

[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 cancer.

5 [0008] In another aspect, there is provided an anti-Agrin agent for use in therapy.

[0009] In another aspect, there is provided a pharmaceutical composition comprising the anti-Agrin agent as described herein and a pharmaceutically-acceptable carrier or diluent.

10 [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 a method of treating a cancer in a subject in need thereof, comprising administering a composition comprising an anti-Agrin agent as defined herein.

[0011] In one example, the subject is a human. In another aspect, there is provided a use of an anti-Agrin agent in the manufacture of a medicament for treating cancer.

15 [0012] In another aspect, there is provided a kit for carrying out the method as defined herein or for the ~~method use~~ 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:

20 [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
30 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
35 (g) shows levels of Agrin in mouse xenografts of indicated cell lines analysed by Western blot

about 80%, or at least about 90% or up to and including a 100% reduction (e.g. substantially complete elimination of tumour), or any decrease between 10-100% as compared to the size of the tumour in the subject before treatment with anti-Agrin. In one example, the inhibitory nucleic acid may include, but is not limited to, antisense RNA, a ribozyme, a short inhibitory RNA (siRNA), and a microRNA (mRNA/miRNA).

[0053] As used herein the term “cancer”, or “tumour”, are well known in the art and refer to the presence of cells possessing characteristics typical of cancer-causing cells, such as uncontrolled proliferation, immortality, metastatic potential, rapid growth and proliferation rate, decreased cell death/apoptosis, and certain characteristic morphological features. For example, cancer cells are often in the form of a solid tumour. In another example, cancer may also include non-solid tumours such as blood tumours, for example leukaemia, where the cells are derived from bone marrow. In one example, the term “cancer” may include pre-malignant cancers. In another example, the term “cancer” may include malignant cancer. In yet another example, the term “cancer” may include both pre-malignant cancer and malignant cancer. In one example, the term cancer may include, but is not limited to, hepatocellular carcinoma, a breast cancer carcinoma and a cholangiocarcinoma.

[0054] As used herein, the term “subject” may be used interchangeably with “patient” and refers to an animal, including mammals, such as human, bovine, porcine, equine, canine, lupine, feline, murine and the like. In one example, the “subject” is a human.

[0055] In one example, the anti-Agrin agent is an antibody. As used herein, the term “antibody” refers to all types of antibodies that specifically bind to Agrin. In one example, the antibody may be an antibody that functionally blocks the c-terminally active signalling component of membrane bound Agrin. In one example, the targeting of C terminal fragment of Agrin (secreted Agrin) may contain the C-terminal fragment portion (C20) 20 kDa protein fragment. The sequence of the 20 kDa fragment bearing the epitopes for the function blocking antibodies is as follows:

[0056] Human and rat Agrin (C20 fragment) sequence showing the epitopes for Agrin monoclonal antibodies D2 (bold) and MAb5204 (underlined) is as follows:

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sp|O00468|AGRIN_HUMAN   GFSGPHCEKGLVEKSAGDVDTLAFDGRTFVEYLVNAVTESELANEIPVPET 1899
sp|P25304|AGRIN_RAT    GFSGLHCEKGLVEKSVGDLETLAFDGRITYEYLVNAVIESELNEIPAPET 1790
*****
sp|O00468|AGRIN_HUMAN   LD SGALH-EKALQSNHFELSLRTEATQGLVLWSGKATERADYVALAIVDG 1948
sp|P25304|AGRIN_RAT    LD SRALFSEKALQSNHFELSLRTEATQGLVLWIGKAAERADYMAIAIVDG 1840
*****
sp|O00468|AGRIN_HUMAN   HLQLSYNLGSQPVVLRSTVPVNTNRWLRVVAHREQREGSLQVGNEAPVTG 1998
sp|P25304|AGRIN_RAT    HLQLSYDLGSQPVVLRSTVKVNTNRWLRIRAHREHREGSLQVGNEAPVTG 1890
*****
sp|O00468|AGRIN_HUMAN   SSPLGATQLD TDGALWLGGLPELPVGPALPKAYGTGFVGCLRDVVVGRHP 2048
sp|P25304|AGRIN_RAT    SSPLGATQLD TDGALWLGGLQKLPVGPALPKAYGTGFVGCLRDVVVGRHQ 1940
*****
sp|O00468|AGRIN_HUMAN   LHLLEDAVTKPELRPCPTP 2067 – SEQ ID NO: 1
sp|P25304|AGRIN_RAT    LHLLEDAVTKPELRPCPTP 1959 – SEQ ID NO: 2

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[00167] **Agrin is frequently overexpressed in hepatocellular carcinoma patients**

