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]), and novel therapeutic strategies using induced pluripotent stem cells. In research for cancers of the digestive tract, we have been developing a novel therapy method using a protease inhibitor.

Research Activities

Novel BMT methods for LSDs

Many LSDs are treated with BMT. However, preconditioning regimens with high-dose chemotherapy and high-dose irradiation worsen the general condition of patients. Thus, we have been developing a safer preconditioning regimen. Hematopoietic stem cells (HSCs) in recipients of BMT must be killed by preconditioning. However, most HSCs are not dividing and are resistant to chemotherapy and radiation. We hypothesized that if HSCs were dividing, the doses of chemotherapy and irradiation could be reduced. We focused on bone marrow niche, inducer of type 1 interferon, and anti-cKit antibody were used for preconditioning for BMT in mucopolysaccharidosis mice. As a result, engraftment of a sufficient number of donor cells was achieved and exhibited a therapeutic effect without significant side effects.

Immune tolerance induction of ERT for LSDs

ERT is extremely effective for Pompe disease. However, its efficacy is decreased by antibodies against infused enzymes. We have previously shown that oral administration of an enzyme induced immune tolerance against the enzyme (Ohashi T, et al. *Mol Genet Metab*, 2011). In this study we immunized mice with intraperitoneal injections of an enzyme and adjuvant. To assess the possible clinical application of this strategy, we immunized mice with an infusion regimen similar to the clinical protocol with biweekly intravenous infusion of enzymes (20 mg/kg). In this immunization protocol, oral administration of enzyme reduced the titer of antibodies against the enzyme and prevented lethal allergic reaction to the enzyme.

Gene therapy for LSDs

We studied the therapeutic effect of gene therapy in a neonatal mouse model of Krabbe disease, a progressive demyelinating disease. We injected a recombinant lentiviral vec-

tor including the therapeutic gene expressing galactocerebrosidase (GALC) into the facial vein of neonatal mice and detected significant effects, including reduced substrate accumulation, decreased pathological findings in Schwann cells, delayed symptom onset, and increased life span. We also investigated neonatal gene therapy for mucopolysaccharidosis (MPS) type VII (Sly disease) which resulted in efficient enzyme expression in the brain. We are attempting *ex vivo* gene therapy using a lentivirus system for MPS II (Hunter disease) and attempting to transduce cells from a patient with Pompe disease into induced pluripotent stem cells. We also studied the synergic effect with substrate reduction therapy and are preparing a homologous recombination system using Zinc-finger nuclease.

Novel therapy for Pompe disease with 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. In this study, we focused on the pathogenic mechanism by which mutant GAA is degraded by endoplasmic reticulum-associated protein degradation in some patients with Pompe disease and investigated whether 2 proteasome inhibitors (bortezomib and MG132) restore the function of mutant GAA in fibroblasts from patient with Pompe disease. Each proteasome inhibitor promoted the stabilization of patient GAA and the processing of them to mature forms at any concentration tested. In addition, lower concentrations of bortezomib and MG132 showed no cytotoxic effects in patient fibroblasts. Increased colocalization of GAA with the lysosomal marker lysosome-associated membrane protein 2 were observed in patient fibroblasts treated with proteasome inhibitors. Furthermore, proteasome inhibitors also increased enzyme activity in patient fibroblasts. In particular, bortezomib was more effective than MG132 in enhancing GAA activity in patient fibroblasts (about 4-fold and 2-fold increases of residual activity, respectively). These results suggest that bortezomib is a novel drug for treating patients with Pompe disease.

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

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. Recently we have investigated the antitumor efficacy of combination therapy with nafamostat mesilate and

radiation for pancreatic cancer.

Recent studies have found that human CD40 ligand (CD40L) gene delivery has direct an antitumor effect via CD40-CD40L interaction. However, CD40L enhances activation of NF-κB. We have previously reported that nafamostat mesilate inhibits NF-κB activation and enhances apoptosis caused by adenovirus vector-mediated tumor necrosis factor α in pancreatic and hepatocellular carcinoma. 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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Reviews and Books

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