A Method for Screening Cancer

BACKGROUND OF THE INVENTION

1. FIELD OF THE INVENTION

The invention relates to the cancer screening method, cancer screening biomarker, and use therof. In particular, to the cancer screening method using methylated DNA as the biomarker.

2. DESCRIPTION OF THE PRIOR ART

Cervical cancer has been one of the main causes of death in females worldwide and in Taiwan. Based on the statistical survey by the World Health Organization (WHO) in 2002, cervical cancer was the second major disease responsible for the death of women worldwide, second to breast cancer. Regular cervical cancer screening is the best way to prevent cervical cancer. Conventional cervical cancer screening includes two approaches: the most commonly used Pap smear, and human papilloma virus testing (HPV testing). Pap smear consists of sampling secreta from cervix uteri, examining under microscope whether there is cancerous pathological change in the exfoliated epithelial cell, so as to detect cervical cancer early. On the other hand, HPV testing consists of examining whether human papilloma virus (HPV) is present in the specimen by using polymerase chain reaction (PCR) or Hybrid Capture.

There are, however, many undesired properties of Pap smear. For one, it requires sampling by a physician, and analysis by a medical

examiner/pathologist, which is a high cost of manpower that poses difficulty on promoting the test in many developing countries. Also, Pap smear has a high false negative rate which delays diagnosis and proper treatment prior to cancerous pathological change. As for HPV testing, although it is highly sensitive, it tends to create a high false positive rate, which not only leaves patients worry in vain, but also wastes much medical resources in examinations follow up to those false positive patients. Accordingly, one of the important topics in promoting cervical cancer examination relies on increasing the accuracy and convenience of cervical cancer examination method.

Genomic deletions have long been considered to be an important factor in tumorigenesis. For a long time, we have been accustomed to the idea that the coding potential of the genome lies within the arrangement of the four A, T, G, C bases. The two-hit theory proposed as early as in 1970s indicates concomitant mutations or deletions of some homologous tumor suppressor genes may cause or predispose to cancer development. However, additional information that affects phenotype can be stored in the modified base 5-methylcytosine. 5-Methylcytosine is found in mammals in the context of the palindromic sequence 5'-CpG-3'. Most CpG dinucleotide pairs are methylated in mammalian cells except some areas called "CpG island." CpG islands are GC- and CpG-rich areas of approximately 1 kb, usually located in the vicinity of genes and often found near the promoter of widely expressed genes. Cytosine methylation occurs after DNA synthesis, by enzymatic transfer of a methyl group from the methyl donor *S*-adenosylmethionine to the carbon-5 position of cytosine. The enzymatic reaction is performed by DNA

methyltransferases (DNMTs). DNMT1 is the main methyltransferase in mammals, and is responsible for the post-replicative restoration of hemi-methylated sites to full methylation, referred to as maintenance methylation, whereas DNMT3A and DNMT3B are thought to be involved primarily in methylating new sites, a process called *isolated* methylation.

Loss of methylation at CpG dinucleotides, i.e., general hypomethylation, was the first epigenetic abnormalities identified in cancer cells. However, during the past few years, it has become increasing apparent that site-specific hypermethylation, e.g., some tumor suppressor genes, is associated with loss of function which may provide selective advantages during carcinogenesis. Dense methylation of CpG islands at promoter regions can trigger chromatin remodeling through histone modifications with subsequent gene silencing. Therefore, in addition to chromosomal deletions or genetic mutations, epigenetic silencing of tumor suppressor genes by promoter hypermethylation is commonly seen in human cancer.

Epidemiologic studies have recently shown the correlation of serum folate level, a major source of methyl group, with the infection and clearance of HPV. Genetic polymorphisms of enzymes in the metabolism of methyl cycle were also reported to be associated with the development of cervical intraepithelial lesions. As the concept of epigenetics evolves, studies exploring the association between DNA methylation and cervical cancer are also booming. Studies of DNA methylation in cervical cancer are accumulating, which showed the potential of using methylation as markers in cervical screening. With the nature of the interface between genetics and environment, the

prevalence of methylation in tumor suppressor genes varies in different genes and different populations. The concept of methylator phenotypes with different disease behaviors was proposed with controversy. The methylator phenotype of cervical cancer and its interaction with HPV genotypes still remains unknown. What genes are specifically methylated in cervical cancer and how many genes are required to achieve clinical application will remain a blossoming issue in the coming future.

The inventor of this application had filed relative patent applications in Taiwan (TW Pat. Pub. No. 200831900)、China(CN Appl. No. 200810094659.2)、Malaysia(UI20085354) and America (US Pat. Pub. No. 20080311570) (Hereinafter referred as prior applications). Comparing with prior applications, the inventor had found some novel cancer screening biomarker and cancer screening method using the same.

