Institute of DNA Medicine
Department of Molecular Cell Biology

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General Summary

Our department uses molecular approaches to analyze physiological and pathological cellular events. Molecular cell biology techniques are practical methods for assessing cellular events. Our methods include modification of nucleic acid transcription and expression by transfection of DNA or short interfering RNA. Also, labeling of molecules with fluorescent nanoparticles, conjugation to sensors, and amplification with radiolabelled materials are our specialties. By introducing these techniques, we are helping to solve clinical problems.

Research Activities

Application of therapeutic ultrasound for delivering nucleic acids to malignant glioma cells

Glioma is an intractable disease of the central nervous system. Because the prognosis after surgical removal remains poor, alternative therapies, such as chemotherapy, radiotherapy, and immunotherapy, have been developed. Despite the poor prognosis, metastasis outside the central nervous system is rare and the cause of death in most cases is local recurrence. Thus, long-term survival might be possible if a more effective local therapy were developed. We have developed such a therapy using therapeutic ultrasound irradiation and have reported the beneficial effect of therapeutic insonation in combination with microbubbles. In the present study, to enhance the therapeutic efficacy, an additional nucleic acid delivery system was developed. This year, we accomplished the experimental production of the device.

Transcription of urocortin and corticotropin-releasing factors in gastric carcinoma cells

Urocortin (Ucn) and corticotropin-releasing factors (CRFs) and their receptors are expressed in many organs, including those of the central nervous system. Previously, we demonstrated the expression of messenger (m) RNAs of Ucn I, II, III, and CRF and CRF receptors (CRFR) 1 and 2 in malignant glioma cell lines. This year, we examined transcription in the STKM gastric carcinoma cell line. This cell line expressed mRNAs of UCN 1 and 2 and CRFR2. However, unlike malignant glioma cells, they did not express the mRNAs of UCN3 and CRFR1. The transcription pattern was not affected by growth or cytotoxic signals.

Peptide-tracking of Ucn I in human glioblastoma cells using the Ucn I-fluorescent protein hybrid protein

Due to the lack of knowledge about the secretion mechanism of Ucn I, we investigated

the secretory pathway of Ucn I using human glioblasotoma cells (A172 and U138-MG cells) and the Ucn I-fluorescent protein hybrid protein-expressing plasmid. Immunocytochemical studies revealed immunoreactivity for Ucn I, CRFR1, and CRFR2 in human gliblastoma cells, but the expression patterns of immunoreactivity were different. Next, a Ucn I-fluorescent protein hybrid protein-expressing plasmid was constructed and introduced into A172 human glioblastoma cells, and the intracellular dynamics of Ucn I was tracked with fluorescent microscopy. Fluorescent microscopy suggested that Ucn I is secreted via the constitutive pathway. This result also suggests that the secretion of Ucn I is correlated with the level of mRNA production.

Development of an in-vitro brain model for nano-brain toxicology assay

Recent technical innovations have enabled mass production of various nano materials. Although nano materials are used for foods and cosmetics owing to their improving quality, their safety is still under investigation. Recently, several studies have shown that nano materials (< 100 nm) penetrate brain tissue. However, whether the penetrating nanoparticles affect neuronal activity is unknown. Direct *in-vivo* assay to reveal the toxic neuronal effects and mechanisms of nanoparticles may be difficult, because most particles are distributed to other organs, and penetrating particles localize in small areas of the brain. Therefore, to understand the toxic effects of nanoparticles we have developed an *in-vitro* brain model for use with cellular assays. Our bottom-up model includes 1) a blood-brain barrier model, which indicates the apparent permeability coefficient, and 2) cellular assays. In our concept, nanoparticles that were able to penetrate were selected by means of the blood-brain barrier model, and then possible toxic effects and mechanisms were assessed with integration of the cellular toxicological results using the penetrating particles. With this novel model, we examined the toxic effects of nanoparticles and microparticles on human neural stem cells. We found that 30-nm silica nanoparticles at high concentration (0.1 mg/mL) affected the viability and differentiation of human neural stem cells. This concentration may be helpful for considering the maximal dose for *in-vivo* administration.

Publications

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