Review Article

Critical Update for the Treatment of Anemia by using Advanced Genome Editing Crispr Cas Technology

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Abstract | Anemia is the condition which results in formation of unhealthy red blood cells which results lack of oxygen supply towards tissues of body. It is common and worldwide problem that associates with all ages like pregnant women, children and aged people. Anemia associated with numerous infectious and continual problems such as persistent kidney disease, cancer, ischemic heart ailment and inflammatory bowel disorder. However, with the development in generation at genome editing level have made feasible to accurate mutations in human genome. A site oriented specific break in double stranded DNA is induced by CRISPR/Cas9, whereas different oligonucleotides are provided to make accurate DNA template for genome correction. It is of importance to knowledge about types of CRISPR to treat anemia. Cas 9 showed one of best gene editing reactions than others. Recent data gives authentic applications of CRISPR use in anemia which provides potential for usage of gene editing technique for different types of anemia. In evaluation we defined currently developed genome editing device update used for the remedy of anemia, its mechanism of movement and sickle cellular mutation corrections.

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1. Introduction

Anemia disease is nutritional deficiency disorder and it is widely spread in people. It causes many health problems in the people of developing countries and developed states. According to world health organization 2005 anemia cause many health consequences and their social improvement. In South Asian countries about half 50 percent death rate is due to anemia and 80 percent death rate in India. In North America people death rate is less than 40 percent. The dominant causative agents or etiologies are different in various region of the world (Ezzati *et al.*, 2002).

Engineered proteins are produced rapidly and utilized to target locus of genome, provide base for excellent genome editing. Transcription activator-like effector nucleases (TALENs), CRISPR/Cas9 (bundle of regular inter spaced repeats) and zinc finger nucleases (ZFN) are classified as recent chief applicants to intervene the genome modifications. The nonspecific FokInuclease domain is linked with DNA binding elements such as ZFNs and TALENs and these elements also give specificity. The double standard DNA break (DSB) is produced by the dimerization of the complex at the target site. The assembly of specialized materials and technology is required for the production of ZFNs but at the

same time, usability for various demands is restricted as compared to ZFNs, the method of reagents assembly for TALEN complexes is uncomplicated and starting materials are publicly accessible. The recently portrayed Streptococcus pyogenes CRISPR/Cas9 stage is likewise profoundly easy to understand and contains two parts: The Cas9 nuclease and a guide RNA (gRNA). The gRNA is a short transcript that can be intended for a one of a kind genomic locus having a GN20GG succession theme and that serves to enlist the Cas9 protein to the objective site where it prompts a DSB (Deans *et al.*, 2016; Mali *et al.*, 2013; Bak *et al.*, 2018). gRNAs direct Cas9 utilizing complementarity between the 5¢-most 20 nts and the objective site, which must have a protospacer contiguous theme (PAM) arrangement of the structure NGG (Mali *et al.*, 2013). Feasible applications for quality altering reagents to accomplish quality amendment incorporate (Ezzati *et al.*, 2002) the presentation of a full-length cDNA at an alleged genomic safe harbor, or (Kassebaum *et al.*, 2015) valid in situ transformation explicit focusing on. The AAVS1 locus on chromosome 19 was recognized as a combination hotspot for wild-type AAV and as encoding the PPP1R12C quality that capacities as a subunit of myosin phosphatase. This locus has been focused for joining of hereditary material that at that point controlled by the PPP1R12C advertiser or foreign advertiser included in the focusing on develop.

CRISPER_Cas9 is a technique of gene editing by adding, removing, altering the part of gene sequences. It is simplest, most versatile, precise method of gene manipulation. It made broad improvement in gene sequence because of its speed, expertise, easiness, and cost. CRISPER is abbreviation of "clustered regularly interspaced short palindromic repeats" it specifies the sequence of DNA of different microorganisms like bacteria and different microbial springs. The gene sequence which is linked to the sequence of next gene is called CRISPER associated gene. CRISPER associated gene can also develop immunity in the body of host against different viruses. The three main purpose of CRISPER system is to first recognize the sequence of DNA then cut the sequence and destroy external DNA. CRISPER system is five type. CRISPER_Cas9 is most deliberate type. Scientist developed that the editing of genome of plant, animal, microorganism is more effective through CRISPER_Cas9 is (Gupta *et al.*, 2019).

