CLAIMS

WHAT IS CLAIMED IS:

1. A DNA fragment for constructing an mRNA targeting molecule comprising an N-acetylgalactosamine binding polypeptide, comprising a promoter, a target gene, a specific protease cleavage sequence, and a polynulcleotide sequence encoding a GBD capable of binding to N-acetylgalactosamine, that are sequentially connected.

2. The DNA fragment for constructing an mRNA targeting molecule comprising an N-acetylgalactosamine binding polypeptide according to claim 1, wherein the polynulcleotide sequence encoding a GBD capable of binding to N-acetylgalactosamine is one or a combination of more than one of SEQ ID NOs.1-5.

3. The DNA fragment for constructing an mRNA targeting molecule comprising an N-acetylgalactosamine binding polypeptide according to claim 2, wherein the sequence of the target gene is set forth in SEQ ID NO. 6 or SEQ ID NO. 7.

4. The DNA fragment for constructing an mRNA targeting molecule comprising an N-acetylgalactosamine binding polypeptide according to any one of claims 1 to 3, wherein the specific protease cleavage sequence is one or more of T2A, P2A, E2A, F2A, TEV, VLP1 and SUMO specific protease cleavage sequences with a GBD sequence.

5. The DNA fragment for constructing an mRNA targeting molecule comprising an N-acetylgalactosamine binding polypeptide according to claim 4, wherein the specific protease cleavage sequence is set forth in SEQ ID NO. 9 or SEQ ID NO. 10.

6. The DNA fragment for constructing an mRNA targeting molecule comprising an N-acetylgalactosamine binding polypeptide according to any one of claims 1 to 4, wherein the promoter is T3, T7 or SP6 promoter.

7. An mRNA targeting molecule comprising an N-acetylgalactosamine binding polypeptide, comprising an mRNA molecule, wherein the mRNA molecule is obtained by *in vitro* transcription using a plasmid containing the DNA fragment according to any one of claims 1 to 5;

the sequence of the mRNA molecule sequentially comprises a 5' cap, a target gene sequence, a specific protease cleavage sequence and a polypeptide GBD protein;

the polypeptide GBD protein is obtained by ribosomal translation of the GBD sequence; and

the GBD sequence end of the mRNA molecule is connected to the GBD protein through puromycin, and the GBD protein is connected to N-acetylgalactosamine through an enzymatic reaction.

8. A method for preparing the mRNA targeting molecule comprising an N-acetylgalactosamine binding polypeptide according to claim 7, comprising the following steps:

step S1, selecting a specific cell surface receptor according to the tissue, organ or cell to which the mRNA is delivered, designing a polynulcleotide sequence encoding a GBD capable of binding to N-acetylgalactosamine, and cloning a combination of a promoter sequence, a target gene sequence, a specific protease cleavage sequence, and a GBD sequence into a plasmid vector to obtain a plasmid DNA; and

step S2, performing *in vitro* transcription using the plasmid DNA of step S1 as a template to obtain an mRNA sequence comprising a 5' cap, a target gene sequence, a specific protease cleavage sequence, and a GBD sequence.

9. Use of the mRNA targeting molecule comprising an N-acetylgalactosamine binding polypeptide according to claim 7 in the preparation of an mRNA drug for specific drug delivery, wherein N-acetylgalactosamine is connected to the 3' end, and by specifically binding to the asialoglycoprotein receptor on a surface of liver cells through the N-acetylgalactosamine, endocytosis is induced, which allows an mRNA to enter the cell for expression.

10. A method for preparing an mRNA-puromycin-GBD-GalNAc complex, comprising the following steps:

step S1, selecting a specific cell surface receptor according to the tissue, organ or cell to which the mRNA is delivered, designing a polynulcleotide sequence encoding a GBD capable of binding to N-acetylgalactosamine, and cloning a combination of a promoter sequence, a target gene sequence, a specific protease cleavage sequence, and a GBD sequence into a plasmid vector to obtain a plasmid DNA;

step S2, performing *in vitro* transcription using the plasmid DNA of step S1 as a template to obtain an mRNA sequence comprising a 5' cap, a target gene sequence, a specific protease cleavage sequence, and a GBD sequence;

step S3, under the action of T4 ligase, binding the mRNA molecule to a DNA-puromycin linker to form an mRNA-puromycin complex;

step S4, *in vitro* translating the mRNA-puromycin complex obtained in step S3, wherein the mRNA-puromycin complex is translated by a ribosome into a fusion protein sequence of gene function protein-specific protease cleavage sequence-GBD;

step S5, at the end of translation, connecting the puromycin to the tail of the antibody through the A-site of the ribosome to form an mRNA-puromycin-GBD-specific protease cleavage sequence-gene function protein complex;

step S6, cleaving the product obtained in step S5 by a specific protease, wherein the part of the specific protease cleavage sequence-gene function protein in the mRNA-puromycin-GBD-specific protease cleavage sequence-gene function protein complex is cleaved to obtain an mRNA-puromycin-GBD complex; and

step S7, under the action of N-acetylgalactosamine transferase, specifically binding N-acetylgalactosamine to the GBD protein sequence to form an mRNA-puromycin-GBD-GalNAc complex.

