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 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 SEQ ID NO.: 2. 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 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 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. 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 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 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 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. 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.

[0026] In some examples, the kit comprises 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. In some examples, each of the first pair of primers and first probe comprises a contiguous sequence of at least 16 nucleotides that is identical to, complementary to, or hybridizes under stringent hybridization conditions to SEQ ID NO.: 1,

[0027] In some examples, the kit comprises 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. In some examples, each of the second pair of primers and second probe comprises a contiguous sequence of at least 16 nucleotides that is identical to, complementary to, or hybridizes under stringent hybridization conditions to SEQ ID NO.: 2,

[0028] In some examples, the first pair of primers and the first probe are selected from the group consisting of:

[0029] 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;

[0030] 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

[0031] 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;

[0032] In some examples, wherein the second first pair of primers and the second probe are selected from the group consisting of:

[0033] 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;

[0034] 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

[0035] 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;

[0036] In some examples, 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.

[0037] In some examples, 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.

[0038] In some examples, 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.

[0039] In some examples, both the first probe and the second probe comprise a fluorescent donor and an acceptor fluorophore.

[0040] In some examples, the first probe and the second probe are TAQMAN® probes.

[0041] In some examples, 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.

[0042] In some examples, 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).

[0043] In some examples, the means for detecting the presence or absence of hemoglobin in the biological sample comprises an anti-hemoglobin antibody.

[0044] In some examples, 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.

[0045] In some examples, the antibody is a colloidal gold-conjugated antibody.

In some examples, the kit further comprises means for amplifying an internal quality control gene. An internal control can detect (1) inhibition contamination from sample or extraction method, (2) detect instrument malfunction, (3) chemistry failures (e.g., expired or degraded kit or components, or false combination of reagents), and (4) human error. In some examples, the internal control gene is a positive control, such as a gene in a positive control sample that has been determined to have methylation. In some examples, the internal control gene is a negative control, such as a gene in a negative control sample that has been determined not to have methylation.

[0046] In some examples, the kit further comprises instructions for use and/or interpretation of a test result obtained by using the kit.

[0047] In some examples, the kit further comprises means to detect a complex formed by the antibody and the hemoglobin in the biological sample.

[0048] In some examples, the biological sample obtained from the patient is a fecal sample.

[0049] In some examples, 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.

[0050] In some examples, instead of using bisulfite, the kit further comprises a methylation sensitive restriction enzyme reagent.

[0051] In some examples, 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.

[0052] In some examples, the positive standard for detecting BMP3 methylation comprises a polynucleotide sequence of:

GTTAGTTTGGTCGGGTGTTTTTAAAAATAAAGCGAGGAGGGAAGGTATAGATAGATTTT
GAAAATATTCGGGTTATATACGTCGCGATTTATAGTTTTTTTTTAGCGTTGGAGTGGAGA
CGGCGTTCGTAGCGTTTTGCGCGGGTGAGGTTCGCGTAGTTGTTGGGGAAGAGTTTATT

TGTTAGGTTGCGTTGGGTTAGCGTAGTAAGTGGGGTTGGTCGTTATTTCGTTGTATTCGG
TCGCGTTTCGGGTTTCGTGCGTTTTCGTTTTAG (SEQ ID NO: 67);

[0053] In some examples, the negative standard for detecting BMP3 methylation comprises a polynucleotide sequence of:

GTTAGTTTGGTTGGGTGTTTTTAAAAATAAAGTGAGGAGGGAAGGTATAGATAGATTTT
GAAAATATTTGGGTTATATATGTTGTGATTTATAGTTTTTTTTTAGTGTTGGAGTGGAGA
TGGTGTTTGTAGTGTTTTGTGTGGGTGAGGTTTGTGTAGTTGTTGGGGAAGAGTTTATTT
GTTAGGTTGTGTTGGGTTAGTGTAGTAAGTGGGGTTGGTTGTTATTTTGTTGTATTTGGT
TGTGTTTTGGGTTTTGTGTGTTTTTGTTTTAG (SEQ ID NO: 68);

