

Institute of DNA Medicine

Department 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 research for 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 research for 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

ERT is extremely effective for Pompe disease. However, its efficacy is decreased by antibodies against infused enzyme. We have previously shown that parenteral administration of an antibody against murine CD3 induced immune tolerance against the enzyme (Ohashi T, et al. *Mol Ther*, 2012). In a previous study we administered an anti-murine CD3 antibody in a murine model of Pompe disease. This antibody did not react with human CD3 antigens. To assess the possible clinical application of this strategy, we administered an anti-human CD3 antibody (otelixizumab) to wild-type mice expressing human CD3 by means of an infusion regimen similar to that used in the previous study. In this immunization protocol, parenteral administration of anti-human CD3 antibody also reduced the titer of antibodies against the enzyme.

Comparison of therapeutic effects of BMT, ERT, and combination therapy with ERT and BMT for LSDs

We have performed BMT, ERT, and combination therapy with both treatments in a murine model of mucopolysaccharidosis (MPS) type II and compared the efficacy of each treatment by means of an assay for tissue glycosaminoglycans (GAGs), which are storage materials in MPS II. The ERT reduced GAGs more profoundly than did BMT. Moreover, ERT had an additive effect to BMT. However, none of treatments reduced GAGs in the brain.

Disease modeling of late-onset Pompe disease-specific iPS cells

As in infantile Pompe disease, cardiovascular complications have been documented in late-onset Pompe disease. To clarify the mechanisms of cardiac involvement in late-onset Pompe disease, we have generated late-onset Pompe disease iPS cells and differentiated them into cardiomyocytes for disease modeling. Cardiomyocytes derived from

late-onset Pompe disease iPS cells exhibited disease-specific hallmarks, such as massive glycogen accumulation and lysosomal enlargement. Our results suggest that pathological changes in differentiated cardiomyocytes might explain the cardiovascular complications in late-onset Pompe disease.

Novel therapy for Pompe disease by using an enzyme-stabilizing agent

An ERT with recombinant human acid alpha-glucosidase (GAA) was recently approved for treating Pompe disease. This ERT prolongs survival and decreases cardiac muscle pathology, but has several problems, such as resistance in skeletal muscles and production of antibodies against recombinant human GAA. Therefore, an alternative method of addressing GAA deficiency is needed for the effective treatment of patients with Pompe disease. We have previously shown that the proteasome inhibitor bortezomib can exert a positive effect on mutant GAA in fibroblasts from a patient with Pompe disease carrying the specific mutation. However, bortezomib has not been fully characterized as an enzyme-enhancement molecule that is effective for multiple GAA mutations. In this study, we investigated the effect of bortezomib treatment on mutant GAAs in patient fibroblasts and transiently expressed cells. Bortezomib increased the maturation and residual activity of GAA in patient fibroblasts carrying the M519V and C647W mutations. Enhanced colocalization of GAA with lysosomal marker lysosome-associated membrane protein 2 was also observed in patient fibroblasts after treatment with bortezomib. When mutant GAAs were overexpressed in HEK293T cells, bortezomib increased the activity of M519V and C647W in these cells (by factors of 1.3 and 5.9, respectively). These results indicate that bortezomib enhances the activity of some GAA mutants, such as M519V and C647W.

Analysis of α -galactosidase A gene mutations from patients with Fabry disease by complementary DNA analysis and multiplex ligation-dependent probe amplification

Background: Fabry disease is characterized by the deficient activity of the enzyme α -galactosidase A (GLA). We revealed 4 new mutations of the *GLA* gene in 5 families using multiplex ligation-dependent probe amplification (MLPA) and *GLA* complementary (c) DNA analysis.

Results: Mutation analysis of *GLA* cDNA showed: 1) insertion of intron 3 (1 family), 2) insertion of intron 4 (2 families), 3) exon-skipping of exon 4 (1 family), and 4) exon-skipping of from exons 2 to 5 (1 family). Analysis of genomic DNA showed: 1) an IVS3+395 (G>C) point mutation, 2) an IVS4+330(113b) insertion mutation, 3) a L1 gene insertion mutation (exon 4 #745), and 4) a Del 5.5-kb (Int1-Ex5) deletion mutation.

Discussion: 1) The IVS3+395 (G>C) might induce alternative splicing. 2) This 113-base pair insertion might function as a splicing enhancer. 3) The exon-skipping by L1 has been reported in some genetic diseases. 4) An inverted repeat sequence is present within the deletion region of the 5'- and 3'-ends of DNA. This sequence might be involved in the deletion mechanism. The MLPA method and cDNA analysis method are useful for *GLA* gene mutation analysis, especially in large deletion/insertion mutations and functional gene mutations in introns.

Gene therapy using a lentiviral vector system and homologous recombination using zinc finger methods for LSDs

We are investigating the effects 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 treatment 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 including the iduronate sulfatase (IDS) gene. 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. 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 tried the zinc finger system for site-specific homologous recombination in the *GALC* gene *in vitro* and succeeded in exchanging a specific sequence of the *GALC* gene 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.

Antitumor effect and application to gene therapy of nafamostat mesilate for cancers of the digestive tract

Recent studies have demonstrated that nuclear factor (NF)- κ B plays important roles in the regulation of cell apoptosis, inflammation, and oncogenesis. Inhibition of NF- κ B is a potential new strategy for the treatment of cancers. We have previously reported that nafamostat mesilate, a serine-protease inhibitor, inhibits NF- κ B 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- κ B 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. Now we are investigating triple combination therapy with gemcitabine, nanoparticle albumin-bound paclitaxel, and nafamostat mesilate, which is the next standard therapy for pancreatic cancer. Recently we have investigated the antitumor efficacy of combination therapy with nafamostat mesilate and radiation for pancreatic cancer. In addition to inducing apoptosis, nafamostat mesilate was found to promote the efficacy of cell arrest at the G2/M checkpoint. Recent studies have found that human CD40 ligand (CD40L) gene delivery has a direct antitumor effect via CD40-CD40L interaction. However, CD40L enhances activation of NF- κ B. We have previously reported that nafamostat mesilate inhibits NF- κ B activation and enhances apoptosis caused by adenovirus vector-mediated tumor necrosis factor α in pancreatic and hepatocellular carcinomas. Therefore, we have investigated the efficacy of combination therapy with nafamostat mesilate and adenovirus vector-mediated CD40L gene therapy for pancreatic cancer.

Publications

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