(ii) each cell having at least two clearly distinct nuclei;
(iii) each cell having a major axis of greater than about 10 µm;
(iv) expression of endothelial cell genes or proteins;
(v) non-expression of leukocyte-specific genes or proteins; and
(vi) non-expression of megakaryocyte or platelets-specific genes or proteins.

[0010] In one example, there is provided a method for detecting the isolated cell population as described herein in a sample of a subject, the method comprising:

(a) capturing and retrieving the cells from the sample using the apparatus as described herein or the method as described herein.

[0010a] In another aspect, the present disclosure refers to a method for detecting an isolated population of cell clusters in a sample of a subject, the method comprising:

(a) capturing and retrieving the cells from the sample using the apparatus as described herein, or the method as described herein;

wherein the isolated population of cell clusters is selected from the group consisting of:

(A) an isolated population of cell clusters, each cell cluster comprising at least two cells and having the following characteristics:

(i) being endothelial ~~cell~~cell clusters derived from blood vessel of a colorectal tumor and isolated from blood;
(ii) each cell cluster having at least two clearly distinct nuclei;
(iii) each cell cluster having a major axis of greater than ~~about~~ 10 µm;
(iv) expression of endothelial cell genes or proteins, comprising *FOLH1*;
(v) non-expression of leukocyte-specific genes or proteins; ~~and~~
(vi) non-expression of megakaryocyte or platelets-specific genes or proteins; and
(vii) being cytomorphologically consistent with malignancy but not having primary tumor mutations or chromosomal abnormalities;

and

(B) an isolated population of cell clusters as defined in (A), which expresses endothelial cell genes selected from the group consisting of *PECAM1*, *VWF* and *CDH5*; and which optionally expresses leukocyte and megakaryocytic or platelet-specific genes selected from the group consisting of *PTPRC*, *ITGA2B* and *GP1BA*.

[0011] In one example, the method of the disclosure further comprises the steps of:

(b) contacting the cells from step (a) with at least one antibody coupled to a detectable label to allow binding of the antibody to one or more target biomarkers expressed on the cells;
(c) removing unbound antibody from the sample; and
(d) detecting and analyzing the detectable label bound to the antibody to detect the isolated population of cells.

[0012] In another example, the method of the disclosure further comprises the steps of:

(b) lysing the cells from step (a);
(c) contacting the lysed cell sample from step (b) with a reverse primer from a first primer pair, the reverse primer from the first primer pair being directed to a target RNA region, and a reverse transcriptase to effect reverse transcription of the RNA into cDNA;
(d) subsequently contacting the sample from step (c) with:
(i) a forward primer from the first primer pair, the forward primer from the first primer pair being directed to a target cDNA region,
(ii) a reverse primer and a forward primer from a second primer pair, the reverse primer and forward primer from the second primer pair being directed to a target DNA region, and
(iii) a DNA polymerase

to simultaneously amplify the target cDNA region and the target DNA region in a pre-amplification step; and

responded positively to the treatment indicates that the cancer patient will respond negatively to the treatment.

[0022] In one example, there is provided a method for analyzing blood vessel characteristics of a tumor in a subject, the method comprising analyzing a sample from the subject for determining the number of the isolated population of cells as described herein, wherein an increased number of the isolated population of cells compared to a baseline sample indicates that the tumor has larger blood vessels compared to the baseline sample, and wherein a reduced number of the isolated population of cells compared to a baseline sample indicates that the tumor has smaller blood vessels compared to the baseline sample.

[0023] In one example, there is provided a kit for use in the disclosed methods, the kit comprising:

(a) the apparatus as described herein.

[0023a] In yet another aspect, the present disclosure refers to a kit for use in:

(A) a method of diagnosing a colorectal cancer in a subject, comprising analyzing a sample from the subject for presence of the isolated population of cell clusters as defined herein, wherein presence of the isolated population of cell clusters indicates that the subject has colorectal cancer; or

(B) a method for monitoring and/or predicting the response to treatment of a colorectal cancer patient, the method comprising analyzing a sample obtained from the patient after treatment for determining the number of the isolated population of cell clusters as defined herein, wherein a reduction in the number of the isolated population of cell clusters compared to the number of the isolated population of cell clusters in a baseline sample obtained from the patient prior to treatment indicates that the patient is responding positively to the treatment; or

