

Development of *in vitro* gene editing therapy in disease treatment

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Abstract. Contemporarily, multitudinous people have been suffering genetic diseases that caused by deficiency of genes. Numerous research has been carried out and many therapies have been proposed for this sort of diseases, such as cell therapy, gene-editing therapy. *In vitro* gene-editing therapy which refers to both gene-editing and cell therapy has become a promising therapeutic strategy for many genetic diseases. This review mainly focuses on several hereditary diseases relevant to blood, liver and pancreas, systematically summarizes the development of *in vitro* gene-editing therapy in anemia, congenital liver metabolic diseases and diabetes caused by gene deficiency and discusses the strengthens and limitations of this protocol.

Keywords: *In vitro* gene-editing therapy; gene editing; cell therapy; diseases.

1. Introduction

Cell therapy, also known as cell transplantation, is a kind of therapeutic strategy including cell injection or transplantation. It originated in the 19th century, among which blood transfusion and bone marrow transplantation are the two that we are most familiar with. Recently, several types of cell therapy have attracted much attention, such as adoptive T cell therapy for anti-tumor immunity (CAR-T therapy), hepatocyte transplantation, and islet β -cell transplantation.

Gene editing technology can correct genes of somatic cells and restore the normal function of cells. Therefore, this technique can be applied to clinical treatment. In 2012, the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) system was discovered by Jennifer Dudner and her colleagues, and since then a huge breakthrough has been made in gene-editing field. Classic CRISPR gene-editing systems include Cas protein (such as Cas9), sgRNA (single-guide RNA that consists of crRNA (CRISPR RNA), tracrRNA (trans-activating CRISPR RNA)) and repair template. sgRNA and Cas proteins constitute effector complexes, and then under the guidance of sgRNA, the effector is able to target the specific sequence. Then, Cas protein completes the cleavage of the target sequence, and finally the gene editing process is completed by homologous recombination repair or non-homologous recombination repair. Although there are still delivery difficulties and off-target problems, CRISPR-based gene-editing tools have the advantages of accurate editing, low cost, and simple operation. At present, scientists have developed a variety of gene-editing tools based on the CRISPR system such as tools for gene expression regulation: CRISPRi and CRISPRa, epigenome editing tools, RNA editing tools and base editing tools and so on [1]. These tools also promote the application of gene-editing in the field of clinical treatment for genetic diseases.

Both gene-editing and cell transplantation still have their drawbacks. For one hand, allogeneic cell transplantation will lead to immune rejection. For the other hand, *in vivo* gene editing may result in the consequence that non-target tissue cells may be edited. However, *in vitro* gene editing combines cell therapy and gene editing, which can edit the patient's own cells *in vitro* and then transplant them

back into the patient's body. It can not only reduce immune rejection but also avoid the risk of editing non-targeted cells, which is a promising treatment plan.

Since *in vitro* gene-editing therapy includes the process of cell transplantation, it can be applied into treatments of genetic diseases which needs cell transplantation. This review systematically introduces *in vitro* gene editing in the treatment of three main groups of diseases: anemia caused by abnormalities in hemoglobin subunits, congenital hepatic metabolism disease and gene mutation-caused diabetes.

2. Blood

During fetal life, γ -globin gene expression is active while β -globin gene is inactive. Therefore, the hemoglobin at this stage is mainly fetal hemoglobin (HbF, [$\alpha 2\gamma 2$]). In the first year after birth, γ -globin gene expression decreases, and β -globin gene expression increases. Ultimately, in adults, adult normal hemoglobin (HbA, [$\alpha 2\beta A 2$]) is the major hemoglobin, and HbF levels are typically about 1% of total hemoglobin [2]. Blood disorders are caused when the β -globin gene is mutated, resulting in a reduced amount or structural abnormality of β -globin. Two typical blood disorders that result from abnormal β -globin are sickle cell anemia and β -thalassemia.

