SUMMARY OF THE INVENTION

The present invention is based, in part, on the discovery that glypican-1 heparan sulfate proteoglycan (GPC-1) levels are elevated in the body fluids or tissues of prostate cancer patients. The present inventors have discovered that glypican-1 is a new marker for prostate cancer. Accordingly, the present invention provides for methods of detecting glypican-1 to determine the existence of prostate cancer in patients.

In one aspect, the present invention provides a method of detecting prostate cancer in a patient, the method comprising measuring the level of soluble glypican-1 in a body fluid sample from a patient and determining that said patient has prostate cancer or an increased likelihood of developing prostate cancer based upon the level of soluble glypican-1 in the body fluid sample.

In one example ~~embodiment~~, the disclosure ~~invention~~ provides for methods for detecting prostate cancer in a patient comprising obtaining a body fluid or tissue sample from a patient, contacting said sample with an anti-glypican-1 antibody, determining that said patient has prostate cancer or an increased likelihood of developing prostate cancer based upon binding of said anti-glypican-1 antibody to said body fluid or tissue sample. In some examples ~~embodiments~~ the levels of one or more additional prostate markers are measured in the body fluid or tissue sample, and the determination that the patient has cancer is based upon the levels of glypican-1 and the levels of the one or more additional markers in the patient body fluid or tissue sample. In some examples ~~embodiments~~, the cancer is prostate cancer, and the additional marker is PSA. In some examples ~~embodiments~~, the anti-glypican-1 antibody is MIL-38. In other examples ~~embodiments~~, the anti-glypican-1 antibody is not MIL-38. In some examples ~~embodiments~~, the anti-glypican-1 antibody is an antibody fragment or recombinant antibody capable of binding glypican-1. In some examples ~~embodiments~~, the anti-glypican-1 antibody is labeled for easy detection. In some examples ~~embodiments~~ the antibody label can be a fluorescent label, a biotin-avidin amplification system, a chemiluminescence system, microspheres or colloidal gold among others.

In some examples ~~embodiments~~ the body fluid sample obtained from the patient is a blood, serum, plasma, or urine sample.

In one example ~~embodiment~~, the anti-glypican-1 antibody binding to the patient's body fluid or tissue sample is compared to the level of anti-glypican-1 antibody binding of a control sample; wherein increased anti-glypican-1 antibody binding of the body fluid or tissue sample over the control sample is associated with the presence of prostate cancer. In

some examples ~~embodiments~~, said control sample comprises the body fluid from an age-matched prostate cancer-free patient.

In other examples ~~embodiments~~, the level of anti-glypican-1 antibody binding to the patient's body fluid or tissue is compared to the level of anti-glypican-1 antibody binding to a reference standard, wherein increased anti-glypican-1 antibody binding of the body fluid or tissue sample over the reference standard sample is associated with the presence of prostate

cancer. In some examples ~~embodiments~~, said reference standard comprises a sample with known glypican-1 content. In some examples ~~embodiments~~, the comparison of the anti-glypican-1 binding to the body fluid or tissue is compared to the anti-glypican-1 binding to the glypican-1 standards to quantify the amount of glypican-1 in said body fluid.

In some examples ~~embodiments~~, glypican-1 content higher than about: 0.1ng/ml, 0.5ng/ml, 1ng/ml, 5ng/ml, 10ng/ml, 15ng/ml or 20ng/ml in the body fluid sample is indicative of prostate cancer.

The diagnostic methods of the disclosure ~~invention~~ may further comprise administering one or more prostate cancer treatments to a patient, and following changes in the level of glypican-1 in body fluids or tissues as a mechanism to monitor patient recovery or responses to the prostate cancer treatments. In some examples ~~embodiments~~, the anti-glypican-1 antibody binding is detected via techniques such as immunofluorescence, radiolabeling, immunoblotting, enzyme-linked immunoassay, flow cytometry, optical density, and chemiluminescence.

In another aspect, the present invention provides use of a kit comprising: a first anti-glypican-1 antibody, and glypican-1 standards; for detecting prostate cancer in the body fluid of a patient, wherein the first anti-glypican-1 antibody is used to detect soluble glypican-1 in the body fluid.

The present disclosure ~~invention~~ also includes kits for detecting glypican-1 in the body fluids or tissues of patients. In one example ~~embodiment~~ the kit for detecting prostate cancer comprises a first anti-glypican-1 antibody, a pharmaceutically acceptable carrier, and glypican-1 standards; wherein said kit is capable of detecting glypican-1 in the body fluid or tissue of a patient. In some examples ~~embodiments~~ the kit further comprises a secondary ligand. In some examples ~~embodiments~~ the secondary ligand is a second anti-glypican-1 antibody. In one example ~~embodiment~~, the second anti-glypican-1 antibody is the same as the first anti-glypican-1 antibody.

In some examples ~~embodiments~~, the secondary ligand is conjugated to a label for rapid detection of said ligand.

