

identifying fetal cells in a maternal blood sample and also discloses new antigens for this purpose.

In one aspect of the disclosure, there is provided a method of isolating a fetal cell in a maternal blood sample, said method comprising the steps of:

- 5 a. Permeabilizing cells in a maternal blood sample or a fraction thereof,
- b. Contacting the sample with a hybridization probe comprising at least 10 contiguous nucleotides complementary to a gene encoding an endothelial cell marker or a ligand directed to an endothelial cell marker, wherein the endothelial cell marker is selected from the group consisting of CD105,
- 10 CD146 or CD141, Vimentin, VCAM, ICAM, VEGFR-1, VEGFR-2, VEGFR-3, ITGA5, ITGB5, CDH11 or CDH3,
- c. Selecting the cells specific for said endothelial cell marker thereby enriching the sample for cells with endothelial phenotype,
- d. Contacting the cells selected in c) demonstrating an endothelial phenotype
- 15 with a hybridization probe comprising at least 10 contiguous nucleotides complementary to a gene encoding an epithelial cell marker or a ligand directed to an epithelial cell marker, wherein the epithelial cell marker is selected from the group consisting of CK1, CK2, CK3, CK4, CK5, CK6, CK7, CK8, CK9, CK10, CK13, CK14, CK15, CK16, CK17, CK18 or CK19,
- 20 e. Detecting the cells with endothelial phenotype also binding the epithelial cell marker of step d), and
- f. Optionally, diagnosing and/or predicting the genetic content of the cells detected in e),

wherein step a)-e) may be performed in any order.

- 25 In another aspect, the present disclosure refers to a method comprising the steps of

- a. Permeabilizing cells in a maternal blood sample or a fraction thereof,
- b. Contacting the sample with
 - 30 i. a hybridization probe comprising at least 10 contiguous nucleotides complementary to a gene encoding an endothelial cell marker or
 - ii. a ligand directed to an endothelial cell marker
 - , wherein the endothelial cell marker is selected from the group consisting of CD105, CD146 or CD141, Vimentin, VCAM, ICAM, VEGFR-1, VEGFR-2, VEGFR-3, ITGA5, ITGB5, CDH11 or CDH3,
- 35 c. Selecting cells that bind the hybridization probe or the ligand of the previous step and thereby enriching the sample for cells that bind the hybridization probe or the ligand of the previous step, and
- d. Contacting the (enriched) sample with

- i. a hybridization probe comprising at least 10 contiguous nucleotides complementary to a gene encoding an epithelial cell marker or
- ii. a ligand directed to an epithelial cell marker,

wherein the epithelial cell marker is selected from the group consisting of
 5 CK1, CK2, CK3, CK4, CK5, CK6, CK7, CK8, CK9, CK10, CK13, CK14, CK15,
 CK16, CK17, CK18 or CK19.

In a preferred first example of the disclosure the maternal blood sample is contacted with an endothelial cell marker and the cells with endothelial phenotype is thereby enriched by selecting the cells specific for said endothelial cell marker.

10 Such a method of identifying a fetal cell in a maternal blood sample comprises the steps of:

- a. Providing a maternal blood sample or a fraction thereof
- b. Contacting the sample with a hybridization probe comprising at
 15 least 10 contiguous nucleotides complementary to a gene encoding an endothelial cell marker or a ligand binding to an endothelial cell marker and
- c. enriching the cells specific for said endothelial cell marker.
- d. Contacting the cells selected in b) demonstrating an endothelial
 20 phenotype with a hybridization probe comprising at least 10 contiguous nucleotides complementary to a gene encoding an epithelial cell marker or a ligand directed to an epithelial cell marker
- e. Detecting the cells with endothelial phenotype also binding the
 25 epithelial cell marker of step c).
- f. Optionally, diagnosing and/or predicting the genetic content of the cells detected in d)

wherein step b-e may be performed in any order

30 **Brief description of the figures**

Figure 1. Male fetal cell identified by X and Y specific probes. The arrow indicates the fetal cell.

Figure 2. Frequency of fetal cell per ml of maternal blood.