Currently, the application of hematopoietic stem cell transplantation (HSCT) is limited by the lack of proper donors [3]. Other treatment options, including blood transfusion and drug therapy, cannot fundamentally solve the cause or completely improve the patient's condition [4]. Therefore, scientists are trying to use gene editing technology to genetically correct patient-derived hematopoietic stem cells (HSCs) and then transplant them back into patients to treat sickle cell anemia and β -thalassemia. Theoretically, diseases result from abnormal β -globin could be treated either by repairing the β -globin gene to rescue the expression of correct β -globin, or by upregulating expression of γ -globin which replaces β -globin to make up hemoglobin.

2.1. Sickle cell anemia

Sickle cell anemia is caused by sickle hemoglobin (hemoglobin S, HbS) encoded by mutant β -globin gene. The mutation of thymine at position 17 of the gene to adenine results in the substitution of glutamic acid at amino acid position 6 of the encoded protein by valine. HbS polymerization changes the shape of erythrocyte to form a fragile and inflexible sickle shape. Patients with sickle cell disease suffer from a series of complications including hemolysis, anemia and major organ infarction [2].

Human γ -globin and δ -globin can inhibit the polymerization of HbS. As early as 1995, the Philippe Leboulch's team designed a fusion protein of β -globin and δ -globin, which retained the overall structure of β -globin and incorporated amino acid residues of δ -globin. The amino acid residues would help δ -globin to enhance its anti-sickling properties. And they tried to validate the function of this fusion protein in animal models [5]. In 2001, they further verified this strategy in a mic model. They designed a variant of human normal β -globin, β^{A-T87Q} , whose threonine at position 87 was changed to γ -Glutamine which play a role of anti-sickling properties. Then, they corrected HSCs with lentiviral vectors, transplanted the edited HSCs into mice and finally, acquired a certain therapeutic effect [6]. Until 2017, they applied the lentiviral vector-mediated anti-sickling β -globin gene to clinical treatment, and successfully treated the patient [7]. In 2022, they reported the long-term outcome of this therapy that therapeutic effects could be maintained, and conditions of patients were improved [8].

Some researchers have also tried to treat sickle cell anemia by upregulating γ -globin gene. Scientists have known that BCL11A, a zinc finger transcription factor, can reduce the content of fetal hemoglobin in erythrocytes through inhibiting the expression of γ -globin. In 2020, two research teams achieved the treatment of this disease by transplanting gene-edited hematopoietic stem cells whose expression of BCL11A was reduced and the expression of γ -globin was increased. Thereinto, the S. Corbacioglu team suppressed the expression of BCL11A by knocking out the enhancer of *BCL11A*

with CRISPR/Cas9 gene editing tool [4]. The David A. Williams, M.D. team used RNA interference (RNAi) technology. They used the BCH-BB694 BCL11A shmiR lentiviral vector to reduce the expression of BCL11A at the post-transcriptional level [9].

2.2. β -thalassemia

β -thalassemia is caused by the reduction or deletion of the β -subunit due to mutations of β -globin gene. Patients with this disease require long-term blood transfusions for treatment. Likewise, HSCT can treat the disease, but the lack of donors and immune rejection limit the application of this therapy.

Researchers have been promoting *in vitro* gene-editing therapy for β -thalassemia. In 2010, Philippe Leboulch's team tried to use a lentiviral vector that expressed β^{A-T87Q} to edit genes of HSCs isolated from a β -thalassemia patient who was dependent on monthly transfusions. Then, they transplanted edited cells into the patient and helped this patient become transfusion independent for the past 21 months [10]. Then in 2018, they reported that they successfully harvested CD34+ cells from patients and edited the cells *in vitro* with a vector that encodes β^{A-T87Q} [3]. In 2022, they reported the long-term outcome of this therapy they applied that the patients are all transfusion free with improvement of symptoms [8]. In 2021, Franco Locatelli's team reported that they used an *in vivo* gene-editing vector containing the gene encoding β^{A-T87Q} to treat β -thalassemia [11]. In addition, in 2020, the S. Corbacioglu team inhibited the expression of BCL11A by knocking out the enhancer of BCL11A to increase the expression of γ -globin, instead of β -globin to function and free the patient from blood transfusion [4].

