Most oncogenic proteins are intracellular proteins (such as intracellular phosphatases, intracellular kinases, transcription factors, etc), and have remained under-explored by the approach of antibody therapies. The long held view that antibodies are too large to penetrate cell membrane has hampered the technology of antibody therapy used in targeting intracellular proteins.

We previously showed that three different antibodies could target three intracellular proteins respectively: PRL-3 (phosphatase of regenerating liver 3), a cancer-associated phosphatase; EGFP (enhanced green fluorescent protein), a general reporter; and mT (polyomavirus middle T), the polyomavirus middle T oncoprotein (WO2011/065923). Only PRL-3 intracellular phosphatase (an enzyme) has been linked to human cancer metastasis (see Saha et al., Science 294; 1343 (2001) and Wang et al., Cancer cell 18; 52-63 (2010)), the other two intracellular proteins (EGFP and middle T) were used to elucidate the general phenomena that antibodies can target intracellular proteins.

Summary

The inventors have uncovered the presence of several oncoproteins preferentially in the urine samples or urine exosomes of cancer patients as compared to those of normal controls. In this study, we tested several intracellular and extracellular proteins: PRL-3, PRL-1, VHZ, myc, ras, actin, GAPDH, p53, N-Cadherin, Her2, EGFR, FLT, Estrogen Receptor, PDGF, PDGF-a. Analysis was performed with western blot. Hundreds of cancer urine samples have been examined, including bladder cancer, lung cancer, lymphoma, prostatic adenocarcinoma, breast cancer, stomach cancer or gastric cancer, nasopharynx cancer and nasopharyngeal carcinoma (NPC) and compared with control normal urine samples. The levels of these oncoproteins in the urine are associated with cancer stages such as initial and later stages of bladder cancer. These discoveries offer a simple test for cancer, by determining the presence of urinary oncoprotein using various immunological assays. Our data show that intracellular oncoproteins can be unconventionally secreted out through exosomes to travel long distance within body fluid (blood, urine, saliva, etc). Disclosed herein are urine tests to detect the presence of oncoproteins for diagnostics, cancer staging, cancer therapy, and monitoring anti-cancer therapy.

The data provided herewith show that, surprisingly, biomarkers found within the cells of a tumor or cancer may be present at adequate levels within the patient's urine to enable detection. Moreover, the inventors have surprisingly found that certain oncoproteins may

be detected in urine at a very early stage in the development of the cancer. It is surprising that sufficient amounts of oncoprotein to enable detection and diagnosis are present distant from the tumor, in the patients urine, during these initial stages.

We found secreted PRL3 oncoprotein in 62% of multiple types of human cancer urine and in 100% of cancer urine derived from PRL3+ (but not PRL3-) tumor bearing mice. Urinary PRL3 levels were significantly reduced after effective treatment with anti-PRL3 antibody. Urinary PRL3 is therefore proposed as a novel diagnostic and surrogate biomarker for therapeutic response monitoring of anti-PRL3 antibody therapy in cancer.

Using bodily fluid samples such as urine may be particularly advantageous where the cancer is inaccessible.

Thus, in one aspect, the present invention provides a method of determining whether a patient does, or does not, have cancer, comprising determining the presence or absence of PRL3 in a urine sample from the patient, wherein the presence of PRL3 in the urine sample indicates that the patient has cancer. In another aspect, the present invention provides a method of determining whether a patient does, or does not, have bladder cancer, the method comprising determining the presence or absence of PRL3 in a sample containing exosomes obtained from a bodily fluid from the patient, the sample having been enriched for exosomes, and/or containing substantially only exosomes, wherein the sample is, or is derived from, urine. In still another aspect, the present invention provides a method of selecting a patient for an anti-cancer therapy, the method comprising detecting the presence of PRL3 in a sample of, or derived from, bodily fluid from the patient, and selecting a treatment based on PRL3 determined to be present, where the bodily fluid is urine. In yet another aspect, the present invention provides a method for diagnosing bladder cancer in a patient, the method comprising isolating exosomes from a urine sample from the patient and determining the presence of PRL3 in the exosomes, wherein the presence of PRL3 in the exosomes is indicative that the patient has bladder cancer. In one other aspect, the present invention provides the use of an anti-cancer therapy antibody in the manufacture of a medicament for treating cancer in a patient, wherein the patient has been selected for treatment by a method comprising determining, in a sample of urine from the patient, the presence of PRL3.

