

[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 example of the present 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.

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

10 In one 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 20 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 25 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 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 example, the biological sample may be one selected from the group consisting of tissue, cells, blood, plasma, stool and urine derived from a patient with  
5 suspected liver cancer or a subject diagnosed with liver cancer.

In one example, 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  
10 DNA-specific binding protein, quantitative PCR, DNA chip assay, pyrosequencing and bisulfite sequencing.

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

According to another example of the present disclosure, a kit for diagnosing a risk of the onset of liver cancer, which includes a probe binding to a CpG site at a  
15 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  
20 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 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 risk of developing liver cancer, comprising:

(a) isolating DNA from a biological sample isolated from a subject; and

5       (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.

[Claim 2]

The method of claim 1, wherein the (b) further measures a methylation level  
10 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  
15 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  
20 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 3]

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

[Claim 4]

5           The method of claim 2, 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  
10 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 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  
15 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  
20 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 5]

The method of claim 2, wherein a CpG site of the sequence from 47998899 to  
5 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  
10 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 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  
15 site of the sequence from 98289604 to 98290404 on chromosome #8 is located at  
98290148 of chromosome #8, a CpG site 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  
20 76556079 on chromosome #4 is located at 76555832 of chromosome #4, a CpG site  
of the sequence from 63782394 to 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 chromosome #14.

[Claim 6]

- 5           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.

[Claim 7]

- 10           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 assay, pyrosequencing and bisulfite sequencing.

[Claim 8]

- 15           The method of claim 1, further comprising:  
            after step (b), (c) comparing the methylation level with a methylation level in a normal control.