SUMMARY OF THE INVENTION

One object of the invention is to provide a cervical cancer screening method as the first line cervical cancer screening.

Another object of the invention is to provide a cervical cancer screening method, characterized in that said method can be used not only in the first line cervical cancer screening, but also in the second line cervical cancer screening to assist HPV testing or uncertain smear result in order to achieve more accurate cervical cancer screening effect.

Yet another object of the invention is to provide a cancer screening method, characterized in that said method can be used in the

screening of cervical cancer as well as in the screening of other cancer (for example: ovarian cancer, colon cancer and the like) to assist the diagnosis of abnormal specimen.

Another object of the invention is to provide the cancer screening biomarker, or a kit containing said biomarker to screen the risk of having cancer.The cancer screening method that can achieve the above-mentioned objects of the invention comprises of detecting the methylation rate of the target gene (biomarker) of the test specimen and use the testing result as a screening index to indicate the risk of having cancer, and said method comprises following steps:

step 1: providing a isolated test specimen;

step 2: detecting the methylation rate of CpG sequence in at least one target gene within the genomic DNA of said test specimen, wherein said target gene comprising DBC1, PDE8B, PTPRR and ZNF582; and

step 3: determining whether there is cancer or pre-cancerous pathological change or not in the specimen based on the methylation rate in the target gene, or using said methylation rate of target gene as prognosis marker;

wherein said test specimen comprising a cervical scraping, a ovarian cancer tissue, ascites, blood, urine, feces, sputum, oral mucous membrane cell, gastric juices, bile, cervical epithelial cell, and post-surgery cancer tissue;

wherein testing methods for the methylation state of CpG sequence in said target gene comprising methylation-specific

polymerase chain reaction (MSP), quantitative methylation-specific polymerase chain reaction (QMSP), bisulfite sequencing (BS), microarrays, mass spectrometry, denaturing high-performance liquid chromatography (DHPLC);

wherein the target gene DBC1 has the nucleotide sequence as depicted in SEQ ID No: 1;

wherein the target gene PDE8B has the nucleotide sequence as depicted in SEQ ID No: 2;

wherein the target gene PTPRR has the nucleotide sequence as depicted in SEQ ID No: 3; and

wherein the target gene ZNF582 has the nucleotide sequence as depicted in SEQ ID No: 4.

In addition, the above-described screening index and screening method can be used further in the screening of cervical cancer, and colon cancer.

The invention provides further a ovarian cancer screening method, characterized in that said method tests the methylation state of the target gene of test specimen, and uses said methylation state as a screening index to determine the risk of having ovarian cancer, and said method comprises following steps:

step 1: providing a isolated test specimen;

step 2: detecting the methylation rate of CpG sequence in at least one target gene within the genomic DNA of said test specimen, wherein said target gene comprising DBC1, PTPRR and ZNF582; and,

step 3: determining whether there is ovarian cancer or cancerous pathological change or not in the specimen based on the methylation rate in the target gene, or using said methylation rate of target gene as prognosis marker;

wherein said test specimen comprising ovarian cancer tissue, ascites, blood, urine, and post-surgery cancer tissue;

wherein testing methods for the methylation state of CpG sequence in said target gene comprising methylation-specific polymerase chain reaction (MSP), quantitative methylation-specific polymerase chain reaction (QMSP), bisulfite sequencing (BS), microarrays, mass spectrometry, denaturing high-performance liquid chromatography (DHPLC), and pyrosequencing.

wherein the target gene DBC1 has the nucleotide sequence as depicted in SEQ ID No: 1;

wherein the target gene PTPRR has the nucleotide sequence as depicted in SEQ ID No: 3; and

wherein the target gene ZNF582 has the nucleotide sequence as depicted in SEQ ID No: 4.

Term "test specimen" refers herein to isolated test specimen, and said isolated test specimen comprising the above-described cervical scraping, ascites, blood, urine, feces, sputum, oral mucous membrane cell, gastric juices, bile, cervical epithelial cell, post-surgery cancer tissue, or other suitable specimen. The inventive cancer screening method is useful to test the methylation state of target genes in said isolated test specimens, and uses said methylation state of biomarker as a screening index of various types of cancer. The cancer screening

method and the screening index of biomarker provided by the invention can be employed by the researcher to perform the test in the laboratory.

Wherein, "the risk of having cancer" refers herein to the percentage of being cancer, the risk of getting cancer in future, or the presence or not of cancer.

Wherein when the methylation state of target gene (biomarker) being higher compare with suitable control, the risk of having cancer would be higher.

According said screening method, the invention provides further the cancer screening biomarker or a kit containg said biomarker to screen the methylation state of target gene (biomarker) in isolated test specimen for determining the risk of having cancer.