In general, the two current approaches for *in vitro* gene-editing therapy for sickle cell anemia and β -thalassemia are: 1) to express the β -globin variant β^{A-T87Q} in erythrocytes; 2) to increase expression of γ -globin in erythrocytes.

3. Liver

Liver, as the largest digestive organ of human being, plays a key role in maintaining individual life activities. Contemporarily, liver transplantation is the only way to cure end-stage liver disease. However, liver transplantation has several limitations. (1) Donor sources are inadequate. Some patients may have no opportunities to accept liver transplantation because of the limitation of donor liver. (2) Inevitable immune rejection. The patients who have undergone liver transplantation need to take immunosuppressive drug for life. (3) The surgery is complicated, and the wound is large, which will cause high risk and cost. Therefore, scientists have been exploring other alternative therapies for liver transplantation. Hepatocyte transplantation is one of the promising therapies right now. Compared to liver transplantation, hepatocyte transplantation has a couple of advantages. (1) More donor sources; (2) Surgery is simple with lower risk; (3) Lower cost. Even for patients with some genetic diseases, donor cells can be provided from themselves since doctors can correct the mutated gene with gene-editing technology. After gene-editing correction, these cells can amplify to achieve suitable quantities for clinical treatment *in vitro*. Thus, hepatocyte transplantation combined with gene-editing technology has more advantages in treatment of congenital metabolic disease of liver.

3.1. Hereditary Tyrosinemia type 1 (HT1)

HT1 is triggered by the mutation of *FAH* gene, encoding a type of metabolic enzyme, fumarylacetoacetate hydrolase, which hydrolyzes fumarylacetoacetate to fumarate and acetoacetate. Tyrosine and its metabolites accumulate abnormally in *FAH* deficient individuals because of the deficiency of function for metabolizing tyrosine, which will cause both severe liver diseases and damage of other organs.

Although HT1 caused by *FAH* deficiency is rare, *FAH*^{-/-} animal models are good choices for liver research and *in vitro* gene-editing therapy for liver disease. In 2015, Raymond D. Hickey's group reported that transplantation of hepatocytes from WT mice can correct metabolic diseases of *FAH*^{-/-} mice [12]. Moreover, they have reported a series of *in vitro* gene-editing therapy in animal models

with *FAH* deficiency including mouse models and pig models since 2016 [13-17]. They used lentiviral vector or AAV vector to deliver Cas9 nuclease, gRNA and homology template to rescue liver function of *FAH*^{-/-} mice successfully [13, 14]. The strategy they used to treat *FAH*^{-/-} pigs includes three steps: 1) isolating primary hepatocytes from *FAH*^{-/-} pigs; 2) isolated hepatocytes were edited with lentiviral vectors *in vitro*; 3) transplantation of corrected autologous hepatocytes by portal vein injection [15-17]. With this therapy, they corrected metabolic disorders of *FAH*^{-/-} pigs, which may also provide a reasonable therapy for other inborn liver metabolic diseases.

3.2. Familial hypercholesterolemia (FH)

There are several types of gene mutations that can lead to familial hypercholesterolemia (FH), including gene mutations of *ApoB* (apolipoprotein B), *PCSK9* (proprotein convertase subtilisin/kexin type 9), *LDLR* (low-density lipoprotein receptor) and so on. However, most of FH are caused by the deficiency of *LDLR*. The endocytosis of low-density lipoprotein is mainly mediated in liver through *LDLR*. *LDLR* located in the cytomembrane will diminish if *LDLR* gene mutate. The deficiency of *LDLR* gene can damage the capability of hepatocytes to absorb low-density lipoprotein cholesterol, which will lead to an increase of plasma cholesterol, and result in the occurrence of cardiovascular disease.

From 2017 to 2019, the observation that iPSCs derived from *LDLR* deficient patients were differentiated into hepatocyte-like cells (HLCs) after gene correction was reported by three groups respectively. After gene correction, the ability of these cells to absorb low-density lipoprotein cholesterol was improved [18-20]. In 2019, Chandan Guha and his colleagues combined irradiation and GC-1, a thyroid hormone receptor- β agonist mimetic, to enhance the ability for engraftment and repopulation of donor hepatocytes. Based on this strategy, they transplanted wild type human hepatocytes into the liver of *ApoE*^{-/-} mouse and observed robust liver repopulation at 24 weeks after hepatocyte transplantation [21]. Their research provided a new strategy for *in vitro* gene editing therapy of congenital liver metabolic diseases.

