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GENE TRANSDUCTION INTO HUMAN HEPATOCYTES TRANSPLANTED INTO A CHIMERIC MOUSE BY USING SELF-COMPLEMENTARY RECOMBINANT AAV8 VECTORS

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[Background] By transplantation of human hepatocytes into albumin enhancer/promoter-driven urokinase plasminogen activator transgenic/severe combined immunodeficiency disease (SCID) (uPA/SCID) mice, whose liver degenerate and induce the proliferation of xenogenic hepatocytes, we developed chimeric mice in which almost all the liver cells were replaced with human hepatocytes. In the chimeric mouse liver, the original characteristics of human hepatocytes are well maintained, and therefore, the chimeric mice can be used as an animal model for not only drug metabolism but also for infections with hepatitis B and C viruses. In this study, we attempted gene transfer into the human hepatocytes that were transplanted into the chimeric mouse by using self-complementary recombinant adeno-associated virus type 8 (scAAV8) vectors.

[Methods] At 9–11 weeks after transplantation, 9 chimeric mice with human hepatocytes and an estimated replacement index of over 80%, which was calculated based on the concentration of blood human albumin, were selected. The vehicles or scAAV8 vectors ($10^{11–12}$ genome copies/animal) encoding cytomegalovirus (CMV) promoter-driven green fluorescent protein (GFP) were injected into these mice via the tail vein. Thereafter, the concentration of blood human albumin was monitored once a week, and all the mice were necropsied at 4 weeks after the inoculation. For *in vitro* gene transfer, human hepatocytes were isolated from the chimeric mouse by using collagenase perfusion method and treated with scAAV8 vectors at $10^{3–6}$ genome copies/hepatocyte. At 7 days after the inoculation, the efficiency of the gene transfer was examined using fluorescence-activated cell sorter.

[Results] In the chimeric mouse injected with 10^{12} genome copies, intense GFP signals were ubiquitously detected in the liver. Approximately 90% of the human hepatocytes were GFP-positive and histological analysis did not reveal any significant abnormality in the liver. With regard to the *in vitro* gene transfer, approximately 50% of the human hepatocytes treated with scAAV8 vectors at 10^6 genome copies/hepatocyte expressed GFP.

[Conclusions] In both the *in vitro* and *in vivo* experiments, the scAAV8 vector efficiently transferred the transgene into the human hepatocytes. The chimeric mouse serves as a new useful animal model to examine the efficacy and safety of gene therapy for human liver and also as a transgenic animal model.