These features and advantages of the present invention will be fully understood and appreciated from the following detailed description of the accompanying Drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A shows the result obtained by performing bisulfite sequencing (BS) analysis on various cervical specimens using a target gene PTPRR in the inventive cancer screening method;

Figure 1B shows the result obtained by performing bisulfite sequencing (BS) analysis on various cervical specimens using a target gene ZNF582 in the inventive cancer screening method;

Figure 1C shows the result obtained by performing bisulfite sequencing (BS) analysis on various cervical specimens using a target

gene PDE8B in the inventive cancer screening method; and

Figure 1D shows the result obtained by performing bisulfite sequencing (BS) analysis on various cervical specimens using a target gene DBC1 in the inventive cancer screening method.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The invention will be illustrated more detailed with the following examples, but the invention is not limited thereto.

Example 1 Materials and Methods

1. Materials

Test materials comprises a series of full cervical lesion specimens, including: squamous cell carcinoma (SCC, n = 20), adenocarcinoma (AC, n=20), and normal cervical specimen (n=10). All of these cervical specimen, ovarian specimen, and colon cancer specimen were obtained from Tri-service General Hospital, Taipei, ROC. Each specimen was subjected to genomic DNA extraction by means of QIAamp DNA Kit (QIAGEN), and was used in the comparison and analysis of DNA methylation condition within genome-wide DNA methylation. Prior to the analysis, the quality of genomic DNA was checked by Bioanalyzer (Agilent). In this example, 10μg of fragmented DNA was subjected to MeDIP (Methyl DNA IP) assay.

2. DNA methylation analysis by MeDIP and CpG island-Plus-Promoter arrays

Genomic DNA was fragmented to the size ranging from 300 to 1,000 bp by Bioruptor™ UCS-200 (Diagenode). We immunoprecipitated it for overnight at 4 °C with 30 μl of polyclonal Anti-5'-methyl cytosine antibody (Abcam) in a final volume of 100 μl IP buffer (0.15% SDS, 1% Triton X-100, 150mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 10 mM Tris and 0.1% BSA). We incubated the mixture with 120 μl of Protein G Sepharose (Amersham) for 2 h at 4°C and washed it twice with 1 ml low salt, high salt buffer, lithium chloride and TE buffer. We then treated twice the protein G with elution buffer (1% SDS, 0.1M $NaHCO_3$) for 15 min at room temperature and recovered the Methylated DNA by phenol-chloroform extraction followed by ethanol precipitation. The enriched methylated DNA and input DNA were amplified by Whole Genome Amplification Kit (Sigma). Enriched and total DNA was end-labeled with Cy5 and Cy3, respectively, and co-hybridized to the CpG Island-Plus-Promoter Arrays designed and synthesized by NimbleGen Systems, Inc. This array contained 385,000 50-75 bp oligonucleotides tiled every 100 bp across the 24,659 HG18 RefSeq promoters (800 bp upstream to 200 bp down stream) and 28,226 CpG islands, present in duplicate.

Each feature on the array has a corresponding scaled log2-ratio. The log2-ratio is normalization to center the ratio data around zero. From the scaled log2-ratio data, a fixed-length window (500 bp) is placed around each consecutive probe and the one-sided

Kolmogorov-Smirnov (KS) test is applied to determine whether the probes are drawn from a significantly more positive distribution than those in the rest of the array. The resulting score for each probe is the -log10 p-value from the windowed KS test around that probe (Scacheri et al, 2006). We detected enriched peaks by searching for at least 2 probes above a p-value minimum cutoff (-log10) of 2. Peaks within 300 bp of each other are merged. Differential methylation regions between SCC/AC and normal cervix within 2500 bp upperstream of transcription star sites were selected for future validation. Finally, p-value data was viewed using SignalMap (NimbleGen).

3. Bisulfite modification, methylation-specific polymerase chain reaction (MSP) and bisulfite sequencing (BS)

A DNA modification kit (Chemicon, Ternecula, CA) was used according to the manufacturer's recommendations to convert 1 μg aliquots of genomic DNA with sodium bisulfite to preserve the methylated cytosines. The final precipitate was eluted with 70 μl of pre-warmed (55°C) TE buffer for MSP.

Normal DNA of human peripheral blood was taken and subjected to bisulfite modification, which was used as the control group having un-methylation promoter sequence.

