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(71) Demandeur/Applicant:
TAIHO PHARMACEUTICAL CO., LTD., JP

(72) Inventeurs/Inventors:

(54) Titre : PROTEINE HYBRIDE DE PROTEINE DCTN1 ET DE PROTEINE RET
(54) Title: FUSION PROTEIN OF DCTN1 PROTEIN WITH RET PROTEIN

(57) **Abrégé/Abstract:**

A novel polypeptide wherein a part of a DCTN1 protein is fused with a part of a RET protein; a polynucleotide encoding the polypeptide; a method for detecting the polynucleotide or the polypeptide; a method for screening a compound which inhibits the expression of the polynucleotide or the expression and/or activity of the polypeptide; and a medicinal composition which comprises as an active ingredient a compound inhibiting RET.

(72) Inventeurs(suite)/Inventors(continued): HAYASHI, KOHEI, JP; ISHIDA, KEIJI, JP

(74) Agent: MARKS & CLERK

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(71) 出願人: 大鵬薬品工業株式会社
(**TAIHO PHARMACEUTICAL CO., LTD.**) [JP/
JP]; 〒1018444 東京都千代田区神田錦町
1-27 Tokyo (JP).(72) 発明者: 林 晃平(**HAYASHI, Kohei**); 〒3002611
茨城県つくば市大久保3 大鵬薬品工業株式
会社内 Ibaraki (JP). 石田 圭司(**ISHIDA, Keiji**);
〒3002611 茨城県つくば市大久保3 大鵬薬
品工業株式会社内 Ibaraki (JP).(74) 代理人: 特許業務法人三枝国際特許事務
所(**SAEGUSA & PARTNERS**); 〒5410045 大阪
府大阪府中央区道修町1-7-1 北
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(54) **Title:** FUSION PROTEIN OF DCTN1 PROTEIN WITH RET PROTEIN

(54) 発明の名称 : D C T N 1 タンパク質と R E T タンパク質との融合タンパク質

(57) **Abstract:** A novel polypeptide wherein a part of a DCTN1 protein is fused with a part of a RET protein; a polynucleotide encoding the polypeptide; a method for detecting the polynucleotide or the polypeptide; a method for screening a compound which inhibits the expression of the polynucleotide or the expression and/or activity of the polypeptide; and a medicinal composition which comprises as an active ingredient a compound inhibiting RET.

(57) 要約 : D C T N 1 タンパク質の一部と R E T タンパク質の一部とが融合した新規なポリペプチド、上記ポリペプチドをコードするポリヌクレオチド、上記ポリヌクレオチド又はポリペプチドの検出方法、上記ポリヌクレオチドの発現又はポリペプチドの発現及び／又は活性を抑制する化合物をスクリーニングする方法、及び R E T を阻害する化合物を有効成分とする医薬組成物。

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DESCRIPTION

Title of Invention: FUSION PROTEIN OF DCTN1 PROTEIN WITH RET PROTEIN

5 Technical Field
[0001]

The present invention relates to a polypeptide that is a fusion protein of DCTN1 protein and RET protein; a polynucleotide that encodes the polypeptide; a method for
10 detecting the polypeptide or the polynucleotide; a compound that targets the polypeptide or the polynucleotide; and a method of screening for the compound.

Background Art
15 [0002]

Cancer is the leading cause of death by disease in Japan, and its treatment must be improved. Although the number of individuals affected with thyroid cancer is increasing, appropriate treatment at the initial stage leads to a high
20 survival rate because of the slow progress of the disease in most cases. The disease, however, has almost no subjective symptoms, and early diagnosis is essential for appropriate treatment.
[0003]

Thyroid cancer is divided by histological types into
25 papillary cancer, follicular cancer, medullary cancer, anaplastic cancer, and malignant lymphoma. Papillary cancer accounts for about 80% of thyroid cancer, and anaplastic cancer, which has a lower incidence though, is known to have a very poor prognosis (Non-patent Literature 1).

30 [0004]

Papillary cancer is known to develop largely because of the activation of oncogenes, and mutually exclusive genetic abnormalities, such as BRAF mutation gene (50 to 60%), RAS mutation gene (10 to 20%), and RET fusion gene (5 to 10%), have
35 been revealed to occur. Studies also report that in non-small-

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cell lung cancer as well, RET fusion gene is present at a frequency of 1 to 2% mutually exclusively with other driver mutation genes, such as EGFR mutation gene (Non-patent Literature 2 to 5).

5 [0005]

Drug treatment is the dominant mode of advanced thyroid cancer treatment, and a variety of multikinase inhibitors have been approved. However, medicinal agents that exhibit an effect specific to driver mutation genes remain unapproved. A study
10 reports that RET fusion gene-positive patients with lung cancer show benefit from inhibiting RET (Non-patent Literature 6); it is necessary in thyroid cancer, as well, to identify gene abnormalities, such as mutation genes or fusion genes, which can be an indicator of the effect of medicinal agents.

15 [0006]

There has been a strong desire to identify mutation genes (mutation proteins), fusion genes (fusion proteins), etc., which can be drivers of cancer; this is because such identification will elucidate the nature of cancer, and
20 significantly contribute to the development of novel cancer treatment drugs or testing methods that target these mutation genes or fusion genes. However, mutation genes, fusion genes, etc., which can be drivers of cancer development, have yet to be fully elucidated, and identifying gene abnormalities that may be
25 associated with therapeutic effects of medicinal agents will be highly significant.

Citation List

Non-patent Literature

30 [0007]

Non-patent Literature 1: Cancer, 115 (16), pp. 3801-7 (2009)

Non-patent Literature 2: Oncogene, 22 (29), pp. 4578-80 (2003)

Non-patent Literature 3: Cell, 159 (3), pp. 676-90 (2014)

Non-patent Literature 4: Cancer Discov., 3 (6), pp. 630-5 (2013)

35 Non-patent Literature 5: Nature, 511 (7511), pp. 543-50 (2014)

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Non-patent Literature 6: Lancet Respir Med., 5 (1), pp. 42-50
(2017)

Summary of Invention

5 Technical Problem

[0008]

An object of the present invention is to provide a novel polypeptide that is a fusion protein incorporating at least a portion of RET protein; a polynucleotide encoding the
10 polypeptide; a method for detecting the polypeptide or the polynucleotide; a compound targeting the polypeptide or the polynucleotide; and a method of screening for the compound.

Solution to Problem

15 [0009]

The present inventors conducted extensive research to achieve the object, and identified a novel polypeptide in which a portion of DCTN1 protein is fused to a portion of RET protein, and a polynucleotide encoding the polypeptide in the cells
20 derived from thyroid cancer patients. The inventors also found a method for detecting the polynucleotide or polypeptide of the present invention in cancer cells, and a method of screening for a compound that inhibits the expression of the polynucleotide or the expression and/or activity of the polypeptide. It is a novel
25 finding and cannot be predicted from the prior art that, among a wide range of proteins, a fusion protein containing a combination of the N-terminal portion of DCTN1 protein and the C-terminal portion of RET protein naturally occurs intracellularly; and that because the fusion gene of DCTN1 and RET functions as a cancer
30 driver, the fusion protein is useful in cancer diagnosis. They further found a pharmaceutical composition that contains a compound that inhibits RET as an active ingredient and that is for use in the treatment of cancer patients who have the expression of the polypeptide and/or the polynucleotide, and
35 completed the present invention.

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[0010]

Specifically, the present invention provides the following subject matter.

[0011]

5 Item 1.

A polypeptide wherein an N-terminal portion of DCTN1 protein is fused to a C-terminal portion of RET protein.

[0012]

Item 2.

10 The polypeptide according to Item 1, which is selected from the following (a) to (c):

[0013]

(a) a polypeptide comprising the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID
15 NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, or SEQ ID NO: 24;

[0014]

(b) a polypeptide comprising an amino acid sequence wherein one or several amino acids are substituted, deleted, or added in the
20 amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, or SEQ ID NO: 24; and

[0015]

25 (c) a polypeptide comprising an amino acid sequence that has at least 90% identity with the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, or SEQ ID NO: 24.

30 [0016]

Item 3.

A polynucleotide encoding the polypeptide according to Item 1 or 2.

[0017]

35 Item 4.

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The polynucleotide according to Item 3, which is selected from the following (d) to (f):

[0018]

(d) a polynucleotide encoding a polypeptide comprising the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, or SEQ ID NO: 24;

[0019]

(e) a polynucleotide encoding a polypeptide comprising an amino acid sequence wherein one or several amino acids are substituted, deleted, or added in the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, or SEQ ID NO: 24; and

[0020]

(f) a polynucleotide encoding a polypeptide comprising an amino acid sequence that has at least 90% identity with the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, or SEQ ID NO: 24.

[0021]

Item 5.

The polynucleotide according to Item 3, which is selected from the following (g) to (i):

[0022]

(g) a polynucleotide comprising the base sequence represented by SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, or SEQ ID NO: 23;

[0023]

(h) a polynucleotide hybridizing under stringent conditions with a polynucleotide comprising a base sequence complementary to the base sequence represented by SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID

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NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, or SEQ ID NO: 23; and

[0024]

- 5 (i) a polynucleotide having at least 90% identity with the base sequence represented by SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, or SEQ ID NO: 23.

10 [0025]

Item 6.

An expression vector comprising the polynucleotide according to any one of Items 3 to 5.

[0026]

15 Item 7.

A cell transfected with the polynucleotide according to any one of Items 3 to 5.

[0027]

Item 8.

- 20 An antibody that specifically binds to the polypeptide according to Item 1 or 2.

[0028]

Item 9.

- 25 A method for detecting the presence of the polypeptide according to Item 1 or 2 in a sample.

[0029]

Item 10.

- 30 A primer or probe for detecting the presence of the polynucleotide according to any one of Items 3 to 5 in a sample, the primer or probe being a polynucleotide selected from the following (j) to (l):

[0030]

- (j) a polynucleotide that is at least one probe selected from the group consisting of probes hybridizing with a polynucleotide encoding DCTN1 protein, and probes hybridizing with a
- 35

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polynucleotide encoding RET protein;

[0031]

(k) a polynucleotide that is a probe that hybridizes to a point of fusion between a polynucleotide encoding DCTN1 protein and a

5 polynucleotide encoding RET protein; and

[0032]

(l) a polynucleotide that is a set of a sense primer and an antisense primer designed to sandwich a point of fusion between a polynucleotide encoding DCTN1 protein and a polynucleotide

10 encoding RET protein.

[0033]

Item 11.

A method for detecting the presence of the polynucleotide according to any one of Items 3 to 5 in a sample.

15 [0034]

Item 12.

A method for diagnosing cancer in a patient when the presence of the polypeptide according to Item 1 or 2 or the polynucleotide according to any one of Items 3 to 5 is detected in a sample derived from the patient by the detection method according to Item 9 or 11.

20

[0035]

Item 13.

A pharmaceutical composition for treating cancer that is positive for a fusion gene of DCTN1 gene and RET gene and/or positive for a fusion protein of DCTN1 protein and RET protein, the composition comprising a compound that inhibits RET as an active ingredient.

25

[0036]

30 Item 14.

A method of screening for a compound that inhibits expression and/or activity of the polypeptide according to Item 1 or 2 or expression of the polynucleotide according to any one of Items 3 to 5, the method comprising the following steps (1) and

35 (2):

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[0037]

(1) the step of bringing the polypeptide according to Item 1 or 2, a cell expressing the polypeptide according to Item 1 or 2 or the polynucleotide according to any one of Items 3 to 5, or the cell according to Item 7 into contact with a test compound; and

[0038]

(2) the step of measuring whether expression and/or activity of the polypeptide according to Item 1 or 2 or expression of the polynucleotide according to any one of Items 3 to 5 is inhibited in step (1), or the step of measuring whether growth of the cell in step (1) is inhibited.

[0039]

Item 15.

A method using the polypeptide according to Item 1 or 2 or the polynucleotide according to any one of Items 3 to 5 as an indicator for determining whether a chemotherapy using a compound that inhibits RET is effective,

the method comprising determining that the chemotherapy using the compound that inhibits RET is effective when the polypeptide according to Item 1 or 2 is detected in a sample by the detection method according to Item 9, and/or when the presence of the polynucleotide according to any one of Items 3 to 5 is detected in a sample by the detection method according to Item 11.

[0040]

Item 16.

A biomarker for detecting cancer, the biomarker comprising at least one member selected from the group consisting of polypeptides wherein an N-terminal portion of DCTN1 protein is fused to a C-terminal portion of RET protein, and polynucleotides encoding the polypeptides.

[0041]

Item 17.

A method for treating cancer, the method comprising giving a chemotherapy using a compound that inhibits

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RET to a cancer patient who is positive for a fusion gene of DCTN1 gene and RET gene, and/or positive for a fusion protein of DCTN1 protein and RET protein.

[0042]

5 Item 18.

A method for treating cancer, the method comprising detecting the presence of the polypeptide according to Item 1 or 2, and/or the presence of the polynucleotide according to any one of Items 3 to 5 in a sample derived from a test

10 subject, and

giving a chemotherapy using a compound that inhibits RET to the test subject when the presence of the polypeptide according to Item 1 or 2 has been detected, and/or the presence of the polynucleotide according to any one of Items 3 to 5 has

15 been detected.

[0043]

Item 19.

A compound that inhibits RET for use in the treatment of a cancer patient who is positive for a fusion gene of DCTN1 gene and RET gene, and/or positive for a fusion protein of DCTN1 protein and RET protein.

20

[0044]

Item 20.

Use of a compound that inhibits RET in the production of a pharmaceutical composition for cancer treatment for treating a cancer patient who is positive for a fusion gene of DCTN1 gene and RET gene, and/or positive for a fusion protein of DCTN1 protein and RET protein.

25

[0045]

30 Item 21.

A method for producing a reagent for determining whether a chemotherapy using a compound that inhibits RET is effective, using a means to detect the presence of the polypeptide according to Item 1 or 2 in a sample, and/or a means to detect the presence of the polynucleotide according to any one

35

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of Items 3 to 5 in a sample.

[0046]

Item 22.

5 A combination of an anti-DCTN1 antibody and an anti-RET antibody for detecting the presence of the polynucleotide according to any one of Items 3 to 5.

[0047]

Item 23.

10 Use of the antibody according to Item 8, the combination of the antibodies according to Item 22, or the primer or probe according to Item 10 in the production of a detection reagent for detecting the presence of the polypeptide according to Item 1 or 2, or the presence of the polynucleotide according to any one of Items 3 to 5.

15

Advantageous Effects of Invention

[0048]

The present invention has demonstrated that the polynucleotide and/or polypeptide of the present invention is
20 specifically expressed in a cancer cell. The polynucleotide, polypeptide, and cell expressing the polynucleotide and/or polypeptide of the present invention can be used in a method of screening for a compound that inhibits the expression of the polynucleotide, or the expression and/or activity of the
25 polypeptide of the present invention. The use of the presence of the polynucleotide and/or polypeptide of the present invention as an indicator enables detection of a target positive for a fusion gene of DCTN1 gene and RET gene, and/or a target positive for a fusion protein of DCTN1 protein and RET protein. A compound that
30 inhibits RET is useful as a pharmaceutical composition for treating cancer that is positive for a fusion gene of DCTN1 gene and RET gene, and/or positive for a fusion protein of DCTN1 protein and RET protein.

35 Brief Description of Drawings

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[0049]

Fig. 1: Confirmation of the expression of DCTN1-RET fusion gene and GAPDH in thyroid cancer tissue using Droplet Digital PCR (ddPCR).

5

Fig. 2: Confirmation of the expression of DCTN1-RET fusion gene and GAPDH in a normal thyroid tissue using Droplet Digital PCR (ddPCR).

10 Fig. 3: Confirmation of the expression of a full-length DCTN1-RET fusion gene in a normal thyroid tissue and a thyroid cancer tissue.

Fig. 4: Confirmation of the expression of DCTN1-RET fusion
15 protein in DCTN1-RET fusion gene-expressing NIH/3T3 cells:
a) Detection of a DCTN1-RET fusion protein using an anti-phosphorylated RET antibody;
b) Detection of a DCTN1-RET fusion protein using an anti-RET antibody; and
20 c) Detection of a DCTN1-RET fusion protein using an anti-DCTN1 antibody.

Fig. 5: Confirmation of the growth of NIH/3T3 cells expressing a DCTN1-RET fusion gene in a 3-dimensional culture.
25 N=3, average + SD.

Fig. 6: Confirmation of tumorigenicity of NIH/3T3 cells expressing a DCTN1-RET fusion gene *in vivo*.

30 Fig. 7: Confirmation of the inhibition of expression of phosphorylated RET by RET siRNA in NIH/3T3 cells expressing a DCTN1-RET fusion gene.

Fig. 8: Confirmation of the growth inhibitory effect on NIH/3T3
35 cells expressing a DCTN1-RET fusion gene by RET siRNA.

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Fig. 9: Confirmation of the inhibition of expression of phosphorylated RET in NIH/3T3 cells expressing a DCTN1-RET fusion gene by compounds that inhibit RET.

5

Description of Embodiments

[0050]

The present invention relates to a novel polynucleotide or polypeptide; a method for detecting the polynucleotide or polypeptide; a compound targeting the polynucleotide or polypeptide; and a method of screening for the compound.

[0051]

The present invention provides a polypeptide in which the N-terminal portion of DCTN1 protein is fused to the C-terminal portion of RET protein (which may be hereinafter referred to as the "polypeptide of the present invention"). The present invention also provides a polynucleotide encoding the polypeptide (which may be hereinafter referred to as the "polynucleotide of the present invention").

20 [0052]

"DCTN1 (Dynactin Subunit 1) protein" in the present invention is also called 150 kDa dynein-associated polypeptide protein or DAP-150 protein, and includes human or non-human mammal DCTN1 protein, with human DCTN1 protein being preferable. DCTN1 protein is encoded by a gene located on 2p13.1 in humans. In the present invention, "DCTN1 protein" includes isoforms (its splice variants), and examples of human-derived DCTN1 protein includes polypeptides comprising the amino acid sequence represented by GenPept accession number NP_004073, NP_075408, NP_001128512, NP_001128513, NP_001177765, or NP_001177766. More specifically, examples include polypeptides comprising the amino acid sequence represented by SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, or SEQ ID NO: 30. Additionally, "N-terminal portion of DCTN1 protein" in the present invention refers to a polypeptide containing part of or

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the entire coiled-coil domain that is in the N-terminal side of DCTN1 protein, and preferably a polypeptide containing the entire coiled-coil domain that is in the N-terminal side of DCTN1 protein.

5 [0053]

"RET protein" in the present invention is also called RET proto-oncogene protein, RET receptor tyrosine kinase protein, or protein rearranged during transfection; and includes human or non-human mammal RET protein, with human RET protein being
10 preferable. RET protein is encoded by a gene located on 10q11.2 in humans. In the present invention, "RET protein" includes isoforms (its splice variants), and examples of human-derived RET protein includes polypeptides comprising the amino acid sequence represented by GenPept accession number NP_066124 or NP_065681.
15 More specifically, examples include polypeptides comprising the amino acid sequence represented by SEQ ID NO: 31 or SEQ ID NO: 32. Additionally, "C-terminal portion of RET protein" in the present invention refers to a polypeptide containing a kinase domain that is in the C-terminal side of RET protein.

20 [0054]

"A polypeptide in which the N-terminal portion of DCTN1 protein is fused to the C-terminal portion of RET protein" in the present invention is a polypeptide in which the polypeptide containing part of or the entire coiled-coil domain that is in
25 the N-terminal side of DCTN1 protein is fused to the polypeptide containing a kinase domain that is in the C-terminal side of the RET protein, preferably a polypeptide in which the polypeptide containing the entire coiled-coil domain that is in the N-terminal side of DCTN1 protein is fused to the polypeptide
30 containing a kinase domain that is in the C-terminal side of the RET protein, and more preferably a polypeptide selected from the following (a) to (c). These polypeptides preferably have kinase activity and/or a cell-proliferating effect.

[0055]

35 (a) A polypeptide comprising the amino acid sequence represented

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by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, or SEQ ID NO: 24.

[0056]

- 5 (b) A polypeptide comprising an amino acid sequence wherein one or several amino acids are substituted, deleted, or added in the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, or SEQ ID NO: 24.

[0057]

- (c) A polypeptide comprising an amino acid sequence that has at least 90% identity with the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, or SEQ ID NO: 24.

[0058]

- More preferably, the polypeptide is selected from the following (a) to (c). These polypeptides preferably have kinase activity or a cell-proliferating effect.

[0059]

- (a) A polypeptide comprising the amino acid sequence represented by SEQ ID NO: 18.

[0060]

- 25 (b) A polypeptide comprising an amino acid sequence wherein one or several amino acids are substituted, deleted, or added in the amino acid sequence represented by SEQ ID NO: 18.

[0061]

- (c) A polypeptide comprising an amino acid sequence that has at least 90% identity with the amino acid sequence represented by SEQ ID NO: 18.

[0062]

- "A polypeptide in which the N-terminal portion of DCTN1 protein is fused to the C-terminal portion of RET protein" in the present invention includes polypeptides comprising an

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amino acid sequence wherein one or several amino acids are substituted, deleted, or added in the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, or SEQ ID NO: 24 (item (b) above). Examples of the polypeptides comprising such an amino acid sequence wherein the N-terminal portion of DCTN1 protein is fused to the C-terminal portion of RET protein include isoforms of polypeptides comprising the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, or SEQ ID NO: 24 wherein the N-terminal portion of DCTN1 protein is fused to the C-terminal portion of RET protein. These polypeptides preferably have kinase activity or a cell-proliferating effect. As used herein, "several amino acids that are substituted, deleted, or added" means, for example, preferably 1 to 10 amino acids, and more preferably 1 to 5 amino acids. The "addition" includes addition of one to several amino acids to the N-terminal or C-terminal, or addition of one to several amino acids to both terminals.

[0063]

Examples of the polypeptide wherein one or several amino acids are substituted include polypeptides in which valine at position 804 (at position 1325 in SEQ ID NO: 2 and SEQ ID NO: 4, at position 1191 in SEQ ID NO: 6 and SEQ ID NO: 8, at position 1300 in SEQ ID NO: 10 and SEQ ID NO: 12, at position 1186 in SEQ ID NO: 14 and SEQ ID NO: 16, at position 1283 in SEQ ID NO: 18 and SEQ ID NO: 20, at position 1318 in SEQ ID NO: 22 and SEQ ID NO: 24), which is at the gatekeeper site of RET protein comprising the amino acid sequence represented by GenPept accession number: NP_066124 (SEQ ID NO: 31) or NP_065681 (SEQ ID NO: 32), is substituted with leucine, methionine, or glutamic acid; and polypeptides in which tyrosine at position 806 (at position 1327 in SEQ ID NO: 2 and SEQ ID NO: 4, at position 1193

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in SEQ ID NO: 6 and SEQ ID NO: 8, at position 1302 in SEQ ID NO: 10 and SEQ ID NO: 12, at position 1188 in SEQ ID NO: 14 and SEQ ID NO: 16, at position 1285 in SEQ ID NO: 18 and SEQ ID NO: 20, at position 1320 in SEQ ID NO: 22 and SEQ ID NO: 24) is substituted with cysteine, glutamic acid, serine, histidine, or asparagine.

