COMPOSITIONS AND METHODS FOR THE TREATMENT OF HEMOGLOBINOPATHIES

RELATED APPLICATIONS

This application claims priority to U.S. Provisional patent application number 62/271,968, filed December 28, 2015, and U.S. Provisional patent application number 62/347,484, filed June 8, 2016. The entire contents of these applications are incorporated herein by reference.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on January 26, 2017, is named PAT057179-WO-PCT_SL.txt and is 1,115,217 bytes in size.

BACKGROUND

CRISPRs (Clustered Regularly Interspaced Short Palindromic Repeats) evolved in bacteria as an adaptive immune system to defend against viral attack. Upon exposure to a virus, short segments of viral DNA are integrated into the CRISPR locus of the bacterial genome. RNA is transcribed from a portion of the CRISPR locus that includes the viral sequence. That RNA, which contains sequence complimentary to the viral genome, mediates targeting of a Cas9 protein to the sequence in the viral genome. The Cas9 protein cleaves and thereby silences the viral target.

Recently, the CRISPR/Cas system has been adapted for genome editing in eukaryotic cells. The introduction of site-specific single (SSBs) or double strand breaks (DSBs) allows for target sequence alteration through, for example, non-homologous end-joining (NHEJ) or homology-directed repair (HDR).

SUMMARY OF THE INVENTION

Without being bound by theory, the invention is based in part on the discovery that CRISPR systems, e.g., Cas9 CRISPR systems, e.g., as described herein, can be used to modify cells (e.g., hematopoietic stem and progenitor cells (HSPCs)) to increase fetal hemoglobin (HbF) expression and/or decrease expression of beta globin (e.g., a beta globin gene having a disease-causing mutation), and that such cells may be used to treat hemoglobinopathies, e.g., sickle cell disease and beta thalassemia.

Thus, in an aspect, the invention provides CRISPR systems (e.g., Cas CRISPR systems, e.g., Cas9 CRISPR systems, e.g., *S. pyogenes* Cas9 CRISPR systems) comprising one or more, e.g., one, gRNA molecule as described herein. Any of the gRNA molecules described herein may be used in such systems, and in the methods and cells described herein.

DNA bands were estimated by the ImageJ software (http://rsb.info.nih.gov/ij/) to determine the percent of edited alleles. Names of gRNAs used and corresponding gene editing efficiencies determined from mismatch detection assay are also shown in the table below the gel image.

Figure 20: Percent allele editing by next generation sequencing. Labels are as described for Figures 18 and 19.

Figure 21. Colony forming cell (CFC) unit assay showing colony number and types of colonies observed for five select gRNAs. The HSPCs genome edited at the +58 erythroid enhancer were plated in methylcellulose and clonal colonies classified and counted based on the number and types of mature cells using morphological and phenotypic criteria using STEMvision. The colonies were classified into colony forming unit-erythroid (CFU-E), burst forming unit-erythroid (BFU-E), colony-forming unit - granulocyte/macrophage (CFU-GM) and colony-forming unit - granulocyte/erythrocyte/macrophage/megakaryocyte (CFU-GEMM).

Figure 22. Kinetics of cell division during erythroid differentiation. Total number of cells determined on day 0, 7, 14 and 21 during erythroid differentiation and the fold cell expansion calculated.

Figure 23. BCL11A, gamma and beta- globin mRNA levels in genome edited and erythroid differentiated HSC. Relative mRNA expression of BCL11A, gamma- and beta-globin chains in unilineage erythroid cultures were quantified by real time PCR. Transcript levels were normalized against human GAPDH transcript levels.

Figure 24A. Efficient editing of HSC obtained with dual gRNA/cas9 system for disruption of BCL11a enhancer (+58) resulted in high levels of induction of HbF. The HSC 48 hours after electroporation were subjected to *in vitro* erythroid differentiation and HbF expression was measured. The percent of HbF positive cells (F-cells) was monitored by FACS analysis at days 7, 14 and 21. Data shown in this figure was generated with dgRNA systems where the dgRNA included the indicated targeting domain.

Figure 24B. Efficient editing of HSC obtained with sgRNA/cas9 system for disruption of BCL11a enhancer (+58) resulted in high levels of induction of HbF. The HSC 48 hours after electroporation were subjected to *in vitro* erythroid differentiation and HbF expression was measured. The percent of HbF positive cells (F-cells) was monitored by FACS analysis at days 7, 14 and 21. Data shown in this figure was generated with sgRNA systems where the sgRNA included the indicated targeting domain.

Figure 25: Indel pattern produced in HSPCs by gRNAs including the indicated targeting domain. Figure discloses SEQ ID NOS 2956-2968, respectively, in order of appearance.