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]

Disclosed is 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. Also disclosed is 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. Also disclosed is 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. Also disclosed is 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. In one aspect, the present invention provides a method for parallel determination of multiple splicing variants produced by alternative splicing of the protein associated with the production of new blood vessels or the growth of vascular endothelial cells using mass spectrometry, comprising: designing peptides obtained by protease digestion of all variants of the protein in a sample; protease-digesting the protein; detecting, using mass spectrometry, among the peptides designed, 2 or more kinds of peptides having an amino acid sequence specific to each of the splicing variants and a peptide having an amino acid sequence common to 2 or more splicing variants among the multiple splicing variants; and determining an amount of each of the splicing variants in the sample based on a result of the mass spectrometry wherein the protein is a vascular endothelial growth factor (VEGF)-A. 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

Claims

[Claim 1]

A method for parallel determination of multiple splicing variants produced by alternative splicing of the protein associated with the production of new blood vessels or the growth of vascular endothelial cells using mass spectrometry, comprising:

designing peptides obtained by protease digestion of all variants of the protein in a sample;

protease-digesting the protein;

detecting, using mass spectrometry, among the peptides designed, 2 or more kinds of peptides having an amino acid sequence specific to each of the splicing variants and a peptide having an amino acid sequence common to 2 or more splicing variants among the multiple splicing variants; and

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

wherein the protein is a vascular endothelial growth factor (VEGF)-A.

[Claim 2]

The method according to claim 1 wherein the splicing 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 3]

The method according to claim 1 or 2, wherein 1 or more peptides having amino acid sequences shown in SEQ ID NOs: 11 – 26 are detected.

[Title of the Invention]

METHOD FOR PARALLEL QUANTIFICATION OF PROTEIN VARIANT

[Document Name] Abstract

There are numerous proteins having different in vivo effects depending on variants. 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.

Provided is a method for performing parallel quantification of variants of a protein having 2 or more variants using mass spectrometry. The method includes: protease-digesting a protein in a sample; detecting, using mass spectrometry, among peptides obtained by the protease digestion, peptides having amino acid sequences specific to the variants without separating the peptides; and determining amounts of the variants in the sample based on results of the detection.

[No Suitable Figure]