[0064]

Examples also include amino acids located at a position other than the gatekeeper site, but are not limited to,

polypeptides in which glutamic acid at position 768 (at position 1289 in SEQ ID NO: 2 and SEQ ID NO: 4, at position 1155 in SEQ ID NO: 6 and SEQ ID NO: 8, at position 1264 in SEQ ID NO: 10 and SEQ ID NO: 12, at position 1150 in SEQ ID NO: 14 and SEQ ID NO: 16, at position 1247 in SEQ ID NO: 18 and SEQ ID NO: 20, at position 1282 in SEQ ID NO: 22 and SEQ ID NO: 24) is substituted with aspartic acid; polypeptides in which alanine at position 883 (at position 1404 in SEQ ID NO: 2 and SEQ ID NO: 4, at position 1270 in SEQ ID NO: 6 and SEQ ID NO: 8, at position 1379 in SEQ ID NO: 10 and SEQ ID NO: 12, at position 1265 in SEQ ID NO: 14 and SEQ ID NO: 16, at position 1362 in SEQ ID NO: 18 and SEQ ID NO: 20, at position 1397 in SEQ ID NO: 22 and SEQ ID NO: 24) is substituted with phenylalanine or serine; polypeptides in which glutamic acid at position 884 (at position 1405 in SEQ ID NO: 2 and SEQ ID NO: 4, at position 1271 in SEQ ID NO: 6 and SEQ ID NO: 8, at position 1380 in SEQ ID NO: 10 and SEQ ID NO: 12, at position 1266 in SEQ ID NO: 14 and SEQ ID NO: 16, at position 1363 in SEQ ID NO: 18 and SEQ ID NO: 20, at position 1398 in SEQ ID NO: 22 and SEQ ID NO: 24) is substituted with valine; polypeptides in which serine at position 891 (at position 1412 in SEQ ID NO: 2 and SEQ ID NO: 4, at position 1278 in SEQ ID NO: 6 and SEQ ID NO: 8, at position 1387 in SEQ ID NO: 10 and SEQ ID NO: 12, at position 1273 in SEQ ID NO: 14 and SEQ ID NO: 16, at position 1370 in SEQ ID NO: 18 and SEQ ID NO: 20, at position 1405 in SEQ ID NO: 22 and SEQ ID NO: 24) is substituted with alanine or leucine; and polypeptides in which methionine at

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position 918 (at position 1439 in SEQ ID NO: 2 and SEQ ID NO: 4,
at position 1305 in SEQ ID NO: 6 and SEQ ID NO: 8, at position
1414 in SEQ ID NO: 10 and SEQ ID NO: 12, at position 1300 in SEQ
ID NO: 14 and SEQ ID NO: 16, at position 1397 in SEQ ID NO: 18
5 and SEQ ID NO: 20, at position 1432 in SEQ ID NO: 22 and SEQ ID
NO: 24) is substituted with threonine.
[0065]

The polypeptide in which the N-terminal portion of
DCTN1 protein is fused to the C-terminal portion of RET protein
10 of the present invention includes polypeptides comprising an
amino acid sequence that has at least 90% identity with the amino
acid sequence represented by any of SEQ ID NO: 2, SEQ ID NO: 4,
SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID
NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO:
15 22, or SEQ ID NO: 24, upon appropriate alignment thereof (item
(c) above). These polypeptides preferably have kinase activity or
a cell-proliferating effect.
[0066]

The identity with the amino acid sequence represented
20 by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID
NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO:
18, SEQ ID NO: 20, SEQ ID NO: 22, or SEQ ID NO: 24 is preferably
at least 90%, more preferably at least 95%, and still more
preferably at least 98%. The identity of the amino acid sequence
25 can be calculated by a commonly used method.
[0067]

The polypeptide of the present invention may comprise
amino acids constituting a protein tag in addition to the amino
acid sequence constituting the polypeptide of the present
30 invention. Examples of usable tags include those well known to a
person skilled in the art; for example, usable tags include tags
for improving expression efficiency and tags for improving
purification efficiency, such as His tag, Myc tag, and FLAG tag.
[0068]

35 The polynucleotide of the present invention encodes the

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polypeptide in which the N-terminal portion of DCTN1 protein is fused to the C-terminal portion of RET protein, and is preferably a polynucleotide selected from the following (d) to (i). These polynucleotides preferably encode polypeptides that have kinase
 5 activity or a cell-proliferating effect.

[0069]

(d) A polynucleotide encoding a polypeptide comprising the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14,
 10 SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, or SEQ ID NO: 24.

[0070]

(e) A polynucleotide encoding a polypeptide comprising an amino acid sequence wherein one or several amino acids are substituted,
 15 deleted, or added in the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, or SEQ ID NO: 24.

[0071]

(f) A polynucleotide encoding a polypeptide comprising an amino acid sequence that has at least 90% identity with the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, or SEQ ID
 20 NO: 24.
 25

[0072]

(g) A polynucleotide comprising the base sequence represented by SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, or SEQ ID NO: 23.
 30

[0073]

(h) A polynucleotide hybridizing under stringent conditions with a polynucleotide comprising a base sequence complementary to the base sequence represented by SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13,
 35

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SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, or
SEQ ID NO: 23.

[0074]

(i) A polynucleotide that has at least 90% identity with the base
5 sequence represented by SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5,
SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID
NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, or SEQ ID
NO: 23.

[0075]

10 More preferably, the polynucleotide of the present
invention is selected from the following (d) to (i). These
polynucleotides preferably encode polypeptides that have kinase
activity or a cell-proliferating effect.

[0076]

15 (d) A polynucleotide encoding a polypeptide comprising the amino
acid sequence represented by SEQ ID NO: 18.

[0077]

(e) A polynucleotide encoding a polypeptide comprising an amino
acid sequence wherein one or several amino acids are substituted,
20 deleted, or added in the amino acid sequence represented by SEQ
ID NO: 18.

[0078]

(f) A polynucleotide encoding a polypeptide comprising an amino
acid sequence that has at least 90% identity with the amino acid
25 sequence represented by SEQ ID NO: 18.

[0079]

(g) A polynucleotide comprising the base sequence represented by
SEQ ID NO: 17.

[0080]

30 (h) A polynucleotide hybridizing under stringent conditions with
a polynucleotide comprising a base sequence complementary to the
base sequence represented by SEQ ID NO: 17.

[0081]

(i) A polynucleotide that has at least 90% identity with the base
35 sequence represented by SEQ ID NO: 17.

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[0082]

The polynucleotide of the present invention includes not only its double-stranded DNA but also various types of single-stranded DNA and RNA that constitute the double-stranded DNA, such as sense strands and antisense strands. The antisense strands can be used as probes and the like. DNA includes those obtained by cloning, chemical synthesis, or a combination thereof, such as cDNA and genomic DNA. Additionally, base sequences, such as untranslated region (UTR) sequences, may be added to the polynucleotide of the present invention, in addition to the base sequence to encode the polypeptide of the present invention.

[0083]

Stringent conditions as used herein include, for example, conditions described in Molecular Cloning: A Laboratory Manual (Second Edition, J. Sambrook et al., 1989). Specifically, stringent conditions include conditions such that a solution containing 6×SSC (1×SSC composition: 0.15 M sodium chloride, 0.015 M sodium citrate, pH 7.0), 0.5% SDS, 5×Denhalt, and 100 mg/mL herring sperm DNA is subjected to isothermal treatment together with a probe at 65°C for 8 to 16 hours to perform hybridization.

[0084]

The identity with the base sequence represented by SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, or SEQ ID NO: 23 is preferably at least 90%, more preferably at least 95%, and still more preferably at least 98%. The identity of the base sequence can be calculated by a commonly used method.

[0085]

In this specification, the phrase "have kinase activity" in "have kinase activity or a cell-proliferating effect" means having an enzymatic activity to phosphorylate tyrosine. Additionally, the phrase "have a cell-proliferating

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effect" in "have kinase activity or a cell-proliferating effect" means that transfection of the polynucleotide and/or polypeptide of the present invention into a cell improves the cellular proliferative ability, compared with a cell to which the polynucleotide and/or polypeptide is not transfected. This effect can be confirmed, for example, as follows: the polynucleotide and/or polypeptide is transfected into a cell line that cytokine-dependently proliferates, and if the cell line proliferates cytokine-independently, the polynucleotide and/or polypeptide has a cell-proliferating effect.

[0086]

The polynucleotide of the present invention can be extracted, for example, using a cDNA library or genomic DNA library prepared from thyroid cancer etc. that retains a fusion gene of DCTN1 gene and RET gene, using a primer that specifically hybridizes with part of the base sequence of the polynucleotide of the present invention. For this primer, any primer of any sequence and any length can be used, as long as the primer specifically hybridizes with at least a portion of the polynucleotide of the present invention or its antisense strand. A method for artificially synthesizing a polynucleotide may also be used (Nat. Methods, 11: 499-507, 2014).

[0087]

The expression vector of the present invention is not particularly limited as long as the expression vector comprises the polynucleotide of the present invention, and allows for the expression of the polypeptide of the present invention. Examples include expression vectors obtained by inserting the polynucleotide of the present invention into a known expression vector suitably selected according to the host for use.

[0088]

The host is not particularly limited as long as the host is a living cell that can undergo transformation, and examples include bacteria, such as *E. coli* and *Bacillus subtilis*; true fungi, such as yeast and filamentous fungi; insect cells,

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such as Sf9 cells; insects, such as silkworm; animal cells; and plants or plant-derived cells.

[0089]

The vector for inserting the polynucleotide of the present invention is not particularly limited as long as the vector is replicable in the host. The vector can be suitably selected in accordance with, for example, the type of introduced host, and the introduction method. Examples include plasmid DNA, phage DNA, and virus vectors. For vector DNA for use in construction of an expression vector, a widely prevalent and readily available vector DNA can be used. Examples include pUC19 (Takara Bio Inc.), pTV118N (Takara Bio Inc.), pMAMneo (Clontech Laboratories, Inc.), pGEX (GE Healthcare), pET160 (Invitrogen), pDEST (Invitrogen), pIEx (Merck Millipore), and pBacPAK (Clontech Laboratories, Inc.). Examples of virus vectors include DNA viruses and RNA viruses, such as baculovirus vectors, retroviral vectors, lentiviral vectors (e.g., human immunodeficiency virus or HIV), adenovirus vectors, adeno-associated virus vectors (AAV vector), herpes virus, vaccinia virus, poxvirus, poliovirus, Sindbis virus, Sendai virus, and simian virus-40 (SV-40).

[0090]

Transformation of a host using the expression vector can be performed, for example, by the protoplast method, competent cell method, or electroporation method. The obtained transformant may be cultured under suitable conditions in a medium containing a carbon source, a nitrogen source, metal salts, vitamins, etc., that can be utilized by the host.

[0091]

Examples of the cell transfected with the polynucleotide according to the present invention include cells transformed by the expression vector of the present invention, and cells to which the polynucleotide of the present invention has been introduced by genome editing. The cell for use includes the host cells listed above. Examples of the method for confirming whether the cell has been transformed by the

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expression vector include the method for detecting the presence of the polypeptide of the present invention, and the method for detecting the presence of the polynucleotide of the present invention.

5 [0092]

“Cells to which the polynucleotide of the present invention has been introduced by genome editing” are preferably cells having a gene obtained by fusing independently present DCTN1 gene and RET gene by genome editing, and more preferably
10 cells having a gene obtained by fusing exon 27 of DCTN1 and exon 12 of RET in respective independently present DCTN1 gene and RET gene by genome editing. These cells can be prepared by a commonly used method, and examples include a method described in Cell Rep., 9(4), pp. 1219-1227 (2014), Nat. Commun., 5, 3728 (2014).
15 Examples of the method for confirming whether the cell is a cell to which the polynucleotide of the present invention has been introduced by genome editing include the method for detecting the presence of the polypeptide of the present invention, and the method for detecting the presence of the polynucleotide of the
20 present invention.

[0093]

The polypeptide of the present invention can be obtained by preparing a culture solution and/or cells by culturing cells transformed by the expression vector of the
25 present invention in a medium suitable for cell culture under suitable conditions, and then collecting and purifying the protein from the culture solution and/or cells by a typical method. The polypeptide of the present invention can also be obtained by incorporating the expression vector containing the
30 polynucleotide of the present invention, or template RNA or template DNA that encodes the polynucleotide of the present invention, into a cell-free protein synthesis system (e.g., human cell line-derived cell extract, rabbit reticulocyte extract, wheat germ extract, and *E. coli* extract); incubating the result
35 under suitable conditions; and collecting and purifying the

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protein from the obtained reaction solution by a typical method.
[0094]

In the present invention, the antibody that specifically binds to the polypeptide of the present invention
5 includes an antibody that specifically binds to the point of fusion of the N-terminal portion of DCTN1 protein and the C-terminal portion of RET protein. The antibody refers to an antibody that specifically binds to the point of fusion of the N-terminal portion of DCTN1 protein and the C-terminal portion of
10 RET protein, but that binds to none of wild-type DCTN1 and wild-type RET protein.
[0095]

In the present invention, the phrase "the point of fusion" in "the point of fusion of the N-terminal portion of
15 DCTN1 protein and the C-terminal portion of RET protein" refers to the point at which a polypeptide derived from the N-terminal portion of DCTN1 protein is fused to a polypeptide derived from the C-terminal portion of RET protein. The point of fusion in SEQ ID NO: 2 is the point at which a polypeptide having the amino
20 acid sequence of positions 1-1233 of SEQ ID NO: 2, which is derived from the N-terminal portion of DCTN1, is fused to a polypeptide having the amino acid sequence of positions 1234-1635 of SEQ ID NO: 2, which is derived from the C-terminal portion of RET. The point of fusion in SEQ ID NO: 4 is the point at which a
25 polypeptide having the amino acid sequence of positions 1-1233 of SEQ ID NO: 4, which is derived from the N-terminal portion of DCTN 1, is fused to a polypeptide having the amino acid sequence of positions 1234-1593 of SEQ ID NO: 4, which is derived from the C-terminal portion of RET. The point of fusion in SEQ ID NO: 6 is
30 the point at which a polypeptide having the amino acid sequence of positions 1-1099 of SEQ ID NO: 6, which is derived from the N-terminal portion of DCTN1, is fused to a polypeptide having the amino acid sequence of positions 1100-1501 of SEQ ID NO: 6, which is derived from the C-terminal portion of RET. The point of
35 fusion in SEQ ID NO: 8 is the point at which a polypeptide having

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the amino acid sequence of positions 1-1099 of SEQ ID NO: 8, which is derived from the N-terminal portion of DCTN1, is fused to a polypeptide having the amino acid sequence of positions 1100-1459 of SEQ ID NO: 8, which is derived from the C-terminal portion of RET. The point of fusion in SEQ ID NO: 10 is the point at which a polypeptide having the amino acid sequence of positions 1-1208 of SEQ ID NO: 10, which is derived from the N-terminal portion of DCTN1, is fused to a polypeptide having the amino acid sequence of positions 1209-1610 of SEQ ID NO: 10, which is derived from the C-terminal portion of RET. The point of fusion in SEQ ID NO: 12 is the point at which a polypeptide having the amino acid sequence of positions 1-1208 of SEQ ID NO: 12, which is derived from the N-terminal portion of DCTN1, is fused to a polypeptide having the amino acid sequence of positions 1209-1568 of SEQ ID NO: 12, which is derived from the C-terminal portion of RET. The point of fusion in SEQ ID NO: 14 is the point at which a polypeptide having the amino acid sequence of positions 1-1094 of SEQ ID NO: 14, which is derived from the N-terminal portion of DCTN1, is fused to a polypeptide having the amino acid sequence of positions 1095-1496 of SEQ ID NO: 14, which is derived from the C-terminal portion of RET. The point of fusion in SEQ ID NO: 16 is the point at which a polypeptide having the amino acid sequence of positions 1-1094 of SEQ ID NO: 16, which is derived from the N-terminal portion of DCTN1, is fused to a polypeptide having the amino acid sequence of positions 1095-1454 of SEQ ID NO: 16, which is derived from the C-terminal portion of RET. The point of fusion in SEQ ID NO: 18 is the point at which a polypeptide having the amino acid sequence of positions 1-1191 of SEQ ID NO: 18, which is derived from the N-terminal portion of DCTN1, is fused to a polypeptide having the amino acid sequence of positions 1192-1593 of SEQ ID NO: 18, which is derived from the C-terminal portion of RET. The point of fusion in SEQ ID NO: 20 is the point at which a polypeptide having the amino acid sequence of positions 1-1191 of SEQ ID NO: 20, which is derived from the N-terminal portion of

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DCTN1, is fused to a polypeptide having the amino acid sequence of positions 1192-1551 of SEQ ID NO: 20, which is derived from the C-terminal portion of RET. The point of fusion in SEQ ID NO: 22 is the point at which a polypeptide having the amino acid sequence of positions 1-1226 of SEQ ID NO: 22, which is derived from the N-terminal portion of DCTN1, is fused to a polypeptide having the amino acid sequence of positions 1227-1628 of SEQ ID NO: 22, which is derived from the C-terminal portion of RET. The point of fusion in SEQ ID NO: 24 is the point at which a polypeptide having the amino acid sequence of positions 1-1226 of SEQ ID NO: 24, which is derived from the N-terminal portion of DCTN1, is fused to a polypeptide having the amino acid sequence of positions 1227-1586 of SEQ ID NO: 24, which is derived from the C-terminal portion of RET.

15 [0096]

Examples of the antibody include immunoglobulins (e.g., IgA, IgD, IgE, IgG, IgM, and IgY), Fab fragments, F(ab')₂ fragments, single-stranded antibody fragments (scFv), single-domain antibodies, and diabodies (Nat. Rev. Immunol., 6: 343-357, 2006). These include, but are not limited to, monoclonal antibodies and polyclonal antibodies of, for example, human antibodies, humanized antibodies, chimeric antibodies, mouse antibodies, llama antibodies, and chicken antibodies.

[0097]

25 The antibody can be prepared by various known methods, and the preparation method is not particularly limited. The known methods include a method in which the polypeptide of the present invention, a polypeptide fragment containing the point of fusion of the N-terminal portion of DCTN1 protein and the C-terminal portion of RET protein, or the like is inoculated into an immunized animal to activate the immune system of the animal, and the serum of the animal is collected to obtain a polyclonal antibody; and a method for obtaining a monoclonal antibody, for example, by the hybridoma method and phage display method.

35 [0098]

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The method of screening for a compound that inhibits expression and/or activity of the polypeptide of the present invention or expression of the polynucleotide of the present invention can be performed by the method comprising the following steps (1) and (2).

[0099]

Specifically, the screening method of the present invention is performed by the method comprising:

(1) the step of bringing the polypeptide of the present invention, or a cell expressing the polypeptide and/or the polynucleotide of

the present invention into contact with a test compound; and

[0100]

(2) the step of measuring whether expression and/or activity of the polypeptide of the present invention or expression of the polynucleotide of the present invention is inhibited in step (1), or the step of measuring whether growth of the cell in step (1) is inhibited.

[0101]

More preferably, the screening method of the present invention is a method comprising the following steps (1) and (2).

[0102]

(1) The step of bringing a cell expressing the polypeptide and/or polynucleotide of the present invention into contact with a test compound.

[0103]

(2) The step of measuring whether growth of the cell in step (1) is inhibited.

[0104]

The method of screening for a compound that inhibits expression and/or activity of the polypeptide of the present invention or expression of the polynucleotide of the present invention can be performed by the method comprising the following steps (1) to (3).

[0105]

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Specifically, the screening method of the present invention is performed by the method comprising:

- (1) the step of bringing the polypeptide of the present invention or a cell expressing the polypeptide and/or the polynucleotide of the present invention into contact with a test compound;
[0106]
- (2) the step of measuring whether expression and/or activity of the polypeptide of the present invention, or expression of the polynucleotide of the present invention is inhibited in step (1),
10 or the step of measuring whether growth of the cell in step (1) is inhibited; and
[0107]
- (3) the step of determining that the test compound inhibits expression and/or activity of the polypeptide of the present
15 invention or expression of the polynucleotide of the present invention when expression and/or activity of the polypeptide of the present invention or expression of the polynucleotide of the present invention is inhibited in step (2), or when growth of the cell in step (1) is inhibited in step (2).
20 [0108]

More preferably, the screening method of the present invention is a method comprising the following steps (1) to (3).
[0109]

- (1) The step of bringing a cell expressing the polypeptide and/or
25 the polynucleotide of the present invention into contact with a test compound.
[0110]
- (2) The step of measuring whether growth of the cell in step (1) is inhibited.
30 [0111]
- (3) The step of determining that the test compound inhibits expression and/or activity of the polypeptide of the present invention, or expression of the polynucleotide of the present invention, when growth of the cell in step (1) is inhibited in
35 step (2).

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[0112]

"A cell expressing the polypeptide and/or the polynucleotide of the present invention" includes cells transformed by the expression vector of the present invention, cells introducing the polynucleotide of the present invention by genome editing, primary culture cells expressing the polypeptide and/or polynucleotide of the present invention, cell lines expressing the polypeptide and/or polynucleotide of the present invention, and cancer patient-derived cells expressing the polypeptide and/or polynucleotide of the present invention. Examples of the method for confirming whether the cell is expressing the polypeptide and/or polynucleotide of the present invention include the method for detecting the presence of the polypeptide of the present invention, and the method for detecting the presence of the polynucleotide of the present invention.

[0113]

In the present invention, "expression of the polypeptide of the present invention or expression of the polynucleotide of the present invention is inhibited" in the phrase "expression and/or activity of the polypeptide of the present invention or expression of the polynucleotide of the present invention is inhibited" means, for example, as follows. A cell expressing the polypeptide and/or polynucleotide of the present invention is brought into contact with a test compound, and the expression level of the polypeptide or polynucleotide of the present invention in the cell is evaluated using the method for detecting the presence of the polypeptide or polynucleotide of the present invention. When the cell brought into contact with the test compound exhibits a statistically significantly lowered expression level of the polypeptide or polynucleotide of the present invention than a cell that has not been brought into contact with the test compound, expression of the polypeptide or polynucleotide of the present invention is determined to be inhibited.

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[0114]

The phrase "activity of the polypeptide of the present invention is inhibited" in the phrase "expression and/or activity of the polypeptide of the present invention or expression of the polynucleotide of the present invention is inhibited" means, for example, as follows. When the polypeptide of the present invention or a cell expressing the polypeptide of the present invention that has been brought into contact with a test compound results in a statistically significantly lowered tyrosine phosphorylation percentage than a polypeptide or cell that has not been brought into contact with the test compound, activity of the polypeptide of the present invention is determined to be inhibited.

[0115]

When a cell expressing the polypeptide of the present invention that has been brought into contact with a test compound exhibits statistically significantly inhibited cell growth compared with a cell that has not been brought into contact with the test compound, activity of the polypeptide of the present invention is determined to be inhibited.

