Research Center for Medical Sciences
Division of Gene Therapy

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

General Summary

We mainly studied gene therapy for lysosomal storage disease such as mucopolysaccharidosis type II, Krabbe diease and Fabry disease, using various viral vector. In addition, we also developing small molecule therapy for Pompe disease and mucopolysaccharidosis type II. Regarding cancer, we studied anticancer effect of nafamostat mesilate, a serine-protease inhibitor, for various type of cancer. Our main mission is to translate these observation to clinic.

Research Activities

Gene therapy for lysosomal storage disease using lentivirus vector and genome editing
We investigated the bone system in mucopolysaccharidosis type II (MPS II), and detected increasing of bone mass, trabecular bone, bone density, and bone strength comparing to normal group. We generated lentiviral vector expressing iduronate-2-sulfatese and carried out hematopoietic stem cell targeted *ex-vivo* gene therapy for MPS II model mouse. The improvement of bone involvement was observed. And we also investigated the effect of newborn gene therapy, combination with substrate reduction therapy (SRT), and *in vitro* gene editing (using Zinc Finger system) for Krabbe disease model mouse.

Development of novel therapy for Pompe disease by using proteasome inhibitor
We evaluated the bortezomib-induced blood toxicity in murine model of Pompe disease. As a result, no significant difference was observed in the number of both leukocytes and platelets between Pompe mice treated with or without bortezomib. This result suggests that bortezomib may exert a positive effect on α-glucosidase activity in Pompe mice without induction of blood toxicity.

Effect of sulfated disaccharides on mutated IDS in Mucopolysaccharidosis type II
We investigated the effect of sulfated disaccharides on mutated iduronate-2-sulfatase (IDS) by using cell-based assay. Sulfated disaccharides improved the enzyme activity of several types of mutated IDS which have an amino acid substitution around of active site.

Improvement of peripheral neuropathy in Fabry disease mouse by AAV9 vector
Fabry disease (FD) is a genetic disorder caused by mutation of the *GLA* gene, resulting in accumulation of globotriaosyl ceramide (Gb3) in various tissues including dorsal root ganglia (DRG). FD patients have peripheral neuropathy from childhoods. We injected rAAV9 encoding hGLA (rAAV2/9-hGLA) intrathecally (i.t.) to reduce the Gb3 accumulation in DRG. The GLA enzyme activity in the lumber DRG of rAAV-FD mice was

increased compare to wild type mice. FD mice showed a thermal hypoalgesia in hot-plate test, and the level of thermal hypoalgesia in the rAAV-FD mice recovered to the level of wild type mice. As a conclusion, the i.t. administration of rAAV2/9-hGLA transduced neural cells of DRG and improves the peripheral neuropathy of FD.

Enhancement of antitumor effect by NF-κB inhibitor for digestive cancers and treatment of cancer pain

We have reported that nafamostat mesilate, a serine-protease inhibitor, inhibits NF-κB activation and enhances anti-tumor effects for pancreatic cancer. The clinical usefulness of the combination chemotherapy of gemcitabine with nacamostat mesilate was examined in phase II study. Moreover, thalidomide, the first generation of IMiDs, has anti-tumor effects for myeloma and colorectal cancer cells. Recently, standard chemotherapies for unresectable pancreatic cancer are gemcitabine/S-1 or gemcitabine/nab-paclitaxel, thus we investigated combination chemotherapy of these agents with IMiDs. Ionizing radiation enhances epithelial-mesenchymal transition (EMT) and cancer metastasis. We examined whether NF-κB inhibitor suppresses EMT in chemoradiation for colorectal cancer. Cancer pain affects QOL of patients with cancer. We are investigating the mechanism of cancer pain and a new treatment strategy.

Publications

Shirai Y, Uwagawa T, Shiba H, Shimada Y, Horiuchi T, Saito N, Furukawa K, Ohashi T, Yanaga K. Recombinant thrombomodulin suppresses tumor growth of pancreatic cancer by blocking thrombin-induced PAR1 and NF-κB activation. *Surgery.* 2017 Jun; **161:** 1675-82.

Matsui M, Inoue K, Sakoda S, Matsumura T, Hamazaki T, Kosuga M, Okuyama T, Shimada Y, Ohashi T, Nishino I. Screening for late-onset Pompe disease in undiagnosed myopathies. *Neur Clin Neurosci.* 2017; **5:** 60-4.

