relevant to CRC and AA. Therefore, there remains a need to determine the exact location of the hypermethylated CpG sites of BMP3 and NDRG4 genes in the Asian population, and to design and optimize kits based on these methylated CpG sites, making the detection of AA more sensitive.

SUMMARY

[0018] The present disclosure provides DNA sequences comprising of hypermethylated CpG sites in the promoter region of BMP3 and NDRG4 genes.

[0019] The present disclosure also provides preferred primers and probes for detecting methylation of BMP3 or NDRG4 genes, and combinations thereof for detecting methylation of both BMP3 and NDRG4 genes.

[0020] The present disclosure further provides a kit for detecting CRC and AA of the Asian population. The DNA sequences comprising of hypermethylated CpG sites in the promoter region of BMP3 and NDRG4 genes can be used as markers for CRC and/or AA detection in Asian population.

[0021] Compared to other primers and probes, the pairs of preferred primers and probes for detecting methylation levels of BMP3 and/or NDRG4 genes have surprisingly higher sensitivity and specificity to detect tumor tissue, such as CRC and AA, and especially AA. In addition, the combinations of these preferred primers and probes of the present disclosure also achieve surprisingly higher sensitivity and specificity to detect tumor tissue, such as CRC and AA, and especially AA.

[0022] A kit for detecting CRC and AA of Asian population based on above preferred primer and probe combinations is also provided.

In one aspect, the present disclosure refers to a kit for detecting the presence or the absence of a colorectal cancer (CRC) cell or an advanced adenoma (AA) cell in a patient in need thereof, comprising: a) a first pair of primers and a first probe for detecting the methylation state or level of at least one CpG dinucleotide of the BMP3 gene in a biological sample obtained from the patient, wherein the first pair of primers and first probe each comprises a contiguous sequence of at least 16 nucleotides that is identical to, complementary to, or hybridizes under stringent hybridization conditions to a fragment of SEQ ID NO.: 1, b) a second pair of primers and a second probe for detecting the methylation state or level of at least one CpG dinucleotide of the NDRG4 gene in a biological sample obtained from the patient, wherein the second pair of primers and second probe each comprises a contiguous sequence of at least 16 nucleotides that is identical to, complementary to, or hybridizes under stringent hybridization conditions to a fragment of SEQ ID NO wherein the first pair of primers and the first probe are selected from the group consisting of:

i) a forward primer comprising SEQ ID NO.: 3, a reverse primer comprising SEQ ID NO.: 4, and a probe comprising SEQ ID NO.: 5;

ii) a forward primer comprising SEQ ID NO.: 9, a reverse primer comprising SEQ ID NO.: 10, and a probe comprising SEQ ID NO.: 11; and

iii) a forward primer comprising SEQ ID NO.: 15, a reverse primer comprising SEQ ID NO.: 16, and a probe comprising SEQ ID NO.: 17;

or wherein the second pair of primers and the second probe are selected from the group consisting of:

iv) a forward primer comprising SEQ ID NO.: 6, a reverse primer comprising SEQ ID NO.: 7, and a probe comprising SEQ ID NO.: 8;

v) a forward primer comprising SEQ ID NO.: 12, a reverse primer comprising SEQ ID NO.: 13, and a probe comprising SEQ ID NO.: 14; and

vi) a forward primer comprising SEQ ID NO.: 18, a reverse primer comprising SEQ ID NO.: 19, and a probe comprising SEQ ID NO.: 20. In another aspect, the present disclosure

refers to an *ex vivo* method for detecting the presence or absence of a colorectal cancer (CRC) cell or an advanced adenoma (AA) cell in a patient in need thereof, comprising: a) treating a genomic DNA obtained from a biological sample of the patient, or a fragment thereof, with one or more reagents to convert cytosine bases that are unmethylated thereof to uracil or another base that is detectably dissimilar to cytosine in terms of hybridization properties; b) contacting the treated genomic DNA, or the treated fragment thereof, with a first pair of primers for detecting the presence or absence of methylation sites of a gene encoding bone morphogenetic protein 3 (BMP3) in the patient, and a second pair of primers for detecting the presence or absence of methylation sites of a gene encoding NDRG family member 4 protein (NDRG4) in the patient, wherein the first pair of primers comprise a contiguous sequence of at least 9 nucleotides that is identical to, complementary to, or hybridizes under stringent hybridization conditions to a fragment of SEQ ID NO.: 1, and wherein the second pair of primers comprise a contiguous sequence of at least 9 nucleotides that is complementary to, or hybridizes under stringent hybridization conditions to a fragment of SEQ ID NO.: 2, wherein the treated genomic DNA or the fragment thereof is either amplified to produce at least one amplificate by the first pair of primers or the second pair of primers, or is not amplified; and c) determining the presence or absence of the CRC cell or the AA cell in the patient, based on a presence or absence of said amplificate, the methylation state or level of at least one CpG dinucleotide of the BMP3 gene and the NDRG4 gene in the patient using a first probe for detecting the presence or absence of methylation sites of BMP3 gene and a second probe for detecting the presence or absence of methylation sites of NDRG4 gene,

wherein the first pair of primers and the first probe are selected from the group consisting of:

i) a forward primer comprising SEQ ID NO.: 3, a reverse primer comprising SEQ ID NO.: 4, and a probe comprising SEQ ID NO.: 5;

ii) a forward primer comprising SEQ ID NO.: 9, a reverse primer comprising SEQ ID NO.: 10, and a probe comprising SEQ ID NO.: 11; and

iii) a forward primer comprising SEQ ID NO.: 15, a reverse primer comprising SEQ ID NO.: 16, and a probe comprising SEQ ID NO.: 17;

or wherein the second pair of primers and the second probe are selected from the group consisting of:

iv) a forward primer comprising SEQ ID NO.: 6, a reverse primer comprising SEQ ID NO.: 7, and a probe comprising SEQ ID NO.: 8;

v) a forward primer comprising SEQ ID NO.: 12, a reverse primer comprising SEQ ID NO.: 13, and a probe comprising SEQ ID NO.: 14; and

vi) a forward primer comprising SEQ ID NO.: 18, a reverse primer comprising SEQ ID NO.: 19, and a probe comprising SEQ ID NO.: 20.