1.1 CRISPER_Cas9 technology mechanism

CRISPER_Cas9 is also an enzyme that cut the DNA and guide the RNAs to involve the insertion or deletion of uridine residue into mitochondrial mRNAs. Guide RNA also involve in the editing of gene also involve in CRISPER_Cas9. The guide RNA targets the specific site of DNA molecule and recognizes the specific region for break down through CRISPER_Cas9 enzyme. The broken part of DNA is condensed, injected and edited by DNA sequences and then modified end S are ligated. According to scientist guide RNA can be deliberated to any DNA sequence. It has various wide applications in various field through editing of genome of plant, animal, microbial (Charpentier and Doudna, 2013). However, CRISPER_Cas9 may change particular cell function by knowing preferred gene sequence and its general mechanism is shown in Figure 1.

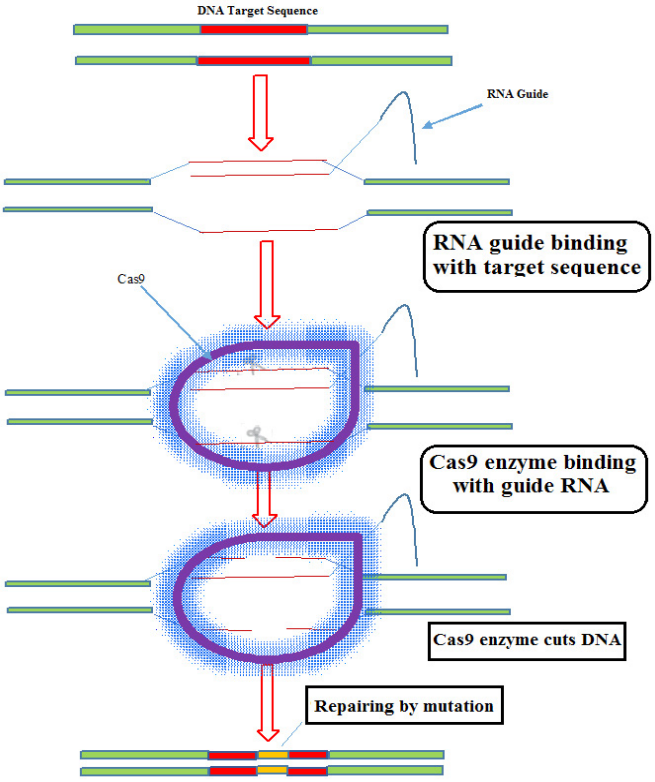

Figure 1: General mechanism of CRISPR Cas9 technique of genome editing (Akbar *et al.*, 2020).

1.2 Versatile tool

In 1987, clustered often interspersed brief palindromic repeats (CRISPR) become first time informed for the duration of a research on *E. coli* genome and after that it was also recognized in lots of other bacterial strains. The position of short palindromic sequences became unsure for many years. The investigations were extended to locate the reason of CRISPR and

Cas genes associated with adaptive immunity towards foreign DNA. The kind II device is a subgroup of CRISPR systems which be determined by on a Cas protein to cut a specific DNA sequence. For the first time in 2013 type II Cas protein isolated from S. Pyogenes was practical for DNA lysis in animal cells guided from RNA (Cong *et al.*, 2013; Mali *et al.*, 2013). Before DNA lysis conformation of Cas9 nuclease modifies with the binding of sg RNA and then it's miles adapted to its target collection region (Jinek *et al.*, 2012). In Figure 2 patient's unedited and edited hematopoietic stem cells of sickle cell anemia disease are shown.

