

invasive biopsy and then to make an subjective~~objective~~ judgment based on a pathologic image obtained by a microscope. If it is possible to assess a degree of progression of prostate cancer at an early stage, e.g., at
5 the stage of blood test, this will significantly reduce the burden on a patient and allow prompt decision of a therapeutic strategy for the patient based on the
| objectives~~subjective~~ criteria. Thus, there is also a demand for development of a method for assessing a degree of
10 progression of prostate cancer which method can be carried out at an early stage.

[0008]

Non-Patent Literature 5 describes the expression amount of NPY in the prostate tissues. However, Non-Patent
15 Literature 5 does not indicate an existing amount of NPY in other samples (e.g., blood or urine). With regard to various proteins, the fact is known that there is no correlation between (i) an expression amount of a protein in tissues and (ii) an existing amount of the protein in
20 samples derived from others. Furthermore, Non-Patent Literature 5 indicates that an expression amount of NPY in prostate tissues has no correlation with a degree of progression of prostate cancer. In addition, Patent Literature 1 does not describe a relationship between an
25 amount of NPY peptide and a degree of progression of prostate cancer. Moreover, none of Non-Patent Literature 5

organism (subject). Examples of the sample derived from the blood include whole blood, a serum, and plasma. Among these, in order to measure an amount of NPY or an amount of PSA, the "sample" is preferably whole blood, a serum, or
5 plasma, more preferably a serum or plasma. Among those listed above, in order to measure an amount of mRNA of TMPRSS2:ERG or an amount of PCA3 RNA, the "sample" is preferably urine.

[0034]

10 The subject is, for example, a human, a mouse, a rat, a rabbit, or a monkey, and preferably a human. A site from which blood is collected is, for example, a median cubital
| vein, a cephalic vein, or a basilica vein. In order to measure amounts of various kinds of prostate cancer
15 markers, either the same sample or different samples may be used.

[0035]

Further, the subject can be the one who has been found to have prostate cancer or the one whose status as to
20 whether he/she has prostate cancer is unknown. For the subject who has been found to have prostate cancer, it is possible to assess an extent of development of the prostate cancer. Meanwhile, for the subject whose status as to whether he/she has prostate cancer is unknown, it is
25 possible to determine whether or not the subject has

cancer, the test ~~kit~~^{kind} including: an antibody or a peptide probe each for measuring an amount of neuropeptide Y in a sample derived from blood or urine obtained from a living organism; and an antibody or a peptide probe each
5 for measuring an amount of prostate-specific antigen in a sample derived from blood or urine obtained from the living organism.

[0073]

As needed, the test kit for detecting prostate cancer
10 may further include at least one of, e.g., (i) any of various reagents (e.g., a secondary antibody, a reporter molecule, and a buffer) and various instruments (e.g., a plate and a pipet) each for immunologically detecting the antibody or the peptide probe, (ii) an instruction manual
15 of the test kit, (iii) a sample serving as a control to be used in measurement, and (iv) data serving as a control to be used in analysis of a result of the measurement. Note that the instruction manual of the test kit for detecting prostate cancer includes the description about the
20 detection method according to the present invention explained in [2. A method for detecting prostate cancer] above.

[0074]

Each of the test kit for assessing a degree of
25 progression of prostate cancer and the test kit for detecting prostate cancer is preferably, but is not

[0101]

(LC/MS/MS analysis)

After the above-described QUEST-MS purification, 1 μ l of sample was analyzed by a mass spectrometer (LTQ-
5 Orbitrap-Velos mass spectrometer, available from Thermo Fisher Scientific Inc.) equipped to Ultimate 3000 nano-flow HPLC (available from Thermo Fisher Scientific Inc.). Using 0.1% formic acid as solvent-A and 0.1% formic acid in acetonitrile as solvent-B, peptides were separated from the
10 sample on C18 Chip-column (75 μ m $\times$ 200 mm, available from Nikkyo Technos), where (i) a flow rate was 250 nL/min and (ii) a gradient of solvent-B was 2% to 30% for 95 minutes and 30% to 95% for 15 minutes. The peptides thus eluted were ionized with a spray voltage of 2000V, and MS data was
15 obtained by a data-dependent fragmentation method in which the survey scan was carried out between m/z 400 and m/z 1600 at a resolution of 60,000 with automatic gain control (AGC) target value of 1.0×10^6 ion counts. The top-20 intense precursor ions in each survey scan were subjected
20 to low-resolution MS/MS obtainment in a normal CID scan mode with AGC target value of 5,000 ion counts in the linear ion trap.