In another aspect, the present disclosure refers to an *ex vivo* method for detecting the presence or absence of a colorectal cancer (CRC) cell or an advanced adenoma (AA) cell in a patient in need thereof, comprising using a kit as disclosed herein. In anther aspect, the present disclosure refers to an *ex vivo* method for detecting the presence or absence of a colorectal cancer (CRC) cell or an advanced adenoma (AA) cell in a patient in need thereof, comprising: a) treating a genomic DNA obtained from a fecal sample of the patient, or a fragment thereof, with one or more reagents to convert cytosine bases that are unmethylated thereof to uracil or another base that is detectably dissimilar to cytosine in terms of hybridization properties; b) performing a quantitative PCR (qPCR) using the treated genomic DNA of a) as a template, and determining the Ct value of BMP3 gene subtracted to that of a first reference gene in the patient as △Ct1; c) performing a qPCR using the treated genomic DNA of a) as a template, and determining the Ct value of NDRG4 gene subtracted to that of a first reference gene in the patient as △Ct2; d) performing a qPCR using the untreated genomic DNA as a template, and determining the Ct value of a mutant KRAS gene subtracted to that of a second reference gene in the patient as △Ct3; e) performing a fecal immunochemical test of hemoglobin protein in the fecal sample and determining a score as FIT; f) determining the value of K, wherein K = a*△Ct1+b*△Ct2+c*△Ct3+d*FIT+X, wherein a, b, c, d, X are clinical constants; and g) determining the value of a comprehensive index P, wherein $P = e^K/(1+e^K)$, wherein e is the natural constant, wherein when P is equal or more than a

predetermined threshold, the patient is determined to have a CRC cell and/or a AA cell, and when P is less than the threshold, the patient is determined to be free of a CRC cell and/or a AA cell, wherein the qPCR for amplifying BMP3 gene comprises a first pair of primers and a first probe selected from the group consisting of:

i) a forward primer comprising SEQ ID NO.: 3, a reverse primer comprising SEQ ID NO.: 4, and a probe comprising SEQ ID NO.: 5;

ii) a forward primer comprising SEQ ID NO.: 9, a reverse primer comprising SEQ ID NO.: 10, and a probe comprising SEQ ID NO.: 11; and

iii) a forward primer comprising SEQ ID NO.: 15, a reverse primer comprising SEQ ID NO.: 16, and a probe comprising SEQ ID NO.: 17;

or wherein the qPCR for amplifying NDRG4 gene comprises a second pair of primers and a second probe selected from the group consisting of:

iv) a forward primer comprising SEQ ID NO.: 6, a reverse primer comprising SEQ ID NO.: 7, and a probe comprising SEQ ID NO.: 8;

v) a forward primer comprising SEQ ID NO.: 12, a reverse primer comprising SEQ ID NO.: 13, and a probe comprising SEQ ID NO.: 14; and

vi) a forward primer comprising SEQ ID NO.: 18, a reverse primer comprising SEQ ID NO.: 19, and a probe comprising SEQ ID NO.: 20. In yet another aspect, the present disclosure refers to an *ex vivo* method for identifying the presence or absence of a colorectal cancer (CRC) cell and/or an advanced adenoma (AA) cell in a patient in need thereof, comprising determining the presence or absence of a CRC cell and/or an AA cell in the patient by using a method as disclosed herein. In another aspect, the present disclosure refers to an *ex vivo* method for selecting a patient in need thereof suitable for treatment of colorectal cancer (CRC) and/or advanced adenoma (AA), wherein the method comprises determining the presence or absence of a CRC cell and/or an AA cell in the patient by using a kit as disclosed herein. In yet another aspect, the present disclosure refers to an *ex vivo* method for selecting a patient in need thereof suitable for treatment of colorectal cancer (CRC) and/or advanced adenoma (AA), wherein the method comprises determining the presence or absence of a CRC cell and/or an AA cell in the patient by using a method as disclosed herein.

[0023] In some examples, the kit comprises: (1) a preferred combination of pairs of primer and probe and corresponding qPCR reagents；(2) primers and probes for detecting seven mutations () in a coding region of KRAS gene and corresponding qPCR reagents; (3) reagents for detecting hemoglobin in feces.

[0024] In some examples, results obtained from an assay using the kit are corrected and analyzed according to a logistic regression formula. In some examples, the formula is used to calculate a value for determining the presence or absence of CRC and/or AA. In some examples, the formula is $P=e^K/(1+e^K)$, where P is a comprehensive index, and K= a*△Ct1+b*△Ct2+c*△Ct3+d*FIT+X, wherein e is a natural constant, and a, b, c, d, X are clinical constant. In some examples, when the P value is equal or more than a predetermined threshold, the result indicates a positive detection of CRC and/or AA in the patient. In some examples, when the P value is less than the threshold, the result indicates a negative detection of CRC and/or AA in the patient, and the patient is determined to be healthy.