(C) a method for predicting the response to treatment of a colorectal cancer patient, the method comprising analyzing a sample obtained from the cancer patient before treatment for determining the number of the isolated population of cell clusters as defined herein, wherein an equal or higher number of the isolated population of cell clusters compared to the number of the isolated population of cell clusters in a sample obtained before treatment from a patient or a group of patients that have responded positively to the treatment indicates that the colorectal cancer patient will respond positively to the treatment, and wherein a lower number of the isolated population of cell clusters compared to the number of the isolated population of cell clusters in a sample obtained before treatment from a patient or a group of patients that have responded positively to the treatment indicates that the colorectal cancer patient will respond negatively to the treatment; or

(D) a method for analyzing blood vessel characteristics of a colorectal tumor in a subject, the method comprising analyzing a sample from the subject for determining the number of the isolated population of cell clusters as defined herein, wherein an increased number of the isolated population of cell clusters compared to a baseline sample indicates that the colorectal tumor has larger blood vessels compared to the baseline sample, and wherein a reduced number of the isolated population of cell clusters compared to a baseline sample indicates that the colorectal tumor has smaller blood vessels compared to the baseline sample; or

(E) the method as described herein,

the kit comprising:

(a) the apparatus as described herein.

[0024] In one example, the kit of the disclosure further comprises one or more of the following:

(b) one or more cell lysis buffers;

(c) a primer selected from the group consisting of:

i. the reverse primer of step (c) of the disclosed method,

ii. the forward primer of step (d)(i) of the disclosed method,

iii. the primer pair of step (d)(ii) of the disclosed method, and

iv. the nested primer and nested primer pair of the disclosed method;

(d) one or more reagents, selected from the group consisting of:

i. a reverse transcriptase and one or more suitable reaction buffers for the reverse transcription in step (c) of the disclosed method,

ii. a DNA polymerase and one or more suitable reaction buffers for the amplification in step (d) of the disclosed method or the semi-nested or nested PCR of the disclosed method, and

iii. one or more labelled or unlabelled deoxyribonucleotides selected from the group consisting of dATP, dCTP, dGTP, and dTTP or dUTP; and

(e) an antibody capable of specific binding to a protein selected from the group consisting of PAI-1, Vimentin, FOXC1, keratin-8, keratin-18, keratin-19, Ep-CAM, CD45, VWF, PECAM-1, CD146, CD41, CD34, PSMA, CD105, CD309, CD144, CD202B and

Claims:

1. An apparatus for capturing and retrieving a cell from a sample, comprising at least one column, the column comprising:

(i) an inner wall defining an inner chamber, the inner chamber having an inlet opening at a first end of the column for receiving the sample, and an outlet opening at a second end of the column;

(ii) a perforated plug disposed within the inner chamber adjacent to the second end of the column;

(iii) a sleeve insert having an opening at a first end and an opening at a second end, the sleeve insert comprising a channel tapered at the second end and disposed within the inner chamber with its second end adjacent to the perforated plug; and

(iv) a filtering means housed within the sleeve insert, the filtering means comprising a sieve sandwiched between two sealing means.

2. The apparatus of claim 1, comprising one column, or

two or more columns; wherein optionally

the second end of the column is adapted for connection to one or more pumps for controlling flow-rate of the sample through the column; wherein optionally

the pump is adapted for passing the sample at a flow-rate selected from the group consisting of at least ~~about~~ 0.05 mL/min, at least ~~about~~ 0.10 mL/min, at least ~~about~~ 0.15 mL/min, at least ~~about~~ 0.20 mL/min, at least ~~about~~ 0.25 mL/min, at least ~~about~~ 0.30 mL/min, at least ~~about~~ 0.40 mL/min, and at least ~~about~~ 0.50 mL/min; wherein optionally

the sieve comprises non cell-adhesive material; wherein optionally

the non cell-adhesive material is selected from the group consisting of silicon, silicon dioxide, silicon nitride, epoxy-based negative photoresist and ceramic; wherein optionally

the epoxy-based negative photoresist comprises SU-8; and wherein optionally

the sieve comprises a plurality of pores having a diameter selected from the group consisting of at least ~~about~~ 6 µm, at least ~~about~~ 7 µm, at least ~~about~~ 8 µm, at least ~~about~~ 9 µm, at least ~~about~~ 10 µm, at least ~~about~~ 12 µm, at least ~~about~~ 14 µm, at least ~~about~~ 16 µm, at least ~~about~~ 18 µm and at least ~~about~~ 20 µm.