Horiuchi T, Uwagawa T, Shirai Y, Saito N, Iwase R, Haruki K, Shiba H, Ohashi T, Yanaga K. New treatment strategy with nuclear factor-κB inhibitor for pancreatic cancer. *J Surg Res.* 2016 Nov; **206:** 1-8.

Kono Y, Wakabayashi T, Kobayashi M, Ohashi T, Eto Y, Ida H, Iguchi Y. Characteristics of cerebral microbleeds in patients with Fabry Disease. *J Stroke Cerebrovasc Dis.* 2016 Jun; **25:** 1320-5.

Yokoi T, Yokoi K, Akiyama K, Higuchi T, Shimada Y, Kobayashi H, Sato T, Ohteki T, Otsu M, Nakauchi H, Ida H, Ohashi T. Non-myeloablative preconditioning with ACK2 (anti-c-kit antibody) is efficient in bone marrow transplantation for murine models of mucopolysaccharidosis type II. *Mol Genet Metab.* 2016 Nov; **119:** 232-8.

Sato Y, Kobayashi H, Higuchi T, Shimada Y, Ida H, Ohashi T. TFEB overexpression promotes glycogen clearance of Pompe disease iPSC-derived skeletal muscle. *Mol Ther Methods Clin Dev.* 2016 Aug 10; **3:** 16054.

Sato Y, Kobayashi H, Higuchi T, Shimada Y, Ida H, Ohashi T. Metabolomic profiling of pompe disease-induced pluripotent stem cell-derived cardiomyocytes reveals that oxidative stress Is associated with cardiac and skeletal muscle pathology. *Stem Cells Transl Med*. Epub 2016 Aug 18. pii: sctm. 2015-0409.

Limkul J, Iizuka S, Sato Y, Misaki R, Ohashi T, Ohashi T, Fujiyama K. The production of human glucocerebrosidase in glyco-engineered Nicotiana benthamiana plants. *Plant Biotechnol J.* 2016 Aug; **14:** 1682-94.

Hughes DA, Nicholls K, Shankar SP, Sunder-Plassmann G, Koeller D, Koeller K, Vockley G, Hamazaki T, Lachmann R, Ohashi T, Olivotto I, Sakai, N, Deegan P, Dimmock D, Eyskens F, Germain DP, Goker-Alpan O, Hachulla E, Jovanovic, A, Lourenco CM, Narita I, Thomas M, Wilcox WR, Bichet DG, Schiffmann R, Ludington E, Viereck C, Kirk J, Yu J, Johnson F, Boudes P, Benjamin ER, Lockhart DJ, Barlow, C, Skuban N, Castelli JP, Barth J, Feldt-Rasmussen U. Oral pharmacological chaperone migalastat compared with enzyme replacement therapy in Fabry disease: 18-month results from the randomised phase III ATTRACT study. *J Med Genet.* 2017 Apr; **54:** 288-96.

Kopajtich R, Murayama K, Janecke AR, Haack TB, Breuer M, Knisely AS, Harting I, Ohashi T, Okazaki Y, Watanabe D, Tokuzawa Y, Kotzaeridou U, Kölker S, Sauer S, Carl M, Straub S, Entenmann A, Gizewski E, Feicht-

inger RG, Mayr JA, Lackner K, Strom TM, Meitinger T, Müller T, Ohtake A, Hoffmann GF, Prokisch H, Staufner C. Biallelic IARS mutations cause growth retardation with prenatal onset, intellectual disability, muscular hypotonia, and infantile hepatopathy. *Am J Hum Genet.* 2016 Aug; **99:** 414–22.

Kato S, Yabe H, Takakura H, Mugishima H, Ishige M, Tanaka A, Kato K, Yoshida N, Adachi S, Sakai N, Hashii Y, Ohashi T, Sasahara Y, Suzuki Y, Tabuchi K. 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 Mar; **20:** 203–14.

Shimada Y, Wakabayashi T, Akiyama K, Hoshina H, Higuchi T, Kobayashi H, Eto Y, Ida H, Ohashi T. A method for measuring disease-specific iduronic acid from the non-reducing end of glycosaminoglycan in mucopolysaccharidosis type II mice. *Mol Genet Metab.* 2016; **117:** 140–3.

Higuchi T, Kobayashi M, Ogata J, Kaneshiro E, Shimada Y, Kobayashi H, Eto Y, Maeda S, Ohtake A, Ida H, Ohashi T. Identification of cryptic novel α-galactosidase A gene mutations: abnormal mRNA splicing and large deletions. *JIMD Rep.* 2016; **30:** 63–72.