

[Disclosure]

[Technical Problem]

To solve the above-described problems of the prior art, the present invention is directed to providing a method of diagnosing the risk or prognosis of liver cancer by measuring a methylation level of a specimen using a specific probe which shows low methylation in normal tissue or blood but shows a high methylation level only in liver cancer tissue to find the risk of liver cancer early.

[Technical Solution]

According to one ~~aspect~~example of the present ~~invention~~disclosure, a method of assessing the prognosis or risk of liver cancer, which includes: (a) isolating DNA from a biological sample of a subject; and (b) measuring a methylation level of a CpG site at a location selected from the group consisting of the sequence from 25438725 to 25439276 on chromosome #2, the sequence from 95941906 to 95942979 on chromosome #12, the sequence from 134597357 to 134602649 on chromosome #10, the sequence from 144649774 to 144651774 on chromosome #8, the sequence from 47998899 to 47999517 on chromosome #1, the sequence from 26394102 to 26396102 on chromosome #2, the sequence from 104510870 to 104513913 on chromosome #8, the sequence from 98289604 to 98290404 on chromosome #8, the sequence from 63281034 to 63281347 on chromosome #2, the sequence from 67873388 to 67875600 on chromosome #8, the sequence from 76555366 to 76556079 on chromosome #4, the sequence from 63782394 to 63790471 on chromosome #1, the sequence from 7849945 to 7850439 on chromosome #5, the sequence from

39186777 to 39187968 on chromosome #2, and the sequence from 74207665 to 74208665 on chromosome #14 in the isolated DNA, is provided.

In one aspect, the present disclosure refers to a method of assessing the risk of developing liver cancer, comprising: (a) isolating DNA from a biological sample isolated from a subject; and (b) measuring a methylation level of a CpG site consisting of the sequence from 47998899 to 47999517 on chromosome #1 in the isolated DNA obtained from a biological sample of a subject.

In one embodiment example, the method may measure a methylation level at two or more CpG sites.

In one embodiment example, the sequence from 25438725 to 25439276 on chromosome #2 may have the base sequence of SEQ ID NO: 1, the sequence from 95941906 to 95942979 on chromosome #12 may have the base sequence of SEQ ID NO: 2, the sequence from 134597357 to 134602649 on chromosome #10 may have the base sequence of SEQ ID NO: 3, the sequence from 144649774 to 144651774 on chromosome #8 may have the base sequence of SEQ ID NO: 4, the sequence from 47998899 to 47999517 on chromosome #1 may have the base sequence of SEQ ID NO: 5, the sequence from 26394102 to 26396102 on chromosome #2 may have the base sequence of SEQ ID NO: 6, the sequence from 104510870 to 104513913 on chromosome #8 may have the base sequence of SEQ ID NO: 7, the sequence from 98289604 to 98290404 on chromosome #8 may have the base sequence of SEQ ID NO: 8, the sequence from 63281034 to 63281347 on chromosome #2 may have the base sequence of SEQ ID NO: 9, the sequence from 67873388 to 67875600 on chromosome #8 may have the base sequence of SEQ ID NO: 10, the sequence from 76555366 to 76556079 on chromosome #4 may have the base sequence of SEQ ID NO: 11, the sequence from 63782394 to 63790471 on chromosome #1 may have the base sequence of SEQ ID NO: 12, the sequence from 7849945 to 7850439 on chromosome #5 may have the base sequence of SEQ ID NO: 13, the sequence from 39186777 to 39187968 on chromosome #2 may have the base sequence of SEQ ID

NO: 14, and the sequence from 74207665 to 74208665 on chromosome #14 may have the base sequence of SEQ ID NO: 15.