Also described herein is a method for determining whether an individual does, or does not, have cancer, the method comprises determining the

presence or absence of an oncoprotein in a sample of a bodily fluid from the individual. The presence of the oncoprotein may be indicative that the individual has cancer. In some cases, the amount of oncoprotein is quantified. The amount of oncoprotein present in the sample may be indicative that the individual has cancer. For example, a higher level of oncoprotein may indicate the presence of a cancer. The amount of oncoprotein may indicate the stage of the cancer, such as early, mid or late stage. The method may involve the detection of exosomal oncoprotein. That is, the detection of oncoprotein within or attached to, exosomes, within the bodily fluid.

The methods disclosed herein may be used to monitor the response of a patient to a therapeutic treatment. For example, the presence or level of oncoprotein in urine may be determined before and after a therapeutic treatment is administered. The therapeutic treatment may be chemotherapy, radiotherapy or other therapeutic treatment. A reduction in the level of oncoprotein may indicate that the therapy is having an anti-cancer effect.

The methods disclosed herein may be used to select a patient for treatment with anti-cancer therapy, or to determine that a patient is suitable for such treament. In some cases, methods disclosed herein relate to the treatment of patients, determined to be suitable for treatment by the methods disclosed herein. Agents for use in such methods are also disclosed.

Samples of bodily fluid useful in the methods described herein include urine, saliva, blood or plasma, or any other bodily fluid, including breast milk. Preferably, the bodily fluid is urine.

The oncoprotein may be present in exosomes in the sample or may be soluble. The method may involve the isolation of exosomes from the sample, and determining the presence or absence of an oncoprotein in the exosomes. The method may involve a step of concentrating the exosomes, relative to the sample isolated from the individual. The sample of exosomes may or may not be treated to release the contents of the exosomes, such as lysing or bursting the exosomes. The method may be performed on a sample of exosomes that has been previously isolated from a sample of bodily fluid. In particular, the presence of oncoprotein in exosomes in the sample may indicate that the patient has bladder cancer. In some cases, the oncoprotein is soluble, such as soluble PRL3. Soluble oncoprotein may not be associated with, or present in, exosomes in the sample.

The presence or absence of the oncoprotein may involve an immunoassay, such as an ELISA or western blot based method. The method may involve the use of antibodies or aptamers. The antibodies may be any antibody that is capable of binding specifically to the oncoprotein. Antibodies may be monoclonal or polyclonal, they may be human, rabbit or mouse antibodies, or from any suitable mammal, or may be produced in cell culture. The antibodies may be humanised or chimeric. The antibody may be Antibody 223 or 318 as discussed in Li et al (Clin.Canc.Res. (2005) 11:2195-204). The antibody may bind to a region of an oncoprotein that is normally presented within the cell, such as within the plasma membrane, cytoplasm or nucleus. In some cases, the antibody does not bind to an extracellular portion of an oncoprotein, such as a portion of the oncoprotein normally presented on the surface of a cell.

Oncoproteins useful in the methods disclosed herein are upregulated or overexpressed in cancers as compared to non-cancerous tissues. In some cases the oncoprotein carries a mutation as compared to the protein that is expressed in non-cancerous tissue. Oncoproteins as discussed herein may be intracellular or extracellular. That is to say that their expression is substantially within the cell, such as in the nucleus, cytoplasm, or internal side of the cell membrane, or substantially on the outside of the cell, such as on the cell surface. Preferably the oncoprotein is an intracellular oncoprotein.