MSP was performed according to prior art. In short, 1 μl of modified DNA was amplified using MSP primers (table 1) that specifically recognized the methylated gene sequences present in the bisulfite-converted DNA. Methylation-specific PCR was done in a

total volume of 25 μl containing 1 μl of modified template DNA, 1.5 pmol of each primer, 0.2 mmol/L deoxynucleotide triphosphates, and 1 unit of Gold *Taq* DNA polymerase (Applied Biosystems, Foster City, CA). MSP reactions were subjected to an initial incubation at 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, and annealing at the appropriate temperature for 30 s and at 72°C for 30 s. The final extension was done at 72°C for 5 min. Amplification products were visualized on 2.5% agarose gel containing ethidium bromide and illuminated under UV light.

Table 1. The sequences of MSP primers

Gene		Primer	Sequence	
DBC1	M	Forward (F')	5' gttttcgtcgtttttcgttcggagatc 3'	(SEQ ID No: 5)
		Reverse (R')	5' gctctcgctctcgctattactcgct 3'	(SEQ ID No: 6)
PDE8B	M	Forward (F')	5' tgtgtatgcgcgtttttttcgttc 3'	(SEQ ID No: 7)
		Reverse (R')	5' acctatatatccgccgctccgtc 3'	(SEQ ID No: 8)
PTPRR	M	Forward (F')	5' cggcgttgggtatgtttagtagtc 3'	(SEQ ID No: 9)
		Reverse (R')	5' aattacgaataaaaaaaacaaaaacgctc 3'	(SEQ ID No: 10)
ZNF582	M	Forward (F')	5' tgacggttttttgtttattcggttattc 3'	(SEQ ID No: 11)
		Reverse (R')	5' cgaacgcaaacgtacctacgc 3'	(SEQ ID No: 12)

M: The primers can specifically recognize the methylated gene sequences present in the bisulfite-converted DNA.

All MSP data were derived from at least two independent modifications of DNA. The absence of signal in duplicate experiments was scored as negative methylation. PCR product obtained by using MSP primer (M) that can recognize specifically methylation gene sequence was constructed into a pCR4-TOPO vector (Invitrogen, Carlsbad, CA). 5 independent clones were selected and were subjected

to bisulfite sequencing (BS). Primers used in bisulfite sequencing (BS) were listed in Table 2. Bisulfite sequencing was carried out using 377 Auto-sequencer (Applied Biosystems, Foster City, CA). Sequenced results were shown in Sequence List. Sequence numbers in bisulfite sequencing were: DBC1_BS (SEQ ID No: 21), PDE8B_BS (SEQ ID No: 22), PTPRR_BS (SEQ ID No: 23) and ZNF582_BS (SEQ ID No: 24), respectively.

Table 2. The sequences of bisulfite sequencing primers

Gene	Primer	Sequence	
DBC1	Forward (F')	5'ggttaagttttttttyggygtagtt 3'	(SEQ ID No: 13)
	Reverse (R')	5'tactccctctacctcccractctctc 3'	(SEQ ID No: 14)
PDE8B	Forward (F')	5'ttgtggygtagaggattattagtttggt 3'	(SEQ ID No: 15)
	Reverse (R')	5'ctaaaaacrcaacccatcccte 3'	(SEQ ID No: 16)
PTPRR	Forward (F')	5'ggaattttattttgaaatttttttgtt 3'	(SEQ ID No: 17)
	Reverse (R')	5'ccccacttcaaataaaatactattaaaaaaaac 3'	(SEQ ID No: 18)
ZNF582	Forward (F')	5'tagtgayggttttttgtttattyggttatt 3'	(SEQ ID No: 19)
	Reverse (R')	5'taaacrtaaaaacaacaacccracct 3'	(SEQ ID No: 20)

Example 2 Screening of methylated target gene in cervical cancer

After screened by CpG island-Plus-Promoter arrays, four target genes that might have been highly methylated in the cervical cancer cell were screened, namely, DBC1 (SEQ ID No: 1), PDE8B (SEQ ID No: 2), PTPRR (SEQ ID No: 3) and ZNF582 (SEQ ID No: 4), respectively. Their detailed information was shown in Table 3. It could be seen from Table 3 that, among these four genes, except DBC1 that

was known to be associated with bladder cancer, little study up to date had revealed the relation of those genes to cervical cancer.

Table 3. Characteristics of methylated genes in cervical cancer that were identified using a CpG island-Plus-Promoter microarray.