In one embodiment example, a CpG site of the sequence from 25438725 to 25439276 on chromosome #2 may be located at 25439110 of chromosome #2, a CpG site of the sequence from 95941906 to 95942979 on chromosome #12 may be located at 95941988 of chromosome #12, a CpG site of the sequence from 134597357 to 134602649 on chromosome #10 may be located at 134599823 of chromosome #10, a CpG site of the sequence from 144649774 to 144651774 on chromosome #8 may be located at 144651002 of chromosome #8, a CpG site of the sequence from 47998899 to 47999517 on chromosome #1 may be located at 47999163 of chromosome #1, a CpG site of the sequence from 26394102 to 26396102 on chromosome #2 may be located at 26395458 of chromosome #2, a CpG site of the sequence from 104510870 to 104513913 on chromosome #8 may be located at 104512877 of chromosome #8, a CpG site of the sequence from 98289604 to 98290404 on chromosome #8 may be located at 98290148 of chromosome #8, a CpG site of the sequence from 63281034 to 63281347 on chromosome #2 may be located at 63281139 of chromosome #2, a CpG site of the sequence from 67873388 to 67875600 on chromosome #8 may be located at 67874178 of chromosome #8, a CpG site of the sequence from 76555366 to 76556079 on chromosome #4 may be located at 76555832 of chromosome #4, a CpG site of the sequence from 63782394 to 63790471 on chromosome #1 may be located at 63789278 of chromosome #1, a CpG site of the sequence from 7849945 to 7850439 on chromosome #5 may be located at 7850070 of chromosome #5, a CpG site of the sequence from 39186777 to 39187968 on chromosome #2 may be located

at 39187533 of chromosome #2, and a CpG site of the sequence from 74207665 to 74208665 on chromosome #14 may be located at 74208165 of chromosome #14.

In one embodimentexample, the biological sample may be one selected from the group consisting of tissue, cells, blood, plasma, stool and urine derived from a patient with suspected liver cancer or a subject diagnosed with liver cancer.

In one embodimentexample, step (b) may be performed by one method selected from the group consisting of PCR, methylation-specific PCR, real-time methylation-specific PCR, MethyLight PCR, MethyLight digital PCR, EpiTYPER, PCR using methylated DNA-specific binding protein, quantitative PCR, DNA chip assay, pyrosequencing and bisulfite sequencing.

In one embodimentexample, the method may further include (c) comparing the methylation level with a methylation level in a normal control.

According to another aspectexample of the present inventiondisclosure, a kit for diagnosing a risk of the onset of liver cancer, which includes a probe binding to a CpG site at a location selected from the group consisting of the sequence from 25438725 to 25439276 on chromosome #2, the sequence from 95941906 to 95942979 on chromosome #12, the sequence from 134597357 to 134602649 on chromosome #10, the sequence from 144649774 to 144651774 on chromosome #8, the sequence from 47998899 to 47999517 on chromosome #1, the sequence from 26394102 to 26396102 on chromosome #2, the sequence from 104510870 to 104513913 on chromosome #8, the sequence from 98289604 to 98290404 on chromosome #8, the sequence from 63281034 to 63281347 on chromosome #2, the sequence from 67873388 to 67875600 on chromosome #8, the sequence from 76555366 to

76556079 on chromosome #4, the sequence from 63782394 to 63790471 on chromosome #1, the sequence from 7849945 to 7850439 on chromosome #5, the sequence from 39186777 to 39187968 on chromosome #2, and the sequence from 74207665 to 74208665 on chromosome #14, is provided.

5 In one ~~embodiment~~example, the diagnostic kit may include two or more probes binding to CpG sites.

[Advantageous Effects]

According to one aspect of the present invention, the possibility of the onset of liver cancer can be effectively predicted using a clinical specimen in which normal
10 tissue is mixed by measuring methylation of a specific CpG site showing a different methylation level from most normal cells including blood as well as cancer and normal tissues.

It should be understood that the effect of the present invention is not limited to the above-described effects, and includes all effects that can be deduced from the
15 configuration of the present invention described in the detailed description or claims of the present invention.

[Description of Drawings]

FIG. 1 illustrates a liver cancer diagnostic marker selection pipeline of the present invention.

20 FIG. 2 is a set of graphs showing the distribution of liver cancer patients before (left) and after (right) normalization of DNA methylation data according to an exemplary embodiment of the present invention, respectively.

