

fragment thereof has a sequence of SEQ ID NO: 8, 9, 10 or 11. The target DNA sequence methylation specific probe for *BEND5* or a fragment thereof has a sequence with identity of at least 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% to SEQ ID NO: 12 or 13. In a further embodiment, the target DNA sequence methylation specific probe for *BEND5* or a fragment thereof has a sequence of SEQ ID NO: 12 or 13.

[0026] In some embodiments, the target DNA sequence methylation specific primer for *SMAD3* or a fragment thereof described herein has a sequence with identity of at least 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% to SEQ ID NO: 14 or 15. In some further embodiments, the target DNA sequence methylation specific primer for *SMAD3* or a fragment thereof has a sequence of SEQ ID NO: 14 or 15. The target DNA sequence methylation specific probe for *SMAD3* or a fragment thereof has a sequence with identity of at least 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% to SEQ ID NO: 16. In a further embodiment, the target DNA sequence methylation specific probe for *SMAD3*~~*SMAD6*~~ or a fragment thereof has a sequence of SEQ ID NO: 16.

[0027] In a further embodiment, determining the methylation status further comprises a step of measuring the specificity and sensitivity by a weighted sum score analysis. In some further embodiments, determining the methylation status of the combination of target *TMEM240*, *MROH6*, *BEND5* and *SMAD3* or a fragment thereof ~~or target *TMEM240*, *MROH6*, *BEND5* and *SMAD3* or a fragment thereof~~ has about 100% of sensitivity and about 100% specificity and about 100% of accuracy.

[0028] In a further embodiment, the method described herein further comprises a step of administering an anti-colorectal cancer agent to the subject.

[0029] The present disclosure provides a method for detecting a predisposition to colorectal cancer or predicting likelihood, treatment response, prognosis or recurrence of colorectal cancer in a subject, comprising (a) providing a biological sample from the subject, and (b) determining a methylation status of a target DNA sequence comprising *TMEM240* or a fragment thereof and *MROH6* or a fragment thereof in the biological sample; wherein the presence of hypermethylation or hypomethylation in the target DNA sequence of the subject is indicative of the predisposition to, likelihood, poor treatment response, poor prognosis or recurrence of, colorectal cancer.

[0030] In some embodiments of the disclosure, the method comprises determining the methylation status of *TMEM240* or a fragment thereof using a target DNA sequence methylation

1E: TMEM240 of TCGA samples; FIG. 1F: ~~MROH6~~~~MRTH6~~ of TCGA samples; FIG. 1G: BEND5 of TCGA samples; FIG. 1H: SMAD3 of TCGA samples).

[0041] Figures 2A to 2E shows the difference in early detection of the methylation status of epigenetic biomarkers of target genes in plasma samples of healthy subjects and colorectal cancer patients (FIG. 2A: TMEM240; FIG. 2B: MROH6; FIG. 2C: BEND5; FIG. 2D: SMAD3; FIG. 2E: the DNA methylation levels for TMEM240; MROH6 and BEND5).

[0042] Figure 3 shows the sum of DNA methylation levels (scores) of epigenetic biomarkers of target genes in plasma samples of healthy subjects and colorectal cancer patients.

[0043] Figure 4 shows Receiver Operating Characteristic (ROC) Curve Analysis exhibiting the early detection of the methylation status of epigenetic biomarkers of target genes in colorectal cancer patients and healthy subjects.

Detailed Description of the Invention

[0044] It is understood that this invention is not limited to the particular materials and methods described herein. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments and is not intended to limit the scope of the present invention, which will be limited only by the appended claims.

[0045] It must be noted that, as used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a biomarker" includes a mixture of two or more biomarkers, and the like.

[0046] The term "AUC" as used herein is an abbreviation for the area under a curve. In particular it refers to the area under a Receiver Operating Characteristic (ROC) curve. The ROC curve is a plot of the true positive rate against the false positive rate for the different possible cut points of a diagnostic test. It shows the trade-off between sensitivity and specificity depending on the selected cut point (any increase in sensitivity will be accompanied by a decrease in specificity). The area under an ROC curve (AUC) is a measure for the accuracy of a diagnostic test (the larger the area the better, optimum is 1, a random test would have a ROC curve lying on the diagonal with an area of 0.5; for reference: J. P. Egan. Signal Detection Theory and ROC Analysis, Academic Press, New York, 1975).

