Journal of Advances in Biology & Biotechnology

14(3): 1-11, 2017; Article no.JABB.35111
ISSN: 2394-1081

Hemophilia A and Induced Pluripotent Stem Cells

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Author's contribution

Author MLN conceived of the study, and participated in its design and helped to draft the manuscript, and involved in revising the manuscript critically for significant intellectual need. The author read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2017/35111
Editor(s):
(1) Maria Serrano, Department of Applied Biology, EPSO, University Miguel Hernandez, Orihuela, Alicante, Spain.
Reviewers:
(1) Mehmet Can Ugur, Izmir Suat Seren Education and Training Hospital, Turkey.
(2) Neema Tiwari, Eras Lucknow Medical College and Hospital, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/20369

Review Article

Received 28th June 2017
Accepted 27th July 2017
Published 5th August 2017

ABSTRACT

Hemophilia A is a hemorrhage disorder inherited according to the X-linked inheritance pattern. It affects about 1 in 4,000 to 10,000 males. Permanent changes in the *F8* gene result in hemophilia A. These changes result in an abnormal version of coagulation factor VIII. This abnormal condition determines the kinds of the disorder, namely, severe and mild or moderate hemophilia A. Currently, to treat hemophilia A, infusions of plasma-derived or recombinant factor VIII can be used. However, the cost is extremely high. It makes heavy burdens on the patients, their families or the health care system. Therefore, advanced therapy as iPSCs technique has attracted attention of researchers to research for curing this disorder. For example, to obtain edited DNA segments due to hemophilia A, this technique can be used. Research results in animal models have shown important advances.

Keywords: F8; hemophilia A; hemorrhage; iPSCs.

1. INTRODUCTION

There are three kinds of hemophilia disorders. These comprise the following: hemophilia A, hemophilia B or Christmas disorder, and hemophilia C. Both hemophilia A and hemophilia B are X-linked recessive disorders [1]. Hemophilia C is an autosomal recessive disorder that can trigger abnormal hemorrhage. In addition, there is hemophilia A called acquired

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hemophilia A; it will not be discussed further here.

Hemophilia A is a hemorrhage disorder caused a deficiency in the activity of coagulation factor VIII [2-5]. Factor VIII is a glycoprotein cofactor that acts as an important element in the formation of blood coagulation factor [5]. Hemophilia results in prolonged hemorrhage after injuries. These injuries can include tooth extractions or surgery, and recurrent hemorrhage before to complete injury curing [6-7]. Hemophilia A commonly occurs in approximately 1 in 4,000 to 5,000, 1 in 8,500, or 1 in 10,000 males [2-3,5,7]. The severity of the disorder relies on factor VIII of < 1% normal, moderate disorder 1-5% [3-4,6], and mild disorder between 5% and 40% [4]. Severe hemophilia A occurs from 40 to 50% of all patients [8]. Females are rarely affected, although can be carriers [9]. Carriers can reach approximately 10% [7].

In severe cases, continuous hemorrhage occurs after a small injury or even in the absence of injury. Critical complications can result from hemorrhage into the brain, joints, muscles, or other internal organs [7] after surgical procedure [3,7]. Milder forms do not necessarily involve spontaneous hemorrhage. The condition may be invisible until abnormal hemorrhage occurs after surgery or a serious injury [7]. Hemophilia A life expectancy in the developing world is increasing [3].

Patients with hemophilia A typically exist during the first two years of life following hemorrhage from small mouth wounds and big wounds. Without prophylactic therapy, patients may average up to two to five spontaneous hemorrhage incidents every month, including spontaneous joint hemorrhages, and prolong hemorrhage and inflammation of small wounds, operation, and tooth extractions. Patients with moderate hemophilia A seldom have spontaneous hemorrhage. However, they have expanded oozing after relatively minor trauma and are usually detected before age five to six years; the frequencies of hemorrhage incidents vary, typically from one time a month to one time a year. Patients with mild hemophilia A do not have spontaneous hemorrhage incidents; however, without pre- and postoperative cure, abnormal hemorrhage happens with operation; the frequency of hemorrhage incidents fluctuates extensively, typically from one time a year to one time every ten years. Patients with mild hemophilia A are frequently not detected until afterward in life [6].