[0054] In some examples, the positive standard for detecting NDRG4 methylation comprises a polynucleotide sequence of:

TGAGAAGTCGGCGGGGGCGCGGATCGATCGGGGTGTTTTTTAGGTTTCGCGTCGCGGTT
TTCGTTCGTTTTTTCGTTCGTTTATCGGGTATTTTAGTCGCGTAGAAGGCGGAAGTTACG
CGCGAGGGATCGCGGTTCGTTCGGGATTAGTTTTAGGTTCGGTATCGTTTCGCGGGTCG
AGCGTTTATATTCGTTAAATTTACGCGGGTACGTTTTCGCGGCGTATCGTTTTTAGTT
(SEQ ID NO.: 69).

[0055] In some examples, the negative standard for detecting NDRG4 methylation comprises a polynucleotide sequence of:

TGAGAAGTTGGTGGGGGTGTGGATTGATTGGGGTGTTTTTTAGGTTTTGTGTTGTGGTTT
TTGTTTGTTTTTTTGTTTGTTTATTGGGTATTTTAGTTGTGTAGAAGGTGGAAGTTATGTG
TGAGGGATTGTGGTTTGTTTGGGATTAGTTTTAGGTTTGGTATTGTTTTGTGGGTTGAGT
GTTTATATTTGTTAAATTTATGTGGGTATGTTTTTGTGGTGTATTGTTTTTAGTT (SEQ ID NO.: 70).

[0056] Also provided is a method for detecting the presence or absence of colorectal cancer (CRC) or advanced adenoma (AA) in a patient in need thereof.

[0057] In some examples, the method comprises a) obtaining genomic DNA from a biological sample of the patient.

[0058] In some examples, the method further comprises b) treating the genomic DNA of a), 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.

[0059] In some examples, the method further comprises c) 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. In some examples, the method further comprises contacting the treated genomic DNA, or a fragment thereof, with 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.

[0060] In some examples, 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 SEQ ID NO.: 1. In some examples, 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 SEQ ID NO.: 2.

[0061] In some examples, 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.

[0062] In some examples, the method further comprises d) determining the presence or absence of CRC or AA 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.

[0063] In some examples, a quantitative PCR is used to amplify the methylated BMP3 gene in the sample. In some examples, a quantitative PCR is used to amplify the methylated NDRG4 gene in the sample.

[0064] In some examples, the method also comprises using primers for amplifying a reference gene (a.k.a., normalizer, housekeeping gene, or endogenous control). In some examples, a quantitative PCR is used to amplify the reference gene in the sample.

[0065] In some examples, 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.

[0066] In some examples, 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.

[0067] In some examples, 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.

[0068] In some examples, 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.

[0069] In some examples, 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.

[0070] In some examples, both the first probe and the second probe comprise a fluorescent donor and an acceptor fluorophore. In some examples, the first probe and the second probe are TAQMAN® probes.

[0071] In some examples, 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.

[0072] In some examples, the method further comprises a step of detecting the presence or absence of hemoglobin in a biological sample obtained from the patient. In some examples, the step of detecting the presence or absence of hemoglobin in the biological sample comprises using an anti-hemoglobin antibody. In some examples, the antibody is a colloidal gold-conjugated antibody.

[0073] In some examples, 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). In some examples, 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.

[0074] In some examples, 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.

[0075] In some examples, the KRAS probe for the qPCR comprises SEQ ID NO.: 46.

[0076] In some examples, the amplification of BMP3 gene is performed in a quantitative PCR (qPCR), and the method further comprises amplifying a first reference gene (i.e., a first reference gene) to determining the Ct value of the BMP3 amplification as ΔCt1.

[0077] In some examples, the amplification of NDRG4 gene is performed in a quantitative PCR (qPCR), and the method further comprises amplifying a second reference gene (i.e., a second reference gene) to determining the Ct value of the NDRG4 amplification as ΔCt2.

