# Research Center for Medical Sciences
# Division of Gene Therapy

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## General Summary

*Education and research outline*
In terms of education, graduate students were instructed in basic gene manipulation techniques by group, undergraduate students were instructed in reading medical English specialized literature, and in the lab, were instructed in practical research for 6 weeks. In terms of research, the entire laboratory is working on the practical application of gene therapy targeting hematopoietic stem cells (HSCs) of mucopolysaccharidosis type II (MPS II). Receiving budget allocation from Japan Agency for Medical Research and Development, we confirmed a significant effect in model mice by gene transfer using lentiviral vector, and applied for a patent. In addition, the lab members' own research was conducted on themes of lysosomal diseases such as MPS II, GM1 gangliosidosis, Fabry disease, and malignant tumors.

## Research Activities

*Establishment of gene therapy protocol to human HSCs using lentiviral vector*
In the practical application research of the gene therapy method targeting HSCs of MPS II, a third-generation lentiviral vector (MPSV LTR, NCR deleted, dl587 PBS [MND] vector) that was outsourced to Takara Bio Inc. Gene transfer was performed *in vitro* with KG1a cells (acute myeloid leukemia-derived lymphocyte line) and cells positive for human CD34 (derived from healthy human bone marrow). As a result, significant increases in iduronate 2-sulfatase (IDS) enzyme activity and provirus copy number were observed in a dose-dependent manner. In addition, the *Escherichia coli* master cell bank of each plasmid, which is the material for preparing the third-generation lentiviral vector, was also prepared at the guanosine monophosphate level in anticipation of preclinical studies.

*Ex-vivo HSC gene therapy for murine MPS II model*
We generated a new type of third-generation self-inactivating lentiviral vector with a MND promoter to treat a mouse model of MPS II with *ex-vivo* HSC gene therapy. The mouse HSC was transduced with the lentiviral vector and transplanted to MPS II mice. Our lentiviral vector achieved high-level IDS enzyme activity and a significant reduction of glycosaminoglycan storage in both peripheral tissues and the central nervous system (CNS) of the MPS II mouse.

*$CD34^+$ HSC gene therapy for MPS II*
This year we transplanted human $CD34^+$ cells, transfected with a lentiviral vector carry-

ing human (h) IDS, to NOG/MPS II mice, and analysed them. Increased enzymatic activity of IDS and decreased levels of glycosaminoglycans in several tissues were observed in the gene therapy group. This result indicates that $CD34^+$ cells transfected with our lentiviral vector can bring therapeutic efficacy to an animal model of MPS II.

*Development of HSC-targeted gene therapy for GM1 gangliosidosis*
GM1 gangliosidosis is characterized by deficient activity of β-galactosidase, resulting in accumulation of GM1 ganglioside and causing CNS disease. Because no effective treatments are available, we aimed to develop HSC-targeted gene therapy. We constructed a lentiviral vector expressing β-galactosidase under control of the MND promoter and performed gene therapy in a mouse model of GM1 gangliosidosis. The results showed a significant elevation of β-galactosidase activity in serum but only limited elevation in CNS tissue.

*Pathological and behavioral analysis of a mouse model of GM1 gangliosidosis*
We revealed the precise pathological condition of GM1 gangliosidosis in the brain by using a mouse model. Microglia, resident immune cells of the brain, were increased in number and most of them were activated. Astrocytes also increased, indicating inflammation was induced in the brain. Interestingly, the neural stem cells in the hippocampus severely decreased. The mouse model of GM1 gangliosidosis showed a loss of motor function in the rotarod performance test and also showed demyelination in the brain.

*Development of a novel preconditioning method for HSC-targeted gene therapy for Fabry disease*
Fabry disease is a hereditary X-linked metabolic disorder characterized by a deficiency or absence of lysosomal α-galactosidase A activity. It causes accumulation of globotriaosylceramide. Because preconditioning with chemotherapy is essential for HSC-targeted gene therapy, we aimed to develop a new preconditioning method that was safer and had effects equivalent to those of chemotherapy. This time, we used an antibody-based method. Six months after treatment, we observed a 77.2% ± 14.3% engraftment rate and a 33.0% ± 7.3% vector transduction rate.

