed with 62.5 to 500 μg of GM1; also sETA toxicity was complete abolished when 4.5 μg of sETA was incubated with 1.25 mg of GM1. Affinities between sETA and GM1 were determined with a Biacore 3000 surface plasmon resonance analysis system (GE Healthcare, Chalfont St. Giles, UK) with a CM5 sensor chip on which sETA was immobilized through amine-coupling chemistry. The association and dissociation rates and affinity constants of GM1 were calculated using the BIAevaluation software kit, version 3.1 (GE Healthcare). Kinetic analysis showed that the association and dissociation rate

constants of the binding interaction of GM1 to sETA were 5.6×10^3 $M^{-1}s^{-1}$ and 5.6×103 $M^{-1}s^{-1}$, respectively, and the dissociation constant was 4.6 nM at 25°C. The present results demonstrate the high specificity of the interaction between GM1 and sETA.

Publications

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