[0078] In some examples, the amplification of mutant KRAS gene is performed in a quantitative PCR (qPCR), and the method further comprises amplifying a third reference gene (i.e., a third reference gene) to determining the Ct value of the mutant KRAS amplification as ΔCt3.

[0079] In some examples, the first and the second reference genes are the same. In some examples, the same reference gene is a B2M gene.

[0080] In some examples, the third reference gene is an ACTB gene. In some examples, qPCR primers for amplifying ACTB gene comprise SEQ ID NOs.: 43 and 44, and the probe comprise SEQ ID NO.: 46.

[0081] In some examples, 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.

[0082] In some examples, the positive standard for detecting BMP3 methylation comprises a polynucleotide sequence of:

GTTAGTTTGGTCGGGTGTTTTTAAAAATAAAGCGAGGAGGGAAGGTATAGATAGATTTT
GAAAATATTCGGGTTATATACGTCGCGATTTATAGTTTTTTTTTAGCGTTGGAGTGGAGA
CGGCGTTCGTAGCGTTTTGCGCGGGTGAGGTTCGCGTAGTTGTTGGGGAAGAGTTTATT
TGTTAGGTTGCGTTGGGTTAGCGTAGTAAGTGGGGTTGGTCGTTATTTCGTTGTATTCGG
TCGCGTTTCGGGTTTCGTGCGTTTTCGTTTTAG (SEQ ID NO: 67);

[0083] In some examples, the negative standard for detecting BMP3 methylation comprises a polynucleotide sequence of

GTTAGTTTGGTTGGGTGTTTTTAAAAATAAAGTGAGGAGGGAAGGTATAGATAGATTTT
GAAAATATTTGGGTTATATATGTTGTGATTTATAGTTTTTTTTTAGTGTTGGAGTGGAGA
TGGTGTTTGTAGTGTTTTGTGTGGGTGAGGTTTGTGTAGTTGTTGGGGAAGAGTTTATTT
GTTAGGTTGTGTTGGGTTAGTGTAGTAAGTGGGGTTGGTTGTTATTTTGTTGTATTTGGT
TGTGTTTTGGGTTTTGTGTGTTTTTGTTTTAG (SEQ ID NO: 68);

[0084] In some examples, the positive standard for detecting NDRG4 methylation comprises a polynucleotide sequence of

TGAGAAGTCGGCGGGGGCGCGGATCGATCGGGGTGTTTTTTAGGTTTCGCGTCGCGGTT
TTCGTTCGTTTTTTCGTTCGTTTATCGGGTATTTTAGTCGCGTAGAAGGCGGAAGTTACG
CGCGAGGGATCGCGGTTCGTTCGGGATTAGTTTTAGGTTCGGTATCGTTTCGCGGGTCG
AGCGTTTATATTCGTTAAATTTACGCGGGTACGTTTTCGCGGCGTATCGTTTTTAGTT
(SEQ ID NO.: 69).

[0085] In some examples, the negative standard for detecting NDRG4 methylation comprises a polynucleotide sequence of

[0086] TGAGAAGTTGGTGGGGGTGTGGATTGATTGGGGTGTTTTTTAGGTTTTGTGTTGT
GGTTTTTGTTTGTTTTTTTGTTTGTTTATTGGGTATTTTAGTTGTGTAGAAGGTGGAAGTT
ATGTGTGAGGGATTGTGGTTTGTTTGGGATTAGTTTTAGGTTTGGTATTGTTTTGTGGGT
TGAGTGTTTATATTTGTTAAATTTATGTGGGTATGTTTTTGTGGTGTATTGTTTTTAGTT
(SEQ ID NO.: 70).

In some examples, the method comprises amplifying a quality control standard.

[0087] In some examples, a method for detecting the presence or absence of colorectal cancer (CRC) or advanced adenoma (AA) in a patient in need thereof, comprising using a kit of the present disclosure.

