

CLAIMS

What is claimed is:

1. Use of COL4A1 gene in the preparation of tumor markers;
preferably, the sequence of COL4A1 gene has at least 97.8%, at least 98.9%, at least 99.9% or 100% identity to the sequence shown in Genebank Accession No. NC_000013.11;
preferably, the tumor is colorectal tumor;
preferably, the tumor is colorectal cancer or adenoma;
preferably, the specimen to be detected targeted by the marker is tissue, body fluid or excreta;
preferably, the tissue is intestinal tissue;
preferably, the body fluid is blood, serum, plasma, extracellular fluid, tissue fluid, lymph fluid, cerebrospinal fluid or aqueous humor;
preferably, the excreta is sputum, saliva, urine or feces.

2. Use of a methylation detection reagent of COL4A1 gene in the preparation of a tumor detection reagent or a kit;
preferably, the sequence of COL4A1 gene has at least 97.8%, at least 98.9%, at least 99.9% or 100% identity to the sequence shown in Genebank Accession No. NC_000013.11;
preferably, the tumor is colorectal tumor;
preferably, the tumor is colorectal cancer or adenoma;
preferably, the specimen to be detected targeted by the detection reagent is tissue, body fluid or excreta;
preferably, the tissue is intestinal tissue;
preferably, the body fluid is blood, serum, plasma, extracellular fluid, tissue fluid, lymph fluid, cerebrospinal fluid or aqueous humor;
preferably, the excreta is sputum, urine, saliva or feces.

3. A capture sequence having any one of the nucleotide sequences as shown below:
 - I. a nucleotide sequence shown in SEQ ID NO: 1;
 - II. a nucleotide sequence obtained by modifying, substituting, deleting or adding one or more bases to the nucleotide sequence shown in SEQ ID NO: 1, or a functionally similar nucleotide sequence obtained from CpG islands of the nucleotide sequence shown in SEQ ID NO: 1;
 - III. a sequence having at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identity to the nucleotide sequence shown in SEQ ID NO: 1 or a functionally similar nucleotide sequence obtained from CpG islands of the nucleotide sequence shown in SEQ ID NO: 1;
 - IV. complementary sequences of the sequences shown as I, II or III.

4. A primer pair, wherein the upstream primer has any one of nucleotide sequences set forth below:

V. a nucleotide sequence shown in SEQ ID NO: 2;

VI. a nucleotide sequence obtained by modifying, substituting, deleting or adding one or more bases to the nucleotide sequence shown in SEQ ID NO: 2;

VII. a nucleotide sequence having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identity to the nucleotide sequence shown in SEQ ID NO: 2, or a functionally similar nucleotide sequence obtained from CpG islands of the nucleotide sequence shown in SEQ ID NO: 2;

VIII. complementary sequences of the sequences shown in V, VI or VII;

the downstream primer has any one of the nucleotide sequences shown below:

IX. a nucleotide sequence shown in SEQ ID NO: 3;

X. a nucleotide sequence obtained by modifying, substituting, deleting or adding one or more bases to the nucleotide sequence shown in SEQ ID NO: 3;

XI. a nucleotide sequence having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identity to the nucleotide sequence set forth in SEQ ID NO: 3, or a functionally similar nucleotide sequence obtained from CpG islands of the nucleotide sequence shown in SEQ ID NO: 3;

XII. complementary sequences of the sequences shown in IX, X or XI.

5. A probe having any one of the following nucleotide sequences:

XIII. a nucleotide sequence shown in SEQ ID NO: 4;

XIV. a nucleotide sequence obtained by modifying, substituting, deleting or adding one or more bases to the nucleotide sequence shown in SEQ ID NO: 4;

XV. a sequence having at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identity to the nucleotide sequence shown in SEQ ID NO: 4, or a functionally similar nucleotide sequence obtained from CpG islands of the nucleotide sequence shown in SEQ ID NO: 4;

