

domains. To date, promoter alterations in cancer have been largely studied on a gene-by-gene basis, and very little is known about the global extent of promoter-level diversity in GC and other solid malignancies.

[0005] Accordingly, there is a need for a method of profiling promoter elements in cancer.

5

SUMMARY

[0005a] In one aspect there is provided a method for determining the presence or absence of at least one promoter in a cancerous biological sample relative to a non-cancerous biological sample, comprising: contacting the cancerous biological sample with at least one antibody specific for histone modification H3K4me3 and at least one antibody specific for histone modification H3K4me1; isolating nucleic acid from the cancerous biological sample having a signal ratio of H3K4me3 relative to H3K4me1 greater than 1, wherein the isolated nucleic acid comprises at least one region specific to said histone modifications; detecting a signal intensity of H3K4me3 in the isolated nucleic acid; and determining the presence or absence of at least one promoter in the cancerous biological sample based on the change in the signal intensity of H3K4me3 relative to the signal intensity of H3K4me3 in a non-cancerous biological sample.

[0006] In one aspect there is provided a method for determining the presence or absence of at least one promoter in a cancerous biological sample relative to a non-cancerous biological sample, comprising: contacting the cancerous biological sample with at least one antibody specific for histone modifications H3K4me3 and H3K4me1; isolating nucleic acid from the cancerous biological sample having a signal ratio of H3K4me3 relative to H3K4me1 greater than 1, wherein the isolated nucleic acid comprises at least one region specific to said histone modifications; detecting a signal intensity of H3K4me3 in the isolated nucleic acid; and determining the presence or absence of at least one promoter in the cancerous biological sample based on the change in the signal intensity of H3K4me3 relative to the signal intensity of H3K4me3 in a non-cancerous biological sample.

[0006a] In another aspect there is provided a method for determining the prognosis of cancer in a subject, comprising, contacting a cancerous biological sample obtained from the subject with at least one antibody specific for histone modification H3K4me3 and at least one antibody specific for histone modification H3K4me1; isolating nucleic acid from the

30

cancerous biological sample having a signal ratio of H3K4me3 relative to H3K4me1 greater than 1, wherein the isolated nucleic acid comprises at least one region specific to said histone modifications; detecting a signal intensity of H3K4me3 in the isolated nucleic acid; and determining the presence or absence of at least one cancer-associated promoter in the cancerous biological sample based on the change in the signal intensity of H3K4me3 relative to the signal intensity of H3K4me3 in a reference nucleic acid sequence, wherein the presence or absence of the at least one cancer-associated promoter in the cancerous biological sample is indicative of the prognosis of the cancer in the subject.

[0007] In another aspect there is provided a method for determining the prognosis of cancer in a subject, comprising, contacting a cancerous biological sample obtained from the subject with at least one antibody specific for histone modification H3K4me3 and H3K4me1; isolating nucleic acid from the cancerous biological sample having a signal ratio of H3K4me3 relative to H3K4me1 greater than 1, wherein the isolated nucleic acid comprises at least one region specific to said histone modifications; detecting a signal intensity of H3K4me3 in the isolated nucleic acid; and determining the presence or absence of at least one cancer-associated promoter in the cancerous biological sample based on the change in the signal intensity of H3K4me3 relative to the signal intensity of H3K4me3 in a reference nucleic acid sequence, wherein the presence or absence of the at least one cancer-associated promoter in the cancerous biological sample is indicative of the prognosis of the cancer in the subject.

[0008] In another aspect there is provided a biomarker for detecting cancer in a subject, the biomarker comprising at least one promoter having a change in signal intensity of H3K4me3 in a cancerous biological sample relative to a non-cancerous biological sample.

[0009] In another aspect there is provided a method for modulating the activity of at least one cancer-associated promoter in a cell, comprising administering an inhibitor of EZH2 to the cell.

5 [0010] In another aspect there is provided a method for modulating the immune response of a subject to cancer, comprising administering to the subject an inhibitor of EZH2, wherein the EZH2 is associated with at least one cancer-associated promoter in the subject.

[0010a] In another aspect there is provided a method for determining the presence or absence of at least one cancer-associated promoter in a cancerous biological sample relative to a non-cancerous biological sample, comprising: contacting the cancerous biological sample with at least one antibody specific for histone modification H3K4me3 and at least one antibody specific for histone modification H3K4me1; isolating nucleic acid from the cancerous biological sample having a signal ratio of H3K4me3 relative to H3K4me1 greater than 1, wherein the isolated nucleic acid comprises at least one region specific to said histone modifications; detecting a signal intensity of H3K4me3 in the isolated nucleic acid at a read depth of 20M; and determining the presence or absence of at least one cancer-associated promoter in the cancerous biological sample based on the change in the signal intensity of H3K4me3 relative to the signal intensity of H3K4me3 in a non-cancerous biological sample.