[0088] The present disclosure further provide a method for detecting the presence or absence of colorectal cancer (CRC) or advanced adenoma (AA) in a patient in need thereof, comprising:

a) obtaining an untreated genomic DNA from a fecal sample of the patient;

b) treating the genomic DNA of a), 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;

c) performing a quantitative PCR (qPCR) using the treated genomic DNA of b) as a template, and determining the Ct value of BMP3 gene in the patient as △Ct1;

d) performing a qPCR using the treated genomic DNA of b) as a template, and determining the Ct value of NDRG4 gene in the patient as △Ct2;

e) performing a qPCR using the untreated genomic DNA as a template, and determining the Ct value of a mutant KRAS gene in the patient as △Ct3;

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

g) 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

h) determining the value of a comprehensive index P, wherein $P = e^K/(1+e^K)$, wherein e is the natural constant.

[0089] The clinical constants a, b, c, d, and X can be determined by analyzing clinical data distribution among a patient population.

[0090] In some examples, when P is equal or more than a predetermined threshold value, the patient is determined to have CRC and/or AA, and when P is less than the predetermined threshold value, the patient is determined to be health.

[0091] In some examples, the predetermined threshold value is calculated from clinical data distribution, such as clinical data obtained from patients that have been determined to have CRC and/or AA, and patients that have been determined not to have CRC and/or AA.

[0092] In some examples, the qPCR for amplifying BMP3 gene comprises a first pair of primers and a first probe, 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.

[0093] In some examples, the qPCR for amplifying NDRG4 gene comprises a second pair of primers and a second probe, 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.

[0094] In some examples, 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.

[0095] In some examples, 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.

[0096] In some examples, 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.

[0097] In some examples, both the first probe and the second probe comprise a fluorescent donor and an acceptor fluorophore. In some examples, the first probe and the second probe are TAQMAN® probes.

[0098] In some examples, the mutant KRAS gene comprising at least one KRAS mutation selected from the group consisting G12D, G12V, G12C, G13D, G12A, G12R, G12S, and G13C.

[0099] In some examples, the fecal immunochemical test comprises a colloidal gold-conjugated antibody.

[00100] In some examples, step c) and step d) of the method comprises using B2M gene as a reference gene.

[00101] In some examples, 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.

[00102] In some examples, the positive standard for detecting BMP3 methylation comprises a polynucleotide sequence of

GTTAGTTTGGTCGGGTGTTTTTAAAAATAAAGCGAGGAGGGAAGGTATAGATAGATTTT
GAAAATATTCGGGTTATATACGTCGCGATTTATAGTTTTTTTTTAGCGTTGGAGTGGAGA
CGGCGTTCGTAGCGTTTTGCGCGGGTGAGGTTCGCGTAGTTGTTGGGGAAGAGTTTATT
TGTTAGGTTGCGTTGGGTTAGCGTAGTAAGTGGGGTTGGTCGTTATTTCGTTGTATTCGG
TCGCGTTTCGGGTTTCGTGCGTTTTCGTTTTAG (SEQ ID NO: 67).

[00103] In some examples, the negative standard for detecting BMP3 methylation comprises a polynucleotide sequence of

GTTAGTTTGGTTGGGTGTTTTTAAAAATAAAGTGAGGAGGGAAGGTATAGATAGATTTTGA
AAATATTTGGGTTATATATGTTGTGATTTATAGTTTTTTTTTAGTGTTGGAGTGGAGATGGTG
TTTGTAGTGTTTTGTGTGGGTGAGGTTTGTGTAGTTGTTGGGGAAGAGTTTATTTGTTAGGTT
GTGTTGGGTTAGTGTAGTAAGTGGGGTTGGTTGTTATTTTGTTGTATTTGGTTGTGTTTTGGG
TTTTGTGTGTTTTTGTTTTAG (SEQ ID NO: 68).

[00104] In some examples, the positive standard for detecting NDRG4 methylation comprises a polynucleotide sequence of

TGAGAAGTCGGCGGGGGCGCGGATCGATCGGGGTGTTTTTTAGGTTTCGCGTCGCGGTTTTC
GTTCGTTTTTTCGTTCGTTTATCGGGTATTTTAGTCGCGTAGAAGGCGGAAGTTACGCGCGA
GGGATCGCGGTTCGTTCGGGATTAGTTTTAGGTTCGGTATCGTTTCGCGGGTCGAGCGTTTA
TATTCGTTAAATTTACGCGGGTACGTTTTCGCGGCGTATCGTTTTTAGTT (SEQ ID NO.: 69).

