

Description

Title of Invention:

ANTI-GPR20 ANTIBODY AND ANTI-GPR20 ANTIBODY-DRUG
CONJUGATE

Technical Field

[0001] The present invention relates to an anti-GPR20 antibody binding to GPR20 and having an internalization effect, a method for producing the anti-GPR20 antibody, an antibody-drug conjugate comprising the antibody, an antitumor agent comprising the antibody-drug conjugate, and the like.

Background Art

[0002] Cancers rank high in causes of death. Although the number of cancer patients is expected to increase with aging of the population, treatment needs have not yet been sufficiently satisfied. The problems of conventional chemotherapeutics are that: due to their low selectivity, these chemotherapeutics are toxic not only to tumor cells but also to normal cells and thereby have adverse reactions; and the chemotherapeutics cannot be administered in sufficient amounts and thus cannot sufficiently produce their effects. Hence, in recent years, more highly selective molecular targeted drugs or antibody drugs have been developed, which target

molecules that exhibit mutations or high expression characteristics of cancer cells, or specific molecules involved in malignant transformation of cells.

[0003] Gastrointestinal stromal tumor (GIST) is a mesenchymal tumor that develops in the gastrointestinal tract from the esophagus to the rectum and the mesenterium, and its incidence is reportedly 1 to 2 persons per 100,000 per year (Non Patent Literature 1). Activating mutations in the receptor tyrosine kinase KIT or PDGFRA are found in approximately 86% of GIST patients, and these mutations contribute to the proliferation of tumor cells. GIST treatment is based on surgical resection. Meanwhile, tyrosine kinase inhibitors (TKIs) such as imatinib, sunitinib, or regorafenib are prescribed for unresectable and progressive or metastatic GIST (Non Patent Literature 2). These TKIs often exhibit significant efficacy for GIST having the above-described mutations, but need to be continuously administered. In addition, in many cases, GIST cannot be completely eradicated by TKIs, and eventually becomes unresponsive to the drugs due to secondary mutations in the target KIT or PDGFRA, activating mutations in RAS, BRAF, and the like, and activation of other signaling pathways, so that the disease progresses. Furthermore, these TKIs rarely exhibit therapeutic effects on wild type GIST found to have no mutation in KIT or PDGFRA, albeit such wild type GIST arise in only a small number of cases (Non Patent

Literature 3). Hence, there has been a demand for the development of treatment methods effective for TKI-resistant GIST.

[0004] GPR20 (G protein-coupled receptor 20) is a seven-transmembrane protein composed of 358 amino acids, which belongs to class A of the G protein-coupled receptor (GPCR) family, and this protein has N-terminal extracellular and C-terminal intracellular domains. The human GPR20 gene was cloned for the first time in 1997 (Non Patent Literature 4). Its putative amino acid sequence was then found to differ partially from that encoded by the human GPR20 gene cloned by another researcher in 2008 (Non Patent Literature 5). The latter sequence, which is identical to a sequence registered in the NCBI database of human complete genomic sequence analysis, is currently disclosed as the DNA sequence encoding human GPR20, together with the amino acid sequence thereof, in a public database. The DNA sequence and the amino acid sequence can be referred to under, for example, accession Nos. NM_005293 and NP_005284 (NCBI).

[0005] GPR20 has an amino acid sequence similar to that of the GPCR family, members of which recognize a nucleotide or a lipid. However, neither physiological functions nor *in vivo* ligands have been identified for GPR20. From an experiment in which GPR20 was exogenously expressed in HEK293 cells, it has been reported that GPR20 constitutively activates G_i trimeric G proteins

under conditions without ligand stimulation (Non Patent Literature 5).

[0006] GPR20 has been confirmed to express messenger RNA (mRNA) in the heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, rectum, and leukocytes, and, in particular, its high expression in the small intestine has been reported (Non Patent Literature 5). In brain, the expression in thalamus, putamen, and caudate nuclei has been reported (Non Patent Literature 4). GPR20-deficient mice have exhibited the phenotype of hyperactivity disorder characterized by increase in total distance travelled in open field tests, suggesting that GPR20 is associated with spontaneous activity in the central nervous system (Patent Literature 1). It has also been reported that GPR20 is highly expressed in GIST (Non Patent Literature 6). It has been reported that the expression of GPR20 is controlled by ETS variant 1 (ETV1), which is a major transcriptional factor of GIST (Non Patent Literature 7).

[0007] Antibodies are highly stable in blood, and specifically bind to their target antigens. For these reasons, a reduced level of adverse reactions is expected, and a large number of antibody drugs targeting molecules, which are highly expressed on the surface of cancer cells, have been developed. Examples of the mechanism of action of antibody drugs directly targeting tumor cells include

antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), blocking of signals of receptors involved in tumor growth, and apoptosis induction.

[0008] One of the products using the antigen-binding ability of antibodies is an antibody-drug conjugate (ADC). ADC for cancer is an antibody conjugated to a cytotoxic drug in which the antibody has an ability to bind to an antigen expressed on the surface of a cancer cell and to internalize into the cell through said binding to the antigen. ADC for cancer can efficiently deliver the drug to cancer cells, and can thereby be expected to kill the cancer cells by accumulating the drug in the cancer cells (Non Patent Literature 8). With regard to ADC, for example, Adcetris (brentuximab vedotin) comprising an anti-CD30 monoclonal antibody conjugated to monomethyl auristatin E has been approved as a therapeutic drug for Hodgkin's lymphoma and anaplastic large cell lymphoma. Also, Kadcyla (trastuzumab emtansine) comprising an anti-HER2 monoclonal antibody conjugated to emtansine is used in the treatment of HER2-positive progressive or recurrent breast cancer.

[0009] The features of the target antigen suitable for ADC as an antitumor drug are that: the antigen is specifically highly expressed on the surface of cancer cells but has low expression or is not expressed in normal cells; the antigen can be internalized into cells;

the antigen is not secreted from the cell surface; etc. Important features of the antibody suitable for ADC are that the antibody specifically binds to the target antigen as well as that it has high internalization ability. The internalization ability of the antibody depends on the properties of both the target antigen and the antibody. It is difficult to predict an antigen-binding site suitable for internalization from the molecular structure of a target or easily to predict an antibody having high internalization ability from binding strength, physical properties, and the like of the antibody. Hence, an important challenge in developing ADC having high efficacy is obtaining an antibody having high internalization ability against the target antigen (Non Patent Literature 9).

[0010] No antitumor therapeutic drug targeting GPR20 has been known so far. Furthermore, there have been no reports of an anti-GPR20 antibody binding to GPR20 expressed on the cell membrane surface and having internalization activity, or of ADC containing such an antibody.

Citation List

Patent Literature

[0011]

Patent Literature 1: US 2003/0018989

Non Patent Literature

[0012]

Non Patent Literature 1: Corless C. L., et al., Nat Rev Cancer (2011) 11, 865-878

Non Patent Literature 2: Demetri G. D., et al., NCCN Task Force report, J Natl Compr Canc Netw. (2010) 8, Suppl 2: S1-41

Non Patent Literature 3: Bauer S., Joensuu H., Drugs. (2015) 75, 1323-1334

Non Patent Literature 4: O'Dowd B. F., Gene 187 (1997) 75-81

Non Patent Literature 5: Hase M., et al., J Biol Chem. (2008) 283, 12747-12755

Non Patent Literature 6: Allander S. V., et al., CANCER RESEARCH (2001) 61, 8624-8628

Non Patent Literature 7: Chi P., et al., Nature. (2010) 467 (7317): 849-853

Non Patent Literature 8: Heidi L. Perez, et al., Drug Discov. Today (2014) 19, 869-881

Non Patent Literature 9: Peters C., Brown S., Bioscience Reports (2015) 35, e00225

Summary of Invention

Technical Problem

[0013] It is an object of the present invention to provide an antibody specifically binding to GPR20-positive tumor cells such as GIST, an antibody-drug conjugate comprising the antibody, a pharmaceutical

product comprising the antibody-drug conjugate and having therapeutic effects on a tumor, a method for treating a tumor using the aforementioned pharmaceutical product, methods for producing the antibody and the antibody-drug conjugate, and the like.

Solution to Problem

[0014] The present inventors have conducted intensive studies directed towards achieving the above-described object, and hypothesized that GPR20 is one of the molecules characterizing GIST, and this molecule is capable of serving as a therapeutic target specific for GIST. As a result of examining the internalization activity and binding pattern of an anti-human GPR20 antibody, the inventors have found an anti-GPR20 antibody having high internalization activity among antibodies that exhibit a specific binding pattern. The inventors have further found that an anti-GPR20 antibody-drug conjugate comprising the aforementioned anti-GPR20 antibody conjugated to a drug exerting toxicity in cells via a linker having a specific structure exerts an antitumor effect on a GPR20-positive malignant tumor, such as GIST, expressing GPR20, thereby completing the present invention. Specifically, the present invention includes the following aspects of the invention.

[0015] Specifically, the invention of the present application provides:

(1) an antibody or a functional fragment of the antibody having the following properties (a) and (b):

(a) specifically binding to GPR20, and

(b) having internalization ability that permits cellular uptake after binding to GPR20;

(2) the antibody or the functional fragment of the antibody according to (1), wherein the GPR20 is a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 1;

(3) the antibody or the functional fragment of the antibody according to (1) or (2), which specifically binds to a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 1 and does not specifically bind to a polypeptide with the amino acid Y (tyrosine) at amino acid position 113 substituted with a different amino acid;

(4) the antibody or the functional fragment of the antibody according to any one of (1) to (3), which specifically binds to a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 1 and does not specifically bind to a polypeptide with the amino acid Y (tyrosine) at amino acid position 113 substituted with F (phenylalanine);

(5) the antibody or the functional fragment of the antibody according to any one of (1) to (4), which specifically binds to a conformation consisting of the amino acid sequence at amino acid positions 1 to 48 and

the amino acid sequence at amino acid positions 108 to 125 in SEQ ID NO: 1;

(6) the antibody or the functional fragment of the antibody according to any one of (1) to (5), which specifically binds to at least one amino acid residue selected from the amino acid sequence at amino acid positions 1 to 48 and at least one amino acid residue selected from the amino acid sequence at amino acid positions 108 to 125 in SEQ ID NO: 1;

(7) the antibody or the functional fragment of the antibody according to any one of (1) to (6), wherein at least one of the amino acids to which the antibody or the functional fragment specifically binds is the amino acid at amino acid position 113 of SEQ ID NO: 1;

(8) the antibody or the functional fragment of the antibody according to any one of (1) to (7), which has competitive inhibitory activity, for binding to GPR20, against any one antibody selected from the group consisting of the following antibodies (a) to (c):

(a) an antibody having a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 475 of SEQ ID NO: 2 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 233 of SEQ ID NO: 7,

(b) an antibody having a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 475 of SEQ ID NO: 12 and a light chain consisting of the amino

acid sequence at amino acid positions 21 to 233 of SEQ ID NO: 17, and

(c) an antibody having a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 475 of SEQ ID NO: 22 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 233 of SEQ ID NO: 27;

(9) the antibody or the functional fragment of the antibody according to any one of (1) to (8), which comprises CDRH1, CDRH2 and CDRH3 in any one combination selected from the group consisting of the following combinations (a) to (c), and CDRL1, CDRL2 and CDRL3 in any one combination selected from the following combinations (d) to (h):

(a) CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 4, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 5, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 6,

(b) CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 14, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 15, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 16,

(c) CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 24, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 25, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 26,

(d) CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 9, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 10, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 11,

(e) CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 9, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 92, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 11,

(f) CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 9, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 93, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 11,

(g) CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 19, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 20, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 21, and

(h) CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 29, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 30, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 31;

(10) the antibody or the functional fragment of the antibody according to any one of (1) to (9), which comprises CDRH1, CDRH2 and CDRH3, and CDRL1, CDRL2 and CDRL3 in any one combination selected from the following combinations (a) to (e):

(a) CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 4, CDRH2 consisting of the amino acid sequence

shown in SEQ ID NO: 5, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 6, and CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 9, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 10, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 11,

(b) CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 4, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 5, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 6, and CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 9, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 92, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 11,

(c) CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 4, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 5, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 6, and CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 9, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 93, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 11,

(d) CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 14, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 15, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 16, and CDRL1 consisting of the amino acid sequence shown in SEQ ID NO:

19, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 20, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 21, and

(e) CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 24, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 25, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 26, and CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 29, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 30, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 31;

(11) the antibody or the functional fragment of the antibody according to any one of (1) to (10), which has any one heavy chain variable region selected from the group consisting of the following variable regions (a) to (c), and any one light chain variable region selected from the following variable regions (d) to (h):

a heavy chain variable region selected from the group consisting of

(a) a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 3,

(b) a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 13, and

(c) a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 23,

(d) a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 8,

(e) a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 62,

(f) a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 64,

(g) a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 18, and

(h) a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 28;

(12) the antibody or the functional fragment of the antibody according to any one of (1) to (11), which comprises a heavy chain variable region and a light chain variable region in any one combination selected from the following combinations (a) to (e):

(a) a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 3, and a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 8,

(b) a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 3, and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 62,

(c) a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 3, and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 64,

(d) a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 13, and a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 18, and

(e) a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 23, and a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 28;

(13) the antibody or the functional fragment of the antibody according to any one of (1) to (12), wherein the constant region is a human-derived constant region;

(14) the antibody or the functional fragment of the antibody according to (13), which comprises a heavy chain consisting of the amino acid sequence shown in SEQ ID NO: 44 and a light chain consisting of the amino acid sequence shown in SEQ ID NO: 45;

(15) the antibody or the functional fragment of the antibody according to any one of (1) to (14), which is humanized;

(16) the antibody or the functional fragment of the antibody according to (15), which has a heavy chain variable region consisting of any one amino acid sequence selected from the group consisting of the following amino acid sequences (a) to (h), and a light chain variable region consisting of any one amino acid sequence selected from the group consisting of the following amino acid sequences (i) to (o):

- (a) the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 48,
- (b) the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 50,
- (c) the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 52,
- (d) the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 54,
- (e) the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 56,
- (f) the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 44,
- (g) an amino acid sequence having a homology of at least 95% or more to the sequence of a framework region other than at each CDR sequence in the sequences of (a) to (f),
- (h) an amino acid sequence comprising a deletion, substitution or addition of one or several amino acids in the sequence of a framework region other than at each CDR sequence in the sequences of (a) to (g),
- (i) the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 58,
- (j) the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 60,
- (k) the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 62,
- (l) the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 64,

(m) the amino acid sequence at amino acid positions 21 to 128 in SEQ ID NO: 45,

(n) an amino acid sequence having a homology of at least 95% or more to the sequence of a framework region other than at each CDR sequence in the sequences of (i) to (m), and

(o) an amino acid sequence comprising a deletion, substitution or addition of one or several amino acids in the sequence of a framework region other than at each CDR sequence in the sequences of (i) to (n);

(17) the antibody or the functional fragment of the antibody according to (16), which comprises a heavy chain variable region and a light chain variable region in any one combination selected from the group consisting of the following combinations (a) to (t):

(a) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 48 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 58,

(b) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 48 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 60,

(c) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID

NO: 48 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 62,

(d) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 48 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 64,

(e) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 50 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 58,

(f) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 50 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 60,

(g) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 50 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 62,

(h) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 50 and a light chain variable region consisting of

the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 64,

(i) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 52 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 58,

(j) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 52 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 60,

(k) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 52 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 62,

(l) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 52 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 64,

(m) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 54 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 58,

(n) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 54 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 60,

(o) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 54 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 62,

(p) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 54 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 64,

(q) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 56 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 58,

(r) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 56 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 60,

(s) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID

NO: 56 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 62, and

(t) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 56 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 64;

(18) the antibody or the functional fragment of the antibody according to (16) or (17), which comprises a heavy chain and a light chain in any one combination selected from the following combinations (a) to (x):

(a) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 48 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 58,

(b) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 48 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 60,

(c) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 48 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 62,

(d) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 48 and a

light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 64,

(e) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 50 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 58,

(f) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 50 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 60,

(g) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 50 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 62,

(h) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 50 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 64,

(i) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 52 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 58,

(j) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 52 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 60,

(k) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 52 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 62,

(l) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 52 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 64,

(m) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 54 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 58,

(n) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 54 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 60,

(o) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 54 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 62,

(p) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 54 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 64,

(q) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 56 and a

light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 58,

(r) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 56 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 60,

(s) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 56 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 62,

(t) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 56 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 64,

(u) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 44 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 58,

(v) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 44 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 60,

(w) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 44 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 62; and

(x) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 44 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 64;

(19) the functional fragment of the antibody according to any one of (1) to (18), wherein the functional fragment is selected from the group consisting of Fab, F(ab)₂, Fab' and Fv;

(20) a polynucleotide encoding the antibody or the functional fragment of the antibody according to any one of (1) to (19);

(21) the polynucleotide according to (20), which comprises polynucleotides in any one combination selected from the group consisting of the following combinations (a) to (e):

(a) a polynucleotide encoding CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 4, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 5 and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 6, and a polynucleotide encoding CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 9, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 10 and CDRL consisting of the amino acid sequence shown in SEQ ID NO: 11,

(b) a polynucleotide encoding CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 4, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO:

5 and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 6, and a polynucleotide encoding CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 9, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 92 and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 11,

(c) a polynucleotide encoding CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 4, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 5 and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 6, and a polynucleotide encoding CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 9, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 93 and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 11,

(d) a polynucleotide encoding CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 14, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 15 and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 16, and a polynucleotide encoding CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 19, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 20 and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 21, and

(e) a polynucleotide encoding CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 24, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO:

25 and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 26, and a polynucleotide encoding CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 29, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 30 and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 31;

(22) the polynucleotide according to (20) or (21), which comprises polynucleotides in any one combination selected from the group consisting of the following combinations

(a) to (e):

(a) a polynucleotide encoding a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 3, and a polynucleotide encoding a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 8,

(b) a polynucleotide encoding a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 3, and a polynucleotide encoding a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 62,

(c) a polynucleotide encoding a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 3, and a polynucleotide encoding a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 64,