[0025] The present disclosure provides a kit for detecting the presence or the absence of colorectal cancer (CRC) or advanced adenoma (AA) in a patient in need thereof. A patient in need thereof is a patient suspected to have CRC and/or AA, such as a patient having at least one sign of developing CRC and/or AA, or a patient having a risk of developing CRC and/or AA, or a subject having a routine medical checkup but otherwise having no sign or risk.

CLAIMS

1. A kit for detecting the presence or the absence of a colorectal cancer (CRC) cell or an advanced adenoma (AA) cell in a patient in need thereof, comprising:

a) a first pair of primers and a first probe for detecting the methylation state or level of at least one CpG dinucleotide of the BMP3 gene in a biological sample obtained from the patient, wherein the first pair of primers and first probe each comprises a contiguous sequence of at least 16 nucleotides that is identical to, complementary to, or hybridizes under stringent hybridization conditions to a fragment of SEQ ID NO.: 1,

b) a second pair of primers and a second probe for detecting the methylation state or level of at least one CpG dinucleotide of the NDRG4 gene in a biological sample obtained from the patient, wherein the second pair of primers and second probe each comprises a contiguous sequence of at least 16 nucleotides that is identical to, complementary to, or hybridizes under stringent hybridization conditions to a fragment of SEQ ID NO.: 2,

wherein the first pair of primers and the first probe are selected from the group consisting of:

i) a forward primer comprising SEQ ID NO.: 3, a reverse primer comprising SEQ ID NO.: 4, and a probe comprising SEQ ID NO.: 5;

ii) a forward primer comprising SEQ ID NO.: 9, a reverse primer comprising SEQ ID NO.: 10, and a probe comprising SEQ ID NO.: 11; and

iii) a forward primer comprising SEQ ID NO.: 15, a reverse primer comprising SEQ ID NO.: 16, and a probe comprising SEQ ID NO.: 17;

or wherein the second pair of primers and the second probe are selected from the group consisting of:

iv) a forward primer comprising SEQ ID NO.: 6, a reverse primer comprising SEQ ID NO.: 7, and a probe comprising SEQ ID NO.: 8;

v) a forward primer comprising SEQ ID NO.: 12, a reverse primer comprising SEQ ID NO.: 13, and a probe comprising SEQ ID NO.: 14; and

vi) a forward primer comprising SEQ ID NO.: 18, a reverse primer comprising SEQ ID NO.: 19, and a probe comprising SEQ ID NO.: 20.

2. The kit of claim 1, comprising:

the first pair of primers and the first probe; and

the second pair of primers and the second probe.

3. The kit of claim 1, wherein the kit comprises:
i) a forward primer comprising SEQ ID NO.: 3, a reverse primer comprising SEQ ID NO.: 4, and a probe comprising SEQ ID NO.: 5, for detecting the methylation state or level of at least one CpG dinucleotide of the BMP3 gene in the biological sample obtained from the patient, and
ii) a forward primer comprising SEQ ID NO.: 6, a reverse primer comprising SEQ ID NO.: 7, and a probe comprising SEQ ID NO.: 8, for detecting the methylation state or level of at least one CpG dinucleotide of the NDRG4 gene in the biological sample obtained from the patient.

4. The kit of claim 1, wherein the kit comprises:
i) a forward primer comprising SEQ ID NO.: 9, a reverse primer comprising SEQ ID NO.: 10, and a probe comprising SEQ ID NO.: 11, for detecting the methylation state or level of at least one CpG dinucleotide of the BMP3 gene in the biological sample obtained from the patient, and
ii) a forward primer comprising SEQ ID NO.: 12, a reverse primer comprising SEQ ID NO.: 13, and a probe comprising SEQ ID NO.: 14, for detecting the methylation state or level of at least one CpG dinucleotide of the NDRG4 gene in the biological sample obtained from the patient.

5. The kit of claim 1, wherein the kit comprises:
i) a forward primer comprising SEQ ID NO.: 15, a reverse primer comprising SEQ ID NO.: 16, and a probe comprising SEQ ID NO.: 17, for detecting the methylation state or level of at least one CpG dinucleotide of the BMP3 gene in the biological sample obtained from the patient, and
ii) a forward primer comprising SEQ ID NO.: 18, a reverse primer comprising SEQ ID NO.: 19, and a probe comprising SEQ ID NO.: 20, for detecting the methylation state or level of at least one CpG dinucleotide of the NDRG4 gene in the biological sample obtained from the patient.

6. The kit of any one of claims 1 to 5, wherein both the first probe and the second probe comprise a fluorescent donor and an acceptor fluorophore.

7. The kit of any one of claims 1 to 6, wherein the kit further comprises:
(1) means for detecting the presence or absence of at least one mutation in the KRAS gene in the patient; and
(2) means for detecting the presence or absence of hemoglobin in a biological sample obtained from the patient.

8. The kit of claim 7, wherein the means for detecting the presence or absence of at least one mutation in the KRAS gene in the patient comprises at least one pair of primers capable of amplifying the Exon 12 and/or Exon 13 region of the KRAS gene in a polymerase chain reaction (PCR).

9. The kit of claim 7, wherein the means for detecting the presence or absence of hemoglobin in the biological sample comprises an anti-hemoglobin antibody.

10. The kit of claim 8, wherein the primers are capable of amplifying a KRAS gene region comprising at least one KRAS mutation selected from the group consisting G12D, G12V, G12C, G13D, G12A, G12R, G12S, and G13C.

11. The kit of claim 9, wherein the antibody is a colloidal gold-conjugated antibody.

12. The kit of any one of claims 1 to 11, wherein the kit further comprises means for amplifying a reference gene for quantification.

