

BIOMARKERS TO DETECT AND CHARACTERISE CANCER

CROSS-REFERENCE TO RELATED APPLICATIONS

5 [001] This application claims the benefit of priority of SG provisional application No. 10201708183V, filed 4 October 2017, the contents of it being hereby incorporated by reference in its entirety for all purposes.

FIELD OF THE INVENTION

[002] The present invention relates generally to the field of molecular biology. In particular, the
10 present invention relates to the use of biomarkers for the detection and characterisation of cancer.

BACKGROUND OF THE INVENTION

[003] An invasive tumour phenotype drives faster tumour growth and is often correlated with the formation of metastases and poor prognosis. For most patients with cancers, metastasis is the ultimate
15 cause of mortality. Detection of cancers at an early stage is difficult due to the lack of sensitivity of current methods, as well as the lack of targets available to allow such detection. Most cancers can only be detected at later stages, and, sometimes, at a time when the disease is no longer curable or the symptoms no longer treatable.

[004] Thus there is an unmet need for methods which allow early detection and characterisation of
20 cancer.

SUMMARY

[005] In one aspect, the present disclosure refers to a method of detecting the presence or absence of cancer, wherein the method comprises the steps of: (i) detecting the level of *O*-glycosylation of one or more endoplasmic reticulum (ER)- resident proteins in a sample obtained from a subject; (ii) comparing
25 the level of *O*-glycosylation of one or more endoplasmic reticulum (ER)- resident proteins in step (i) with the level of *O*-glycosylation of one or more endoplasmic reticulum (ER)-resident proteins in a control group; wherein an increase in the level of *O*-glycosylation of one or more endoplasmic reticulum (ER)-resident proteins present in the sample compared to the control group is indicative of the presence of cancer. In one example, the present disclosure refers to a method of detecting the presence or absence of
30 cancer, wherein the method comprises the steps of (i) obtaining a sample from a subject; (ii) detecting the level of *O*-glycosylation of one or more endoplasmic reticulum (ER)-resident proteins in the sample obtained in step (ii); (iii) comparing the level of *O*-glycosylation of one or more endoplasmic reticulum (ER)-resident proteins in step (ii) with the level of *O*-glycosylation of one or more endoplasmic reticulum (ER)-resident proteins in a control group; wherein an increase in the level of *O*-glycosylation of one or
35 more endoplasmic reticulum (ER)-resident proteins present in the sample compared to the control group is indicative of the presence of cancer.

[006] In another aspect, the present disclosure refers to a method of determining the risk of a subject developing cancer, wherein the method comprises the steps of: (i) detecting the level of *O*-glycosylation of one or more endoplasmic reticulum (ER)- resident proteins in a sample obtained from a subject; (ii) comparing the level of *O*-glycosylation of one or more endoplasmic reticulum (ER)- resident proteins in step (i) with the level of *O*-glycosylation of one or more endoplasmic reticulum (ER)-resident proteins in a control group; wherein an at least 4-fold increase in the level of *O*-glycosylation of one or more endoplasmic reticulum (ER)-resident proteins present in the sample compared to the control group is indicative that the subject is suffering from cancer. In another example, the present disclosure refers to a method of determining the risk of a subject developing cancer, wherein the method comprises the steps of (i) obtaining a sample from a subject; (ii) detecting the level of *O*-glycosylation of one or more endoplasmic reticulum (ER)-resident proteins in the sample; (iii) comparing the level of *O*-glycosylation of one or more endoplasmic reticulum (ER)-resident proteins in step (ii) with the level of *O*-glycosylation of one or more endoplasmic reticulum (ER)-resident proteins in a control group; wherein an at least 4-fold increase in the level of *O*-glycosylation of one or more endoplasmic reticulum (ER)-resident proteins present in the sample compared to the control group is indicative that the subject is suffering from cancer.

