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Research Highlight

Reduction of BCL11A in hematopoietic stem cells through gene editing: new strategy to ameliorate the severe β-globin disorders sickle cell disease

Weiqi Hong, Mengyuan Huang, Yuquan Wei, Xiawei Wei *

Laboratory of Aging Research and Cancer Drug Target, State Key Laboratory of Biotherapy, National Clinical Research Center for Geriatrics, West China Hospital, Sichuan University, Chengdu 610041, China

Site-specific gene editing is of great importance in precise medicine. Two conventional genome editing methods, Zine finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), are based on protein-DNA recognition, with tedious work in constructing target protein [1,2]. Developed from immune response of bacteria, CRISPR/Cas9 has been widely investigated as a promising tool for therapeutic genome editing in clinical settings nowadays [3,4]. This system succeeds in gene deletion, insertion and frameshift mutations with higher efficiency, less cost, improved flexibility and simplified designing process [5]. There are two crucial components in the CRISPR/Cas9 system: Cas9 endonuclease and single-stranded guide RNA (gRNA). A guide RNA (gRNA) is composed of a constant tracrRNA and a crRNA, forming base pairs with site-specific DNA sequences by the first changeable 20 nts in gRNA. After pairing, Cas9 "cut" the targeted DNA fragment and generate double stand break (DSB). Then, non-homologous end-joining (NHEJ) and homology directed repair (HDR) occur to repair DNA double stands with random base insertion or precise modification in presence of donor DNA templates [2].

Mutating or correcting related genes by CRISPR-Cas9 is now a hot topic in treatment of various diseasewith heritable and precisely edited genotype in various species, which is widely applied in biomedical fields [3,6,7].

A recent study published in *Nature Medicine* by Wu et al. [8] proposed a potential way for reduction of BCL11A expression, and induction of HbF through CRISPR–Cas9-mediated gene modification in hematopoietic stem cells (HSCs) to ameliorate severe β-globin disorders sickle cell disease (SCD) and β-thalassemia. The clinical trials of BCL11A enhancer editing via Cas9 (NCT03655678) and finger nucleases (NCT03432364) developed by pharmaceutical company Vertex/CRISPR therapeutics have been approved by FDA in 2018. Currently, phase I/II clinical trials have been conducted in US and Europe. This study would contribute to the success of the clinical trials by improving the efficiency of CRIPSP/Cas9 (Fig. 1).

Sickle-cell anemia is a prototypical monogenic disorder caused by mutation of β-globin subunit. It is a promising therapy strategy to induct fetal hemoglobin (HbF, $\alpha 2\gamma 2$) by re-expressing the paralogous γ-globin genes (HBG1/2) for severe β-globin disorders sickle cell disease (SCD) and β-thalassemia [9]. Researches in the past have shown that the core of the +58 erythroid enhancer of BCL11A was crucial for repression of HBF in adult stage erythroid. Wu *et al* found that chemically modified synthetic sgRNAs (MS-sgRNAs) was more efficient than *in vitro* transcribed sgRNAs. Targeting the core of the +58 erythroid enhancer of BCL11A in $CD34^+$ hematopoietic stem and progenitor cells (HSPCs) with MS-sgRNAs led to reduction of BCL11A expression and induction of γ-globin and HbF. $CD34^+$ HSPCs from patients with β-thalassemia of varying genotypes were also edited, and terminal erythroid maturation was improved. The edited human $CD34^+$ HSPCs were then engrafted into immunodeficient NBSGW mice. However the indel frequencies in the engrafting cells was reduced after 16weeks, which was possibly caused by the presence of long-term engrafting HSCs. To maximize genome editing efficiency, they added a c-Myc-like NLS to the amino (N) terminus and both SV40 and nucleoplasmin NLSs to the C terminus of SpCas9 (3xNLS-Cas9). 3xNLS-Cas9 RNP electroporation achieves up to 98.1% indels in $CD34^+$ HSPCs. Edited human $CD34^+$ HSPCs were engrafted into immunodeficient NBSGW mice and the indel frequencies in engrafting cells didn't significantly decrease after 16 weeks. BCL11A expression was reduced and γ-Globin was elevated in edited erythroid cells. The researchers also proved that BCL11A enhancer editing was effective in SCD through efficiently editing $CD34^+$ cell from patients. The level of HbF was improved in SCD patient's cells, which were resistant to sickling. Quiescent and engrafting HSCs are more inclined to implement NHEJ instead of MMEJ repair. The safety of gene editing in HPCs was proven by testing the specificity of the RNP sgRNA and no malignancy-associated mutations was identified.