To keep hemostasis in hemophilia A, the recombinant antihemophilic factor (rAHF) can be used. However, it is problematic to use rAHF or plasma-derived concentrate for treatment of hemophilia A. This method needs to repeated venous access. It only has availability restricted of factor VIII concentrates. This method spends high costs for the treatment needs [10]. However, this method makes serious problems of the hemophilia patients, their families or the health care system. To handle such problems, induced pluripotent stem cells (iPSCs) technique can be used. This technique can correct error segments in the *F8* gene. The iPSCs are relevant to the disease treatment needs such as disease modeling and stem cell therapy.

In this article, the author draws the advances in the study of hemophilia A focusing on the genetic aspects. It comprises the *F8* gene, mutations in the gene, gene editing techniques, and iPSCs technology.

2. GENE IN HEMOPHILIA A

A gene is the basic physical and functional unit of heredity. Genes made up of DNA act as instructions to make proteins. Genes are mutable. A gene mutation is a permanent change in the DNA. It can cause the protein to malfunction condition. A condition caused by mutations in at least one gene is called a genetic disorder [11]. A genetic disorder can be such as alkaptonuria and hemophilia A. In hemophilia A, mutations in the *F8* gene reduce the amount of coagulation factor VIII. Mutation types in hemophilia A can comprise such as deletion, duplication, and inversion. Mutation effects may comprise such as frameshift and large deletion.

2.1 The F8 Gene

The valid name of the gene is “coagulation factor VIII, procoagulant component.” *F8* is the gene’s valid symbol. Other symbols for the *F8* gene include AHF, antihemophilic factor; coagulation factor VIII; procoagulant component (hemophilia A); DXS1253E; F8_HUMAN; Factor VIIIF8B; FVIII; and HEMA [12]. The human *F8* gene occupies chromosome Xq28 in the chromosome map [1].The cytogenetic location of the *F8* gene is on the long arm of the X chromosome [12-13] at position 28 (Fig. 1) [12]. It occupies a region from base pair 154,064,062 to base pair 154,250,997.

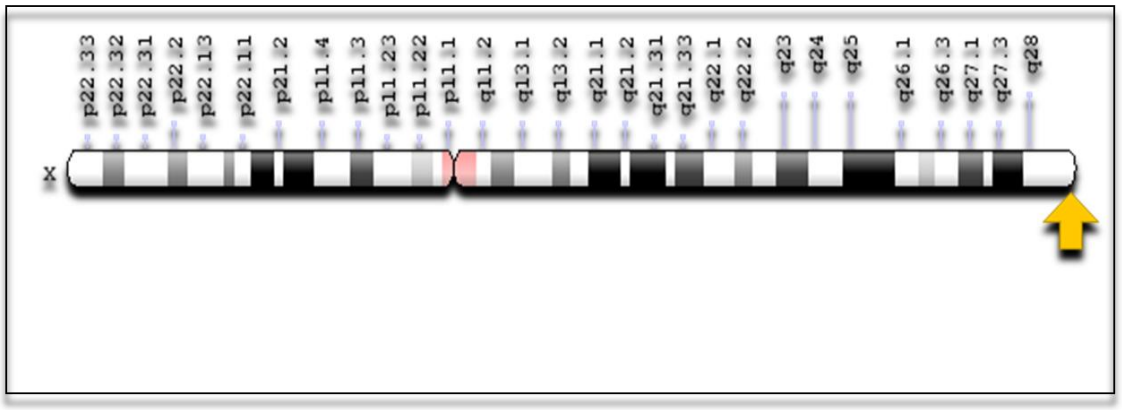

Fig. 1. The *F8* gene; The *F8* gene location on the chromosome Xq28 (from reference 12)

The *F8* gene spans over 180 kbp [5] or 186 kbp [13], and comprises 26 exons. It encodes a polypeptide chain of 2,351 amino acids [5] (69 – 3.106 kbp) and 25 [14] introns (from 207 base to 32.4 kbp [13]. Product of factor VIII protein has no enzyme activity. An open reading frame encodes the 19 amino acid. This amino acid leads to the passage of factor VIII protein through hepatocytes to blood vessels [14]. The total span of the coding sequence of the *F8* gene is 9 kbp [13].

The mature factor VIII polypeptide includes three A domains, two C domains and a single B domain. These domains are crucial for factor VIII function [14-15]. These domains show A1-A2-B-A3-C1-C2 structure [13] from the N-terminus to the C-terminus [14]. There are three acidic subs-domains. These include a1-a3-A1 (a1) -A2 (a2) -B-(a3) A3-C1-C2. These occupy at the boundaries of A domains. All play a crucial role in the interaction between factor VIII and other proteins, particularly thrombin. Mutations in these sub-domains decrease the scale of factor VIII activation that is triggered by thrombin [13].