Gene	UniGene	Chromosomal location	Full name	SEQ ID No
DBC1	NM_014618	9q32-q33	deleted in bladder cancer 1	SEQ ID No: 1
PDE8B	NM_003719	5q14.1	phosphodiesterase 8B	SEQ ID No: 2
PTPRR	NM_002849	12q15	protein tyrosine phosphatase, receptor type, R	SEQ ID No: 3
ZNF582	NM_144690	19q13.43	zinc finger protein 582	SEQ ID No: 4

Example 3 The analysis of methylation state of target gene in cervical lesion specimen by bisulfite sequencing (BS)

The target gene : PTPRR

Test specimen groups:

1. HeLa_0: HeLa cervical cancer cell line;
2. HeLa_10D: HeLa cervical cancer cell line (HeLa_0) treated with 10 μM DNA methyltransferase inhibitor 5'-aza-2'-deoxycytidine (AZC);
3. HeLa_DT: HeLa cervical cancer cell line (HeLa_0) treated with both of 10 μM DNA methyltransferase inhibitor 5'-aza-2'-deoxycytidine (AZC) and 0.33 μM TSA (Sigma Chemical Co., St. Louis, MO);
4. AC: Cervical adenocarcinoma specimen;
5. SCC: Cervical squamous cell carcinoma specimen;

6. Control group (normal): normal cervical blood DNA as the control group without methylation.

Each of those test specimens described above was subjected to bisulfite modification, and then bisulfite sequencing (BS) to analyze whether the target gene (PTPRR) in each test specimen has been hypermethylated or not. The result was shown in Fig. 1, where black indicated methylation region, and white indicated un-methylation region. The target gene PTPRR had not been methylated in the control group and the adenocarcinoma, while the target gene PTPRR had been hypermethylated in HeLa cervical cancer cell line (HeLa_0) and cervical squamous cell carcinoma specimen (SCC). Consequently, the methylation rate of PTPRR could be used as biomarker to screen whether there was cervical cancer or not.

In addition, in order to ascertain whether methylation rate of the target gene in cervical cancer specimen could be regulated through DNA methylation, HeLa cervical cancer cell line was treated with 10 μM DNA methyltransferase inhibitor 5'-aza-2'-deoxycytidine (AZC) (Sigma Chemical Co.). The DNA specimen extracted from said cell was subjected to bisulfite modification and then bisulfite sequencing (BS). Result shown in Fig. 1A indicated that, compared with the cervical squamous cell carcinoma specimen (SCC) and HeLa cervical cancer cell line (HeLa_0), the target gene PTPRR in the AZC-treated HeLa cervical cancer cell line (HeLa_10D) had part of region been de-methylated.

Trichostatin A (TSA) is histone deacetylase (HDAC) inhibitors, and can be used to lower or degrade methylation rate. HeLa cervical cancer cell line (HeLa_DT) has been treated with both AZC and TSA.

The result shown in Fig. 1 indicated that, compared with cervical squamous cell carcinoma specimen (SCC) and HeLa cervical cancer cell line (HeLa_0), the target gene PTPRR in the AZC- and TSA-treated HeLa cervical cancer cell line (HeLa_DT) had been highly de-methylated.

In summary, the above-described results had confirmed that the target gene PTPRR in cervical cancer specimen had been methylated through DNA methylation.

The target gene : ZNF582

Test specimen groups:

1. HeLa_0: HeLa cervical cancer cell line;
2. HeLa_10D: the HeLa cervical cancer cell line (HeLa_0) treated with 10 μM DNA methyltransferase inhibitor 5'-aza-2'-deoxycytidine (AZC);
3. HeLa_DT: the HeLa cervical cancer cell line (HeLa_0) treated with both of 10 μM DNA methyltransferase inhibitor 5'-aza-2'-deoxycytidine (AZC) and 0.33 μM TSA;
4. SCC 1: Cervical squamous cell carcinoma specimen 1;
5. SCC 2: Cervical squamous cell carcinoma specimen 2;
6. The control group (normal): normal cervical blood DNA as the un-methylation control group;

Wherein cervical squamous cell carcinoma specimen 1 and specimen 2 were specimens obtained from different patients.

Those test specimens mentioned above were subjected to bisulfite sequencing (BS) to analyze whether the target gene (ZNF582) in each test specimen had been hypermethylated or not. The result shown in

Fig. 1B indicated that, compared with the control group, the target gene ZNF582 in cervical squamous cell carcinoma specimen 1 (SCC), cervical squamous cell carcinoma specimen 2 (SCC) and HeLa cervical cancer cell line (HeLa_0) had been hypermethylated. Consequently, the target gene ZNF582 in cervical cancer specimens would be highly methylated.

The target gene : PDE8B

Test specimen groups:

1. SiHa_0: SiHa cervical cancer cell line;
2. SiHa_10D: SiHa cervical cancer cell line (SiHa_0) treated with 10 μM DNA methyltransferase inhibitor 5'-aza-2'-deoxycytidine (AZC);
3. SiHa_DT: SiHa cervical cancer cell line (SiHa_0) treated with both of 10 μM DNA methyltransferase inhibitor 5'-aza-2'-deoxycytidine(AZC) and 0.33 μM TSA;
4. SCC 1: Cervical squamous cell carcinoma specimen 1;
5. SCC 2: Cervical squamous cell carcinoma specimen 2;
6. The control group (normal): normal cervical blood DNA used as un-methylation control group;

wherein cervical squamous cell carcinoma specimen 1 and specimen 2 were specimens obtained from different patients.