13. The kit of any one of claims 1 to 11, wherein the kit further comprises instructions for use and/or interpretation of a test result obtained by using the kit.

14. The kit of claim 9, wherein the kit further comprises means to detect a complex formed by the antibody and the hemoglobin in the biological sample.

15. The kit of any one of claims 1 to 14, wherein the biological sample obtained from the patient is a fecal sample.

16. The kit of any one of claims 1 to 15, wherein the kit further comprises a bisulfite reagent, and a container suitable for mixing the bisulfite reagent and the biological sample of the patient, or polynucleotides obtained from the biological sample.

17. The kit of any one of claims 1 to 16, wherein the kit further comprises a methylation sensitive restriction enzyme reagent.

18. The kit of any one of claims 1 to 17, wherein the kit further comprises

(1) a positive standard and a negative standard for detecting BMP3 methylation in the biological sample, and

(2) a positive standard and a negative standard for detecting NDRG4 methylation in the biological sample.

19. The kit of claim 18, wherein

the positive standard for detecting BMP3 methylation comprises a polynucleotide sequence of GTTAGTTTGGTCGGGTGTTTTTAAAAATAAAGCGAGGAGGGAAGGTATAGATAGATTTTG AAAATATTCGGGTTATATACGTCGCGATTTATAGTTTTTTTTTAGCGTTGGAGTGGAGACG GCGTTCGTAGCGTTTTGCGCGGGTGAGGTTCGCGTAGTTGTTGGGGAAGAGTTTATTTGT TAGGTTGCGTTGGGTTAGCGTAGTAAGTGGGGTTGGTCGTTATTTCGTTGTATTCGGTCGC GTTTCGGGTTTCGTGCGTTTTCGTTTTAG (SEQ ID NO: 67);

the negative standard for detecting BMP3 methylation comprises a polynucleotide sequence of GTTAGTTTGGTTGGGTGTTTTTAAAAATAAAGTGAGGAGGGAAGGTATAGATAGATTTTG AAAATATTTGGGTTATATATGTTGTGATTTATAGTTTTTTTTTAGTGTTGGAGTGGAGATGG TGTTTGTAGTGTTTTGTGTGGGTGAGGTTTGTGTAGTTGTTGGGGAAGAGTTTATTTGTTA GGTTGTGTTGGGTTAGTGTAGTAAGTGGGGTTGGTTGTTATTTTGTTGTATTTGGTTGTGT TTTGGGTTTTGTGTGTTTTTGTTTTAG (SEQ ID NO: 68);

the positive standard for detecting NDRG4 methylation comprises a polynucleotide sequence of TGAGAAGTCGGCGGGGGCGCGGATCGATCGGGGTGTTTTTTAGGTTTCGCGTCGCGGTTT TCGTTCGTTTTTTCGTTCGTTTATCGGGTATTTTAGTCGCGTAGAAGGCGGAAGTTACGCG CGAGGGATCGCGGTTCGTTCGGGATTAGTTTTAGGTTCGGTATCGTTTCGCGGGTCGAGC GTTTATATTCGTTAAATTTACGCGGGTACGTTTTCGCGGCGTATCGTTTTTAGTT (SEQ ID NO.: 69); and

the negative standard for detecting NDRG4 methylation comprises a polynucleotide sequence of TGAGAAGTTGGTGGGGGTGTGGATTGATTGGGGTGTTTTTTAGGTTTTGTGTTGTGGTTT TTGTTTGTTTTTTTGTTTGTTTATTGGGTATTTTAGTTGTGTAGAAGGTGGAAGTTATGTGT GAGGGATTGTGGTTTGTTTGGGATTAGTTTTAGGTTTGGTATTGTTTTGTGGGTTGAGTGT TTATATTTGTTAAATTTATGTGGGTATGTTTTTGTGGTGTATTGTTTTTAGTT (SEQ ID NO.: 70).

20. The kit of claim 12, wherein the means for amplifying an internal control gene comprises primers for amplifying a positive control gene and/or a negative control gene.

21. An *ex vivo* method for detecting the presence or absence of a colorectal cancer (CRC) cell or an advanced adenoma (AA) cell in a patient in need thereof, comprising:

a) treating a genomic DNA obtained from a biological sample of the patient, or a fragment thereof, with one or more reagents to convert cytosine bases that are unmethylated thereof to uracil or another base that is detectably dissimilar to cytosine in terms of hybridization properties;

b) contacting the treated genomic DNA, or the treated fragment thereof, with a first pair of primers for detecting the presence or absence of methylation sites of a gene encoding bone morphogenetic protein 3 (BMP3) in the patient, and a second pair of primers for detecting the presence or absence of methylation sites of a gene encoding NDRG family member 4 protein (NDRG4) in the patient, wherein the first pair of primers comprise a contiguous sequence of at least 9 nucleotides that is identical to, complementary to, or hybridizes under stringent hybridization conditions to a fragment of SEQ ID NO.: 1, and wherein the second pair of primers comprise a contiguous sequence of at least 9 nucleotides that is complementary to, or hybridizes under stringent hybridization conditions to a fragment of SEQ ID NO.: 2, wherein the treated genomic DNA or the fragment thereof is either amplified to produce at least one amplificate by the first pair of primers or the second pair of primers, or is not amplified; and

c) determining the presence or absence of the CRC cell or the AA cell in the patient, based on a presence or absence of said amplificate, the methylation state or level of at least one CpG dinucleotide of the BMP3 gene and the NDRG4 gene in the patient using a first probe for detecting the presence or absence of methylation sites of BMP3 gene and a second probe for detecting the presence or absence of methylation sites of NDRG4 gene,