*Artificial intelligence research on Fabry disease*
We are extracting RNA from peripheral blood of a male patient with Fabry disease and will measure promoter activity by comprehensively identifying the transcription start site with an outsourced cap analysis of gene expression system. By dividing the male patient group into 2 groups with and without afferent cardiac hypertrophy, we will identify the gene groups that show significant changes in promoter activity and input image data, such as myocardial magnetic resonance imaging and T1 mapping, and blood test data to the computer. By deep learning, we analyze the correlation between changes in the genetic environment other than pathogenic genes and cardiac hypertrophy and fibrosis and aim to create a diagnostic algorithm for Fabry disease. Currently, 24 patients and 5 normal blood samples have been collected, and RNA extraction is in progress. Data conversion and analysis will be gradually started.

*Administrative study of lysosomal disease*

With funding by the Intractable Diseases Policy Research Program (Ministry of Health, Labour and Welfare), we created clinical practice guidelines for lysosomal disease and constructed a disease registry. In particular, we have enrolled more than 100 patients in a Fabry disease registry.

*New strategy of cancer gene therapy with suppression of lysosome enzymes*

Lysosomes are involved in cancer proliferation and survival through various mechanisms, such as autophagy. Lysosomes contain several enzymes, which might be novel therapeutic targets in cancers. We investigate the antitumor effects induced by knocking down several lysosomal enzymes in pancreatic cancer cell lines and a mouse model of subcutaneous tumors.

## Publications

***Nojiri A, Anan I, Morimoto S, Kawai M, Sakuma T, Kobayashi M, Kobayashi H, Ida H, Ohashi T, Eto Y, Shibata T, Yoshimura M, Hongo K.*** Clinical findings of gadolinium-enhanced cardiac magnetic resonance in Fabry patients. *J Cardiol.* 2020 Jan; **75**(1): 27-33. doi: 10.1016/j.jjcc.2019.09.002. Epub 2019 Oct 15. PMID: 31623930.

***Narita I, Ohashi T, Sakai N, Hamazaki T, Skuban N, Castelli JP, Lagast H, Barth JA.*** Efficacy and safety of migalastat in a Japanese population: a subgroup analysis of the ATTRACT study. *Clin Exp Nephrol.* 2020 Feb; **24**(2): 157-166. doi: 10.1007/s10157-019-01810-w. Epub 2019 Dec 30. PMID: 31889231; PMCID: PMC7007427.

***Saito N, Uwagawa T, Hamura R, Takada N, Sugano H, Shirai Y, Shiba H, Ohashi T, Yanaga K.*** Prevention of early liver metastasis after pancreatectomy by perioperative administration of a nuclear factor-κB inhibitor in mice. *Surgery.* 2019 Dec; **166**(6): 991-996. doi: 10.1016/j.surg.2019.05.044. Epub 2019 Jul 26. PMID: 31353078.

***Sakurai K, Ohashi T, Shimozawa N, Joo-Hyun S, Okuyama T, Ida H.*** Characteristics of Japanese patients with X-linked adrenoleukodystrophy and concerns of their families from the 1st registry system. *Brain Dev.* 2019 Jan; **41**(1): 50-56. doi: 10.1016/j.braindev.2018.07.007. Epub 2018 Aug 1. PMID: 30077509.

***Kobayashi M, Ohashi T, Kaneshiro E, Higuchi T, Ida H.*** Mutation spectrum of α-Galactosidase gene in Japanese patients with Fabry disease. *J Hum Genet.* 2019 Jul; **64**(7): 695-699. doi: 10.1038/s10038-019-0599-z. Epub 2019 Apr 15. PMID: 30988410.

## Review and Books

***Ohashi T.*** Gene therapy for lysosomal storage diseases and peroxisomal diseases. *J Hum Genet.* 2019 Feb; **64**(2): 139-143. doi: 10.1038/s10038-018-0537-5. Epub 2018 Nov 29. PMID: 30498239.

***Kobayashi H.*** Recent trends in mucopolysaccharidosis research. *J Hum Genet*. 2019 Feb; **64**(2): 127-137. doi: 10.1038/s10038-018-0534-8. Epub 2018 Nov 19. PMID: 30451936.