XVI. complementary sequences shown in XIII, XIV or XV.

6. A methylation detection reagent of COL4A1 gene, comprising a capture sequence, a primer and/or a probe for detection of methylation of COL4A1 gene;

preferably, comprising a capture sequence, a primer and/or a probe obtained from CpG islands of COL4A1 gene;

preferably, comprising a capture sequence, a primer and/or a probe obtained from CpG islands of a genome, an intergenic region, a promoter region or a region near the promoter region of COL4A1 gene;

preferably, the capture sequence has any of the nucleotide sequences shown below:

I. a nucleotide sequence shown in SEQ ID NO: 1;

II. a nucleotide sequence obtained by modifying, substituting, deleting or adding one or more bases to the nucleotide sequence shown in SEQ ID NO: 1, or a functionally similar nucleotide sequence obtained from CpG islands of the nucleotide sequence shown in SEQ ID NO: 1;

III. a sequence having at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identity to the nucleotide sequence shown in SEQ ID NO: 1 or a functionally similar nucleotide sequence obtained from CpG islands of the nucleotide sequence shown in SEQ ID NO: 1;

IV. complementary sequences of sequences shown in I, II or III;

preferably, the upstream primer in the primer pair has any one of the nucleotide sequences shown below:

V. a nucleotide sequence shown in SEQ ID NO: 2;

VI. a nucleotide sequence obtained by modifying, substituting, deleting or adding one or more bases to the nucleotide sequence shown in SEQ ID NO: 2;

VII. a nucleotide sequence having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identity to the nucleotide sequence shown in SEQ ID NO: 2, or a functionally similar nucleotide sequence obtained from CpG islands of the nucleotide sequence shown in SEQ ID NO: 2;

VIII. complementary sequences of the sequences shown in V, VI or VII;

the downstream primer in the primer pair has any one of the nucleotide sequences shown below:

IX. a nucleotide sequence shown in SEQ ID NO: 3;

X. a nucleotide sequence obtained by modifying, substituting, deleting or adding one or more bases to the nucleotide sequence shown in SEQ ID NO: 3;

XI. a nucleotide sequence having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identity to the nucleotide sequence set forth in SEQ ID NO: 3, or a functionally similar nucleotide sequence obtained from CpG islands of the nucleotide sequence shown in SEQ ID NO: 3;

XII. complementary sequences of the sequences shown in IX, X or XI;

preferably, the probe has any one of the nucleotide sequences shown below:

XIII. a nucleotide sequence shown in SEQ ID NO: 4;

~~XIV. a nucleotide sequence obtained by modifying, substituting, deleting or adding one or more bases to the nucleotide sequence shown in SEQ ID NO: 4;~~

~~XV. a sequence having at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identity to the nucleotide sequence shown in SEQ ID NO: 4, or a functionally similar nucleotide sequence obtained from CpG islands of the nucleotide sequence shown in SEQ ID NO: 4; and~~

~~XVI. complementary sequences shown in XIII, XIV or XV.~~

1. A primer pair, wherein the upstream primer has a nucleotide sequence shown in SEQ ID NO: 2, and the downstream primer has a nucleotide sequence shown in SEQ ID NO: 3.

2. A nucleic acid fragment having a nucleotide sequence shown in SEQ ID NO: 1.

3. A probe having a nucleotide sequence shown in SEQ ID NO: 4.

4. A methylation detection reagent of COL4A1 gene,
comprising a nucleic acid fragment, a primer and/or a probe for detection of methylation of COL4A1 gene,

the nucleic acid fragment has a nucleotide sequence shown in SEQ ID NO: 1,

the upstream primer in the primer has a nucleotide sequence shown in SEQ ID NO: 2, and
the upstream primer in the primer has a nucleotide sequence shown in SEQ ID NO: 3, and
the probe has a nucleotide sequence shown in SEQ ID NO: 4.

~~75. A kit comprising the capture sequence nucleic acid fragment of claim ~~32~~, or the primer pair of claim ~~41~~, or the probe of claim ~~53~~, or the methylation detection reagent of claim ~~64~~;~~

6. The kit according to claim 5, wherein preferably, the kit comprising: a first container comprising a capture sequence the nucleic acid fragment ; a second container comprising a the primer pair for amplification; and a third container comprising a the probe.