15 [0011] In another aspect there is provided a method for determining the presence or absence of at least one cancer-associated promoter in a cancerous biological sample relative to a non-cancerous biological sample, comprising: contacting the cancerous biological sample with at least one antibody specific for histone modifications H3K4me3 and H3K4me1; isolating nucleic acid from the cancerous biological sample having a signal ratio of H3K4me3 relative to H3K4me1 greater than 1, wherein the isolated nucleic acid comprises at least one region specific to said histone modifications; detecting a signal intensity of H3K4me3 in the isolated nucleic acid at a read depth of 20M; and determining the presence or absence of at least one cancer-associated promoter in the cancerous biological sample based on the change in the signal intensity of H3K4me3 relative to the signal intensity of H3K4me3 in a non-cancerous biological sample.

[0012] In one aspect, there is provided a biomarker comprising at least one promoter having a change in signal intensity of H3K4me3 in a cancerous biological sample relative to a non-cancerous biological sample for use in detecting cancer in a subject.

[0013] In one aspect, there is provided a use of a biomarker comprising at least one promoter having a change in signal intensity of H3K4me3 in a cancerous biological sample relative to a non-cancerous biological sample in the manufacture of a medicament for detecting cancer in a subject.

[0014] In one aspect, there is provided an inhibitor of EZH2 for use in modulating the activity of at least one cancer-associated promoter in a cell.

[0015] In one aspect, there is provided a use of an inhibitor of EZH2 in the manufacture of a medicament for modulating the activity of at least one cancer-associated promoter in a cell.

Claims

1. A method for determining the presence or absence of at least one promoter in a cancerous biological sample relative to a non-cancerous biological sample, comprising:
 - contacting the cancerous biological sample with at least one antibody specific for histone modification H3K4me3 and at least one antibody specific for histone modification H3K4me1;
 - isolating nucleic acid from the cancerous biological sample having a signal ratio of H3K4me3 relative to H3K4me1 greater than 1, wherein the isolated nucleic acid comprises at least one region specific to said histone modifications;
 - detecting a signal intensity of H3K4me3 in the isolated nucleic acid; and
 - determining the presence or absence of at least one promoter in the cancerous biological sample based on the change in the signal intensity of H3K4me3 relative to the signal intensity of H3K4me3 in a non-cancerous biological sample.
2. The method of claim 1, wherein the cancerous and non-cancerous biological sample comprises a single cell, multiple cells, fragments of cells, body fluid or tissue.
3. The method of any one of claims 1-2, wherein the cancerous and non-cancerous biological sample is obtained from the same subject.
4. The method of any one of claims 1-3, wherein the cancerous and non-cancerous biological sample are each obtained from different subjects.
5. The method of any one of claims 1-4, wherein the contacting step comprises the immunoprecipitation of chromatin with the antibodies specific for the histone modifications.
6. The method of any one of claims 1-5, further comprising mapping at least one promoter from the cancerous biological sample against at least one reference nucleic acid sequence to identify a gene transcript associated with the at least one promoter.
7. The method of claim 6, wherein the at least one reference nucleic acid sequence comprises a nucleic acid sequence derived from:
 - i) an annotated genome sequence;

- ii) a *de novo* transcriptome assembly; and/or
 - iii) a non-cancerous nucleic acid sequence library or database.
8. The method of claim 1, wherein the change of signal intensity of H3K4me3 is greater than a 1.5 fold increase or decrease relative to the signal intensity of H3K4me3 in the non-cancerous biological sample.
 9. The method of claim 8, wherein a change of signal intensity of H3K4me3 greater than a 1.5 fold increase relative to the signal intensity of H3K4me3 in a non-cancerous biological sample, correlates to the presence of at least one cancer-associated promoter in the cancerous biological sample.
 10. The method of claim 9, wherein the activity of the at least one cancer-associated promoter correlates with an increase of SUZ12 or EZH2 binding sites relative to the total promoter population.
 11. The method of claim 10, wherein the increase of SUZ12 or EZH2 binding sites correlates with an upregulation of activity of the at least one cancer-associated promoter.
 12. The method of claim 10, wherein the increase of SUZ12 or EZH2 binding sites correlates with a downregulation of activity of the at least one cancer-associated promoter.
 13. The method of claim 12, wherein the gene transcript start site is associated with one or more of a cell-type specification gene, a cell adhesion gene, a cell mediated immunity gene, a gastric cancer-associated or deregulated gene, a PRC2 target gene or a transcription factor.
 14. The method of claim 13, wherein the gene transcript start site is associated with an oncogene.

