experimental conditions our Cas9 nuclease is efficient at cleaving the target sequence, and the TYMP cDNA is correctly inserted and expressed.

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Specific gene correction of the AGXT gene and direct cell reprogramming for the treatment of Primary Hyperoxaluria Type 1

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Primary Hyperoxaluria Type 1 (PH1) is an inherited rare metabolic liver disease caused by the deficiency in the alanine: glyoxylate aminotransferase enzyme (AGXT), involved in the glyoxylate metabolism. The only potentially curative treatment is organ transplantation. Thus, the development of new therapeutic approaches for the treatment of these patients appears as a priority. We propose the combination of site-specific gene correction and direct cell reprogramming for the generation of autologous phenotypically healthy induced hepatocytes (iHeps) from skin-derived fibroblast of PH1 patients. For the correction of AGXT mutations, we have designed specific gene editing tools to address gene correction by two different strategies, assisted by CRISPR/Cas9 system. Accurate specific point mutation correction (c.853T-C) has been achieved by homology-directed repair (HDR) with ssODN harbouring wild-type sequence. In the second strategy, an enhanced version of AGXT cDNA has been inserted near the transcription start codon of the endogenous gene, constituting an almost universal correction strategy for PH1 mutations. Direct reprogramming of fibroblasts has been conducted by overexpression of hepatic transcription factors and in vitro culture in defined media. In vitro characterization of healthy induced hepatocytes (iHeps) has demonstrated hepatic function of the reprogrammed cells. PH1 patient fibroblasts and the gene edited counterparts have also been reprogrammed to iHeps, showing similar general hepatic characteristics as healthy iHeps. Restoration of the glyoxylate metabolism in the gene edited and reprogrammed iHeps is being demonstrated. The development of these advanced therapies will be useful as alternative cellular source to replace endogenous deficient hepatocytes with functional corrected cells.

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Cell therapy for Fabry disease using CellSaic technology

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Fabry disease is caused by decrease or loss in the activity of α-galactosidase (GLA), and its substrate globotriaosylceramide (Gb-3) accumulates in cells throughout the body, resulting in kidney injury and heart failure due to hypertrophy. Enzyme replacement therapy (ERT) has been used as a standard therapy, but it is a significant financial burden and the regular administration schedule is inconvenient for patients. In this study, we examined whether GLA secreted from donor cells could be an alternative to ERT. First, we established Gla-knockout (Gla-KO) mice (C57BL/6 background) by CRISPR. Gla-KO mice exhibited no Gla activity and accumulation of Gb-3, which is not recognized in the previous reported Gla-KO mice. Next, syngeneic wild-type mouse embryonic fibroblasts were cultured with a recombinant collagen peptide, called μ-piece, on 96U-bottom plate to produce cellular spheroid (hereafter referred to as CellSaic), and transplanted under the renal capsule of Gla-KO mouse. CellSaic protected central necrosis of cellular spheroid in long-term culture. One month after transplantation, CellSaic was clearly visible with vascular networks, and did not significantly show TUNEL-positive cells. LysoGb-3 in liver of transplanted mice significantly decreased depending on the number of CellSaic (Control vs 20 CellSaic: $p = 0.0002$, vs 40 CellSaic: $p = 0.0004$), suggesting that Gla secreted from CellSaic enters into the circulation and degrades Gb-3 in the liver. These results indicate the effectiveness of CellSaic to treat Fabry disease. We're planning ex vivo gene therapy in combination with CellSaic technology to enable the strategy practicable with a reasonable cell number.

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HNF4a promotes haepatic differentiation of human adipose tissue-derived stem cells

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Objective: Due to the high proliferation rate along with affinity to acquire hepatocyte functions, human adipose tissue-derived stem cells (hASCs) have been considering as an important candidate for cell therapy of liver diseases. In addition, hepatocyte nuclear factor a (HNF4a) has been redundantly reported as a key transcription factor during early liver development. This factor is essential for both hepatocyte differentiation and the maintenance of hepatic functions. Therefore, the present study seeks whether HNF4a can enhance hepatic differentiation of hASCs in the absence of any other stimulators.

Methods: Lentiviral transduction was applied to overexpress HNF4a in hASCs for up to 21 days. Then hepatic functionality was