(d) a polynucleotide encoding a heavy chain variable region consisting of the amino acid sequence shown in SEQ

ID NO: 13, and a polynucleotide encoding a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 18, and

(e) a polynucleotide encoding a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 23, and a polynucleotide encoding a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 28;

(23) an expression vector comprising the polynucleotide according to any one of (20) to (22);

(24) a host cell transformed with the expression vector according to (23);

(25) the host cell according to (23), wherein the host cell is a eukaryotic cell;

(26) a method for producing an antibody of interest or a functional fragment of the antibody, which comprises a step of culturing the host cell according to (24) or (25), and a step of collecting an antibody of interest or a functional fragment of the antibody from the culture obtained by the aforementioned step;

(27) an antibody or a functional fragment of the antibody obtained by the production method according to (26);

(28) the functional fragment of the antibody according to (27), wherein the functional fragment is selected from the group consisting of Fab, F(ab)₂, Fab' and Fv;

(29) the antibody or the functional fragment of the antibody according to any one of (1) to (19), (27) and

(28), which comprises one or two or more modifications selected from the group consisting of N-linked glycosylation, O-linked glycosylation, N-terminal processing, C-terminal processing, deamidation, isomerization of aspartic acid, oxidation of methionine, addition of a methionine residue to the N-terminus, amidation of a proline residue, and a heavy chain comprising a deletion of one or two amino acids at the carboxyl terminus;

(30) the antibody according to (29), wherein one or two amino acids are deleted at the carboxyl terminus of a heavy chain thereof;

(31) the antibody according to (30), wherein one amino acid is deleted at each of the carboxyl termini of both of the heavy chains thereof;

(32) the antibody according to any one of (29) to (31), wherein a proline residue at the carboxyl terminus of a heavy chain thereof is further amidated;

(33) the antibody or the functional fragment of the antibody according to any one of (1) to (19) and (26) to (31), wherein sugar chain modification is regulated in order to enhance antibody-dependent cellular cytotoxic activity;

(34) an antibody-drug conjugate comprising the antibody or the functional fragment of the antibody according to any one of (1) to (19) and (27) to (33) conjugated to a drug;

(35) the antibody-drug conjugate according to (34),

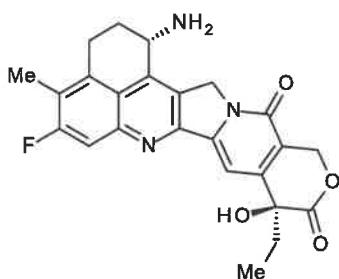
wherein the drug is an antitumor compound;

(36) the antibody-drug conjugate according to (35),

wherein the antitumor compound is an antitumor compound represented by the following formula:

[0016]

[Formula 1]



[0017]

(37) the antibody-drug conjugate according to (35) or (36), wherein the antibody is conjugated to the antitumor compound via a linker having a structure represented by any of the following formulas (a) to (f):

(a) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-$,

(b) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-$,

(c) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2-\text{O}-\text{CH}_2-\text{C}(=\text{O})-$,

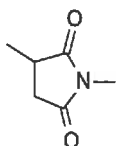
(d) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2\text{CH}_2-\text{O}-\text{CH}_2-\text{C}(=\text{O})-$,

(e) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{NH}-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-$, and

(f) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{NH}-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-$,
 wherein the antibody is connected to the terminus of $-(\text{Succinimid-3-yl-N})$, the antitumor compound is connected to the carbonyl group of the $-(\text{CH}_2)_n-\text{C}(=\text{O})-$ moiety with the nitrogen atom of the amino group at position 1 as a connecting position, GGFG represents an amino acid sequence consisting of glycine-glycine-phenylalanine-glycine linked through peptide bonds, and $-(\text{Succinimid-3-yl-N})-$ has a structure represented by the following formula:

[0018]

[Formula 2]



[0019]

which is connected to the antibody at position 3 thereof and is connected to a methylene group in the linker structure containing this structure on the nitrogen atom at position 1;

(38) the antibody-drug conjugate according to any one of (34) to (37), wherein the linker is represented by any formula selected from the following formulas (a) to (c):

(a) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2-\text{O}-\text{CH}_2-\text{C}(=\text{O})-$,

(b) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2\text{CH}_2-\text{O}-\text{CH}_2-\text{C}(=\text{O})-$, and

(c) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{NH}-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-$.

[0020]

(39) the antibody-drug conjugate according to any one of (34) to (38), wherein the linker is represented by the following formula (a) or (b):

(a) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2-\text{O}-\text{CH}_2-\text{C}(=\text{O})-$, and

(b) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{NH}-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-$.

[0021]

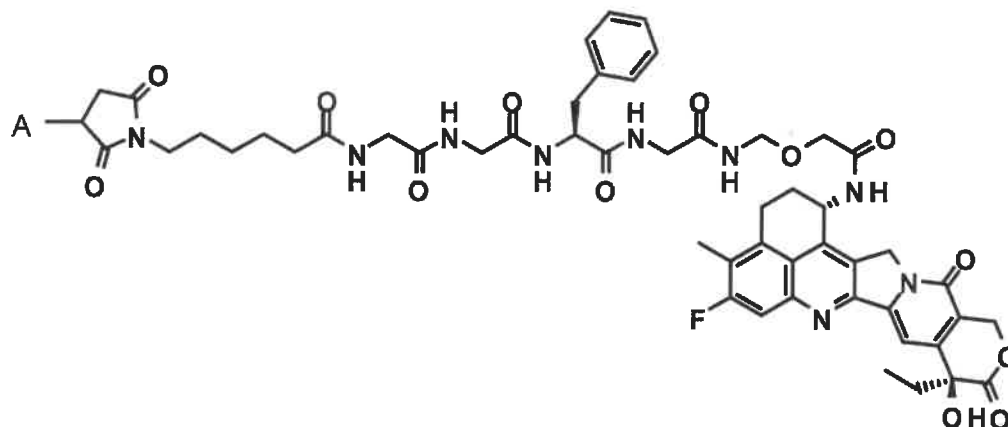
(40) the antibody-drug conjugate according to any one of (34) to (39), wherein the linker is represented by the following formula (a):

(a) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2-\text{O}-\text{CH}_2-\text{C}(=\text{O})-$;

(41) the antibody-drug conjugate according to any one of (34) to (40), wherein the antibody is conjugated to a drug linker structure represented by the following formula [Formula 3] (wherein A represents a connecting position to the antibody) by a thioether bond:

[0022]

[Formula 3]



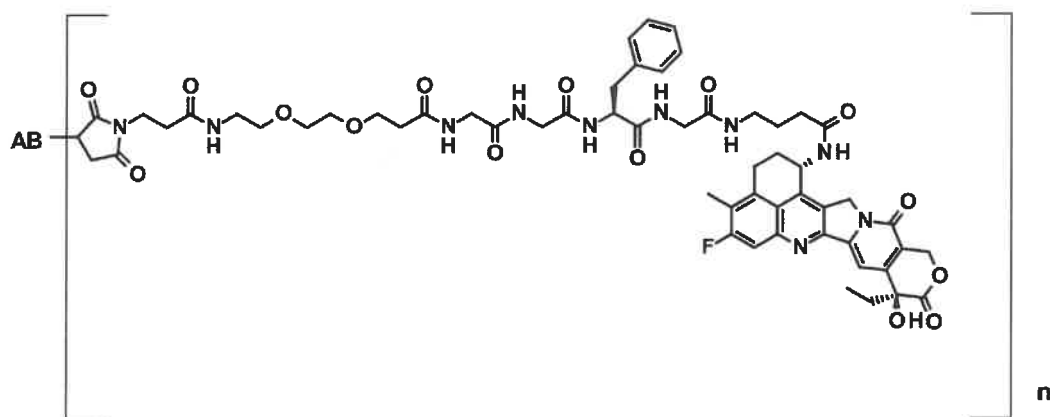
[0023]

(42) the antibody-drug conjugate according to any one of (34) to (40), which has a structure represented by the following formula [Formula 4],

wherein AB represents the antibody or the functional fragment of the antibody, n represents an average number of units of the drug-linker structure conjugated to the antibody per antibody, and the antibody is connected to the linker via a sulfhydryl group derived from the antibody:

[0024]

[Formula 4]



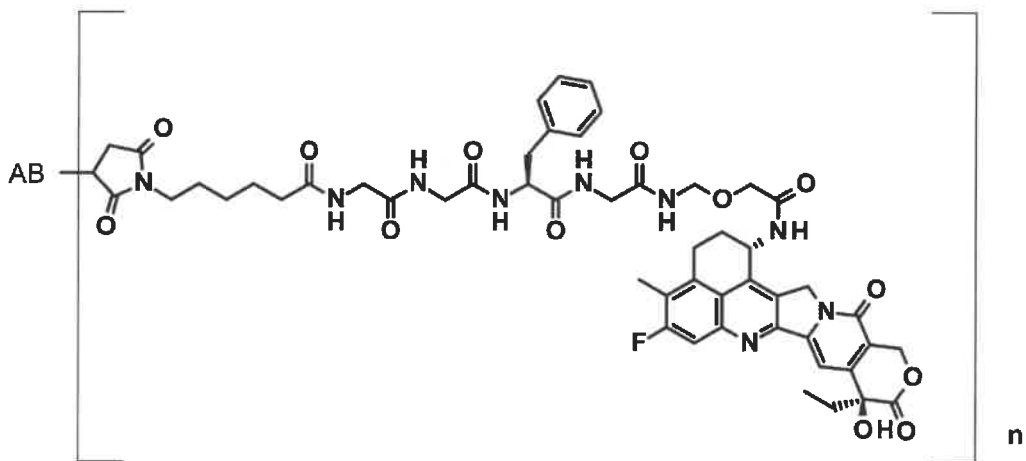
[0025]

(43) the antibody-drug conjugate according to any one of (34) to (39), which is represented by the following formula [Formula 5],

wherein AB represents the antibody or the functional fragment of the antibody, n represents an average number of units of the drug-linker structure conjugated to the antibody per antibody, and the antibody is connected to the linker via a sulfhydryl group derived from the antibody:

[0026]

[Formula 5]



(44) the antibody-drug conjugate according to any one of (41) to (43), wherein the antibody is an antibody comprising a heavy chain and a light chain in any one combination selected from the group consisting of the following combinations (a) to (x), or a functional fragment of the antibody:

- (a) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 48, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 58,
- (b) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 48, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 60,
- (c) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 48, and a

light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 62,

(d) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 48, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 64,

(e) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 50, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 58,

(f) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 50, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 60,

(g) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 50, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 62,

(h) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 50, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 64,

(i) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 52, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 58,

- (j) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 52, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 60,
- (k) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 52, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 62,
- (l) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 52, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 64,
- (m) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 54, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 58,
- (n) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 54, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 60,
- (o) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 54, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 62,
- (p) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 54, and a

light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 64,

(q) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 56, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 58,

(r) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 56, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 60,

(s) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 56, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 62,

(t) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 56, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 64,

(u) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 44, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 58,

(v) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 44, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 60,

(w) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 44, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 62, and

(x) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 44, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 64;

(45) the antibody-drug conjugate according to (44), wherein the heavy chain of the antibody comprises one or two or more modifications selected from the group consisting of N-linked glycosylation, O-linked glycosylation, N-terminal processing, C-terminal processing, deamidation, isomerization of aspartic acid, oxidation of methionine, addition of a methionine residue to the N-terminus, amidation of a proline residue, and a heavy chain comprising a deletion of one or two amino acids at the carboxyl terminus;

(46) the antibody-drug conjugate according to any one of (34) to (45), wherein the average number of the selected drug-linker structure conjugated per antibody is in a range of from 1 to 10;

(47) the antibody-drug conjugate according to any one of (34) to (46), wherein the average number of the selected drug-linker structure conjugated per antibody is in a range of from 2 to 8;

(48) the antibody-drug conjugate according to any one of (34) to (47), wherein the average number of the selected drug-linker structure conjugated per antibody is in a range of from 3 to 8;

(49) the antibody-drug conjugate according to any one of (34) to (48), wherein the average number of the selected drug-linker structure conjugated per antibody is in a range of from 7 to 8;

(50) the antibody-drug conjugate according to any one of (32) to (49), wherein the average number of the selected drug-linker structure conjugated per antibody is 8;

(51) a pharmaceutical composition comprising any component selected from the antibodies or the functional fragments of the antibodies according to (1) to (19) and (27) to (33) and the antibody-drug conjugates according to (34) to (50), a salt of the component, or a hydrate of the component or the salt;

(52) the pharmaceutical composition according to (51), which is an antitumor drug;

(53) the antitumor drug according to (52), wherein the tumor is a tumor expressing GPR20;

(54) the antitumor drug according to (52) or (53), wherein the tumor is gastrointestinal stromal tumor;

(55) the pharmaceutical composition or the antitumor drug according to any one of (51) to (54), further comprising an additional antitumor drug;

(56) a method for treating a tumor, which comprises administering any component selected from the antibodies or the functional fragments of the antibodies according to (1) to (19) and (27) to (33), the antibody-drug conjugates according to (34) to (50), salts of these components, and hydrates of these components or the salts to an individual;

(57) the treatment method according to (56), wherein the tumor is gastrointestinal stromal tumor;

(58) the treatment method according to (56) or (57), wherein the tumor is a tumor that exhibits resistance to a tyrosine kinase inhibitor;

(59) a method for treating a tumor, which comprises administering a pharmaceutical composition comprising at least one component selected from the antibodies or the functional fragments of the antibodies according to (1) to (19) and (27) to (33), the antibody-drug conjugates according to (34) to (50), salts of these components, and hydrates of these components or the salts, and at least one antitumor drug to an individual, simultaneously, separately, or continuously;

(60) the method for treating a tumor according to (59), wherein the antitumor drug is a tyrosine kinase inhibitor;

(61) the method for treating a tumor according to (60), wherein the tyrosine kinase inhibitor is at least one selected from sunitinib, imatinib, and regorafenib;

(62) the method for treating a tumor according to any one of (56) to (61), wherein the tumor is gastrointestinal stromal tumor that exhibits resistance to a tyrosine kinase inhibitor; and

(63) a method for producing an antibody-drug conjugate, which comprises a step of culturing the host cell according to (24) or (25), a step of collecting an antibody of interest or a functional fragment of the antibody from the culture obtained by the aforementioned step, and a step of reacting the antibody or the functional fragment of the antibody obtained by the aforementioned step with a drug-linker intermediate compound.

Advantageous Effects of Invention

[0027] Features of the anti-GPR20 antibody of the present invention are to recognize a conformation consisting of two extracellular regions having the amino acid sequence at positions 1 to 48 and the amino acid sequence at positions 108 to 125, respectively, from the N-terminus of GPR20, and to have internalization activity. An anti-GPR20 antibody-drug conjugate comprising the anti-GPR20 antibody of the present invention conjugated to a drug, which exerts toxicity in cells, via a linker having a specific structure can be expected to achieve an excellent antitumor effect and be safe when administered to patients having cancer cells expressing GPR20.

Specifically, the anti-GPR20 antibody-drug conjugate of the present invention is useful as an antitumor agent.

Brief Description of Drawings

[0028]

[Figure 1] Figure 1 shows the amino acid sequence (SEQ ID NO: 2) of the heavy chain of rat anti-GPR20 antibody 04-046 and the nucleotide sequence (SEQ ID NO: 32) of cDNA encoding the heavy chain.

[Figure 2] Figure 2 shows the amino acid sequence (SEQ ID NO: 7) of the light chain of the rat anti-GPR20 antibody 04-046 and the nucleotide sequence (SEQ ID NO: 34) of cDNA encoding the light chain.

[Figure 3] Figure 3 shows the amino acid sequence (SEQ ID NO: 12) of the heavy chain of rat anti-GPR20 antibody 04-079 and the nucleotide sequence (SEQ ID NO: 36) of cDNA encoding the heavy chain.

[Figure 4] Figure 4 shows the amino acid sequence (SEQ ID NO: 17) of the light chain of the rat anti-GPR20 antibody 04-079 and the nucleotide sequence (SEQ ID NO: 38) of cDNA encoding the light chain.

[Figure 5] Figure 5 shows the amino acid sequence (SEQ ID NO: 22) of the heavy chain of rat anti-GPR20 antibody 04-126 and the nucleotide sequence (SEQ ID NO: 40) of cDNA encoding the heavy chain.

[Figure 6] Figure 6 shows the amino acid sequence (SEQ ID NO: 27) of the light chain of the rat anti-GPR20 antibody

04-126 and the nucleotide sequence (SEQ ID NO: 42) of cDNA encoding the light chain.

[Figure 7] Figure 7 shows the amino acid sequence (SEQ ID NO: 44) of the heavy chain of human chimeric anti-GPR20 antibody 04-046Ch and the nucleotide sequence (SEQ ID NO: 46) of cDNA encoding the heavy chain.

[Figure 8] Figure 8 shows the amino acid sequence (SEQ ID NO: 45) of the light chain of the human chimeric anti-GPR20 antibody 04-046Ch and the nucleotide sequence (SEQ ID NO: 47) of cDNA encoding the light chain.

[Figure 9] Figure 9 shows the amino acid sequence (SEQ ID NO: 48) of a humanized h046-H4b type heavy chain.

[Figure 10] Figure 10 shows the amino acid sequence (SEQ ID NO: 50) of a humanized h046-H4e type heavy chain.

[Figure 11] Figure 11 shows the amino acid sequence (SEQ ID NO: 52) of a humanized h046-H5b type heavy chain.

[Figure 12] Figure 12 shows the amino acid sequence (SEQ ID NO: 54) of a humanized h046-H8 type heavy chain.

[Figure 13] Figure 13 shows the amino acid sequence (SEQ ID NO: 56) of a humanized h046-H10 type heavy chain.

[Figure 14] Figure 14 shows the amino acid sequence (SEQ ID NO: 58) of a humanized h046-L1 type light chain.

[Figure 15] Figure 15 shows the amino acid sequence (SEQ ID NO: 60) of a humanized h046-L2 type light chain.