Overall, using CRISP/Cas9 system to edit BCL11A enhancer in hematopoietic stem cells from patients with β-thalassemia and β-globin disorders sickle cell disease and improving editing efficiency of CRISPR/Cas9 via optimizing gRNA and Cas9, resulted in erythroid cells being normal with high proportion of HbF. Thus it is possible to completely cure this family of diseases.

* Corresponding author.
E-mail address: xiaweiwei@scu.edu.cn (X. Wei).

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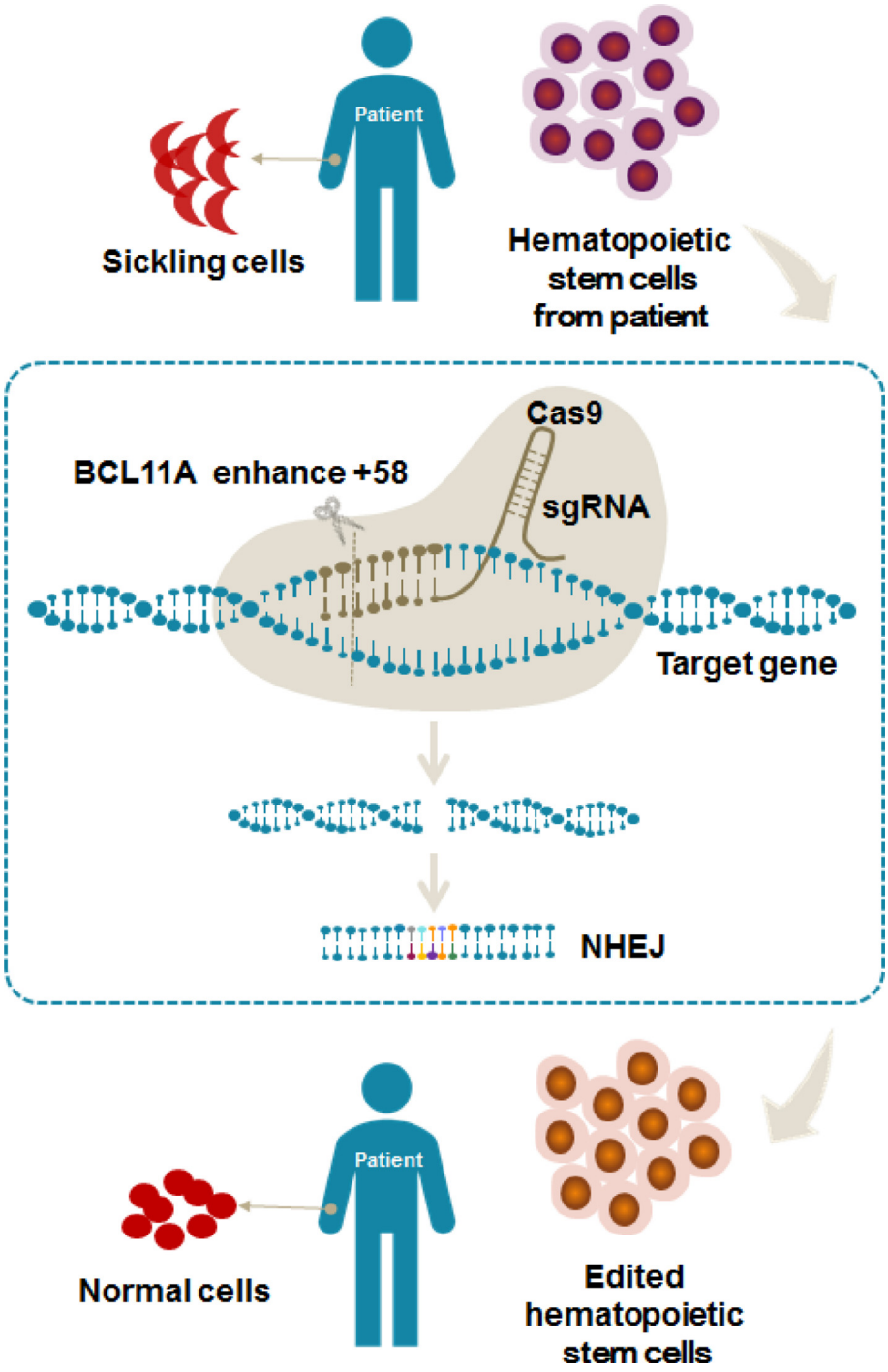