The *F8* gene has a crucial role in making coagulation factor VIII. Factor VIII acts as a cofactor for factor IXa to convert factor X to the activated form. It changes into factor Xa [16-18]. The conversion occurs in the presence of Ca^{++} and phospholipid. In the *F8* gene, isoform b is transcript variant 2 that encodes a putative small protein. It is made mainly of domain of factor VIIIc that bind phospholipid. Isoform b is important for coagulation activity [16]. NCBI Gene includes the factor VIIIc as the same as FVIII and FVIIIC, for example. Transcript variant 2 comprises primarily of the phospholipid-binding domain factor VIII.

2.2 Mutations in F8 Gene

The commonly F8 abnormality to cause hemophilia A is an inversion of intron 1 (5%) or intron 22 (50%) [13-14]. Remaining hemophilia A cases are various point mutations spread throughout the gene. These comprise the following: missense, nonsense, splice, frameshift, gross deletion [14], and silent. As indicated by Nair et al., Human Gene Mutation Database (HGMD) reported 2,572 mutations in which 1,514 are missense and nonsense, 168 splice, 426 small deletions, 140 small insertions, 32 small indels, 288 gross deletions, 38 gross insertions, 19 complex rearrangements, and 7 regulatory mutations [19]. Currently, HGMD lists 2,863 mutations; those are responsible for hemophilia A disorder.

Geoffrey showed that over 50 type's nonsense point mutation associated with severe hemophilia A. Over 40 types, various missense point mutations were in exon 14. Most of them occur in codons 1,680 and 1,689; cause moderate hemophilia A [5]. Intron-22 inversion mutations cause around 45-50% of severe hemophilia A patients. The intron-1 inversion mutations cause 5% of patients with severe phenotype [20].

Vogel concluded that the mutation rate, causing hemophilia A is higher in males than in females. Winter et al. got a likelihood estimate of 9.6 for the ratio of male to female mutation. Bernardi et al. found a higher mutation in males than in females in families with hemophilia A [MIM: #360700; 1].

3. ADVANCED THERAPY AS CHOICE FOR *F8* GENE MUTATIONS CORRECTION

To correct mutations in the *F8* gene, advanced therapy can be used. Advanced therapy is one of the methods to treat hemophilia A. The method can comprise gene therapy, cell therapy, or tissue engineering. Viral vectors are useful in advanced therapy. Viral vectors used in advanced therapy may comprise such as lentiviral, adeno-associated virus (AAV) and Sendai virus. Base of the cell therapy for hemophilia is, mainly, in the use of iPSCs techniques to replace damaged cells with healthy cells. In 2011, Liras showed that in the iPSCs technique, endothelial progenitor cells from hemophilia patients could be used. These cells can result in the factor VIII protein effectively. Advanced therapy is a promising tool to treat Mendelian disorders [4,21] such as albinism, and hemophilia A. To date, scientists have adopted the iPSCs technique as a tool to fight hemophilia, including hemophilia A.

4. INDUCED PLURIPOTENT STEM CELLS (iPSCs)

IPSC is a genetic manipulation method to have a stage as embryonic growth and properties using transcription factors. Several methods have been developed so far. These include the use of RNA viruses, transposon insertions, and small molecules.

4.1 Progresses of iPSCs Methods

There are three important categories associated with the genetic manipulation methods. These comprise the following: Embryonic stem cells (ESCs), mesenchymal stem cells (MSCs), and iPSCs. ESCs derived from the blastocyst and have high quality. Unfortunately, ESCs destruct the blastocyst. Their use is disturbed over ethical issues. MSCs derived from materials beyond the blastocyst such as blood and cord blood. Thus, their use is undisturbed over ethical issues. However, to use MSCs, it still needs further development to increase in cell numbers they produce [22]. Finally, the iPSCs have similar pluripotency to ESCs and undisturbed over ethical issues.