[Figure 16] Figure 16 shows the amino acid sequence (SEQ ID NO: 62) of a humanized h046-L6 type light chain.

[Figure 17] Figure 17 shows the amino acid sequence (SEQ ID NO: 64) of a humanized h046-L7 type light chain.

[Figure 18] Figure 18 shows flow cytometry analysis results when a culture supernatant of a hybridoma producing an anti-human GPR20 antibody was reacted with a cell line transiently expressing human GPR20.

[Figure 19-1] Figure 19-1 shows the concentration-dependent binding of a rat anti-human GPR20 antibody to a human GPR20-expressing cell line in flow cytometry analysis.

[Figure 19-2] Figure 19-2 shows the concentration-dependent binding of a rat anti-human GPR20 antibody to a human GPR20-expressing cell line in flow cytometry analysis.

[Figure 19-3] Figure 19-3 shows the concentration-dependent binding of a rat anti-human GPR20 antibody to a human GPR20-expressing cell line in flow cytometry analysis.

[Figure 20] Figure 20 shows the internalization ability of a rat anti-GPR20 antibody. The ordinate depicts a survival rate (relative ratio to a cell survival rate without antibody addition defined as 100%) when the rat anti-GPR20 antibody and a saporin-labeled anti-rat IgG antibody were added to a human GPR20-expressing cell line.

[Figure 21] Figure 21 shows a diagram of the comparison between the amino acid sequences of human GPR20 and mouse GPR20. The amino acid sequences indicated by EC1, EC2,

EC3, and EC4 represent extracellular regions, and the numbers within the parentheses represent regional sites based on human GPR20 amino acid positions. The shaded characters represent the same amino acids between humans and mice.

[Figure 22-a] Figure 22-a shows the binding properties of various anti-GPR20 antibodies. This drawing shows the binding activity of each anti-GPR20 antibody against human GPR20, mouse GPR20, and human/mouse chimeric GPR20 proteins in which one of the extracellular regions EC1, EC2, EC3, and EC4 of human GPR20 was substituted with the corresponding sequence of mouse GPR20.

[Figure 22-b] Figure 22-b shows the binding properties of various anti-GPR20 antibodies. This drawing shows the binding activity of each anti-GPR20 antibody against human GPR20, mouse GPR20, and human/mouse chimeric GPR20 proteins in which one of the extracellular regions EC1, EC2, EC3, and EC4 of human GPR20 was substituted with the corresponding sequence of mouse GPR20.

[Figure 23-a] Figure 23-a shows the human GPR20-specific binding of the human chimeric anti-GPR20 antibody 04-046Ch.

[Figure 23-b] Figure 23-b shows the human GPR20-specific binding of the human chimeric anti-GPR20 antibody 04-046Ch.

[Figure 24] Figure 24 shows the GPR20-binding activity of a humanized anti-GPR20 antibody.

[Figure 25] Figure 25 shows the GPR20-binding activity of a humanized anti-GPR20 antibody and an antibody-drug conjugate.

[Figure 26] Figure 26 shows the *in vitro* cell proliferation-suppressive activity of an antibody-drug conjugate (1) against GPR20-expressing cells.

[Figure 27] Figure 27 shows the *in vitro* cell proliferation-suppressive activity of antibody-drug conjugates (7), (8), and (9) against GPR20-expressing cells.

[Figure 28] Figure 28 shows the antitumor effects of antibody-drug conjugates (1) and (2) in subcutaneously GIST-T1/GPR20 cell-transplanted nude mouse models.

[Figure 29] Figure 29 shows the antitumor effects of antibody-drug conjugates (1) and (2) in subcutaneously GIST430 cell-transplanted nude mouse models.

[Figure 30] Figure 30 shows the antitumor effects of antibody-drug conjugates (6) and (9) in subcutaneously GIST-T1/GPR20 cell-transplanted nude mouse models.

[Figure 31] Figure 31 shows the antitumor effects of antibody-drug conjugates (3), (5), (6), (10), (11), and (12) in subcutaneously GIST-T1/GPR20 cell-transplanted nude mouse models.

[Figure 32] Figure 32 shows the antitumor effects of antibody-drug conjugate (13) in subcutaneously GIST-T1/GPR20 cell-transplanted nude mouse models.

[Figure 33] Figure 33 shows the antitumor effects of antibody-drug conjugates (4) and (13) in subcutaneously GIST020-transplanted nude mouse models.

[Figure 34] Figure 34 shows the antitumor effect of antibody-drug conjugate (13) in subcutaneously GIST1-transplanted nude mouse models.

[Figure 35] Figure 35 shows the antitumor effects of antibody-drug conjugate (14) and an antibody-drug conjugate targeting HER2 in subcutaneously stomach-cancer-cell-line-NCI-N87-cell-transplanted nude mouse models.

[Figure 36] Figure 36 shows the antitumor effect of antibody-drug conjugate (14) in models prepared by subcutaneously transplanting, into nude mice, a tumor GIST074 derived from a patient with gastrointestinal stromal tumor in the stomach that had become unresponsive to regorafenib treatment.

[Figure 37] Figure 37 shows the effect of combined use of antibody-drug conjugate (3) and sunitinib in models prepared by subcutaneously transplanting, into nude mice, a human gastrointestinal stromal tumor cell line GIST430/654 having an imatinib-resistant mutation.

Description of Embodiments

[0029] Hereinafter, the preferred embodiments for carrying out the present invention will be described with reference to the drawings. It is to be noted that the

embodiments described below merely illustrate the representative embodiments of the present invention, and the scope of the present invention shall not be narrowly interpreted due to these examples.

[0030] In the present description, the term "cancer" is used to have the same meaning as that of the term "tumor".

[0031] In the present description, the term "gene" is used to include not only DNA but also its mRNA and cDNA, and cRNA thereof.

In the present description, the term "polynucleotide" is used to have the same meaning as that of a nucleic acid, and also includes DNA, RNA, a probe, an oligonucleotide, and a primer.

[0032] In the present description, the terms "polypeptide" and "protein" are used without being distinguished from each other.

[0033] In the present description, the term "cell" includes cells in an individual animal, and cultured cells.

[0034] In the present description, the term "GPR20" is used to have the same meaning as that of GPR20 protein.

[0035] In the present description, the term "cytotoxic activity" is used to mean that a pathologic change is caused to cells in any given way. The term not only means a direct trauma, but also means all types of structural or functional damage caused to cells, such as DNA cleavage, formation of a base dimer, chromosomal

cleavage, damage to cell mitotic apparatus, and a reduction in the activities of various types of enzymes.

[0036] In the present description, the phrase "exerting toxicity in cells" is used to mean that toxicity is exhibited in cells in any given way. The term not only means a direct trauma, but also means all types of structural, functional, or metabolic influences caused to cells, such as DNA cleavage, formation of a base dimer, chromosomal cleavage, damage to cell mitotic apparatus, a reduction in the activities of various types of enzymes, and suppression of effects of cell growth factors.

[0037] In the present description, the term "epitope" is used to mean the partial peptide or partial three-dimensional structure of GPR20, to which a specific anti-GPR20 antibody binds. Such an epitope, which is the above-described partial peptide of GPR20, can be determined by a method well known to a person skilled in the art, such as an immunoassay. First, various partial structures of an antigen are produced. As regards production of such partial structures, a known oligopeptide synthesis technique can be applied. For example, a series of polypeptides, in which GPR20 has been successively truncated at an appropriate length from the C-terminus or N-terminus thereof, are produced by a genetic recombination technique well known to a person skilled in the art. Thereafter, the reactivity of an antibody to such polypeptides is studied, and recognition

sites are roughly determined. Thereafter, further shorter peptides are synthesized, and the reactivity thereof to these peptides is then studied, so as to determine an epitope. When an antibody binding to a membrane protein having a plurality of extracellular domains is directed to a three-dimensional structure composed of a plurality of domains as an epitope, a domain to which the antibody binds can be determined by modifying the amino acid sequence of a specific extracellular domain, and thereby modifying the three-dimensional structure. The epitope, which is a partial three-dimensional structure of an antigen that binds to a specific antibody, can also be determined by specifying the amino acid residues of the antigen adjacent to the above-described antibody by X-ray structural analysis.

[0038] In the present description, the phrase "antibodies binding to the same epitope" is used to mean antibodies that bind to a common epitope. If a second antibody binds to a partial peptide or a partial three-dimensional structure to which a first antibody binds, it can be determined that the first antibody and the second antibody bind to the same epitope. In addition, by confirming that a second antibody competes with a first antibody for the binding of the first antibody to an antigen (i.e., a second antibody interferes with the binding of a first antibody to an antigen), it can be determined that the first antibody and the second

antibody bind to the same epitope, even if the specific sequence or structure of the epitope has not been determined. Furthermore, when a first antibody and a second antibody bind to the same epitope and further, the first antibody has special effects such as antitumor activity or internalization activity, the second antibody can be expected to have the same activity as that of the first antibody. Accordingly, by confirming that the second anti-GPR20 antibody competes with the first anti-GPR20 antibody for the binding of the first anti-GPR20 antibody to a partial peptide of GPR20, it can be determined that the first antibody and the second antibody are antibodies binding to the same epitope of GPR20.

[0039] In the present description, the term "CDR" is used to mean a complementarity determining region. It is known that the heavy chain and light chain of an antibody molecule each have three CDRs. Such a CDR is also referred to as a hypervariable region, and is located in the variable regions of the heavy chain and light chain of an antibody. These regions have a particularly highly variable primary structure and are separated into three sites on the primary structure of the polypeptide chain in each of the heavy chain and light chain. In the present description, with regard to the CDR of an antibody, the CDRs of a heavy chain are referred to as CDRH1, CDRH2 and CDRH3, respectively, from the amino-

terminal side of the amino acid sequence of the heavy chain, whereas the CDRs of a light chain are referred to as CDRL1, CDRL2 and CDRL3, respectively, from the amino-terminal side of the amino acid sequence of the light chain. These sites are located close to one another on the three-dimensional structure, and determine the specificity of the antibody to an antigen to which the antibody binds.

[0040] In the present invention, the phrase "hybridizing under stringent conditions" is used to mean that hybridization is carried out in the commercially available hybridization solution ExpressHyb Hybridization Solution (manufactured by Clontech Laboratories, Inc.) at 68°C, or that hybridization is carried out under conditions in which hybridization is carried out using a DNA-immobilized filter in the presence of 0.7 to 1.0 M NaCl at 68°C, and the resultant is then washed at 68°C with a 0.1- to 2-fold concentration of SSC solution (wherein 1-fold concentration of SSC consists of 150 mM NaCl and 15 mM sodium citrate), or conditions equivalent thereto, for identification.

[0041] 1. GPR20

GPR20 is a seven-pass transmembrane protein composed of 358 amino acids, which belongs to class A of the G protein-coupled receptor (GPCR) family, and this protein has N-terminal extracellular and C-terminal intracellular domains.

[0042] The GPR20 protein used in the present invention can be directly purified from the GPR20-expressing cells of a human or a non-human mammal (e.g., a rat or a mouse) and can then be used, or a cell membrane fraction of the aforementioned cells can be prepared and can be used as the GPR20 protein. Alternatively, GPR20 can also be obtained by synthesizing it *in vitro*, or by allowing host cells to produce GPR20 by genetic manipulation.

According to such genetic manipulation, the GPR20 protein can be obtained, specifically, by incorporating GPR20 cDNA into a vector capable of expressing the GPR20 cDNA, and then synthesizing GPR20 in a solution containing enzymes, substrate and energetic materials necessary for transcription and translation, or by transforming the host cells of other prokaryotes or eukaryotes, so as to allow them to express GPR20. Also, the GPR20-expressing cells based on the above-described genetic manipulation, or a cell line expressing GPR20 may be used as the GPR20 protein. Alternatively, the expression vector into which GPR20 cDNA has been incorporated can be directly administered to an animal to be immunized, and GPR20 can be expressed in the body of the animal thus immunized.

[0043] The DNA sequence and amino acid sequence of human GPR20 are disclosed in a public database, and can be referred to under, for example, accession Nos. NM_005293 and NP_005284 (NCBI).

[0044] Moreover, a protein which consists of an amino acid sequence comprising a substitution, deletion and/or addition of one or several amino acids in the above-described amino acid sequence of GPR20, and has a biological activity equivalent to that of the GPR20 protein, is also included in GPR20.

[0045] The amino acid sequence of the human GPR20 protein is shown in SEQ ID NO: 1 in the sequence listing. The extracellular regions are constituted by an extracellular domain consisting of amino acid positions 1 to 48 (EC1), an extracellular domain consisting of amino acid positions 108 to 125 (EC2), an extracellular domain (EC3) consisting of amino acid positions 190 to 196, and an extracellular domain (EC4) consisting of amino acid positions 260 to 275, in SEQ ID NO: 1 in the sequence listing.

[0046] 2. Production of anti-GPR20 antibody

One example of the anti-GPR20 antibody of the present invention can include an anti-GPR20 antibody which recognizes a conformation consisting of two extracellular regions having the amino acid sequence at positions 1 to 48 and the amino acid sequence at positions 108 to 125, respectively, from the N-terminus of GPR20 shown in SEQ ID NO: 1 in the sequence listing, and has internalization activity.

[0047] Another example of the anti-GPR20 antibody of the present invention can include an anti-GPR20 antibody

which recognizes a conformation consisting of two extracellular regions having the amino acid sequence at positions 1 to 48 and the amino acid sequence at positions 108 to 125, respectively, from the N-terminus of GPR20 shown in SEQ ID NO: 1 in the sequence listing, binds to at least tyrosine at position 113, and has internalization activity.

[0048] A further example of the anti-GPR20 antibody of the present invention can include an anti-GPR20 antibody which specifically binds to a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 1 in the sequence listing, does not bind to a polypeptide with the amino acid tyrosine at amino acid position 113 of SEQ ID NO: 1 in the sequence listing substituted with a different amino acid (e.g., phenylalanine), and has internalization activity.

[0049] The anti-GPR20 antibody of the present invention may be derived from any species. Preferred examples of the species can include humans, rats, mice and rabbits. When the anti-GPR20 antibody of the present invention is derived from a species other than human, it is preferred to chimerize or humanize the anti-GPR20 antibody by a well-known technique. The antibody of the present invention may be a polyclonal antibody or may be a monoclonal antibody, and a monoclonal antibody is preferred.

[0050] The anti-GPR20 antibody of the present invention is an antibody that can target tumor cells. Specifically, the anti-GPR20 antibody of the present invention possesses a property of being able to recognize tumor cells, a property of being able to bind to tumor cells, a property of being internalized into tumor cells by cellular uptake, and the like. Accordingly, the anti-GPR20 antibody of the present invention can be conjugated to a compound having antitumor activity via a linker to prepare an antibody-drug conjugate.

[0051] The binding activity of an antibody against tumor cells can be confirmed by flow cytometry. The uptake of an antibody into tumor cells can be confirmed by (1) an assay of visualizing a cellularly taken-up antibody under a fluorescent microscope using a secondary antibody (fluorescently labeled) binding to a therapeutic antibody (Cell Death and Differentiation (2008) 15, 751-761), (2) an assay of measuring the amount of cellularly taken-up fluorescence using a secondary antibody (fluorescently labeled) binding to a therapeutic antibody (Molecular Biology of the Cell Vol. 15, 5268-5282, December 2004) or (3) Mab-ZAP assay using an immunotoxin binding to a therapeutic antibody, wherein the toxin is released upon cellular uptake, so as to suppress cell proliferation (Bio Techniques 28: 162-165, January 2000). A recombinant complex protein of a catalytic region of

diphtheria toxin and protein G may be used as the immunotoxin.

[0052] In the present description, the term "high internalization ability" is used to mean that the survival rate (which is the ratio relative to a cell survival rate without antibody addition which is defined as 100%) of GPR20-expressing cells to which the aforementioned antibody and a saporin-labeled anti-rat IgG antibody have been administered is preferably 70% or less, and more preferably 60% or less.

[0053] The antitumor antibody-drug conjugate of the present invention comprises a conjugated compound exerting an antitumor effect. Therefore, it is preferred, but not essential, that the antibody itself should have an antitumor effect. For the purpose of specifically and selectively exerting the cytotoxicity of the antitumor compound in tumor cells, it is important and preferred that the antibody should have a property of being internalized and transferred into tumor cells.

[0054] The anti-GPR20 antibody can be obtained by immunizing an animal with a polypeptide serving as an antigen by a method usually performed in this field, and then collecting and purifying an antibody produced in the living body thereof. Since GPR20 is a seven-pass transmembrane protein, it is preferred to use GPCR retaining a three-dimensional structure as an antigen.

Examples of such a method can include a DNA immunization method.

[0055] The origin of the antigen is not limited to a human, and thus, an animal can also be immunized with an antigen derived from a non-human animal such as a mouse or a rat. In this case, an antibody applicable to the disease of a human can be selected by examining the cross-reactivity of the obtained antibody binding to the heterologous antigen with the human antigen.

[0056] Furthermore, antibody-producing cells that produce an antibody against the antigen can be fused with myeloma cells according to a known method (e.g., Kohler and Milstein, *Nature* (1975) 256, p. 495-497; and Kennet, R. ed., *Monoclonal Antibodies*, p. 365-367, Plenum Press, N. Y. (1980)) to establish hybridomas, so as to obtain a monoclonal antibody.

[0057] Hereinafter, the method for obtaining an antibody against GPR20 will be specifically described.

(1) Preparation of antigen

The antigen can be obtained by allowing host cells to produce a gene encoding the antigen protein according to genetic manipulation. Specifically, a vector capable of expressing the antigen gene is produced, and the vector is then introduced into host cells, so that the gene is expressed therein, and thereafter, the expressed antigen may be purified. The antibody can also be obtained by a method of immunizing an animal with the

antigen-expressing cells based on the above-described genetic manipulation, or a cell line expressing the antigen.

[0058] Alternatively, the antibody can also be obtained, without the use of the antigen protein, by incorporating cDNA of the antigen protein into an expression vector, then administering the expression vector to an animal to be immunized, and expressing the antigen protein in the body of the animal thus immunized, so that an antibody against the antigen protein is produced therein.