[0116]

In the present invention, "tyrosine phosphorylation" includes not only phosphorylation of tyrosine in RET protein (including RET protein fused to other protein), but also phosphorylation of tyrosine in a protein on signaling downstream of RET. Examples of proteins on downstream signaling of RET include STAT, AKT, and ERK. Tyrosine phosphorylation is preferably phosphorylation of tyrosine in RET protein (including RET protein fused to other protein).

[0117]

"Tyrosine phosphorylation percentage" can be measured using, for example, a phosphorylated RET-specific antibody by Western blotting, immunoprecipitation, immunohistochemistry, ELISA, or flow cytometry.

[0118]

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In the present invention, "sample" includes not only biological samples (e.g., cells, tissues, organs, body fluids (e.g., blood and lymph), digestive juice, and urine), but also nucleic acid extracts (e.g., genomic DNA extracts, mRNA extracts, and cDNA preparations and cRNA preparations prepared from mRNA extracts) and protein extracts obtained from these biological samples. The sample may be those subjected to formalin fixation, alcohol fixation, freeze treatment, or paraffin embedding. The biological sample for use may be those collected from a living body, and is preferably a sample derived from a cancer patient, and more preferably a sample containing tumor cells. The method for collecting a biological sample can be suitably selected depending on the type of biological sample.

[0119]

The present invention encompasses a method for detecting the presence of the polypeptide of the present invention in a sample.

[0120]

In the present invention, the method for detecting the presence of the polypeptide of the present invention in a sample includes detection methods in accordance with commonly used methods, such as ELISA, Western blotting, or immunohistochemical staining, using an antibody that specifically binds to the polypeptide of the present invention; and FRET (fluorescence resonance energy transfer) using an antibody that specifically binds to DCTN1 protein, and an antibody that specifically binds to RET protein. The detection method is preferably ELISA, Western blotting, or immunohistochemical staining, using an antibody that specifically binds to the polypeptide of the present invention.

[0121]

The antibody that specifically binds to DCTN1 protein and the antibody that specifically binds to RET protein are preferably an antibody that binds to an N-terminal portion from the point of fusion in DCTN1 protein, and an antibody that binds to a C-terminal portion from the point in fusion of RET protein.

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These antibodies may be commercially available products, or prepared by a typical known method.

[0122]

In the present invention, the method for detecting the presence of the polypeptide of the present invention in a sample preferably comprises the step of detecting the polypeptide of the present invention using an antibody that specifically binds to the polypeptide of the present invention, or an antibody that specifically binds to DCTN1 protein and an antibody that specifically binds to RET protein; and more preferably comprises the step of detecting the polypeptide of the present invention using an antibody that specifically binds to the polypeptide of the present invention. The means to detect the presence of the polypeptide of the present invention is not particularly limited, and examples thereof include a combination of an antibody that specifically binds to DCTN1 protein and an antibody that specifically binds to RET protein; and an antibody that specifically binds to the polypeptide of the present invention.

[0123]

The present invention encompasses a primer or probe for detecting the presence of the polynucleotide of the present invention in a sample. In the present invention, the means to detect the presence of the polypeptide of the present invention is not particularly limited, and examples thereof include a primer or probe for detecting the presence of the polynucleotide of the present invention.

[0124]

The primer or probe includes polynucleotides selected from the following (j) to (l):

(j) a polynucleotide that is at least one probe selected from the group consisting of probes hybridizing with a polynucleotide encoding DCTN1 protein, and probes hybridizing with a polynucleotide encoding RET protein;

[0125]

(k) a polynucleotide that is a probe that hybridizes to the point

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of fusion between a polynucleotide encoding DCTN1 protein and a polynucleotide encoding RET protein; and

[0126]

- (1) a polynucleotide that is a set of a sense primer and an antisense primer designed to sandwich the point of fusion between a polynucleotide encoding DCTN1 protein and a polynucleotide encoding RET protein.

[0127]

In the present invention, "the point of fusion" in the phrase "the point of fusion between a polynucleotide encoding DCTN1 protein and a polynucleotide encoding RET protein" refers to the point at which a polynucleotide encoding DCTN1 protein is fused to a polynucleotide encoding RET protein. The point of fusion in SEQ ID NO: 1 is the point at which a polynucleotide having the base sequence of positions 1-3699 of SEQ ID NO: 1, which is derived from the polynucleotide encoding DCTN1, is fused to a polynucleotide having the base sequence of positions 3700-4905 of SEQ ID NO: 1, which is derived from the polynucleotide encoding RET. The point of fusion in SEQ ID NO: 3 is the point at which a polynucleotide having the base sequence of positions 1-3699 of SEQ ID NO: 3, which is derived from the polynucleotide encoding DCTN1, is fused to a polynucleotide having the base sequence of positions 3700-4779 of SEQ ID NO: 3, which is derived from the polynucleotide encoding RET. The point of fusion in SEQ ID NO: 5 is the point at which a polynucleotide having the base sequence of positions 1-3297 of SEQ ID NO: 5, which is derived from the polynucleotide encoding DCTN1, is fused to a polynucleotide having the base sequence of positions 3298-4503 of SEQ ID NO: 5, which is derived from the polynucleotide encoding RET. The point of fusion in SEQ ID NO: 7 is the point at which a polynucleotide having the base sequence of positions 1-3297 of SEQ ID NO: 7, which is derived from the polynucleotide encoding DCTN1, is fused to a polynucleotide having the base sequence of positions 3298-4377 of SEQ ID NO: 7, which is derived from the polynucleotide encoding RET. The point of fusion in SEQ ID NO: 9

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is the point at which a polynucleotide having the base sequence of positions 1-3624 of SEQ ID NO: 9, which is derived from the polynucleotide encoding DCTN1, is fused to a polynucleotide having the base sequence of positions 3625-4830 of SEQ ID NO: 9, which is derived from the polynucleotide encoding RET. The point of fusion in SEQ ID NO: 11 is the point at which a polynucleotide having the base sequence of positions 1-3624 of SEQ ID NO: 11, which is derived from the polynucleotide encoding DCTN1, is fused to a polynucleotide having the base sequence of positions 3625-4704 of SEQ ID NO: 11, which is derived from the polynucleotide encoding RET. The point of fusion in SEQ ID NO: 13 is the point at which a polynucleotide having the base sequence of positions 1-3282 of SEQ ID NO: 13, which is derived from the polynucleotide encoding DCTN1, is fused to a polynucleotide having the base sequence of positions 3283-4488 of SEQ ID NO: 13, which is derived from the polynucleotide encoding RET. The point of fusion in SEQ ID NO: 15 is the point at which a polynucleotide having the base sequence of positions 1-3282 of SEQ ID NO: 15, which is derived from the polynucleotide encoding DCTN1, is fused to a polynucleotide having the base sequence of positions 3283-4362 of SEQ ID NO: 15, which is derived from the polynucleotide encoding RET. The point of fusion in SEQ ID NO: 17 is the point at which a polynucleotide having the base sequence of positions 1-3573 of SEQ ID NO: 17, which is derived from the polynucleotide encoding DCTN1, is fused to a polynucleotide having the base sequence of positions 3574-4779 of SEQ ID NO: 17, which is derived from the polynucleotide encoding RET. The point of fusion in SEQ ID NO: 19 is the point at which a polynucleotide having the base sequence of positions 1-3573 of SEQ ID NO: 19, which is derived from the polynucleotide encoding DCTN1, is fused to a polynucleotide having the base sequence of positions 3574-4653 of SEQ ID NO: 19, which is derived from the polynucleotide encoding RET. The point of fusion in SEQ ID NO: 21 is the point at which a polynucleotide having the base sequence of positions 1-3678 of SEQ ID NO: 21, which is derived from the polynucleotide encoding DCTN1, is fused

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to a polynucleotide having the base sequence of positions 3679-4884 of SEQ ID NO: 21, which is derived from the polynucleotide encoding RET. The point of fusion in SEQ ID NO: 23 is the point at which a polynucleotide having the base sequence of positions 1-3678 of SEQ ID NO: 23, which is derived from the polynucleotide encoding DCTN1, is fused to a polynucleotide having the base sequence of positions 3679-4758 of SEQ ID NO: 23, which is derived from the polynucleotide encoding RET.

[0128]

10 In the present invention, the primer or probe is prepared as a polynucleotide that specifically hybridizes with the polynucleotide of the present invention on the basis of the sequence information of the polynucleotide of the present invention, in accordance with a typical known method. The number of bases for the primer or probe is 10 to 50, preferably 15 to 50, and more preferably 18 to 35.

[0129]

The primer or probe does not need to be completely complementary, as long as the primer or probe specifically hybridizes with the polynucleotide of the present invention. The primer or probe is a polynucleotide that has at least 70% identity, preferably at least 80% identity, more preferably at least 90% identity, more preferably at least 95% identity, and more preferably at least 98% identity with the corresponding base sequence.

[0130]

The primer or probe of the present invention is preferably a polynucleotide represented by (i) SEQ ID NO: 69, (ii) SEQ ID NO: 70, or (iii) SEQ ID NO: 71, more preferably a polynucleotide that is a set of a sense primer and an antisense primer represented by (iv) SEQ ID NO: 69 and SEQ ID NO: 70, and more preferably a polynucleotide that is a set of a sense primer, an antisense primer, and a probe represented by (v) SEQ ID NO: 69, SEQ ID NO: 70, and SEQ ID NO: 71.

35 [0131]

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The present invention encompasses a method for detecting the presence of the polynucleotide of the present invention in a sample.

[0132]

5 In the present invention, the method for detecting the presence of the polynucleotide of the present invention in a sample is a detection method in accordance with a commonly used detection method, such as Northern blotting, Southern blotting, RT-PCR, real-time PCR, digital PCR, DNA microarray, *in situ* hybridization, and sequence analysis.

[0133]

In the present invention, the method for detecting the presence of the polynucleotide of the present invention in a sample also includes a method for detecting the presence of a polynucleotide of RET fusion gene including the polynucleotide of the present invention. The method includes a method in which a PCR product amplified by the 5' RACE technique using a primer that hybridizes with a polynucleotide encoding RET protein (e.g., a primer that hybridizes with a sequence of the 3' side from the RET kinase domain) is subjected to sequence analysis.

[0134]

In the present invention, the method for detecting the presence of the polynucleotide of the present invention in a sample preferably comprises the step of detecting the polynucleotide of the present invention using the primer or probe of the present invention.

[0135]

The present invention encompasses a pharmaceutical composition for treating cancer that is positive for a fusion gene of DCTN1 gene and RET gene and/or positive for a fusion protein of DCTN1 protein and RET protein, the composition comprising a compound that inhibits RET as an active ingredient.

[0136]

More preferably, the present invention encompasses a pharmaceutical composition for treating cancer that is positive

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for a fusion gene of DCTN1 gene and RET gene and/or positive for a fusion protein of DCTN1 protein and RET protein, the composition comprising a compound that inhibits expression and/or activity of the polypeptide of the present invention or
5 expression of the polynucleotide of the present invention as an active ingredient.

[0137]

In the present invention, "cancer that is positive for a fusion gene of DCTN1 gene and RET gene" in the phrase "cancer
10 that is positive for a fusion gene of DCTN1 gene and RET gene and/or positive for a fusion protein of DCTN1 protein and RET protein" refers to cancer that expresses the polynucleotide of the present invention, and preferably to cancer in which the polynucleotide of the present invention has been detected using
15 the method for detecting the presence of the polynucleotide of the present invention.

[0138]

In the present invention, "cancer that is positive for a fusion protein of DCTN1 protein and RET protein" in the phrase
20 "cancer that is positive for a fusion gene of DCTN1 gene and RET gene and/or positive for a fusion protein of DCTN1 protein and RET protein" refers to cancer that expresses the polypeptide of the present invention, and preferably to cancer in which the polypeptide of the present invention has been detected using the
25 method for detecting the presence of the polypeptide of the present invention.

[0139]

The active ingredient of the pharmaceutical composition for cancer treatment according to the present invention is a
30 compound that inhibits RET, and more preferably a compound that inhibits expression and/or activity of the polypeptide of the present invention, or expression of the polynucleotide of the present invention. A compound selected by the screening method of the present invention may also be used as an active ingredient.
35 For example, a compound known to inhibit RET can be used as an

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active ingredient of the pharmaceutical composition of the present invention. The compound that inhibits RET may be a compound that inhibits expression and/or activity of other tyrosine kinases, as long as the compound can inhibit the expression and/or activity of RET, and more preferably a compound that can inhibit the activity of RET and expression and/or activity of other tyrosine kinases. Examples of such compounds include vandetanib, sorafenib, sunitinib, motesanib, cabozantinib, lenvatinib, and compounds described in the WO2016/127074 pamphlet, WO2017/043550 pamphlet, WO2017/011776 pamphlet, and WO2017/146116 pamphlet.

The active ingredient for a pharmaceutical composition for treating cancer positive for a fusion gene of DCTN1 gene and RET gene, and/or positive for a fusion protein of DCTN1 protein and RET protein is a compound that inhibits RET; more preferably vandetanib, cabozantinib, lenvatinib, the fused pyrimidine compound represented by formula (1) disclosed in the WO2017/043550 pamphlet, and the fused pyrimidine compound represented by formula (1) disclosed in the WO2017/146116 pamphlet; more preferably vandetanib, cabozantinib, lenvatinib, Example Compounds 1 to 90 disclosed in the WO2017/043550 pamphlet, and Example Compounds 1 to 207 disclosed in the WO2017/146116 pamphlet; still more preferably vandetanib, cabozantinib, lenvatinib, 4-amino-1-(tert-butyl)-N-(5-methyl-1H-pyrazol-3-yl)-1H-pyrazolo[3,4-d]pyrimidine-3-carboxamide, 4-amino-7-(tert-butyl)-N-(5-methyl-1H-pyrazol-3-yl)-7H-pyrrolo[2,3-d]pyrimidine-5-carboxamide, 4-amino-7-(1-fluoro-2-methylpropan-2-yl)-N-(5-methyl-1H-pyrazol-3-yl)-7H-pyrrolo[2,3-d]pyrimidine-5-carboxamide, 4-amino-N-(5-methyl-1H-pyrazol-3-yl)-7-(1-methylcyclopropyl)-7H-pyrrolo[2,3-d]pyrimidine-5-carboxamide, 4-amino-7-(2-cyclopropylpropan-2-yl)-N-(5-methyl-1H-pyrazol-3-yl)-7H-pyrrolo[2,3-d]pyrimidine-5-carboxamide, 4-amino-N-[4-(methoxymethyl)phenyl]-7-(1-methylcyclopropyl)-6-(3-morpholinopro-1-pyn-1-yl)-7H-pyrrolo[2,3-d]pyrimidine-5-carboxamide, 4-amino-N-[4-(methoxymethyl)phenyl]-7-(1-

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methylcyclopropyl)-6-((tetrahydro-2H-pyran-4-yl)ethynyl)-7H-
 pyrrolo[2,3-d]pyrimidine-5-carboxamide, (R)-4-amino-N-[4-
 (methoxymethyl)phenyl]-7-(1-methylcyclopropyl)-6-
 ((tetrahydrofuran-2-yl)methoxy)-7H-pyrrolo[2,3-d]pyrimidine-5-
 5 carboxamide, and 4-amino-N-[4-(methoxymethyl)phenyl]-6-((1-
 methyl-1H-pyrazol-4-yl)ethynyl)-7-(1-methylcyclopropyl)-7H-
 pyrrolo[2,3-d]pyrimidine-5-carboxamide; and particularly
 preferably 4-amino-1-(tert-butyl)-N-(5-methyl-1H-pyrazol-3-yl)-
 1H-pyrazolo[3,4-d]pyrimidine-3-carboxamide, 4-amino-7-(tert-
 10 butyl)-N-(5-methyl-1H-pyrazol-3-yl)-7H-pyrrolo[2,3-d]pyrimidine-
 5-carboxamide, 4-amino-7-(1-fluoro-2-methylpropan-2-yl)-N-(5-
 methyl-1H-pyrazol-3-yl)-7H-pyrrolo[2,3-d]pyrimidine-5-
 carboxamide, 4-amino-N-(5-methyl-1H-pyrazol-3-yl)-7-(1-
 methylcyclopropyl)-7H-pyrrolo[2,3-d]pyrimidine-5-carboxamide, 4-
 15 amino-7-(2-cyclopropylpropan-2-yl)-N-(5-methyl-1H-pyrazol-3-yl)-
 7H-pyrrolo[2,3-d]pyrimidine-5-carboxamide, 4-amino-N-[4-
 (methoxymethyl)phenyl]-7-(1-methylcyclopropyl)-6-(3-
 morpholinopro-1-pyn-1-yl)-7H-pyrrolo[2,3-d]pyrimidine-5-
 carboxamide, 4-amino-N-[4-(methoxymethyl)phenyl]-7-(1-
 20 methylcyclopropyl)-6-((tetrahydro-2H-pyran-4-yl)ethynyl)-7H-
 pyrrolo[2,3-d]pyrimidine-5-carboxamide, (R)-4-amino-N-[4-
 (methoxymethyl)phenyl]-7-(1-methylcyclopropyl)-6-
 ((tetrahydrofuran-2-yl)methoxy)-7H-pyrrolo[2,3-d]pyrimidine-5-
 carboxamide, and 4-amino-N-[4-(methoxymethyl)phenyl]-6-((1-
 25 methyl-1H-pyrazol-4-yl)ethynyl)-7-(1-methylcyclopropyl)-7H-
 pyrrolo[2,3-d]pyrimidine-5-carboxamide.
 [0140]

In the present invention, "can inhibit the expression
 of RET" in the phrase "the compound can inhibit the expression
 30 and/or activity of RET" means, for example, as follows. A cell
 expressing the polypeptide and/or polynucleotide of RET is
 brought into contact with a test compound, and the expression
 level of the polypeptide or polynucleotide of RET in the cell is
 detected. When the cell brought into contact with the test
 35 compound exhibits a lowered expression level of the polypeptide

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or polynucleotide of RET than a cell that has not been brought into contact with the test compound, the expression of RET is determined to be inhibited. Such compounds include compounds described above, siRNA, miRNA, and nucleic acid (DNA, RNA)

5 aptamers. Examples of siRNA include CACAUGUCAUCAAUUGUATT (SEQ ID NO: 74), GGAUUGAAAACAAACUCUATT (SEQ ID NO: 75), and GCUUGUCCCGAGAUGUUUATT (SEQ ID NO: 76); and siRNA is preferably CACAUGUCAUCAAUUGUATT (SEQ ID NO: 74) or GGAUUGAAAACAAACUCUATT (SEQ ID NO: 75).

10 [0141]

Whether a compound "can inhibit the activity of RET" in the phrase "the compound can inhibit the expression and/or activity of RET" can be determined using tyrosine phosphorylation as an indicator. Examples of the method for measuring tyrosine phosphorylation include a method described in Test Example 1 in

15 WO2017/043550 pamphlet.

[0142]

Additionally, a compound can be determined as being able to inhibit the activity of RET using a cell expressing the polypeptide and/or polynucleotide of RET, with the cell growth inhibitory effect being an indicator. Examples of a method for measuring the cell growth inhibitory effect include a method described in Test Example 3 and Test Example 4 of WO2017/043550 pamphlet.

20

25 [0143]

The cancer targeted by the pharmaceutical composition of the present invention is not particularly limited, as long as the cancer expresses the polynucleotide and/or the polypeptide of the present invention, and examples include head and neck cancer, thyroid cancer, gastroenterological cancer (e.g., esophageal cancer, stomach cancer, duodenal cancer, liver cancer, biliary tract cancer (e.g., gallbladder cancer and bile duct cancer), pancreas cancer, small intestine cancer, bowel cancer (e.g., colorectal cancer, colon cancer, rectal cancer), and

30

35 gastrointestinal stromal tumor), lung cancer (non-small-cell lung

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cancer, small-cell lung cancer), breast cancer, ovarian cancer, uterus cancer (e.g., cervical cancer, endometrial cancer), kidney cancer, bladder cancer, prostate cancer, and skin cancer. The cancer is preferably thyroid cancer or lung cancer (non-small-
5 cell lung cancer, small-cell lung cancer). The cancer as used herein includes not only primary tumors, but also cancer that has spread to other organs (e.g., liver).

[0144]

A preparation containing a compound that inhibits
10 expression and/or activity of the polypeptide of the present invention or expression of the polynucleotide of the present invention as an active ingredient can be prepared in the form of a pharmaceutical composition containing a pharmaceutical carrier so as to suit a variety of dosage forms. Examples of dosage forms
15 include oral agents, injections, suppositories, ointments, and patches. These dosage forms can be prepared by preparation methods known and common to a person skilled in the art.

[0145]

The pharmaceutical carrier for use includes various
20 organic or inorganic carrier substances commonly used as preparation materials, and these are added as excipients, binders, disintegrators, lubricants, coating agents, and the like for solid formulations; and solvents, solubilizing agents, suspending agents, tonicity agents, pH regulators and buffers,
25 soothing agents, and the like for liquid formulations. Optionally usable are additives for formulations, such as preservatives, antioxidants, colorants, flavorings, and stabilizers.

[0146]

In preparing an oral solid formulation, an excipient,
30 optionally with an excipient, a binder, a disintegrator, a lubricant, a colorant, a flavoring, etc., is added to the compound of the present invention, and then tablets, coated tablets, granules, powders, capsules, and the like are produced in accordance with an ordinary method.

35 [0147]

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In preparing an oral liquid formulation, a pH regulator and a buffer, a stabilizer, a flavoring, and the like are added to the compound of the present invention, and an internal liquid medicine, a syrup medicine, an elixir, and the like are produced
5 in accordance with an ordinary method.

[0148]

In preparing an injections, a pH regulator and a buffer, a stabilizer, a tonicity agent, a local anesthetic, and the like are added to the compound of the present invention, and
10 then a subcutaneously, intramuscularly, or intravenously injection is produced in accordance with an ordinary method.

[0149]

The present invention encompasses a method for diagnosing cancer when the presence of the polypeptide of the present invention or the polynucleotide of the present invention
15 is detected in a sample by the method for detecting the presence of the polypeptide of the present invention, or by the method for detecting the presence of the polynucleotide of the present invention. The cancer diagnosed in this invention includes those
20 listed as targets of the pharmaceutical composition of the present invention. As described above, the use of the polypeptide or polynucleotide of the present invention enables diagnosis of cancer. Thus, the polypeptide and the polynucleotide of the present invention can be used as a biomarker for detecting
25 cancer.

[0150]

The present invention encompasses a method using the polypeptide of the present invention or the polynucleotide of the present invention as an indicator for determining whether a
30 chemotherapy using a compound that inhibits RET is effective, the method determining that the chemotherapy using the compound that inhibits RET is effective when the polypeptide of the present invention is detected in a sample by the detection method of the present invention, and/or when the presence of the polynucleotide
35 of the present invention is detected in a sample by the detection

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method of the present invention.

[0151]

More preferably, the present invention encompasses a method using the polypeptide of the present invention or the polynucleotide of the present invention as an indicator for determining whether a chemotherapy using a compound that inhibits expression and/or activity of the polypeptide of the present invention, or expression of the polynucleotide of the present invention, is effective, the method determining that the chemotherapy using the compound that inhibits expression and/or activity of the polypeptide of the present invention, or expression of the polynucleotide of the present invention, is effective when the polypeptide of the present invention is detected in a sample by the detection method of the present invention, and/or when the presence of the polynucleotide of the present invention is detected in a sample by the detection method of the present invention.