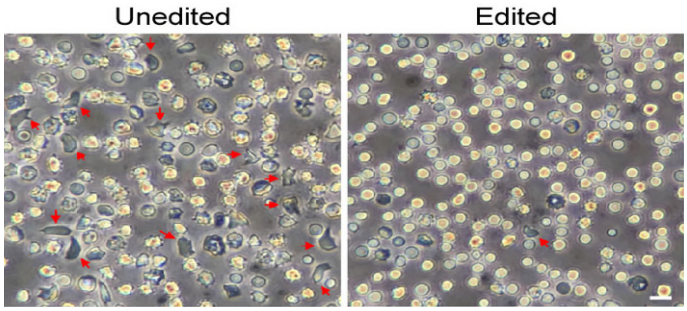

Figure 2: Red blood cells from patient with sickle cell disease. The cells were differentiated from bone marrow with unedited and edited hematopoietic stem cells, and the red arrows show the sickled cells (Posted on April 2nd, 2019 by Dr. Francis Collins on NIH Director's Blog and Credit: Wu et al. Nature Medicine. March 25, 2019).

1.3 Different CRISPR systems

CRISPR system has different chief classes like the type III CRISPR structures of Archaea are identified in type I of class 1. Although the type II, IV, V, and VI CRISPR structures are found in class 2. The simplest NGG PAM series of *Streptococcus pyogenes* are involved in condensation of several genome CRISPR/Cas structures on the daily used CRISPR-Cas9 kind II system (Makarova *et al.*, 2015). Acidaminococcussp and Lachnospiraceae bacterium are identified as sources of Cpf1 proteins and more than 10 CRISPR/Cas proteins have been discovered in some former years (Yamano *et al.*, 2016). The calling of Cas9 calls for RNAs along with the Cpf1 requirement for simplest one sg RNA (unmarried guide RNA). The adeno connected viruses along with Cas9 (huge protein) can create several issues in packaging and delivery (Fonfara *et al.*, 2016).

1.4 Genome editing in sickle cell disease (SCD)

The monogenic problem like SCD are repaired through genotypic and phenotypic possibilities. The pathophysiology is added into the infection by process of exertion which introduce basic characteristics and chromosomal zones. Different types of protein behave antagonistic to the sickling properties. For instance, the extra aggressive behavior towards the sickling characteristics is observed by wild sort β-globin altered the β-globin (T87Q). Some other types like β-globin or β/γ half are found to utilize the distinct viral builds after their transfer to sickle HSPCs. The competence and security benefits are attained after their utilization in clinical prolegomenon (Demirci *et al.*, 2018). The production of erasures and defensive addition make the Genome alerting tool a fascination source of universal elimination and repetition of harmful changes. The DNA fix system is utilized to make two-fold DSB (strand breaks) at specific genome locus by the programmable nucleases enzyme. The involvement DNA fix system includes two systems either through the HDR or homology coordinated fix (which consume homologous arrangements of homologous chromosomes, extrachromosomal benefactor DNA lineage adapted to reviewing aims to the DSB place) and siter chromatids, and the other system non-homologous end-joining (NHEJ). Three significant nucleases were analyzed as homing nucleases including mega nucleases before three years back (Humbert *et al.*, 2018).

The huge creature models with indel proportions and following clinical trials with large group of patients are considered as important factors for the beneficial and achievement purposes of the BCL11A knockdown which is as yet expecting. The focusing BCL11A in patients with serious SCD is now developing by the lentiviral quality replacement of vector, encoding a micro RNA maintained small barrette RNA called as (shRNAmiR). This exchange proved as instigation in clinical trials. The progress of this process is seen in predominant patient displaying (NCT03282656) 23% HbF (Shim *et al.*, 2017; Esrick and Bauer, 2018). Another alternate technology was also introduced by Daniel Bauer and his colleagues. This technique performs as an erythroid explicit enhancer through focus on the +58 intronic site of the BCL11A by creating the matching degrees of HbF in CD34+ cells (Bauer *et al.*, 2013; Canver *et al.*, 2015).

On the other hand, it was observed that organized disturbances in enhancer site activate the increase in HbF articulation in mice by minimizing the

Bcl11a articulation in erythrocyte cells, whereas the non-erythroid genealogies did not show impacts of articulation (Smith and Orkin, 2016) Ancestor cells of patients with β- Thalassemia major (Psatha *et al.*, 2018) and its erythroid cells concludes the accommodative behavior of disturbing enhancer system in clinical and pre-clinical trials and would be beneficial for clinical usability for cases. It is now achievable to search about novel genomic destinations/qualities controlling HbF articulation through the basics of RNA screening models.