[0059] (2) Production of anti-GPR20 monoclonal antibody

The anti-GPR20 antibody used in the present invention is not particularly limited. For example, an antibody specified by an amino acid sequence shown in the sequence listing of the present application can suitably be used. The anti-GPR20 antibody used in the present invention is desirably an antibody having the following properties:

- (1) an antibody having the following properties:
 - (a) specifically binding to GPR20, and
 - (b) having the activity of being internalized into GPR20-expressing cells by binding to GPR20;
- (2) the antibody according to the above (1) or the aforementioned antibody, wherein the GPR20 is human GPR20; and
- (3) recognizing a conformation consisting of two extracellular regions having the amino acid sequence at

positions 1 to 48 and the amino acid sequence at positions 108 to 125, respectively, from the N-terminus of human GPR20, and having internalization activity.

[0060] The method for obtaining the antibody against GPR20 of the present invention is not particularly limited as long as the anti-GPR20 antibody can be obtained. Since GPR20 is a transmembrane protein, it is preferred to use GPR20 retaining the conformation as an antigen.

[0061] One preferred example of the method for obtaining the antibody can include a DNA immunization method. The DNA immunization method is an approach which involves transfecting an animal (e.g., mouse or rat) individual with an antigen expression plasmid, and then expressing the antigen in the individual to induce immunity against the antigen. The transfection approach includes a method of directly injecting the plasmid into the muscle, a method of injecting a transfection reagent such as a liposome or polyethylenimine into a vein, an approach using a viral vector, an approach of injecting gold particles attached to the plasmid using a gene gun, a hydrodynamic method of rapidly injecting a plasmid solution in a large amount into a vein, and the like. With regard to the transfection method of injecting the expression plasmid into the muscle, a technique called *in vivo* electroporation, which involves applying electroporation to the intramuscular injection site of

the plasmid, is known as an approach for improving expression levels (Aihara H, Miyazaki J. Nat Biotechnol. 1998 Sep; 16 (9): 867-70 or Mir LM, Bureau MF, Gehl J, Rangara R, Rouy D, Caillaud JM, Delaere P, Branellec D, Schwartz B, Scherman D. Proc Natl Acad Sci U S A. 1999 Apr 13; 96 (8): 4262-7). This approach further improves expression levels by treating the muscle with hyaluronidase before the intramuscular injection of the plasmid (McMahon JM, Signori E, Wells KE, Fazio VM, Wells DJ., Gene Ther. 2001 Aug; 8 (16): 1264-70). Furthermore, hybridoma production can be performed by a known method, and can also be performed using, for example, the Hybrimmune Hybridoma Production System (Cyto Pulse Sciences, Inc.).

[0062] Specific examples of obtaining a monoclonal antibody can also include the following procedures:

(a) an immune response can be induced by incorporating GPR20 cDNA into an expression vector (e.g., pcDNA3.1; Thermo Fisher Scientific Inc.), and directly administering the vector to an animal (e.g., a rat or a mouse) to be immunized by a method such as electroporation or using a gene gun, so as to express GPR20 in the body of the animal. The administration of the vector by electroporation or the like may be performed one or more times, preferably a plurality of times, if necessary for enhancing antibody titer;

- (b) collection of a tissue (e.g., a lymph node) containing antibody-producing cells from the aforementioned animal in which the immune response has been induced;
- (c) preparation of myeloma cells (hereinafter, referred to as "myelomas") (e.g., mouse myeloma SP2/0-ag14 cells);
- (d) cell fusion between the antibody-producing cells and the myelomas;
- (e) selection of a hybridoma group producing an antibody of interest;
- (f) division into single cell clones (cloning);
- (g) optionally, the culture of hybridomas for the mass production of monoclonal antibodies, or the breeding of animals into which the hybridomas are transplanted; and
- (h) study on the physiological activity (internalization activity) and binding specificity of the monoclonal antibody thus produced, or examination of the properties of the antibody as a labeling reagent.

Examples of the method for measuring the antibody titer used herein can include, but are not limited to, flow cytometry and Cell-ELISA.

[0063] Examples of the hybridoma strain thus established can include anti-GPR20 antibody-producing hybridomas 04-046, 04-079 and 04-126. It is to be noted that, in the present description, an antibody produced by the anti-GPR20 antibody-producing hybridoma 04-046 is referred to as a "04-046 antibody" or simply "04-046", an antibody

produced by the hybridoma 04-079 is referred to as a "04-079 antibody" or simply "04-079", and an antibody produced by the hybridoma 04-126 is referred to as a "04-126 antibody" or simply "04-0126".

[0064] The heavy chain of the 04-046 antibody has the amino acid sequence shown in SEQ ID NO: 2 in the sequence listing. In the heavy chain amino acid sequence shown in SEQ ID NO: 2 in the sequence listing, the amino acid sequence consisting of the amino acid residues at positions 1 to 19 is a signal sequence, the amino acid sequence consisting of the amino acid residues at positions 20 to 142 is a variable region, and the amino acid sequence consisting of the amino acid residues at positions 143 to 475 is a constant region. The aforementioned variable region has CDRH1 consisting of an amino acid sequence consisting of the amino acid residues at positions 45 to 54, CDRH2 consisting of an amino acid sequence consisting of the amino acid residues at positions 69 to 78, and CDRH3 consisting of an amino acid sequence consisting of the amino acid residues at positions 118 to 131, in SEQ ID NO: 2 in the sequence listing. The heavy chain variable region of the 04-046 antibody has the amino acid sequence shown in SEQ ID NO: 3 in the sequence listing. The CDRH1 of the 04-046 antibody has the amino acid sequence shown in SEQ ID NO: 4 in the sequence listing, the amino acid sequence of the CDRH2 has the amino acid sequence shown in SEQ ID NO: 5

in the sequence listing, and the amino acid sequence of the CDRH3 has the amino acid sequence shown in SEQ ID NO: 6 in the sequence listing. Furthermore, the sequence of the heavy chain of the 04-046 antibody is shown in Figure 1.

[0065] The light chain of the 04-046 antibody has the amino acid sequence shown in SEQ ID NO: 7 in the sequence listing. In the light chain amino acid sequence shown in SEQ ID NO: 7 in the sequence listing, the amino acid sequence consisting of the amino acid residues at positions 1 to 20 is a signal sequence, the amino acid sequence consisting of the amino acid residues at positions 21 to 128 is a variable region, and the amino acid sequence consisting of the amino acid residues at positions 129 to 233 is a constant region. The aforementioned variable region has CDRL1 consisting of an amino acid sequence consisting of the amino acid residues at positions 43 to 53, CDRL2 consisting of an amino acid sequence consisting of the amino acid residues at positions 69 to 75, and CDRL3 consisting of an amino acid sequence consisting of the amino acid residues at positions 108 to 116, in SEQ ID NO: 7 in the sequence listing. The light chain variable region of the 04-046 antibody has the amino acid sequence shown in SEQ ID NO: 8 in the sequence listing. The CDRL1 of the 04-046 antibody has the amino acid sequence shown in SEQ ID NO: 9 in the sequence listing, the amino acid sequence of the

CDRL2 has the amino acid sequence shown in SEQ ID NO: 10 in the sequence listing, and the amino acid sequence of the CDRL3 has the amino acid sequence shown in SEQ ID NO: 11 in the sequence listing. Furthermore, the sequence of the light chain of the 04-046 antibody is shown in Figure 2.

[0066] The heavy chain of the 04-079 antibody has the amino acid sequence shown in SEQ ID NO: 12 in the sequence listing. In the heavy chain amino acid sequence shown in SEQ ID NO: 12 in the sequence listing, the amino acid sequence consisting of the amino acid residues at positions 1 to 19 is a signal sequence, the amino acid sequence consisting of the amino acid residues at positions 20 to 142 is a variable region, and the amino acid sequence consisting of the amino acid residues at positions 143 to 475 is a constant region. The aforementioned variable region has CDRH1 consisting of an amino acid sequence consisting of the amino acid residues at positions 45 to 54, CDRH2 consisting of an amino acid sequence consisting of the amino acid residues at positions 69 to 78, and CDRH3 consisting of an amino acid sequence consisting of the amino acid residues at positions 118 to 131, in SEQ ID NO: 12 in the sequence listing. The heavy chain variable region of the 04-079 antibody has the amino acid sequence shown in SEQ ID NO: 13 in the sequence listing. The CDRH1 of the 04-079 antibody has the amino acid sequence shown in SEQ ID NO:

14 in the sequence listing, the amino acid sequence of the CDRH2 has the amino acid sequence shown in SEQ ID NO: 15 in the sequence listing, and the amino acid sequence of the CDRH3 has the amino acid sequence shown in SEQ ID NO: 16 in the sequence listing. Furthermore, the sequence of the heavy chain of the 04-079 antibody is shown in Figure 3.

[0067] The light chain of the 04-079 antibody has the amino acid sequence shown in SEQ ID NO: 17 in the sequence listing. In the light chain amino acid sequence shown in SEQ ID NO: 17 in the sequence listing, the amino acid sequence consisting of the amino acid residues at positions 1 to 20 is a signal sequence, the amino acid sequence consisting of the amino acid residues at positions 21 to 128 is a variable region, and the amino acid sequence consisting of the amino acid residues at positions 129 to 233 is a constant region. The aforementioned variable region has CDRL1 consisting of an amino acid sequence consisting of the amino acid residues at positions 43 to 53, CDRL2 consisting of an amino acid sequence consisting of the amino acid residues at positions 69 to 75, and CDRL3 consisting of an amino acid sequence consisting of the amino acid residues at positions 108 to 116, in SEQ ID NO: 17 in the sequence listing. The light chain variable region of the 04-079 antibody has the amino acid sequence shown in SEQ ID NO: 18 in the sequence listing. The CDRL1 of the 04-079

antibody has the amino acid sequence shown in SEQ ID NO: 19 in the sequence listing, the amino acid sequence of the CDRL2 has the amino acid sequence shown in SEQ ID NO: 20 in the sequence listing, and the amino acid sequence of the CDRL3 has the amino acid sequence shown in SEQ ID NO: 21 in the sequence listing. Furthermore, the sequence of the light chain of the 04-079 antibody is shown in Figure 4.

[0068] The heavy chain of the 04-126 antibody has the amino acid sequence shown in SEQ ID NO: 22 in the sequence listing. In the heavy chain amino acid sequence shown in SEQ ID NO: 22 in the sequence listing, the amino acid sequence consisting of the amino acid residues at positions 1 to 19 is a signal sequence, the amino acid sequence consisting of the amino acid residues at positions 20 to 142 is a variable region, and the amino acid sequence consisting of the amino acid residues at positions 143 to 475 is a constant region. The aforementioned variable region has CDRH1 consisting of an amino acid sequence consisting of the amino acid residues at positions 45 to 54, CDRH2 consisting of an amino acid sequence consisting of the amino acid residues at positions 69 to 78, and CDRH3 consisting of an amino acid sequence consisting of the amino acid residues at positions 118 to 131, in SEQ ID NO: 22 in the sequence listing. The heavy chain variable region of the 04-126 antibody has the amino acid sequence shown in SEQ ID NO:

23 in the sequence listing. The CDRH1 of the 04-126 antibody has the amino acid sequence shown in SEQ ID NO: 24 in the sequence listing, the amino acid sequence of the CDRH2 has the amino acid sequence shown in SEQ ID NO: 25 in the sequence listing, and the amino acid sequence of the CDRH3 has the amino acid sequence shown in SEQ ID NO: 26 in the sequence listing. Furthermore, the sequence of the heavy chain of the 04-126 antibody is shown in Figure 5.

[0069] The light chain of the 04-126 antibody has the amino acid sequence shown in SEQ ID NO: 27 in the sequence listing. In the light chain amino acid sequence shown in SEQ ID NO: 27 in the sequence listing, the amino acid sequence consisting of the amino acid residues at positions 1 to 20 is a signal sequence, the amino acid sequence consisting of the amino acid residues at positions 21 to 128 is a variable region, and the amino acid sequence consisting of the amino acid residues at positions 129 to 233 is a constant region. The aforementioned variable region has CDRL1 consisting of an amino acid sequence consisting of the amino acid residues at positions 43 to 53, CDRL2 consisting of an amino acid sequence consisting of the amino acid residues at positions 69 to 75, and CDRL3 consisting of an amino acid sequence consisting of the amino acid residues at positions 108 to 116, in SEQ ID NO: 27 in the sequence listing. The light chain variable region of the 04-126

antibody has the amino acid sequence shown in SEQ ID NO: 28 in the sequence listing. The CDRL1 of the 04-126 antibody has the amino acid sequence shown in SEQ ID NO: 29 in the sequence listing, the amino acid sequence of the CDRL2 has the amino acid sequence shown in SEQ ID NO: 30 in the sequence listing, and the amino acid sequence of the CDRL3 has the amino acid sequence shown in SEQ ID NO: 31 in the sequence listing. Furthermore, the sequence of the light chain of the 04-126 antibody is shown in Figure 6.

[0070] The heavy chain amino acid sequence of the 04-046 antibody is encoded by the nucleotide sequence shown in SEQ ID NO: 32 in the sequence listing. In the nucleotide sequence shown in SEQ ID NO: 32 in the sequence listing, the nucleotide sequence consisting of the nucleotides at positions 1 to 57 encodes a signal sequence, the nucleotide sequence consisting of the nucleotides at positions 58 to 426 encodes the heavy chain variable region of the 04-046 antibody, and the nucleotide sequence consisting of the nucleotides at positions 427 to 1425 encodes the heavy chain constant region of the 04-046 antibody. The nucleotide sequence encoding the aforementioned variable region has a polynucleotide consisting of the nucleotide sequence at nucleotide positions 133 to 162 encoding CDRH1, a polynucleotide consisting of the nucleotide sequence at nucleotide positions 205 to 234 encoding CDRH2, and a polynucleotide

consisting of the nucleotide sequence at nucleotide positions 352 to 393 encoding CDRH3, in SEQ ID NO: 32. The nucleotide sequence of the heavy chain variable region of the 04-046 antibody is also shown in SEQ ID NO: 33 in the sequence listing. Furthermore, the sequence of SEQ ID NO: 32 is shown in Figure 1.

[0071] The light chain nucleotide sequence of the 04-046 antibody is encoded by the nucleotide sequence shown in SEQ ID NO: 34 in the sequence listing. In the nucleotide sequence shown in SEQ ID NO: 34 in the sequence listing, the nucleotide sequence consisting of the nucleotides at positions 1 to 60 encodes a signal sequence, the nucleotide sequence consisting of the nucleotides at positions 61 to 384 encodes the light chain variable region of the 04-046 antibody, and the nucleotide sequence consisting of the nucleotides at positions 385 to 699 encodes the light chain constant region of the 04-046 antibody. The nucleotide sequence encoding the aforementioned variable region has a polynucleotide consisting of the nucleotide sequence at nucleotide positions 127 to 159 encoding CDRL1, a polynucleotide consisting of the nucleotide sequence at nucleotide positions 205 to 225 encoding CDRL2, and a polynucleotide consisting of the nucleotide sequence at nucleotide positions 322 to 348 encoding CDRL3, in SEQ ID NO: 34. The nucleotide sequence of the light chain variable region of the 04-046 antibody is also shown in SEQ ID NO:

35 in the sequence listing. Furthermore, the sequence of SEQ ID NO: 34 is shown in Figure 2.

[0072] The heavy chain nucleotide sequence of the 04-079 antibody is encoded by the nucleotide sequence shown in SEQ ID NO: 36 in the sequence listing. In the nucleotide sequence shown in SEQ ID NO: 36 in the sequence listing, the nucleotide sequence consisting of the nucleotides at positions 1 to 57 encodes a signal sequence, the nucleotide sequence consisting of the nucleotides at positions 58 to 426 encodes the heavy chain variable region of the 04-079 antibody, and the nucleotide sequence consisting of the nucleotides at positions 427 to 1425 encodes the heavy chain constant region of the 04-079 antibody. The nucleotide sequence encoding the aforementioned variable region has a polynucleotide consisting of the nucleotide sequence at nucleotide positions 133 to 162 encoding CDRH1, a polynucleotide consisting of the nucleotide sequence at nucleotide positions 205 to 234 encoding CDRH2, and a polynucleotide consisting of the nucleotide sequence at nucleotide positions 352 to 393 encoding CDRH3, in SEQ ID NO: 36. The nucleotide sequence of the heavy chain variable region of the 04-079 antibody is also shown in SEQ ID NO: 37 in the sequence listing. Furthermore, the sequence of SEQ ID NO: 36 is shown in Figure 3.

[0073] The light chain nucleotide sequence of the 04-079 antibody is encoded by the nucleotide sequence shown in

SEQ ID NO: 38 in the sequence listing. In the nucleotide sequence shown in SEQ ID NO: 38 in the sequence listing, the nucleotide sequence consisting of the nucleotides at positions 1 to 60 encodes a signal sequence, the nucleotide sequence consisting of the nucleotides at positions 61 to 384 encodes the light chain variable region of the 04-079 antibody, and the nucleotide sequence consisting of the nucleotides at positions 385 to 699 encodes the light chain constant region of the 04-079 antibody. The nucleotide sequence encoding the aforementioned variable region has a polynucleotide consisting of the nucleotide sequence at nucleotide positions 127 to 159 encoding CDRL1, a polynucleotide consisting of the nucleotide sequence at nucleotide positions 205 to 225 encoding CDRL2, and a polynucleotide consisting of the nucleotide sequence at nucleotide positions 322 to 348 encoding CDRL3, in SEQ ID NO: 38. The nucleotide sequence of the light chain variable region of the 04-079 antibody is also shown in SEQ ID NO: 39 in the sequence listing. Furthermore, the sequence of SEQ ID NO: 38 is shown in Figure 4.