[0152]

More preferably, the present invention encompasses a method using the polypeptide of the present invention or the polynucleotide of the present invention as an indicator for determining whether a chemotherapy using a compound obtained in the screening method of the present invention is effective, the method determining that the chemotherapy using the compound obtained in the screening method of the present invention is effective when the polypeptide of the present invention is detected in a sample by the detection method of the present invention, and/or when the presence of the polynucleotide of the present invention is detected in a sample by the detection method of the present invention.

[0153]

The following Examples describe the present invention in detail. However, the present invention is not limited to these Examples.

35

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Examples

[0154]

Example 1: Preparation of Fusion Gene of DCTN1 Gene and RET Gene
(DCTN1-RET Fusion Gene)

5 1-1: Extraction of RNA Derived from Clinical Specimen

RNA was extracted from a human thyroid cancer tissue purchased from Asterand Bioscience using an RNeasy Mini Kit (Qiagen) in accordance with the following method. 600 µL of Buffer RLT was added to the thyroid cancer tissue, and applied to
10 a QIAshredder spin column, followed by centrifugation (16,000 rpm, 2 minutes, room temperature), thereby collecting the filtrate. An equivalent amount of a 70% ethanol aqueous solution was added to the collected filtrate. After they were mixed, the mixture was applied to an RNeasy Mini column, and then
15 centrifuged (10,000 rpm, 15 seconds, room temperature). 700 µL of Buffer RW1 was added to an RNeasy Mini column, and centrifuged (10,000 rpm, 15 seconds, room temperature). 500 µL of Buffer RPE was further added thereto, and centrifuged (10,000 rpm, 15 seconds, room temperature). In the same manner, 500 µL of Buffer
20 RPE was added again, and centrifuged (10,000 rpm, 2 minutes, room temperature). The RNeasy Mini column was centrifuged again (16,000 rpm, 1 minute, room temperature), and the remaining buffer was removed. 40 µL of RNase-free water was applied to the RNeasy Mini column and centrifuged (10,000 rpm, 1 minute, room
25 temperature), thereby collecting the filtrate as total RNA.

[0155]

1-2: Preparation of cDNA Derived from Clinical Specimen

cDNA was synthesized from the total RNA obtained in section 1-1 above using a SuperScript VILO cDNA Synthesis Kit
30 (Invitrogen) in accordance with the following method. 500 ng of total RNA was adjusted with RNase-free water to give an amount of 14 µL, and 4 µL of 5×VILO Reaction Mix and 2 µL of 10×SuperScript Enzyme Mix were added thereto and mixed. The mixture was kept warm at 25°C for 10 minutes, and subsequently kept warm at 42°C
35 for 60 minutes. To stop the reaction, the mixture was finally

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incubated at 85°C for 5 minutes, thereby obtaining cDNA.

[0156]

1-3: Preparation and Purification of Cloning Vector

To amplify DCTN1-RET fusion gene, primers shown in

- 5 Table 1 were designed: primer 1 (SEQ ID NO: 33) as a sense primer and primer 2 (SEQ ID NO: 34) as an antisense primer, as well as primer 3 (SEQ ID NO: 35) as a sense primer and primer 4 (SEQ ID NO: 36) as an antisense primer for use in nested PCR.

[0157]

10 Table 1

Primer 1	5'-TGTCCAGCTTTGTGCCTGATTGATGT-3'	SEQ ID NO: 33
Primer 2	5'-GCTGGGCACTGAAGAGAAAGGAATGC-3'	SEQ ID NO: 34
Primer 3	5'-AGCAGGATGAGTGCGGAGGCAAGC-3'	SEQ ID NO: 35
Primer 4	5'-TTAACTATCAAACGTGTCCATTAATTTTGCCGC-3'	SEQ ID NO: 36

[0158]

- DCTN1-RET fusion gene was amplified with cDNA synthesized in section 1-2 above as a template using these primers and using KOD-Plus-Neo (Toyobo) in accordance with the following method. 2 µL of cDNA, 5 µL of 10×PCR Buffer for KOD-Plus-Neo, 5 µL of 2 mM dNTPs, 3 µL of 25 mM MgSO₄, 1 µL of KOD-Plus-Neo, 1.5 µL of primer 1 (10 µM), 1.5 µL of primer 2 (10 µM), and 31 µL of double distilled water (DDW) were mixed; and PCR was performed. Subsequently, the obtained PCR product was diluted by
- 20 a factor of 100, and 2 µL of the diluted PCR product, 5 µL of 10×PCR Buffer for KOD-Plus-Neo, 5 µL of 2 mM dNTPs, 3 µL of 25 mM MgSO₄, 1 µL of KOD-Plus-Neo, 1.5 µL of primer 3 (10 µM), 1.5 µL of primer 4 (10 µM), and 31 µL of DDW were mixed; and nested PCR was performed.

25 [0159]

The nested PCR product was separated by electrophoresis using 1% agarose gel (Nacalai Tesque), and the PCR product was purified from the gel using a QIAquick Gel Extraction Kit (Qiagen).

30 [0160]

pUC18 DNA (Takara Bio Inc.) cleaved by a restriction enzyme SmaI (NEB), the purified PCR product, T4 DNA ligase (NEB),

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and T4 DNA ligase reaction buffer (NEB) were mixed, and the mixture was incubated at 16°C overnight. The ligation product was treated with SmaI (NEB), and transformation of competent cells was performed by the following method. The ligation product
5 treated with SmaI was added to 50 µL of *E. coli* DH5α competent cells (Takara Bio Inc.), and allowed to stand on ice for 30 minutes. Thereafter, the cells were subjected to heat shock at 42°C for 30 seconds, and allowed to stand on ice for 2 minutes. A SOC medium (Takara Bio Inc.) was added thereto, and the cells
10 were cultured with shaking at 37°C for 1 hour. The culture solution was then applied onto an ampicillin-containing LB agar medium plate (Unitech), and allowed to stand at 37°C overnight. The *E. coli* colonies were suspended in an ampicillin-containing LB medium (InvivoGen), and cultured with shaking at 37°C
15 overnight. Plasmid DNA inserting DCTN1-RET fusion gene was purified from the proliferated *E. coli* using a QIAquick Spin Miniprep Kit (Qiagen) in accordance with the protocol provided with the kit.

[0161]

20 1-4: Determination of Sequence

A sequence reaction was performed with the plasmid DNA obtained in section 1-3 above as a template, using primers 5 to 36 for sequencing shown in Table 2 and using a BigDye Terminator V3.1 Cycle Sequencing Kit; and sequence analysis was performed
25 using an Applied Biosystems 3730xl DNA Analyzer. The results of the sequence analysis revealed that DCTN1-RET fusion gene was a gene (SEQ ID NO: 17) in which exons 12 to 20 of RET variant 2 (GenBank accession number: NM_020975) were fused downstream of the 3' side of exons 1 to 27 of DCTN1 variant 5 (GenBank
30 accession number: NM_001190836).

[0162]

Table 2

Primer 5	5'-AGTACTGGGGTGGCTGGG-3'	SEQ ID NO: 37
Primer 6	5'-CACTTTGGACAAGGAGATG-3'	SEQ ID NO: 38
Primer 7	5'-ACAGAACTGGAGCTGCGG-3'	SEQ ID NO: 39
Primer 8	5'-GGACTGGTGTACTCGCTG-3'	SEQ ID NO: 40
Primer 9	5'-TCCTAGACTGCAGGAAACAC-3'	SEQ ID NO: 41
Primer 10	5'-CATCGAGAAAGTCCAGAC-3'	SEQ ID NO: 42
Primer 11	5'-GCTGCTGGAGACATTGAA-3'	SEQ ID NO: 43
Primer 12	5'-TCACTGCTGCTCAGCTCA-3'	SEQ ID NO: 44
Primer 13	5'-GAGGATCCAAAGTGGGAATT-3'	SEQ ID NO: 45
Primer 14	5'-AGTATCTGGCCGAGATGAAG-3'	SEQ ID NO: 46
Primer 15	5'-GCAAAGACCTGGAGAAGATG-3'	SEQ ID NO: 47
Primer 16	5'-AGGACGTTGAACTCTGACAG-3'	SEQ ID NO: 48
Primer 17	5'-CCTTTGCTTCATCCAGAATC-3'	SEQ ID NO: 49
Primer 18	5'-GATTTTGTGTTTCTCCAGCTCT-3'	SEQ ID NO: 50
Primer 19	5'-CCTGCTTCTCTGAGGAAGAA-3'	SEQ ID NO: 51
Primer 20	5'-GGGCCTTAGTCTCAGCAAAC-3'	SEQ ID NO: 52
Primer 21	5'-GAGCACTCTGCGTGAACCTTA-3'	SEQ ID NO: 53
Primer 22	5'-CAGCTTGTTTCATGGTACTGAT-3'	SEQ ID NO: 54
Primer 23	5'-TGGTGAGTCCTTACCAG-3'	SEQ ID NO: 55
Primer 24	5'-CCTAGAGTTTTTCCAAGAACCA-3'	SEQ ID NO: 56
Primer 25	5'-CATTTAACTGGAATCCGACC-3'	SEQ ID NO: 57
Primer 26	5'-GACTCTCTCCAGGCCAGTTC-3'	SEQ ID NO: 58
Primer 27	5'-GGCTATCAGAAGTAAAACCACC-3'	SEQ ID NO: 59
Primer 28	5'-CGAGAGCTGATGGCACTA-3'	SEQ ID NO: 60
Primer 29	5'-CTTCATCACAAGTGAAGTACTTCC-3'	SEQ ID NO: 61
Primer 30	5'-CGTACTCCACGATGAGGAG-3'	SEQ ID NO: 62
Primer 31	5'-GATTCTGGATGAAGCAAAGG-3'	SEQ ID NO: 63
Primer 32	5'-GGAAGTACTTCACTTGTGATGAAG-3'	SEQ ID NO: 64
Primer 33	5'-CCCAGCCACCCAGTACT-3'	SEQ ID NO: 65
Primer 34	5'-GTAAAACGACGGCCAGT-3'	SEQ ID NO: 66
Primer 35	5'-GTTTTCCAGTCACGAC-3'	SEQ ID NO: 67
Primer 36	5'-CAGGAAACAGCTATGAC-3'	SEQ ID NO: 68

[0163]

5 Example 2: Detection of DCTN1-RET Fusion Gene

cDNA was synthesized from normal human thyroid tissue-derived RNA purchased from Asterand Bioscience and human thyroid cancer tissue-derived RNA obtained in section 1-1 using a SuperScript VILO cDNA Synthesis Kit (Invitrogen) in accordance

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with the following method. 280 ng of total RNA was adjusted with RNase-free water to give an amount of 14 μ L, and 4 μ L of 5 $\times$ VILO Reaction Mix and 2 μ L of 10 $\times$ SuperScript Enzyme Mix were added respectively thereto and mixed. The mixture was kept warm at 25°C for 10 minutes, and subsequently kept warm at 42°C for 60 minutes. To end the reaction, the mixture was finally incubated at 85°C for 5 minutes.

[0164]

To detect DCTN1-RET fusion gene, primers and a probe shown in Table 3 were designed: primer 37 (SEQ ID NO: 69) as a sense primer for detecting DCTN1-RET fusion gene, primer 38 (SEQ ID NO: 70) as an antisense primer for detecting DCTN1-RET fusion gene, and primer 39 (SEQ ID NO: 71) as a probe for detecting DCTN1-RET fusion gene (probe: TaqMan MGB probe; fluorescent dye: FAM (Thermo Fisher Scientific)).

[0165]

Table 3

Primer 37	5'-CTGGAGCCACAGTACCCACT-3'	SEQ ID NO: 69
Primer 38	5'-TCCAAATTCGCCTTCTCCTA-3'	SEQ ID NO: 70
Primer 39	5'-TTCATCAGCCTTCCTCAGGGAGGAT-3'	SEQ ID NO: 71

[0166]

The obtained cDNA was diluted by a factor of 10, and 1.1 μ L thereof was used as a template. 11 μ L of ddPCR Supermix for probe (Bio-Rad), 2 μ L of primer 37 (10 μ M), 2 μ L of primer 38 (10 μ M), 0.6 μ L of primer 39 (10 μ M), and 1.1 μ L of 20 $\times$ HEX assay for detecting GAPDH (Prime PCR ddPCR Expression Probe Assay: GAPDH, Human, Bio-Rad) were mixed; and droplets were prepared using an automated droplet generator (Bio-Rad). PCR was performed on the prepared droplets, and droplets positive for DCTN1-RET and GAPDH were counted with a droplet reader (Bio-Rad). Figs. 1 and 2 show the results.

30 [0167]

DCTN1-RET fusion gene was amplified with the cDNA synthesized above as a template using KOD-Plus-Neo (Toyobo) in

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accordance with the following method. 2 μ L of cDNA, 5 μ L of 10 $\times$ PCR buffer for KOD-Plus-Neo, 5 μ L of 2 mM dNTPs, 3 μ L of 25 mM MgSO₄, 1 μ L of KOD-Plus-Neo, 1.5 μ L of primer 1 (10 μ M), 1.5 μ L of primer 2 (10 μ M), and 31 μ L of DDW were mixed, and PCR was performed. Subsequently, the obtained PCR product was diluted by a factor of 100, and 2 μ L of the diluted PCR product, 5 μ L of 10 $\times$ PCR Buffer for KOD-Plus-Neo, 5 μ L of 2 mM dNTPs, 3 μ L of 25 mM MgSO₄, 1 μ L of KOD-Plus-Neo, 1.5 μ L of primer 3 (10 μ M), 1.5 μ L of primer 4 (10 μ M), and 31 μ L of DDW were mixed; and nested PCR was performed. The nested PCR product was separated by electrophoresis using 1% agarose gel (Nacalai Tesque), and photographed. Fig. 3 shows the results.

[0168]

As shown in Fig. 1, Fig. 2, and Fig. 3, while DCTN1-RET fusion gene was detected in cDNA synthesized from human thyroid cancer tissue-derived RNA, DCTN1-RET fusion gene was not detected in cDNA synthesized from normal human thyroid tissue-derived RNA. These results indicate that DCTN1-RET fusion gene is useful as a biomarker for cancer.

[0169]

Example 3: Construction of Expression Vector for DCTN1-RET Fusion Gene

To construct an expression vector, Primer 40 (SEQ ID NO: 72) as a sense primer and Primer 41 (SEQ ID NO: 73) as an antisense primer were designed as shown in Table 4.

[0170]

Table 4

Primer 40	5'-GGGGACAAGTTTGTACAAAAAGCAGGCTTCGCCACCAGCA GGATGAGTGCGGAGGCAAGCGCCCGG-3'	SEQ ID NO: 72
Primer 41	5'-GGGGACCACTTTGTACAAGAAAGCTGGGTTTAACTATCAAA CGTGTCCATTAATTTTGC-3'	SEQ ID NO: 73

[0171]

A DCTN1-RET fusion gene was amplified using these primers, cDNA synthesized in section 1-2 above as a template, and Prime STAR Max DNA Polymerase (TaKaRa) in accordance with the

-50-

method described below. 1 µL of cDNA, 25 µL of 2 × Prime STAR Max DNA Polymerase, 1 µL of Primer 40 (10 µM), 1 µL of Primer 41 (10 µM), and 22 µL of double-distilled water (DDW) were mixed to perform PCR. The obtained PCR product was separated by
5 electrophoresis using 1% agarose gel (Nacalai Tesque), and the PCR product was purified from the gel using GFX PCR DNA and Gel Band Purification Kit (GE Healthcare). Subsequently, the purified PCR product was inserted into a Gateway pDONR221 Vector using a Gateway BP Clonase II Enzyme Mix (Thermo Fisher) in accordance
10 with the method described below, thereby preparing an entry vector. Specifically, 5.0 µL of the purified PCR product, 3.5 µL of pDONR221 (85 ng/µL), 4.0 µL of BP Clonase II Enzyme Mix, and 7.5 µL of TE were mixed and incubated at 25°C for 90 minutes. After incubation, 1 µL of Proteinase K (2 mg/mL) was added
15 thereto, followed by incubation at 37°C for 10 minutes, thereby preparing the entry vector.

[0172]

The obtained entry vector was added to 50 µL of *E. coli* DH5α Competent Cells (Takara Bio Inc.), and allowed to stand on
20 ice for 30 minutes. Thereafter, the cells were given heat shock at 37°C for 20 seconds, and allowed to stand for 2 minutes on ice. A SOC medium (Takara Bio Inc.) was then added thereto, and the cells were subjected to shaking culture at 37°C for 1 hour. The culture solution was then applied onto a Kanamycin-containing
25 LB agar medium plate, and allowed to stand at 37°C overnight. The *Escherichia coli* colonies were suspended in a Kanamycin-containing LB medium, and subjected to shaking culture at 37°C overnight. From the grown *Escherichia coli*, a DCTN1-RET fusion gene inserted-plasmid DNA (entry vector clone) was purified with
30 a GENE PREP STAR PI-480 automated DNA isolation system (Kurabo Industries Ltd.).

[0173]

The DCTN1-RET fusion gene was inserted into a pJTI Fast DEST vector using the obtained plasmid and a Gateway LR Clonase
35 II Enzyme Mix (Thermo Fisher) in accordance with the method

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described below, thereby preparing an expression vector.

150 ng of the entry vector clone, 1 μ L of a pJTI Fast DEST vector (150 ng/ μ L), 2 μ L of an LR Clonase II Enzyme Mix, and a TE buffer were mixed to give 10 μ L in total, and the mixture was incubated
5 at 25°C for 90 minutes. After incubation, 1 μ L of Proteinase K (2 mg/mL) was added thereto, followed by incubation at 37°C for 10 minutes, thereby obtaining a DCTN1-RET fusion gene-inserted pJTI Fast DEST vector (DCTN1-RET fusion gene expression vector). The obtained DCTN1-RET fusion gene expression vector was added to 50
10 μ L of *E. coli* DH5 α Competent Cells (Takara Bio Inc.), and allowed to stand for 30 minutes on ice. Thereafter, the cells were then given heat shock at 37°C for 20 seconds, and allowed to stand for 2 minutes on ice. A SOC medium (Takara Bio Inc.) was then added thereto, and the cells were subjected to shaking culture at 37°C
15 for 1 hour. The culture solution was applied onto an ampicillin-containing LB agar medium plate, and allowed to stand at 37°C overnight. The *Escherichia coli* colonies were suspended in an ampicillin-containing LB medium, and subjected to shaking culture at 37°C overnight. From the grown *Escherichia coli*, a DCTN1-RET
20 fusion gene-inserted plasmid DNA (DCTN1-RET fusion gene expression vector) was purified using a Plasmid Plus Maxi Kit (QIAGEN).

[0174]

Example 4: Establishment of DCTN1-RET Fusion Gene-Expressing Cell 25 4-1: Establishment of Cell

For the host cell for establishing a DCTN1-RET fusion gene-expressing cell, mouse embryonic fibroblast NIH/3T3 cells (American Type Culture Collection) were chosen, and the cells were transfected with the DCTN1-RET fusion gene-inserted
30 expression vector prepared above, thereby establishing a DCTN1-RET fusion gene-expressing cell. The details of the procedure are as follows. NIH/3T3 cells were prepared by culture in a medium for typical culture (2-dimensional cell culture) at 37°C in 5% CO₂; and the medium for use in the 2-dimensional cell culture was
35 prepared by adding newborn calf serum (NBCS) (Gibco) to D-MEM

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(high glucose) (containing L-glutamine, phenol red, sodium pyruvate, 1500 mg/L sodium hydrogen carbonate) (Wako) to give 10%. On the day before performing transfection, NIH/3T3 cells were seeded onto a 6-well plate (Iwaki) at 1.5×10^5 cells/2 mL, and
5 incubated at 37°C in 5% CO₂ overnight. A ViaFect transfection reagent was added to a mixture solution prepared by mixing 1.5 µg of the DCTN1-RET fusion gene expression vector and 1.5 µg of a pJTI phiC31 integrase vector such that the amount of the ViaFect transfection reagent was six times the amount of the mixture
10 solution; and then Opti-MEM was added thereto to give a total amount of 300 µL, followed by incubation at room temperature for 5 minutes, thereby preparing a transfection solution. From the wells on which NIH/3T3 cells were seeded, 300 µL of the medium was removed, and 300 µL of the prepared transfection solution was
15 added to the wells, followed by incubation at 37°C in 5% CO₂ overnight. The next day, the medium was replaced to remove the transfection solution. When replacing the medium, hygromycin B (Nacalai Tesque) was added to a new medium to give 500 µg/mL. Hygromycin B removed cells that did not transfect the DCTN1-RET
20 fusion gene-inserted expression vector. After transfection, while replacing the medium about twice a week, the cells were cultured until they proliferated. After 22 days from the transfection, the cells were collected with trypsin, and single-cell cloning was performed in accordance with the following method. The number of
25 cells collected was measured, and a medium was added thereto to give 1 cell/200 µL. The cells were seeded onto a 96-well plate (Thermo Fisher) to give 200 µL per well. After seeding, the cells were observed daily, and cells grown from a single cell were taken; these cells were DCTN1-RET fusion gene-expressing cells
30 (DCTN1-RET fusion gene-expressing NIH/3T3 cells).

[0175]

4-2: Confirmation of Expression of Target Protein

The expression of DCTN1-RET fusion protein in the obtained DCTN1-RET fusion gene-expressing NIH/3T3 cells was
35 confirmed by western blotting. Specifically, the medium was

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removed from the culture flask, followed by washing with PBS once. Sample Diluent Concentrate 2 (R&D Systems) containing a phosphatase inhibitor (Roche) and a protease inhibitor (Roche) was added to the culture flask, and the cell lysate was collected with a scraper. From the collected cell lysate, a protein sample was obtained by centrifugation. The protein sample was subjected to protein determination, and the protein concentration was adjusted to a prescribed concentration. A sample buffer solution with reducing reagent (6x) for SDS-PAGE (Nacalai Tesque) was added to the protein sample of a prescribed concentration; and the mixture was incubated at 95°C for 5 minutes to denature the protein, thereby obtaining a sample for use in western blotting. For negative control, a sample for western blotting was obtained using NIH/3T3 cells (parental cell line) in accordance with the same procedure. The expression of the protein was confirmed using the samples in accordance with the method described below. The protein was separated using a 4-15% acrylamide gel (Bio-Rad) and 1 × Tris/Glycine/SDS buffer by SDS-PAGE (for 30 minutes at 200 V). The protein was transferred onto a PVDF membrane using a Trans-Blot Turbo RTA Midi PVDF Transfer Kit (Bio-Rad) and a Trans-Blot Turbo transcription system (Bio-Rad), and the PVDF membrane was immersed in Blocking One-P for 1 hour. A solution was prepared by diluting Blocking One-P with TBS-T to give 10%, and a primary antibody (Phospho-RET (Tyr905) Antibody (CST), Ret (C31B4) Rabbit mAb (CST), and Anti-Dctn1 Antibody (Atlas Antibodies)) was diluted with the prepared solution to give a concentration of 1/1000. The PVDF membrane was immersed in the resulting solution, and incubated at 4°C overnight. After being washed with TBS-T, the PVDF membrane was immersed in a secondary antibody dilution solution that was prepared by diluting an anti-rabbit IgG, HRP-linked antibody (CST) with TBS-T to give a concentration of 1/2000; and incubated at room temperature for 1 hour. After the membrane was washed with TBS-T, the protein was detected using a SuperSignal West Dura Extended Duration Substrate (Thermo Fisher) with an Amersham Imager 600 lumino

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image analyzer (GE Healthcare). The molecular weight of the detected protein was confirmed with Precision Plus Protein Kaleidoscope Prestained Protein Standards (Bio-Rad). As shown in Figs. 4a) and 4b), endogenous RET (150 and 175 kDa) was not
5 detected when the anti-pRET antibody or the anti-RET antibody was used. However, a band that appeared to be of the DCTN1-RET fusion protein was confirmed at around 175 kDa only in the DCTN1-RET fusion gene-expressing NIH/3T3 cells. Additionally, as shown in Fig. 4c), endogenous DCTN1 was detected at around 150 kDa in the
10 use of the anti-DCTN1 antibody. A band was also detected at around 175 kDa above the band of endogenous DCTN1 at around 150 kDa only in the DCTN1-RET fusion gene-expressing NIH/3T3 cells. Specifically, a band at around 175 kDa was detected both in the use of the antibody against RET and the antibody against DCTN1
15 only in the DCTN1-RET fusion gene-expressing NIH/3T3 cells; this clearly indicates that a fusion protein of DCTN1 and RET was expressed in the prepared DCTN1-RET fusion gene-expressing NIH/3T3 cells.