3.3. Ornithine transcarbamylase deficiency (OTCD)

OTCD is one of urea cycle disorders. OTC, an enzyme that only expresses in liver, is able to combine carbamoyl phosphate and ornithine to citrulline. Deficiency of *OTC* can lead to a disruption of the urea cycle, which eventually leads to an increase in blood ammonia and brain disease.

In 2021, Stephen C. Strom's group differentiated iPSCs derived from *OTC* deficient patients into hepatocyte-like cells after *in vitro* gene correction and observed that the function of urea cycle of these cells was rescued to some extent [22]. Apart from that, they transplanted gene-edited hepatocytes isolated from *OTC*^{-/-} patients to *OTC*^{-/-} mice and found that compared to *OTC*^{-/-} mice without hepatocyte transplantation, *OTC*^{-/-} mice transplanted with edited cells had a partial recovery of OTC enzyme activity [23].

3.4. Haemophilia (hemophilia)

Haemophilia is a kind of inherited coagulopathy with which patients have prolonged clotting time. The most common types are haemophilia A and haemophilia B, which are caused by the deficiency of factor VIII gene and factor IX gene respectively. Factor VIII is a kind of glycoprotein, which is mainly synthesized in hepatocyte. Factor IX, an essential protein for coagulation function, is one of the serine proteases of the coagulation system [24]. A large number of researchers have donated themselves in the study of *in vitro* gene-editing therapy for haemophilia right now.

3.4.1 Haemophilia A (hemophilia A)

Until by now, several research groups have successfully corrected iPSCs derived from haemophilia patients through *in vitro* gene editing and differentiated them into endothelial cells after gene correction. All of these groups noticed expression of factor VIII in the endothelial cells they harvested after gene-editing and differentiation [25-29]. Among these groups, Liang Desheng's group

detected factor VIII in the supernatant of differentiated endothelial cells [28]. Jin-Soo Kim's group transplanted endothelial cells differentiated from haemophilia A patients' iPSCs after gene correction to mouse models that suffered from haemophilia A and they found that their coagulation function was partially recovered [29]. K. John Pasi's group tried to utilize *in vivo* gene-editing therapy to treat patients. They injected AAV5 vector encoding an variant of human factor VIII in patients with severe haemophilia A and observed symptoms improvement in patients [30]. Their strategy is useful to some extent and their research may provide some ideas for the further study.

3.4.2 Haemophilia B (hemophilia B)

Among the study of haemophilia B, three research groups have differentiated gene-corrected iPSCs derived from haemophilia B patients into HLCs and observed expression of factor IX [31-33]. Anne Dubart-Kupperschmitt's team transplanted gene-edited HLCs to haemophilia B mouse models and found that the group with transplantation had significant higher level of factor IX compared with the group without transplantation [33]. In 2014, A.C. Nathwani's team found AAV8 vector mediated delivery strategy could achieve long-term therapeutic factor IX expression, which might provide some inspiration for *in vitro* gene-editing therapy for haemophilia B [24].

Theoretically, *in vitro* gene editing could also be applied into curing other congenital metabolic diseases of the liver, such as phenylketonuria (PKU), urea cycle disorder caused by arginase-1 deficiency and alpha1-antitrypsin deficiency (AATD) and so on. However, it is a challenge for doctors to achieve enough cells that are suitable for clinical treatment. In 2014, Hui Lijian's team firstly harvested human HLCs by transdifferentiating from human fibroblast directly and achieved large scale expansion of this kind of cell in 2016 [34, 35]. Then, in 2018, they reported that they successfully amplified human hepatocyte *in vitro* and several other research teams achieved this goal [36]. These achievements offered different sources of human hepatocytes for clinical treatment. With these strategies, scientists acquire more chance to expand patients' hepatocytes after gene-editing and finally harvest adequate cells for treatment. In addition, the engraftment of donor cells should also be considered, which needs more useful transplant and preconditioning strategy to obtain better efficiency.