[0074] The heavy chain nucleotide sequence of the 04-126 antibody is encoded by the nucleotide sequence shown in SEQ ID NO: 40 in the sequence listing. In the nucleotide sequence shown in SEQ ID NO: 40 in the sequence listing, the nucleotide sequence consisting of the nucleotides at positions 1 to 57 encodes a signal sequence, the

nucleotide sequence consisting of the nucleotides at positions 58 to 426 encodes the heavy chain variable region of the 04-126 antibody, and the nucleotide sequence consisting of the nucleotides at positions 427 to 1425 encodes the heavy chain constant region of the 04-126 antibody. The nucleotide sequence encoding the aforementioned variable region has a polynucleotide consisting of the nucleotide sequence at nucleotide positions 133 to 162 encoding CDRH1, a polynucleotide consisting of the nucleotide sequence at nucleotide positions 205 to 234 encoding CDRH2, and a polynucleotide consisting of the nucleotide sequence at nucleotide positions 352 to 393 encoding CDRH3, in SEQ ID NO: 40. The nucleotide sequence of the heavy chain variable region of the 04-126 antibody is also shown in SEQ ID NO: 41 in the sequence listing. Furthermore, the sequence of SEQ ID NO: 40 is shown in Figure 5.

[0075] The light chain nucleotide sequence of the 04-126 antibody is encoded by the nucleotide sequence shown in SEQ ID NO: 42 in the sequence listing. In the nucleotide sequence shown in SEQ ID NO: 42 in the sequence listing, the nucleotide sequence consisting of the nucleotides at positions 1 to 60 encodes a signal sequence, the nucleotide sequence consisting of the nucleotides at positions 61 to 384 encodes the light chain variable region of the 04-126 antibody, and the nucleotide sequence consisting of the nucleotides at positions 385

to 699 encodes the light chain constant region of the 04-126 antibody. The nucleotide sequence encoding the aforementioned variable region has a polynucleotide consisting of the nucleotide sequence at nucleotide positions 127 to 159 encoding CDRL1, a polynucleotide consisting of the nucleotide sequence at nucleotide positions 205 to 225 encoding CDRL2, and a polynucleotide consisting of the nucleotide sequence at nucleotide positions 322 to 348 encoding CDRL3, in SEQ ID NO: 42. The nucleotide sequence of the light chain variable region of the 04-126 antibody is also shown in SEQ ID NO: 43 in the sequence listing. Furthermore, the sequence of SEQ ID NO: 42 is shown in Figure 6.

[0076] Furthermore, also in the case where the steps (a) to (h) in the above "3. Production of anti-GPR20 antibody" are carried out again to obtain a monoclonal antibody separately and in the case where a monoclonal antibody is obtained separately by other methods, an antibody having internalization activity equivalent to that of the 04-046 antibody, the 04-079 antibody or the 04-126 antibody can be obtained. One example of such an antibody can include an antibody binding to the same epitope to which the 04-046 antibody, the 04-079 antibody or the 04-126 antibody binds. If a newly prepared monoclonal antibody binds to a partial peptide or a partial three-dimensional structure to which the 04-046 antibody, the 04-079 antibody or the 04-126 antibody

binds, it can be determined that the monoclonal antibody binds to the same epitope to which the 04-046 antibody, the 04-079 antibody or the 04-126 antibody binds. Moreover, by confirming that the monoclonal antibody competes with the 04-046 antibody, the 04-079 antibody or the 04-126 antibody in the binding of the antibody to GPR20 (i.e., the monoclonal antibody interferes with the binding of the 04-046 antibody, the 04-079 antibody or the 04-126 antibody to GPR20), it can be determined that the monoclonal antibody binds to the same epitope to which the anti-GPR20 antibody binds, even if the specific sequence or structure of the epitope has not been determined. When it is confirmed that the monoclonal antibody binds to the same epitope to which the 04-046 antibody, the 04-079 antibody or the 04-126 antibody binds, then it is strongly expected that the monoclonal antibody should have antigen-binding ability, biological activity and/or internalization activity equivalent to that of the 04-046 antibody, the 04-079 antibody or the 04-126 antibody.

(3) Other antibodies

The antibody of the present invention also includes genetically recombinant antibodies that have been artificially modified for the purpose of reducing heterogenetic antigenicity to humans, such as a chimeric antibody, a humanized antibody and a human antibody, as

well as the above-described monoclonal antibody against GPR20. These antibodies can be produced by known methods.

[0077] Examples of the chimeric antibody can include antibodies in which a variable region and a constant region are heterologous to each other, such as a chimeric antibody formed by conjugating the variable region of a mouse- or rat-derived antibody to a human-derived constant region (see Proc. Natl. Acad. Sci. U.S.A., 81, 6851-6855, (1984)).

[0078] The chimeric antibody derived from the rat anti-human GPR20 antibody 04-046 antibody is an antibody consisting of a heavy chain comprising a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 3, and a light chain comprising a light chain variable region shown in SEQ ID NO: 8, and this chimeric antibody may have any given human-derived constant region.

[0079] The chimeric antibody derived from the rat anti-human GPR20 antibody 04-079 antibody is an antibody consisting of a heavy chain comprising a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 13, and a light chain comprising a light chain variable region shown in SEQ ID NO: 18, and this chimeric antibody may have any given human-derived constant region.

[0080] The chimeric antibody derived from the rat anti-human GPR20 antibody 04-126 antibody is an antibody

consisting of a heavy chain comprising a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 23, and a light chain comprising a light chain variable region shown in SEQ ID NO: 28, and this chimeric antibody may have any given human-derived constant region.

[0081] Specific examples of the chimeric antibody derived from the rat anti-human GPR20 antibody 04-046 antibody can include chimeric antibody 04-046Ch (hereinafter, also referred to as "04-046Ch") derived from the rat anti-human GPR20 antibody 04-046 antibody. The amino acid sequence of the 04-046Ch antibody can be an antibody consisting of a heavy chain having an amino acid sequence consisting of the amino acid residues at positions 20 to 472 of SEQ ID NO: 44 in the sequence listing, and a light chain having an amino acid sequence consisting of positions 21 to 233 of SEQ ID NO: 45 in the sequence listing. It is to be noted that, in the heavy chain sequence shown in SEQ ID NO: 44 in the sequence listing, the amino acid sequence consisting of the amino acid residues at positions 1 to 19 is a signal sequence, the amino acid sequence consisting of the amino acid residues at positions 20 to 142 is a variable region, and the amino acid sequence consisting of the residues at positions 143 to 472 is a constant region. The sequence of SEQ ID NO: 44 is shown in Figure 7. Furthermore, in the light chain sequence shown in SEQ ID NO: 45 in the

sequence listing, the amino acid sequence consisting of the amino acid residues at positions 1 to 20 is a signal sequence, the amino acid sequence consisting of the amino acid residues at positions 21 to 128 is a variable region, and the amino acid sequence consisting of the amino acid residues at positions 129 to 233 is a constant region.

The sequence of SEQ ID NO: 45 is shown in Figure 8.

[0082] The heavy chain amino acid sequence of the 04-046Ch antibody is encoded by the nucleotide sequence shown in SEQ ID NO: 46 in the sequence listing. The nucleotide sequence consisting of the nucleotides at positions 1 to 57 in the nucleotide sequence shown in SEQ ID NO: 46 in the sequence listing encodes the signal sequence of the 04-046Ch antibody. The nucleotide sequence consisting of the nucleotides at positions 58 to 426 in the nucleotide sequence shown in SEQ ID NO: 46 in the sequence listing encodes the heavy chain variable region sequence of the 04-046Ch antibody. The nucleotide sequence consisting of the nucleotides at positions 427 to 1416 in the nucleotide sequence shown in SEQ ID NO: 46 in the sequence listing encodes the heavy chain constant region of the 04-046Ch antibody. The sequence of SEQ ID NO: 46 is shown in Figure 7. The light chain amino acid sequence of the 04-046Ch antibody is encoded by the nucleotide sequence shown in SEQ ID NO: 47 in the sequence listing. The nucleotide sequence consisting of the nucleotides at positions 1 to 60 in the nucleotide

sequence shown in SEQ ID NO: 47 in the sequence listing encodes the signal sequence of the 04-046Ch antibody. The nucleotide sequence consisting of the nucleotides at positions 61 to 384 in the nucleotide sequence shown in SEQ ID NO: 47 in the sequence listing encodes the light chain variable region sequence of the 04-046Ch antibody. The nucleotide sequence consisting of the nucleotides at positions 385 to 699 in the nucleotide sequence shown in SEQ ID NO: 47 in the sequence listing encodes the light chain constant region of the 04-046Ch antibody. The sequence of SEQ ID NO: 47 is shown in Figure 8.

[0083] Examples of the humanized antibody can include an antibody formed by incorporating only complementarity determining regions (CDRs) into a human-derived antibody (see Nature (1986) 321, p. 522-525), an antibody formed by transplanting the amino acid residues in some frameworks, as well as CDR sequences, into a human antibody according to a CDR grafting method (International Publication No. WO90/07861), and an antibody formed by modifying the amino acid sequences of some CDRs while maintaining antigen-binding ability.

[0084] However, the humanized antibody derived from the 04-046 antibody, 04-079, or the 04-126 antibody is not limited to a specific humanized antibody as long as it retains all 6 CDR sequences of the 04-046 antibody, the 04-079 antibody or the 04-126 antibody and has internalization activity. Moreover, this humanized

antibody is not limited to a specific humanized antibody as long as it has internalization activity while the amino acid sequences of some CDRs of the 04-046 antibody, the 04-079 antibody or the 04-126 antibody are modified. [0085] Concrete examples of the humanized antibody of the rat antibody 04-046 can include any given combination of: a heavy chain comprising a heavy chain variable region consisting of any one of (1) an amino acid sequence consisting of the amino acid residues at positions 20 to 142 in SEQ ID NO: 48, 50, 52, 54 or 56 in the sequence listing, (2) an amino acid sequence having a homology of at least 95% or more to the above-described amino acid sequence (1), and (3) an amino acid sequence comprising a deletion, substitution or addition of one or several amino acids in the above-described amino acid sequence (1); and a light chain comprising a light chain variable region consisting of any one of (4) an amino acid sequence consisting of the amino acid residues at positions 21 to 129 in SEQ ID NO: 58, 60, 62 or 64, (5) an amino acid sequence having a homology of at least 95% or more to the above-described amino acid sequence (4), and (6) an amino acid sequence comprising a deletion, substitution or addition of one or several amino acids in the above-described amino acid sequence (4).

Alternatively, an antibody having a humanized heavy chain or light chain and the other chain derived from a rat antibody or a chimeric antibody can also be used.

Examples of such an antibody can include any given combination of: a heavy chain consisting of any one of (1) an amino acid sequence consisting of the amino acid residues at positions 20 to 472 in SEQ ID NO: 44 in the sequence listing, (2) an amino acid sequence having a homology of at least 95% or more to the above-described amino acid sequence (1), and (3) an amino acid sequence comprising a deletion, substitution or addition of one or several amino acids in the above-described amino acid sequence (1); and a light chain comprising a light chain variable region consisting of any one of (4) an amino acid sequence consisting of the amino acid residues at positions 21 to 129 in SEQ ID NO: 58, 60, 62 or 64, (5) an amino acid sequence having a homology of at least 95% or more to the above-described amino acid sequence (4), and (6) an amino acid sequence comprising a deletion, substitution or addition of one or several amino acids in the above-described amino acid sequence (4). Other examples of such an antibody can include any given combination of: a heavy chain comprising a heavy chain variable region consisting of any one of (1) an amino acid sequence consisting of the amino acid residues at positions 20 to 142 in SEQ ID NO: 48, 50, 52, 54 or 56 in the sequence listing, (2) an amino acid sequence having a homology of at least 95% or more to the above-described amino acid sequence (1), and (3) an amino acid sequence comprising a deletion, substitution or addition of one or

several amino acids in the above-described amino acid sequence (1); and a light chain comprising a light chain variable region consisting of any one of (4) an amino acid sequence consisting of the amino acid residues at positions 21 to 233 in SEQ ID NO: 45 in the sequence listing, (5) an amino acid sequence having a homology of at least 95% or more to the above-described amino acid sequence (4), and (6) an amino acid sequence comprising a deletion, substitution or addition of one or several amino acids in the above-described amino acid sequence (4). Further alternative examples thereof can include an antibody consisting of any combination of a heavy chain and a light chain selected from the following combinations (7) to (19): (7) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 44, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 58, (8) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 44, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 60, (9) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 44, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 62, and (10) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO:

44, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 64.

[0086] In SEQ ID NO: 48, the sequence consisting of the amino acid residues at positions 45 to 54 (GYTFTSYIIS) corresponds to CDRH1, the sequence consisting of the amino acid residues at positions 69 to 78 (FINPGSGHTN) corresponds to CDRH2, and the sequence consisting of the amino acid residues at positions 118 to 131 (GAGGFLRIITKFDY) corresponds to CDRH3. In SEQ ID NO: 50, the sequence consisting of the amino acid residues at positions 45 to 54 (GYTFTSYIIS) corresponds to CDRH1, the sequence consisting of the amino acid residues at positions 69 to 78 (FINPGSGHTN) corresponds to CDRH2, and the sequence consisting of the amino acid residues at positions 118 to 131 (GAGGFLRIITKFDY) corresponds to CDRH3. In SEQ ID NO: 52, the sequence consisting of the amino acid residues at positions 45 to 54 (GYTFTSYIIS) corresponds to CDRH1, the sequence consisting of the amino acid residues at positions 69 to 78 (FINPGSGHTN) corresponds to CDRH2, and the sequence consisting of the amino acid residues at positions 118 to 131 (GAGGFLRIITKFDY) corresponds to CDRH3. In SEQ ID NO: 54, the sequence consisting of the amino acid residues at positions 45 to 54 (GYTFTSYIIS) corresponds to CDRH1, the sequence consisting of the amino acid residues at positions 69 to 78 (FINPGSGHTN) corresponds to CDRH2, and

the sequence consisting of the amino acid residues at positions 118 to 131 (GAGGFLRIITKFDY) corresponds to CDRH3. In SEQ ID NO: 56, the sequence consisting of the amino acid residues at positions 45 to 54 (GYTFTSYIIS) corresponds to CDRH1, the sequence consisting of the amino acid residues at positions 69 to 78 (FINPGSGHTN) corresponds to CDRH2, and the sequence consisting of the amino acid residues at positions 118 to 131 (GAGGFLRIITKFDY) corresponds to CDRH3.

[0087] In SEQ ID NO: 58, the sequence consisting of the amino acid residues at positions 44 to 54 (RASKSVSTYIH) corresponds to CDRL1, the sequence consisting of the amino acid residues at positions 70 to 76 (SASNLES) corresponds to CDRL2, and the sequence consisting of the amino acid residues at amino acid positions 109 to 117 (QQINELPYT) corresponds to CDRL3. In SEQ ID NO: 60, the sequence consisting of the amino acid residues at positions 44 to 54 (RASKSVSTYIH) corresponds to CDRL1, the sequence consisting of the amino acid residues at positions 70 to 76 (SASNLES) corresponds to CDRL2, and the sequence consisting of the amino acid residues at amino acid positions 109 to 117 (QQINELPYT) corresponds to CDRL3. In SEQ ID NO: 62, the sequence consisting of the amino acid residues at positions 44 to 54 (RASKSVSTYIH) corresponds to CDRL1, the sequence consisting of the amino acid residues at positions 70 to 76 (SASDRES) corresponds to CDRL2, and the sequence

consisting of the amino acid residues at amino acid positions 109 to 117 (QQINELPYT) corresponds to CDRL3. In SEQ ID NO: 64, the sequence consisting of the amino acid residues at positions 44 to 54 (RASKSVSTYIH) corresponds to CDRL1, the sequence consisting of the amino acid residues at positions 70 to 76 (SAGNLES) corresponds to CDRL2, and the sequence consisting of the amino acid residues at amino acid positions 109 to 117 (QQINELPYT) corresponds to CDRL3.

[0088] It is to be noted that the term "several" is used in the present description to mean 1 to 10, 1 to 9, 1 to 8, 1 to 7, 1 to 6, 1 to 5, 1 to 4, 1 to 3, or 1 or 2.

[0089] The amino acid substitution in the present description is preferably a conservative amino acid substitution. The conservative amino acid substitution is a substitution occurring within an amino acid group associated with amino acid side chains. Preferred amino acid groups are the following: acidic group = aspartic acid and glutamic acid; basic group = lysine, arginine, and histidine; non-polar group = alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, and tryptophan; and uncharged polar family = glycine, asparagine, glutamine, cysteine, serine, threonine, and tyrosine. Other preferred amino acid groups are the following: aliphatic hydroxy group = serine and threonine; amide-containing group = asparagine and glutamine; aliphatic group = alanine, valine, leucine and

isoleucine; and aromatic group = phenylalanine, tryptophan and tyrosine. Such amino acid substitution is preferably carried out without impairing the properties of a substance having the original amino acid sequence.

[0090] Examples of the antibody having a preferred combination of the above-described heavy chains and light chains can include an antibody consisting of a heavy chain having a variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 48, and a light chain having a variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 64, an antibody consisting of a heavy chain having a variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 52, and a light chain having a variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 60, an antibody consisting of a heavy chain having a variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 54, and a light chain having a variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 58, an antibody consisting of a heavy chain having a variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 56, and a light chain having a variable region consisting of the amino acid sequence at amino acid positions 21 to

129 in SEQ ID NO: 58, an antibody consisting of a heavy chain having a variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 56, and a light chain having a variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 62, and an antibody consisting of a heavy chain having a variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 50, and a light chain having a variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 64.

[0091] Examples of the antibody having a more preferred combination can include an antibody consisting of a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 48, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 64, an antibody consisting of a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 52, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 60, an antibody consisting of a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 54, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 58, an antibody consisting of a heavy chain

consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 56, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 58, an antibody consisting of a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 56, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 62, and an antibody consisting of a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 50, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 64.

[0092] By combining together sequences showing a high homology to the above-described heavy chain amino acid sequences and light chain amino acid sequences, it is possible to select an antibody having a biological activity equivalent to that of each of the above-described antibodies. Such a homology is a homology of generally 80% or more, preferably 90% or more, more preferably 95% or more, and most preferably 99% or more. Moreover, also by combining with one another, amino acid sequences comprising a substitution, deletion or addition of one or several amino acid residues with respect to the amino acid sequence of a heavy chain or a light chain, it is possible to select an antibody having a biological

activity equivalent to that of each of the above-described antibodies.