11. The method for preparing an mRNA-puromycin-GBD-GalNAc complex according to claim 10, wherein the sequence of the DNA-puromycin linker is set forth in SEQ ID NO. 8; and the GBD sequence is set forth in any one of SEQ ID NOs. 1-5.

12. The method for preparing an mRNA-puromycin-GBD-GalNAc complex according to claim 10, wherein in step S1, the plasmid vector is modified from pCDNA3.1.

13. The method for preparing an mRNA-puromycin-GBD-GalNAc complex according to claim 10, wherein in step S7, the GBD protein sequence binds to one N-acetylgalactosamine, to two N-acetylgalactosamines, to three N-acetylgalactosamines, or to n N-acetylgalactosamines.

14. A GalNAc-mRNA delivery system, comprising the mRNA-puromycin-GBD-GalNAc complex prepared by the method of any one of claims 10 to 13.

15. Use of the GalNAc-mRNA delivery system according to claim 14 in targeted treatment of a liver disease.

16. The use according to claim 15, wherein the GalNAc-mRNA delivery system is introduced into the body.

CLAIMS

WHAT IS CLAIMED IS:

1. A DNA fragment, the DNA fragment comprising a target sequence and a polynulcleotide sequence encoding a N-acetylgalactosamine (GalNAc) binding domain (GBD) capable of binding to GalNAc.
2. The DNA fragment of claim 1, wherein the GBD comprises one or more Serines, Threonines, or any combination thereof, and optionally wherein the one or more Serines, Threonines, or any combination thereof are separated by glycines.
3. The DNA fragment of claim 1 or 2, wherein the GBD comprises a sequence as set forth in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5.
4. The DNA fragment of one of the preceding claims, wherein the DNA fragment further comprises a second polynucleotide sequence encoding specific protease cleavage sequence, flanked by the target sequence and the first polynucleotide sequence, and optionally wherein the specific protease cleavage sequence comprises one or more of T2A, P2A, E2A, F2A, TEV, VLP1 or SUMO specific protease cleavage sequences.
5. The DNA fragment of claim 4, wherein the specific protease cleavage sequence comprises a sequence as set forth in SEQ ID NO: 9 or SEQ ID NO: 10.
6. The DNA fragment of one of the preceding claims, wherein the DNA fragment further comprises a promoter, and optionally wherein the promoter is T3, T7, or SP6.
7. The DNA fragment of claim 6, wherein the promoter comprises a sequence as set forth in SEQ ID NO: 11.
8. The DNA fragment of one of the preceding claims, wherein the DNA fragment is expressed in a pCDNA3.1 plasmid vector.
9. A polynucleotide-puromycin molecule, wherein the polynucleotide-puromycin molecule comprises a transcription product of the DNA fragment of one of claims 1-8, subsequently with a DNA-puromycin linker and a puromycin.
10. The polynucleotide-puromycin molecule of claim 9, wherein the DNA-puromycin linker comprises a sequence as set forth in SEQ ID NO. 8.
11. A polynucleotide-polypeptide molecule, wherein the polynucleotide-polypeptide molecule comprises a transcription product of the DNA fragment of one of claims 1-8

subsequently with a DNA-puromycin linker, a puromycin, and a translation product of the DNA fragment of one of claims 1-8 in a reverse order.

12. A polynucleotide-polypeptide molecule, wherein the polynucleotide-polypeptide molecule comprises a transcription product of the DNA fragment of one of claims 1-8, subsequently with a DNA-puromycin linker, a puromycin, and the GBD.
13. A molecule with N-acetylgalactosamine (GalNAc), the molecule comprising a transcription product of the DNA fragment of one of claims 1-8, subsequently with a DNA-puromycin linker, a puromycin, and the GBD, wherein one or more GalNAcs bind to the GBD.
14. A method of preparing a molecule, the method comprising
    a. Obtaining the DNA fragment of one of claims 1-8;
    b. *In vitro* transcribing the DNA fragment of one of claims 1-8, thereby obtaining a transcription product of the DNA fragment of one of claims 1-8;
    c. Ligating a puromycin to the transcription product from step b, thereby obtaining a polynucleotide-puromycin molecule;
    d. *In vitro* translating the polynucleotide-puromycin molecule from step c;
    e. conjugating one or more GalNacs
15. The method of claim 14, wherein the method further comprises cleaving the product obtained in step d prior to step e.
16. The method of claim 14, wherein the conjugating is catalyzed by a N-acetylgalactosamine transferase.
17. A method of delivering a target gene sequence to a target cell, the method comprising applying the molecule with GalNAc of claim 13 to the target cell, and wherein the target cell expresses a ligand of GalNAc.
18. The method of claim 17, wherein the target cell is a liver cell.