wherein the first pair of primers and the first probe are selected from the group consisting of:

i) a forward primer comprising SEQ ID NO.: 3, a reverse primer comprising SEQ ID NO.: 4, and a probe comprising SEQ ID NO.: 5;

ii) a forward primer comprising SEQ ID NO.: 9, a reverse primer comprising SEQ ID NO.: 10, and a probe comprising SEQ ID NO.: 11; and

iii) a forward primer comprising SEQ ID NO.: 15, a reverse primer comprising SEQ ID NO.: 16, and a probe comprising SEQ ID NO.: 17;

or wherein the second pair of primers and the second probe are selected from the group consisting of:

iv) a forward primer comprising SEQ ID NO.: 6, a reverse primer comprising SEQ ID NO.: 7, and a probe comprising SEQ ID NO.: 8;

v) a forward primer comprising SEQ ID NO.: 12, a reverse primer comprising SEQ ID NO.: 13, and a probe comprising SEQ ID NO.: 14; and

vi) a forward primer comprising SEQ ID NO.: 18, a reverse primer comprising SEQ ID NO.: 19, and a probe comprising SEQ ID NO.: 20.

22. The method of claim 21, wherein the first pair of primers and the first probe are selected from the group consisting of:
i) a forward primer comprising SEQ ID NO.: 3, a reverse primer comprising SEQ ID NO.: 4, and a probe comprising SEQ ID NO.: 5;
ii) a forward primer comprising SEQ ID NO.: 9, a reverse primer comprising SEQ ID NO.: 10, and a probe comprising SEQ ID NO.: 11; and
iii) a forward primer comprising SEQ ID NO.: 15, a reverse primer comprising SEQ ID NO.: 16, and a probe comprising SEQ ID NO.: 17;
and,
wherein the second first pair of primers and the second probe are selected from the group consisting of:
iv) a forward primer comprising SEQ ID NO.: 6, a reverse primer comprising SEQ ID NO.: 7, and a probe comprising SEQ ID NO.: 8;
v) a forward primer comprising SEQ ID NO.: 12, a reverse primer comprising SEQ ID NO.: 13, and a probe comprising SEQ ID NO.: 14; and
vi) a forward primer comprising SEQ ID NO.: 18, a reverse primer comprising SEQ ID NO.: 19, and a probe comprising SEQ ID NO.: 20.

23. The method of claim 21, wherein the method comprises using
i) a forward primer comprising SEQ ID NO.: 3, a reverse primer comprising SEQ ID NO.: 4, and a probe comprising SEQ ID NO.: 5, for detecting the methylation state or level of at least one CpG dinucleotide of the BMP3 gene in the biological sample obtained from the patient, and
ii) a forward primer comprising SEQ ID NO.: 6, a reverse primer comprising SEQ ID NO.: 7, and a probe comprising SEQ ID NO.: 8, for detecting the methylation state or level of at least one CpG dinucleotide of the NDRG4 gene in the biological sample obtained from the patient.

24. The method of claim 21, wherein the method comprises using
i) a forward primer comprising SEQ ID NO.: 9, a reverse primer comprising SEQ ID NO.: 10, and a probe comprising SEQ ID NO.: 11, for detecting the methylation state or level of at least one CpG dinucleotide of the BMP3 gene in the biological sample obtained from the patient, and

ii) a forward primer comprising SEQ ID NO.: 12, a reverse primer comprising SEQ ID NO.: 13, and a probe comprising SEQ ID NO.: 14, for detecting the methylation state or level of at least one CpG dinucleotide of the NDRG4 gene in the biological sample obtained from the patient.

25. The method of claim 21, wherein the method comprises using
i) a forward primer comprising SEQ ID NO.: 15, a reverse primer comprising SEQ ID NO.: 16, and a probe comprising SEQ ID NO.: 17, for detecting the methylation state or level of at least one CpG dinucleotide of the BMP3 gene in the biological sample obtained from the patient, and
ii) a forward primer comprising SEQ ID NO.: 18, a reverse primer comprising SEQ ID NO.: 19, and a probe comprising SEQ ID NO.: 20, for detecting the methylation state or level of at least one CpG dinucleotide of the NDRG4 gene in the biological sample obtained from the patient.

26. The method of any one of claims 21 to 25, wherein both the first probe and the second probe comprise a fluorescent donor and an acceptor fluorophore.

27. The method of any one of claims 21 to 26, wherein the method further comprises a step of detecting the presence or absence of at least one mutation in the KRAS gene in a biological sample obtained from the patient, and a step of detecting the presence or absence of hemoglobin in a biological sample obtained from the patient.

28. The method of any one of claim 27, wherein the step of detecting the presence or absence of at least one mutation in the KRAS gene in the patient comprises using at least one pair of primers capable of amplifying the Exon 12 and/or Exon 13 region of the KRAS gene in a polymerase chain reaction (PCR).

29. The method of any one of claim 27, wherein the step of detecting the presence or absence of hemoglobin in the biological sample comprises using an anti-hemoglobin antibody.

30. The method of claim 28, wherein the primers are capable of amplifying a KRAS gene region comprising at least one KRAS mutation selected from the group consisting G12D, G12V, G12C, G13D, G12A, G12R, G12S, and G13C.

31. The method of claim 29, wherein the antibody is a colloidal gold-conjugated antibody.

32. The method of any one of claims 21 to 31, wherein the amplification of BMP3 gene is performed in a quantitative PCR (qPCR), and the method further comprises amplifying a first reference gene to determining the Ct value of the BMP3 amplification as ΔCt1.