[0093] The homology between two types of amino acid sequences can be determined using default parameters of Blast algorithm version 2.2.2 (Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schaffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25: 3389-3402). The Blast algorithm can also be used by accessing www.ncbi.nlm.nih.gov/blast through the internet.

[0094] It is to be noted that, in the heavy chain amino acid sequence shown in SEQ ID NO: 48, 50, 52, 54 or 56 in the sequence listing, the amino acid sequence consisting of the amino acid residues at positions 1 to 19 is a signal sequence, the amino acid sequence consisting of the amino acid residues at positions 20 to 142 is a variable region, and the amino acid sequence consisting of the amino acid residues at positions 143 to 472 is a constant region. The sequence of SEQ ID NO: 48 is shown in Figure 9, the sequence of SEQ ID NO: 50 is shown in Figure 10, the sequence of SEQ ID NO: 52 is shown in Figure 11, the sequence of SEQ ID NO: 54 is shown in Figure 12, and the sequence of SEQ ID NO: 56 is shown in Figure 13.

[0095] Furthermore, in the light chain amino acid sequence shown in SEQ ID NO: 58, 60, 62 or 64 in the

sequence listing, the amino acid sequence consisting of the amino acid residues at positions 1 to 20 is a signal sequence, the amino acid sequence consisting of the amino acid residues at positions 21 to 129 is a variable region, and the amino acid sequence consisting of the amino acid residues at positions 130 to 234 is a constant region.

The sequence of SEQ ID NO: 58 is shown in Figure 14, the sequence of SEQ ID NO: 60 is shown in Figure 15, the sequence of SEQ ID NO: 62 is shown in Figure 16, and the sequence of SEQ ID NO: 64 is shown in Figure 17.

[0096] Further examples of the antibody of the present invention can include a human antibody binding to GPR20. The anti-GPR20 human antibody means a human antibody having only the gene sequence of an antibody derived from human chromosomes. The anti-GPR20 human antibody can be obtained by a method using a human antibody-producing mouse having a human chromosomal fragment comprising the heavy chain and light chain genes of a human antibody (see Tomizuka, K. et al., *Nature Genetics* (1997) 16, p. 133-143; Kuroiwa, Y. et al., *Nucl. Acids Res.* (1998) 26, p. 3447-3448; Yoshida, H. et al., *Animal Cell Technology: Basic and Applied Aspects* vol. 10, p. 69-73 (Kitagawa, Y., Matsuda, T. and Iijima, S. eds.), Kluwer Academic Publishers, 1999; Tomizuka, K. et al., *Proc. Natl. Acad. Sci. USA* (2000) 97, p. 722-727; etc.).

[0097] Such a human antibody-producing mouse can be specifically produced by using a genetically modified

animal, the gene loci of endogenous immunoglobulin heavy chain and light chain of which have been disrupted and instead the gene loci of human immunoglobulin heavy chain and light chain have been then introduced using a yeast artificial chromosome (YAC) vector or the like, then producing a knock-out animal and a transgenic animal from such a genetically modified animal, and then breeding such animals with one another.

[0098] Otherwise, the anti-GPR20 human antibody can also be obtained by transforming eukaryotic cells with cDNA encoding each of the heavy chain and light chain of such a human antibody, or preferably with a vector comprising the cDNA, according to genetic recombination techniques, and then culturing the transformed cells producing a genetically modified human monoclonal antibody, so that the antibody can be obtained from the culture supernatant.

[0099] In this context, eukaryotic cells, and preferably, mammalian cells such as CHO cells, lymphocytes or myelomas can, for example, be used as a host.

[0100] Furthermore, a method of obtaining a phage display-derived human antibody that has been selected from a human antibody library (see Wormstone, I. M. et al., *Investigative Ophthalmology & Visual Science*. (2002) 43 (7), p. 2301-2308; Carmen, S. et al., *Briefings in Functional Genomics and Proteomics* (2002), 1 (2), p. 189-203; Siriwardena, D. et al., *Ophthalmology* (2002) 109 (3), p. 427-431; etc.) is also known.

[0101] For example, a phage display method, which comprises allowing the variable regions of a human antibody to express as a single chain antibody (scFv) on the surface of phages, and then selecting a phage binding to an antigen, can be applied (Nature Biotechnology (2005), 23, (9), p. 1105-1116).

[0102] By analyzing the phage gene that has been selected because of its binding ability to the antigen, DNA sequences encoding the variable regions of a human antibody binding to the antigen can be determined.

[0103] Once the DNA sequence of scFv binding to the antigen is determined, an expression vector having the aforementioned sequence is produced, and the produced expression vector is then introduced into an appropriate host and can be allowed to express therein, thereby producing a human antibody (International Publication Nos. WO92/01047, WO92/20791, WO93/06213, WO93/11236, WO93/19172, WO95/01438, and WO95/15388, Annu. Rev. Immunol (1994) 12, p. 433-455, Nature Biotechnology (2005) 23 (9), p. 1105-1116).

[0104] If a newly produced human antibody binds to a partial peptide or a partial three-dimensional structure to which the 04-046 antibody described in the present description binds, it can be determined that the human antibody binds to the same epitope to which the 04-046 antibody, the 04-079 antibody or the 04-126 antibody binds. Moreover, by confirming that the human antibody

competes with the 04-046 antibody, the 04-079 antibody or the 04-126 antibody in the binding of the antibody to GPR20 (i.e., the human antibody interferes with the binding of the 04-046 antibody, the 04-079 antibody or the 04-126 antibody to GPR20), it can be determined that the human antibody binds to the same epitope to which the 04-046 antibody, the 04-079 antibody or the 04-126 antibody binds, even if the specific sequence or structure of the epitope has not been determined. When it is confirmed that the human antibody binds to the same epitope to which the 04-046 antibody, the 04-079 antibody or the 04-126 antibody binds, then it is strongly expected that the human antibody should have a biological activity equivalent to that of the 04-046 antibody, the 04-079 antibody or the 04-126 antibody.

[0105] The chimeric antibodies, the humanized antibodies, or the human antibodies obtained by the above-described methods are evaluated for their binding activity against the antigen according to a known method, etc., so that a preferred antibody can be selected.

[0106] One example of another indicator for comparison of the properties of antibodies can include the stability of an antibody. A differential scanning calorimeter (DSC) is an apparatus capable of promptly and exactly measuring a thermal denaturation midpoint (T_m) serving as a good indicator for the relative structural stability of a protein. By using a DSC to measure T_m values and making

a comparison regarding the obtained values, differences in the thermal stability can be compared. It is known that the preservation stability of an antibody has a certain correlation with the thermal stability of the antibody (Lori Burton, et al., Pharmaceutical Development and Technology (2007) 12, p. 265-273), and thus, a preferred antibody can be selected using thermal stability as an indicator. Other examples of the indicator for selection of an antibody can include high yield in suitable host cells and low agglutination in an aqueous solution. For example, since an antibody with the highest yield does not always exhibit the highest thermal stability, it is necessary to select an antibody most suitable for administration to a human by comprehensively determining it based on the aforementioned indicators.

[0107] The antibody of the present invention also includes a modification of an antibody. The modification is used to mean the antibody of the present invention, which is chemically or biologically modified. Examples of such a chemical modification include the binding of a chemical moiety to an amino acid skeleton, and the chemical modification of an N-linked or O-linked carbohydrate chain. Examples of such a biological modification include antibodies which have undergone a posttranslational modification (e.g., N-linked or O-linked glycosylation, N-terminal or C-terminal processing,

deamidation, isomerization of aspartic acid, and oxidation of methionine), and antibodies, to the N-terminus of which a methionine residue is added as a result of having been allowed to be expressed using prokaryote host cells. In addition, such a modification is meant to also include labeled antibodies for enabling detection or isolation of the antibody of the present invention or an antigen, for example, an enzymatically labeled antibody, a fluorescently labeled antibody, and an affinity-labeled antibody. Such a modification of the antibody of the present invention is useful for the improvement of the stability and retention in blood of an antibody, a reduction in antigenicity, detection or isolation of an antibody or an antigen, etc.

[0108] Moreover, by regulating a sugar chain modification (glycosylation, de-fucosylation, etc.) that binds to the antibody of the present invention, antibody-dependent cellular cytotoxic activity can be enhanced. As techniques of regulating the sugar chain modification of an antibody, those described in International Publication Nos. WO1999/54342, WO2000/61739, and WO2002/31140, etc. are known, though the techniques are not limited thereto. The antibody of the present invention also includes antibodies in which the aforementioned sugar chain modification has been regulated.

[0109] Once an antibody gene is isolated, the gene can be introduced into an appropriate host to produce an

antibody, using an appropriate combination of a host and an expression vector. A specific example of the antibody gene can be a combination of a gene encoding the heavy chain sequence of the antibody described in the present description and a gene encoding the light chain sequence of the antibody described therein. Upon transformation of host cells, such a heavy chain sequence gene and a light chain sequence gene may be inserted into a single expression vector, or these genes may instead each be inserted into different expression vectors.

[0110] When eukaryotic cells are used as hosts, animal cells, plant cells or eukaryotic microorganisms can be used. In particular, examples of the animal cells can include mammalian cells such as COS cells which are monkey cells (Gluzman, Y., Cell (1981) 23, p. 175-182, ATCC CRL-1650), mouse fibroblasts NIH3T3 (ATCC No. CRL-1658), a dihydrofolate reductase-deficient cell line of Chinese hamster ovary cells (CHO cells, ATCC CCL-61) (Urlaub, G. and Chasin, L. A. Proc. Natl. Acad. Sci. U.S.A. (1980) 77, p. 4126-4220), and FreeStyle 293F cells (Invitrogen Corp.).

[0111] When prokaryotic cells are used as hosts, *Escherichia coli* or *Bacillus subtilis* can be used, for example.

[0112] An antibody gene of interest is introduced into these cells for transformation, and the transformed cells are then cultured *in vitro* to obtain an antibody. In the

aforementioned culture, there are cases where yield is different depending on the sequence of the antibody, and thus, it is possible to select an antibody, which is easily produced as a medicament, from antibodies having equivalent binding activity, using the yield as an indicator. Accordingly, the antibody of the present invention also includes an antibody obtained by the above-described method for producing an antibody, which comprises a step of culturing the transformed host cells and a step of collecting an antibody of interest or a functional fragment of the antibody from the culture obtained in the aforementioned step.

[0113] It is known that the lysine residue at the carboxyl terminus of the heavy chain of an antibody produced in cultured mammalian cells is deleted (Journal of Chromatography A, 705: 129-134 (1995)), and also, it is known that the two amino acid residues at the heavy chain carboxyl terminus, glycine and lysine, are deleted, and that the proline residue positioned at the carboxyl terminus is newly amidated (Analytical Biochemistry, 360: 75-83 (2007)). However, such deletion and modification of these heavy chain sequences do not have an influence on the antigen-binding activity and effector function (activation of complement, antibody-dependent cellular cytotoxicity, etc.) of an antibody. Accordingly, the antibody according to the present invention also includes an antibody that has undergone the aforementioned

modification, and a functional fragment of the antibody, and specific examples of such an antibody include a deletion mutant comprising a deletion of 1 or 2 amino acids at the heavy chain carboxyl terminus, and a deletion mutant formed by amidating the aforementioned deletion mutant (e.g., a heavy chain in which the proline residue at the carboxyl-terminal site is amidated). However, deletion mutants involving a deletion at the carboxyl terminus of the heavy chain of the antibody according to the present invention are not limited to the above-described deletion mutants, as long as they retain antigen-binding activity and effector function. Two heavy chains constituting the antibody according to the present invention may be any one type of heavy chain selected from the group consisting of a full-length antibody and the above-described deletion mutants, or may be a combination of any two types selected from the aforementioned group. The ratio of individual deletion mutants can be influenced by the types of cultured mammalian cells that produce the antibody according to the present invention, and the culture conditions. Examples of the main ingredient of the antibody according to the present invention can include antibodies where one amino acid residue is deleted at each of the carboxyl termini of the two heavy chains.

[0114] Examples of the isotype of the antibody of the present invention can include IgG (IgG1, IgG2, IgG3, and IgG4). Among others, IgG1 and IgG2 are preferable.

[0115] Examples of the biological activity of an antibody can generally include antigen-binding activity, the activity of being internalized into cells expressing an antigen by binding to the antigen, the activity of neutralizing the activity of an antigen, the activity of enhancing the activity of an antigen, antibody-dependent cellular cytotoxic (ADCC) activity, complement-dependent cytotoxic (CDC) activity, and antibody-dependent cellular phagocytosis (ADCP). The function of the antibody according to the present invention is binding activity against GPR20 and is preferably the activity of being internalized into GPR20-expressing cells by binding to GPR20. Moreover, the antibody of the present invention may have ADCC activity, CDC activity and/or ADCP activity, as well as cellular internalization activity.

[0116] The obtained antibody can be purified to a homogenous state. For separation and purification of the antibody, separation and purification methods used for ordinary proteins may be used. For example, column chromatography, filtration, ultrafiltration, salting-out, dialysis, preparative polyacrylamide gel electrophoresis, and isoelectric focusing are appropriately selected and combined with one another, so that the antibody can be separated and purified (Strategies for Protein

Purification and Characterization: A Laboratory Course Manual, Daniel R. Marshak et al. eds., Cold Spring Harbor Laboratory Press (1996); and Antibodies: A Laboratory Manual. Ed Harlow and David Lane, Cold Spring Harbor Laboratory (1988)), though examples of the separation and purification methods are not limited thereto.

[0117] Examples of the chromatography can include affinity chromatography, ion exchange chromatography, hydrophobic chromatography, gel filtration chromatography, reverse phase chromatography, and absorption chromatography.

[0118] These chromatographic techniques can be carried out using liquid chromatography such as HPLC or FPLC.

[0119] Examples of the column used in the affinity chromatography can include a Protein A column and a Protein G column. Examples of the column involving the use of Protein A can include Hyper D, POROS, and Sepharose F. F. (Pharmacia).

[0120] Also, using an antigen-immobilized carrier, the antibody can be purified by utilizing the binding activity of the antibody to the antigen.

[0121] 3. Anti-GPR20 antibody-drug conjugate

(1) Drug

The anti-GPR20 antibody obtained in the above "2. Production of anti-GPR20 antibody" can be conjugated to a drug via a linker structure moiety to prepare an anti-GPR20 antibody-drug conjugate. The drug is not

particularly limited as long as it has a substituent or a partial structure that can be connected to a linker structure. The anti-GPR20 antibody-drug conjugate can be used for various purposes according to the conjugated drug. Examples of such a drug can include substances having antitumor activity, substances effective for blood diseases, substances effective for autoimmune diseases, anti-inflammatory substances, antimicrobial substances, antifungal substances, antiparasitic substances, antiviral substances, and anti-anesthetic substances.

[0122] (1)-1 Antitumor compound

An example using an antitumor compound as a compound to be conjugated in the anti-GPR20 antibody-drug conjugate of the present invention will be described below. The antitumor compound is not particularly limited as long as the compound has an antitumor effect and has a substituent or a partial structure that can be connected to a linker structure. Upon cleavage of a part or the whole of the linker in tumor cells, the antitumor compound moiety is released so that the antitumor compound exhibits an antitumor effect. As the linker is cleaved at a connecting position with the drug, the antitumor compound is released in its original structure to exert its original antitumor effect.

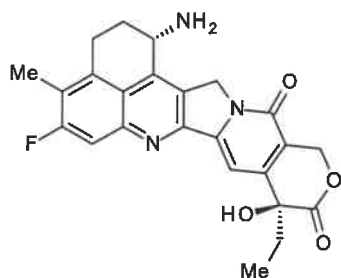
[0123] The anti-GPR20 antibody obtained in the above "2. Production of anti-GPR20 antibody" can be conjugated to

the antitumor compound via a linker structure moiety to prepare an anti-GPR20 antibody-drug conjugate.

[0124] As one example of the antitumor compound used in the present invention, exatecan, a camptothecin derivative ((1S,9S)-1-amino-9-ethyl-5-fluoro-2,3-dihydro-9-hydroxy-4-methyl-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinoline-10,13(9H,15H)-dione represented by the following formula) can be preferably used.

[0125]

[Formula 6]



[0126] The compound can be easily obtained by, for example, a method described in U.S. Patent Publication No. US2016/0297890 or other known methods, and the amino group at position 1 can be preferably used as a connecting position to the linker structure. Further, exatecan may be released in tumor cells while a part of the linker is still attached thereto. However, the compound exerts an excellent antitumor effect even in such a state.

[0127] Since exatecan has a camptothecin structure, it is known that the equilibrium shifts to a structure with a

formed lactone ring (closed ring) in an acidic aqueous medium (e.g., of the order of pH 3) whereas the equilibrium shifts to a structure with an opened lactone ring (open ring) in a basic aqueous medium (e.g., of the order of pH 10). A drug conjugate into which exatecan residues corresponding to such a closed ring structure and an open ring structure have been introduced is also expected to have an equivalent antitumor effect, and it is needless to say that any of such drug conjugates is included in the scope of the present invention.

[0128] Other examples of the antitumor compound can include antitumor compounds described in the literature (Pharmacological Reviews, 68, p. 3-19, 2016). Specific examples thereof can include doxorubicin, calicheamicin, dolastatin 10, auristatins such as monomethyl auristatin E (MMAE) and monomethyl auristatin F (MMAF), maytansinoids such as DM1 and DM4, a pyrrolobenzodiazepine dimer SG2000 (SJG-136), a camptothecin derivative SN-38, duocarmycins such as CC-1065, amanitin, daunorubicin, mitomycin C, bleomycin, cyclocytidine, vincristine, vinblastine, methotrexate, platinum-based antitumor agents (cisplatin and derivatives thereof), and Taxol and derivatives thereof.

