

Therefore, measuring a total amount of VEGF-A in a sample derived from a patient to be treated does not accurately reflect an anticipated medicinal effect. This is the same for other proteins that have different in vivo effects depending on variants.

[0013]

For the reasons described above, in order to gain a more accurate understanding of an in vivo effect of a protein, when the protein in a sample is quantified, it is necessary to measure an amount of each variant that can be present.

[Means for Solving the Problems]

[0014]

Quantitative and structural analysis of a protein using mass spectrometry has been dramatically expanded in its application range, along with developments of mass spectrometry technologies and data analysis server and software. In particular, an absolute quantification technique using mass spectrometry has increased awareness as a method independent of specific antibodies. The present inventors examined possibility of simultaneously quantifying variants of a protein using mass spectrometry.

[0015]

As an example, in order to be able to quantify all VEGF-A variants at once, a peptide fragment obtained by protease digestion from each variant was designed. Then, by selecting a peptide having an amino acid sequence specific to each variant, it was possible to develop an analytical condition that enables an all-at-once quantitative analysis of the variants by detecting them in parallel using mass spectrometry. The method developed by the present inventors is not only applicable to VEGF-A, but is also similarly applicable to a protein having 2 or more variants.

[0016]

In one aspect, the present invention refers to a method for parallel determination of variants of a protein having 2 or more variants using mass spectrometry, comprising: protease-digesting a protein in a sample; detecting, using mass spectrometry, among peptides obtained by the protease digestion, 2 or more kinds of peptides having an amino acid sequence specific to each of the variants; and determining an amount of each of the variants in the sample based on a result of the mass spectrometry, wherein the variants are splicing variants of the protein associated with

the production of new blood vessels or the growth of vascular endothelial cells. In another aspect, the present invention refers to a kit for use in executing the method according to the present invention using high performance liquid chromatograph mass spectrometry (LC-MS) comprising: a protease; a reaction container for digesting the protein by bringing the protein and the protease into contact with each other; a buffer solution for causing a digestion reaction due to the protease to occur; and 1 or more internal standard peptides having amino acid sequences specific to the variants. In still another aspect, the present invention refers to a computer readable recording medium that is for use in the method according to any one of claims 1-5 and in which data for executing mass spectrometry is stored, wherein the data includes data of a parent ion, a fragment ion, an expected retention time, and voltages at triple quadrupoles with respect to 1 or more peptides having amino acid sequences specific to variants. In yet another aspect, the present invention refers to a method package for parallel detection of a protein variant using high performance liquid chromatograph mass spectrometry (LC-MS), comprising: the recording medium according to the present invention; and an instruction manual of the recording medium. Also described herein is the following:.

(1) A method for parallel determination of variants of a protein having 2 or more variants using mass spectrometry, includes: protease-digesting a protein in a sample; detecting, using mass spectrometry, among peptides obtained by the protease digestion, 2 or more kinds of peptides

having an amino acid sequence specific to each of the variants; and determining an amount of each of the variants in the sample based on a result of the mass spectrometry.

(2) The method according to the above-described example (1) includes further detecting a peptide having an amino acid sequence common to 2 or more variants.

(3) In the method according to the above-described example (1) or (2), the variants are splicing variants of the protein.

(4) In the method according to any one of the above-described examples (1) – (3), the protein is a vascular endothelial growth factor (VEGF)-A.

(5) In the method according to the above-described example (4), the variants include 2 or more selected from the following splicing variants of VEGF-A: 206 (SEQ ID NO: 1), 189 (SEQ ID NO: 2), 183 (SEQ ID NO: 3), 165 (SEQ ID NO: 4), 148 (SEQ ID NO: 5), 145 (SEQ ID NO: 6), 121 (SEQ ID NO: 7), 165b (SEQ ID NO: 8), 121b (SEQ ID NO: 9), and 111 (SEQ ID NO: 10).

(6) In the method according to the above-described example (4) or (5), 1 or more peptides having amino acid sequences shown in SEQ ID NOs: 11 – 26 are detected.

(7) A kit for use in executing the method according to any one of examples 1 – 6 using high performance liquid chromatograph mass spectrometry (LC-MS) includes: a protease; a reaction container for digesting the protein by bringing the protein and the protease into contact with each other; a buffer solution for causing a digestion reaction due to the protease to occur; and 1 or more internal standard peptides having amino acid sequences specific to the variants.

(8) The kit according to the above-described example (7) further includes an instruction manual describing a mass spectrometry condition for detecting the variants.

(9) The kit according to the above-described example (7) or (8) includes peptides as internal standard peptides having amino acid sequences shown in SEQ ID NOs: 11 – 26.

