

diagnosis, the main tools for post-treatment follow up or active surveillance also include only imaging studies. Surgery or ablation would result in tissue change and scar formation, causing the detection of local recurrence to be challenging. Lastly, there is also a lack of methods to assess the efficiency of systemic treatments in advanced clear cell renal cell carcinoma patients. In view of the above, there is an unmet need for a method of identifying clear cell renal cell carcinoma, differentiation of benign lesions from malignant tumours, for detection of recurrence after local treatments, and for assessment of systemic treatments.

SUMMARY OF INVENTION

- 10 [006] In one aspect, the present inventiondisclosure refers to a clear cell renal cell carcinoma (ccRCC) biomarker set, wherein the biomarker set comprises at least two biomarkers selected from the group consisting of ZNF395, SMPDL3A, SLC28A1, SLC6A3, VEGFA, EGLN3, wherein one of the at least two biomarkers is SMPDL3A or SLC28A1; wherein the biomarkers are proteins, or nucleic acids encoding the same, or variants thereof.
- 15 [007] In another exampleaspect, the present inventiondisclosure refers to a detection system comprising a) a receiving section to receive a sample from a subject suspected to suffer from clear cell renal cell carcinoma, and wherein the sample is suspected to comprise the biomarker set as disclosed herein; and b) a detection section comprising a substance or substances capable of detecting the biomarker set as disclosed herein.
- 20 [008] In one exampleaspect, the present inventiondisclosure refers to a method of determining whether a subject has or shows recurrence of clear cell renal cell carcinoma, wherein the method comprises obtaining a sample from the subject; detecting the presence of the biomarker set as disclosed herein in the sample using a detection system as disclosed herein, wherein the presence of the biomarker set determines that the subject has or shows recurrence of clear cell renal cell carcinoma.
- 25 [009] In a further exampleaspect, the present inventiondisclosure refers to a method of detecting response of a subject to systemic treatment, the method comprising a) obtaining a sample from the subject; and b) determining the levels of the biomarker set as defined herein in the sample; wherein a decrease in levels or an absence of the biomarker set indicates that the subject is responsive to treatment.
- 30 [009a] In another aspect, the present inventiondisclosure refers to a method of detecting response of a subject to systemic treatment for clear cell renal cell carcinoma, the method comprising ~~a) obtaining a sample from the subject; b)~~ determining the levels of the biomarker set as defined herein in the sample obtained from the subject; wherein a decrease in levels or an absence of the biomarker set indicates that the subject is responsive to treatment.
- 35 [0010] In yet another exampleaspect, the present inventiondisclosure refers to a method of determining whether a renal mass sample is benign or malignant, the method comprising obtaining a sample from the_

renal mass of a subject; determining the levels or the presence or absence of the biomarker set as disclosed herein in the sample; wherein the increase in levels of the biomarker set compared to a benign sample indicates that the sample is malignant.

[0010a] In another aspect, the present disclosure refers to method of determining whether a subject has or shows recurrence of clear cell renal cell carcinoma, wherein the method comprises detecting the presence of the biomarker set as disclosed herein in a sample obtained from the subject, wherein the presence of the biomarker set determines that the subject has or shows recurrence of clear cell renal cell carcinoma.

[0010b] In another aspect, the present disclosure refers to a method of determining whether a renal mass sample is benign or malignant, the method comprising determining the levels or the presence or absence of the biomarker set as disclosed herein in a sample obtained from the renal mass of a subject; wherein the increase in levels of the biomarker set compared to a benign sample indicates that the sample is malignant.

[0011] In another example aspect, the present invention disclosure refers to a kit for carrying out the method as disclosed herein, wherein the kit comprises a detection buffer, a lysis buffer, and a substance or substances as defined herein suitable for the detection of the biomarker set as disclosed herein.

[0012] In a further example aspect, the present invention disclosure refers to a kit as disclosed herein and a detection system as disclosed herein for detecting the biomarker set as defined herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

[0014] **Figure 1** illustrates that von Hippel-Lindau (*VHL*) deficient clear cell renal cell carcinoma tumours exhibit an aberrant cis-regulatory landscape. **Figure 1A** shows a graph that illustrates the percentage of overlap of the histone chromatin immunoprecipitation sequencing (ChIP-seq) (H3K27ac, H3K4me3 and H3K4me1) peaks of normal kidney tissues with peaks from adult kidney tissues in the Epigenomics Roadmap dataset. **Figure 1B** shows a graph illustrating the percentage of overlap of histone ChIP-seq (H3K27ac, H3K4me3 and H3K4me1) peaks between five primary clear cell renal cell carcinoma tumours and cell lines derived from these tumours. **Figure 1C** shows a table and a diagram illustrating that the putative active promoters are defined by co-occurrence of H3K4me3, H3K27ac within 2 kilo bases (kb) proximity to transcription start sites (TSS); and putative active enhancers are defined by the presence of H3K4me1, H3K27ac and the exclusivity with promoters. The table further illustrates the total number of identified putative promoters and putative enhancers; and the total number of gained promoters, lost promoters, gained enhancers and lost enhancers identified in this study. **Figure 1D** shows a graph illustrating principal component analysis (PCA) using all 17,497 promoters and 66,448 enhancers to classify normal samples and tumour samples into distinct clusters. The numbers in the graph depicts patient IDs which are the following 1-12364284; 2-17621953; 3-20431713; 4-40911432; 5-57398667; 6-70528835; 7-74575859; 8-77972083; 9-86049102 and 10-75416923. **Figure 1E** shows graphs illustrating saturation analysis of the total number of predicted promoters or enhancers across increasing number of primary samples. The total number of predicted promoters saturates at 4 or more samples while the total number of predicted enhancers saturates at 16 or more samples. The dotted line indicates the total number of predicted regulatory elements by integrating all 10 normal-tumour pairs (n=20). The whiskers indicate standard deviations. **Figure 1F** shows graphs and tables describing the variances captured by each principle component from normalized H3K27ac signals at promoters or enhancers. The cumulative percentages of variance are indicated in the tables. **Figure 1G** shows a graph of the number of altered promoters and enhancers per patient.