Those test specimens described above were subjected to bisulfite sequencing (BS) to analyze whether the target gene (PDE8B) in each test specimen had been hypermethylated or not. The result shown in Fig. 1C indicated that, compared with the control group, the target gene PDE8B in cervical squamous cell carcinoma specimen 1 (SCC 1),

cervical squamous cell carcinoma specimen 2 (SCC 2) and SiHa cervical cancer cell line (SiHa_0) had been hypermethylated. Consequently, the target gene PDE8B in cervical cancer specimens would be highly methylated.

The target gene : DBC1

Test specimen groups:

1. SiHa_0: SiHa cervical cancer cell line;
2. SiHa_10D: SiHa cervical cancer cell line (SiHa_0) treated with 10 μM DNA methyltransferase inhibitor 5'-aza-2'-deoxycytidine (AZC);
3. SiHa_DT: SiHa cervical cancer cell line (SiHa_0) treated with both of 10 μM DNA methyltransferase inhibitor 5'-aza-2'-deoxycytidine (AZC) and 0.33 μM TSA;
4. SCC 1: Cervical squamous cell carcinoma specimen 1;
5. SCC 2: Cervical squamous cell carcinoma specimen 2;
6. The control group (normal): normal cervical blood DNA as the un-methylation control group;

wherein cervical squamous cell carcinoma specimen 1 及 specimen 2 were specimens obtained from different patients.

Those test specimens described above were subjected to bisulfite sequencing (BS) to analyze whether the target gene (DBC1) in each test specimen had been hypermethylated. The result shown in Fig. 1D indicated that, compared with the control group, the target gene DBC1 in cervical squamous cell carcinoma specimen 1 (SCC 1), cervical squamous cell carcinoma specimen 2 (SCC 2) and SiHa cervical cancer cell line (SiHa_0) had been hypermethylated. Consequently,

the target gene DBC1 in cervical cancer specimens would be highly methylated.

Example 4 Methylation analysis of the target gene in cervical cancer specimens

Methylation-specific PCR (MSP) was used to analyze the methylation state of said four target genes in cervical squamous cell carcinoma (SCC) specimens. The methylation state analysis result shown in Table 4 indicated that, in normal cervical specimen, methylation frequency of DBC1, PDE8B, PTPRR and ZNF582 was 11%, 0%, 9% and 6%, respectively; in cervical squamous cell carcinoma specimen, the methylation frequency of DBC1, PDE8B, PTPRR and ZNF582 was 100%, 47%, 100% and 97%, respectively. It could be known from these results that, in cervical squamous cell carcinoma specimens, said four genes were highly methylated. Consequently, methylation rate of DBC1, PDE8B, PTPRR and ZNF582 could be used indeed as the screening index in screening cervical cancer.

Table 4 Methylation state analysis of the target gene in cervical squamous cell carcinoma specimens

	Target Genes CGI methylation rate (%)	
	Normal (n=54)	SCC tissue (n=30)
DBC1	11%	100%
PDE8B	0%	47%
PTPRR	9%	100%

ZNF582	6%	97%

Example 5 Methylation analysis of the target gene in ovarian tumor specimen

Methylation-specific PCR (MSP) was used to analyze the methylation state of the target gene in ovarian tumor specimens. Methylation state analysis results in Table 5 reported the analysis of methylation states of three genes DBC1, PTPRR and ZNF582 in ovarian malignant tumor specimen and ovarian benign tumor specimen. The results indicated that, the methylation frequency of DBC1, PTPRR and ZNF582 in ovarian malignant tumor specimen was 50.3%, 50.0% and 56.3%, respectively; the methylation frequency of DBC1, PTPRR and ZNF582 in ovarian benign tumor specimen was 2.5%, 0.0% and 12.5%, respectively. The differential methylation level was 53.8%, 50.0% and 43.8%, respectively. Consequently, compared with ovarian benign tumor specimen, methylation levels of said three genes in ovarian malignant tumor specimen were remarkably higher. Therefore, methylation rate of DBC1, PTPRR and ZNF582 could be used indeed as the screening index in screening ovarian cancer.