33. The method of any one of claims 21 to 31, wherein the amplification of NDRG4 gene is performed in a quantitative PCR (qPCR), and the method further comprises amplifying a second reference gene to determining the Ct value of the NDRG4 amplification as ΔCt2.

34. The method of claim 28 or 30, wherein the amplification of mutant KRAS gene is performed in a quantitative PCR (qPCR), and the method further comprises amplifying a third reference gene to determining the Ct value of the mutant KRAS amplification as ΔCt3.

35. The method of claim 32 or 33, wherein the first and the second reference genes are the same.

36. The method of claim 35, wherein the same reference gene is a B2M gene.

37. The method of claim 34, wherein the mutant KRAS gene comprises a mutation selected from the group consisting of G12D, G13D, G12V, G12C, G12S, G12A, and G13R.

38. The method of claim 37, wherein the mutant KRAS gene is amplified by one or more pairs of primers selected from the group consisting of:
(1) a forward primer G12D-F comprising SEQ ID NO.: 35, and a reverse primer Kras-R comprising SEQ ID NO.: 42;
(2) a forward primer G13D-F comprising SEQ ID NO.: 36, and a reverse primer Kras-R comprising SEQ ID NO.: 42;
(3) a forward primer G12V-F comprising SEQ ID NO.: 37, and a reverse primer Kras-R comprising SEQ ID NO.: 42;
(4) a forward primer G12C-F comprising SEQ ID NO.: 38, and a reverse primer Kras-R comprising SEQ ID NO.: 42;
(5) a forward primer G12S-F comprising SEQ ID NO.: 39, and a reverse primer Kras-R comprising SEQ ID NO.: 42;
(6) a forward primer G12A-F comprising SEQ ID NO.: 40, and a reverse primer Kras-R comprising SEQ ID NO.: 42; and

(7) a forward primer G12R-F comprising SEQ ID NO.: 41, and a reverse primer Kras-R comprising SEQ ID NO.: 42,
and wherein the KRAS probe for the qPCR comprises SEQ ID NO.: 45.

39. The method of claim 34, wherein the third reference gene is an ACTB gene.

40. The method of claim 39, wherein qPCR primers for amplifying ACTB gene comprise SEQ ID NOs.: 43 and 44, and the probe comprises SEQ ID NO.: 46.

41. The method of claims 21 to 40, wherein the method comprises using
(1) a positive standard and a negative standard for detecting BMP3 methylation in the sample, and
(2) a positive standard and a negative standard for detecting NDRG4 methylation in the sample.

42. The method of claim 41, wherein
the positive standard for detecting BMP3 methylation comprises a polynucleotide sequence of GTTAGTTTGGTCGGGTGTTTTTAAAAATAAAGCGAGGAGGGAAGGTATAGATAGATTTTG AAAATATTCGGGTTATATACGTCGCGATTTATAGTTTTTTTTTAGCGTTGGAGTGGAGACG GCGTTCGTAGCGTTTTGCGCGGGTGAGGTTCGCGTAGTTGTTGGGGAAGAGTTTATTTGT TAGGTTGCGTTGGGTTAGCGTAGTAAGTGGGGTTGGTCGTTATTTCGTTGTATTCGGTCGC GTTTCGGGTTTCGTGCGTTTTCGTTTTAG (SEQ ID NO: 67);
the negative standard for detecting BMP3 methylation comprises a polynucleotide sequence of GTTAGTTTGGTTGGGTGTTTTTAAAAATAAAGTGAGGAGGGAAGGTATAGATAGATTTTG AAAATATTTGGGTTATATATGTTGTGATTTATAGTTTTTTTTTAGTGTTGGAGTGGAGATGG TGTTTGTAGTGTTTTGTGTGGGTGAGGTTTGTGTAGTTGTTGGGGAAGAGTTTATTTGTTA GGTTGTGTTGGGTTAGTGTAGTAAGTGGGGTTGGTTGTTATTTTGTTGTATTTGGTTGTGT TTTGGGTTTTGTGTGTTTTTGTTTTAG (SEQ ID NO: 68);
the positive standard for detecting NDRG4 methylation comprises a polynucleotide sequence of TGAGAAGTCGGCGGGGGCGCGGATCGATCGGGGTGTTTTTTAGGTTTCGCGTCGCGGTTT TCGTTCGTTTTTTCGTTCGTTTATCGGGTATTTTAGTCGCGTAGAAGGCGGAAGTTACGCG CGAGGGATCGCGGTTCGTTCGGGATTAGTTTTAGGTTCGGTATCGTTTCGCGGGTCGAGC GTTTATATTCGTTAAATTTACGCGGGTACGTTTTCGCGGCGTATCGTTTTTAGTT (SEQ ID NO.: 69); and
the negative standard for detecting NDRG4 methylation comprises a polynucleotide sequence of

TGAGAAGTTGGTGGGGGTGTGGATTGATTGGGGTGTTTTTTAGGTTTTGTGTTGTGGTTT TTGTTTGTTTTTTTGTTTGTTTATTGGGTATTTTAGTTGTGTAGAAGGTGGAAGTTATGTGT GAGGGATTGTGGTTTGTTTGGGATTAGTTTTAGGTTTGGTATTGTTTTGTGGGTTGAGTGT TTATATTTGTTAAATTTATGTGGGTATGTTTTTGTGGTGTATTGTTTTTAGTT (SEQ ID NO.: 70).