The cellular reprogramming reached an important development with the cloning of the first mammal “Dolly the sheep”. Then, Takahashi and Yamanaka introduced their reprogramming method with the introduction of reprogramming factors to generate iPSCs [23]. Reprogramming factors include *Oct4*, *Sox2*, *Klf4*, and *c-Myc* (OSKM) [24-25]. Takahashi and Yamanaka generated the iPSCs from mouse embryonic or adult fibroblasts [26-27]. The authors used OSKM found by Yamanaka et al. [24]. In their study, Takahashi and Yamanaka used retroviral vectors [26]. Wu et al. [28] generated iPSCs from somatic cells with four factors OSKM in mice and human. The authors also generated iPSCs from OS + *Nanog* and *Lin28* (OSNL) in mice and human. However, those iPSCs have weakness in terms of oncogenic transformation into the host genome. Focosi et al. [25] showed many groups have generated less dangerous genes. These genes are useful to avoid the use of the proto-oncogene *c-Myc* as transformation worries. To conquer the low transfection efficiency of primary cells, several retroviral vectors have been used. These retroviral vectors can introduce reprogramming factors into cells. However, the mutagenesis insertion related to retroviral vectors still shows a major weakness of this type of iPSCs approach. *Nanog* iPSCs silenced strongly OSKM. However, one of the chimera *Nanog* iPSC clones generated by Okita et al. [29]. produce about 20% tumors. These tumors are attributable to the *c-myc* transgene reactivation. Focosi et al. [25] showed that prolonged and uncontrollable duration of reprogramming factors influence iPSCs natural properties. The reprogramming silencing and spontaneous reactivation also influences iPSCs natural properties. These influences occur both *in vitro* and *in vivo* [25]. The *c-Myc* retrovirus reactivation raises tumorigenicity in the chimeras [30]. For these reasons, to improve the safety of delivery techniques, numerous efforts have been made. In addition, to provide tightly reprogramming factor controllable expression systems, many efforts have also been made. A wide array of delivery technique has been tested. These include safely integrating (AAV) to non-integrating vectors (as Sendai virus) [25]. It also includes non-viral, such as transposing-based gene delivery methods [31] and episomal reprogramming methods. In addition, a delivery system using adenoviruses have also been developed.

For the gene delivery methods, each method has cargo capacity. For example, AAV vector has around 4.7 kb of the genome [32-33]. It is a small gene delivery vehicle. To lessen this disadvantageous, Choi et al. [36] generated

around 5.2 kb of the genome for AAV vector. Nelwan indicated that there are several gene delivery vehicles [37-38] can be used. These comprise the following: retroviruses, adenovirus, AAV, HSV-1, transposon insertions, episomal, and Sendai virus. The Table 1 shows, viral vectors and non-viral vectors for gene delivery vehicles. The non-integrating vectors are more secure towards such as transgene integration, for example. In addition, there are two big gene delivery vehicles described in the Table 1. These two vehicles are HSV-1 amplicon vectors and episomal vectors. In these two vectors, to purify supercoiled DNA, it is quite cumbersome [33]. Therefore, to get higher efficiency of delivery, it should be done tests to optimize the whole system.

Sendai virus is an essential respiratory pathogen of rats and mice. It efficiently transduces the respiratory tract cells of mice as well as humans [42]. Sendai virus is an RNA virus of the Paramyxoviridae family [43]. The virus replicates in the cytoplasm without integrating into the host genome [43-44]. Fusaki et al. [44] demonstrated that Sendai virus-based vector system provides a critical solution for reprogramming methods. Sendai virus will speed up clinical use purposes. Fusaki et al. [44] generated iPSCs from adult human fibroblast as introduced by Takahashi et al. [45]. To generate iPSCs, a safer technique has also been developed. This technique uses Sendai virus and activated T cells under a defined culture state for clinical application. It has advantages to get genetic markers for application in a clinical research for future regenerative medicine [43]. Researchers have used Sendai virus-based vectors as a safe technique for gene therapy.

The finding of direct cell reprogramming and iPSCs technology resulted in new ways for the use of non-viral techniques. The *Sleeping Beauty* transposon technique is an excellent advancement for unlimited genetic manipulations in mammalian cells. Grabundzija et al. [31] introduced reprogramming of iPSCs from embryonic mouse fibroblasts, and human foreskin. The authors demonstrated a Cre substitute. It was a recombinase-mediated substitute, allowing concurrent elimination of the reprogramming cassette. The substitute targeted knock-in of a demonstration cassette of interest into the transposon-tagged locus in mouse iPSCs. The authors showed that this approach would allow repair of incorrect genes. It will be useful for treatment of various genetic disorders.

