J Human Gen Genom. 2018 July; 2(1):e80764. doi: 10.5812/jhgg.80764.

Published online 2017 July 29. **Review Article**

Hemophilia Gene Therapy; Clinical and Molecular Aspects

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Received 2017 May 10; **Accepted** 2017 July 01.

Abstract

Hemophilia is a coagulation disorder in which bleeding time is prolonged. There are a number of hemophilia subtypes and more than 4,000,000 individuals are suffered worldwide. The most common types of hemophilia are type A and B in which coagulation factor VIII and IX are defected respectively. Type A hemophilia is responsible for 80% to 85% of cases. The genes of 8 and 9 coagulation factors located on the long arm of X chromosome and mutation in these genes causes disturbance in coagulation. This disease is a very good target for gene therapy because if amount of protein production reaches 1% that of normal the disease phenotype is modified. Different methods of hemophilia gene therapy include increased production of coagulation factors via insertion of attributed genes into patient's stem cells by vectors, or insertion of transgenes into differentiated cells with prolonged survival such as muscle or liver cells. One of the most recent advances in hemophilia gene therapy is using induced pluripotent stem cells (iPS) for gene transfer. Hepatocytes are very good candidates for hemophilia gene therapy due to their natural capacity for production of coagulation factors. Myocytes are also suitable for injection of transgene because they are available and have sufficient secretory power. Most important and useful viral vectors for hemophilia are retroviral, lentiviral, and Adeno-Associated viruses. Among these only the retroviral vectors target dividing cells.

Keywords: Hemophilia, Gene Therapy, iPS

1. Background

Hemophilia is a bleeding disorder in which coagulation time is prolonged. In severe cases prolonged bleeding after mild trauma or even spontaneous bleeding occur. Bleeding into joints, muscles and brain is a consequence that may be lethal, however, most patients only experience hemarthrosis (joint bleeding) (1).

There are different types of hemophilia and more than 400,000 individuals are suffered worldwide. Two main types of hemophilia are types A and B, which result from defective coagulation factors VIII and IX. Hemophilia type A is responsible for 80 to 85% of cases. On average, type A hemophilia occurs one in every 1,500 male births and type B hemophilia occurs one in every 30,000 male births (2-4). As mentioned, type A hemophilia or classic hemophilia is the most common type of hemophilia and is a consequence of mutations in coagulation factor VIII gene. Type B hemophilia or Christmas disease is the result of mutations in the factor IX in coagulation cascade. Both of these genes are located on the long arm of chromosome X. Their products are involved in internal pathway of coagulation. Inheritance pattern of hemophilia is X linked.

2. Structure and Function of Hemophilia Associated Genes and Proteins

Coagulation factor VIII gene, with 180 kb length and contains 26 exons is located on the long arm of chromosome X. The functional protein translated from this gene consists of 2,332 amino acids and weighs 300 kilo Daltons.

It is noteworthy that this gene is mainly expressed in endothelial cells and hepatocytes and its product is involved in internal pathway of coagulation cascade (5). This gene transcribes into mRNA and translates into a protein witch becomes active after splicing process is accomplished (6).

Coagulation factor IX is also located on the long arm of chromosome X. Its length is 34 kb and consists of 8 exons. It is expressed into a 57 kilo Dalton glycoprotein. Coagulation factor IX in company with other coagulation factors plays role in coagulation cascade.

3. Hemophilia Clinical Signs

As far as clinical features of type A and type B are concerned, they are indistinguishable.

4. Diagnosis

Diagnosis of hemophilia is based on the result of partial thromboplastin time (PTT) and prothrombin time (PT) tests which measure internal and external pathways respectively (7).

5. Treatment

Currently hemophilia is treated by protein replacement. Although it is a suitable temporary therapy, it is not long lasting and not a kind of cure. Coagulation factor is produced as recombinant protein or come out as concentrate from fresh blood plasma. This treatment has limitations such as the need for repeated injections because of short half-life, production of antibodies against protein leading to declined efficacy, besides not being a definite cure (7, 8).