[0176]

20 Example 5: Confirmation of Growth of DCTN1-RET Fusion Gene-Expressing NIH/3T3 Cells by 3-dimensional Cell Culture

NIH/3T3 cells grow well under 2-dimensional cell culture conditions, but barely grow under 3-dimensional cell culture conditions. However, NIH/3T3 cells are also known to grow
25 under 3-dimensional cell culture conditions when an oncogene is expressed in the cells. Thus, whether the DCTN1-RET fusion gene is an oncogene was confirmed using this character. DCTN1-RET fusion gene-expressing NIH/3T3 cells and NIH/3T3 cells were cultured at 37°C in 5% CO₂ in a 2-dimensional cell culture; and
30 collected with trypsin, followed by counting the number of cells. To perform a 3-dimensional cell culture, a medium for 3-dimensional cell culture was prepared using an FCEM-series Preparation Kit (Nissan Chemical Industries, Ltd.), D-MEM (high glucose) (containing L-glutamine, phenol red, sodium pyruvate,
35 and 1500 mg/L sodium hydrogen carbonate) (Wako), and newborn calf

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serum (NBCS) (Gibco). The cells were suspended in the prepared medium for 3-dimensional cell culture to give 1000 cells/90 μ L, and seeded onto a 96-well clear black round bottom, spheroid microplate (Corning), 90 μ L per well, followed by incubation at 5 37°C in 5% CO₂. The day following seeding (day 1) and after 8 days from seeding (day 8), the luminescence level (counts per second: cps) was measured using a detection reagent for intracellular ATP luminescence (CellTiter-Glo 2.0 reagent, Promega) with a luminometer (EnSpire, PerkinElmer), and the result was determined 10 to be the indicator for viable cell count. The cell growth rate was then calculated from the measurement result on day 1 and the measurement result on day 8 (N=3). As a result, as shown in Fig. 5, while the number of cells in the NIH/3T3 cells on day 8 was 2.4 times the number of cells on day 1, the number of cells in 15 the DCTN1-RET fusion gene-expressing NIH/3T3 cells on day 8 was 20.9 times the number of cells on day 1. Additionally, while no cellular aggregates were formed in the NIH/3T3 cells by the 3-dimensional cell culture, cellular aggregates were confirmed to have formed in the DCTN1-RET fusion gene-expressing NIH/3T3 cells 20 by the 3-dimensional cell culture. This clearly indicates that transfection of the DCTN1-RET fusion gene enhanced cell growth, suggesting that the DCTN1-RET fusion gene is an oncogene. [0177]

Example 6: Confirmation of tumorigenicity in DCTN1-RET Fusion 25 Gene-Expressing NIH/3T3 Cells *in vivo*

To confirm tumorigenicity in DCTN1-RET fusion gene-expressing NIH/3T3 cells *in vivo*, a transplant experiment was performed with nude mice. The NIH/3T3 cell, which is a parental cell line, is commonly known to not grow subcutaneously in nude 30 mice; and it can be confirmed whether the DCTN1-RET fusion gene contributes to tumorigenicity, or whether the DCTN1-RET fusion gene is an oncogene, by subcutaneously transplanting nude mice with the DCTN1-RET fusion gene-expressing NIH/3T3 cells. Nude mice, BALB/cAJcl-nu/nu (CLEA Japan, Inc.) were used for 35 transplantation. The DCTN1-RET fusion gene-expressing NIH/3T3

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cells were collected with trypsin, and suspended in PBS to finally give 1×10^8 cells/mL. An equivalent amount of a Matrigel Basement Membrane Matrix (Corning) was added thereto, and adjusted to give 5×10^7 cells/mL to prepare a cell suspension for
 5 transplantation. 0.1 mL of the cell suspension for transplantation was subcutaneously transplanted into the right-side chest of each nude mouse (N=10) using a 25G injection needle and a 1-mL syringe. On day 10, 13, and 17 after transplantation, the major axis and short axis of a tumor of each mouse was
 10 measured with a digital caliper (Mitutoyo Corporation), and the tumor volume was calculated based on the following equation.

The tumor volume (mm^3) = (major axis: mm) $\times$ (short axis: mm) $\times$ (short axis: mm) / 2

15

Fig. 6 shows the measurement results of tumor volume. The DCTN1-RET fusion gene-expressing NIH/3T3 cells subcutaneously transplanted in nude mice were confirmed to have tumorigenesis and to have grown well; thus, this *in vivo* experiment also
 20 suggests that the DCTN1-RET fusion gene is an oncogene.

[0178]

Example 7: Confirmation of Inhibition of DCTN1-RET Fusion Protein and Cell Growth Inhibitory Effect by siRNA in DCTN1-RET Fusion Gene Expressing NIH/3T3 Cells

25

The effect of siRNA treatment on the DCTN1-RET fusion gene-expressing NIH/3T3 cells was examined. The siRNA for use was as follows: three types of RET siRNA shown in Table 5 below, and Silencer Select Negative Control #1 siRNA (Ambion) as a negative control. The target of three types of RET siRNA is human RET; and
 30 RET siRNA1 and RET siRNA2 contain a sequence that binds to the RET portion of the DCTN1-RET fusion gene, while RET siRNA3 contains no sequence that binds to the DCTN1-RET fusion gene. In other words, RET siRNA1 and RET siRNA2 were assumed to inhibit the expression of the DCTN1-RET fusion gene, and RET siRNA3 would
 35 not. The following describes the method of the experiment using

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siRNA.

[0179]

Table 5

RET siRNA1	CACAUGUCAUCAAUUGUATT	SEQ ID NO: 74
RET siRNA2	GGAUUGAAAACAAACUCUATT	SEQ ID NO: 75
RET siRNA3	CCACUGCUACCACAAGUUUTT	SEQ ID NO: 77

5 [0180]

DCTN1-RET fusion gene-expressing NIH/3T3 cells for use were prepared by culture in a medium for 2-dimensional cell culture at 37°C in 5% CO₂. On the day before siRNA treatment, the cells were seeded onto a 6-well plate (Iwaki), 3×10⁵ cells/2 mL, and incubated at 37°C in 5% CO₂ overnight. 12 µL of each type of siRNA adjusted to 20 µM beforehand using water, 4 µL of a Lipofectamine RNAiMAX Transfection Reagent (Thermo Fisher), and 384 µL of Opti-MEM were mixed; and incubated at room temperature for 15 minutes, thereby preparing siRNA solutions. 400 µL of each siRNA solution was added to the wells in which the cells were seeded, and incubation was performed at 37°C in 5% CO₂ overnight. The following day, a portion of the incubated cells was taken as a sample for use in protein expression analysis; and another portion was also taken and reseeded for use in confirmation of cell growth. The sampling for protein expression analysis and the protein expression analysis were performed in the same manner as in 4-2: Confirmation of Expression of Target Protein described above, except for the use of a Phospho-RET (Tyr905) antibody (CST), Ret (C31B4) Rabbit mAb (CST), and GAPDH (D16H11) XP Rabbit mAb (CST) as primary antibodies. The results indicate, as shown in Fig. 7, that the expression of the DCTN1-RET fusion protein was not inhibited in the cells treated with the negative control siRNA (NC), compared with cells without siRNA treatment (no treatment). When the cells were treated with RET siRNA1 or RET siRNA2, the expression of the DCTN1-RET fusion protein was confirmed to have been inhibited in the DCTN1-RET fusion gene-expressing NIH/3T3 cells, and not inhibited when RET siRNA3 was used.

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Subsequently, to confirm a cell growth inhibitory effect, cells were collected with trypsin from the wells without treatment and wells treated with siRNA, and the number of cells was counted. A 3-dimensional cell culture was then performed in the same manner as in Example 5, and on the day seeding was performed (day 0) and after 4 days from seeding (day 4), the number of viable cells was counted in the same manner as in Example 5. The growth rate of the cells was calculated from the measurement result on day 0 and the measurement result on day 4. The results indicate, as shown in Fig. 8, that cells that were without siRNA treatment (no treatment) and cells that were treated with the negative control siRNA (NC) respectively exhibited the number of cells on day 4 4.9 times and 3.6 times that on day 0; however, cells treated with RET siRNA1 and cells treated with RET siRNA2 merely exhibited growth of about 2.0 times and 2.4 times, respectively, which were both notably lower. The cells treated with RET siRNA3 exhibited growth of 3.7 times, which is substantially equal to the growth of the cells treated with negative control siRNA, rarely showing a decrease in growth. These results reveal that the growth of the DCTN1-RET fusion gene-expressing NIH/3T3 cells is also inhibited when the expression of RET is inhibited by siRNA.

[0181]

Example 8: Cell Growth Inhibitory Effect in DCTN1-RET Fusion Gene-Expressing NIH/3T3 Cells

An *in vitro* cell growth assay was performed on DCTN1-RET fusion gene-expressing NIH/3T3 cells. A 3-dimensional cell culture and seeding were performed in the same manner as in Example 5. After seeding, incubation was performed at 37°C in 5% CO₂ overnight (day 0). Cabozantinib, vandetanib, alectinib, lenvatinib, and fused pyrimidine compounds (Compounds 1 to 9 shown in Table 6), which are reported as capable of inhibiting RET, were dissolved in dimethyl sulfoxide to give a concentration of 10 mmol/L; and further diluted with a medium for 3-dimensional cell culture such that these compounds have a final concentration

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of 1000, 333, 111, 37.0, 12.3, 4.12, 1.37, and 0.457 nmol/L. These diluted compounds were then individually added to each well (0.01 mL per well) of the plate in which the cells were seeded (day 1); and the cells were incubated at 37°C in 5% CO₂ for 7 days. After culture (day 8), an intracellular ATP luminescence detection reagent (CellTiter-Glo 2.0 reagent, Promega) was added to each well, and the luminescence level (counts per second: cps) was measured with a luminometer (EnSpire, PerkinElmer). The growth rate from day 1 of cells treated with the compounds of different concentrations was calculated based on the following equation, according to the value of T_{day8} and C_{day1}, and the concentration of each test compound at which cell growth is inhibited by 50% (GI₅₀(nM)) was determined.

15 1) When $T_{\text{day8}} \geq C_{\text{day1}}$

$$\text{Growth Rate (\%)} = (T_{\text{day8}} - C_{\text{day1}}) / (C_{\text{day8}} - C_{\text{day1}}) \times 100$$

T: cps of a well to which a test compound was added

C: cps of a well to which a test compound was not added

Day 1: the day on which a test compound was added

20 Day 8: the day on which evaluation was performed

2) When $T_{\text{day 8}} < C_{\text{day1}}$

$$\text{Growth Rate (\%)} = (T_{\text{day8}} - C_{\text{day1}}) / (C_{\text{day1}}) \times 100$$

T: cps of a well to which a test compound was added

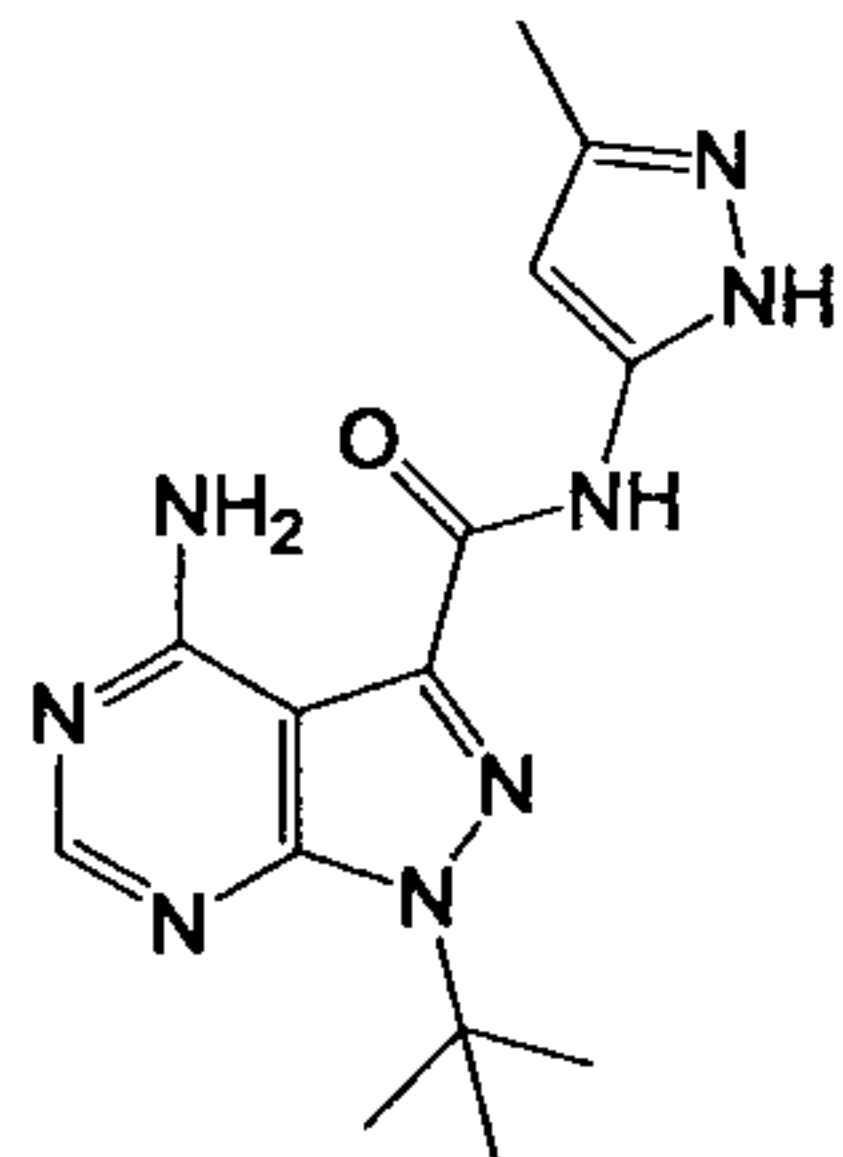
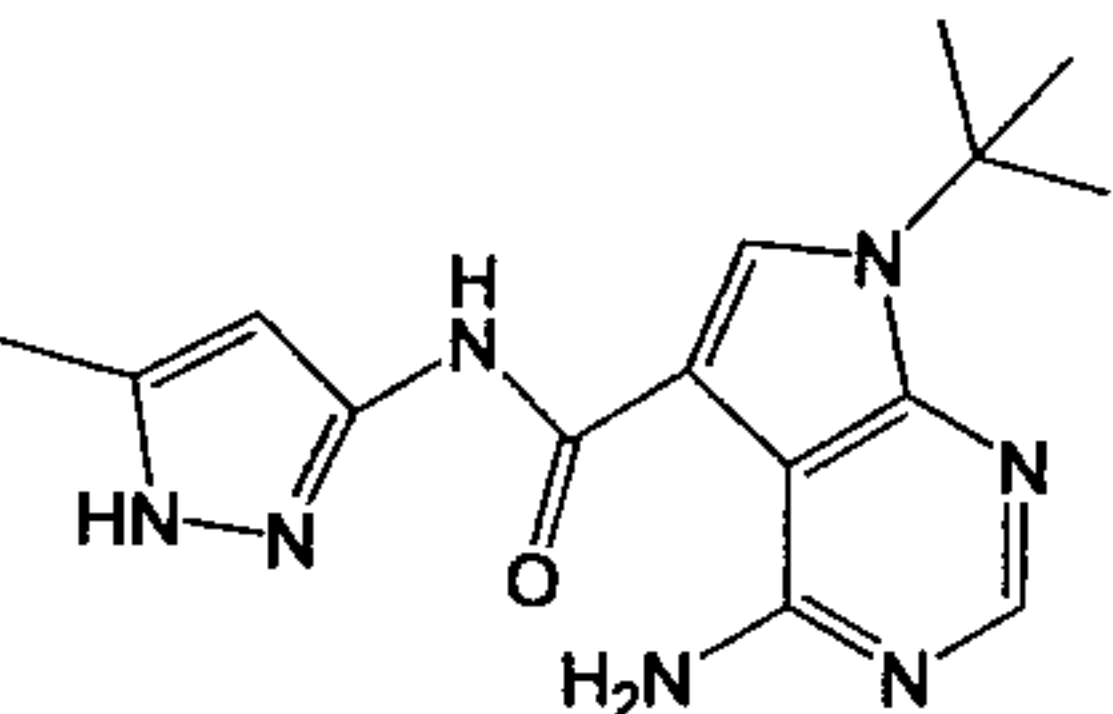
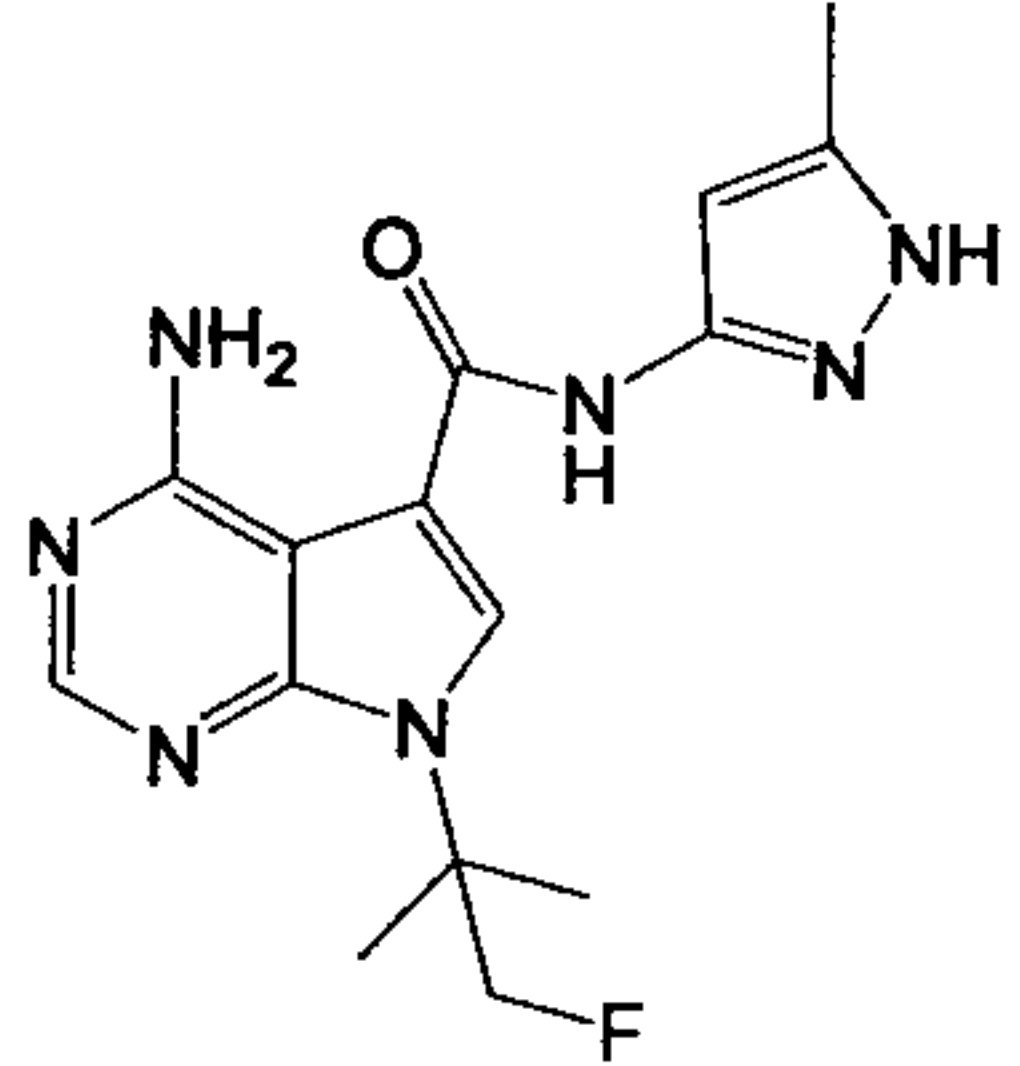
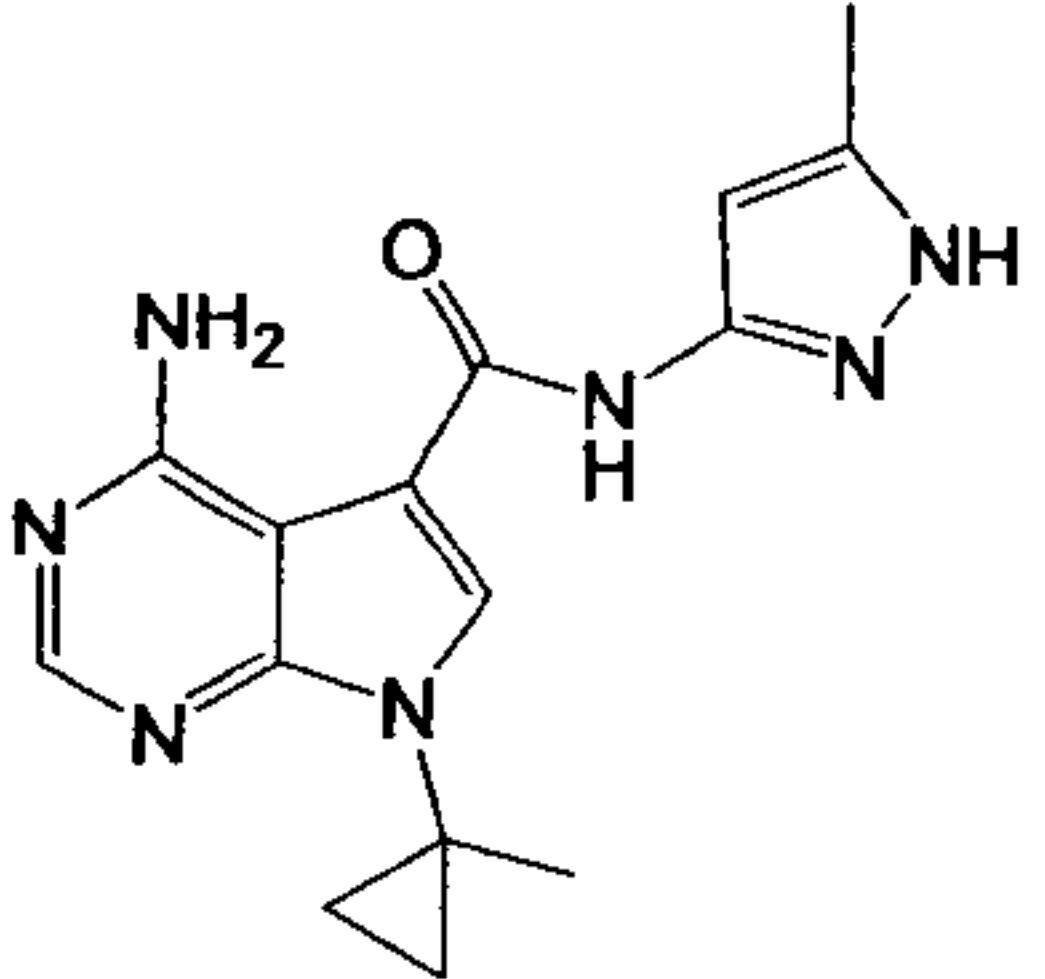
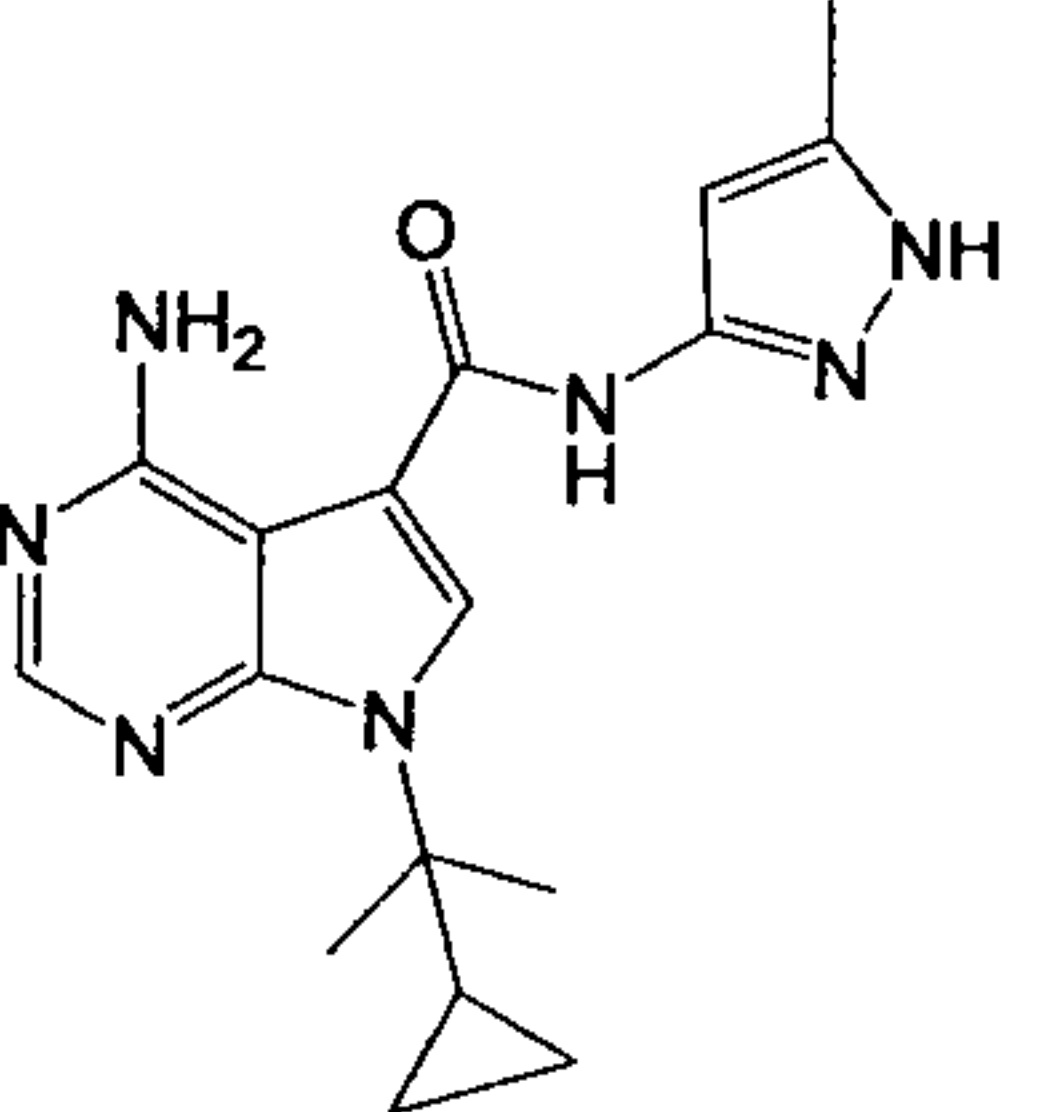
C: cps of a well to which a test compound was not added