15. The method of claim 14, wherein the gene transcript start site is associated with a gene selected from the group consisting of *MYC*, *MET*, *CEACAM6*, *CLDN7*, *CLDN3*, *HOTAIR*, *PVT1*, *HNF4 α* , *RASA3*, *GRIN2D*, *EpCAM* and a combination thereof.
16. The method of any of claims 1-15, wherein the cancer is gastric cancer or colon cancer.
17. The method of any of claims 1-16, wherein the at least one promoter is an alternative promoter that is associated with a canonical promoter, wherein the canonical promoter is present in both the cancerous biological sample and the non-cancerous biological sample, and wherein the alternative promoter is only present in the cancerous biological sample, or wherein the alternative promoter is only absent in the cancerous biological sample.
18. The method of any of claims 1-12, wherein the at least one promoter is an unannotated promoter that is positioned more than 500bp away from a gene transcript start site.
19. The method of claim 17, further comprising:
 - measuring the expression level of the at least one alternative promoter in the cancerous biological sample and non-cancerous biological sample, wherein the measuring comprises digital profiling of reporter probes; and
 - determining the differential expression level of the at least one alternative promoter relative to the non-cancerous biological sample, based on the digital profiling of the reporter probes, to validate the presence or absence of at least one alternative promoter in the cancerous biological sample relative to a non-cancerous biological sample.
20. The method of claim 19, wherein said step of measuring is conducted using a NanoString™ platform.
21. A method for determining the prognosis of cancer in a subject, comprising,

contacting a cancerous biological sample obtained from the subject with at least one antibody specific for histone modification H3K4me3 and at least one antibody specific for histone modification H3K4me1;

isolating nucleic acid from the cancerous biological sample having a signal ratio of H3K4me3 relative to H3K4me1 greater than 1, wherein the isolated nucleic acid comprises at least one region specific to said histone modifications;

detecting a signal intensity of H3K4me3 in the isolated nucleic acid; and

determining the presence or absence of at least one cancer-associated promoter in the cancerous biological sample based on the change in the signal intensity of H3K4me3 relative to the signal intensity of H3K4me3 in a reference nucleic acid sequence, wherein the presence or absence of the at least one cancer-associated promoter in the cancerous biological sample is indicative of the prognosis of the cancer in the subject.

22. The method of claim 21, wherein the at least one cancer-associated promoter is an alternative promoter that is associated with a canonical promoter, wherein the canonical promoter is present in both the cancerous biological sample and the reference nucleic acid sequence, and wherein the alternative promoter is only present in the cancerous biological sample or wherein the alternative promoter is only absent in the cancerous biological sample.
23. The method of claims 22, wherein the presence or absence of the at least one alternative promoter in the cancerous sample is indicative of a poor prognosis of cancer survival in the subject.
24. The method of claim 22, further comprising:
 - measuring the expression level of the at least one alternative promoter in the cancerous biological sample and the reference nucleic acid sequence, wherein the measuring comprises digital profiling of reporter probes; and
 - determining the differential expression level of the at least one alternative promoter relative to the non-cancerous biological sample, based on the digital profiling of the reporter probes, to validate the presence or absence of at least one alternative promoter in the cancerous biological sample relative to the reference nucleic acid sequence.

25. The method of claim 24, wherein said step of measuring is conducted using a NanoString™ platform.
26. A method for determining the presence or absence of at least one cancer-associated promoter in a cancerous biological sample relative to a non-cancerous biological sample, comprising:
- contacting the cancerous biological sample with at least one antibody specific for histone modification H3K4me3 and at least one antibody specific for histone modification H3K4me1;
 - isolating nucleic acid from the cancerous biological sample having a signal ratio of H3K4me3 relative to H3K4me1 greater than 1, wherein the isolated nucleic acid comprises at least one region specific to said histone modifications;
 - detecting a signal intensity of H3K4me3 in the isolated nucleic acid at a read depth of 20M; and
 - determining the presence or absence of at least one cancer-associated promoter in the cancerous biological sample based on the change in the signal intensity of H3K4me3 relative to the signal intensity of H3K4me3 in a non-cancerous biological sample.