Institute of DNA Medicine
Department of Gene Therapy

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General Summary

Our purpose is to develop therapeutic methods for intractable diseases, including genetic diseases, cancer, and diabetes. We performed various studies and investigations this year. Below, we will describe the progress in each of our projects.

Research Activities

Genetics Disease

1. Development of gene therapy for lysosomal storage diseases:

We generated recombinant lentiviral vectors expressing missing enzymes in Krabbe disease and mucopolysaccharidosis type VII and administered these vectors to newborn model mice. For Krabbe disease, we detected increases in body weight but no effects on the progression of symptoms. For mucopolysaccharidosis type VII, effects on body weight and life span were observed, and lentiviral vector DNA copies were detected in organs, including the brain, of neonatal-treated model mice, findings that suggest efficient long-term expression.

2. Pathophysiological analysis of Pompe disease

We analyzed the relationship of autophagy with endoplasmic reticulum stress in fibroblasts derived from patients with late-onset Pompe disease. We found that autophagy is activated in patients' fibroblasts by mutant lysosomal acid/alpha-1,4- glucosidase-induced endoplasmic reticulum stress and is inhibited by treatment with N-butyl-deoxynojirimycin.

3. Immune tolerance induction for enzyme replacement therapy for Pompe disease

Last year, we found that antibodies against infused enzymes in enzyme replacement therapy for Fabry disease decrease clinical efficacy. This year, we developed tolerance-induction therapy for enzyme replacement therapy for Pompe disease using a murine model. The oral administration of enzymes successfully induced immune tolerance. The formation of antibodies against enzymes is dependent upon T cells. Therefore, we administered anti-CD3 antibodies before enzyme infusion and successfully induced immune tolerance against the enzyme. Moreover, this tolerance persisted even if the enzyme was repeatedly infused. We are investigating the mechanism of this tolerance induction.

4. Bone marrow transplantation for lysosomal storage diseases

Although bone marrow transplantation is an effective treatment for lysosomal storage diseases, the minimum number of donor cells required to reduce storage materials remains unknown. To answer this question, we created a Fabry model mouse that carries various numbers of donor cells by the transplantation of various ratios of Fabry

mouse bone marrow cells and wild-type bone marrow cells to lethally irradiated Fabry mice. We found that 30% and 50% donor cells were enough to reduce storage material in the lungs and heart, respectively, and that these reduction values were equivalent to 100% donor cell reconstitution. Therefore, the intensity of the preconditioning regimen can be reduced, and this observation is beneficial for hematopoietic stem cell targeted gene therapy.

5. Antitumor effect and application to gene therapy of nafamostat mesilate for fatal digestive cancers

Recent studies have demonstrated that nuclear factor κB (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 cancer. We have shown that nafamostat mesilate, a serine-protease inhibitor used in Japan for the treatment of pancreatitis and disseminated intravascular coagulation and as an anticoagulant for hemodialysis, inhibits NF-κB activation and induces apoptosis of pancreatic cancer. The combination of nafamostat mesilate and gemcitabine has been shown to be effective against pancreatic cancer in animal experiments and will be examined in clinical trials. We have also shown that the combination of paclitaxel and nafamostat mesilate is effective in models of peritoneal dissemination of pancreatic cancer; the combination is being studied in gastric cancer, which also tends to disseminate to the peritoneal cavity. Gene therapy with an adenoviral vector expressing tumor necrosis factor-alpha (TNF-α) is a new therapeutic approach for pancreatic cancer, for which treatment efficacy is limited, because TNF-α activates NF-κB. We hypothesize that the addition of nafamostat mesilate, an NF-κB inhibitor, may enhance the antitumor effect of adenovirus vector-mediated TNF-α gene therapy for pancreatic cancer.

6. Vitamin D receptor polymorphisms and the prognosis of patients with epithelial ovarian cancer

Recently, the vitamin D receptor (VDR) polymorphism FokI was shown to be associated with increased susceptibility to ovarian cancer. We examined whether VDR FokI polymorphisms affect the prognosis of patients with epithelial ovarian cancer. The VDR polymorphisms from FokI in 101 patients with epithelial ovarian cancer were genotyped by sequencing. Overall survival was compared between FokI single nucleotide polymorphisms by means of Kaplan-Meier survival analysis, log-rank tests, and the Cox proportional hazard model adjusted for International Federation of Gynecology and Obstetrics stages, postoperative chemotherapy, histologic type, and the presence of residual tumor. Hazard ratios, adjusted hazard ratios, and 95% confidence intervals were determined. The FokI C/C genotypes were associated with a better prognosis than were the C/T and T/T genotypes (log-rank test: $P=0.008$; adjusted hazard ratio: 0.16; 95% confidence interval: 0.05 to 0.57; $P=0.004$). Thirty months after surgery, 90% of patients with the FokI C/C genotype were still alive; in contrast, 66% of patients with the C/T or T/T genotype were alive. When the cancer stage of patients was restricted to II to IV, 84% of patients with the FokI C/C genotype were still alive: in contrast, only 50% of patients with the C/T or T/T genotype were alive. These results suggest that the VDR polymorphisms from the FokI genotype are associated with the improved prognosis of patients with epithelial ovarian cancer.

7. Molecular intervention therapy for pancreatic islets

1) We have continued studying molecular intervention therapy for pancreatic islets using an adeno-associated viral vector (serotype 8). We focused on possible mechanisms of beta cell death by islet injury, including oxidative stress, and planned to use several genes expressed in beta cells as therapeutic molecules.

2) To determine the mechanism of islet injury and its prevention, we have started to study the development of islet structures during murine embryogenesis. This year, we have investigated the interaction among islet endocrine cells and peri-islet Schwann cells.

3) We performed a comprehensive study of a novel system for delivering therapeutic molecules, such as insulin and glucose-like peptide 1. For this purpose, the steadiness of insulin injection with an ink-jet printer was studied by investigating glucose uptake into L cells and biochemical characteristics with high-performance liquid chromatography and matrix-assisted laser desorption ionization-time of flight mass spectrometer/spectometry. We also investigated its bioavailability of the ink-jet insulin in vivo in rats.

Publications

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