

Claims

1. A genetically modified red blood precursor cell comprising a genomic modification made following cleavage within the BCL11A gene by a zinc finger nuclease (ZFN), a TALE nuclease (TALEN), and/or a CRISPR/Cas system that binds to a target site of at least 12 base pairs of the sequences of SEQ ID NOs: 1 or 12 such that the BCL11A gene is inactivated.

2. The genetically modified red blood precursor cell of claim 1, wherein the genomic modification is selected from the group consisting of insertions and/or deletions.

3. The genetically modified red blood precursor cell of claim 1 or claim 2, wherein the genomic modification is at or near an endogenous sequence selected from the group consisting of SEQ ID NOs: 1 and 12.

4. The genetically modified red blood precursor cell of claim 1 or claim 2, wherein the cell is a hematopoietic stem cell, and wherein the cell is optionally differentiated into a red blood cell (RBC).

5. The genetically modified red blood precursor cell of claim 1, wherein:

(a) the BCL11A gene is cleaved by a zinc finger nuclease;

(b) the nuclease is introduced into the cell as a polynucleotide; or

(c) the genomic modification comprises integration of a donor polynucleotide encoding a transgene.

6. The genetically modified red blood precursor cell of claim 1, wherein the zinc finger nuclease comprises a zinc finger protein comprising 4, 5, or 6 zinc finger domains comprising a recognition helix and further wherein the zinc finger protein comprises the recognition helix regions of the proteins as follows:

(i) a protein comprising the recognition helix regions as follows:

F1: DQSNLRA (SEQ ID NO:2);

F2: RNFSLTMT (SEQ ID NO:9);

F3: SNGNLRN (SEQ ID NO:10) or STGNLTN (SEQ ID NO:7) or SSYNLAN (SEQ ID NO:11);

F4: TSGSLTR (SEQ ID NO:5);

F5: DQSNLRA (SEQ ID NO:2); and

F6: AQCCLFH (SEQ ID NO:6); or

(ii) a protein comprising the recognition helix regions as follows:

F1: RNDHRTT (SEQ ID NO:19);

F2: QKAHLIR (SEQ ID NO:20);

F3: QKGTLGE (SEQ ID NO:15);

F4: RGRDLSR (SEQ ID NO:21) or LKRTLKR (SEQ ID NO:25); and

F5: RRDNLHS (SEQ ID NO:17); or

(iii) a protein comprising the recognition helix regions as follows:

F1: STGNLTN (SEQ ID NO:7);

F2: TSGSLTR (SEQ ID NO:5);

F3: DQSNLRA (SEQ ID NO:2); and

F4: AQCCLFH (SEQ ID NO:6); or

(iv) a protein comprising the recognition helix regions as follows:

F1: RSDHLTQ (SEQ ID NO:13);

F2: QSGHLAR (SEQ ID NO:14);

F3: QKGTLGE (SEQ ID NO:15);

F4: RHRDLSR (SEQ ID NO:18); and

F5: RRDNLHS (SEQ ID NO:17); or

(v) a protein comprising the recognition helix regions as follows:

F1: DQSNLRA (SEQ ID NO:2);

F2: RPYTLRL (SEQ ID NO:3);

F3: SRGALKT (SEQ ID NO:8);

F4: TSGSLTR (SEQ ID NO:5);

F5: DQSNLRA (SEQ ID NO:2); and

F6: AQCCLFH (SEQ ID NO:6);

(vi) a protein comprising the recognition helix regions as follows:

F1: RSDHLTQ (SEQ ID NO:13);

F2: QRAHLTR (SEQ ID NO:22);

F3: QKGTGLGE (SEQ ID NO:15) or QSGTRNH (SEQ ID NO:24);
 F4: HRNTLVR (SEQ ID NO:23); and
 F5: RRDNLHS (SEQ ID NO:17); or
 (vii) a protein comprising the recognition helix regions as follows:
 F1: RSDHLTQ (SEQ ID NO:13);
 F2: QKAHLIR (SEQ ID NO:20);
 F3: QKGTGLGE (SEQ ID NO:15) or QSGTRNH (SEQ ID NO:24);
 F4: RGRDLSR (SEQ ID NO:21); and
 F5: RRDNLHS (SEQ ID NO:17); or
 (viii) a protein comprising the recognition helix regions as follows:
 F1: RSDHLTQ (SEQ ID NO:13);
 F2: QSGHLAR (SEQ ID NO:14);
 F3: QSGTRNH (SEQ ID NO:24);
 F4: QSSDLSR (SEQ ID NO:16); and
 F5: RRDNLHS (SEQ ID NO:17).

