Division of Gene Therapy

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General Summary

As we did last year, this year we have been studying lysosomal storage diseases (LSDs) and various cancers of the digestive tract. In the research on LSDs, we have been developing novel gene therapy technology, novel strategies to overcome limitations of current therapies (enzyme replacement therapy [ERT] and bone marrow transplantation [BMT]), pathophysiological analysis of LSDs using induced pluripotent stem (iPS) cells and molecular analysis of patients with LSDs. In the research on cancers of the digestive tract, we have been developing a novel gene therapy method using a protease inhibitor.

Research Activities

Immune tolerance induction of ERT for LSDs
Animal and human studies of ERT for Pompe disease have indicated that antibodies generated against infused recombinant human α-glucosidase (GAA) can have a negative effect on the therapeutic outcome and cause hypersensitivity reactions. We have previously shown that oral administration of GAA reduces immune tolerance against GAA. In a previous study, wild-type mice were immunized after receiving only 2 intraperitoneal injections of GAA/adjuvant. To study whether the above approach for inducing immune tolerance can be applied to clinical ERT, we mimicked the clinical protocol. As a result, the oral administration of GAA efficiently prevented lethal hypersensitivity and successfully induced immune tolerance.

BMT for LSDs
Mucopolysaccharidosis (MPS) type II is a lysosomal storage disorder caused by deficient activity of the iduronate-2-sulfatase (IDS). Although BMT has been proposed to have a beneficial effect for patients with MPS II, the requirement for donor-cell chimerism to reduce glycosaminoglycan (GAG) levels is unknown. To address this issue, we transplanted various ratios of normal and MPS II bone marrow cells in a mouse model of MPS II and analyzed GAG accumulation in various tissues. The level of GAG reduction in these tissues depends on the percentage of normal-cell chimerism. These observations suggest that a high degree of chimerism is necessary to achieve the maximum effect of BMT. However, the relation between clinical responses and the level of GAG reduction should be clarified.

Gene therapy using lentiviral vector system and homologous recombination using zinc finger methods for LSDs and the analysis of bone system metabolism in MPS II
We are investigating the effect of gene therapy for MPS VII, MPS II, and Krabbe disease. For MPS VII, we injected a lentiviral vector into newborn mice and found that this treat-

ment increased β-glucuronidase expression and decreased accumulated GAGs in key organs at 20 to 30 weeks, increased the vector copy number in the brain at 30 weeks, and decreased the autophagic buildup in the brain. We performed *ex vivo* gene therapy in a mouse model of MPS II at 8 weeks of age using a recombinant lentiviral vector expressing the IDS gene (*IDS*). This treatment succeeded in establishing long-term overexpression of the IDS enzyme in the circulating blood and efficient IDS expression and decreasing levels of accumulated GAGs in the brain. (Wakabayashi et al. 2015 Human Gene Therapy)
We have begun to analyze bone system metabolism, including factors of signal transduction, in MPS II and will investigate the effect of gene therapy using lentivirus vector.
For Krabbe disease, we performed neonatal gene therapy using a lentiviral vector and succeeded in increasing the expression of β-galactosylceramidase (GALC), decreasing high levels of accumulated psychosine in the brain, improving myelin-forming cells, and increasing life spans. We also attempted to use the zinc finger system for site-specific homologous recombination in the GALC gene (*GALC*) *in vitro* and succeeded in exchanging a specific sequence of *GALC* and increasing GALC expression in the treated cells. These results suggest the efficient effect of the neonatal gene therapy and the zinc finger system for LSDs.

Development of a method for measuring disease-specific iduronic acid from the nonreducing end of GAGs in MPS II
The GAGs are used as a biomarker for analyzing MPSs, including MPS II. However, the conventional assay for total GAGs has low sensitivity and specificity for evaluating abnormalities in tissue extracts of MPS II. To resolve this problem, we developed a novel method of measuring disease-specific GAGs based on the analysis of 2-sulfoiduronic acid levels derived from the nonreducing end of GAGs by using recombinant human IDS and iduronidase. Our method demonstrated that the levels of generated iduronic acid were markedly increased in tissues from MPS II mice, whereas the monosaccharide was not detected in samples from wild-type mice. This result indicates that this assay is useful for analyzing disease-specific GAGs in tissues from MPS II mice.