43. The method of any one of claims 21 to 42, wherein the method comprises amplifying a quality control standard.

44. An *ex vivo* method for detecting the presence or absence of a colorectal cancer (CRC) cell or an advanced adenoma (AA) cell in a patient in need thereof, comprising using a kit of any one of claims 1 to 20.

45. An *ex vivo* method for detecting the presence or absence of a colorectal cancer (CRC) cell or an advanced adenoma (AA) cell in a patient in need thereof, comprising:

a) treating a genomic DNA obtained from a fecal sample of the patient, or a fragment thereof, with one or more reagents to convert cytosine bases that are unmethylated thereof to uracil or another base that is detectably dissimilar to cytosine in terms of hybridization properties;

b) performing a quantitative PCR (qPCR) using the treated genomic DNA of a) as a template, and determining the Ct value of BMP3 gene subtracted to that of a first reference gene in the patient as $\triangle$Ct1;

c) performing a qPCR using the treated genomic DNA of a) as a template, and determining the Ct value of NDRG4 gene subtracted to that of the first reference gene in the patient as $\triangle$Ct2;

d) performing a qPCR using the untreated genomic DNA as a template, and determining the Ct value of a mutant KRAS gene subtracted to that of a second reference gene in the patient as $\triangle$Ct3;

e) performing a fecal immunochemical test of hemoglobin protein in the fecal sample and determining a score as FIT;

f) determining the value of K, wherein $K = a*\triangle Ct1+b*\triangle Ct2+c*\triangle Ct3+d*FIT+X$, wherein a, b, c, d, X are clinical constants; and

g) determining the value of a comprehensive index P, wherein $P = e^K/(1+e^K)$, wherein e is the natural constant, wherein when P is equal or more than a predetermined threshold, the patient is determined to have a CRC cell and/or a AA cell, and when P is less than the threshold, the patient is determined to be free of a CRC cell and/or a AA cell, wherein the qPCR for amplifying BMP3 gene comprises a

first pair of primers and a first probe selected from the group consisting of:

i) a forward primer comprising SEQ ID NO.: 3, a reverse primer comprising SEQ ID NO.: 4, and a probe comprising SEQ ID NO.: 5;

ii) a forward primer comprising SEQ ID NO.: 9, a reverse primer comprising SEQ ID NO.: 10, and a probe comprising SEQ ID NO.: 11; and

iii) a forward primer comprising SEQ ID NO.: 15, a reverse primer comprising SEQ ID NO.: 16, and a probe comprising SEQ ID NO.: 17;

or wherein the qPCR for amplifying NDRG4 gene comprises a second pair of primers and a second probe selected from the group consisting of:

iv) a forward primer comprising SEQ ID NO.: 6, a reverse primer comprising SEQ ID NO.: 7, and a probe comprising SEQ ID NO.: 8;

v) a forward primer comprising SEQ ID NO.: 12, a reverse primer comprising SEQ ID NO.: 13, and a probe comprising SEQ ID NO.: 14; and

vi) a forward primer comprising SEQ ID NO.: 18, a reverse primer comprising SEQ ID NO.: 19, and a probe comprising SEQ ID NO.: 20.

46. The method of claim 45, wherein the qPCR for amplifying BMP3 gene comprises the first pair of primers and the first probe, and wherein the qPCR for amplifying NDRG4 gene comprises the second pair of primers and the second probe.

47. The method of claim 45, wherein the method comprises using

i) a forward primer comprising SEQ ID NO.: 3, a reverse primer comprising SEQ ID NO.: 4, and a probe comprising SEQ ID NO.: 5, for detecting the methylation state or level of at least one CpG dinucleotide of the BMP3 gene in the sample, and

ii) a forward primer comprising SEQ ID NO.: 6, a reverse primer comprising SEQ ID NO.: 7, and a probe comprising SEQ ID NO.: 8, for detecting the methylation state or level of at least one CpG dinucleotide of the NDRG4 gene in the sample.

48. The method of claim 45, wherein the method comprises using

i) a forward primer comprising SEQ ID NO.: 9, a reverse primer comprising SEQ ID NO.: 10, and a probe comprising SEQ ID NO.: 11, for detecting the methylation state or level of at least one CpG dinucleotide of the BMP3 gene in the sample, and

ii) a forward primer comprising SEQ ID NO.: 12, a reverse primer comprising SEQ ID NO.: 13, and a probe comprising SEQ ID NO.: 14, for detecting the methylation state or level of at least one CpG dinucleotide of the NDRG4 gene in the sample.

49. The method of claim 45, wherein the method comprises using
i) a forward primer comprising SEQ ID NO.: 15, a reverse primer comprising SEQ ID NO.: 16, and a probe comprising SEQ ID NO.: 17, for detecting the methylation state or level of at least one CpG dinucleotide of the BMP3 gene in the sample, and
ii) a forward primer comprising SEQ ID NO.: 18, a reverse primer comprising SEQ ID NO.: 19, and a probe comprising SEQ ID NO.: 20, for detecting the methylation state or level of at least one CpG dinucleotide of the NDRG4 gene in the sample.

50. The method of any one of claims 45 to 49, wherein both the first probe and the second probe comprise a fluorescent donor and an acceptor fluorophore.

51. The method of any one of claims 45 to 50, wherein the mutant KRAS gene comprising at least one KRAS mutation selected from the group consisting G12D, G12V, G12C, G13D, G12A, G12R, G12S, and G13C.

52. The method of any one of claims 45 to 51, wherein the fecal immunochemical test comprises a colloidal gold-conjugated antibody.

53. the method of any one of claims 45 to 52, wherein step b) and step c) comprises using B2M gene as the first reference gene.

