Research Center for Medical Sciences
Division of Gene Therapy

Toya Ohashi, *Professor and Director*　　Hiroshi Kobayashi, *Associate Professor*

Research Activities

As we have done for many years, we have been developing novel therapeutic methods to overcome limitation of current therapy for lysosomal storage disease (LSD) and intractable cancer, which has no therapy or only inefficient therapy. Our missions are finding new seeds for such novel therapy and translating new seeds to clinical use.

Induction of immune tolerance in enzyme replacement therapy for LSDs
We have already shown that immune reactions against enzymes in enzyme replacement therapy for LSDs have a negative effect on its efficacy. This year, we found that an anti–B-lymphocyte stimulator antibody, which reacts with B cells and is approved in the United States, induced tolerance against enzymes in enzyme replacement therapy for Fabry disease.

Recombinant lentiviral vector and zinc finger nuclease–mediated gene therapy for LSDs
We investigated the bone system in mucopolysaccharisosis type II (MSP II), and detected increasing of bone mass, trabecular bone, bone density, and bone strength comparing to the normal. We are preparing to investigate changes in these factors by the intervention of gene therapy.

Developing human hematopoietic cells transplantable MSP II mouse model
We are developing hematopoietic stem cell targeted gene therapy for MSP II. To translate this methodology to clinical use, we are developing a human hematopoietic cells transplantable humanized MSP II mouse model. The NOG mice were developed in the Central Institute for Experimental Animals (Kawasaki, Japan) and shows marked engraftment of human hematopoietic stem cells. This year, we knocked out the iduronate 2-sulfatase (IDS) gene (*IDS*) in fertilized eggs from NOG mice by using gene-editing technology (CRISPR/Cas9) and transferred embryos to the uteri of NOG mice. We are now analyzing newborn mice.

Development of novel therapy for Pompe disease by using a proteasome inhibitor
We previously demonstrated that the proteasome inhibitor bortezomib can improve acid α-glucosidase (GAA) activity in patient fibroblasts and cell lines transiently expressing several types of mutants of the GAA gene (*GAA*) mutants. However, the efficacy of bortezomib treatment in an animal model is still unclear. In this study, we generated a missense mouse model of Pompe disease expressing human GAA with M519V amino-acid substitution and analyzed the efficacy of bortezomib treatment on GAA activity in our novel Pompe mice. Bortezomib treatment increased levels of precursor and mature GAA

in the hearts of M519V mice but not in skeletal muscle. Increased GAA activity was also observed in the heart but not in skeletal muscle. These results indicate that bortezomib can work as enzyme-enhancement molecule in an animal model and in cultured cell lines.

Effect of sulfated disaccharides on IDS in MSP II
Pharmacological chaperone therapy has attracted considerable attention as a potential treatment for LSDs accompanying central nervous system lesions. However, a pharmacological chaperone therapy for MSP II has not been developed. In this study, we focused on the sulfated disaccharides derived from heparin, which is a substrate of IDS, and evaluated the efficacy of oligosaccharides as pharmacological chaperones for MSP II. When sulfated disaccharides were incubated with recombinant IDS, thermal degeneration of the enzyme was significantly prevented. In addition, sulfated disaccharides increased IDS activity in fibroblasts from a patients with MSP II. These results suggest that sulfated disaccharides are candidate molecules for pharmacological chaperone therapy for MSP II.

Treatment for Fabry peripheral neuropathy with adeno-associated virus vector in murine Fabry disease model
Fabry disease is an X-linked LSD caused by mutation of the α-galactosidase A (GLA) gene (*GLA*), resulting in deficient activity of GLA. Peripheral neuropathy is a significant symptom of Fabry disease. We treated a murine model of Fabry disease with the human *GLA* gene encoded with an adeno-associated virus (AAV) serotype AAV rh 10 vector. The activity of GLA was increased in the heart, liver, spleen central nervous system and the peripheral nervous system. The murine model of Fabry disease showed dysesthesia compared with wild-type mouse. With the von Frey up-down test, dysesthesia was marginally more improved in AAV-treated mice than in untreated mice with Fabry disease. Our data indicates that gene therapy with AAV rh10 encoding the human *GLA* gene could be a new therapeutic approach for Fabry peripheral neuropathy.

Antitumor effect of nafamostat mesilate for digestive cancer and treatment of cancer pain
We have previously reported that nafamostat mesilate, a serine-protease inhibitor, inhibits the activation of nuclear factor kappa B (NF-κB) 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 and gemcitabine/nab-paclitaxel, we have investigated combination therapy with these anticancer agents and an NF-κB inhibitor. Moreover, we have investigated the antitumor effect of combination therapy with new NF-κB inhibitors, such as pomalidomide and recombinant thrombomodulin, for pancreatic cancer.
Ionizing radiation enhances epithelial-mesenchymal transition (EMT) and cancer metastasis. Neoadjuvant chemoradiation for colorectal cancer also enhances EMT. Therefore, we have suppressed EMT by inhibiting NF-κB or signal transducer and activator of tran-

scription 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

***Kato S*[1]*, Yabe H*[1]*, Takakura H*[1]*, Mugishima H*[2]*, Ishige M*[2]*, Tanaka A*[3]*, Kato K*[4]*, Yoshida N*[4]*, Adachi S*[5]*, Sakai N*[6]*, Hashii Y*[6]*, Ohashi T, Sasahara Y*[7]*, Suzuki Y*[8]*, Tabuchi K*[9] *([1]Tokai Univ, [2]Nihon Univ, [3]Osaka City Univ, [4]Jpn Red Cross Nagoya Hosp, [5]Kyoto Univ, [6]Osaka Univ, [7]Tohoku Univ, [8]Gifu Univ, [9]Komagome Hosp).** Hematopoietic stem cell transplantation for inborn errors of metabolism: A report from the Research Committee on Transplantation for Inborn Errors of Metabolism of the Japanese Ministry of Health, Labour and Welfare and the Working Group of the Japan Society for Hematopoietic Cell Transplantation. *Pediatr Transplant.* 2016; **20:** 203–14.

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