Yu et al. [45]. reported an important development of the episomal reprogramming technique. The authors established feeder-free reprogramming state aided by a small molecule. This technique gives the effective derivation of footprint-free human iPSCs. The authors used chemically defined media in this technique. To generate iPSCs, the authors used skin fibroblasts, adipose tissue-derived cells and cord blood cells. Yu et al. [45]. have effectively obtained ESC-like iPSCs from human neonatal and human adult skin fibroblasts. This technique could be readily adapted to the clinical application in human. It will be interesting to see how this technique and other reprogramming techniques regarding the iPSCs generation. Hou et al. [46]. generated pluripotent stem cells from mouse somatic cells. The authors used a combination of several small molecule compounds in their technique. The iPSCs seems similar to ESCs. The similarity includes their gene expression profiles, epigenetic status, and potential for differentiation and germ line transmission. [46]. This technique has potential use for the treatment of various genetic disorders, including hemophilia A. Kamath et al. [47] generated iPSCs without the need for *l-Mic, c-Myc,* and *Lin28*. The authors used a mixture of reprogramming molecules in combination with episomal vectors. The iPSCs method is virus-free, *Mic-free*, and *Lin28*-free [47].

Stadtfeld et al. [48] generated mouse iPSCs from fibroblasts and liver cells. The authors used non-integrating adenovirus quickly expressing OSKM to generate iPSCs. These adenoviral iPSCs show DNA demethylation typical of reprogrammed cells express endogenous pluripotency genes, from teratomas. The iPSCs supplies the reprogrammed cells to various tissues, including the germ line, in chimeric mice. Stadtfelt et al present strong evidence that insertional mutagenesis is not needed for *in vitro* reprogramming. Adenoviral reprogramming may provide a better method for producing and examining patient-specific stem cells. To evaluate ESCs and iPSCs, the adenoviral reprogramming can also be used.

Mavilio et al had corrected erroneous segments from a patient with an epidermal blistering disorder. The authors used advanced therapy, combined gene/cell therapy, in their study [49]. The authors had an important role in the development of gene therapy and cell therapy. Takahashi and Yamanaka's have an important role in the use of iPSCs technologies to cure

Table 1. Capacity and features of gene delivery vehicles

Vehicle	Cargo capacity	Features	References
Retrovirus	7-10 kb	Integrating	[32]
Adenovirus	~ 36 kb	Non-integrating	[33]
AAV	4.7 kb	Safely integrating	[32, 34]
HSV-1 Amplicon	150 kb	Non-integrating	[37-38]
*Tol*2 Transposon	~ 10 kb	Less integrating	[39]
Episomal	172-660 kb	Non-integrating	[43]
Sendai virus	15,384 kb	Non-integrating	[40-41]

genetic disorders. The iPSCs technology is useful for disease modeling, drug screening and stem cell therapy for hemophilia A. The iPSCs uses for diseases such as hypertension and retinoblastoma in recent clinical trials have been chosen. However, for hemophilia A, this technology has not been chosen.

4.2 Editing Procedures for iPSCs

To edit incorrect genes, four methods can be used (Table 2). These comprise the following: the meganucleases (MNs), zinc finger nucleases (ZFNs) system, transcription activator-like effectors nucleases (TALENs) system and the clustered regularly interspaced short palindromic repeats (CRISPR) system or CRISPR/Cas9 (Cas9, CRISPR associated 9) system. MNs as a gene editing tool have not been widely adopted. Both ZFNs and TALENs have the same techniques. To bind to a unique sequence that linked to FokI nuclease as a pair and cleavage sequence, ZFNs and TALENs can be used [50]. Finally, the CRISPR system gets the benefit of the RNA-guided Cas9 nuclease to produce aimed double-stranded DNA breaks [22].

Table 2. Several gene-editing tools

Platform	Function	Source
MNs	gene-editing	Microbial movable elements
ZFNs	gene-editing	Eu. transcription factors
TALENs	gene-editing	*Xanthomonas*
CRISPR/ Cas9	gene-editing	*Streptococcus pyogene*

The CRISPR/Cas9 system depends on a single catalytic protein. The tracrRNA and crRNA guide Cas9 to a particular sequence. A mixture of the tracrRNA/crRNA into a sgRNA has guided to the gene-editing technique progress. It is particular for any goal inside the genome. The endonuclease needs a protospacer adjacent motif (PAM). The PAM is an invariant part of the DNA goal. It is not present in the sgRNA. The CRISPR/Cas9 system uses sgRNA to recognize the corresponding sequence in the DNA and afterward make DSBs [51]. DSBs are double-strand breaks. Error-prone non-homologous end joining (NHEJ) repairs DSBs through homology-directed repair (HDR). NHEJ introduces indel mutations, leading to knock-out gene function [52,53].