[007] In another aspect, the present disclosure refers to a method of determining the malignancy, grade, or staging of a cancer, the method comprising (i) detecting the level of *O*-glycosylation of one or more endoplasmic reticulum (ER)- resident proteins in a sample obtained from a subject; (ii) comparing the level of *O*-glycosylation of one or more endoplasmic reticulum (ER)- resident proteins in step (i) with the level of *O*-glycosylation of one or more endoplasmic reticulum (ER)-resident proteins in a group defined for each grade of cancer. In yet another example, the present disclosure refers to a method of determining the malignancy, grade, or staging of a cancer, the method comprising (i) obtaining a sample from a subject; (ii) detecting the level of *O*-glycosylation of one or more endoplasmic reticulum (ER)-resident proteins in the sample; (iii) comparing the level of *O*-glycosylation of one or more endoplasmic reticulum (ER)-resident proteins in step (ii) with the level of *O*-glycosylation of one or more endoplasmic reticulum (ER)-resident proteins in a group defined for each grade of cancer.

[008] In a further aspect, the present disclosure refers to a kit comprising a monosaccharide-binding protein capable of binding to one or more *O*-glycosylated endoplasmic reticulum (ER)-resident proteins; a detection agent capable of binding to the monosaccharide-binding protein and/or the one or more *O*-glycosylated endoplasmic reticulum (ER)-resident proteins; and one or more standards, wherein each standard comprises any one of the *O*-glycosylated endoplasmic reticulum (ER)-resident proteins as disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[009] 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:

[0010] **Fig. 1** is a schematic drawing that illustrates N-acetylgalactosamine (GalNAc)-T Activation Pathway (GALA) activation. During GALA activation, polypeptide N-acetylgalactosaminyltransferases

(GALNTs) relocate from the Golgi to the endoplasmic reticulum (ER), which leads to increased *O*-glycosylation of proteins in the ER, including matrix metalloproteinase-14 (MMP14) and protein disulfide isomerase family A member 4 (PDIA4). *O*-glycosylated matrix metalloproteinase-14 (MMP14) leads to increased extra-cellular matrix (ECM) degradation. Cells with high GALA activation during early stage cancer can lead to rapid tumour growth, neighbouring organ invasion and metastasis during late stage cancer. Conversely, cells with low GALA activation during early stage cancer can lead to slow tumour growth, rare invasion and rare metastasis during late stage cancer.

[0011] **Fig. 2** presents data showing results that malignant liver tumours display high Tn staining and glycosylation of the ER-resident protein PDIA4. **(A)** is a vertical scatter plot that shows the quantification of Tn antigen using the intensity of *Vicia Villosa* lectin (VVL) antibody staining levels during human liver tumour progression. Tissue microarrays (TMA) of human liver biopsies, LV8011 and BC03002 that include normal, benign, malignant (with different grades), and metastatic liver tumours were analysed. Horizontal lines indicate the mean of each group. **(B)** shows four images that are close-up images of the representative cores in the tissue microarray (TMA) BC03002 that is stained with *Vicia Villosa* lectin (VVL) antibody. Scale bar, 100 μ m. **(C)** shows two images that are immunohistofluorescence analysis of Tn using *Helix pomatia* lectin (HPL) antibodies to stain mouse hepatocellular carcinoma (HCC). Scale bar, 100 μ m. Asterisks mark background staining of red blood cells. **(D)** shows six images that show co-staining of *Vicia Villosa* lectin (VVL) and ER-marker Calnexin in mouse hepatocellular carcinoma

CLAIMS

1. A method of detecting the presence or absence of cancer, wherein the method comprises the steps of:

- (i) detecting the level of *O*-glycosylation of one or more endoplasmic reticulum (ER)-resident proteins in a sample obtained from a subject;
- (ii) comparing the level of *O*-glycosylation of one or more endoplasmic reticulum (ER)-resident proteins in step (i) with the level of *O*-glycosylation of one or more endoplasmic reticulum (ER)-resident proteins in a control group;

wherein an increase in the level of *O*-glycosylation of one or more endoplasmic reticulum (ER)-resident proteins present in the sample compared to the control group is indicative of the presence of cancer.

2. A method of determining the risk of a subject developing cancer, wherein the method comprises the steps of:

- (i) detecting the level of *O*-glycosylation of one or more endoplasmic reticulum (ER)-resident proteins in a sample obtained from a subject;
- (ii) comparing the level of *O*-glycosylation of one or more endoplasmic reticulum (ER)-resident proteins in step (i) with the level of *O*-glycosylation of one or more endoplasmic reticulum (ER)-resident proteins in a control group;

wherein an at least 4-fold increase in the level of *O*-glycosylation of one or more endoplasmic reticulum (ER)-resident proteins present in the sample compared to the control group is indicative that the subject is suffering from cancer.

