Research Center for Medical Sciences
Division of Gene Therapy

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General Summary

This year, development of two types of gene therapy for mucopolysaccharidosis type II(MPS II) was funded by AMED. Two vector system will be employed, one is lentiviral system, the other is AAV system. The establishment of this year will be followed.

Research Activities

Gene Therapy for Lysosomal storage diseases using Lentiviral vector
MPS II is characterized by deficient activity of iduronate 2-sulfatase (IDS), resulting in accumulation of glycosaminoglycan in various tissues, such as brain, bone, heart.
We investigated the bone system in MPS II mice, and detected increasing of bone mass, tubercular bone, bone density, and bone strength comparing to normal group. We tried hematopoietic stem cell targeted *ex-vivo* gene therapy using recombinant lentiviral vector expressing IDS for MPSII model mouse, and detected the improvement of bone involvement and evaluated the influence of radiation as myeloablative pretreatment.
In this approach, lethal irradiation or administration of anti-cancer drug is mandatory as a preconditioning. This is for open a bone marrow niche. MPS II is not a malignant disease, thus, such strong conditioning should be avoid. Instead of strong preconditioning, we tested anti-cKit antibody with low dose irradiation. This preconditionig seems to be very promising.
And we also investigated the effect of newborn gene therapy with substrate reduction therapy for Krabbe disease model mouse.

Development of novel murine model of MPS II
To establish the MPS II mouse which can be transplanted with human hematopoietic stem cells, we performed the genome editing of iduronate-2-sulfatase (IDS) gene in NOG mouse, which is sever immunodeficient animal, by CRISPR/Cas9 system. As a result, no IDS activity was observed in several NOG littermates. These mice had a deletion of DNA sequence in IDS gene. In addition, accumulation of glycosaminoglycan in tissues was observed in IDS-deficient NOG mice.

Analysis of peripheral neuropathy and treatment for the neuropathy by AAV in Fabry murine model
Fabry disease (FD) is a monogenic disorder caused by mutation of the alfa-galactosidase A (GLA) gene. Many FD patients have peripheral neuropathy. This is caused by accumulation of globotriaosylceramide in dorsal root ganglia (DRG). FD mouse expressed Trpv1 mRNA and the express level was same level as wild type (WT) mice. Unexpectedly, FD

mouse showed hyposensitivity to the hot-plate test compare to WT mouse. There were many myelin-like granules in the cytosol of the DRG neuron. It was difficult to observe "patient-like" peripheral neuropathy in this mouse model. When FD mouse was injected in AAV vector encoded GLA gene intrathecal, the FD mouse hyposensitivity was rescued.

AAV 9 mediated gene therapy for MPS II

To develop AAV mediated gene therapy for MPS II, AAV 9 vector expressing IDS was generated and injected to MPS II model mice intravenously. The IDS activity in serum was increased 100 times more than wild type mice. Currently, we are analyzing storage material of various tissues.

Antitumor effect of inhibitor of nuclear factor κB and new treatment strategy for chemo resistance pancreatic cancer by suppression of Lysosome enzymes

We have previously reported the anti-tumor effect of nafamostat mesilate as inhibitor of NF-κB activation in pancreatic cancer. Moreover, nafamostat mesilate was enhance the anti-tumor effect of chemotherapy and ionizing radiation therapy owing to the inhibition of the NF-κB activation in pancreatic, gastric and gallbladder cancer. Furthermore, we have reported pomalidomide and recombinant thrombomodulin (rTM) enhance the anti-tumor effect of chemotherapy and suppressed the NF-κB activation in pancreatic cancer cells. In addition, the usefulness of gemcitabine in combination with nafamostat mesilate for the patients with unresectable pancreatic cancer has been demonstrated in clinical Phase I and II studies.

Recently, suppression of autophagy is expected to be a new strategy for cancer. Autophagy depend on hydrolysis by lysosome enzymes, so down-regulation of lysosomal enzyme suppressed autophagy. Therefore, we investigate down-regulation of the lysosome enzyme gene and evaluate anti-tumor effect of gemcitabine.

Publications

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