[0047] The term "a biological sample" refers to a sample of tissue, cells, or fluid isolated from a subject, including but not limited to, for example, blood, buffy coat, plasma, serum, blood cells

[00125] β value (T), methylation status of tumor tissues; β value(T) ≥ 0.5 will be calculated as hypermethylation biomarkers and β value(T) ≤ 0.25 will be calculated as hypomethylation biomarker.

[00126] Figure 1 shows the difference of methylation status (β value) of target nucleic acid and genes between tumor tissues and adjacent normal tissues (n=97). Darker color indicates tissues with higher methylation status according to Illumina Methylation 450K array-based data.

Example 2 Early Detection of Methylation Status of Epigenetic Biomarkers of Target Genes in Plasma Samples of Healthy Subjects and Colorectal Cancer Patients

[00127] The circulating cell-free DNA was extracted from plasma. Briefly, 3.5 mL of plasma was isolated immediately from 10 mL of peripheral blood. After circulating cell-free DNA (cfDNA) was extracted from plasma that obtained from colorectal cancer patients and healthy subjects, cfDNA was performed by bisulfite conversion. Probe-based methylation specific real-time PCR (qMSP) was used for cfDNA methylation analyses.

[00128] Data obtained from the qMSP assays was processed according to the following criteria. If the C_t value of qMSP was less than 45 cycles for circulating methylated *TMEM240* gene; less than 40 cycles for circulating methylated *MROH6* gene; less than 45 cycles for circulating methylated *BEND5*; higher than 45 cycles for circulating methylated *SMAD3*~~*SMAD6*~~, a score was defined as 1, respectively. Otherwise, a score was defined as 0.

[00129] The average of the total scores of qMSP in *TMEM240*, *MROH6*, and *BEND5* was higher than 0.2, the human subject was defined as CRC patient.

[00130] Figure 2 shows the difference in early detection of the methylation status of epigenetic biomarkers of target genes in plasma samples of healthy subjects and colorectal cancer patients. Figure 3 shows the sum of DNA methylation levels (scores) of epigenetic biomarkers of target genes in plasma samples of healthy subjects and colorectal cancer patients. In addition, Receiver Operating Characteristic (ROC) Curve Analysis as shown in Figure 4 indicates the early detection of the methylation status of epigenetic biomarkers of target genes in colorectal cancer patients and healthy subjects.

Claims

What is claimed is:

1. A method for detecting a predisposition to colorectal cancer or predicting likelihood, treatment response, prognosis or recurrence of colorectal cancer in a subject, comprising (a) providing a biological sample from the subject, and (b) determining the methylation status of a target DNA sequence comprising ~~*TMEM240* or a fragment thereof or *MROH6* or a fragment thereof~~ in the biological sample, wherein the presence of hypermethylation or hypomethylation in *MROH6* or a fragment thereof the target DNA sequence of the subject is indicative of the predisposition to, likelihood, poor treatment response, poor prognosis or recurrence of, colorectal cancer.

2. The method of claim 1, wherein the target DNA sequence further comprises one or more DNA sequences selected from the group consisting of *TMEM240* or a fragment thereof, *BEND5* or a fragment thereof and *SMAD3* or a fragment thereof, or any of combinations thereof; wherein the presence of hypermethylation in *TMEM240* or a fragment, hypermethylation in *BEND5* or a fragment, or hypomethylation in *SMAD3* or a fragment, of the subject is indicative of the predisposition to, likelihood, poor treatment response, poor prognosis or recurrence of, colorectal cancer.

3. The method of claim 1, wherein the biological sample is a tissue, cell, blood, urine, serum, plasma, stool, ascites, sputum, saliva, gastric juice, bile, or oral mucosa.

4. The method of claim 1, wherein the method further comprises a step of defining a score as 1 if the presence of hypermethylation or hypomethylation of each gene or a fragment thereof and, defining a score as 0 if the absence of hypermethylation or hypomethylation of each gene or a fragment thereof, and summing the score.

5. The method of claim 1, wherein the presence of hypermethylation or hypomethylation in the target DNA sequence of the subject is determined by comparing the methylation status of the target DNA sequence to a methylation status of a control DNA sequence.

