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General Summary

Study of antibody formation during enzyme replacement therapy for Fabry disease
Two enzyme preparations are available for enzyme replacement therapy for Fabry disease. One is agalsidase alfa (α), and the other is agalsidase beta (β). The antibody against β cross-reacted with α to the same extent as to β. The anti-β antibody neutralizes the enzyme activity of both α and β equally and inhibited the cellular uptake of β. The antibody titer assayed with enzyme-linked immunosorbent assay was positively correlated with the neutralizing activity of β and the inhibition of cellular uptake by β.

Neonatal gene therapy for Krabbe disease
We have studied the therapeutic effects of gene therapy in the neonatal mouse model of Krabbe disease, a progressive demyelinating disease. We injected a recombinant lentiviral vector including an enzyme (galactocerebrosidase) expressing the gene for the mouse neonatal facial vein and detected the significant effects of reduced substrate accumulation, improved pathological findings, and increased life span. We are preparing studies of more efficient transduction, *ex-vivo* gene transfer, and a homologous recombination system using the zinc finger method.

Pathophysiological analysis of Pompe disease
We analyzed the signaling pathway of endoplasmic reticulum stress-independent autophagy in fibroblasts derived from patients with Pompe disease. We found decreased levels of phosphorylated Akt and phosphorylated p70 S6 kinase in the fibroblasts. This result suggests that the down-regulated Akt/mammalian target of rapamycin pathway activates autophagy in fibroblasts from patients with Pompe disease.

Antitumor effect and application to gene therapy of nafamostat mesilate for fatal digestive cancer
Recent studies have demonstrated that nuclear factor (NF)-κB plays an important role in the regulation of cell apoptosis, inflammation, and oncogenesis. Inhibition of NF-κB is a potential new strategy for the treatment of cancers. We have previously reported that nafamostat mesilate, a serine-protease inhibitor, inhibits NF-κB activation and induces the apoptosis of pancreatic cancer. Moreover, we have shown that the addition of nafamostat mesilate promotes apoptosis induced by gemcitabine or paclitaxel owing to the inhibition of the NF-κB activation of pancreatic cancer. The clinical usefulness of the combination of gemcitabine and nafamostat mesilate for patients with unresectable pancreatic cancer was examined in a phase II study. Recently, we investigated the antitumor effects of nafamostat mesilate against other digestive cancers. Intraperitoneal combina-

tion therapy with paclitaxel and nafamostat mesilate enhanced the antitumor effect of paclitaxel in a mouse model of gastric cancer with peritoneal dissemination.
Gene therapy using an adenoviral vector expressing tumor necrosis factor-alpha (TNF-α) is a new therapeutic approach for chemoresistant malignancies. However, the efficacy of TNF-α is limited because of the activation of NF-κB. We hypothesized that the addition of nafamostat mesilate would enhance the antitumor effect of TNF-α gene delivery, and we have demonstrated the efficacy of the combination therapy against pancreatic cancer. Recently, we have investigated the efficacy of the combination therapy against hepatocellular carcinoma.

Islet biology and molecular medicine in diabetes
To develop a method for in-vitro observation of isolated islets of the pancreas, we performed animal experiments this year with a completed intercellular matrix. The results were submitted to a journal for publication.
As a clinical research, we performed study on pathophysiology of hypoglycemia by analyzing the timing of spontaneous hypoglycemia and the glucagon response with the continuous glucose monitoring in a patient with frequent hypoglycemia with unknown cause. From the hormone response, we hypothized the patient should have impairment in the process of gluconeogenesis, and have started genetic analysis of candidate genes for the gluconeogenic enzymes including phosphoenolpyruvate carboxykinase, pyruvate kinase, and fructose-1,6-bisphosphatase.

High-risk ovarian cancer based on 126-gene expression signature is uniquely characterized by downregulation of the antigen-presentation pathway
High-grade serous ovarian cancers are heterogeneous both in terms of clinical outcomes and at the molecular level. Our aim was to establish a novel risk-classification system based on gene expression signatures for predicting overall survival which we hope will lead to novel therapeutic strategies for high-risk patients. In this large-scale cross-platform study of 6 microarray data sets from 1,054 patients with ovarian cancer, we developed a gene expression signature for predicting overall survival by applying elastic net and 10-fold cross-validation to Japanese data set A (n=260) and evaluated signatures in 5 other data sets. Subsequently, we investigated differences in the biological characteristics between patients with high- and low-risk ovarian cancers. An elastic net analysis of Japanese data set A identified a 126-gene expression signature for predicting overall survival in patients with ovarian cancer (multivariate analysis, $P=4\times10(-20)$). We validated the predictive ability of the signature through multivariate analysis with 5 other data sets (Tothill's data set, $P=1\times10(-5)$; Bonome's data set, P=0.0033; Dressman's data set, P=0.0016; TCGA data set, P=0.0027; and Japanese data set B, P=0.021). Through gene ontology and pathway analyses, we identified a significant reduction in expression of immune-response-related genes, especially on the antigen-presentation pathway, in patients with high-risk ovarian cancer. This risk classification based on the 126-gene expression signature is an accurate predictor of clinical outcome in patients with advanced-stage high-grade serous ovarian cancer and might lead to new therapeutic strategies for patients with high-grade serous ovarian cancer.

Publications

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