6. Potential of Gene Therapy for Hemophilia

Hemophilia is a suitable target for gene therapy because if the protein concentration reaches 1% that of normal, the disease phenotype will be modified (9). The aim of gene therapy for hemophilia is prolonged expression of related defective genes. There are two ways to reach this goal. First approach is insertion of relevant genes into patient's stem cells via integrating vectors and second approach is delivering vectors into differentiated cells with prolonged survival such as myocyte and hepatocytes.

7. Tissue Targets for Hemophilia Gene Therapy

Liver cells: liver is the main site for coagulation factor synthesis from where the proteins enter the circulation easily (10, 11).

Muscular cells: these cells are readily available however, post translation changes occur better in the liver cells (12).

Endothelial cells: coagulation factors are synthesized in these cells and enter the circulation easily (13).

Hematopoietic stem cells: these cells are readily available for ex-vivo gene therapy. They are especially useful due to their self-renewal ability and their potential to differentiate into various blood cells. This technique also bears more immunologic tolerance (14).

8. Viral Vectors

The most useful viral vectors for hemophilia gene therapy include retroviral, lentiviral, and Adeno-associated viruses.

Retroviral vectors: These vectors unite within the host genome. They only affect the dividing cells and their capacity is 7 kb.

Lentiviral vectors: these vectors unite within the host genome. They infect non-dividing and latent cells. Like retroviral vectors, their capacity is 7 kb.

Adeno-associated viruses: replication of these vectors is dependent. More than 90 percent of them do not enter the host genome and despite long term expression; besides their chance of carcinogenesis is low. The main limitation of these vectors is their low capacity (4.7 kb) (15).

9. Stem Cell Based Gene Transfer

Hematopoietic stem cells could be retrieved from various sources. The self-renewal ability results in long term expression of the transgene even as the stem cell differentiates into various cell types. The stem cells are extracted and infected with the retroviral or lentiviral vectors and then transplanted into the bone marrow. One advantage of this technique is the absence of antibody production against factor VIII. In 2010, the human-pig factor VIII hybrid transgene was designed and attached to a lentiviral vector and transferred into hematopoietic stem cells. The expression level of this hybrid was higher in comparison to human factor which denotes long term expression (14-16).

A recent safe method of stem cell based hemophilia gene therapy is using induced pluripotent stem cell (iPS). For this purpose somatic adult cells are obtained and dedifferentiated to become stem cell by means of growth in cell culture nourished with transcription factors including SOX-2, OCT-4,C-MYC, and KLF-4; then therapeutic gen is inserted into cells via vectors. Finally cells are injected to patients. The important advantage of this technique is that the recipient dose not confront with immunological reactions (17, 18).

10. Hepatocyte Based Gen Transfer

Hepatocytes are the best target for hemophilia gene therapy because of natural ability to synthesis and secretion of coagulation factors. Another reason that make hepatocytes suitable candidate for hemophilia gene therapy is that, post translation changes are done well in hepatocytes. For hepatocytes based gene therapy Lentiviruses and Adeno viruses are used (19).

11. Myocyte Based Gene Transfer

In spite of hepatocytes, muscle cells are not naturally a center for generating coagulation factors. However, myocytes have a good secretory ability of coagulation factors. Unfortunately, post translation changes like glycosylation and proteolysis are not done as well as hepatocytes. For Myocyte based hemophilia gene therapy, therapeutic gene is attached to Adeno-associated viruses followed by injection into muscle cells (20).

12. Conclusions

Due to significant improvement in the clinical symptoms of hemophilia by slight changes in the expression of coagulation Factors VIII and IX, hemophilia considered as one of the most appropriate target for gene therapy. To obtain this goal, modifier genes sould be transfered to the specific tissues such as liver cells, muscular cells, endothelial cells, hematopoietic stem cells and etc. In available approaches modifier genes are inserted into vectors such as retroviral, lentiviral, and Adeno-Associated viruses. However, they may have immunological complications or even hamper the gene expression. The novel approach is based on the type of stem cells, which are called iPS. iPS are derived from somatic adult cells and has no immunological complications. Although gene therapy is a potential and long-lasting treatment for hemophilia, more investigation are required to establish these methods.

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