4. Pancreas

Pancreas is not only a digestive organ but also an endocrine organ, which performs digestive and endocrine functions. As an endocrine gland, its main function is to adjust blood glucose level and secrete several types of hormones, such as insulin and glucagon. As a digestive organ, pancreas secrete pancreatic juice to digest food. Insulin is the only hormone that can reduce the level of blood glucose in human body, which is secreted by islet β cells, an essential kind of cell which located in pancreas. A person will suffer from diabetes if his or her islet β cells are damaged or abnormal.

Diabetes can be divided into several types including type 1 diabetes and type 2 diabetes. The former is a kind of autoimmune disease. Immunity cells of patients will attack their own islet β cells, which damages β cells and finally leads to type 1 diabetes. Additionally, type 2 diabetes is featured with insulin resistance that cells lose normal response to insulin, which is mainly caused by obesity and lack of exercise.

In vitro gene-editing therapy is a promising clinical protocol for diabetes caused by the gene deficiency that impairs the function of insulin secretion. Currently, transplantation of islet β cells is a proven treatment for diabetes caused by insulin deficiency. β cells stem from patients' iPSCs (SC- β cells) is a kind of cell source for *in vitro* gene-editing therapy for diabetes. In 2018, Dieter Egli's group isolated fibroblasts from permanent neonatal diabetes mellitus (PNDM) patients and induced these cells to iPSCs. Then, they corrected the mutated gene of these iPSCs, differentiated them to SC- β cells and then transplanted them into mice. They observed that mice transplanted with mutated cells and mice without cell transplantation suffered diabetes at 7 days after treated with streptozotocin (STZ). However, these mice transplanted with gene-corrected cells maintained a normal blood glucose level [37]. What's more, in 2020, Jeffrey R. Millman's team acquired iPSCs from a Wolfram

syndrome (WS) patient and differentiated them to SC- β cells after gene-editing. They transplanted these SC- β cells into mice with diabetes caused by STZ treatment and the symptoms of diabetes were rescued [38].

Similar to hepatocyte transplantation, adequate cells that suitable for clinical treatment and proper preconditioning strategy are also needed for transplantation of pancreatic β cells. In 2020, Zeng and her team identified a lineage of protein C receptor positive cells (Procr⁺ cells). This cluster of Procr⁺ cells can be cultured to pancreatic islet organoids *in vitro* that can reverse diabetes in mice after transplantation [39]. This study provides a promising cell resource for cell therapy for diabetes.

5. Discussion

Compared to *in vivo* gene-editing therapy, *in vitro* gene-editing therapy has several strengthens because it is combined with cell transplantation. (1) Compared to organ transplantation, *in vitro* gene editing can be done directly with the patient's own cells, without delaying treatment because of a shortage of donors. (2) Donor cells derived from patients will lead to lower immunity response, which will contribute to a better therapeutic outcome. (3) It has higher security compared with *in vivo* gene-editing therapy. Because this therapy avoids the injection of editing vectors and quality testes of donor cells can be applied before clinical treatment, which provides chances to filtrate donor cells to improve the security of this therapy. (4) The operation of surgery is simpler, and the wound is smaller compared with organ transplantation. (5) The cost can be lower because of the simpler surgery operation, lower demand of immunosuppressive drugs and the industrialization of cell transplantation.

However, there are still some limitations and obstacles need to be addressed before this strategy was applied to clinical treatment. (1) Donor cells. Doctors must harvest adequate and high-quality cells that can be used as clinical donor cells. Thus, to achieve the desired therapeutic effect, the problem refers to large scale expansion of donor cells *in vitro* need to be solved. (2) Transplantation strategy. Firstly, the location of transplantation may influence the function of donor cells. For example, during the process of islet β cells transplantation, scientists must considerate whether donor cells can response to glucose fluctuation at the region where they graft. In addition, donor cells may cannot graft into patients and repopulate because of the microenvironment, immunity response and other obstacles like vascular wall. Therefore, preconditioning protocols need be explored to enhance the engraftment and repopulation of donor cells. (3) Off-target. It is necessary to control this problem to guarantee the security of treatment. (4) Delivery efficiency. All vectors have a limited capacity, and the components used to complete gene editing may be larger than the capacity of the vector. Thus, multiple vectors may be needed, which will reduce editing efficiency. Therefore, scientists are also looking for smaller editors and vectors which have larger capacity.

