A DIAGNOSTIC AND THERAPEUTIC TOOL FOR CANCER

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of Singapore patent application No. 10201402829Q, filed 2 June 2014, the contents of it being hereby incorporated by reference in its entirety for all purposes.

TECHNICAL FIELD

[0002] The present invention relates to biochemistry in particular biomarkers. In particular, the present invention relates to a biomarker associated with cancer and methods of using the biomarker to determine the likelihood that a patient suffers from or progressing in cancer.

BACKGROUND ART

[0003] Cancer is a disease caused by the unregulated proliferation of cells due to loss and normal cellular controls. Cancer can develop in any tissue or organ at any age. The most common causes of cancer death are cancers of lung, liver, stomach, colorectal, breast, and oesophageal cancer. In patients with cancer, abnormal cells rapidly grow beyond their usual boundaries and can invade adjoining parts of the body and metastasized. Metastases are the major cause of death from cancer.

[0004] According to the World Health Organisation (WHO), cancer is one of the leading causes of death in the world and with the increase in population growth and aging, the number of new cases is expected to rise by about 70% over the next two years. In fact, it is expected that annual cancer cases will increase from significantly within the next two decades.

[0005] Cell surface molecules defining various signalling pathways are crucial for many cancer progressions. Certain markers or biomarkers for determining the likelihood of a patient suffering from or progressing in cancer is known in the art. It is an object of the present invention to provide further biomarkers, methods and uses thereof suitable for use in cancer.

SUMMARY OF INVENTION

[0006] In one aspect, there is provided method for determining the likelihood of the presence or progression of a hepatocellular carcinoma in a subject comprising determining the level of extracellular form of Agrin in a blood sample obtained from the subject, wherein an increased level of the extracellular form of Agrin in the blood sample is indicative of the presence or progression of the hepatocellular carcinoma, wherein an increased level is determined by comparison of the level of extracellular form of Agrin in the subject with the extracellular form of Agrin in a non-diseased subject. Disclosed is a method for determining the likelihood of the presence or progression of a hepatocellular carcinoma in a subject. The method comprises determining the level of extracellular form of Agrin in blood sample obtained from the subject, wherein an increased level of the extracellular form of Agrin in the blood sample is indicative of the presence or progression of the hepatocellular carcinoma, wherein an increased level is determined by comparison of the level of extracellular form of Agrin in the subject with the extracellular form of Agrin in a non-diseased subject.

[0007] In another aspect, there is provided a method of determining the presence of a breast cancer in a subject comprising determining the presence of membrane-bound Agrin in a tissue sample obtained from the subject, wherein the presence of membrane-bound Agrin in the tissue sample is indicative of the breast cancer.

[0008] Also disclosed is an anti-Agrin agent for use in therapy, wherein the agent is an inhibitory nucleic acid selected from the group consisting of an antisense RNA, a ribozyme, a short inhibitory RNA (siRNA), and a micro RNA (miRNA).

[0009] Also disclosed is a pharmaceutical composition comprising the anti-Agrin agent as described herein and a pharmaceutically-acceptable carrier or diluent.

[0010] In another aspect, there is provided the use of a composition comprising an anti-Agrin agent as defined herein in the manufacture of a medicament for treating a cancer in a subject in need thereof, wherein the cancer is one selected from the group consisting of a hepatocellular carcinoma and a cholangiocarcinoma, wherein the anti-Agrin agent is an anti-Agrin antibody or an inhibitory nucleic acid that specifically inhibits the translation of Agrin in the cancer cell.

[0011] In one example, the subject is a human.

[0012] Also disclosed is a kit for carrying out the method as defined herein or for the use as defined herein.

BRIEF DESCRIPTION OF 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] **Fig.1** shows the process of identifying Agrin as an overexpressed cell surface protein in hepatocellular carcinoma cell lines. In particular, (a) shows a schematic workflow of SILAC (Stable isotope labelling by amino acids in cell culture) mass spectrometry based analysis of cell surface proteins where cells of the cell lines MIHA and Hep3B are labelled with 'heavy' and 'light' amino acids and vice-versa; (b) shows the ratio intensity plot representing protein fold change (SILAC ratio vs corresponding summed peptide intensity distribution) and protein density plot (upper panel). Right, left and centre cluster indicates up-, down- and un-regulated proteins; (c) shows gene ontology Cellular Component analysis (GO) of up-regulated proteins in Hep3B cells. The scale bar (Y-axis right hand side) indicates the P-values, while the numbers on top of each bar denotes the abundance of identified proteins in the particular cellular component; (d) shows Western blot analysis of biotinylated cell surface proteins and total cell lysates from the indicated cell lines; (e) shows Western blot evaluation of Agrin expression in non-tumourigenic, hepatocellular (HCC) and selected breast carcinoma cell lines. β-actin is used as loading control; (f) shows Western blot analysis of supernatants from serum starved (12h) cell lines, which were concentrated and precipitated with Tri-chloroacetic acid (TCA). Total cell lysates from the same cell lines were also probed for Agrin and β-actin as loading control; and (g) shows levels of Agrin in mouse xenografts of indicated cell lines analysed by Western blot

CLAIMS

1. A method for determining the likelihood of the presence or progression of a hepatocellular carcinoma in a subject comprising

   determining the level of extracellular form of Agrin in a blood sample obtained from the subject,

   wherein an increased level of the extracellular form of Agrin in the blood sample is indicative of the presence or progression of the hepatocellular carcinoma,

   wherein an increased level is determined by comparison of the level of extracellular form of Agrin in the subject with the extracellular form of Agrin in a non-diseased subject.

2. The method of claim 1, wherein the extracellular Agrin is a splice variant of Agrin that is secreted in extracellular matrix.

3. The method of claim 1 or 2, wherein the Agrin comprises the C-terminal fragment portion (C20) 20 KDa protein fragment.

4. A method of determining the presence of a breast cancer in a subject comprising determining the presence of membrane-bound Agrin in a tissue sample obtained from the subject, wherein the presence of membrane-bound Agrin in the tissue sample is indicative of the breast cancer.

5. The method of claim 4, wherein the tissue sample is a breast tissue or a lymph node.

6. The method of any one of claims 1 to 5, wherein the subject is a human.

7. Use of a composition comprising an anti-Agrin agent in the manufacture of a medicament for treating a cancer in a subject in need thereof, wherein the cancer is one selected from the group consisting of a hepatocellular carcinoma and a cholangiocarcinoma, wherein the anti-Agrin agent is an anti-Agrin antibody or an inhibitory nucleic acid that specifically inhibits the translation of Agrin in the cancer cell.

8. The use of claim 7, wherein the inhibitory nucleic acid is selected from an antisense RNA, a ribozyme, a short inhibitory RNA (siRNA), and a micro RNA (miRNA).

9. The use of claim 7, wherein the anti-Agrin agent is an anti-Agrin antibody.

10. The use of claim 9, wherein the antibody binds to or recognizes SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4 and/or SEQ ID NO: 5.

11. The use of claim 9 or 10, wherein the antibody is a monoclonal antibody or a polyclonal antibody.

12. The use of claim 11, wherein the antibody is selected from the group consisting of neutralizing antibody such as Agrin antibody (D-2): sc-374117 and anti-Agrin antibody MAB5204.

13. The use of any one of claims 9 to 12, wherein the antibody is provided as a humanized antibody.

14. The use of any one of claims 7 to 13, wherein the subject is a human.