[0102]

(Label-free quantification by Expressionist RefinerMS)

solvent-B (0.1% formic acid in acetonitrile), peptides were separated from the samples on C18 Chip-column (75 μ m $\times$ 200 mm, available from Nikkyo Technos), where (i) a flow rate was 250 nL/min and (ii) a gradient of solvent B was a two-step linear gradient of 2% to 30% for 10 minutes and 30% to 95% for 5 minutes. MRM transitions specific to NPY and BSA (available from KYA technologies) were simultaneously monitored in the MRM mode of Analyst 1.5 software (available from AB Sciex). The instrumental settings were as follows: Ionization spray voltage = 2200V, Curtain gas (N₂) = 12 psi, CAD = 4, Declustering potential = 70V, Entrance potential = 10V, Q1 resolution = HIGH, Q3 resolution = LOW, and Pause in between = 2 msec. The MRM chromatograms thus obtained were analyzed by MultiQuant 2.02 software (available from AB Sciex). The mass chromatogram of each transition was smoothed by 1 pt window, and quantified for a peak area. After collision energy was optimized for 12 NPY transitions, quantification was substantially carried out only with Q1/Q3 = 466.23/272.20, which corresponds to NPY₈₁₋₈₈ peptide, because this transition showed the highest sensitivity. Then, with use of a BSA peptide which was measured in the same manner, an area of NPY₈₁₋₈₈ peak was normalized as follows: Normalized NPY = Area (NPY₈₁₋₈₈, Q1/Q3 = 466.2/272.2) / Area (BSA₆₆₋₇₅, Q1/Q3 = 582.3/951.5) $\times$

Claims

Claim 1

An analysis method for assessing a degree of
5 progression of prostate cancer, comprising the step of:

measuring an amount of neuropeptide Y in a sample
derived from blood or urine obtained from a living
organism.

10 Claim 2

The analysis method as set forth in claim 1, further
comprising the step of:

measuring an amount of at least one of prostate-
specific antigen, mRNA of TMPRSS2:ERG, and PCA3 RNA in a
15 sample derived from blood or urine obtained from the living
organism.

Claim 3

The analysis method as set forth in claim 1, further
20 comprising the step of:

measuring an amount of prostate-specific antigen in a
sample derived from blood or urine obtained from the living
organism.

25 Claim 4

The analysis method as set forth in any one of claims
1 through 3, wherein:

the amount of neuropeptide Y is measured by mass
spectrometry or luminance assay.

30

Claim 5

The analysis method as set forth in any one of claims
1 through 4, wherein:

the sample is whole blood, a serum, or plasma.

Claim 6

The analysis method as set forth in any one of claims
5 1 through 5, wherein:

in the step of measuring the amount of neuropeptide Y,
the sample is subjected to reversed phase extraction twice
or more so that a fraction containing neuropeptide Y is
obtained, and the amount of neuropeptide Y is measured in
10 the fraction thus obtained.

Claim 7

A method for detecting prostate cancer, comprising the
step of:
15 measuring respective amounts of neuropeptide Y and
prostate-specific antigen in a sample derived from blood or
urine obtained from a living organism.

~~Claim 8~~
20 ~~A method for assessing a degree of progression of~~
~~prostate cancer, comprising the step of:~~
~~measuring an amount of neuropeptide Y in a sample~~
~~derived from blood or urine obtained from a living~~
~~organism.~~

25

Claim 89

A test kit for assessing a degree of progression of
prostate cancer, comprising:
a peptide probe for measuring an amount of
30 neuropeptide Y in a sample derived from blood or urine
obtained from a living organism.

Claim 910

The test kit as set forth in claim 89, further comprising:

at least one of (i) an antibody or a peptide probe each for measuring an amount of prostate-specific antigen in a sample derived from blood or urine obtained from the living organism, (ii) a nucleic-acid probe or a nucleic-acid primer each for measuring an amount of mRNA of TMPRSS2:ERG in a sample derived from blood or urine obtained from the living organism, and (iii) a nucleic-acid probe or a nucleic-acid primer each for measuring an amount of PCA3 RNA in a sample derived from blood or urine obtained from the living organism.

Claim ~~1011~~

A test kit for assessing a degree of progression of prostate cancer, comprising:

an antibody for measuring an amount of neuropeptide Y in a sample derived from blood or urine obtained from a living organism; and

at least one of (i) an antibody or a peptide probe each for measuring an amount of prostate-specific antigen in a sample derived from blood or urine obtained from the living organism, (ii) a nucleic-acid probe or a nucleic-acid primer each for measuring an amount of mRNA of TMPRSS2:ERG in a sample derived from blood or urine obtained from the living organism, and (iii) a nucleic-acid probe or a nucleic-acid primer each for measuring an amount of PCA3 RNA in a sample derived from blood or urine obtained from the living organism.

30

~~Claim 12~~

~~A test kit for detecting prostate cancer, comprising:~~

~~— an antibody or a peptide probe each for measuring an amount of neuropeptide Y in a sample derived from blood or urine obtained from a living organism; and~~

5 ~~— an antibody or a peptide probe each for measuring an amount of prostate-specific antigen in a sample derived from blood or urine obtained from the living organism.~~