(10) A computer readable recording medium for use in the method according to any one of the above-described examples (1) – (6) stores data for executing mass spectrometry. The data includes data of a parent ion, a fragment ion, an expected retention time, and voltages at triple quadrupoles with respect to 1 or more peptides having amino acid sequences specific to variants.

(11) A method package for parallel detection of a protein variant using high performance liquid chromatograph mass spectrometry (LC-MS) includes: the recording medium according to the above-described example (10); and an instruction manual of the recording medium.

(12) In the recording medium according to the above-described example (10) or the method package according to the above-described example (11), the data is with respect to 1 or more peptides having amino acid sequences shown in SEQ ID NOs: 11 – 26.

[0017]

The present specification incorporates a disclosure content of Japanese Patent Application No. 2015-153656 on which priority of the present application is based.

[Effect of the Invention]

[0018]

According to the present invention, it is possible to detect, all at once, amounts of variants that are mixed in a sample and have different effects, and it is possible to collectively acquire information that is only partially obtained in the case of using the conventional ELISA.

[0019]

For example, all-at-once quantification of VEGF-A variants becomes possible by setting an optimization condition in mass spectrometry quantification of VEGF-A. By accurately detecting and quantifying each variant, it is possible to gain an understanding of to what extent each individual cancer patient has been exposed to an angiogenesis promoting effect or an angiogenesis inhibiting effect. By regulating the proliferation of cancer, it is possible to establish a more effective dosing strategy. That is, in a case of a highly proliferative cancer, chemotherapy based on neutralization with an antibody can be a first priority and can be a medication method that allows a maximum medicinal efficacy to be obtained. On the other hand, in a case where it is judged that proliferation is not so high, it is preferable to prioritize a low molecular anticancer drug and radiotherapy. Therefore, it is possible to provide an anti-cancer treatment according to each individual patient.

[0020]

Claims

[Claim 1]

A method for parallel determination of variants of a protein having 2 or more variants using mass spectrometry, comprising:

protease-digesting a protein in a sample;

detecting, using mass spectrometry, among peptides obtained by the protease digestion, 2 or more kinds of peptides having an amino acid sequence specific to each of the variants; and

determining an amount of each of the variants in the sample based on a result of the mass spectrometry,

wherein the variants are splicing variants of the protein associated with the production of new blood vessels or the growth of vascular endothelial cells.

[Claim 2]

The method according to claim 1, comprising further detecting a peptide having an amino acid sequence common to 2 or more variants.

[Claim 3]

The method according to claim 1 or 2, wherein the protein is a vascular endothelial growth factor (VEGF)-A.

[Claim 4]

The method according to claim 3 wherein the variants include 2 or more selected from the following splicing variants of VEGF-A: 206 (SEQ ID NO: 1), 189 (SEQ ID NO: 2), 183

(SEQ ID NO: 3), 165 (SEQ ID NO: 4), 148 (SEQ ID NO: 5), 145 (SEQ ID NO: 6), 121 (SEQ ID NO: 7), 165b (SEQ ID NO: 8), 121b (SEQ ID NO: 9), and 111 (SEQ ID NO: 10).

[Claim 5]

The method according to claim 3 or 4, wherein 1 or more peptides having amino acid sequences shown in SEQ ID NOs: 11 – 26 are detected.

[Claim 6]

A kit for use in executing the method according to any one of claims 1-5 using high performance liquid chromatograph mass spectrometry (LC-MS) comprising:

- a protease;
- a reaction container for digesting the protein by bringing the protein and the protease into contact with each other;
- a buffer solution for causing a digestion reaction due to the protease to occur; and
- 1 or more internal standard peptides having amino acid sequences specific to the variants.

[Claim 7]

The kit according to claim 6 further comprising an instruction manual describing a mass spectrometry condition for detecting the variants.

[Claim 8]

The kit according to claim 6 or 7 comprising peptides as internal standard peptides having amino acid sequences shown in SEQ ID NOs: 11 – 26.

[Claim 9]

A computer readable recording medium that is for use in the method according to any one of claims 1-5 and in which data for executing mass spectrometry is stored, wherein the data

includes data of a parent ion, a fragment ion, an expected retention time, and voltages at triple quadrupoles with respect to 1 or more peptides having amino acid sequences specific to variants.

[Claim 10]

A method package for parallel detection of a protein variant using high performance liquid chromatograph mass spectrometry (LC-MS), comprising:

the recording medium according to claim 9; and
an instruction manual of the recording medium.

[Claim 11]

The recording medium according to claim 9 or the method package according to claim 10, wherein the data is with respect to 1 or more peptides having amino acid sequences shown in SEQ ID NOs: 11 – 26.