25 Day 1: the day on which a test compound was added

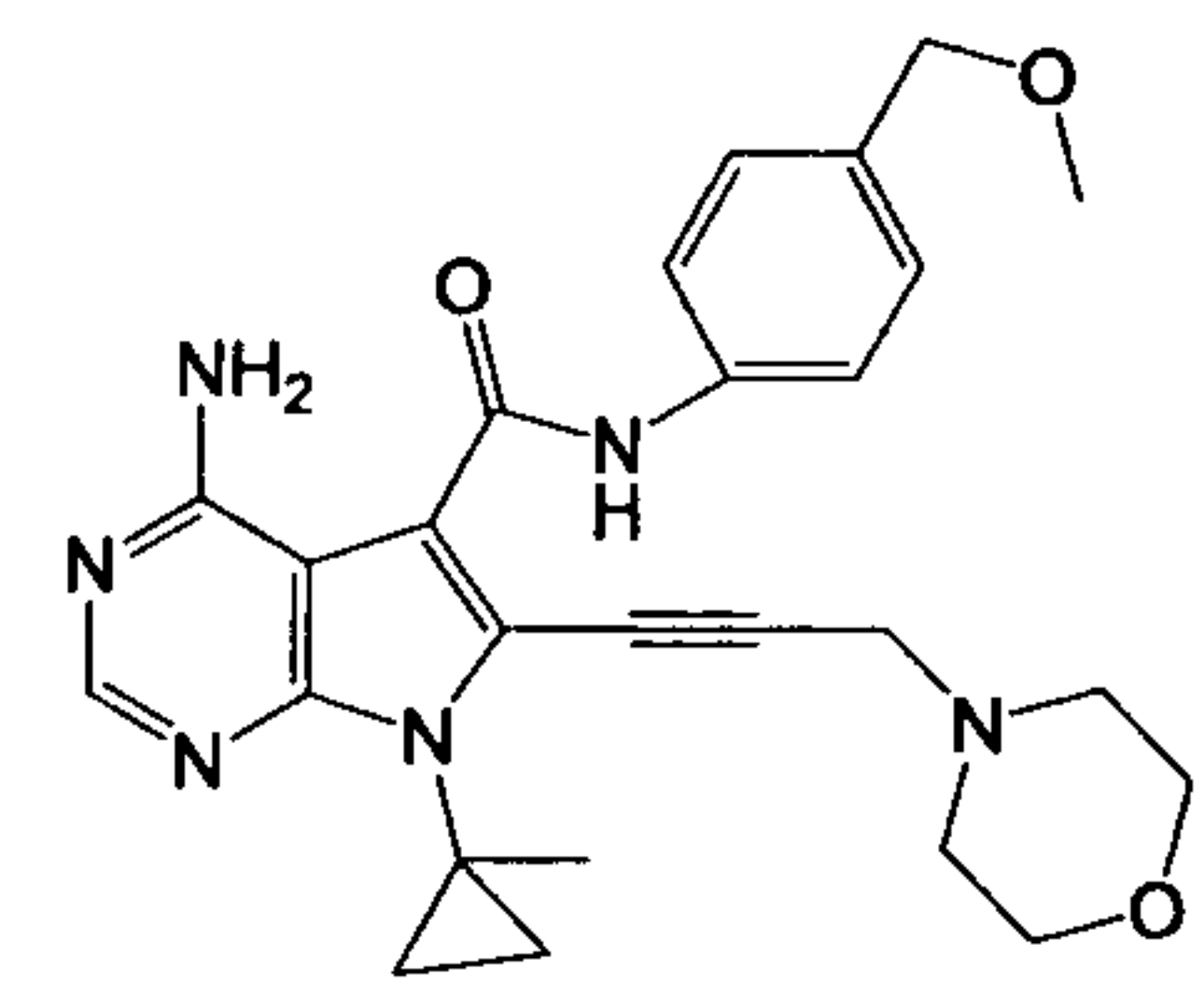
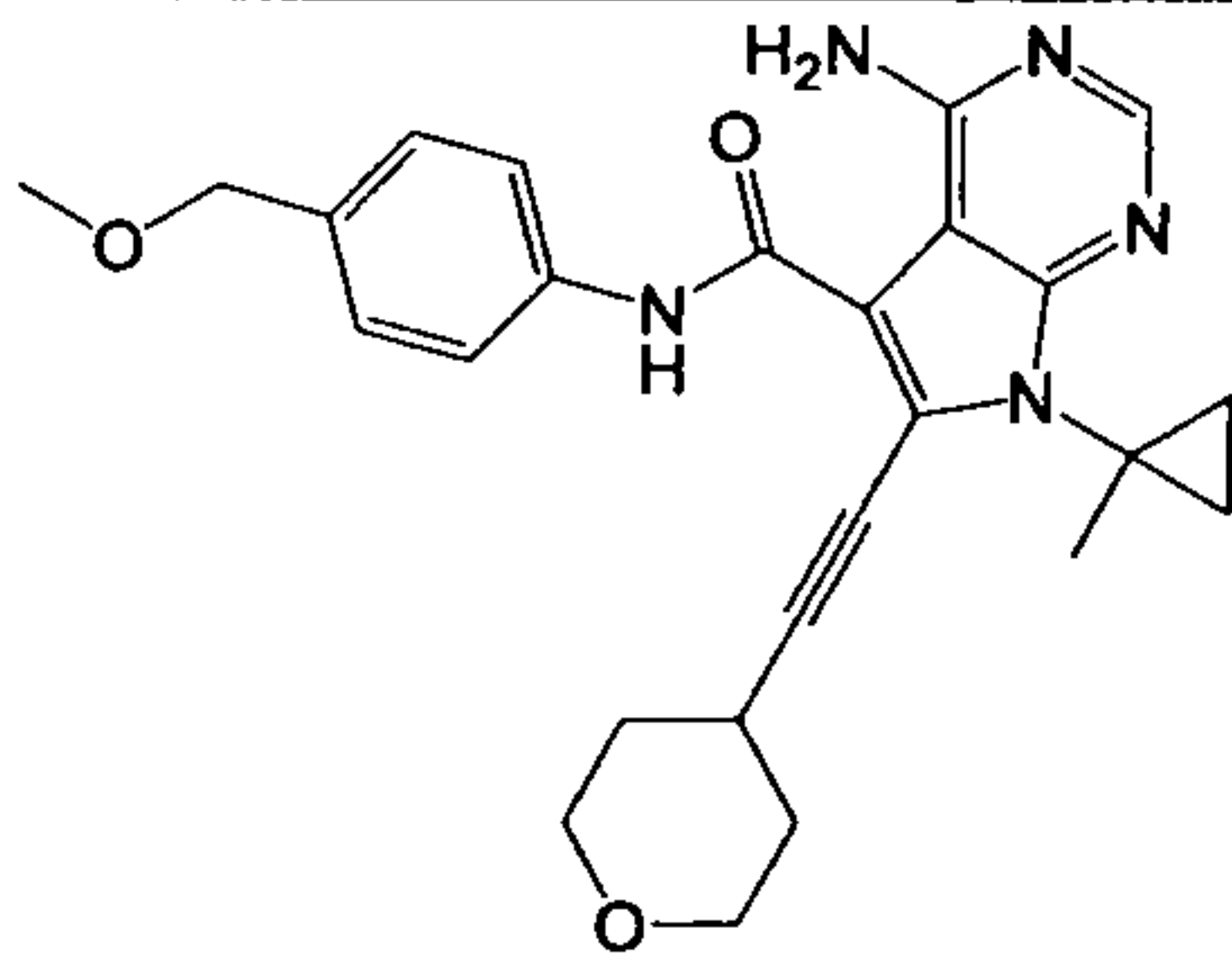
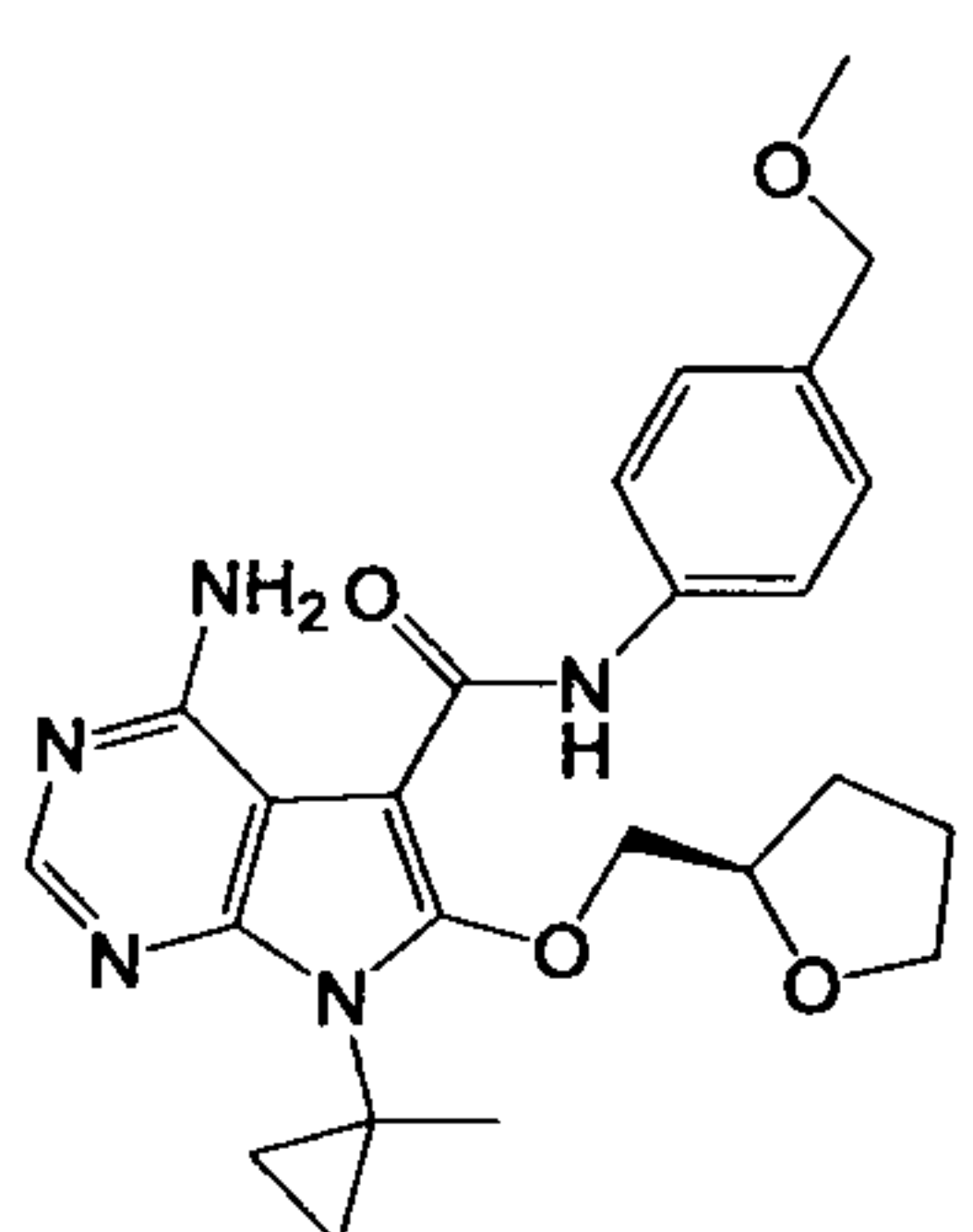
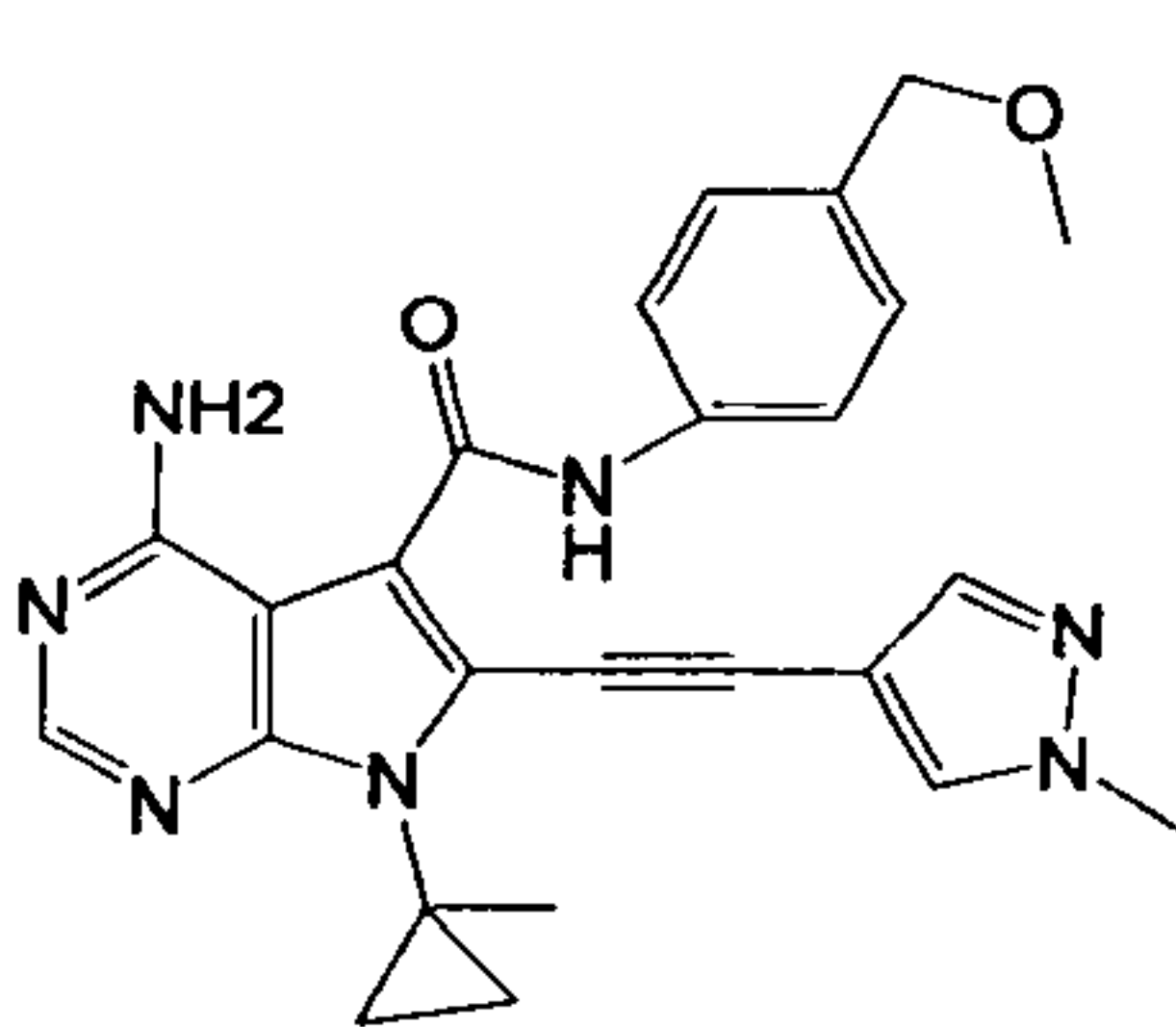
Day 8: the day on which evaluation was performed

[0182]

Table 6

Compound No	Structural Formula	Chemical Name	International Publication No. and Example No.
Compound 1		4-amino-1-(tert-butyl)-N-(5-methyl-1H-pyrazol-3-yl)-1H-pyrazolo[3,4-d]pyrimidine-3-carboxamide	WO2017/043550 Example Compound 34
Compound 2		4-amino-7-(tert-butyl)-N-(5-methyl-1H-pyrazol-3-yl)-7H-pyrrolo[2,3-d]pyrimidine-5-carboxamide	WO2017/043550 Example Compound 48
Compound 3		4-amino-7-(1-fluoro-2-methylpropan-2-yl)-N-(5-methyl-1H-pyrazol-3-yl)-7H-pyrrolo[2,3-d]pyrimidine-5-carboxamide	WO2017/043550 Example Compound 50
Compound 4		4-amino-N-(5-methyl-1H-pyrazol-3-yl)-7-(1-methylcyclopropyl)-7H-pyrrolo[2,3-d]pyrimidine-5-carboxamide	WO2017/043550 Example Compound 51
Compound 5		4-amino-7-(2-cyclopropylpropan-2-yl)-N-(5-methyl-1H-pyrazol-3-yl)-7H-pyrrolo[2,3-d]pyrimidine-5-carboxamide	WO2017/043550 Example Compound 52

(Continued from Table 6)

Compound No	Structural Formula	Chemical Name	International Publication No. and Example No.
Compound 6		4-amino-N-[4-(methoxymethyl)phenyl]-7-(1-methylcyclopropyl)-6-(3-morpholinopropyl)-7H-pyrrolo[2,3-d]pyrimidine-5-carboxamide	WO2017/146116 Example Compound 85
Compound 7		4-amino-N-[4-(methoxymethyl)phenyl]-7-(1-methylcyclopropyl)-6-((tetrahydro-2H-pyran-4-yl)ethynyl)-7H-pyrrolo[2,3-d]pyrimidine-5-carboxamide	WO2017/146116 Example Compound 87
Compound 8		(R)-4-amino-N-[4-(methoxymethyl)phenyl]-7-(1-methylcyclopropyl)-6-((tetrahydrofuran-2-yl)methoxy)-7H-pyrrolo[2,3-d]pyrimidine-5-carboxamide	WO2017/146116 Example Compound 89
Compound 9		4-amino-N-[4-(methoxymethyl)phenyl]-6-((1-methyl-1H-pyrazol-4-yl)ethynyl)-7-(1-methylcyclopropyl)-7H-pyrrolo[2,3-d]pyrimidine-5-carboxamide	WO2017/146116 Example Compound 90

[0183]

The results indicate, as shown in Table 7, that cabozantinib, vandetanib, lenvatinib, and fused pyrimidine compounds (Compounds 1 to 9) inhibited the growth of DCTN1-RET fusion gene-expressing NIH/3T3 cells.