[00105] In some examples, the negative standard for detecting NDRG4 methylation comprises a polynucleotide sequence of

TGAGAAGTTGGTGGGGGTGTGGATTGATTGGGGTGTTTTTTAGGTTTTGTGTTGTGGTTTTTG
TTTGTTTTTTTGTTTGTTTATTGGGTATTTTAGTTGTGTAGAAGGTGGAAGTTATGTGTGAGG
GATTGTGGTTTGTTTGGGATTAGTTTTAGGTTTGGTATTGTTTTGTGGGTTGAGTGTTTATAT
TTGTTAAATTTATGTGGGTATGTTTTTGTGGTGTATTGTTTTTAGTT (SEQ ID NO.: 70).

In some examples, the method comprises amplifying a quality control standard in the step c) and the step d).

[00106] Also provided is a method for diagnosing and treating a colorectal cancer (CRC) and/or advanced adenoma (AA) in a patient in need thereof, comprising determining the presence or absence of CRC and/or AA in the patient by using a kit of the present disclosure, and treating the patient depends on the presence or absence of CRC and/or AA in the patient.

[00107] Also provided is a method for diagnosing and treating a colorectal cancer (CRC) and/or advanced adenoma (AA) in a patient in need thereof, comprising determining the presence or absence of CRC and/or AA in the patient by using a method described herein, and treating the patient depends on the presence or absence of CRC and/or AA in the patient.

BRIEF DESCRIPTION OF THE DRAWINGS

[00108] Figure 1A to Figure 1D depict the results of CpG island prediction and relative position of amplicons of two BMP3 and NDRG4 genes. “Y”, “R” is degenerate bases. Figure 1A - The result of CpG islands prediction of promoter region of BMP3 genes; Figure 1B - The relative position of amplicons of BMP3 gene; Figure 1C - The result of CpG islands prediction of promoter region of NDRG4 genes; Figure 1D - The relative position of amplicons of NDRG4 gene.

[00109] Figure 2A depicts the difference in methylation CpG sites of BMP in the white and Asian populations. Figure 2B depicts the difference in methylation CpG sites of NDRG4 gene in the white and Asian populations.

[00110] Figure 3A depicts the analytical sensitivity amplification curve of BMP3 with the primers and probes in the preferred group 1. Figure 3B depicts the analytical sensitivity amplification curve of NDRG4 with primers and probes in the preferred group 1. Figure 3C depicts the analytical sensitivity amplification curve of BMP3 with the primers and probes in the preferred group 2. Figure 3D depicts the analytical sensitivity amplification curve of NDRG4 with primers and probes in the preferred group 2. Figure 3E depicts the analytical sensitivity amplification curve of BMP3 with the primers and probes in the preferred group 3. Figure 3F depicts the analytical sensitivity amplification curve of NDRG4 with primers and probes in the preferred group 3. Figure 3G depicts the analytical sensitivity amplification curve of BMP3 with the primers and probes in the comparative group 1. Figure 3H depicts the analytical sensitivity amplification curve of NDRG4 with primers and probes in the comparative group 1. Figure 3I depicts the analytical sensitivity amplification curve of BMP3 with the primers and probes in the comparative group 2. Figure 3J depicts the analytical sensitivity amplification curve of NDRG4 with primers and probes in the comparative group 2. Figure 3K depicts the analytical sensitivity amplification curve of BMP3 with the primers and probes in the comparative group 3. Figure 3L depicts the analytical sensitivity amplification curve of NDRG4 with primers and probes in the comparative group 3.

[00111] Figure 4A depicts the analytical specificity amplification curve of BMP3 with the primers and probes in the preferred group 1. Figure 4B depicts the analytical specificity amplification curve of

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 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 SEQ ID NO.: 2.