7. A zinc finger protein comprising 4, 5 or 6 zinc finger domains comprising a recognition helix region, wherein the zinc finger protein comprises the recognition helix regions of the proteins as follows:

(i) a protein comprising the recognition helix regions as follows:

F1: DQSNLRA (SEQ ID NO:2);
 F2: RNFSLTM (SEQ ID NO:9);
 F3: SNGNLRN (SEQ ID NO:10) or STGNLTN (SEQ ID NO:7) or SSYNLAN (SEQ ID NO:11);

F4: TSGSLTR (SEQ ID NO:5);
 F5: DQSNLRA (SEQ ID NO:2); and
 F6: AQCCLFH (SEQ ID NO:6); or

(ii) a protein comprising the recognition helix regions as follows:

F1: RNDHRTT (SEQ ID NO:19);
 F2: QKAHLIR (SEQ ID NO:20);
 F3: QKGTGLGE (SEQ ID NO:15);

F4: RGRDLSR (SEQ ID NO:21) or LKRTLKR (SEQ ID NO:25); and

F5: RRDNLHS (SEQ ID NO:17); or

(iii) a protein comprising the recognition helix regions as follows:

F1: STGNLTN (SEQ ID NO:7);

F2: TSGSLTR (SEQ ID NO:5);

F3: DQSNLRA (SEQ ID NO:2); and

F4: AQCCLFH (SEQ ID NO:6); or

(iv) a protein comprising the recognition helix regions as follows:

F1: RSDHLTQ (SEQ ID NO:13);

F2: QSGHLAR (SEQ ID NO:14);

F3: QKGTLGE (SEQ ID NO:15);

F4: RHRDLSR (SEQ ID NO:18); and

F5: RRDNLHS (SEQ ID NO:17); or

(v) a protein comprising the recognition helix regions as follows:

F1: DQSNLRA (SEQ ID NO:2);

F2: RPYTLRL (SEQ ID NO:3);

F3: SRGALKT (SEQ ID NO:8);

F4: TSGSLTR (SEQ ID NO:5);

F5: DQSNLRA (SEQ ID NO:2); and

F6: AQCCLFH (SEQ ID NO:6);

(vi) a protein comprising the recognition helix regions as follows:

F1: RSDHLTQ (SEQ ID NO:13);

F2: QRAHLTR (SEQ ID NO:22);

F3: QKGTLGE (SEQ ID NO:15) or QSGTRNH (SEQ ID NO:24);

F4: HRNTLVR (SEQ ID NO:23); and

F5: RRDNLHS (SEQ ID NO:17); or

(vii) a protein comprising the recognition helix regions as follows:

F1: RSDHLTQ (SEQ ID NO:13);

F2: QKAHLIR (SEQ ID NO:20);

F3: QKGTLGE (SEQ ID NO:15) or QSGTRNH (SEQ ID NO:24);

F4: RGRDLSR (SEQ ID NO:21); and

F5: RRDNLHS (SEQ ID NO:17); or

(viii) a protein comprising the recognition helix regions as follows:

F1: RSDHLTQ (SEQ ID NO:13);

F2: QSGHLAR (SEQ ID NO:14);

F3: QSGTRNH (SEQ ID NO:24);

F4: QSSDLSR (SEQ ID NO:16); and

F5: RRDNLHS (SEQ ID NO:17).

8. A fusion protein comprising the zinc finger protein of claim 7 and a wild-type or engineered cleavage domain or wild-type or engineered cleavage half-domain.

9. A polynucleotide encoding one or more proteins of claim 7 or claim 8.

10. An isolated cell comprising one or more fusion proteins of claim 8 or one or more polynucleotides of claim 9.

11. The cell of claim 10, wherein the cell is selected from the group consisting of a red blood cell (RBC) or a precursor cell, such as a CD34+ hematopoietic stem cell.

12. A kit comprising the protein of claim 7, the fusion protein of claim 8 or the polynucleotide of claim 9.

13. An *in vitro* method of altering globin gene expression in a cell, the method comprising:
introducing, into the cell, one or more proteins of claim 7 or claim 8 or one or more polynucleotides of claim 9, under conditions such that the one or more proteins are expressed and expression of the globin gene is altered.

14. The one or more proteins of claim 7 or claim 8 or one or more polynucleotides of claim 9 for use in a method of altering globin gene expression in a cell, the method comprising:
introducing, into the cell, the one or more proteins of claim 7 or claim 8 or one or more polynucleotides of claim 9, under conditions such that the one or more proteins are expressed and expression of the globin gene is altered.

15. The method of claim 13 or the one or more proteins or one or more polynucleotides for use of claim 14, wherein:

- (a) the proteins increase expression of a globin gene, such as a gamma globin or beta globin gene;
- (b) the method further comprises integrating a donor sequence into the genome of the cell, for example, by introducing the donor sequence to the cell using a viral vector, an oligonucleotide or a plasmid;
- (c) the cell is a red blood cell (RBC) precursor cell, such as a CD34+ hematopoietic stem cell; and/or
- (d) the donor sequence comprises a transgene under the control of an endogenous promoter or an exogenous promoter.

16. The method of claim 13 or claim 15, or the one or more proteins or one or more polynucleotides for use according to claim 14, wherein globin gene expression is the level of globin mRNA or globin protein produced.

17. A genetically modified red blood precursor cell as claimed in any of claims 1 to 6 for use in the treatment of hemoglobinopathies.

18. Use of a genetically modified red blood precursor cell according to any of claims 1 to 6 in the manufacture of medicament for the treatment of a beta-thalassemia or sickle cell disease wherein upon administration, the medicament increases globin production in a subject.

19. The use of claim 18, wherein the subject is a human and the cell is a human stem cell or human precursor cell.

20. The use of claim 18 or claim 19, wherein the cell is to be administered in a bone marrow transplant whereupon the cell engrafts, differentiates and matures in the subject.