Figure 3. Fetal cell showing pan cytokeratin staining. The arrow indicates the fetal
 35 cell.

Figure 4. Fetal cell showing cytokeratin 7 staining. The arrow indicates the fetal cell.

Figure 5. Fetal cell showing vimentin staining. The arrow indicates the fetal cell.

Claims

1. A method of isolating a fetal cell in a maternal blood sample, said method comprising the steps of:

- 5 a. Permeabilizing cells in a maternal blood sample or a fraction thereof,
- b. Contacting the sample with a hybridization probe comprising at least 10 contiguous nucleotides complementary to a gene encoding an endothelial cell marker or a ligand directed to an endothelial cell marker, wherein the endothelial cell marker is selected from the group consisting of CD105, CD146
- 10 or CD141, Vimentin, VCAM, ICAM, VEGFR-1, VEGFR-2, VEGFR-3, ITGA5, ITGB5, CDH11 or CDH3,
- c. Selecting the cells specific for said endothelial cell marker thereby enriching the sample for cells with endothelial phenotype,
- d. Contacting the cells selected in c) demonstrating an endothelial phenotype
- 15 with a hybridization probe comprising at least 10 contiguous nucleotides complementary to a gene encoding an epithelial cell marker or a ligand directed to an epithelial cell marker, wherein the epithelial cell marker is selected from the group consisting of CK1, CK2, CK3, CK4, CK5, CK6, CK7, CK8, CK9, CK10, CK13, CK14, CK15, CK16, CK17, CK18 or CK19,
- 20 e. Detecting the cells with endothelial phenotype also binding the epithelial cell marker of step d), and
- f. Optionally, diagnosing and/or predicting the genetic content of the cells detected in e),
- wherein step a)-e) may be performed in any order.

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2. The method of claim 1, wherein the target of the endothelial marker is located at the surface of the cell to be identified.

3. The method of claim 1, wherein the target of the epithelial marker is located
- 30 intracellular of the cell to be identified.

4. A method comprising the steps of

- a. Permeabilizing cells in a maternal blood sample or a fraction thereof,
- b. Contacting the sample with

- i. a hybridization probe comprising at least 10 contiguous nucleotides complementary to a gene encoding an endothelial cell marker or
 - ii. a ligand directed to an endothelial cell marker
 - , wherein the endothelial cell marker is selected from the group consisting of CD105, CD146 or CD141, Vimentin, VCAM, ICAM, VEGFR-1, VEGFR-2, VEGFR-3, ITGA5, ITGB5, CDH11 or CDH3,
 - c. Selecting cells that bind the hybridization probe or the ligand of the previous step and thereby enriching the sample for cells that bind the hybridization probe or the ligand of the previous step, and
 - d. Contacting the (enriched) sample with
 - i. a hybridization probe comprising at least 10 contiguous nucleotides complementary to a gene encoding an epithelial cell marker or
 - ii. a ligand directed to an epithelial cell marker,
 - wherein the epithelial cell marker is selected from the group consisting of CK1, CK2, CK3, CK4, CK5, CK6, CK7, CK8, CK9, CK10, CK13, CK14, CK15, CK16, CK17, CK18 or CK19.
5. The method of claim 4 further comprising a step of identifying fetal cells of the sample.
6. The method of any of claims 4 and 5, wherein identification comprises detecting the presence of the ligand or the hybridization probe on or in the fetal cell.
7. The method any of the preceding claims, wherein the maternal blood sample is whole blood.
8. The method of any of the preceding claims, wherein cells in the sample are subjected to a fixation step after the selection/enrichment step.
9. The method of any of the preceding claims, wherein cells in the sample is permeabilized after the fixation step and before being contacted with the epithelial cell marker directed ligand or hybridization probe.
10. The method of any of the preceding claims, wherein the endothelial cell marker is selected from the group consisting of: CD105, CD146 or CD141.

11. The method of any of the preceding claims, wherein the endothelial cell marker is CD105.

5 12. The method of any of the preceding claims, wherein the epithelial cell marker is selected from the group consisting of: CK8, CK18, CK19 or CK7.