6. The method of claim 1, wherein the methylation status is determined by a polymerase chain reaction.

7. The method of claim 1, wherein the hypermethylation is indicated when a C_t value of the polymerase chain reaction for determining the methylation status of *TMEM240* or a fragment thereof in the human subject is less than 50; or the hypermethylation is indicated when a C_t value

of the polymerase chain reaction for determining the methylation status of *MROH6* or a fragment thereof in the human subject is less than 45.

~~78. The method of claim 51, wherein the hypermethylation is indicated when a C_t value of the polymerase chain reaction for determining the methylation status of *TMEM240* or a fragment thereof in the human subject is less than 45; or the hypermethylation is indicated when a C_t value of the polymerase chain reaction for determining the methylation status of *MROH6* or a fragment thereof in the human subject is less than 40.~~

~~89. The method of claim 1, wherein a target DNA sequence methylation specific primer for *TMEM240* or a fragment thereof or *MROH6* or a fragment thereof is used in the methylation determination, wherein the target DNA sequence methylation specific primer for *TMEM240* or a fragment thereof has an identity of about 85% to a sequence selected from the group consisting of SEQ ID NOs: 1, 2, and 3; and the target DNA sequence methylation specific primer for *MROH6* or a fragment thereof has an identity of about 85% to a sequence selected from the group consisting of SEQ ID NOs: 5, and 6.~~

~~910. The method of claim 1, wherein a target DNA sequence methylation specific probe for *TMEM240* or a fragment thereof or *MROH6* or a fragment thereof is used in the methylation determination, wherein the target DNA sequence methylation specific probe for *TMEM240* or a fragment thereof has an identity of about 85% to a sequence of SEQ ID NO: 4; and the target DNA sequence methylation specific probe for *MROH6* or a fragment thereof has an identity of about 85% to a sequence of SEQ ID NO: 7.~~

~~10. The method of claim 1, wherein the target DNA sequence further comprises one or more DNA sequences selected from the group consisting of *BEND5* or a fragment thereof and *SMAD3* or a fragment thereof, or any of combinations thereof.~~

~~11. The method of claim 240, wherein the target DNA sequence comprises any of the following combinations of the DNA sequences: *TMEM240* or a fragment thereof and *MROH6* or a fragment thereof; *TMEM240* or a fragment thereof, *MROH6* or a fragment thereof and *BEND5* or a fragment thereof; *TMEM240* or a fragment thereof, *MROH6* or a fragment thereof, *BEND5* or a fragment thereof and *SMAD3* or a fragment thereof; *TMEM240* or a fragment thereof, *BEND5* or a fragment thereof and *SMAD3* or a fragment thereof; *TMEM240* or a fragment thereof, *MROH6* or a fragment thereof, and *SMAD3* or a fragment thereof; *MROH6* or a fragment thereof and~~

BEND5 or a fragment thereof; and *MROH6* or a fragment thereof, *BEND5* or a fragment thereof and *SMAD3* or a fragment thereof.

12. The method of claim ~~10 or 11~~2, wherein the methylation status is determined by a polymerase chain reaction, and the hypermethylation is indicated when a C_t value of the polymerase chain reaction for determining the methylation status of *TMEM240* or a fragment thereof in the human subject is less than 50; the hypermethylation is indicated when a C_t value of the polymerase chain reaction for determining the methylation status of *BEND5* or a fragment thereof in the human subject is less than 50; or the hypomethylation is indicated when a C_t value of the polymerase chain reaction for determining the methylation status of *SMAD3* or a fragment thereof in the human subject is higher than 40.

13. The method of claim ~~10 or 11~~2, wherein the methylation status is determined by a polymerase chain reaction, and the hypermethylation is indicated when a C_t value of the polymerase chain reaction for determining the methylation status of *TMEM240* or a fragment thereof in the human subject is less than 45; the hypermethylation is indicated when a C_t value of the polymerase chain reaction for determining the methylation status of *BEND5* or a fragment thereof in the human subject is less than 45; or the hypomethylation is indicated when a C_t value of the polymerase chain reaction for determining the methylation status of *SMAD3* or a fragment thereof in the human subject is higher than 45.