Table 5 Methylation state analysis of the target gene in ovarian tumor

specimens

	Malignance_Ov (n=28)	Benign_Ov (n=28)	Differential Methylation_level
DBC1	56.3%	2.5%	53.8%
PTPRR	50.0%	0.0%	50.0%
ZNF582	56.3%	12.5%	43.8%

Example 6 Methylation analysis of the target gene in colon cancer specimen

Methylation-specific PCR (MSP) was used to analyze the methylation state of the target gene in colon cancer specimen. The methylation state analysis result in Table 6 reported the methylation state of four genes, DBC1, PDE8B, PTPRR and ZNF582, in colon cancer specimen. The result indicated that, methylation frequencies of DBC1, PDE8B, PTPRR and ZNF582 in colon cancer specimen was 100.0%, 100.0, 100.0% and 100.0%, respectively; while methylation frequencies of DBC1, PDE8B, PTPRR and ZNF582 in normal colon tissue specimen were 25.0%, 25.0%, 25.0% and 25.0%, respectively. Consequently, compared with normal colon tissue specimen, methylation levels of said four genes in colon cancer specimen were remarkably higher. Therefore, methylation rates of DBC1, PDE8B, PTPRR and ZNF582 could be used indeed as the screening index in

screening colon cancer.

Table 6 Methylation state analysis of the target gene in colon cancer specimen

	Target Genes CGI methylation rate (%)	
	Normal colon tissue specimen (n=24)	Colon cancer specimen (n=20)
DBC1	25%	100%
PDE8B	25%	100%
PTPRR	25%	100%
ZNF582	25%	100%

The cancer screening method provided by the invention has following advantages over the above-described conventional techniques:

1. The cancer screening method, biomarker and use therof, provided by the invention uses the methylation rate of a particular gene in a isolated specimen as the biomarker to determine the risk of having cancer, and as compared with conventional methods such as cervical scraping and human papilloma virus testing (HPV testing), both of sensitivity and specificity of the inventive cancer screening method are higher than those of the two above-described methods.

2. The cancer screening method, biomarker and use therof, provided by the invention can be used not only as the first line cervical cancer screening, but also in combination with or assisting human papilloma virus testing (HPV testing) as the second line

cervical cancer screening, so as to achieve more accurate cervical cancer screening effect.

3. The cancer screening method, biomarker and use therof, provided by the invention can be applied on not only the screening of cervical cancer, but also on the screening of other cancer (for example: ovarian cancer, colon cancer and the like) to assist the diagnosis of abnormal specimen.

Many changes and modifications in the above described embodiment of the invention can, of course, be carried out without departing from the scope thereof. Accordingly, to promote the progress in science and the useful arts, the invention is disclosed and is intended to be limited only by the scope of the appended claims.

SEQUENCE LISTING

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attgaaatat atatatatta 5000

What is claimed is:

1. A cancer screening method, based on detecting the methylation rate of target gene in test specimen, and using the methylation rate as the screening index to determine the risk of having cancer, the method comprising following steps:

step 1: providing a test specimen;

step 2: detecting the methylation rate of CpG sequence in at least one target gene of said test specimen, wherein the target gene comprising, PDE8B, PTPRR and ZNF582; and

step 3: determining whether there is cancer or pre-cancerous pathological change or not in the specimen based on the methylation rate in the target gene, or using the methylation rate of target gene as prognosis marker.

2. A cancer screening method as recited in claim 1, wherein the test specimen is an isolated specimen selected from the group consisting of cervical smear, ascites, blood, urine, feces, sputum, oral mucous membrane cell, gastric juices, bile, cervical epithelial cell, and post-surgery cancer tissue.

3. A cancer screening method as recited in claim 1, wherein the testing method for the methylation state of CpG sequence of the target gene comprising methylation-specific polymerase chain

reaction (MSP), quantitative methylation-specific polymerase chain reaction (QMSP), bisulfite sequencing (BS), microarrays, mass spectrometry, denaturing high-performance liquid chromatography (DHPLC), and pyrosequencing.

4. A cancer screening method as recited in claim 1, wherein the target gene PDE8B has nucleotide sequence as depicted in SEQ ID No: 2, the target gene PTPRR has nucleotide sequence as depicted in SEQ ID No: 3, and the target gene ZNF582 has nucleotide sequence as depicted in SEQ ID No: 4.

5. A cervical cancer screening method, based on detecting the methylation rate of target gene in test specimen, and using the methylation rate as the screening index to determine the risk of having cervical cancer, the method comprising following steps:

step 1: providing a test specimen;

step 2: detecting the methylation rate of CpG sequence in at least one target gene of the test specimen, wherein the target gene comprising DBC1, PDE8B, PTPRR and ZNF582; and

step 3: determining whether there is cervical cancer or cancerous pathological change or not in the specimen based on the methylation rate in the target gene, or using the

methylation rate of target gene as prognosis marker.

6. A cervical cancer screening method as recited in claim 5, wherein the test specimen is an isolated specimen selected from the group consisting of cervical smear, blood, urine, cervical epithelial cell, and post-surgery cancer tissue.