References

- [1] M. Nishiga et al., The use of new CRISPR tools in cardiovascular research and medicine. *Nature Reviews Cardiology* 19, 505-521 (2022).
- [2] S.-R. Pasricha et al., Hemoglobinopathies in the fetal position. *New England Journal of Medicine* 379, 1675-1677 (2018).
- [3] A. A. Thompson et al., Gene therapy in patients with transfusion-dependent beta-thalassemia. *New England Journal of Medicine* 378, 1479-1493 (2018).
- [4] H. Frangoul et al., CRISPR-Cas9 gene editing for sickle cell disease and beta-thalassemia. *New England Journal of Medicine* 384, 252-260 (2021).
- [5] K. J. Takekoshi et al., Retroviral transfer of a human beta-globin delta-globin hybrid gene linked to beta-locus-control region hypersensitive site-2 aimed at the gene-therapy of sickle-cell disease. *Proceedings of the National Academy of Sciences of the United States of America* 92, 3014-3018 (1995).
- [6] R. Pawliuk et al., Correction of sickle cell disease in transgenic mouse models by gene therapy. *Science* 294, 2368-2371 (2001).

- [7] J.-A. Ribeil et al., Gene therapy in a patient with sickle cell disease. *New England Journal of Medicine* 376, 848-855 (2017).
- [8] E. Magrin et al., Long-term outcomes of lentiviral gene therapy for the beta-hemoglobinopathies: the HGB-205 trial. *Nature Medicine* 28, 81-+ (2022).
- [9] E. B. Esrick et al., Post-transcriptional genetic silencing of BCL11A to treat sickle cell disease. *New England Journal of Medicine* 384, 205-215 (2021).
- [10] M. Cavazzana-Calvo et al., Transfusion independence and HMGA2 activation after gene therapy of human beta-thalassaemia. *Nature* 467, 318-U394 (2010).
- [11] F. Locatelli et al., Betibeglogene autotemcel gene therapy for non-beta(0)/beta(0) genotype beta-thalassemia. *New England Journal of Medicine* 386, 415-427 (2022).
- [12] R. D. Hickey et al., Noninvasive 3-dimensional imaging of liver regeneration in a mouse model of hereditary tyrosinemia type 1 using the sodium iodide symporter gene. *Liver Transplantation* 21, 442-453 (2015).
- [13] C. VanLith et al., Curative ex vivo hepatocyte-directed gene editing in a mouse model of hereditary tyrosinemia type 1. *Human Gene Therapy* 29, 1315-1326 (2018).
- [14] C. J. VanLith et al., Ex vivo hepatocyte reprogramming promotes homology-directed DNA repair to correct metabolic disease in mice after transplantation. *Hepatology Communications* 3, 558-573 (2019).
- [15] R. D. Hickey et al., Curative ex vivo liver-directed gene therapy in a pig model of hereditary tyrosinemia type 1. *Science Translational Medicine* 8, (2016).
- [16] R. D. Hickey et al., Autologous gene and cell therapy provides safe and long-term curative therapy in a large pig model of hereditary tyrosinemia type 1. *Cell Transplantation* 28, 79-88 (2019).
- [17] R. A. Kaiser et al., Lentiviral vector-mediated gene therapy of hepatocytes ex vivo for autologous transplantation in swine. *Jove-Journal of Visualized Experiments*, (2018).
- [18] J. Caron et al., Low-density lipoprotein receptor-deficient hepatocytes differentiated from induced pluripotent stem cells allow familial hypercholesterolemia modeling, CRISPR/Cas-mediated genetic correction, and productive hepatitis C virus infection. *Stem Cell Research & Therapy* 10, (2019).
- [19] H. Okada et al., Function and immunogenicity of gene-corrected iPSC-derived hepatocyte-like cells in restoring low density lipoprotein uptake in homozygous familial hypercholesterolemia. *Scientific Reports* 9, (2019).
- [20] L. Omer et al., CRISPR correction of a homozygous low-density lipoprotein receptor mutation in familial hypercholesterolemia induced pluripotent stem cells. *Hepatology Communications* 1, 886-898 (2017).
- [21] M. Barahman et al., Radiation-primed hepatocyte transplantation in murine monogenic dyslipidemia normalizes cholesterol and prevents atherosclerosis. *Journal of Hepatology* 70, 1170-1179 (2019).
- [22] M. Zabulica et al., Correction of a urea cycle defect after ex vivo gene editing of human hepatocytes. *Molecular Therapy* 29, 1903-1917 (2021).
- [23] M. Zabulica et al., Gene editing correction of a urea cycle defect in organoid stem cell derived hepatocyte-like cells. *International Journal of Molecular Sciences* 22, (2021).
- [24] A. C. Nathwani et al., Long-term safety and efficacy of factor IX gene therapy in hemophilia B. *New England Journal of Medicine* 371, 1994-2004 (2014).
- [25] C.-Y. Park et al., Targeted inversion and reversion of the blood coagulation factor 8 gene in human iPS cells using TALENs. *Proceedings of the National Academy of Sciences of the United States of America* 111, 9253-9258 (2014).
- [26] C.-Y. Park et al., Universal correction of blood coagulation factor VIII in patient-derived induced pluripotent stem cells using CRISPR/Cas9. *Stem Cell Reports* 12, 1242-1249 (2019).
- [27] J. J. Sung et al., Restoration of FVIII expression by targeted gene insertion in the FVIII locus in hemophilia A patient-derived iPSCs. *Experimental & molecular medicine* 51, 1-9 (2019).
- [28] Y. Wu et al., In situ genetic correction of F8 intron 22 inversion in hemophilia A patient-specific iPSCs. *Scientific Reports* 6, (2016).
- [29] S. Rangarajan et al., AAV5-factor VIII gene transfer in severe hemophilia A. *New England Journal of Medicine* 377, 2519-2530 (2017).

