

Claims

1. A fusion protein or a protein complex comprising a DNA binding protein (DnaBP), a nucleobase modifying protein (NMP), and a Base Excision Repair associated protein (BERAP); wherein the fusion protein or protein complex does not comprise a Uracil binding protein or a catalytically active DNA polymerase.
2. The fusion protein or protein complex according to claim 1, wherein the nucleobase modifying protein (NMP) is a cytosine deaminase domain.
3. The fusion protein or protein complex according to claim 1 or 2, wherein the BERAP is selected from the group consisting of an AP endonuclease, an end processing enzyme, a catalytically inactive DNA polymerase, a lyase domain, a Flap endonuclease, a DNA ligase, and a scaffold protein involved in the BER pathway.
4. The fusion protein or protein complex according to any of claims 1-3, wherein the BERAP is selected from the group consisting of: a DNA ligase III (LIG3), an XRCC1, a DNA binding or lyase domain of DNA Polymerase beta, a DNA binding or lyase domain of DNA Polymerase delta, a DNA binding or lyase domain of DNA Polymerase epsilon, an AP endonuclease (APE1), Proliferating cell nuclear antigen (PCNA), DNA-(apurinic or apyrimidinic site) lyase (APEX), Poly (ADP-ribose) polymerase (PARP), Flap endonuclease 1 (FEN1), and DNA ligase I (LIG1).
5. The fusion protein or protein complex according to any of claims 1-4, wherein the BERAP is an XRCC1.
6. The fusion protein or protein complex according to any of claims 1-4, wherein the BERAP is a DNA binding or lyase domain of DNA Polymerase beta.
7. The fusion protein or protein complex according to claim 6, wherein the DNA binding or lyase domain of DNA Polymerase beta corresponds to a region contained within amino acids 1-140, 1-120, 1-100, or 1-87 of a full DNA Polymerase beta sequence (SEQ ID NO: 12 or 13).
8. The fusion protein or protein complex according to any of claims 1-4, wherein the BERAP is a DNA Ligase III (LIG3).

9. The fusion protein or protein complex according to any of claims 1-8, wherein the DnaBP comprises a CRISPR-associated (Cas) domain.

10. The fusion protein or protein complex according to claim 9, wherein the Cas domain is selected from the group consisting of a Cas3, a Cas9, a xCas9, a HF-Cas9, a Cas9-NG, a SpRY Cas9, a circularly permuted Cas9, a Cas10 and a Cas12 (also known as Cpf1), a Cas14, a CasX, a CasΦ, and variants thereof.

11. The fusion protein or protein complex according to claim 9 or 10, wherein the Cas domain is a Cas nickase (nCas).

12. The fusion protein or protein complex according to claim 9 or 10, wherein the Cas domain is a nuclease inactive Cas (dCas).

13. The fusion protein or protein complex according to any of claims 2-12, wherein the cytosine deaminase domain is a deaminase domain from an apolipoprotein B mRNA-editing complex (APOBEC) family deaminase.

14. The fusion protein or protein complex according to of any of claims 1-13, wherein the DNA binding protein (DnaBP) is a nickase Cas protein; wherein Base Excision Repair associated protein (BERAP) is selected from the group consisting of an XRCC1, a DNA Ligase III (LIG3), and a DNA binding or lyase domain of DNA Polymerase beta; wherein the nucleobase modifying protein (NMP) is a deaminase domain from an apolipoprotein B mRNA-editing complex (APOBEC) family deaminase.

15. The fusion protein according to of any of claims 1-14, wherein the orientation of the DnaBP, NMP and BERAP within the fusion protein is selected from the group consisting of: [NMP]-[DnaBP]-[BERAP], [NMP]-[BERAP]-[DnaBP] and [BERAP]-[NMP]-[DnaBP]; wherein each instance of “]-[” comprises an optional linker.

16. The fusion protein according to claim 15, wherein the orientation of the DnaBP, NMP and BERAP within the fusion protein is [NMP]-[DnaBP]-[BERAP].

17. A fusion protein comprising:

- a first amino acid sequence that is at least 80% identical to the amino acid sequence of any one of SEQ ID NOs: 1-2,

- a second amino acid sequence that is at least 80% identical to the amino acid sequence of SEQ ID NO: 3,
- a third amino acid sequence that is at least 80% identical to the amino acid sequence of SEQ ID NO: 4-10.

18. A fusion protein comprising a sequence of any one of SEQ ID NOs: 42 to 72.

19. The fusion protein of claim 18, wherein the sequence is SEQ ID NO: 44 or SEQ ID NO: 69.

20. A protein complex comprising:

- a first protein comprising an amino acid sequence that is at least 80% identical to the amino acid sequence of any one of SEQ ID NOs: 1-2,
- a second protein comprising an amino acid sequence that is at least 80% identical to the amino acid sequence of SEQ ID NO: 3,
- a third protein comprising an amino acid sequence that is at least 80% identical to the amino acid sequence of SEQ ID NO: 4-11.

21. A protein-nucleic acid complex comprising a nucleic acid molecule and any one of:
the fusion protein or protein complex according to any one of claims 1-14,
the fusion protein according to any one of claims 15-19; and
the protein complex of claim 20.

22. A pharmaceutical composition comprising the fusion protein or protein complex of any one of claims 1-14, the fusion protein according to any one of claims 15-19, the protein complex of claim 20, or the protein-nucleic acid complex of claim 21.

23. The pharmaceutical composition of claim 22, further comprising a pharmaceutically acceptable excipient.

24. A method of replacing a cytosine with a guanine on a DNA strand in a cell, said method comprises introducing to the cell the fusion protein or protein complex of any one of claims 1-13, the fusion protein according to any one of claims 15-19, the protein complex of claim 20, or the protein-nucleic acid complex of claim 21.

25. A polynucleotide encoding the fusion protein or protein complex of any one of claims 1-14, the fusion protein according to any one of claims 15-19, the protein complex of claim 20, or the protein-nucleic acid complex of claim 21.

26. A vector comprising the polynucleotide of claim 25.

27. A cell comprising the fusion protein or protein complex of any one of claims 1-14, the fusion protein according to any one of claims 15-19, the protein complex of claim 20, or the protein-nucleic acid complex of claim 21.

28. Use of ~~A method of treating a subject having or suspected of having a disease or disorder comprising administering~~ the fusion protein or protein complex of any one of claims 1-14, the fusion protein according to any one of claims 15-19, the protein complex of claim 20, the protein-nucleic acid complex of claim 21, the pharmaceutical composition of any one of claims 22-23, the polynucleotide of claim 25, or the vector of claim 26 in the manufacture of a medicament for treating a subject having or suspected of having a disease or disorder.
~~to the subject.~~

29. The ~~method-use~~ of claim 28, wherein the disease or disorder comprises one or more C>G and/or one or more G>C mutations.

30. The ~~method-use~~ of claim 29, wherein the disease or disorder is selected from a group consisting of skin fibrosis, bladder cancer, liver cancer, Myasthenic syndrome, Spondyloepimetaphyseal dysplasia, Parkinson's disease, Deafness, blood disorders, and Schnyder crystalline corneal dystrophy.