In preferred methods described herein, the oncoprotein is selected from PRL3, PRL1, VHZ, c-myc, H-ras, AKT-1, p53, Rac1, FAK, Runx1, Estrogen Receptor (ER), PTEN, b-actin or GAPDH. The p53 may be mutant p53. For example, the p53 protein may have a proline residue instead of an arginine residue at position 72. Preferably the oncoprotein is PRL3, PRL1 or VHZ. In some cases the oncoprotein is a form of GAPDH or b-Actin which is mutated relative to the form found in non-cancerous tissues. In some cases, the presence or absence of more than one oncoprotein is determined. The oncoprotein may be Her2, N-Cadherin, PDGF receptor (alpha), FLT-3 or p-EGFR.

Cancers detectable by the methods disclosed herein include gastric cancer, bladder cancer, lung cancer, breast cancer, stomach cancer, nasopharyngeal cancer, prostate cancer (such as prostatic adenocarcinoma or prostatic hyperplasia, particularly being prostatic hyperplasia). The cancer may be distant from the source of the sample. The cancer may be one that is difficult and/or invasive to access for example to sample or biopsy.

In some cancers the methods involve determining whether the level of oncoprotein in the sample is higher or lower than the level in a sample obtained from an individual who does not have cancer. In some cases, a higher level of oncoprotein relative to a non-cancerous individual is indicative that the individual has cancer. In some cases, the level of expression is indicative of the stage of the cancer. A high level of expression may indicate that the individual has a late stage, or advanced cancer.

The individual is preferably a human individual, and the oncoprotein detected is preferably a human oncoprotein.

In some cases, the oncoprotein is not Her2, N-Cadherin, PDGF receptor (alpha), FLT-3 or p-EGFR. In some cases, the cancer is not bladder cancer.

In some cases the methods described herein involve determining the stage of the cancer in the individual. That is, the presence or absence of certain oncoproteins in the sample may be indicative that the cancer is early, mid, or late stage. The presence or absence of a certain oncoprotein may be indicative that the cancer is, or will be metastatic. In some cases, the amount of oncoprotein is indicative of the stage of the cancer.

The methods may involve a comparison step in which the level of biomarker in a sample from an individual is compared to the level of the biomarker in a sample obtained for a non-cancerous individual. The level may be compared directly with a sample from the non-cancerous individual (e.g. a control sample), or may be compared with a database of one or more reference values.

Bodily fluid samples may be obtained from an individual by any suitable method known in the art. The sample may have been obtained prior to commencing the methods disclosed herein, or may be obtained as part of that method. The bodily fluid may be a fluid in which exosomes are present. The method may involve the detection of oncoproteins in the sample such as soluble oncoprotein. The method may involve the isolation of exosomes from the sample, or concentration of exosomes within the sample, and the detection of oncoproteins in the exosomes.

Methods for isolating exosomes from samples are known in the art. For example, the EXOQUICK-TC™ process is a commercially available technique in which a sample is incubated in a precipitation solution overnight prior to centrifugation to form a solid precipitate containing exosomes. Other methods, for isolating or concentrating exosomes include ultra-configuration.

Exosomes are cell derived vesicles. They may have a diameter of 30-100nm. They are membrane bound, and released from the plasma membrane. Exosomes contain various molecular constituents, including miRNA and protein. There is increasing clinical interest in exosomes, particularly in their use in diagnosis, prognosis and therapy. Exosomes have been identified in a variety of biological fluids, including urine, blood, serume, saliva and breast milk.

Exosomes isolated by methods described herein may be ruptured prior to the detection of oncoproteins. The methods disclosed herein may additionally involve the detection of exosomal markers, such as CD63, or other proteins or RNA sequences that are characteristic of exosomes.