[0129] In the antibody-drug conjugate, the number of conjugated drug molecules per antibody molecule is a key factor having an influence on the efficacy and safety thereof. The production of the antibody-drug conjugate

is carried out by specifying reaction conditions such as the amounts of starting materials and reagents used for reaction, so as to attain a constant number of conjugated drug molecules. Unlike the chemical reaction of a low-molecular-weight compound, a mixture containing different numbers of conjugated drug molecules is usually obtained. The number of conjugated drug molecules per antibody molecule is defined and indicated as an average value, i.e., the average number of conjugated drug molecules. Unless otherwise specified, i.e., except in the case of representing an antibody-drug conjugate having a specific number of conjugated drug molecules that is included in an antibody-drug conjugate mixture having different numbers of conjugated drug molecules, the number of conjugated drug molecules according to the present invention means an average value as a rule. The number of exatecan molecules conjugated to an antibody molecule is controllable, and as an average number of conjugated drug molecules per antibody, approximately 1 to 10 exatecan molecules can be conjugated. The number of exatecan molecules is preferably 2 to 8, more preferably 5 to 8, further preferably 7 to 8, still further preferably 8. It is to be noted that a person skilled in the art can design a reaction for conjugating a required number of drug molecules to an antibody molecule based on the description of the Examples of the present

application, and can obtain an antibody-drug conjugate with a controlled number of conjugated exatecan molecules.

[0130] (2) Linker structure

The linker structure which conjugates the drug to the anti-GPR20 antibody in the anti-GPR20 antibody-drug conjugate of the present invention will now be described.

[0131] In the antibody-drug conjugate of the present application, the linker structure which conjugates the anti-GPR20 antibody to the drug is not particularly limited as long as the resulting antibody-drug conjugate can be used. The linker structure can be appropriately selected and used according to the purpose of use. One example of the linker structure can include a linker described in known literature (Pharmacol Rev 68: 3-19, January 2016, Protein Cell DOI 10.1007/s13238-016-0323-0, etc.). Further specific examples thereof can include VC (valine-citrulline), MC (maleimidocaproyl), SMCC (succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate), SPP (N-succinimidyl 4-(2-pyridyldithio)pentanoate, SS (disulfide), SPDB (N-succinimidyl 4-(2-pyridyldithio)butyrate, SS/hydrazone, hydrazone and carbonate.

[0132] Another example of the linker structure can include a linker structure described in U.S. Patent Publication No. US2016/0297890 (as one example, those described in paragraphs [0260] to [0289]). Any linker structure given below can preferably be used. It is to

be noted that the left terminus of the structure is a connecting position to the antibody, and the right terminus thereof is a connecting position to the drug. Furthermore, GGFG in the linker structures given below represents an amino acid sequence consisting of glycine-glycine-phenylalanine-glycine linked through peptide bonds.

- (Succinimid-3-yl-N) -CH₂CH₂-C(=O) -GGFG-NH-CH₂CH₂CH₂-C(=O) -,

- (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O) -GGFG-NH-CH₂CH₂CH₂-C(=O) -,

- (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O) -GGFG-NH-CH₂-O-CH₂-C(=O) -,

- (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O) -GGFG-NH-CH₂CH₂-O-CH₂-C(=O) -,

- (Succinimid-3-yl-N) -CH₂CH₂-C(=O) -NH-CH₂CH₂O-CH₂CH₂O-CH₂CH₂-C(=O) -GGFG-NH-CH₂CH₂CH₂-C(=O) -, and

- (Succinimid-3-yl-N) -CH₂CH₂-C(=O) -NH-CH₂CH₂O-CH₂CH₂O-CH₂CH₂O-CH₂CH₂-C(=O) -GGFG-NH-CH₂CH₂CH₂-C(=O) -.

[0133] More preferred are the following:

- (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O) -GGFG-NH-CH₂-O-CH₂-C(=O) -,

- (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O) -GGFG-NH-CH₂CH₂-O-CH₂-C(=O) -, and

- (Succinimid-3-yl-N) -CH₂CH₂-C(=O) -NH-CH₂CH₂O-CH₂CH₂O-CH₂CH₂-C(=O) -GGFG-NH-CH₂CH₂CH₂-C(=O) -.

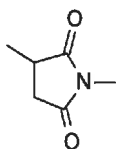
Still more preferred are the following:

-(Succinimid-3-yl-N)-CH₂CH₂CH₂CH₂CH₂-C(=O)-GGFG-NH-CH₂-O-CH₂-C(=O)-, and
 -(Succinimid-3-yl-N)-CH₂CH₂-C(=O)-NH-CH₂CH₂O-CH₂CH₂O-CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂CH₂-C(=O)-.

The antibody is connected to the terminus of -(Succinimid-3-yl-N) (e.g., a bond opposite (left bond) to the bond to which "-CH₂CH₂CH₂CH₂CH₂-" is connected in "(Succinimid-3-yl-N)-CH₂CH₂CH₂CH₂CH₂-C(=O)-GGFG-NH-CH₂-O-CH₂-C(=O)-"), and the antitumor compound is connected to a terminus opposite to the terminus which -(Succinimid-3-yl-N) forms (the carbonyl group of CH₂-O-CH₂-C(=O)- at the right terminus in the above-described example). "(Succinimid-3-yl-N)-" has a structure represented by the following formula:

[0134]

[Formula 7]



[0135] Position 3 of this partial structure is the connecting position for the anti-GPR20 antibody. This connection to the antibody at position 3 is characterized by forming a thioether bond. The nitrogen atom at position 1 of this structure moiety is connected to the carbon atom of methylene which is present within the linker including the structure.

[0136] In the antibody-drug conjugate of the present invention having exatecan as the drug, a drug-linker structure moiety having any structure given below is preferred for the conjugation to the antibody. For these drug-linker structure moieties, the average conjugated number per antibody may be 1 to 10 and is preferably 2 to 8, more preferably 5 to 8, further preferably 7 to 8, and still further preferably 8.

-(Succinimid-3-yl-N)-CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂CH₂-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-CH₂CH₂CH₂CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂CH₂-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-CH₂CH₂CH₂CH₂CH₂-C(=O)-GGFG-NH-CH₂-O-CH₂-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-CH₂CH₂CH₂CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂-O-CH₂-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-CH₂CH₂-C(=O)-NH-CH₂CH₂O-CH₂CH₂O-CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂CH₂-C(=O)-(NH-DX), and

-(Succinimid-3-yl-N)-CH₂CH₂-C(=O)-NH-CH₂CH₂O-CH₂CH₂O-CH₂CH₂O-CH₂CH₂O-CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂CH₂-C(=O)-(NH-DX).

[0137] More preferred are the following:

-(Succinimid-3-yl-N)-CH₂CH₂CH₂CH₂CH₂-C(=O)-GGFG-NH-CH₂-O-CH₂-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-CH₂CH₂CH₂CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂-O-CH₂-C(=O)-(NH-DX), and

-(Succinimid-3-yl-N)-CH₂CH₂-C(=O)-NH-CH₂CH₂O-CH₂CH₂O-CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂CH₂-C(=O)-(NH-DX).

[0138] Still more preferred are the following:

-(Succinimid-3-yl-N)-CH₂CH₂CH₂CH₂CH₂-C(=O)-GGFG-NH-CH₂-O-CH₂-C(=O)-(NH-DX), and
-(Succinimid-3-yl-N)-CH₂CH₂-C(=O)-NH-CH₂CH₂O-CH₂CH₂O-CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂CH₂-C(=O)-(NH-DX).

[0139] Further preferred is the following:

-(Succinimid-3-yl-N)-CH₂CH₂CH₂CH₂CH₂-C(=O)-GGFG-NH-CH₂-O-CH₂-C(=O)-(NH-DX)

[0140] (3) Method for producing antibody-drug conjugate

The antibody that can be used in the antibody-drug conjugate of the present invention is not particularly limited as long as it is an anti-GPR20 antibody having internalization activity or a functional fragment of the antibody, described in the above section "2. Production of anti-GPR20 antibody" and in the Examples.

[0141] Next, the method for producing the antibody-drug conjugate of the present invention will be described with reference to specific examples. It is to be noted that, in the description below, a compound No. shown in each reaction scheme is used to represent a compound.

Specifically, each compound is referred to as a "compound of the formula (1)", "compound (1)", or the like. The same holds true for the other compound Nos.

[0142] (3)-1 Production method 1

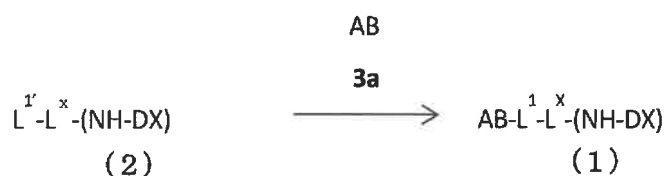
The antibody-drug conjugate represented by the formula (1) given below in which the anti-GPR20 antibody is connected to the linker structure via a thioether can

be produced by reacting an antibody having a sulfhydryl group converted from a disulfide bond by the reduction of the anti-GPR20 antibody, with the compound (2) obtainable by a known method (e.g., obtainable by a method described in the patent publication literature US2016/297890 (e.g., a method described in paragraphs [0336] to [0374])).

This antibody-drug conjugate can be produced by the following method, for example.

[0143]

[Formula 8]



[0144]

wherein AB represents an antibody with a sulfhydryl group,

wherein

L¹ has a structure represented by -(Succinimid-3-yl-N)-,

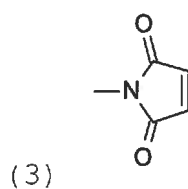
and

L^{1'} represents a maleimidyl group represented by the

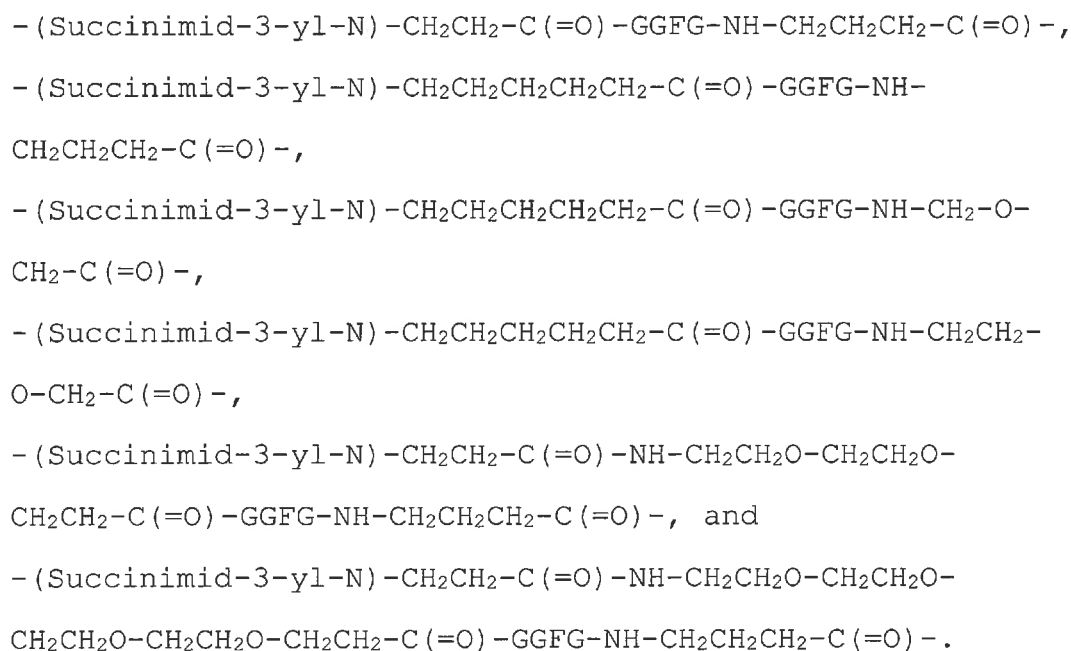
following formula.

[0145]

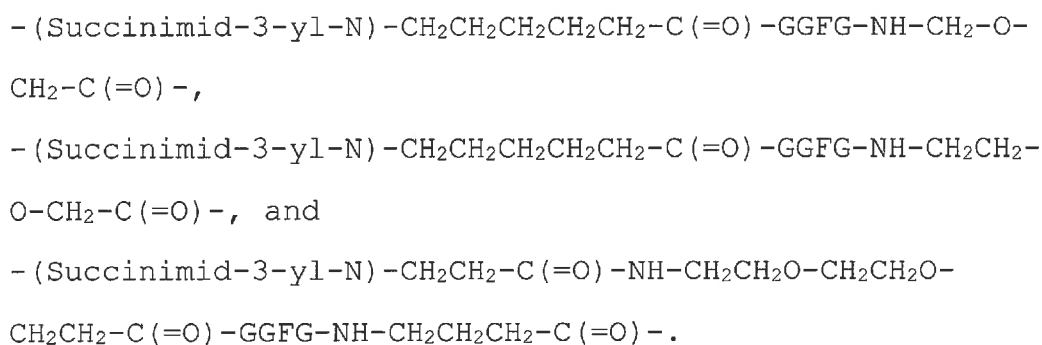
[Formula 9]



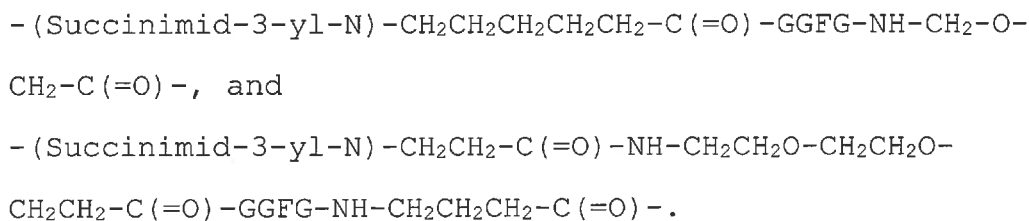
[0146] $-L^1-L^X$ has a structure represented by any of the following formulas:



[0147] Among them, more preferred are the following:



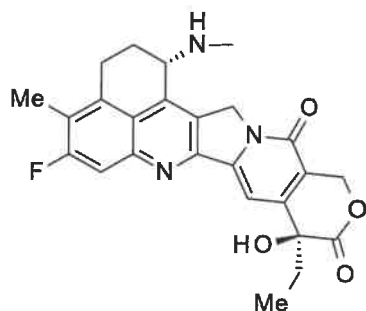
[0148] Further preferred are the following:



[0149] $-(\text{NH-DX})$ has a structure represented by the following formula:

[0150]

[Formula 10]



[0151] and it represents a group that is derived by removing one hydrogen atom of the amino group at position 1 of exatecan. In the above-described reaction scheme, the compound of formula (1) can be interpreted as having a structure in which one structure moiety from the drug to the linker terminus is connected to one antibody. However, this description is given for the sake of convenience, and there are actually many cases in which a plurality of the aforementioned structure moieties is connected to one antibody molecule. The same holds true for the explanation of the production method described below.

[0152] Specifically, the antibody-drug conjugate (1) can be produced by reacting the compound (2) obtainable by a known method (e.g., obtainable by a method described in the patent publication literature US2016/297890 (e.g., a method described in paragraphs [0336] to [0374])), with the antibody (3a) having a sulfhydryl group.

[0153] Provision of the sulfhydryl group on the antibody (3a) can be accomplished by a method well known to a person skilled in the art (Hermanson, G.T, Bioconjugate Techniques, pp. 56-136, pp. 456-493, Academic Press (1996)). Examples of the method can include, but are not limited to: Traut's reagent is reacted with the amino group of the antibody; N-succinimidyl S-acetylthioalkanoates are reacted with the amino group of the antibody followed by reaction with hydroxylamine; N-succinimidyl 3-(pyridyldithio)propionate is reacted with the antibody, followed by reaction with a reducing agent; the antibody is reacted with a reducing agent such as dithiothreitol, 2-mercaptoethanol, or tris(2-carboxyethyl)phosphine hydrochloride (TCEP) to reduce the interchain disulfide bond in the antibody, so as to form a sulfhydryl group.

[0154] Specifically, an antibody with interchain disulfide bonds partially or completely reduced can be obtained by using 0.3 to 3 molar equivalents of TCEP as a reducing agent per interchain disulfide bond in the antibody, and reacting the reducing agent with the antibody in a buffer solution containing a chelating agent. Examples of the chelating agent can include ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA). The chelating agent can be used at a concentration of 1 mM to 20 mM. A solution of sodium phosphate, sodium borate, sodium

acetate, or the like can be used as the buffer solution. As a specific example, the antibody (3a) having partially or completely reduced sulfhydryl groups can be obtained by reacting the antibody with TCEP at 4°C to 37°C for 1 to 4 hours.

[0155] It is to be noted that by carrying out an addition reaction of a sulfhydryl group to a drug-linker moiety, the drug-linker moiety can be conjugated by a thioether bond.

[0156] Then, using 2 to 20 molar equivalents of the compound (2) per antibody (3a) having a sulfhydryl group, the antibody-drug conjugate (1) in which 2 to 8 drug molecules are conjugated per antibody can be produced. Specifically, a solution containing the compound (2) dissolved therein may be added to a buffer solution containing the antibody (3a) having a sulfhydryl group for the reaction. In this context, a sodium acetate solution, sodium phosphate solution, sodium borate solution, or the like can be used as the buffer solution. pH for the reaction is 5 to 9, and more preferably, the reaction may be performed near pH 7. An organic solvent such as dimethyl sulfoxide (DMSO), dimethylformamide (DMF), dimethylacetamide (DMA), or N-methyl-2-pyrrolidone (NMP) can be used as a solvent for dissolving the compound (2). The reaction may be performed by adding the solution containing the compound (2) dissolved in the organic solvent at 1 to 20% v/v to a buffer solution

containing the antibody (3a) having a sulfhydryl group. The reaction temperature is 0 to 37°C, more preferably 10 to 25°C, and the reaction time is 0.5 to 2 hours. The reaction can be terminated by deactivating the reactivity of unreacted compound (2) with a thiol-containing reagent. The thiol-containing reagent is, for example, cysteine or N-acetyl-L-cysteine (NAC). More specifically, the reaction can be terminated by adding 1 to 2 molar equivalents of NAC to the compound (2) used, and incubating the obtained mixture at room temperature for 10 to 30 minutes.

