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

Yoshinobu Manome, *Professor and Director*
Takeo Iwanoto, *Professor*
Keiichi Ikeda, *Assistant Professor*
Akito Tsubota, *Professor*
Toshiaki Tachibana, *Professor*
Kouki Fujioka, *Assistant Professor*

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).

Research Activities

Cell membrane perforation with photosensitizer and a brush-shaped soft-polymer sheet and apoptosis of colon and lung cancer cells by microRNA-203 and Puma expression

Transduction of foreign molecules into cells is an important technique for investigating the functions of corresponding molecules or targets or both. Recently, a mass-producible nanoprinting perforator was devised to enable large-scale, high-performance drugs or nucleic acids to be transferred into cells without causing damage. Therefore, we investigated the effects of this perforator on a malignant glioma cell line. Photosensitization transduced fluorescein isothiocyanate-conjugated albumin into cells. The trypan blue inclusion test demonstrated membrane disintegration by the procedure, and scanning electron microscopy disclosed perforation of the cell membrane. A local oxidation reaction during nanoprinting caused reversible membrane perforation; therefore, the specific printing system might be convenient for the transduction of foreign molecules into malignant glioma cells.

The relationship between microRNA-203 (miR-203) and the p53 upregulated modulator of apoptosis (Puma) was investigated in colon and lung-cancer cell lines. Although p53 downregulation decreased both miR-203 and Puma expression, miR-203 overexpression increased Puma expression. These findings suggest that activated p53 increases both miR-203 and Puma expression and that Puma expression remains elevated in cells with miR-203 overexpression in the presence of p53 downregulation. Our data suggest that p53 increases Puma expression directly and may also do so through miR-203. This functional study revealed that miR-203 overexpression induces apoptosis and inhibits cell invasiveness.

Chimeric model mice of hepatitis infection with human hepatocytes, intrahepatic cellular localization of ATP7B, gene mutation in the treatment of chronic hepatitis C virus infection, comprehensive gene expression profiling analysis of microRNA/messenger RNA, and gene delivery and immunomodulatory effects of plasmid DNA associated with branched amphiphilic peptide capsules

We have established the human hepatocyte chimeric mouse as an animal model for investigating hepatitis B or C virus infection and are aggressively researching the efficacy of novel antiviral agents, the infection mechanism, and the ultrastructural alteration of intrahepatocellular organelles after viral eradication.

In collaboration with the University of Barcelona (Spain), we are investigating the protein ATPase copper transporting beta (ATP7B), which balances the copper level by excreting excess copper into bile and plasma, because the exact localization of ATP7B in the hepatocyte remains to be determined.

We are investigating the association of single nucleotide polymorphisms of the genes with the serum drug concentration, treatment response, and liver damage induced by directly acting antiviral agents in the treatment of hepatitis C virus infection. Resistance-associated variants are also being investigated in detail.

We have found the novel interaction between microRNA and messenger RNA in the replication and life cycle of hepatitis B virus and investigated the association of the serum microRNA expression level with treatment outcomes and prognosis in patients with hepatocellular carcinoma who were treated with transcatheter arterial chemoembolisation and radiofrequency ablation. In addition we have reported on a new class of branched amphiphilic peptides associated with double-stranded DNA and promoted *in vitro* transfection of eukaryotic cells, yielding high transfection rates and minimal cytotoxicity and representing a new and promising nonviral DNA/gene delivery approach for DNA vaccines.

Matrix-remodeling response of human periodontal tissue cells toward fibrosis upon nicotine exposure

Fibrosis is frequently observed in the gingiva of smokers. However, the mechanisms by which smoking results in pathological changes in periodontal tissue and lead to fibrosis are not entirely clear. Our former report showed that type I collagen synthesis is promoted by nicotine via CCN family protein 2 in human periodontal tissue cells. Here, we evaluated other aspects of nicotine function from the viewpoint of extracellular matrix remodeling. Human gingival fibroblasts ($n = 4$) and periodontal ligament cells ($n = 3$) were isolated. The cells were treated with various concentrations of nicotine for 12 to 48 hours. Modulators of matrix remodeling were measured with enzyme-linked immunosorbent assays. Cell migration and morphology were also evaluated. After treatment with 1 μg/ml nicotine, significant increases ($p < 0.05$) were observed of tissue inhibitor of metalloproteinase 1 and transforming growth factor β1 production in both cell lysates and supernatants and of matrix metalloproteinase 1 production in cell lysates. Cell migration was significantly inhibited ($p < 0.005$) by nicotine in a time-dependent manner. Electron microscopic analysis revealed vacuoles in nicotine-treated cells. These results indicate that nicotine impairs fibroblast motility, induces cellular degenerative changes, and alters the extracellular matrix remodeling systems of periodontal cells. Induction of matrix

remodeling molecules, combined with type I collagen accumulation, may account for the molecular mechanism of nicotine-induced periodontal fibrosis.

Control of insulin secretion by urocortin III under hyperglycemic condition

Insulin secretion from pancreatic β cells is reported to be disturbed under hyperglycemic conditions. A recent study has found that release of urocortin III, a specific antagonist of corticotropin-releasing factor receptor type 2, both stimulates insulin release and is stimulated by elevation of extracellular glucose. Therefore, the effect of urocortin III at higher glucose levels on insulin release was investigated with the pancreatic β cells of MIN6 mice. The addition of urocortin III (10^{-7} M) resulted in a gradual increase of insulin release but in a decrease in a culture medium with 90 mM glucose. We will further investigate the mechanism of urocortin III–induced insulin release under such hyperglycemic conditions.

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

Recent technical innovations have allowed various nanomaterials to be mass-produced. Although nanomaterials are used for daily-use materials, such as foods and cosmetics, because of improved quality, nanomaterials are still being investigated for their safety. Recently, we have investigated the effect of nanoparticles on several brain cells. This year, we investigated the penetration mechanism of nanoparticles into the blood brain barrier using endothelial cells of capillary vessel. Our data showed that the cell index (electrical resistance value) of endothelial cells was decreased with the addition of nanoparticles, although the observation images of the cells were apparently unaffected. This result suggests that the barrier function of endothelial cells is affected by the nanoparticles and allows the particles to penetrate. Because the cell index seems to have higher sensitivity than does the observation of cell images, we will use the index to screen for the penetration of particles.

Publications

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