The present disclosure ~~invention~~ thus relates at least to the following series of numbered examples ~~embodiments~~ below:

Example ~~Embodiment~~ 1: A method of detecting prostate cancer in a patient, the method comprising measuring the level of glypican-1 in a body fluid sample from a patient

and determining that said patient has prostate cancer or an increased likelihood of developing prostate cancer based upon the level of glypican-1 in the body fluid sample.

Example ~~Embodiment~~ 2: The method of detecting prostate cancer in a patient of example ~~embodiment~~ 1, comprising the steps of:

(a) obtaining a body fluid sample from a patient;

(b) contacting said body fluid sample with an anti-glypican-1 antibody; and

(c) determining that said patient has prostate cancer or an increased likelihood of developing prostate cancer based upon binding of said anti-glypican-1 antibody to said body fluid sample.

Example ~~Embodiment~~ 3: The method of example ~~embodiment~~ 2, wherein said anti-glypican-1 antibody is MIL-38.

Example ~~Embodiment~~ 4: The method of example ~~embodiment~~ 2, wherein said body fluid sample is contacted with a population of antibodies, wherein:

antibodies of the population comprise:

(a) a heavy chain variable region comprising:

a complementarity determining region 1 (CDR1) comprising or consisting of an amino acid sequence defined by positions 50-54 of **SEQ ID NO: 10**;

a complementarity determining region 2 (CDR2) comprising or consisting of an amino acid sequence defined by positions 69-85 of **SEQ ID NO: 10**;

a complementarity determining region 3 (CDR3) comprising or consisting of an amino acid sequence defined by positions 118-126 of **SEQ ID NO: 10**; and

(b) a light chain variable region comprising:

a complementarity determining region 1 (CDR1) comprising or consisting of an amino acid sequence defined by positions 44-54 of **SEQ ID NO: 11**;

a complementarity determining region 2 (CDR2) comprising or consisting of an amino acid sequence defined by positions 70-76 of **SEQ ID NO: 11**;

a complementarity determining region 3 (CDR3) comprising or consisting of an amino acid sequence defined by positions 109-117 of **SEQ ID NO: 11**; and

antibodies of the population do not comprise a light chain variable region comprising:

a complementarity determining region 1 (CDR1) comprising or consisting of an amino acid sequence defined by positions 48-58 of **SEQ ID NO: 12**;

a complementarity determining region 2 (CDR2) comprising or consisting of an amino acid sequence defined by positions 74-80 of **SEQ ID NO: 12**;

a complementarity determining region 3 (CDR3) comprising or consisting of an amino acid sequence defined by positions 113-121 of **SEQ ID NO: 12**.

Example ~~Embodiment~~ 5: The method of example ~~embodiment~~ 4, wherein the antibody population is produced by or otherwise identical to an antibody population as generated by hybridoma cells

deposited on 22 August 2014 at CellBank Australia (CBA) under accession number CBA20140026.

Example ~~Embodiment~~ 6: The method of example ~~embodiment~~ 2, wherein said anti-glypican-1 antibody is not MIL-38.

Example ~~Embodiment~~ 7: The method of any one of examples ~~embodiments~~ 2 to 4, wherein said anti-glypican-1 antibody is an antibody fragment or recombinant antibody capable of binding glypican-1.

Example ~~Embodiment~~ 8: The method of any one of examples ~~embodiments~~ 2 to 7, wherein said anti-glypican-1 antibody is labeled.

Example ~~Embodiment~~ 9: The method of example ~~embodiment~~ 8, wherein said label is chosen from a group consisting of a radiolabel, a fluorescent label, a biotin-avidin amplification system, a chemiluminescence system, microspheres, and colloidal gold.

Example ~~Embodiment~~ 10: The method of any one of examples ~~embodiments~~ 2 to 9, wherein anti-glypican-1 antibody binding is detected via a technique selected from the group consisting of immunofluorescence, radiolabeling, immunoblotting, Western blotting, enzyme-linked immunosorbent assay (ELISA), flow cytometry, immunoprecipitation, immunohistochemistry, biofilm test, affinity ring test, antibody array optical density test, and chemiluminescence.

Example ~~Embodiment~~ 11: The method of example ~~embodiment~~ 1, wherein said level of glypican-1 in the body fluid sample from a patient is compared to the level of glypican-1 in a control sample; wherein increased anti-glypican-1 antibody binding of the body fluid sample over the control sample is associated with the presence of prostate cancer.

Example ~~Embodiment~~ 12: The method of example ~~embodiment~~ 11, wherein a 50% or more increase in the level of glypican-1 of said body fluid sample over the level of glypican-1 in the control sample is indicative of prostate cancer.

Example ~~Embodiment~~ 13: The method of any one of examples ~~embodiments~~ 2 to 10, wherein anti-glypican-1 antibody binding to said body fluid sample is compared to anti-glypican-1 antibody binding of a control sample; wherein increased anti-glypican-1 antibody binding of the body fluid sample over the control sample is associated with the presence of prostate cancer.

Example ~~Embodiment~~ 14: The method of example ~~embodiment~~ 13, wherein a 50% or more increase in the anti-glypican-1 antibody binding to said body fluid sample over the level of anti-glypican-1 antibody binding of the control sample is indicative of prostate cancer.