[00168] Finally to establish a clinical relevance of Agrin's role in HCC, microarray datasets generated from a cohort of liver cancer patients were analysed. Consistent with the findings described above, a 3.5 and 3.9 fold up-regulation of Agrin (AGRN) mRNA and miRNA were observed in HCC patients (compared to normal liver tissues) in two independent datasets, with P values of 6.22e-6 and 1.22e-7, respectively (Fig.8a). Western blot analysis of matched normal (nontumour; N) and HCC (liver-tumour; T) samples of HCC patients revealed a significant upregulation of Agrin in 9 out of 11 HCC patients (Fig.8b). Similarly compared to normal liver tissues, Agrin overexpression was also observed across various stages of HCC (Fig.16). Furthermore, circulating levels of plasma Agrin were also majorly increased in HCC patients when compared to normal individuals (Fig.8c). Taken together, these clinical evidences confirmed that Agrin is overexpressed in HCC and associated with increased levels of circulating Agrin.

[00169] Comprehensive lines of evidence presented here identify a unique role of extracellular matrix and cell surface Agrin in providing oncogenic cues that activate focal adhesions to drive liver tumourigenesis. The present disclosure demonstrates Agrin's role in *in vitro* and *in vivo* liver tumourigenic potential by manipulating cellular migration and adhesion dynamics. Together with gaining insights into the molecular mechanisms of Agrin function in cellular adhesion and invasion, the present Example section also validated the frequent up-regulation of Agrin in clinical hepatocellular carcinoma (HCC) patient samples. Since cell surface proteins are the best known therapeutic targets in most cancers, the results described above shows that targeting Agrin suppresses liver carcinogenesis through decreased focal adhesion activity, improper adhesion dynamics, loss of cell migration and invasiveness.

[00170] Proteomic screens offer robust and unbiased approach to screen candidates in many cancer types. It is known that instances of quantitative screening of cell membrane proteins in liver cancer are rare. Therefore, SILAC screen identifying several differentially expressed cell surface proteins in Hep3B cells compared to MIHA cells was designed accordingly. Apart from the global identification of several unique candidates, some identified proteins are established key players in hepatocellular carcinoma and other cancers. Through the surprising finding of the inventors, Agrin has been demonstrated to be secreted and enriched in lipid rafts from where it can be actively endocytosed. Such endocytosis of over expressed receptors is often associated with signal amplification in many cancers, such as hepatocellular carcinoma, breast cancer and cholangiocarcinoma.

[00171] EMT program in cancer is achieved when invading tumour cells breach through the underlying basement membrane and ECM. However, the integrity of basement membrane

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 an extracellular fluid obtained from the subject, wherein an increased level of the extracellular form of Agrin in the extracellular fluid is indicative of the presence or progression of the cancer, 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. The method of any one of claims 1 to 3, wherein the extracellular fluid is whole blood.
5. 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 cancer.
6. The method of claim 5, wherein the tissue sample is a breast tissue or a lymph node.
7. The method of any one of claims 1 to 6, wherein the subject is a human.
8. An anti-Agrin agent for use in therapy.
9. The anti-Agrin agent of claim 8 is an anti-Agrin antibody or an inhibitory nucleic acid that specifically inhibits the translation of Agrin in a cancer cell.
10. The anti-Agrin agent of claim 9, wherein the inhibitory nucleic acid is selected from an antisense RNA, a ribozyme, a short inhibitory RNA (siRNA), and a micro RNA (miRNA).
11. The anti-Agrin agent of claim 8, wherein the anti-Agrin agent is an antibody.
12. The anti-Agrin agent of claim 11, wherein the anti-Agrin antibody is an antibody functionally blocks the c-terminally active signaling component of membrane bound Agrin.
13. The anti-Agrin agent of claim 11 or 12, wherein the antibody functionally blocks the binding of Agrin to Lipoprotein related receptor 4 (Lrp4) and muscle specific receptor tyrosine kinases (MuSK) complex.
14. The anti-Agrin agent of any one of claims 11 to 13, 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.
15. The anti-Agrin agent of any one of claims 11 to 14, wherein the antibody is a monoclonal antibody or a polyclonal antibody.