~~87. Use of the capture sequence nucleic acid fragment of claim ~~32~~, or the primer pair of claim ~~41~~, or the probe of claim ~~53~~, or the methylation detection reagent of claim ~~64~~, in the preparation of a kit in detecting tumors;~~

~~preferably, the tumor is colorectal tumor.~~

~~preferably, the tumor is colorectal cancer or adenoma;~~

~~preferably, the specimen to be detected for detection is tissue, body fluid or excreta;~~

~~preferably, the tissue is intestinal tissue;~~

~~preferably, the body fluid is blood, serum, plasma, extracellular fluid, tissue fluid, lymph fluid, cerebrospinal fluid or aqueous humor;~~

~~preferably, the excreta is sputum, urine, saliva or feces.~~

98. Use of the ~~capture sequence~~ nucleic acid fragment of claim 32, or the primer pair of claim 41, or the probe of claim 53, or the methylation detection reagent of claim 64, or the kit of claim 75 in detecting tumors;

~~preferably, the tumor is colorectal tumor;~~

9. The use of claim 7 or claim 8, wherein ~~preferably,~~ the tumor is colorectal cancer or adenoma;

10. The use of claim 7 or claim 8, wherein ~~preferably,~~ the specimen to be detected is tissue, body fluid or excreta;

11. The use of claim 10, wherein ~~preferably,~~ the tissue is intestinal tissue;

12. The use of claim 10, wherein ~~preferably,~~ the body fluid is blood, serum, plasma, extracellular fluid, tissue fluid, lymph fluid, cerebrospinal fluid or aqueous humor;

13. The use of claim 10, wherein ~~preferably,~~ the excreta is sputum, urine, saliva or feces.

14. A method for ~~detecting~~ determining the risk of a tumor *in vitro*, comprising:

(1) using the nucleic acid fragment of claim 2, or the primer pair of claim 1, or the probe of claim 3, or the methylation detection reagent of claim 4, or the kit of claim 5 to detecting the methylation level of COL4A1 gene of a sample obtained from ~~of~~ a subject;

(2) comparing the methylation level of COL4A1 gene of ~~a the~~ subject with the methylation level of a normal control specimen; and

(3) indicating that the subject has or is at a risk of developing a tumor so as to distinguish a normal specimen from a tumor specimen, according to the increase in the methylation level of COL4A1 gene of the subject compared with the methylation level of the normal control specimen; and

wherein the tumor is colorectal tumor.

~~preferably, a normal specimen is distinguished from a tumor specimen by detecting methylation levels in genosome, an intergenic region or a promoter region and a region near the promoter region of COL4A1 gene;~~

~~preferably, a normal specimen is distinguished from a tumor specimen by detecting methylation levels of a promoter region and a region near the promoter region of COL4A1 gene;~~

~~preferably, the methylation level is detected by methylation-specific PCR, or methylation-specific quantitative PCR, or PCR of methylated DNA-specific binding protein PCR, quantitative PCR, and DNA chips, or methylation-sensitive restriction endonucleases, or bisulfite sequencing, or pyrosequencing;~~

~~preferably, the methylation level is detected by methylation-specific quantitative PCR;~~

~~preferably, the methylation level is detected using the capture sequence of claim 3, or the primer pair of claim 4, or the probe of claim 5, or the methylation detection reagent of claim 6, or the kit of claim 7;~~

15. The method of claim 14, wherein, preferably, in step (1), detecting the methylation level of COL4A1 gene of the subject comprising the steps of:

step a) of extracting DNA of a specimen to be detected by adopting a magnetic bead capture method;

step b) of converting the DNA of the specimen to be detected by hydrosulphite, bisulfite or hydrazine salt; and

step c) of performing methylation-specific quantitative PCR detection;

16. The method of claim 15, wherein, preferably, in the step a), extracting the DNA of the specimen to be detected by adopting a magnetic bead capture method comprising the following steps of:

mixing and grinding a specimen to be detected in a protective liquid, followed by centrifuging, and taking supernatant;

centrifuging the supernatant, taking the supernatant, adding a lysis solution and magnetic beads with specific complementary oligonucleotide-capture sequences nucleotide acid fragments into the supernatant for incubation;

discarding part of the supernatant, washing off the magnetic beads and transferring to a clean centrifuge tube, adding washing liquid, performing incubation under 100-2000 rpm at room temperature for 0.5-5 min, placing on a magnetic frame and pipetting the supernatant, and repeating 3 times; and

and eluting the target gene DNA with a buffer.