Example ~~Embodiment~~ 15: The method of any one of examples ~~embodiments~~ 2 to 10, 13 or 14 wherein anti-glypican-1 antibody binding to said body fluid sample is compared to anti-glypican-1 antibody binding to one or more glypican-1 standards; wherein the anti-glypican-1 antibody binding of the standards is used to quantify the amount of glypican-1 in said body fluid sample.

Example ~~Embodiment~~ 16: The method of any one of examples ~~embodiments~~ 1 to 15, wherein a glypican-1 content higher than about 10ng/ml in the body fluid sample is indicative of prostate cancer.

Example ~~Embodiment~~ 17: The method of any one of examples ~~embodiments~~ 1 to 16, further comprising:

measuring the level of prostate-specific antigen (PSA) in a body fluid sample from the patient, and

determining that said patient has prostate cancer or an increased likelihood of developing prostate cancer based upon (i) the level of PSA measured in the body fluid sample, and (ii) binding of said anti-glypican-1 antibody to said body fluid sample.

Example ~~Embodiment~~ 18: The method of example ~~embodiment~~ 17, wherein the level of prostate-specific antigen (PSA) is measured in a blood sample from the patient.

Example ~~Embodiment~~ 19: The method of example ~~embodiment~~ 17 or example ~~embodiment~~ 18, wherein the level of prostate-specific antigen (PSA) in the body fluid sample measured is compared to the level of PSA measured in a control sample; wherein increased PSA levels in the body fluid sample over the control sample is associated with the presence of prostate cancer.

Example ~~Embodiment~~ 20: The method of any one of examples ~~embodiments~~ 1 to 19, wherein said body fluid is selected from the group consisting of blood, serum, plasma, and urine.

Example ~~Embodiment~~ 21: A kit for detecting prostate cancer comprising a first anti-glypican-1 antibody, a pharmaceutically acceptable carrier, and glypican-1 standards; wherein said kit is capable of detecting glypican-1 in the body fluid of a patient.

Example ~~Embodiment~~ 22: The kit of example ~~embodiment~~ 21, wherein the anti-glypican-1 antibody is not MIL-38.

Example ~~Embodiment~~ 23: The kit of example ~~embodiment~~ 21, wherein the anti-glypican-1 antibody is MIL-38.

Example ~~Embodiment~~ 24: The kit of example ~~embodiment~~ 21, wherein the anti-glypican-1 antibody is the antibody referred to in any one of examples ~~embodiments~~ 4, 5 or 7.

Example ~~Embodiment~~ 25: The kit of any one of examples ~~embodiments~~ 21 to 24, further comprising a secondary ligand.

Example ~~Embodiment~~ 26: The kit of example ~~embodiment~~ 25, wherein said secondary ligand is a second anti-glypican-1 antibody or an aptamer capable of binding to glypican-1; wherein said second anti-glypican-1 antibody can be the same as the first anti-glypican-1 antibody.

Example ~~Embodiment~~ 27: The kit of example ~~embodiment~~ 25 or example ~~embodiment~~ 26, wherein said secondary ligand is conjugated to a label for rapid detection of said ligand.

Example ~~Embodiment~~ 28: The kit of example ~~embodiment~~ 27, wherein said label is for use in a detection method selected from the group consisting of immunofluorescence, radiolabeling, immunoblotting, Western blotting, enzyme-linked immunosorbent assay (ELISA), flow cytometry, immunoprecipitation, immunohistochemistry, biofilm test, affinity ring test, antibody array optical density test, and chemiluminescence.

Example ~~Embodiment~~ 29: The kit of any one of examples ~~embodiments~~ 22 to 28, wherein the kit comprises components for conducting an ELISA.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Characterization of the cell bound MIL-38 glypican-1 antigen.

DU-145 prostate cancer cells were processed with a Membrane Protein Extraction Kit (MPEK, Merck) and incubated with the MIL-38 antibody attached to magnetic beads. The antigen was then immunoprecipitated and washed on the magnetic beads before eluting the antigen and subjecting said antigen to mass spectrometry analysis. Results of mass spec analysis are shown identifying glypican-1 as the MIL-38 antigen. The 18 unique peptide sequences spanning the glypican-1 protein are underlined.

Figure 2. MIL-38 immunoprecipitation and size-exclusion chromatography.

DU-145 prostate cancer cell membrane extracts were immunoprecipitated using MIL-38 and run through a size-exclusion chromatography column. The figure shows western blot analyses of even numbered chromatography fractions using either MIL-38 antibody or secondary antibody control. The MIL-38 antigen is shown at 60Kd in fractions A28 and A30.

Figure 3. Mass spectrometry of size-exclusion chromatography purified MIL-38 antigen.

Fraction number A29 from size-exclusion chromatography separation of Figure 2 was analyzed via mass spectrometry. Results of mass spec analysis are shown identifying glypican-1 as the MIL-38 antigen with 8 unique peptide sequences spanning the glypican-1 protein underlined.