CLAIMS

1. A clear cell renal cell carcinoma (ccRCC) biomarker set, wherein the biomarker set comprises at least two biomarkers selected from the group consisting of ZNF395, SMPDL3A, SLC28A1, SLC6A3, VEGFA, EGLN3, wherein one of the at least two biomarkers is SMPDL3A or SLC28A1; wherein the biomarkers are proteins, or nucleic acids encoding the same, or variants thereof.

2. The clear cell renal cell carcinoma biomarker set of claim 1, wherein the biomarker set comprises at least three biomarkers.

3. The clear cell renal cell carcinoma biomarker set of claim 1 or 2, wherein the biomarker set consists of ZNF395, SMPDL3A and SLC28A1.

~~4. A detection system comprising a) a receiving section to receive a sample from a subject suspected to suffer from clear cell renal cell carcinoma, and wherein the sample is suspected to comprise the biomarker set according to claim 3 and b) a detection section comprising a substance or substances capable of detecting the biomarker set according to claim 3.~~

~~5. The detection system of claim 4, wherein the substance is a bio-specific capture reagent selected from the group consisting of antibodies, or antigen binding fragments thereof, interacting fusion proteins, aptamers, and affibodies.~~

~~6. The detection system of claim 4, wherein the system is selected from the group consisting of a biochip, a test strip, a polymerase chain reaction (PCR) apparatus and a microtiter plate.~~

~~7.4.~~ A method of determining whether a subject has or shows recurrence of clear cell renal cell carcinoma, wherein the method comprises:

~~a) obtaining a sample from the subject;~~

~~b) detecting the presence of the biomarker set according to any one of claims 1 to 3 in the sample obtained from the subject using a detection system according to claims 4 to 6,~~

wherein the presence of the biomarker set determines that the subject has or shows recurrence of clear cell renal cell carcinoma.

~~8.5.~~ A method of detecting response of a subject to systemic treatment for clear cell renal cell carcinoma, the method comprising

~~a. obtaining a sample from the subject;~~

~~b. determining the levels of the biomarker set according to any one of claims 1 to 3 in the sample obtained from the subject;~~

wherein a decrease in levels or an absence of the biomarker set indicates that the subject is responsive to treatment.

~~9.6.~~ A method of determining whether a renal mass sample is benign or malignant, the method comprising

~~a. obtaining a sample from the renal mass of a subject;~~

~~b.~~ determining the levels or the presence or absence of the biomarker set according to any one of claims 1 to 3 in ~~the~~ sample obtained from the renal mass of a subject;

wherein the increase in levels of the biomarker set compared to a benign sample indicates that the sample is malignant.

~~10.7.~~ The method of any one of claims ~~47~~ to ~~69~~, wherein detection is carried out using ~~and/or the detection system uses~~, one or more molecular biological methods.

~~11.8.~~ The method of claim ~~710~~, wherein the one or more molecular biological methods are selected from the group consisting of polymerase chain reaction (PCR), quantitative polymerase chain reaction (qPCR), Western Blot, dot blot, mass spectrometry, nucleic acid sequencing and immunological methods.

~~12.9.~~ The method of any one of claims ~~47~~ to ~~811~~, wherein the determination as to whether the subject has or shows recurrence of clear cell renal cell carcinoma is made based on comparison of the same biomarker set with a control group.

~~13.10.~~ The method of claim ~~912~~, wherein the control group comprises one or more samples obtained from disease-free subjects and/or samples from non-diseased areas of the same or different subjects suffering from clear cell renal cell carcinoma.

~~14.11.~~ ~~The detection system of claims 4 to 6, or t~~The method of claims ~~47~~ to ~~103~~, wherein the sample is solid sample or fluid sample.

~~15.12.~~ ~~The detection system or t~~The method of claim ~~114~~, wherein the solid sample is solid tumour biopsy.

~~16.13.~~ ~~The detection system or t~~The method of claim ~~114~~, wherein the fluid sample is liquid tumour biopsy, urine sample, blood sample, sputum sample or cell culture medium.

~~17. The detection system of any one of claims 14 or 16, wherein the fluid sample contains exosomes suspected to comprise the biomarker set as defined in claim 3.~~

~~18.14.~~ The method of any one of claims ~~114~~ or ~~136~~, wherein the fluid sample contains exosomes suspected to comprise the biomarker set as defined in claims 1 to 3.

~~19. The detection system or method of claim 17 or 18, wherein the exosomes are detected using quantitative polymerase chain reaction (qPCR).~~

~~20. A kit for carrying out the method of any one of claims 7 to 16, 18 or 19, wherein the kit comprises a detection buffer, a lysis buffer, and a substance or substances as defined in claim 5 suitable for the detection of the biomarker set according to any one of claims 1 to 3.~~

~~21. A kit according to claim 20 and a detection system according to any one of claims 4 to 6, 14 to 17 or 19 for detecting the biomarker set of claim 3.~~