3. A method of capturing and retrieving a cell from a sample, comprising the steps of:

(a) introducing the sample to the inlet opening of the apparatus of claim 1 or 2 to allow the sample to flow through the sleeve insert and filtering means of the apparatus; and

(b) collecting the residue retained on the surface of the sieve in the filtering means of the apparatus.

4. The method of claim 3, wherein the sample comprises a biological fluid optionally

selected from the group consisting of whole blood, blood serum, blood plasma, cerebrospinal fluid, lymph fluid, cystic fluid, sputum, stool, pleural effusion, mucus, ascitic fluid and urine; wherein optionally

the sample comprises a single cell optionally selected from the group consisting of a suspected cancer cell, a suspected tumor-derived cell, a suspected cell derived from an embryo or a foetus, and a cell from a pathogenic organism; or

a plurality of cells wherein at least some of the plurality of cells are optionally selected from the group consisting of: suspected cancer cells, suspected tumor-derived cells, suspected cells derived from an embryo or a foetus, and cells from a pathogenic organism; wherein optionally

two or more of the plurality of cells form a cell cluster; wherein optionally

the single cell or at least some of the plurality of cells is multinucleated; wherein optionally

the sample is a blood sample, and the cell captured and retrieved therefrom comprises at least two clearly distinct nuclei; wherein optionally

the major axis of the cell is at least 10 μm; wherein optionally

the cell expresses a gene selected from the group consisting of *PECAM1, VWF* and *CDH5*; wherein optionally

the cell does not express a gene selected from the group consisting of *PTPRC, ITGA2B* and *GP1BA*; and wherein optionally

collecting the residue in step (b) comprises using a pipette to retrieve the residue.

5. A method for detecting an isolated population of cell clusters in a sample of a subject, the method comprising:

(a) capturing and retrieving the cells from the sample using the apparatus of claim 1 or 2, or the method of claim 3 or 4;

wherein the isolated population of cell clusters is selected from the group consisting of:
(A) an isolated population of cell clusters, each cell cluster comprising at least two cells and having the following characteristics:

(i) being endothelial ~~cell~~cell clusters derived from blood vessel of a colorectal tumor and isolated from blood;

(ii) each cell cluster having at least two clearly distinct nuclei;

(iii) each cell cluster having a major axis of greater than ~~about~~ 10 µm;

(iv) expression of endothelial cell genes or proteins, comprising *FOLH1*;

(v) non-expression of leukocyte-specific genes or proteins; ~~and~~

(vi) non-expression of megakaryocyte or platelets-specific genes or proteins; and

(vii) being cytomorphologically consistent with malignancy but not having primary tumor mutations or chromosomal abnormalities;

and

(B) an isolated population of cell clusters as defined in (A), which expresses endothelial cell genes selected from the group consisting of *PECAM1*, *VWF* and *CDH5*; and which optionally expresses leukocyte and megakaryocytic or platelet-specific genes selected from the group consisting of *PTPRC*, *ITGA2B* and *GP1BA*.

6. The method of claim 5, further comprising the steps of:

(b) contacting the cells from step (a) with at least one antibody coupled to a detectable label to allow binding of the antibody to one or more target biomarkers expressed on the cells;

(c) removing unbound antibody from the sample; and

(d) detecting and analyzing the detectable label bound to the antibody to detect the isolated population of cell clusters.

7. The method of claim 6, wherein the antibody is capable of specific binding to a target biomarker selected from the group consisting of PAI-1, Vimentin, FOXC1, keratin-8, keratin-18, keratin-19, Ep-CAM, CD45, VWF, PECAM-1, CD146, CD41, CD34, PSMA, CD105, CD309, CD144, CD202B and Angiopoietin 2; wherein optionally

the detectable label is selected from the group consisting of a fluorescent group, a radioisotope, a stable isotope, an enzymatic group, a chemiluminescent group and a biotinyl group.