[0184]

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Table 7

Compound	GI ₅₀ (nmol/L)
Compound 1	8.50
Compound 2	5.80
Compound 3	5.40
Compound 4	5.70
Compound 5	4.20
Compound 6	3.60
Compound 7	11.40
Compound 8	17.90
Compound 9	4.80
Cabozantinib	91.10
Vandetanib	68.70
Lenvatinib	50.30
Alectinib	>1000

[0185]

The results above suggest the potential usefulness of these RET inhibitors as a therapeutic agent for cancer in which the DCTN1-RET fusion gene has been detected. The results also suggest that the use of the DCTN1-RET fusion gene-expressing NIH/3T3 cells enables screening for a compound that inhibits DCTN1-RET.

10 [0186]

Example 9: Inhibition of RET Phosphorylation in DCTN1-RET Fusion Gene-Expressing Cells

The following examined whether RET phosphorylation in DCTN1-RET fusion gene-expressing cells is inhibited by existing medicinal agents that are reported as inhibiting RET in accordance with the method described below. The DCTN1-RET fusion gene-expressing NIH/3T3 cells for use were prepared by culture in a medium for 2-dimensional cell culture at 37°C in 5% CO₂. On the day before treating the cells with a medicinal agent, the cells were seeded onto a 6-well plate (Iwaki), 3×10⁵ cells/2 mL, and incubated at 37°C in 5% CO₂ overnight. Cabozantinib, vandetanib, alectinib, and lenvatinib were dissolved in dimethyl sulfoxide to give a concentration of 10 mmol/L, and further diluted with PBS such that these compounds have a final concentration of 1000, 100, and 10 nmol/L. The diluted compounds were individually added

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to each well onto which the cells were previously seeded, 20 μ L per well (day 1), and incubated at 37°C in 5% CO₂ for 1 hour. After incubation, a sample of the cells for protein expression analysis was taken in the same manner as in Example 7, and
5 protein expression was analyzed. The results indicate, as shown in Fig. 9, that phosphorylated RET levels in the DCTN1-RET fusion gene-expressing NIH/3T3 cells notably decreased by cabozantinib and lenvatinib. Additionally, the inhibition of RET
10 phosphorylation by the fused pyrimidine compounds was also evaluated in the same manner as above, and RET phosphorylation was also confirmed to have notably decreased by the fused pyrimidine compounds. These results suggest that a medicinal agent capable of notably decreasing the phosphorylated RET levels would be a compound that can inhibit the growth of the DCTN1-RET
15 fusion gene-expressing NIH/3T3 cells, and that the compound is potentially useful as a therapeutic agent for cancer in which the DCTN1-RET fusion gene has been detected. The results also suggest that the use of phosphorylated RET levels in DCTN1-RET fusion gene-expressing NIH/3T3 cells enables screening for a RET
20 inhibitor.

Sequence List Free Text

[0187]

SEQ ID NO: 1 shows the base sequence of the polynucleotide
25 encoding a fusion peptide of DCTN1 variant 1 (v1) (a portion of SEQ ID NO: 25) and RET variant 2 (v2) (a portion of SEQ ID NO: 31).

SEQ ID NO: 2 shows the amino acid sequence of a fusion peptide of DCTN1 v1 and RET v2.

30 SEQ ID NO: 3 shows the base sequence of the polynucleotide encoding a fusion peptide of DCTN1 v1 and RET variant 4 (v4) (a portion of SEQ ID NO: 32).

SEQ ID NO: 4 shows the amino acid sequence of a fusion peptide of DCTN1 v1 and RET v4.

35 SEQ ID NO: 5 shows the base sequence of the polynucleotide

-64-

- encoding a fusion peptide of DCTN1 variant 2 (v2) (a portion of SEQ ID NO: 26) and RET v2.
- SEQ ID NO: 6 shows the amino acid sequence of a fusion peptide of DCTN1 v2 and RET v2.
- 5 SEQ ID NO: 7 shows the base sequence of the polynucleotide encoding a fusion peptide of DCTN1 v2 and RET v4.
- SEQ ID NO: 8 shows the amino acid sequence of a fusion peptide of DCTN1 v2 and RET v4.
- SEQ ID NO: 9 shows the base sequence of the polynucleotide
- 10 encoding a fusion peptide of DCTN1 variant 3 (v3) (a portion of SEQ ID NO: 27) and RET v2.
- SEQ ID NO: 10 shows the amino acid sequence of a fusion peptide of DCTN1 v3 and RET v2.
- SEQ ID NO: 11 shows the base sequence of the polynucleotide
- 15 encoding a fusion peptide of DCTN1 v3 and RET v4.
- SEQ ID NO: 12 shows the amino acid sequence of a fusion peptide of DCTN1 v3 and RET v4.
- SEQ ID NO: 13 shows the base sequence of the polynucleotide encoding a fusion peptide of DCTN1 variant 4 (v4) (a portion of
- 20 SEQ ID NO: 28) and RET v2.
- SEQ ID NO: 14 shows the amino acid sequence of a fusion peptide of DCTN1 v4 and RET v2.
- SEQ ID NO: 15 shows the base sequence of the polynucleotide encoding a fusion peptide of DCTN1 v4 and RET v4.
- 25 SEQ ID NO: 16 shows the amino acid sequence of a fusion peptide of DCTN1 v4 and RET v4.
- SEQ ID NO: 17 shows the base sequence of the polynucleotide encoding a fusion peptide of DCTN1 variant 5 (v5) (a portion of SEQ ID NO: 29) and RET v2.
- 30 SEQ ID NO: 18 shows the amino acid sequence of a fusion peptide of DCTN1 v5 and RET v2.
- SEQ ID NO: 19 shows the base sequence of the polynucleotide encoding a fusion peptide of DCTN1 v5 and RET v4.
- SEQ ID NO: 20 shows the amino acid sequence of a fusion peptide
- 35 of DCTN1 v5 and RET v4.

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- SEQ ID NO: 21 shows the base sequence of the polynucleotide encoding a fusion peptide of DCTN1 v6 and RET v2.
- SEQ ID NO: 22 shows the amino acid sequence of a fusion peptide of DCTN1 v6 and RET v2.
- 5 SEQ ID NO: 23 shows the base sequence of the polynucleotide encoding a fusion peptide of DCTN1 v6 and RET v4.
- SEQ ID NO: 24 shows the amino acid sequence of a fusion peptide of DCTN1 v6 and RET v4.
- SEQ ID NO: 33 shows the base sequence of a primer.
- 10 SEQ ID NO: 34 shows the base sequence of a primer.
- SEQ ID NO: 35 shows the base sequence of a primer.
- SEQ ID NO: 36 shows the base sequence of a primer.
- SEQ ID NO: 37 shows the base sequence of a primer.
- SEQ ID NO: 38 shows the base sequence of a primer.
- 15 SEQ ID NO: 39 shows the base sequence of a primer.
- SEQ ID NO: 40 shows the base sequence of a primer.
- SEQ ID NO: 41 shows the base sequence of a primer.
- SEQ ID NO: 42 shows the base sequence of a primer.
- SEQ ID NO: 43 shows the base sequence of a primer.
- 20 SEQ ID NO: 44 shows the base sequence of a primer.
- SEQ ID NO: 45 shows the base sequence of a primer.
- SEQ ID NO: 46 shows the base sequence of a primer.
- SEQ ID NO: 47 shows the base sequence of a primer.
- SEQ ID NO: 48 shows the base sequence of a primer.
- 25 SEQ ID NO: 49 shows the base sequence of a primer.
- SEQ ID NO: 50 shows the base sequence of a primer.
- SEQ ID NO: 51 shows the base sequence of a primer.
- SEQ ID NO: 52 shows the base sequence of a primer.
- SEQ ID NO: 53 shows the base sequence of a primer.
- 30 SEQ ID NO: 54 shows the base sequence of a primer.
- SEQ ID NO: 55 shows the base sequence of a primer.
- SEQ ID NO: 56 shows the base sequence of a primer.
- SEQ ID NO: 57 shows the base sequence of a primer.
- SEQ ID NO: 58 shows the base sequence of a primer.
- 35 SEQ ID NO: 59 shows the base sequence of a primer.

-66-

- SEQ ID NO: 60 shows the base sequence of a primer.
 SEQ ID NO: 61 shows the base sequence of a primer.
 SEQ ID NO: 62 shows the base sequence of a primer.
 SEQ ID NO: 63 shows the base sequence of a primer.
 5 SEQ ID NO: 64 shows the base sequence of a primer.
 SEQ ID NO: 65 shows the base sequence of a primer.
 SEQ ID NO: 66 shows the base sequence of a primer.
 SEQ ID NO: 67 shows the base sequence of a primer.
 SEQ ID NO: 68 shows the base sequence of a primer.
 10 SEQ ID NO: 69 shows the base sequence of a primer.
 SEQ ID NO: 70 shows the base sequence of a primer.
 SEQ ID NO: 71 shows the base sequence of a primer.
 SEQ ID NO: 72 shows the base sequence of a primer.
 SEQ ID NO: 73 shows the base sequence of a primer.
 15 SEQ ID NO: 74 shows the base sequence of RET siRNA.
 SEQ ID NO: 75 shows the base sequence of RET siRNA.
 SEQ ID NO: 76 shows the base sequence of RET siRNA.
 SEQ ID NO: 77 shows the base sequence of RET siRNA.

20 Sequence Table

P17-158WOPCT_DCTN1 PROTEIN_20180820_115457_8.txt

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CLAIMS

[Claim 1]

A polypeptide wherein an N-terminal portion of DCTN1 protein is fused to a C-terminal portion of RET protein.

5

[Claim 2]

The polypeptide according to claim 1, which is selected from the following (a) to (c):

(a) a polypeptide comprising the amino acid sequence represented
10 by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID
NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO:
18, SEQ ID NO: 20, SEQ ID NO: 22, or SEQ ID NO: 24;

(b) a polypeptide comprising an amino acid sequence wherein one
or several amino acids are substituted, deleted, or added in the
15 amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4,
SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID
NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO:
22, or SEQ ID NO: 24 ; and

(c) a polypeptide comprising an amino acid sequence that has at
20 least 90% identity with the amino acid sequence represented by
SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID
NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO:
18, SEQ ID NO: 20, SEQ ID NO: 22, or SEQ ID NO: 24.

25 [Claim 3]

A polynucleotide encoding the polypeptide according to
claim 1 or 2.

[Claim 4]

30 The polynucleotide according to claim 3, which is
selected from the following (d) to (f):

(d) a polynucleotide encoding a polypeptide comprising the amino
acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID
NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14,
35 SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, or

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SEQ ID NO: 24;

(e) a polynucleotide encoding a polypeptide comprising an amino acid sequence wherein one or several amino acids are substituted, deleted, or added in the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, or SEQ ID NO: 24; and

(f) a polynucleotide encoding a polypeptide comprising an amino acid sequence that has at least 90% identity with the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, or SEQ ID NO: 24.

[Claim 5]

The polynucleotide according to claim 3, which is selected from the following (g) to (i):

(g) a polynucleotide comprising the base sequence represented by SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, or SEQ ID NO: 23;

(h) a polynucleotide hybridizing under stringent conditions with a polynucleotide comprising a base sequence complementary to the base sequence represented by SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, or SEQ ID NO: 23; and

(i) a polynucleotide having at least 90% identity with the base sequence represented by SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, or SEQ ID NO: 23.

[Claim 6]

An expression vector comprising the polynucleotide

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according to any one of claims 3 to 5.

[Claim 7]

A cell transfected with the polynucleotide according to
5 any one of claims 3 to 5.

[Claim 8]

An antibody that specifically binds to the polypeptide
according to claim 1 or 2.
10

[Claim 9]

A method for detecting the presence of the polypeptide
according to claim 1 or 2 in a sample.

15 [Claim 10]

A primer or probe for detecting the presence of the
polynucleotide according to any one of claims 3 to 5 in a sample,
the primer or probe being a polynucleotide selected from the
following (j) to (l):

20 (j) a polynucleotide that is at least one probe selected from
the group consisting of probes hybridizing with a polynucleotide
encoding DCTN1 protein, and probes hybridizing with a
polynucleotide encoding RET protein;

(k) a polynucleotide that is a probe that hybridizes to a point
25 of fusion between a polynucleotide encoding DCTN1 protein and a
polynucleotide encoding RET protein; and

(l) a polynucleotide that is a set of a sense primer and an
antisense primer designed to sandwich a point of fusion between a
polynucleotide encoding DCTN1 protein and a polynucleotide
30 encoding RET protein.

[Claim 11]

A method for detecting the presence of the
polynucleotide according to any one of claims 3 to 5 in a sample.

35

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[Claim 12]

A method for diagnosing cancer in a patient when the presence of the polypeptide according to claim 1 or 2 or the polynucleotide according to any one of claims 3 to 5 is detected
5 in a sample derived from the patient by the detection method according to claim 9 or 11.

[Claim 13]

A pharmaceutical composition for treating cancer that
10 is positive for a fusion gene of DCTN1 gene and RET gene and/or positive for a fusion protein of DCTN1 protein and RET protein, the composition comprising a compound that inhibits RET as an active ingredient.

15 [Claim 14]

A method of screening for a compound that inhibits expression and/or activity of the polypeptide according to claim 1 or 2 or expression of the polynucleotide according to any one of claims 3 to 5, the method comprising the following steps (1)
20 and (2):

(1) the step of bringing the polypeptide according to claim 1 or 2, a cell expressing the polypeptide according to claim 1 or 2 or the polynucleotide according to any one of claims 3 to 5, or the cell according to claim 7 into contact with a test compound; and
25 (2) the step of measuring whether expression and/or activity of the polypeptide according to claim 1 or 2 or expression of the polynucleotide according to any one of claims 3 to 5 is inhibited in step (1), or the step of measuring whether growth of the cell in step (1) is inhibited.

30

[Claim 15]

A method using the polypeptide according to claim 1 or 2 or the polynucleotide according to any one of claims 3 to 5 as an indicator for determining whether a chemotherapy using a
35 compound that inhibits RET is effective,

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the method comprising determining that the chemotherapy using the compound that inhibits RET is effective when the polypeptide according to claim 1 or 2 is detected in a sample by the detection method according to claim 9, and/or when the presence of the polynucleotide according to any one of claims 3 to 5 is detected in a sample by the detection method according to claim 11.

[Claim 16]

10 A biomarker for detecting cancer, the biomarker comprising at least one member selected from the group consisting of polypeptides wherein an N-terminal portion of DCTN1 protein is fused to a C-terminal portion of RET protein, and polynucleotides encoding the polypeptides.

15

[Claim 17]

A method for treating cancer, the method comprising giving a chemotherapy using a compound that inhibits RET to a cancer patient who is positive for a fusion gene of DCTN1 gene and RET gene, and/or positive for a fusion protein of DCTN1 protein and RET protein.

20

[Claim 18]

25 A method for treating cancer, the method comprising detecting the presence of the polypeptide according to claim 1 or 2, and/or the presence of the polynucleotide according to any one of claims 3 to 5 in a sample derived from a test subject, and

giving a chemotherapy using a compound that inhibits RET to the test subject when the presence of the polypeptide according to claim 1 or 2 has been detected, and/or the presence of the polynucleotide according to any one of claims 3 to 5 has been detected.

30

[Claim 19]

35

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A compound that inhibits RET for use in the treatment of a cancer patient who is positive for a fusion gene of DCTN1 gene and RET gene, and/or positive for a fusion protein of DCTN1 protein and RET protein.

5

[Claim 20]

Use of a compound that inhibits RET in the production of a pharmaceutical composition for cancer treatment for treating a cancer patient who is positive for a fusion gene of DCTN1 gene and RET gene, and/or positive for a fusion protein of DCTN1 protein and RET protein.

10

[Claim 21]

A method for producing a reagent for determining whether a chemotherapy using a compound that inhibits RET is effective, using a means to detect the presence of the polypeptide according to claim 1 or 2 in a sample, and/or a means to detect the presence of the polynucleotide according to any one of claims 3 to 5 in a sample.

15
20

[Claim 22]

A combination of an anti-DCTN1 antibody and an anti-RET antibody for detecting the presence of the polynucleotide according to any one of claims 3 to 5.

25

[Claim 23]

Use of the antibody according to claim 8, the combination of the antibodies according to claim 22, or the primer or probe according to claim 10 in the production of a detection reagent for detecting the presence of the polypeptide according to claim 1 or 2, or the presence of the polynucleotide according to any one of claims 3 to 5.