2. The kit of claim 1,
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.

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 claim 6, wherein the first probe and the second probe are TAQMAN® probes.

8. The kit of any one of claims 1 to 7, 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.

9. The kit of claim 8, 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).

10. The kit of claim 8, wherein the means for detecting the presence or absence of hemoglobin in the biological sample comprises an anti-hemoglobin antibody.

11. The kit of claim 9, 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.

12. The kit of claim 10, wherein the antibody is a colloidal gold-conjugated antibody.

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

14. The kit of any one of claims 1 to 12, wherein the kit further comprises instructions for use and/or interpretation of a test result obtained by using the kit.

15. The kit of claim 10, wherein the kit further comprises means to detect a complex formed by the antibody and the hemoglobin in the biological sample.

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

17. The kit of any one of claims 1 to 16, 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.

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

19. The kit of any one of claims 1 to 18, 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.

20. The kit of claim 19, 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).

21. The kit of claim 13, wherein the means for amplifying an internal control gene comprises primers for amplifying a positive control gene and/or a negative control gene.

22. 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 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 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.

23. The method of claim 22, 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.

24. The method of claim 22, 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.

25. The method of claim 22, 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.

26. The method of claim 22, 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.

27. The method of any one of claims 22 to 26, wherein both the first probe and the second probe comprise a fluorescent donor and an acceptor fluorophore.

28. The method of any one of claims 22 to 26, wherein the first probe and the second probe are TAQMAN® probes.

29. The method of any one of claims 22 to 28, 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.

30. The method of any one of claim 29, 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).

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

32. The method of claim 30, 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.

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

34. The method of any one of claims 22 to 33, 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.

35. The method of any one of claims 22 to 33, 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.

36. The method of any one of claims 30 to 33, 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.

37. The method of claim 34 or 35, wherein the first and the second reference genes are the same.

38. The method of claim 37, wherein the same reference gene is a B2M gene.

39. The method of claim 36, wherein the mutant KRAS gene comprises a mutation selected from the group consisting of G12D, G13D, G12V, G12C, G12S, G12A, and G13R.

40. The method of claim 39, 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.: 46.

41. The method of claim 36, wherein the third reference gene is an ACTB gene.

42. The method of claim 41, wherein qPCR primers for amplifying ACTB gene comprise SEQ ID NOs.: 43 and 44, and the probe comprises SEQ ID NO.: 46.

43. The method of claims 22 to 42, 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.

44. The method of claim 43, 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).

45. The method of any one of claims 22 to 44, wherein the method comprises amplifying a quality control standard.

46. 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 21.

47. 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 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 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 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.

48. The method of claim 47, wherein the qPCR for amplifying BMP3 gene comprises a first pair of primers and a first probe, 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 qPCR for amplifying NDRG4 gene comprises a second pair of primers and a second probe, 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.

49. The method of claim 47, 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.

50. The method of claim 47, 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.

51. The method of claim 47, 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.

52. The method of any one of claims 47 to 51, wherein both the first probe and the second probe comprise a fluorescent donor and an acceptor fluorophore.

53. The method of any one of claims 47 to 52, wherein first probe and the second probe are TAQMAN® probes.

54. The method of any one of claims 47 to 53, 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.

55. The method of any one of claims 47 to 54, wherein the fecal immunochemical test comprises a colloidal gold-conjugated antibody.

56. the method of any one of claims 47 to 55, wherein step b) and step c) comprises using B2M gene as a reference gene.

57. The method of claim 54, 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.: 46.

58. The method of claim 57, wherein ACTB gene is used as a reference gene in the qPCR for amplifying the mutant KRAS gene.

59. The method of claim 58, 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.

60. The method of claims 47 to 59, 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.

61. The method of claim 60, 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).

62. The method of any one of claims 47 to 61, wherein the method comprises amplifying a quality control standard in the step b) and the step c).

63. 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 22 to 62.

64. 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 21.

65. 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 22 to 62.