Research Center for Medical Sciences
Division of Gene Therapy

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General Summary

The optimal lentivirus vector was selected in hematopoietic stem cell (HSC)-targeted gene therapy using a lentivirus vector for mucopolysaccharidosis type II (MPS II), which was funded by the Agency for Medical Research and Development. In another MPS II gene-therapy project funded by this agency, significant reductions of accumulated compounds in the brain were observed with intravenous administration of adeno-associated virus (AAV) vectors. In addition, we have started to develop gene therapy for GM1 gangliosidosis and an artificial intelligence (AI) study of Fabry disease.

Research Activities

Summary of proceedings in ex vivo HSC gene therapy for MPS II
To develop gene therapy for MPS II, we have reported efficient substrate reduction with a lentiviral vector in the central nervous system (CNS) of a mouse model of MPS II (2015). We have changed the construction of this vector to the 3rd generation, similar to a vector needed for clinical trials, and succeeded in substrate reduction in important organs and behavioral tests. We applied for a patent of these results and are preparing for nonclinical tests and clinical trials.

Ex vivo HSC gene therapy for a mouse model of MPS II
We compared several lentiviral vectors with a different promoter (phosphoglycerate kinase, CD11b, or MPSV LTR, NCR deleted, dl587 PBS [MND]) for the treatment of a mouse model of MPS II. The HSC was transduced by each lentiviral vector and transplanted to the mice. Lentiviral vectors with the MND promoter achieved high levels of iduronate 2-sulfatase (IDS) enzyme activity and a significant reduction of glycosaminoglycan (GAG) storage, both in peripheral tissue and the CNS.

$CD34^+$ HSC gene therapy for MPS II
This year we optimized the lentiviral transfection method for $CD34^+$ cells *in vitro* and transplanted the cells to NOG-MPS II mice. With the newly established method the copy number of $CD34^+$ cells was approximately 2.5 times as high as with our previous method. The analysis of $CD34^+$ cell-transplanted mice is ongoing. Additionally, we have developed an antitransferrin antibody-fused IDS, which has high cell-penetrating ability.

Lethal preconditioning needed to ameliorate CNS involvement of MPS II
We have previously reported that HSC-mediated gene therapy using lentiviral vector ameliorates CNS involvement. We compared 3 conditioning procedures before gene ther-

apy. The results showed that only a lethal conditioned group achieved behavior correction providing an elevation of IDS activity and a reduction of GAG in CNS tissue.

AAV vector-based gene therapy for murine Fabry model
We intrathecally injected the rAAVPHP.eB vector encoding Gla into a mouse model of Fabry disease and bred the mice for 7 months. In the AAV-gene therapy group, the dorsal root ganglion tissue Gla enzyme activity level was 88% greater than in wild-type control mice. With hot-plate analysis at 52°C, the AAV-gene therapy group showed a significant improvement in thermal hypoalgesia.

Development of 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. We aimed to develop HSC-targeted gene therapy. We constructed a lentiviral vector expressing β-galactosidase under control of a phosphoglycerate kinase promoter and performed gene therapy in a mouse model of GM1 gangliosidosis. Increases in enzyme activity and vector copy number were observed in peripheral organs but not in the brain.

AAV-mediated gene therapy for MPS II
We intravenously administered the amount of 1.0×10^{11} vector genomes/mouse of the AAV9 vector expressing the optimized IDS gene to 8-week-old MPS II mice. The results showed a significant elevation of IDS activity and a reduction of GAG accumulation in homogenates of the cerebrum and cerebellum 16 weeks after treatment.

Gene therapy for bone complication of MPS II, gene therapy for Krabbe disease, and the construction of an AI-mediated database of Fabry disease
We investigated the bone complications in MPS II mice, detected density and strength greater than those in normal mice, and succeeded in decreasing the changes with *ex vivo* gene therapy. For Krabbe disease, we studied the effects of neonatal *in vivo* gene therapy with lentiviral vectors and succeeded in substrate reduction in the CNS, a delay of the onset time point, and an improvement of survival rate. We planned the study for Fabry disease, aiming at a new diagnostic system by AI-mediated deep learning of the relation between secondary genomic change and magnetic resonance imaging or blood analysis, and applying to the institutional review board.

New strategy of gene therapy with suppression of lysosome enzymes
Suppression of lysosome function is expected to be a new strategy for treating chemoresistant cancer. We hypothesized that down-regulation of lysosomal enzymes induces lysosomal dysfunction and enhances chemosensitization. We now knock down several lysosomal enzymes and are clarifying the role of each enzyme's activity in cancer.

Publications

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