30

Fig. 1

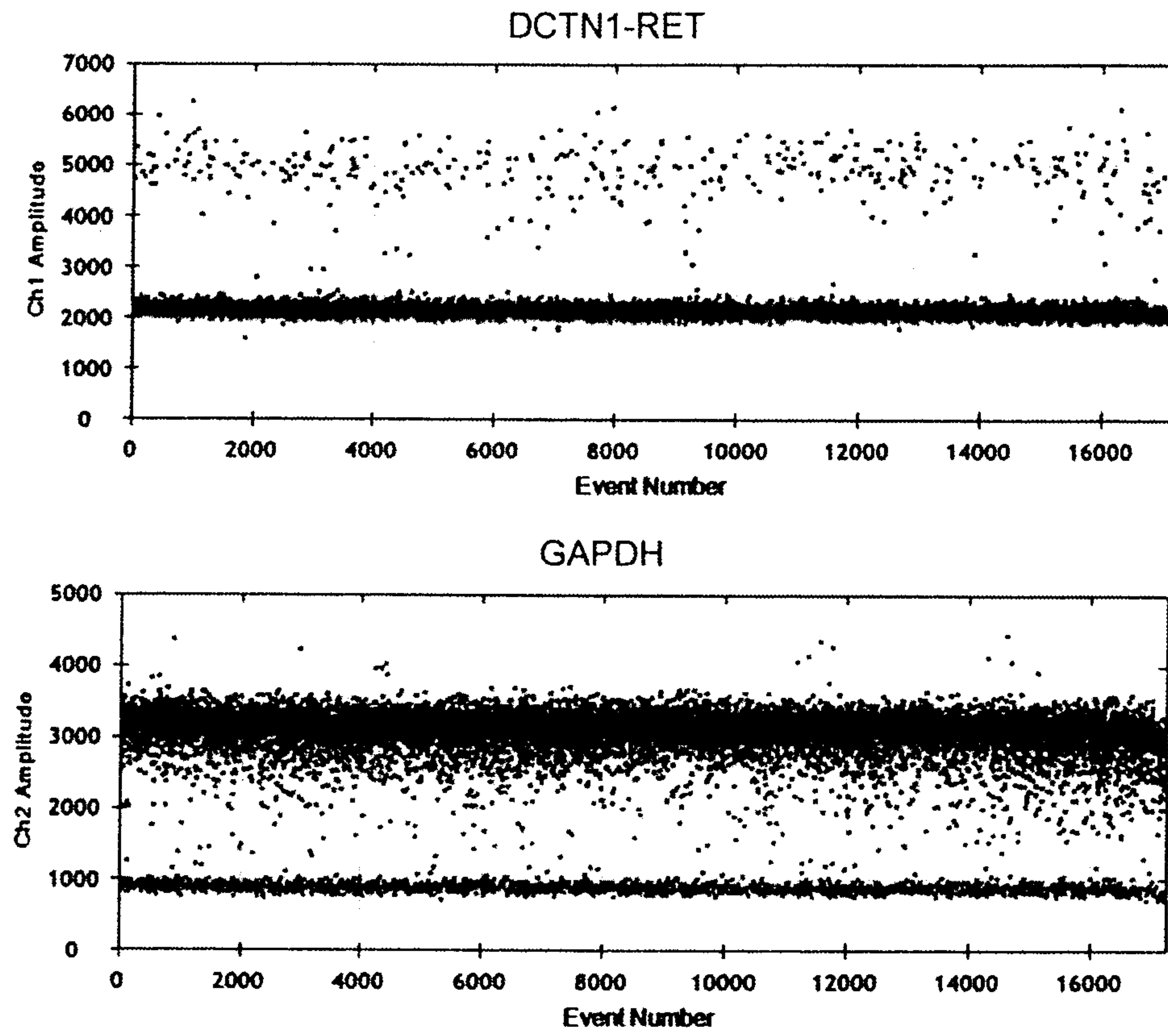


Fig. 2

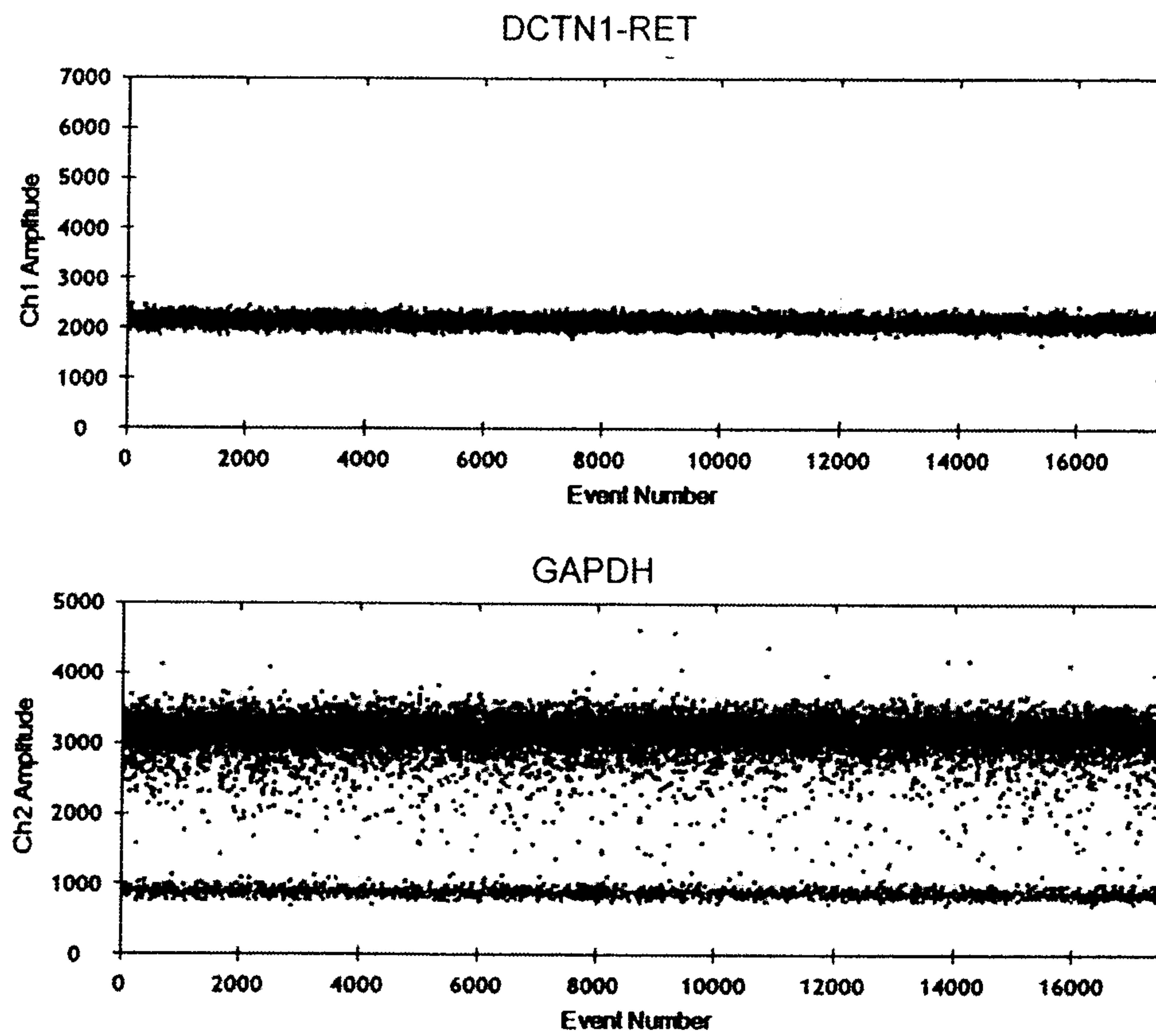


Fig.3

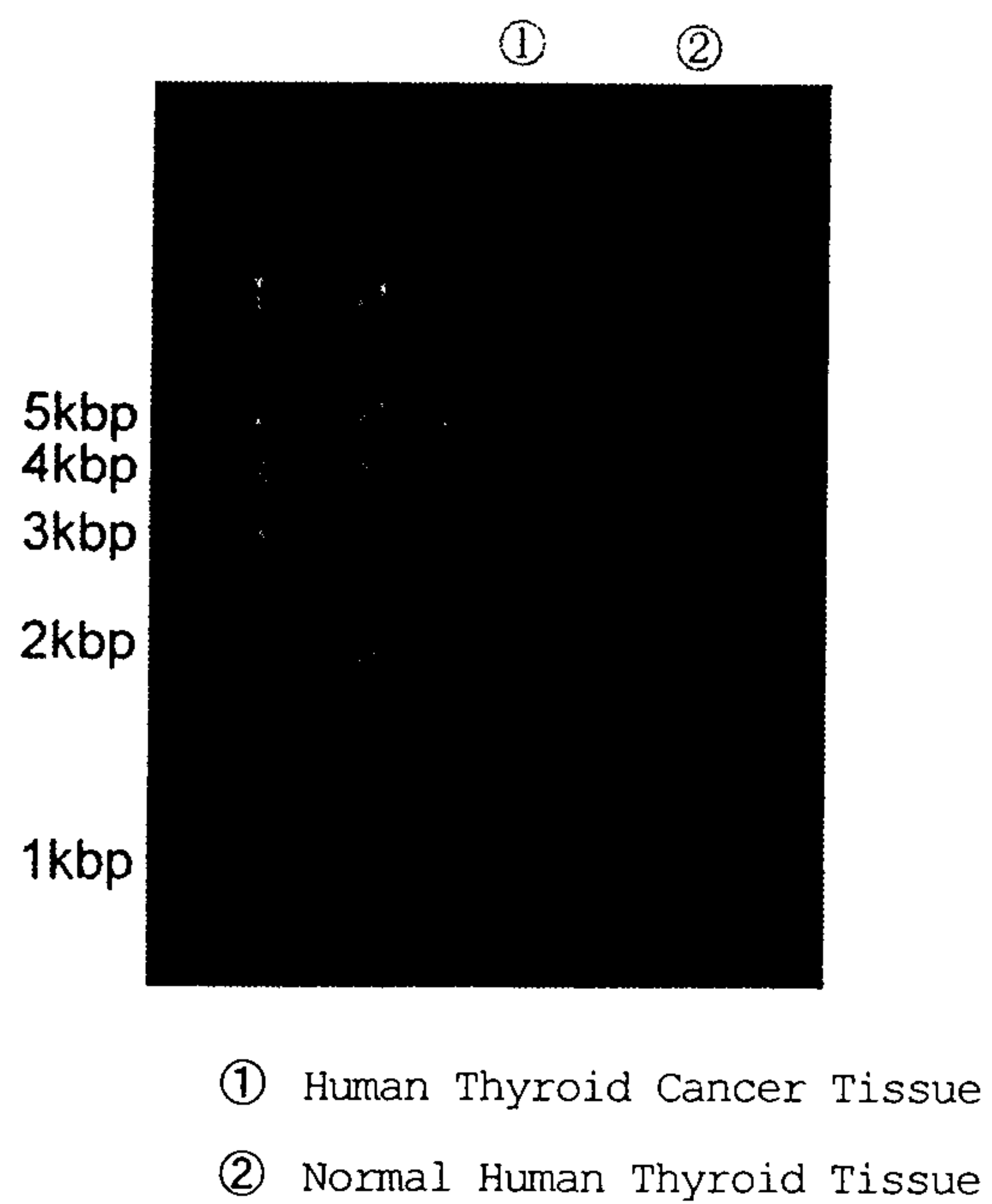


Fig. 4

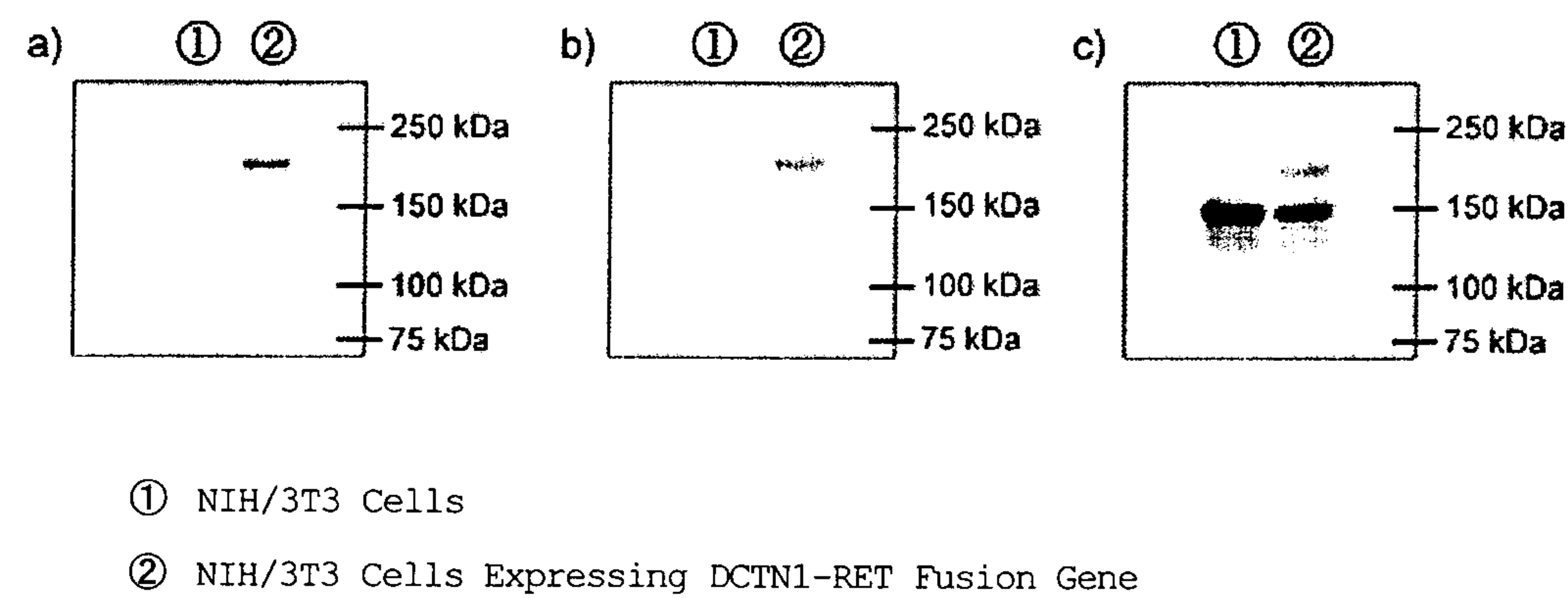


Fig. 5

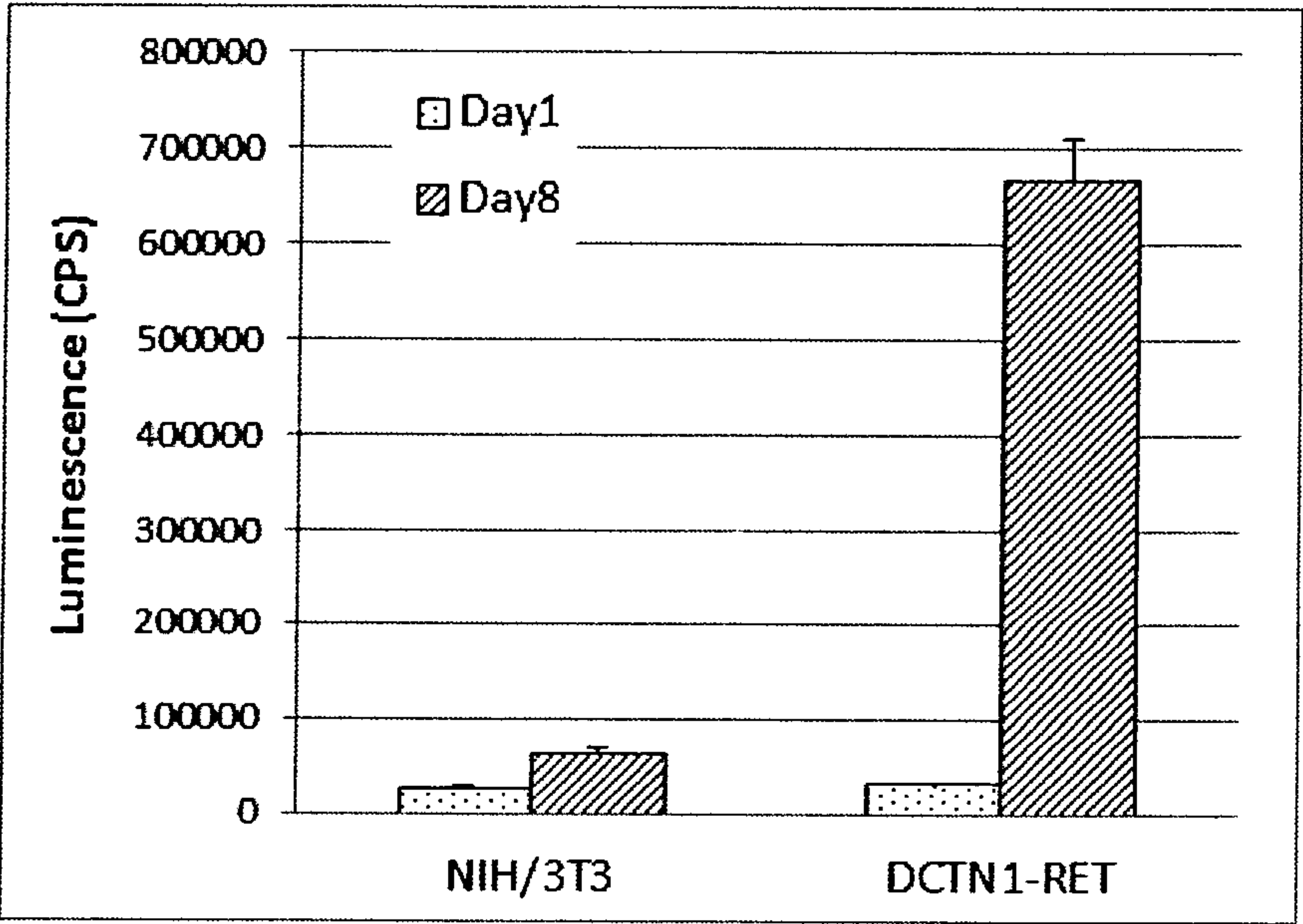


Fig. 6

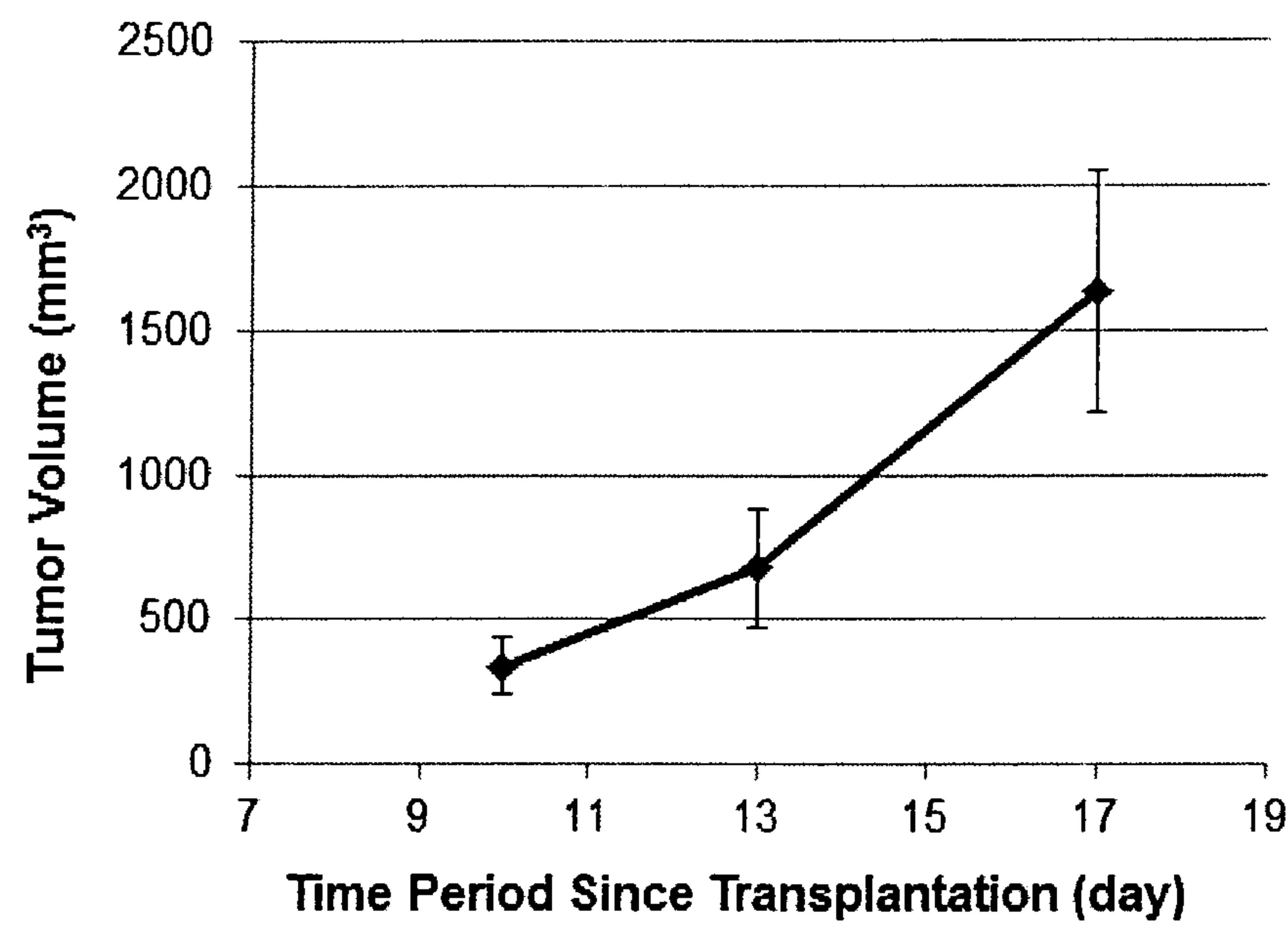


Fig. 7

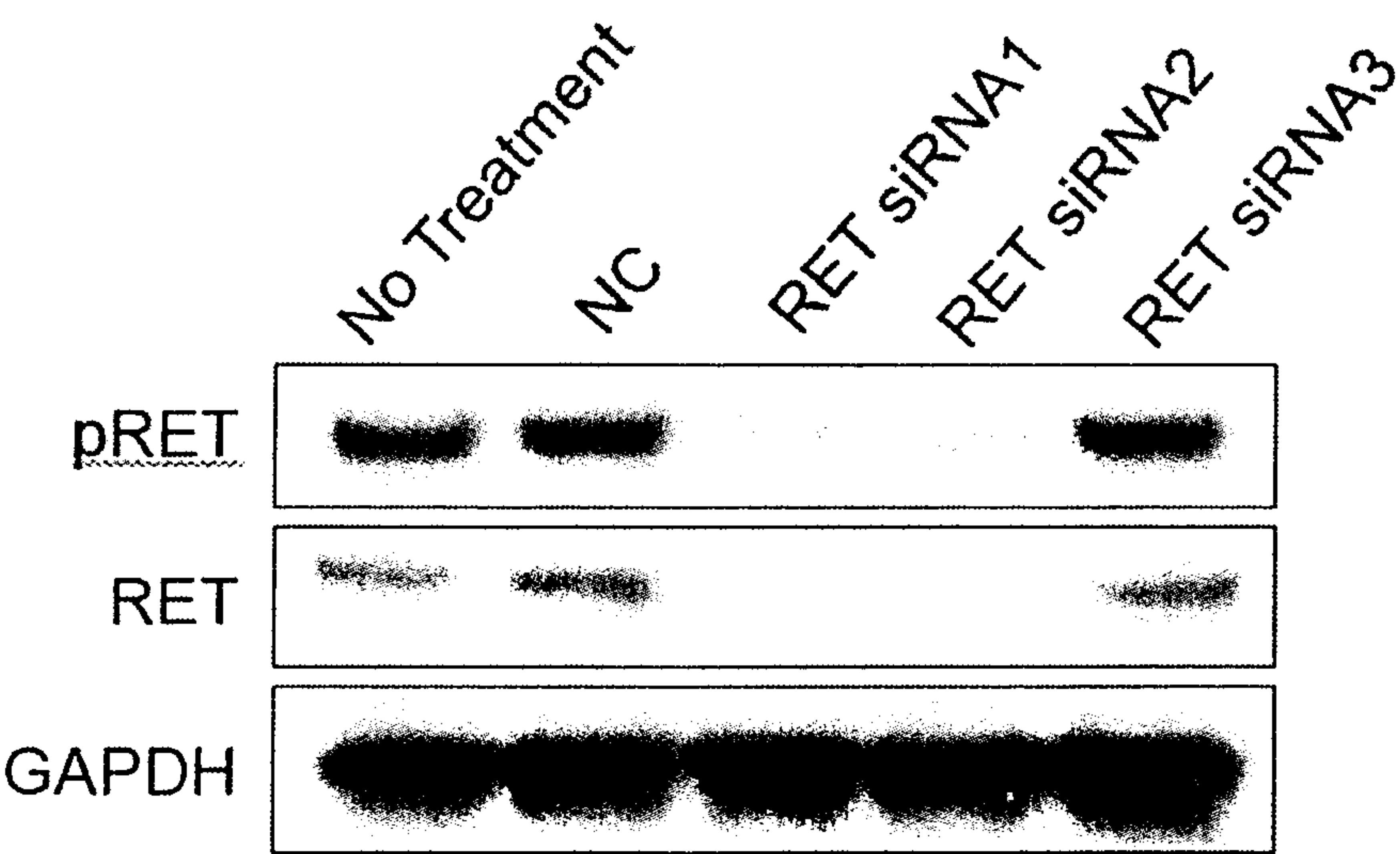


Fig. 8

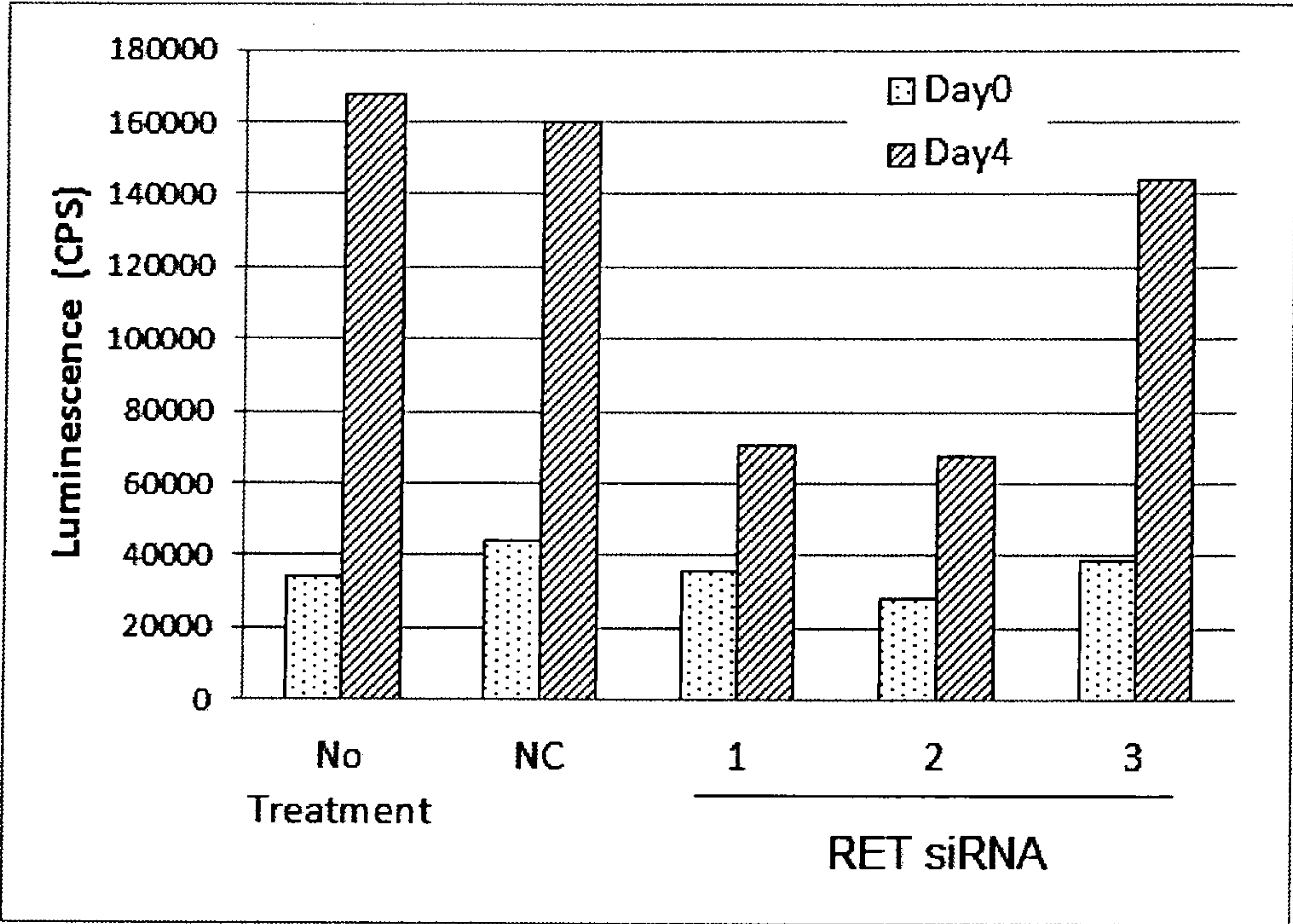


Fig. 9

