Successful correction of hemophilia by CRISPR/Cas9 genome editing in vivo: delivery vector and immune responses are the key to success

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Hemophilia B is a serious hemostasis disorder due to mutations of the factor IX gene in the X chromosome. Gene therapy has gained momentum in recent years as a therapeutic option for hemophilia B. In hemophilia, reconstitution with a mere 1–2% of the clotting factor improves the quality of life, while 5–20% suffices to ameliorate the bleeding disorder. A paper by Guan et al (2016) in this issue of EMBO Molecular Medicine reports on the direct CRISPRs/Cas9-mediated correction in the liver of a hemophilia-causing point mutation in FIX.

See also: Y Guan et al (May 2016)

The main therapeutic goal, as with other monogenic disorders, is to restore gene function permanently, while limiting cell toxicity, genome alterations or induction of harmful immune responses against the components of the gene therapy system used and against the products of the transgene.

To avoid genotoxicity and achieve physiological levels of transgene expression, gene-specific genome editing or site-specific gene addition is preferable to the random integration of expression cassettes, which could result in insertional mutagenesis and cancer as well as silencing of transgene expression.

Gene therapy without genome editing using recombinant adeno-associated virus (rAAV) containing an expression cassette has been tested in clinical gene therapy trials to treat hemophilia B with excellent results at 5-year post-treatment follow-up (Nathwani et al, 2014). As rAAV largely persists in an episomal state, the long-lasting therapeutic effects observed in the clinical trial are probably due to the fact that recruited patients are adults and thus the liver is quiescent and consequently hepatocyte renewal is very slow. The pre-existence or the appearance of anti-AAV cellular immunity is an obstacle to the persistence of genetically corrected cells (Manno et al, 2006).

Repair of the defective gene or gene replacement by targeted integration into the genome would allow long-term expression of the introduced sequence, but this would depend on the type of delivery method used. Gene-specific nucleases such as ZFNs, TALENs, or CRISPR/Cas9 upon DNA cleavage of the targeted sequence allow a severalfold increase in the insertion into the cleavage point of DNA repairing sequences that are delivered simultaneously to the nucleases.

In this respect, rAAV were used for hepatic delivery in adult and newborn mice of ZFNs to intron 1 of the factor 9 gene (F9) and as DNA donor an acceptor splicing sequence and the cDNA of exons 2–8 to obtain production of factor IX in a humanized murine model of hemophilia B (Li et al, 2011).

More recently, rAAV were used for hepatic delivery in hemophilia B mice of a promoter-less F9 cDNA preceded by a 2A-peptide coding sequence flanked by homology arms to integrate the repairing DNA just upstream of the stop codon of albumin, a highly expressed gene in the liver. The target genomic sequence was not cleaved by nucleases (nuclease-free approach), and the F9 cDNA was integrated just by means of spontaneous homologous recombination possibly favored by the rAAV flanking sequences (Barzel et al, 2015). On-target in the albumin locus was achieved at a level of up to 0.5% and restored 7–20% of normal FIX expression. This approach thus avoids potential off-effects of nucleases.

A recent report involving instead site-specific insertion in the liver described the correction of a mutation causing fumarylacetatoacetase hydrolase (FAH) deficiency, which at variance with hemophilia, confers a selective advantage to hepatocytes in which correction has taken place. In this paper, genome editing was performed using CRISPR/Cas and ssODNs that directly introduced the appropriate sequence into the defective gene (Yin et al, 2014).

As mentioned above, the paper by Guan et al (2016) in this issue of EMBO Molecular Medicine reports on the direct CRISPRs/
Cas9-based correction in the liver of a hemophilia-causing point mutation using an approach similar to the one used to correct FAH deficiency but without a selective advantage for corrected hepatocytes. The authors first describe a male hemophilia B patient with a novel F9 missense mutation [g.31094T>G (p.Tyr371Asp)]. The authors then elegantly demonstrate that this mutation is indeed responsible for hemophilia by generating a mouse strain containing the mutation and presenting with normal levels of F9 but with the hemostasis defect. Finally, the authors correct the mouse mutation in vivo by hydrodynamic tail injection of a plasmid encoding Cas9 and the sgRNA along with either ssODN (120 nt) or long donor DNA (with homology arms of 0.4 kb) containing the corrected sequence. Using both DNA donors, hemostasis was significantly corrected with up to 0.56 and 1.5% of hepatocytes displaying a corrected genotype using ssODNs and the long DNA, respectively. A higher proportion of hepatocytes showed indels without correction indicating DNA cleavage with non-homologous end joining repair and without insertion of the donor DNA. This treatment did not induce hepatocyte damage. The authors then moved to the simultaneous use of two adeno-viral vectors, one encoding Cas9 and the second one the sgRNA and the long DNA donor. Despite obtaining a high frequency of hepatocyte correction (5.5%) at early time points, no correction of hemostasis was observed at later time points. This lack of biological response despite genomic correction is indeed responsible for hemophilia by off-target effects by in silico definition of potential targets followed by the T7 assay. It has been shown that more and new off-targets can be identified when other methods are used (Gabriel et al, 2011) and T7 analysis is not very sensitive when compared to techniques such as NGS analysis. Therefore, potential off-target effects need to be better defined before going into clinical trials.

Previous reports have shown that ZFNs (Li et al, 2011) and even a nuclease-free approach (Barzel et al, 2015) are also efficient and since they insert F9 cDNA under the control of the albumin or F9 promoters, they have potential application in any case of hemophilia B regardless of the mutation. The genome editing strategy using CRISPR/Cas and ssODNs described in the manuscript by Guan et al (2016) would have to be tailored to each mutation and therefore depends on the generation of different sgRNAs that may have different efficacies.

All the above-mentioned strategies have the clinical potential to also treat hemophilia A, which is more common than hemophilia B, as well as several liver genetic diseases. The paper by Guan et al (2016) is therefore important and encouraging and illustrates how the gene delivery vehicle and the role of immune responses are of key importance in the efficacy, biosafety, and duration of successful therapeutically editing.

Acknowledgements

The authors apologize for not having been able to individually cite all the relevant original papers due to space constraints. Research activity in the authors’ laboratory is supported by the three projects of the “Investissements d’Avenir” French Government program, managed by the French National Research Agency (ANR): (i) IHU-CESTI (ANR-10-IBHU-005 and by Nantes Métropole and Région Pays de la Loire); (ii) Labex IGO project (no ANR-11-LABX-0016-01); and (iii) “TEFOR” project (ANR-11-INSB-0014) as well as by other ANR projects (14-CE16-0026, 13-LAB3-001-01 and 11-BSV1-009).

References


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