- [30] C.-Y. Park et al., Functional correction of large factor VIII gene chromosomal inversions in hemophilia A patient-derived iPSCs using CRISPR-Cas9. *Cell Stem Cell* 17, 213-220 (2015).
- [31] Q. He et al., Genetic correction and hepatic differentiation of hemophilia B-specific human induced pluripotent stem cells. *Chinese Medical Sciences Journal* 32, 135-144 (2017).
- [32] C. Lyu et al., Targeted genome engineering in human induced pluripotent stem cells from patients with hemophilia B using the CRISPR-Cas9 system. *Stem Cell Research & Therapy* 9, (2018).
- [33] E. Luce et al., In vitro recovery of FIX clotting activity as a marker of highly functional hepatocytes in a hemophilia B iPSC model. *Hepatology* 75, 866-880 (2022).
- [34] P. Huang et al., Direct reprogramming of human fibroblasts to functional and expandable hepatocytes. *Cell Stem Cell* 14, 370-384 (2014).
- [35] X.-L. Shi et al., Improved survival of porcine acute liver failure by a bioartificial liver device implanted with induced human functional hepatocytes. *Cell Research* 26, 206-216 (2016).
- [36] K. Zhang et al., In Vitro expansion of primary human hepatocytes with efficient liver repopulation capacity. *Cell Stem Cell* 23, 806-+ (2018).
- [37] S. Ma et al., Beta cell replacement after gene editing of a neonatal diabetes-causing mutation at the insulin locus. *Stem Cell Reports* 11, 1407-1415 (2018).
- [38] K. G. Maxwell et al., Gene-edited human stem cell-derived beta cells from a patient with monogenic diabetes reverse preexisting diabetes in mice. *Science Translational Medicine* 12, (2020).
- [39] D. Wang et al., Long-term expansion of pancreatic islet organoids from resident Procr(+) progenitors. *Cell* 180, 1198-+ (2020).