~~11. The method of claim 10, wherein the detection criteria is as follows: interpreting a tumor specimen and a normal specimen according to a threshold value, wherein the threshold value of the Ct value in the fecal specimen is 32-42, if the Ct value of the fecal specimen is less than the threshold value of the Ct value, it is interpreted as a tumor specimen, and if the Ct value of the fecal specimen is greater than or equal to the threshold value of the Ct value, then it is interpreted as a normal specimen;~~

~~the threshold value of the methylation level value in the tissue specimen is 1-10, if the methylation level value of the tissue specimen is greater than the threshold value of the methylation level value, it is interpreted as a tumor specimen; if the methylation level value is less than or equal to the threshold value of the methylation level value, it is interpreted as a normal specimen.~~

~~12~~17. A tumor detection system, comprising the following components:

- (1) a methylation detection component of COL4A1 gene;
- (2) a data processing component; and
- (3) a result output ~~member~~ component;

wherein the methylation detection component further comprises the nucleic acid fragment of claim 2, or the primer pair of claim 1, or the probe of claim 3, or the methylation detection reagent of claim 4, or the kit of claim 5; and

the tumor is colorectal tumor.

18. The tumor detection system of claim 17, wherein preferably, the methylation detection component comprises one or more of a fluorescence quantitative PCR instrument, a PCR instrument and a sequencer;

preferably, the methylation detection component further comprises the capture sequence of claim 3, or the primer pair of claim 4, or the probe of claim 5, or the methylation detection reagent of claim 6, or the kit of claim 7;

19. The tumor detection system of claim 17, wherein preferably, the data processing component is configured to

- a. receive test data of a specimen to be tested and a normal control specimen;
- b. store test data of a specimen to be tested and a normal control specimen;
- c. compare test data of a specimen to be tested of the same type and a normal control specimen;

and

d. respond to the probability or possibility that a subject suffers from a tumor according to the comparison results;

20. The tumor detection system of claim 17, wherein preferably, the result output component is used for outputting the probability or possibility that a subject suffers from a tumor;

~~preferably, the judgment criteria of the data processing component is as follows: interpreting a tumor specimen and a normal specimen according to a threshold value;~~

~~wherein the threshold value of the Ct value in the fecal specimen is 32-42, if the Ct value of the fecal specimen is less than the threshold value of the Ct value, the fecal specimen is interpreted as a tumor specimen, and if the Ct value of the fecal specimen is greater than or equal to the threshold value of the Ct value, the fecal specimen is interpreted as a normal specimen;~~

~~the threshold value of the methylation level value in the tissue specimen is 1-10, if the methylation level value of the tissue specimen is greater than the threshold value of the methylation level value, it is interpreted as a tumor specimen; if the methylation level value is less than or equal to the threshold value of the methylation level value, it is interpreted as a normal specimen.~~

~~1321. The method of any one of claims 14 to 16-10-11, or the system of claim 12, wherein the tumor is colorectal tumor;~~

~~preferably, the tumor is colorectal cancer or adenoma.~~

~~1422. The method of any one of claims 14 to 16-10-11, or the system of claim 12, wherein the system or method detects the specimen type as tissue, body fluid or excreta;~~

23. The method of claim 22, wherein preferably, the tissue is intestinal tissue;

24. The method of claim 22, wherein preferably, the body fluid is blood, serum, plasma, extracellular fluid, tissue fluid, lymph fluid, cerebrospinal fluid or aqueous humor;

25. The method of claim 22, wherein preferably, the excreta is sputum, urine, saliva or feces.