Identification of cryptic novel α-galactosidase a gene mutations: Abnormal messenger RNA splicing and large deletions
Fabry disease is an X-linked lysosomal storage disorder caused by mutation of the galactosidase alpha (GLA) gene (*GLA*) and resulting in the deficient activity of GLA. This deficient activity causes various glycolipids, such as globotriaosylceramide, to be stored in many tissues. The main clinical symptoms of Fabry disease are neuropathic pain, hypohydrosis, and cerebrovascular, renal, and cardiac disease. Although Fabry disease is inherited in an X-linked recessive manner, clinical symptoms often develop in heterozygous female patients. In male patients, enzyme analysis is usually sufficient for diagnosis. However in female patients, especially those without a family history of the disease, a definitive diagnosis often requires the pathologic *GLA* mutation to be detected. More than 700 variants and mutations have been identified in *GLA* from patients with Fabry disease. In the present study, we examined *GLA* from patients with Fabry disease who did not

carry mutations in exons or exon/intron boundaries. We found some novel cryptic mutations related to Fabry disease: a multiple-exon deletion mutation, an insertion mutation of splicing enhancer sequence, an insertion mutation of exon-skipping element by long interspersed nuclear element 1 retrotransposon element, and a point mutation in a non-protein coding region by MLPA (multiplex ligation-dependent probe amplification) method and analyzing cDNA.

Gene transfer and metabolic profiling of Pompe disease induced pluripotent stem cells
We have investigated late-onset Pompe disease-specific induced pluripotent stem cells and have already shown that cardiac differentiation is useful in terms of the disease modeling. We have generated a third-generation lentiviral vector expressing missing enzyme and confirmed the dose-dependent expression in patient-specific induced pluripotent stem cells. Moreover, the enzyme activity and glycogen accumulation also improve according to the lentiviral gene transfer. Therapeutic efficacy is maintained even after the cardiac differentiation. Next, we have quantified the metabolic status of Pompe disease-specific induced pluripotent stem cell-derived cardiomyocyte by "metabolomic analysis." Pompe disease-induced pluripotent stem cell-derived cardiomyocytes show the oxidative stress. We have also validated that such change occurs in the cardiomyocytes and skeletal muscle in the mouse model of Pompe disease. Oxidative stress might be associated with the mechanism of Pompe disease *in vitro* and *in vivo*. In the future we plan to assess the efficacy of antioxidant in the pluripotent stem cell-derived cardiomyocytes and model mice.

Antitumor effect of nafamostat mesilate for digestive cancer and treatment of cancer pain
Recent studies have demonstrated that nuclear factor (NF)-κB plays important roles 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, gastric, and gallbladder cancers. The clinical usefulness of the combination of gemcitabine and nafamostat mesilate for patients with unresectable pancreatic cancer was examined in a phase II study. Because the standard therapies for unresectable pancreatic cancer are gemcitabine/S-1 or gemcitabine/nanoparticle albumin-bound paclitaxel, we investigated the combination therapy of these anticancer agents and NF-κB inhibitor. Moreover, we have investigated the antitumor effect of combination therapy with nafamostat mesilate and radiation for pancreatic cancer.
Ionizing radiation enhances epithelial-mesenchymal transition (EMT) and cancer metastasis. Neoadjuvant chemoradiation for colorectal cancer also enhanced EMT. Therefore, we have suppressed EMT by inhibiting NF-κB or signal transducer and activator of transcription 3 in chemoradiation for colorectal cancer.
Cancer pain worsens the quality of life of patients with unresectable pancreatic cancer. We have shown the mechanism of cancer pain and investigated a new treatment strategy.

Publications

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