CRISPR/Cas9 system in bacteria and archea use RNA-guided nucleases related to degradation of foreign nucleic. To exploit helper-dependent adenoviral vector and for gene targeting in iPSCs at multiple loci simultaneously, that activity is used [25]. CRISPR/Cas9 and TALEN-mediated knock-in mice have been created. In animal models, to treat hemophilia A, these two systems along with iPSCs technique has been used.

4.3 The iPSCs for Hemophilia A

The use of cell therapy in the cure of hemophilia has consisted mainly in the transplantation of normal cells. It is an effort to correct a coagulation factor deficiency. Use of these techniques is with adult stem cells and with progenitor cells partially differentiated from iPSCs [4]. Hemophilia is a monogenic recessive disorder which requires low circulating levels of coagulation factor. It does not need gene regulation to attain a moderate phenotype. In addition, a large diversity of pathological animal models is obtainable for experimentation. The iPSCs technology may offer countless clinical applications for the cure of hemophilia. In the case of hemophilia A, iPSCs technique have shown as a potential technique to cure the disorder [54]. In addition, uses of iPSCs technique along with gene editing technologies have shown remarkable results.

Xu et al. succeeded to differentiate mouse iPSCs from fibroblast into endothelial cells and their precursors. These iPSCs-derived cells show

specific membrane markers for these cells, factor VIII in particular. The factor VIII survived the tail-clip hemorrhage assay for more than three months in mice. The plasma levels of the factor increased to about 10%. Yadav et al. generated factor VIII in a hemophilic mouse model after transplantation of iPSC-derived endothelial progenitor cells. Alipio et al. obtained this factor VIII during one year after transplantation of iPSCs-derived endothelial cells [4,54]. Jia et al. [55] generated iPSCs from urine collected from hemophilia A patients. The authors used non-integrating episomal vectors containing human genes such as *Oct4* and *SV40LT* to generate iPSCs. The authors differentiated the iPSCs into hepatocyte-like cells. The phenotype of the *F8* genes and factor VIII activity was available in the patient-specific iPSCs-derived hepatic cells. Jia et al. differentiated the hemophilia A patient-specific iPSCs into functional hepatocyte-like cells. These cells did not produce factor VIII. It shows that hemophilia A patient-specific iPSCs provide an effective venue for modeling hemophilia A *in vitro.* These results suggest that iPSCs technique can be helpful to overcome hemophilia A.

The iPSCs technique can edit incorrect gene in hemophilia A by using MNs, ZFNs, TALENs and CRISPR (Fig. 2). In case of these techniques, they cleave the chromosomal DNA in a targeted site and produce DNA double-strand breaks. The NHEJ will correct the nick. Finally, it will result in the correction of the incorrect gene such as deletions and inversions. These gene-corrected are differentiated into suitable somatic cells. Then, patients can get the corrected-gene by delivering to patients. iPScs can derive from urine, peripheral blood or fibroblast, for example.

Liver sinusoidal endothelial cells (LSECs) are the only source of factor VIII production in the fetal human liver. These cells continue to produce factor VIII after transplantation into mice. Therefore, fetal LSECs could be a potential cell source for human transplantation for treatment of hemophilia A. However, size and gestational age limit the production of LSECs [56]. To obtain the healthy gene from the fetal human liver, the iPSCs technique can be used. Then, healthy gene iPSCs are differentiated before distribution into mice.