14. The method of claim ~~10 or 11~~2, wherein a target DNA sequence methylation specific primer for *TMEM240* or a fragment thereof, *BEND5* or a fragment thereof, or *SMAD3* or a fragment thereof or a combination thereof is used in the methylation determination, and wherein the target DNA sequence methylation specific primer for *TMEM240* or a fragment thereof has an identity of about 85% to a sequence selected from the group consisting of SEQ ID NOs: 1, 2, and 3; the target DNA sequence methylation specific primer for *BEND5* or a fragment thereof has an identity of about 85% to a sequence selected from the group consisting of SEQ ID NOs: 8 to 11; and the target DNA sequence methylation specific primer for *SMAD3* or a fragment thereof has an identity of about 85% to a sequence selected from the group consisting of SEQ ID NOs: 14 to 15.

15. The method of claim ~~10 or 11~~2, wherein a target DNA sequence methylation specific probe for *TMEM240* or fragment thereof, *BEND5* or a fragment thereof, or *SMAD3* or a fragment thereof or a combination thereof is used in the methylation determination, and wherein the target DNA sequence methylation specific probe for *TMEM240* or a fragment thereof has an identity of

about 85% to a sequence of SEQ ID NO: 4; the target DNA sequence methylation specific probe for *BEND5* or a fragment thereof has an identity of about 85% to a sequence selected from the group consisting of SEQ ID NOs: 12 to 13; and the target DNA sequence methylation specific probe for *SMAD3* or a fragment thereof has an identity of about 85% to a sequence of SEQ ID NO: 16.

16. The method of any one of claims 1 to 15, wherein determining the methylation status further comprises a step of measuring the specificity and sensitivity by a weighted sum score analysis.

17. An isolated nucleic acid molecule having a sequence selected from the group consisting of: SEQ ID Nos: 1 to 16.

18. A kit for detecting a predisposition to colorectal cancer or predicting likelihood, treatment response, prognosis or recurrence of colorectal cancer in a subject, which comprises an target DNA sequence methylation specific primer pair for detection of a methylation status of a target DNA sequence comprising ~~*TMEM240* or a fragment thereof or *MROH6* or a fragment thereof~~.

19. The kit of claim 18, further comprising a target DNA sequence methylation specific probe for detection of a methylation status of a target DNA sequence comprising ~~*TMEM240* or a fragment thereof or *MROH6* or a fragment thereof~~.

20. The kit of claim 18, wherein ~~the target DNA sequence methylation specific primer pair for *TMEM240* or a fragment thereof comprises SEQ ID NOs: 1 and 2 or 1 and 3; and the target DNA sequence methylation specific primer pair for *MROH6* or a fragment thereof comprises SEQ ID NOs: 5 and 6.~~

21. The kit of claim 19, wherein ~~the target DNA sequence methylation specific probe for *TMEM240* or a fragment thereof comprises SEQ ID NO: 4; and the target DNA sequence methylation specific probe for *MROH6* or a fragment thereof comprises SEQ ID NO: 7~~

22. The kit of any of claims 18 to 21, wherein the target DNA sequence further comprises *TMEM240* or a fragment thereof, *BEND5* or a fragment thereof, or *SMAD3* or a fragment thereof, and the kit further comprises one or more target DNA sequence methylation specific primer pairs for detection of a methylation status of *TMEM240* or a fragment thereof, *BEND5* or a fragment thereof, or *SMAD3* or a fragment thereof, or any combination thereof.

23. The kit of claim 22, wherein the target DNA sequence methylation specific primer pair for *TMEM240* or a fragment thereof comprises SEQ ID NOs: 1 and 2 or 1 and 3; the target DNA sequence methylation specific primer pair for *BEND5* or a fragment thereof comprises SEQ ID Nos. 8 and 9 or 10 and 11; the target DNA sequence methylation specific primer pair for *SMAD3* or a fragment thereof comprises SEQ ID Nos. 14 and 15.

24. The kit of claim 22, wherein the kit further comprises one or more target DNA sequence methylation specific probe for detection of a methylation status of *TMEM240* or a fragment thereof, *BEND5* or a fragment thereof, or *SMAD3* or a fragment thereof, or any combination thereof, and wherein the target DNA sequence methylation specific probe for *TMEM240* or a fragment thereof comprises SEQ ID NO: 4; the target DNA sequence methylation specific probe for *BEND5* or a fragment thereof comprises SEQ ID NO: 12 or 13; and the target DNA sequence methylation specific probe for *SMAD3*~~*SMAD6*~~ or a fragment thereof comprises SEQ ID NO: 16.