[CLAIMS]

[Claim 1]

A method of assessing the ~~prognosis or~~ risk of developing liver cancer, comprising:

- 5 (a) isolating DNA from a biological sample isolated from ~~of~~ a subject; and
- (b) measuring a methylation level of a CpG site consisting of the sequence
from 47998899 to 47999517 on chromosome #1 in the isolated DNA obtained from a
biological sample of a subject~~at a location selected from the group consisting of the~~
~~sequence from 25438725 to 25439276 on chromosome #2, the sequence from~~
10 ~~95941906 to 95942979 on chromosome #12, the sequence from 134597357 to~~
~~134602649 on chromosome #10, the sequence from 144649774 to 144651774 on~~
~~chromosome #8, the sequence from 47998899 to 47999517 on chromosome #1, the~~
~~sequence from 26394102 to 26396102 on chromosome #2, the sequence from~~
~~104510870 to 104513913 on chromosome #8, the sequence from 98289604 to~~
15 ~~98290404 on chromosome #8, the sequence from 63281034 to 63281347 on~~
~~chromosome #2, the sequence from 67873388 to 67875600 on chromosome #8, the~~
~~sequence from 76555366 to 76556079 on chromosome #4, the sequence from~~
~~63782394 to 63790471 on chromosome #1, the sequence from 7849945 to 7850439~~
~~on chromosome #5, the sequence from 39186777 to 39187968 on chromosome #2,~~
20 ~~and the sequence from 74207665 to 74208665 on chromosome #14 in the isolated~~
~~DNA.~~

[Claim 2]

The method of claim 1, wherein the (b) further measures a methylation level of a CpG site selected from the group consisting of the sequence from 25438725 to 25439276 on chromosome #2, the sequence from 95941906 to 95942979 on chromosome #12, the sequence from 134597357 to 134602649 on chromosome #10, the sequence from 144649774 to 144651774 on chromosome #8, the sequence from 26394102 to 26396102 on chromosome #2, the sequence from 104510870 to 104513913 on chromosome #8, the sequence from 98289604 to 98290404 on chromosome #8, the sequence from 63281034 to 63281347 on chromosome #2, the sequence from 67873388 to 67875600 on chromosome #8, the sequence from 76555366 to 76556079 on chromosome #4, the sequence from 63782394 to 63790471 on chromosome #1, the sequence from 7849945 to 7850439 on chromosome #5, the sequence from 39186777 to 39187968 on chromosome #2, and the sequence from 74207665 to 74208665 on chromosome #14 in the isolated DNA.

[Claim 32]

The method of claim 24, wherein levels of methylation at 2 or more CpG sites are measured.

[Claim 43]

The method of claim 24, wherein the sequence from 47998899 to 47999517 on chromosome #1 has the base sequence of SEQ ID NO: 5, the sequence from 25438725 to 25439276 on chromosome #2 has the base sequence of SEQ ID NO: 1, the sequence from 95941906 to 95942979 on chromosome #12 has the base sequence of SEQ ID NO: 2, the sequence from 134597357 to 134602649 on

chromosome #10 has the base sequence of SEQ ID NO: 3, the sequence from 144649774 to 144651774 on chromosome #8 has the base sequence of SEQ ID NO: 4, the sequence from ~~47998899 to 47999517 on chromosome #1 has the base sequence of SEQ ID NO: 5, the sequence from~~ 26394102 to 26396102 on chromosome #2 has the base sequence of SEQ ID NO: 6, the sequence from 104510870 to 104513913 on chromosome #8 has the base sequence of SEQ ID NO: 7, the sequence from 98289604 to 98290404 on chromosome #8 has the base sequence of SEQ ID NO: 8, the sequence from 63281034 to 63281347 on chromosome #2 has the base sequence of SEQ ID NO: 9, the sequence from 67873388 to 67875600 on chromosome #8 has the base sequence of SEQ ID NO: 10, the sequence from 76555366 to 76556079 on chromosome #4 has the base sequence of SEQ ID NO: 11, the sequence from 63782394 to 63790471 on chromosome #1 has the base sequence of SEQ ID NO: 12, the sequence from 7849945 to 7850439 on chromosome #5 has the base sequence of SEQ ID NO: 13, the sequence from 39186777 to 39187968 on chromosome #2 has the base sequence of SEQ ID NO: 14, and the sequence from 74207665 to 74208665 on chromosome #14 has the base sequence of SEQ ID NO: 15.