7. A cervical cancer screening method as recited in claim 5, wherein the test specimen is the abnormal cervical smear.

8. A cervival cancer screening method as recited in claim 5, wherein the test specimen is the HPV(human papilloma virus)-positive cervical cell specimen.

9. A cervical cancer screening method as recited in claim 5, wherein the testing method for methylation state of CpG sequence of the target gene comprising methylation-specific polymerase chain reaction (MSP), quantitative methylation-specific polymerase chain reaction (QMSP), bisulfite sequencing (BS), microarrays, mass spectrometry, denaturing high-performance liquid chromatography (DHPLC), and pyrosequencing.

10. A cervical cancer screening method as recited in claim 5, wherein the target gene DBC1 has nucleotide sequence as depicted in SEQ ID No: 1, the target gene PDE8B has nucleotide sequence as depicted in SEQ ID No: 2, the target gene PTPRR has nucleotide sequence as depicted in SEQ ID No: 3, and the target gene

ZNF582 has nucleotide sequence as depicted in SEQ ID No: 4.

11. An ovarian cancer screening method, based on detecting the methylation rate of target gene of test specimen, and using the methylation rate as the screening index to determine the risk of having ovarian cancer, the method comprising following steps:

step 1: providing a test specimen;

step 2: detecting the methylation rate of CpG sequence in at least one target gene of the test specimen, wherein the target gene comprising DBC1, PTPRR and ZNF582; and

step 3: determining whether there is ovarian cancer or cancerous pathological change or not in the specimen based on the methylation rate in the target gene, or using the methylation rate of target gene as prognosis marker.

12. An ovarian cancer screening method as recited in claim 11, wherein the test specimen is an isolated specimen selected from the group consisting of ovarian cancer tissue, ascites, blood, urine, and post-surgery cancer tissue.

13. An ovarian cancer screening method as recited in claim 11, wherein the testing method for the methylation state of CpG sequence of said target gene comprising methylation-specific polymerase chain reaction (MSP), quantitative methylation-specific polymerase chain reaction (QMSP), bisulfite

sequencing (BS), microarrays, mass spectrometry, denaturing high-performance liquid chromatography (DHPLC), and pyrosequencing.

14. An ovarian cancer screening method as recited in claim 11, wherein the target gene DBC1 has nucleotide sequence as depicted in SEQ ID No: 1, the target gene PTPRR has nucleotide sequence as depicted in SEQ ID No: 3, and the target gene ZNF582 has nucleotide sequence as depicted in SEQ ID No: 4.

15. A colon cancer screening method, based on detecting the methylation rate of target gene of test specimen, and using the methylation rate as the screening index to determine the risk of having colon cancer, said method comprising following steps:

step 1: providing a test specimen;

step 2: detecting the methylation rate of CpG sequence in at least one target gene of the test specimen, wherein the target gene comprising DBC1, PDE8B, PTPRR and ZNF582; and

step 3: determining whether there is colon cancer or cancerous pathological change or not in the specimen based on the methylation rate in the target gene, or using the methylation rate of target gene as prognosis marker.

16. A colon cancer screening method as recited in claim 13, wherein

the test specimen is an isolated specimen selected from the group consisting of ascites, feces, blood, urine, and post-surgery cancer tissue.

17. A colon cancer screening method as recited in claim 13, wherein the testing method for the methylation state of CpG sequence of the the target gene comprising methylation-specific polymerase chain reaction (MSP), quantitative methylation-specific polymerase chain reaction (QMSP), bisulfite sequencing (BS), microarrays, mass spectrometry, denaturing high-performance liquid chromatography (DHPLC), and pyrosequencing.

18. A colon cancer screening method as recited in claim 13, wherein the target gene DBC1 has nucleotide sequence as depicted in SEQ ID No: 1, the target gene PDE8B has nucleotide sequence as depicted in SEQ ID No: 2, the target gene PTPRR has nucleotide sequence as depicted in SEQ ID No: 3, and the target gene ZNF582 has nucleotide sequence as depicted in SEQ ID No: 4.

ABSTRACT OF THE DISCLOSURE

A method for screening cancer comprises the following steps: (1) providing a test specimen; (2) detecting the methylation rate of the CpG sequence in at least one target gene within the genomic DNA of the test specimen, wherein the target genes are consisted of PTPRR, ZNF582, PDE8B and DBC1; and (3) determining whether there is cancer or cancerous pathological change in the specimen based on the presence or absence of the methylation state in at least one target gene; wherein the method for detecting methylation state is methylation-specific PCR (MSP), quantitative methylation-specific PCR (QMSP), bisulfite sequencing (BS), microarrays, mass spectrometer, denaturing high-performance liquid chromatography (DHPLC), and the like.