8. The method of claim 5, further comprising the steps of:

(b) lysing the cells from step (a);

(c) contacting the lysed cell sample from step (b) with a reverse primer from a first primer pair, the reverse primer from the first primer pair being directed to a target RNA region, and a reverse transcriptase to effect reverse transcription of the RNA into cDNA;

(d) subsequently contacting the sample from step (c) with:

(i) a forward primer from the first primer pair, the forward primer from the first primer pair being directed to a target cDNA region,

(ii) a reverse primer and a forward primer from a second primer pair, the reverse primer and forward primer from the second primer pair being directed to a target DNA region, and

(iii) a DNA polymerase

to simultaneously amplify the target cDNA region and the target DNA region in a pre-amplification step; and

(e) analyzing the amplified target cDNA region and/or the amplified target DNA region.

9. The method of claim 8, further comprising: subjecting the sample from step (d) to a semi-nested PCR using the reverse primer in step (c) or the forward primer in step (d)(i), and a nested primer that binds within the amplified target cDNA region, or subjecting the sample from step (d) to a nested PCR using a nested primer pair that binds within the amplified target DNA region; wherein optionally steps (c) and (d) are conducted in the same reaction mixture; wherein optionally the analysis in step (e) comprises analyzing the amplified target cDNA for gene expression; and wherein optionally the analysis in step (e) comprises analyzing the amplified target cDNA for mutations.

10. The method of any one of claims 8-9, wherein the first or the second primer pair is selected from the group consisting of: (i) SEQ ID NO: 1 and SEQ ID NO: 2; (ii) SEQ ID NO: 3 and SEQ ID NO: 4; (iii) SEQ ID NO: 5 and SEQ ID NO: 6; (iv) SEQ ID NO: 7 and SEQ

ID NO: 8; (v) SEQ ID NO: 9 and SEQ ID NO: 10; (vi) SEQ ID NO: 11 and SEQ ID NO: 12; (vii) SEQ ID NO: 13 and SEQ ID NO: 14; (viii) SEQ ID NO: 15 and SEQ ID NO: 16; (ix) SEQ ID NO: 17 and SEQ ID NO: 18; (x) SEQ ID NO: 19 and SEQ ID NO: 20; (xi) SEQ ID NO: 21 and SEQ ID NO: 22; (xii) SEQ ID NO: 23 and SEQ ID NO: 24; (xiii) SEQ ID NO: 25 and SEQ ID NO: 26; (xiv) SEQ ID NO: 27 and SEQ ID NO: 28; (xv) SEQ ID NO: 29 and SEQ ID NO: 30; (xvi) SEQ ID NO: 31 and SEQ ID NO: 32; (xvii) SEQ ID NO: 33 and SEQ ID NO: 34; (xviii) SEQ ID NO: 52 and 53; and (xix) SEQ ID NO: 54 and 55; wherein optionally

the primer pair for semi-nested or nested PCR is selected from the group consisting of: (i) SEQ ID NO: 35 and SEQ ID NO: 2; (ii) SEQ ID NO: 3 and SEQ ID NO: 36; (iii) SEQ ID NO: 5 and SEQ ID NO: 37; (iv) SEQ ID NO: 38 and SEQ ID NO: 8; (v) SEQ ID NO: 39 and SEQ ID NO: 10; (vi) SEQ ID NO: 40 and SEQ ID NO: 12; (vii) SEQ ID NO: 41 and SEQ ID NO: 14; (viii) SEQ ID NO: 42 and SEQ ID NO: 16; (ix) SEQ ID NO: 17 and SEQ ID NO: 43; (x) SEQ ID NO: 44 and SEQ ID NO: 20; (xi) SEQ ID NO: 45 and SEQ ID NO: 22; (xii) SEQ ID NO: 46 and SEQ ID NO: 24; (xiii) SEQ ID NO: 25 and SEQ ID NO: 47; (xiv) SEQ ID NO: 27 and SEQ ID NO: 48; (xv) SEQ ID NO: 49 and SEQ ID NO: 30; (xvi) SEQ ID NO: 31 and SEQ ID NO: 50; (xvii) SEQ ID NO: 51 and SEQ ID NO: 34; (xviii) SEQ ID NO: 56 and SEQ ID NO: 57; and (xix) SEQ ID NO: 58 and SEQ ID NO: 55.

