Institute of DNA Medicine
Department of Molecular Cell Biology

Yoshinobu Manome, *Associate Professor and Director*
Toru Obata, *Associate Professor*
Hiroyuki Sasaki, *Associate Professor*
Michiko Watanabe, *Lecturer*

General Summary

Our research activities include analysis and visualization of cell biological events under physiological and pathological conditions. For this purpose molecular biological, fine morphological, and biochemical techniques were applied.

Research Activities

Development of sonodynamic therapy for malignant glioma

Ultrasound is widely used in clinical medicine, and, thus, its safety is well established. In addition to diagnostic applications, therapeutic applications are also possible. When combined with a contrast agent containing microbubbles, such as Levovist (Schering AG, Berlin, Germany), ultrasound can induce cell death in nearby tissues by the cavitation effect. Using this effect, we established a new therapeutic application of ultrasound for central nervous system malignancies, such as malignant glioma. We found 210 kHz and 2.61 W/cm^2 of insonation could efficiently induce cell death when combined with 30 mg/ml of Levovist. Moreover, the effect was also confirmed with *in vivo* animal experiments. We are now proposing therapeutic/diagnostic, or theragnostic, applications of ultrasound and are attempting to refine the system.

Soft ionization

Soft ionization processes, such as electrospray ionization, in mass spectrometry, have used proton transition from water molecules. This condition may cause proton substitution in the target molecule. The isotope dilution method of quantification is commonly used in mass spectrometry. This method is based on the stability of the labeled compound during the preparation and assay processes. The study of stability check with labeled compounds during soft ionization shows the possibility of substitution of deuterium label to proton in water molecules.

Functional analysis of tight junctions

Tight junctions (TJs) are the most apical components of junctional complexes and play a vital role in cell-cell adhesion in epithelial and endothelial cells. Evidence is accumulating that TJs in the granular layer of the epidermis contribute to the epidermal barrier. To further clarify the role of TJs in the barrier function of human epidermal keratinocytes, we investigated the behavior of TJ components by means of a keratinocyte culture system in which differentiation was induced by transfer to a high-calcium

medium. TJ-related molecules became localized at the cell membrane, and transepithelial resistance was elevated with the progression of keratinocyte differentiation. The epidermal barrier function was clearly suppressed when the expression of claudin-1 or occludin was blocked by RNA interference. Furthermore, suppression of claudin-1 inhibited occludin expression in the cell membrane, whereas suppression of occludin did not affect the localization of claudin-1.

Morphological analysis with a 3-dimensional cell-culture system

To clarify the *in vivo* behavior of malignant glioma cells, we investigated morphological changes in a novel 3-dimensional (3D) cell-culture system by means of scanning electron microscopy. Four glioma cell lines, T98G, U118MG, A172 and KNS42, were used. When cells were cultivated in 3D, their morphologies changed markedly. Both A172 and KNS42 cells showed self-adherence, aggregated, and formed balloon-like structure, whereas T98G cells were partly conglomerated. The tight attachment of U118MG cells to the scaffold was observed. Although overall characteristics of these cells were similar to those of cells in ordinary two-dimensional cell culture and each line was used as a standard model for malignant brain tumor the morphology and adherence or fiber formation differed in the 3D cell culture system. This observation might be related to proliferation signals, such as phosphorylation of Akt, which were enhanced in A172 cells.

Publications

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