Fig. 1. Editing $CD34^+$ hematopoietic stem cells by CRISPR/Cas9 system.

CRISPR/Cas9 systems are important in gene editing for therapy of genetic diseases because of their highly programmable DNA binding and cleavage activity. With the development of CRISPR/Cas9 system in biomedical field, researchers used CRISPR/Cas9 to successfully correct human genetic diseases ranging from single base mutations to large insertions and it is also applied in therapy of chromosomal inversion [2]. As cancer is thought to be a disorder disease in genetic alterations and multiple mutations, CRISPR/Cas9 system has also been applied in cancer research and treatment. Scientists use CRISPR/Cas9 to generate cancer model, screen functional genes and diagnose genes. In addition, over ten CRISPR/Cas9 systems have come to clinical trials [10]. Combined with program cell death-1 (PD-1) as target site, PD-1 knockout trials are being conducted in advanced esophageal cancer, castration resistant prostate cancer, muscle-invasive bladder cancer, metastatic non-small cell lung cancer and so on. CRISPR/Cas9 gene therapy has also been used in leukemia, lymphoma, malignant neoplasm, myeloma and nervous system for phase I or II clinical trial.

However, there are still issues remaining to be solved. For instance, improvement of editing efficiency, accuracy and safety should be paid persistent attention when applying in human. Appropriate delivery systems with low toxicity and high efficiency, including viral and non-viral gene vectors, are the most vital part for both *in vivo* and *ex vivo* delivery. Cas9 nuclease can be

optimized to orientate various target gene. Furthermore, new systems of CRISPR/Cas should be explored. Recently, Type V systems containing the signature DNA-targeting Cas12 effectors and Cas13 effectors have been discovered, these systems may edit gene with higher efficiency [11,12]. In addition, the emergence of base editing makes gene editing more precise. Conclusively, CRISPR/Cas9 systems provide us with a powerful tool to research and treat various diseases from DNA and gene, the original part in central dogma. Despite huge challenges and ethical issues, we should be cognizant of the positive effects of CRISPR/Cas9, and come up with better approaches for human genetic diseases and formulate unified standard for further clinical application.

Conflict of interest

The authors declare that they have no conflict of interest.

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Weiqi Hong is a M.D. candidate at Key Laboratory of Biotherapy of West China Hospital, Sichuan University. He obtained B.S. degree from West China School of Pharmacy, Sichuan University. His current research aims to treat diverse neurogenic diseases via small molecular drugs or gene therapy, and look for some natural products to delay the aging process.

Xiawei Wei is a Director and Professor in the Lab of Nanotoxicology, National/State Key Laboratory of Biotherapy. Her major research interests include cancer gene therapy and immunotherapy by nanoparticles and viral vectors as well as the mechanisms by which nanoparticles cause the toxicity and/or adverse immune responses induced by the vectors.