11. A kit for use in:

(A) a method of diagnosing a colorectal cancer in a subject, comprising analyzing a sample from the subject for presence of the isolated population of cell clusters as defined in claim 5, wherein presence of the isolated population of cell clusters indicates that the subject has colorectal cancer; or

(B) a method for monitoring and/or predicting the response to treatment of a colorectal cancer patient, the method comprising analyzing a sample obtained from the patient after treatment for determining the number of the isolated population of cell clusters as defined in claim 5, wherein a reduction in the number of the isolated population of cell clusters compared to the number of the isolated population of cell clusters in a baseline sample obtained from the patient prior to treatment indicates that the patient is responding positively to the treatment; or

(C) a method for predicting the response to treatment of a colorectal cancer patient, the method comprising analyzing a sample obtained from the cancer patient before treatment for determining the number of the isolated population of cell clusters as defined in claim 5, wherein an equal or higher number of the isolated population of cell clusters compared to the number of the isolated population of cell clusters in a sample obtained before treatment from a patient or a group of patients that have responded positively to the treatment indicates that the colorectal cancer patient will respond positively to the treatment, and wherein a lower number of the isolated population of cell clusters compared to the number of the isolated

population of cell clusters in a sample obtained before treatment from a patient or a group of patients that have responded positively to the treatment indicates that the colorectal cancer patient will respond negatively to the treatment; or

(D) a method for analyzing blood vessel characteristics of a colorectal tumor in a subject, the method comprising analyzing a sample from the subject for determining the number of the isolated population of cell clusters as defined in claim 5, wherein an increased number of the isolated population of cell clusters compared to a baseline sample indicates that the colorectal tumor has larger blood vessels compared to the baseline sample, and wherein a reduced number of the isolated population of cell clusters compared to a baseline sample indicates that the colorectal tumor has smaller blood vessels compared to the baseline sample; or

(E) the method of any one of claims 3-10,

the kit comprising:

(a) the apparatus of claim 1 or 2.

12. The kit of claim 11, ~~wherein the cancer in the method in (A) is selected from the group consisting of carcinoma, sarcoma, lymphoma, germ cell tumor, blastoma, colon cancer, rectal cancer, breast cancer, prostate cancer, renal cell cancer, transitional cell carcinoma, lung cancer, cholangiocarcinoma, brain cancer, non-small cell lung cancer, pancreatic cancer, gastric carcinoma, bladder cancer, esophageal cancer, mesothelioma, melanoma, thyroid cancer, head and neck cancer, osteosarcoma, hepatocellular carcinoma, carcinoma of unknown primary, ovarian carcinoma, endometrial carcinoma, glioblastoma, neuroblastoma, Hodgkin lymphoma and non-Hodgkin lymphomas;~~ wherein: ~~optionally~~

the colorectal cancer is an invasive and/or metastatic cancer; or

a stage I cancer, stage II cancer, stage III cancer or stage IV cancer; or

an early stage cancer.

13. The kit of claim 11, further comprising one or more of the following:

(b) one or more cell lysis buffers;

(c) a primer selected from the group consisting of:

i. the reverse primer of step (c) of the method of any one of claims 8-10

ii. the forward primer of step (d)(i) of the method of any one of claims 8-10

iii. the primer pair of step (d)(ii) of the method of any one of claims 8-10

iv. the nested primer and nested primer pair of the method of any one of claims 8-10

(d) one or more reagents, selected from the group consisting of:

i. a reverse transcriptase and one or more suitable reaction buffers for the reverse transcription in step (c) of the method of any one of claims 8-10

ii. a DNA polymerase and one or more suitable reaction buffers for the amplification in step (d) of the method of any one of claims 8-10or the semi-nested or nested PCR of the method of claim 9 or 10, and

iii. one or more labelled or unlabelled deoxyribonucleotides selected from the group consisting of dATP, dCTP, dGTP, and dTTP or dUTP; and

(e) an antibody capable of specific binding to a protein selected from the group consisting of PAI-1, Vimentin, FOXC1, keratin-8, keratin-18, keratin-19, Ep-CAM, CD45, VWF, PECAM-1, CD146, CD41, CD34, PSMA, CD105, CD309, CD144, CD202B and Angiopoietin 2, wherein the antibody is coupled to a detectable label; and optionally means for detecting the detectable label.