1.5 SCD mutation correction

The sickle cell disorder (SCD) is now completely differentiated because of the pathologic alternation and changes in SCD create complications but these issues are easily resolved by the achievable and favorable Cas9 technique. This technique produces cuts of sickle β-globin and these cuts are fixed with homology arms to the DSB margined with typical β-globin arrangement. The specific locus on the genome provide the right order for genotypic adjustment and foreign transgene actuation is not required further. The adjustment of SCD modifications in different cell types is carried out by utilizing increase number of analysts which perform alternation in quality advancement and assure the relevant rectification. As compared to quality modification tools like TALENs, the CRISPR/Cas9 is vastly utilized because of its incredible competence and less OTEs (Bak *et al.*, 2018; Hoban *et al.*, 2016). In present time, HSPC is considered as the vastly used genome modifying concern, with truly bone marrow implied CD34+ HSPCs. A new CD34+ HSPCs with plerixafor is discovered by chance which proved to appear beneficial impacts in SCD patients by stimulating the element in granulocyte area of these infected people. The saturation is used to modify the CD34+ cells and their transfer back into infected people.

The adjusted pathways of the cells might get disturb after the DSB supply during the defense from efficient quality modification. This disturbance is caused by less resistance in cell cycle or may be due to definite proximity of nucleases (Lomova *et al.*, 2018). Therefore, the conveyance of the CRISPR/Cas9 framework with the giver DNA is achieved and enhanced by the process of electroporation with an Adeno-related infection (AAV) 6 viral vector. It is observed from few examinations that focused and profound sequencing is utilized to analyze the treatment for SCD change.

Conclusions and Recommendations

Each region of world is unusually dominant with anemia. This disease is interlinked with increased medical prices and infuriating clinical conclusions. Hematological disorders can be efficiently treated with the utilization of genome editing strategies. Illusory antigen receptor T-cell immune therapy (CAR-T) is considered as most necessary discovery. The treatment of malignant tumors and extreme constant lymphoid leukemia with CAR-T expose its charm and powerful curable effects (Singh *et al.*, 2017; González-Romero *et al.*, 2019). There is need to stimulate the extraordinary vaults forward inside the following century with the increased advancement including the blend of CAR-T and CRISPR. Recently Emmanuelle Charpentier and Jennifer A. Doudna got 2020 Noble prize in Chemistry for their great discovery of this gene editing technique that will attract scientists to work on this technique for the application invarious fields like clinical and agriculture as this method allow the researchers to cut the DNA at their desired site by guide RNA containing desired DNA template sequence.

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Novelty Statement

This review article describes the novel ideas of CRISPR Cas9 regarding rapid and permanent treatment of sickle cell Anemia.

Author's Contribution

Conceived and designed the experiment: Ghulam Akbar, Ali Ahmad, Neha Arooj, Muhammad Anjum Zia, Amna Rafique, Sania Riasat, Mohsin Raza, Mahpara qamar, Shahneela Nusrat and Shakila Haneef. Performed the experiment: Ghulam Akbar, Ali Ahmad, Neha Arooj, Muhammad Anjum Zia, Amna Rafique, Sania Riasat, Mohsin Raza, Mahpara qamar, Shahneela Nusrat and Shakila Haneef. Analyzed the data: Ghulam Akbar, Ali Ahmad, Neha Arooj, Muhammad Anjum Zia, Amna Rafique, Sania

Riasat, Mohsin Raza, Mahpara qamar, Shahneela Nusrat and Shakila Haneef. Contributed reagents/ materials/ analysis tools: Ghulam Akbar, Ali Ahmad, Neha Arooj, Muhammad Anjum Zia, Amna Rafique, Sania Riasat, Mohsin Raza, Mahpara qamar, Shahneela Nusrat and Shakila Haneef.

Conflict of interest

The authors have declared no conflict of interest.

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