[Claim 54]

The method of claim 21, wherein a CpG site of the sequence from 47998899 to 47999517 on chromosome #1 is located at 47999163 of chromosome #1, a CpG site of the sequence from 25438725 to 25439276 on chromosome #2 is located at 25439110 of chromosome #2, a CpG site of the sequence from 95941906 to 95942979 on chromosome #12 is located at 95941988 of chromosome #12, a CpG

site of the sequence from 134597357 to 134602649 on chromosome #10 is located at 134599823 of chromosome #10, a CpG site of the sequence from 144649774 to 144651774 on chromosome #8 is located at 144651002 of chromosome #8, a CpG site of the sequence from ~~47998899 to 47999517 on chromosome #1 is located at~~
5 ~~47999163 of chromosome #1, a CpG site of the sequence from~~ 26394102 to 26396102 on chromosome #2 is located at 26395458 of chromosome #2, a CpG site of the sequence from 104510870 to 104513913 on chromosome #8 is located at 104512877 of chromosome #8, a CpG site of the sequence from 98289604 to 98290404 on chromosome #8 is located at 98290148 of chromosome #8, a CpG site
10 of the sequence from 63281034 to 63281347 on chromosome #2 is located at 63281139 of chromosome #2, a CpG site of the sequence from 67873388 to 67875600 on chromosome #8 is located at 67874178 of chromosome #8, a CpG site of the sequence from 76555366 to 76556079 on chromosome #4 is located at 76555832 of chromosome #4, a CpG site of the sequence from 63782394 to
15 63790471 on chromosome #1 is located at 63789278 of chromosome #1, a CpG site of the sequence from 7849945 to 7850439 on chromosome #5 is located at 7850070 of chromosome #5, a CpG site of the sequence from 39186777 to 39187968 on chromosome #2 is located at 39187533 of chromosome #2, and a CpG site of the sequence from 74207665 to 74208665 on chromosome #14 is located at 74208165 of
20 chromosome #14.

[Claim ~~65~~]

The method of claim 1, wherein the biological sample is one selected from the group consisting of tissue, cells, blood, plasma, stool and urine derived from a patient with suspected liver cancer or a subject diagnosed with liver cancer.

5 [Claim ~~76~~]

The method of claim 1, wherein the step (b) is performed by one method selected from the group consisting of PCR, methylation-specific PCR, real-time methylation-specific PCR, MethyLight PCR, MethyLight digital PCR, EpiTYPER, PCR using methylated DNA-specific binding protein, quantitative PCR, DNA chip
10 assay, pyrosequencing and bisulfite sequencing.

[Claim ~~87~~]

The method of claim 1, further comprising:

after step (b), (c) comparing the methylation level with a methylation level in a normal control.

15 ~~[Claim 8]~~

~~A kit for diagnosing a risk of the onset of liver cancer, comprising:~~

~~a probe binding to a CpG site at a location selected from the group consisting of the sequence from 25438725 to 25439276 on chromosome #2, the sequence from 95941906 to 95942979 on chromosome #12, the sequence from 134597357 to
20 134602649 on chromosome #10, the sequence from 144649774 to 144651774 on chromosome #8, the sequence from 47998899 to 47999517 on chromosome #1, the sequence from 26394102 to 26396102 on chromosome #2, the sequence from 104510870 to 104513913 on chromosome #8, the sequence from 98289604 to~~

~~98290404 on chromosome #8, the sequence from 63281034 to 63281347 on chromosome #2, the sequence from 67873388 to 67875600 on chromosome #8, the sequence from 76555366 to 76556079 on chromosome #4, the sequence from 63782394 to 63790471 on chromosome #1, the sequence from 7849945 to 7850439 on chromosome #5, the sequence from 39186777 to 39187968 on chromosome #2, and the sequence from 74207665 to 74208665 on chromosome #14.~~

[Claim 9]

~~The kit of claim 8, which comprises two or more probes binding to the CpG sites.~~