16. The anti-Agrin agent of any one of claims 11 to 15, wherein the antibody is provided as a humanized antibody.
17. A pharmaceutical composition comprising the anti-Agrin agent as claimed in any one of claims 8 to 16 and a pharmaceutically-acceptable carrier or diluent.
18. Use of a composition comprising an anti-Agrin agent as claimed in any one of claims 8 to 16 in the manufacture of a medicament for treating a cancer in a subject in need thereof.
19. The use of claim 18, wherein the anti-Agrin agent is an anti-Agrin antibody or an inhibitory nucleic acid that specifically inhibits the translation of Agrin in a cancer cell.
20. The use of claim 19, wherein the inhibitory nucleic acid is selected from an antisense RNA, a ribozyme, a short inhibitory RNA (siRNA), and a micro RNA (miRNA).
21. The use of claim 19, wherein the anti-Agrin agent is an anti-Agrin antibody.
22. The use of claim 21, wherein the antibody functionally blocks the c-terminally active signaling component of membrane-bound Agrin.
23. The use of claim 21 or 22, wherein the antibody functionally blocks the binding of Agrin to muscle specific receptor tyrosine kinases (MuSK).
24. The use of any one of claims 21 to 23, 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.
25. The use of any one of claims 21 to 24, wherein the antibody is a monoclonal antibody or a polyclonal antibody.
26. The use of claim 25, 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.
27. The use of any one of claims 21 to 26, wherein the antibody is provided as a humanized antibody.
28. The use of any one of claims 18 to 27, wherein the cancer is a hepatocellular carcinoma, a breast cancer carcinoma and a cholangiocarcinoma.
29. The use of any one of claims 18 to 28, wherein the subject is a human.
30. A kit for carrying out the method of any one of claims 1 to 7 or for the use of claim 18 to 29.

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 an extracellular fluid obtained from the subject, wherein an increased level of the extracellular form of Agrin in the extracellular fluid is indicative of the presence or progression of the cancer, 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. The method of any one of claims 1 to 3, wherein the extracellular fluid is whole blood.
5. 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 cancer.
6. The method of claim 5, wherein the tissue sample is a breast tissue or a lymph node.
7. The method of any one of claims 1 to 6, wherein the subject is a human.
8. An anti-Agrin agent for use in therapy.
9. The anti-Agrin agent of claim 8 is an anti-Agrin antibody or an inhibitory nucleic acid that specifically inhibits the translation of Agrin in a cancer cell.
10. The anti-Agrin agent of claim 9, wherein the inhibitory nucleic acid is selected from an antisense RNA, a ribozyme, a short inhibitory RNA (siRNA), and a micro RNA (mRNA).
11. The anti-Agrin agent of claim 10, wherein the anti-Agrin agent is an antibody.
12. The anti-Agrin agent of claim 11, wherein the anti-Agrin antibody is an antibody functionally blocks the c-terminally active signaling component of membrane bound Agrin.
13. The anti-Agrin agent of claim 11 or 12, wherein the antibody functionally blocks the binding of Agrin to Lipoprotein related receptor 4 (Lrp4) and muscle specific receptor tyrosine kinases (MuSK) complex.
14. The anti-Agrin agent of any one of claims 11 to 13, 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.
15. The anti-Agrin agent of any one of claims 11 to 14, wherein the antibody is a monoclonal antibody or a polyclonal antibody.

16. The anti-Agrin agent of any one of claims 11 to 15, wherein the antibody is provided as a humanized antibody.
17. A pharmaceutical composition comprising the anti-Agrin agent as claimed in any one of claims 8 to 16 and a pharmaceutically-acceptable carrier or diluent.
- 5 18. A method of treating a cancer in a subject in need thereof, comprising administering a composition comprising an anti-Agrin agent as claimed in any one of claims 8 to 16.
19. The method of claim 18, wherein the anti-Agrin agent is an anti-Agrin antibody or an inhibitory nucleic acid that specifically inhibits the translation of Agrin in a cancer cell.
- 10 20. The method of claim 19, wherein the inhibitory nucleic acid is selected from an antisense RNA, a ribozyme, a short inhibitory RNA (siRNA), and a micro RNA (mRNA).
21. The method of claim 19, wherein the anti-Agrin agent is an anti-Agrin antibody.
22. The method of claim 21, wherein the antibody functionally blocks the c-terminally active signaling component of membrane-bound Agrin.
- 15 23. The method of claim 21 or 22, wherein the antibody functionally blocks the binding of Agrin to muscle specific receptor tyrosine kinases (MuSK).
24. The method of any one of claims 21 to 23, 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.
25. The method of any one of claims 21 to 24, wherein the antibody is a monoclonal antibody or a polyclonal antibody.
- 20 26. The method of claim 25, 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.
27. The method of any one of claims 21 to 26, wherein the antibody is provided as a humanized antibody.
- 25 28. The method of any one of claims 18 to 27, wherein the cancer is a hepatocellular carcinoma, a breast cancer carcinoma and a cholangiocarcinoma.
29. The method of any one of claims 18 to 28, wherein the subject is a human.
30. Use of an anti-Agrin agent in the manufacture of a medicament for treating cancer.
- 30 31. A kit for carrying out the method of any one of claims 1 to 7 or for the method of claim 18 to 29.