54. The method of claim 51, wherein the mutant KRAS gene is amplified by one or more pairs of primers selected from the group consisting of:
(1) a forward primer G12D-F comprising SEQ ID NO.: 35, and a reverse primer Kras-R comprising SEQ ID NO.: 42;
(2) a forward primer G13D-F comprising SEQ ID NO.: 36, and a reverse primer Kras-R comprising SEQ ID NO.: 42;
(3) a forward primer G12V-F comprising SEQ ID NO.: 37, and a reverse primer Kras-R comprising SEQ ID NO.: 42;

(4) a forward primer G12C-F comprising SEQ ID NO.: 38, and a reverse primer Kras-R comprising SEQ ID NO.: 42;
(5) a forward primer G12S-F comprising SEQ ID NO.: 39, and a reverse primer Kras-R comprising SEQ ID NO.: 42;
(6) a forward primer G12A-F comprising SEQ ID NO.: 40, and a reverse primer Kras-R comprising SEQ ID NO.: 42; and
(7) a forward primer G12R-F comprising SEQ ID NO.: 41, and a reverse primer Kras-R comprising SEQ ID NO.: 42,
and wherein the KRAS probe for the qPCR comprises SEQ ID NO.: 45.

55. The method of claim 54, wherein ACTB gene is used as the second reference gene in the qPCR for amplifying the mutant KRAS gene.

56. The method of claim 55, wherein the qPCR primers for amplifying ACTB gene comprise SEQ ID NOs.: 43 and 44, and the qPCR probe for ACTB gene comprises SEQ ID NO.: 46.

57. The method of claims 45 to 56, wherein the method comprises using
(1) a positive standard and a negative standard for detecting BMP3 methylation in the sample, and
(2) a positive standard and a negative standard for detecting NDRG4 methylation in the sample.

58. The method of claim 57, wherein
the positive standard for detecting BMP3 methylation comprises a polynucleotide sequence of GTTAGTTTGGTCGGGTGTTTTTAAAAATAAAGCGAGGAGGGAAGGTATAGATAGATTTTG AAAATATTCGGGTTATATACGTCGCGATTTATAGTTTTTTTTTAGCGTTGGAGTGGAGACG GCGTTCGTAGCGTTTTGCGCGGGTGAGGTTCGCGTAGTTGTTGGGGAAGAGTTTATTTGT TAGGTTGCGTTGGGTTAGCGTAGTAAGTGGGGTTGGTCGTTATTTCGTTGTATTCGGTCGC GTTTCGGGTTTCGTGCGTTTTCGTTTTAG (SEQ ID NO: 67);
the negative standard for detecting BMP3 methylation comprises a polynucleotide sequence of GTTAGTTTGGTTGGGTGTTTTTAAAAATAAAGTGAGGAGGGAAGGTATAGATAGATTTTG AAAATATTTGGGTTATATATGTTGTGATTTATAGTTTTTTTTTAGTGTTGGAGTGGAGATGG TGTTTGTAGTGTTTTGTGTGGGTGAGGTTTGTGTAGTTGTTGGGGAAGAGTTTATTTGTTA GGTTGTGTTGGGTTAGTGTAGTAAGTGGGGTTGGTTGTTATTTTGTTGTATTTGGTTGTGT TTTGGGTTTTGTGTGTTTTTGTTTTAG (SEQ ID NO: 68);
the positive standard for detecting NDRG4 methylation comprises a polynucleotide sequence of

TGAGAAGTCGGCGGGGGCGCGGATCGATCGGGGTGTTTTTTAGGTTTCGCGTCGCGGTTT
TCGTTCGTTTTTTCGTTCGTTTATCGGGTATTTTAGTCGCGTAGAAGGCGGAAGTTACGCG
CGAGGGATCGCGGTTCGTTCGGGATTAGTTTTAGGTTCGGTATCGTTTCGCGGGTCGAGC
GTTTATATTCGTTAAATTTACGCGGGTACGTTTTCGCGGCGTATCGTTTTTAGTT (SEQ ID
NO.: 69); and
the negative standard for detecting NDRG4 methylation comprises a polynucleotide sequence of
TGAGAAGTTGGTGGGGGTGTGGATTGATTGGGGTGTTTTTTAGGTTTTGTGTTGTGGTTT
TTGTTTGTTTTTTTGTTTGTTTATTGGGTATTTTAGTTGTGTAGAAGGTGGAAGTTATGTGT
GAGGGATTGTGGTTTGTTTGGGATTAGTTTTAGGTTTGGTATTGTTTTGTGGGTTGAGTGT
TTATATTTGTTAAATTTATGTGGGTATGTTTTTGTGGTGTATTGTTTTTAGTT (SEQ ID NO.:
70).

59. The method of any one of claims 45 to 58, wherein the method comprises amplifying a quality control standard in the step b) and the step c).

60. An *ex vivo* method for identifying the presence or absence of a colorectal cancer (CRC) cell and/or an advanced adenoma (AA) cell in a patient in need thereof, comprising determining the presence or absence of a CRC cell and/or an AA cell in the patient by using a method of any one of claims 21 to 59.

61. An *ex vivo* method for selecting a patient in need thereof suitable for treatment of colorectal cancer (CRC) and/or advanced adenoma (AA), wherein the method comprises determining the presence or absence of a CRC cell and/or an AA cell in the patient by using a kit of any one of claims 1 to 20.

62. An *ex vivo* method for selecting a patient in need thereof suitable for treatment of colorectal cancer (CRC) and/or advanced adenoma (AA), wherein the method comprises determining the presence or absence of a CRC cell and/or an AA cell in the patient by using a method of any one of claims 21 to 59.