3. The method according to any one of claims 1 to 2, wherein the detection of the level of *O*-glycosylation of one or more endoplasmic reticulum (ER)-resident proteins comprises contacting the sample with a monosaccharide-binding protein.

4. The method according to any one of the preceding claims, wherein the one or more endoplasmic reticulum (ER)-resident proteins is selected from the group consisting of protein disulfide isomerase family A member 4 (PDIA4), calnexin (CANX), protein disulfide isomerase family A member 3 (PDIA3), Endoplasmic Reticulum Lectin 1 (ERLEC1) and heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa) (HSPA5/GRP78/Bip).

5. The method according to claim 4, wherein the one or more endoplasmic reticulum (ER)-resident proteins is protein disulfide isomerase family A member 4 (PDIA4) and/or calnexin (CANX).

6. The method according to claim 3, wherein the monosaccharide-binding protein is an N-acetylgalactosamine binding protein.

7. The method according to claim 6, wherein the N-acetylgalactosamine binding protein is selected from the group consisting of *Vicia villosa* lectin (VVL), *Helix pomatia* lectin A (HPL), ricin (RCA), peanut agglutinin (PNA) and jacalin (AIL).

8. The method according to any one of claims 6 to 7, wherein the N-acetylgalactosamine binding protein is either *Vicia villosa* lectin (VVL) or *Helix pomatia* lectin A (HPL).

9. The method of any one of the preceding claims, wherein the cancer is selected from the group consisting of liver cancer, breast cancer, lung cancer, hepatocellular carcinoma (HCC), hepatocellular adenoma (HCA), fibrolamellar hepatocellular carcinoma (FHCC), hepatoblastoma, focal nodular hyperplasia (FNH), nodular regenerative hyperplasia, ductal carcinoma in situ (DCIS), Paget's disease of the breast, comedocarcinoma, invasive ductal carcinoma (IDC), intraductal papilloma, lobular carcinoma in situ (LCIS), invasive lobular carcinoma (ILC), medullary carcinoma, inflammatory breast cancer, non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC).

10. The method of any one of the preceding claims, wherein the cancer is malignant.

11. The method of any one of the preceding claims, wherein the control group is a disease-free group.

12. A method of determining the malignancy, grade, or staging of a cancer, the method comprising

- (i) detecting the level of *O*-glycosylation of one or more endoplasmic reticulum (ER)-resident proteins in a sample obtained from a subject;
- (ii) comparing the level of *O*-glycosylation of one or more endoplasmic reticulum (ER)-resident proteins in step (i) with the level of *O*-glycosylation of one or more endoplasmic reticulum (ER)-resident proteins in a group defined for each grade of cancer.

13. A kit comprising:

- (i) a monosaccharide-binding protein capable of binding to one or more *O*-glycosylated endoplasmic reticulum (ER)-resident proteins;
- (ii) a detection agent capable of binding to the monosaccharide-binding protein and/or the one or more *O*-glycosylated endoplasmic reticulum (ER)-resident proteins; and
- (iii) one or more standards, wherein each standard comprises any one of the *O*-glycosylated endoplasmic reticulum (ER)-resident proteins according to any one of claims 4 to 5.

14. The kit according to claim 13, wherein the kit is used to determine the level of the one or more *O*-glycosylated endoplasmic reticulum (ER)-resident proteins in a sample according to the methods as defined in any one of the preceding claims; and/or compare the level of the one or more *O*-glycosylated endoplasmic reticulum (ER)-resident proteins according to the method as defined in any one of the preceding claims to a baseline level provided by the standard.

15. The kit according to any one of claims 13 to 14, wherein the kit is an enzyme-linked immunosorbent assay (ELISA).

16. The kit of claim 15, wherein the ELISA kit comprises:

- (i) a microwell plate;
- (ii) a sample diluent;
- (iii) a wash buffer;
- (iv) a substrate solution that can be detected using the detection agent; and
- (v) a stop solution capable of reacting with the substrate solution and allowing visualisation.