Also disclosed herein are kits for determining the presence or absence of an oncoprotein in a sample. The kit may comprise an antibody that is specific to an oncoprotein.

In some cases, the ELISA may be performed using a solid support which is immersible in the biological fluid, or in the sample of exosomes obtained from a biological fluid. The solid support may be a plate, dipstick or bead, or other support known in the art.

Also included herein is a kit for detecting or measuring oncoprotein in a bodily fluid. The kit may include a lateral flow test device. The kit may include instructions for use. The lateral flow test may include a reaction zone, a test zone, and optionally a control zone.

Suitable antibodies for use in an ELISA based method for detecting an oncoprotein include any anti-oncoprotein antibody known in the art. For detecting PRL3, the antibody may be monoclonal antibodies 223 or 318 as disclosed in Li et al, or polyclonal antibody serum from rabbit #1992 or #1993. Any combination of antibodies may be used as capture or detection agent. Preferably however the capture and detection agents are different. In the case of PRL3, a particularly preferred combination is the use of monoclonal antibody 223 as capture antibody. Rabbit antiserum #1992 is preferred as detector. The detector may be linked to gold particles to allow for detection.

Methods described herein may include the following steps:

a) providing a biological sample obtained from an individual;

b) exposing the biological sample to a solid support on which an oncoprotein binding agent is immobilised, such that oncoprotein within the biological sample is immobilised to the solid support;

c) partitioning the oncoprotein from the biological sample;

d) detecting the oncoprotein.

The presence of the oncoprotein may be indicative that the individual has cancer. The method may involve quantifying the amount of oncoprotein detected. The method may involve one or more washing steps, such as following immobilisation of the oncoprotein on the solid support. The method may involve a comparison step in which the result obtained in step (d) is compared to the results obtained from an individual known to not have cancer. The comparison may involve comparison with known values in a database.

In some cases, the solid support is a nitrocellulose membrane, particularly a nitrocellulose AE99. Preferably a monoclonal antibody is bound to the solid support as the capture antibody. Preferably the antibody is antibody 223. The assay may involve exposing the

Claims

1. A method of determining whether a patient does, or does not, have cancer, comprising determining the presence or absence of PRL3 in a urine sample from the patient, wherein the presence of PRL3 in the urine sample indicates that the patient has cancer.

2. The method of claim 1, wherein the cancer is located at a site distant from the source of the sample.

3. The method of claim 1 or 2 further comprising quantifying the level of PRL3 in the sample.

4. The method of any one of claims 1 to 3 wherein the cancer is an initial or early stage cancer.

5. The method of any one of the preceding claims wherein the cancer is selected from bladder, lung, breast, stomach, nasopharangeal or prostate cancer.

6. A method of determining whether a patient does, or does not, have bladder cancer, the method comprising determining the presence or absence of PRL3 in a sample containing exosomes obtained from a bodily fluid from the patient, the sample having been enriched for exosomes, and/or containing substantially only exosomes, wherein the sample is, or is derived from, urine.

7. The method of any one of the preceding claims wherein the PRL3 is present within the exosomes in the sample.

8. A method of selecting a patient for an anti-cancer therapy, the method comprising detecting the presence of PRL3 in a sample of, or derived from, bodily fluid from the patient, and selecting a treatment based on PRL3 determined to be present, where the bodily fluid is urine.

9. A method for diagnosing bladder cancer in a patient, the method comprising isolating exosomes from a urine sample from the patient and determining the presence of PRL3 in the exosomes, wherein the presence of PRL3 in the exosomes is indicative that the patient has bladder cancer.

10. Use of an anti-cancer therapy antibody in the manufacture of a medicament for treating cancer in a patient, wherein the patient has been selected for treatment by a method comprising determining, in a sample of urine from the patient, the presence of PRL3.

11. The use according to claim 10 wherein the anti-cancer therapy is an antibody therapy.

12. The use according to claim 11 wherein the antibody therapy is anti-PRL3 antibody therapy.