Park et al. showed that to generate disease models in iPSCs and to generate correct alleles by inversions, TALENs could be used. The authors used TALENs to invert the normal *F8* gene for hemophilia A model in human iPSCs. Then, the authors reversed the error segment to the WT situation in the hemophilia model iPSCs [57]. Park et al. used RT-PCR, quantitative PCR, and T7E1 analysis to confirm the research results. Wu et al. did a TALEN-mediated correction of 22 inversions by targeting a complemented sequence at the junction of exon 22 and intron 22 with a high efficiency,

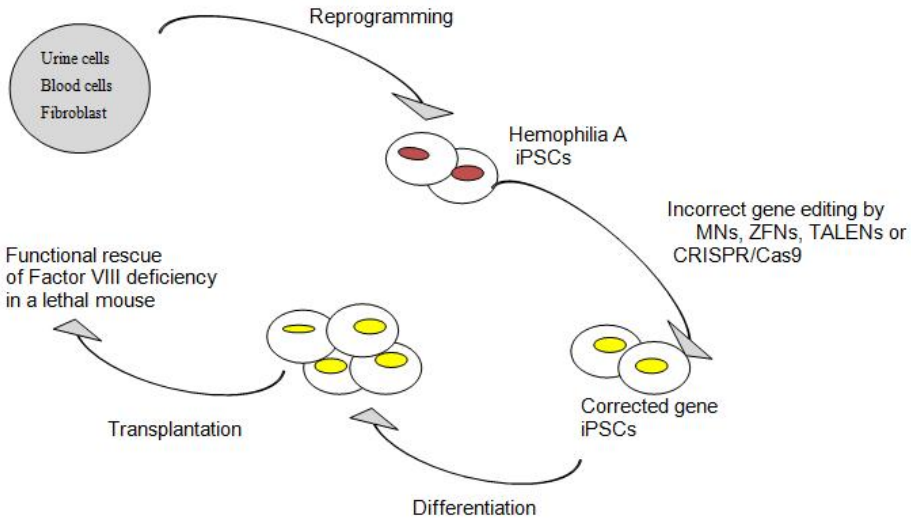

Fig. 2. Treatment of hemophilia A using iPSCs; Incorrect gene correction in iPSCs via MNs, ZFNs, TALENs, or CRISPR/Cas9 and transplantation as functional safe of factor VIII deficiency in a lethal mouse

62.5% and 52.9%, in human hemophilia A iPSCs. After elimination of drug selection cassette by using a Cre-LoxP system, the karyotipe was normal. Both *F8* transcript and factor VIII secretion were rescued in the endothelial cells (ECs) and MSCs taken from the gene-edited iPSCs. In their study, Wu et al. did an efficient *in situ* genetic correction in the error segment in the *F8* gene [58]. Pang et al. generated iPSCs from patients with severe hemophilia A. The authors used TALENickases to target the *F8* gene at the multi-copy ribosomal DNA (rDNA) in hemophilia A iPSCs. It aims to save the deficiency of factor VIII protein. In their study, the exogenous F8 and factor VIII protein was detected in targeted hemophilia A iPSCs after differentiated into endothelial cells [59]. These results show the potential of iPSCs technique and gene editing with TALENs to cure hemophilia A disorder.

Park et al. obtained iPSCs from patients with inversion genotypes. The authors used CRISPR/Cas9 nucleases to fix these mutations back to the WT situation. Park et al. isolated the normal chromosome segment with frequencies up to 6.7%. Endothelial cells from edited iPSCs produced the *F8* gene. It practically rescued the factor VIII deficiency in a lethal mouse model of hemophilia. It suggests potential therapeutic applications with the iPSCs technique and CRISPR/Cas9 techniques for treatment of hemophilia A [60].

It seems that iPSCs technique will be very useful to treat hemophilia A. This method can edit erroneous segments in the *F8* gene to the normal segments. In addition, the iPSCs technique along with genome editing techniques can cause the correct *F8* gene to incorrect gene. Also, it can make back to the WT situation. It shows that hemophilia A came from mutations or DNA changes in the *F8* gene in the past. To overcome the disadvantages in patient-specific iPSCs for the cure of hemophilia A, TALENs and CRISPR/Cas9 systems can be used.

5. CONCLUSION

Hemophilia A is a monogenic recessive disorder that can cause hemorrhage to the people with hemophilia A. Mutations in the *F8* gene can result in hemorrhage in the hemophilia A patients. These mutations can include such as deletion and inversion. To correct mutations in incorrect genes, the gene-editing tools along with iPSCs technique can be used. In hemophilia A, gene editing with TALENs and CRISPR/Cas9 systems in patient-specific iPSCs have shown important progresses. The iPSCs in combination with CRISPR/Cas9 system have shown important progress to treat hemophilia A. These tools succeeded to rescue the factor VIII deficiency in animal models of hemophilia A.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Peer-review history:
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