[0157] (4) Identification of antibody-drug conjugate

The produced antibody-drug conjugate (e.g., antibody-drug conjugate (1)) can be subjected to concentration, buffer exchange, purification, and measurement of antibody concentration and an average number of conjugated drug molecules per antibody molecule according to common procedures described below, to identify the antibody-drug conjugate (1).

[0158] (4)-1 Common procedure A: Concentration of aqueous solution of antibody or antibody-drug conjugate

To Amicon Ultra (50,000 MWCO, Millipore Corporation) container, a solution of an antibody or an antibody-drug conjugate was added, and the solution of the antibody or the antibody-drug conjugate was concentrated by centrifugation (centrifugation at 2000 G to 3800 G for 5

to 20 minutes) using a centrifuge (Allegra X-15R, Beckman Coulter, Inc.)

[0159] (4)-2 Common procedure B: Measurement of antibody concentration

Using a UV detector (Nanodrop 1000, Thermo Fisher Scientific Inc.), measurement of the antibody concentration was carried out according to the method defined by the manufacturer. In this respect, 280 nm absorption coefficient differing among antibodies ($1.3 \text{ mLmg}^{-1}\text{cm}^{-1}$ to $1.8 \text{ mLmg}^{-1}\text{cm}^{-1}$) was used.

[0160] (4)-3 Common procedure C: Buffer exchange for antibody

A NAP-25 column (Cat. No. 17-0852-02, GE Healthcare Japan Corporation) using Sephadex G-25 carrier was equilibrated with a phosphate buffer (50 mM, pH 6.0) (referred to as PBS6.0/EDTA in the present description) containing sodium chloride (50 mM) and EDTA (2 mM) according to the method defined by the manufacturer. An aqueous solution of the antibody was applied in an amount of 2.5 mL per NAP-25 column, and thereafter, a fraction (3.5 mL) eluted with 3.5 mL of PBS6.0/EDTA was collected. This fraction was concentrated by common procedure A. After measurement of the concentration of the antibody using common procedure B, the antibody concentration was adjusted to 20 mg/mL using PBS6.0/EDTA.

[0161] (4)-4 Common procedure D: Purification of antibody-drug conjugate

A NAP-25 column was equilibrated with any commercially available buffer solution such as an acetate buffer containing sorbitol (5%) (10 mM, pH 5.5; referred to as ABS in the present description). An aqueous reaction solution of the antibody-drug conjugate (approximately 2.5 mL) was applied to the NAP-25 column, and thereafter, elution was carried out with the buffer solution in an amount defined by the manufacturer, so as to collect an antibody fraction. A gel filtration purification process, in which the collected fraction was applied again to the NAP-25 column, and elution was carried out with the buffer solution, was repeated a total of 2 or 3 times to obtain the antibody-drug conjugate excluding non-conjugated drug linker and low-molecular-weight compounds (tris(2-carboxyethyl)phosphine hydrochloride (TCEP), N-acetyl-L-cysteine (NAC), and dimethyl sulfoxide).

[0162] (4)-5 Common procedure E: Measurement of antibody concentration in antibody-drug conjugate and average number of conjugated drug molecules per antibody molecule

The conjugated drug concentration in the antibody-drug conjugate can be calculated by measuring UV absorbance of an aqueous solution of the antibody-drug conjugate at two wavelengths of 280 nm and 370 nm, and thereafter performing a calculation shown below.

[0163] The total absorbance at any given wavelength is equal to the sum of the absorbance of all light-absorbing

chemical species that are present in a system [additivity of absorbance]. Therefore, based on the hypothesis that the molar absorption coefficients of the antibody and the drug do not vary between before and after conjugation between the antibody and the drug, the antibody concentration and the drug concentration in the antibody-drug conjugate are represented by the following equations. [0164]

$$A_{280} = A_{D,280} + A_{A,280} = \epsilon_{D,280}C_D + \epsilon_{A,280}C_A \text{ Equation (1)}$$

$$A_{370} = A_{D,370} + A_{A,370} = \epsilon_{D,370}C_D + \epsilon_{A,370}C_A \text{ Equation (2)}$$

[0165] In this context, A_{280} represents the absorbance of an aqueous solution of the antibody-drug conjugate at 280 nm, A_{370} represents the absorbance of an aqueous solution of the antibody-drug conjugate at 370 nm, $A_{A,280}$ represents the absorbance of the antibody at 280 nm, $A_{A,370}$ represents the absorbance of the antibody at 370 nm, $A_{D,280}$ represents the absorbance of a conjugate precursor at 280 nm, $A_{D,370}$ represents the absorbance of the conjugate precursor at 370 nm, $\epsilon_{A,280}$ represents the molar absorption coefficient of the antibody at 280 nm, $\epsilon_{A,370}$ represents the molar absorption coefficient of the antibody at 370 nm, $\epsilon_{D,280}$ represents the molar absorption coefficient of the conjugate precursor at 280 nm, $\epsilon_{D,370}$ represents the molar absorption coefficient of the conjugate precursor at 370 nm, C_A represents the antibody concentration in the antibody-drug conjugate, and C_D

represents the drug concentration in the antibody-drug conjugate.

[0166] In this context, with regard to $\epsilon_{A,280}$, $\epsilon_{A,370}$, $\epsilon_{D,280}$, and $\epsilon_{D,370}$, preliminarily prepared values (estimated values based on calculation or measurement values obtained by UV measurement of the compound) are used. For example, $\epsilon_{A,280}$ can be estimated from the amino acid sequence of the antibody by a known calculation method (Protein Science, 1995, vol. 4, 2411-2423). $\epsilon_{A,370}$ is generally zero. $\epsilon_{D,280}$ and $\epsilon_{D,370}$ can be obtained according to Lambert-Beer's law (Absorbance = Molar concentration $\times$ Molar absorption coefficient $\times$ Cell path length) by measuring the absorbance of a solution in which the conjugate precursor used is dissolved at a certain molar concentration. C_A and C_D can be determined by measuring A_{280} and A_{370} of an aqueous solution of the antibody-drug conjugate, and then solving the simultaneous equations (1) and (2) by substitution of these values. Further, by dividing C_D by C_A , the average number of conjugated drug molecules per antibody can be determined.

[0167] (4)-6 Common procedure F: Measurement of average number of conjugated drug molecules per antibody molecule in antibody-drug conjugate - (2)

The average number of conjugated drug molecules per antibody molecule in the antibody-drug conjugate can also be determined by high-performance liquid chromatography (HPLC) analysis using the following method, in addition

to the aforementioned "(4)-5 Common procedure E". Hereinafter, the method for measuring the average number of conjugated drug molecules by HPLC when the antibody is conjugated to the drug linker by a disulfide bond will be described. A person skilled in the art is capable of appropriately measuring the average number of conjugated drug molecules by HPLC, depending on the connecting pattern between the antibody and the drug linker, with reference to this method.

[0168] F-1. Preparation of sample for HPLC analysis
(Reduction of antibody-drug conjugate)

An antibody-drug conjugate solution (approximately 1 mg/mL, 60 μ L) is mixed with an aqueous solution of dithiothreitol (DTT) (100 mM, 15 μ L). By incubating the mixture at 37°C for 30 minutes, the disulfide bond between the light chain and heavy chain of the antibody-drug conjugate is cleaved. The resulting sample is used in HPLC analysis.

[0169] F-2. HPLC analysis

The HPLC analysis is carried out under the following measurement conditions.

[0170] HPLC system: Agilent 1290 HPLC system (Agilent Technologies, Inc.)

Detector: Ultraviolet absorption spectrometer
(measurement wavelength: 280 nm)

Column: ACQUITY UPLC BEH Phenyl (2.1 $\times$ 50 mm, 1.7 μ m, 130 angstroms; Waters Corp., P/N 186002884)

Column temperature: 80°C

Mobile phase A: Aqueous solution containing 0.10% trifluoroacetic acid (TFA) and 15% 2-propanol

Mobile phase B: Acetonitrile solution containing 0.075% TFA and 15% 2-propanol

Gradient program: 14%-36% (0 min-15 min), 36%-80% (15 min-17 min), 80%-14% (17 min-17.01 min.), and 14% (17.01 min-25 min)

Sample injection: 10 μ L

or

HPLC system: Agilent 1290 HPLC system (Agilent Technologies, Inc.)

Detector: Ultraviolet absorption spectrometer (measurement wavelength: 280 nm)

Column: PLRP-S (2.1 $\times$ 50 mm, 8 μ m, 1000 angstroms; Agilent Technologies, Inc., P/N PL1912-1802)

Column temperature: 80°C

Mobile phase A: 0.04% aqueous TFA solution

Mobile phase B: Acetonitrile solution containing 0.04% TFA

Gradient program: 29%-36% (0 min-12.5 min), 36%-42% (12.5 min-15 min), 42%-29% (15 min-15.1 min), and 29%-29% (15.1 min-25 min)

Sample injection: 15 μ L

[0171] F-3. Data analysis

F-3-1. The light chain and heavy chain of the antibody are represented by L_i and H_i , respectively,

according to the number of conjugated drug molecules (wherein i represents the number of conjugated drug molecules, i.e., the number of conjugated drug molecules according to the present invention is represented by L_0 , L_1 , H_0 , H_1 , H_2 , H_3 , etc.)

Compared with non-conjugated antibody light (L_0) and heavy (H_0) chains, a light chain bound to one drug molecule (L_1), a heavy chain bound to one drug molecule (H_1), a heavy chain bound to two drug molecules (H_2), and a heavy chain bound to three drug molecules (H_3) exhibit higher hydrophobicity in proportion to the number of conjugated drug molecules and thus have a larger retention time. These chains are therefore eluted in the order of L_0 and L_1 or H_0 , H_1 , H_2 , and H_3 . Detection peaks can be assigned to any of L_0 , L_1 , H_0 , H_1 , H_2 , and H_3 by the comparison of retention times with L_0 and H_0 .

[0172] F-3-2. Since the drug linker absorbs UV, peak area values are corrected in response to the number of conjugated drug linker molecules according to the following expression using the molar absorption coefficients of the light chain or heavy chain and the drug linker.

[0173]

[Expression 1]

Corrected value of peak area of light chain bound to i drug molecule(s) (A_{Li}) = Peak area

$$\times \frac{\text{Molar absorption coefficient of light chain}}{\text{Molar absorption coefficient of light chain} + \text{The number of conjugated drug molecules (i)} \times \text{Molar absorption coefficient of drug linker}}$$

[0174]

[Expression 2]

Corrected value of peak area of heavy chain bound to i drug molecule(s) (A_{Hi}) = Peak area

$$\times \frac{\text{Molar absorption coefficient of heavy chain}}{\text{Molar absorption coefficient of heavy chain} + \text{The number of conjugated drug molecules (i)} \times \text{Molar absorption coefficient of drug linker}}$$

[0175] In this context, a value estimated from the amino acid sequence of the light chain or heavy chain of each antibody by a known calculation method (Protein Science, 1995, vol. 4, 2411-2423) can be used as the molar absorption coefficient (280 nm) of the light chain or heavy chain of the antibody. In the case of h046-H4e/L7, a molar absorption coefficient of 26210 and a molar absorption coefficient of 68990 were used as estimated values for the light chain and heavy chain, respectively, according to the amino acid sequence of the antibody. The actually measured molar absorption coefficient (280 nm) of a compound in which the maleimide group has been converted to succinimide thioether by the reaction of each drug linker with mercaptoethanol or N-acetylcysteine was used as the molar absorption coefficient (280 nm) of the drug linker. The wavelength for absorbance

measurement can be appropriately set by a person skilled in the art, but is preferably a wavelength at which the peak of the antibody can be measured, and more preferably 280 nm.

[0176] F-3-3. The peak area ratio (%) of each chain is calculated for the total of the corrected values of peak areas according to the following expression.

[0177]

[Expression 3]

$$\text{Peak area ratio of light chain bound to } i \text{ drug molecule(s)} = \frac{A_{Li}}{A_{L0} + A_{L1}} \times 100$$

$$\text{Peak area ratio of heavy chain bound to } i \text{ drug molecule(s)} = \frac{A_{Hi}}{A_{H0} + A_{H1} + A_{H2} + A_{H3}} \times 100$$

A_{Li} and A_{Hi} : Corrected values of peak areas of L_i and H_i , respectively

[0178]

F-3-4. The average number of conjugated drug molecules per antibody molecule in the antibody-drug conjugate is calculated according to the following expression.

[0179] Average number of conjugated drug molecules = (L_0 peak area ratio x 0 + L_1 peak area ratio x 1 + H_0 peak area ratio x 0 + H_1 peak area ratio x 1 + H_2 peak area ratio x 2 + H_3 peak area ratio x 3) / 100 x 2

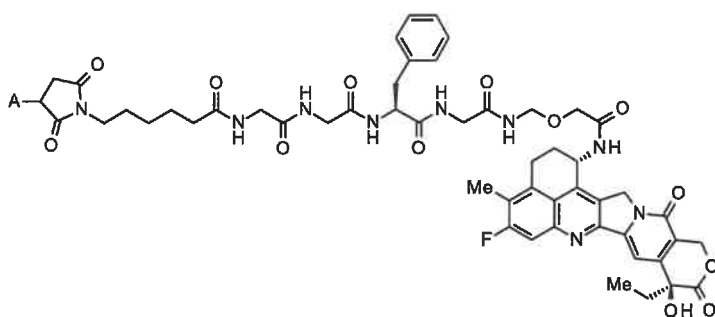
The compound represented by the formula (2) in production method 1 is a compound of any of the following formulas:

[0180] It is to be noted that, in order to secure the amount of the conjugate, a plurality of conjugates having almost the same average number of conjugated drug molecules (e.g., on the order of ± 1), which have been produced under similar conditions, can be mixed to prepare a new lot. In this case, the average number of drug molecules falls between the average numbers of drug molecules before mixing.

[0181] One specific example of the antibody-drug conjugate used in the present invention can include an antibody-drug conjugate in which a drug-linker structure moiety represented by the formula:

[0182]

[Formula 11]



[0183]

wherein A represents a connecting position to the antibody,
is conjugated to the anti-GPR20 antibody disclosed in the present description by a thioether bond.

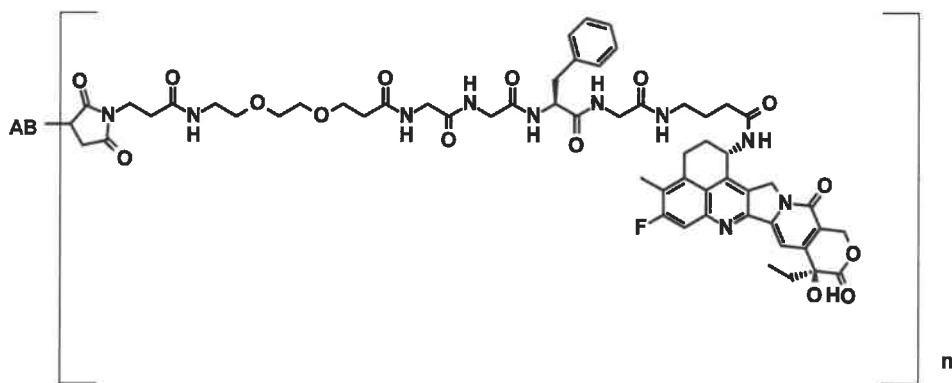
In the present invention, the partial structure consisting of the linker and the drug in the antibody-

drug conjugate is also referred to as a "drug-linker structure", a "drug-linker structure moiety" or a "drug linker". This drug linker is connected to a thiol group (in other words, a sulfur atom of a cysteine residue) formed at an interchain disulfide bond site (two heavy chain-heavy chain positions and two heavy chain-light chain positions) of the antibody.

[0184] One specific example of the antibody-drug conjugate of the present invention can include an antibody-drug conjugate having a structure represented by the following formula:

[0185]

[Formula 12]

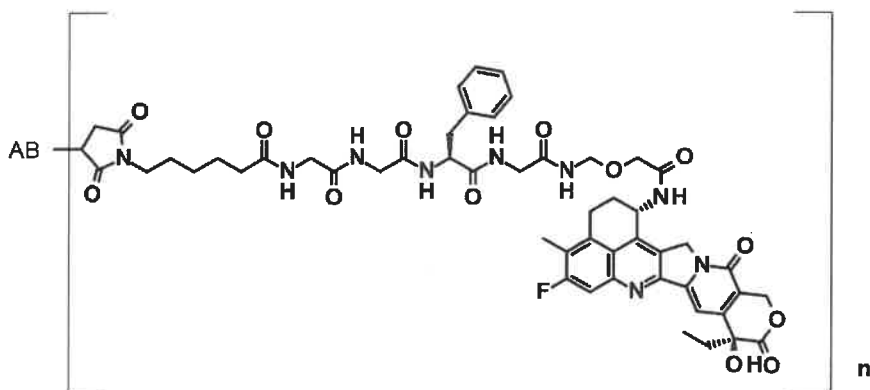


[0186]

or the following formula:

[0187]

[Formula 13]



[0188]

[0189] In this context, AB represents the anti-GPR20 antibody disclosed in the present description, and the antibody is conjugated to the drug linker via a sulfhydryl group derived from the antibody. In this context, n has the same meaning as that of so-called DAR (drug-to-antibody Ratio), and represents a drug-to-antibody ratio per antibody. Specifically, n represents the number of conjugated drug molecules per antibody molecule, which is a numeric value defined and indicated as an average value, i.e., the average number of conjugated drug molecules. In the case of the antibody-drug conjugate represented by [Formula 5] or [Formula 6] of the present invention, n can be 2 to 8 and is preferably 5 to 8, more preferably 7 to 8, and still more preferably 8, in measurement by common procedure F.

[0190] One example of the antibody-drug conjugate of the present invention can include an antibody-drug conjugate having a structure represented by the above-described

formula [Formula 10] or [Formula 11] wherein the antibody represented by AB is an antibody having a heavy chain and a light chain in any one combination selected from the group consisting of the following combinations (a) to (y), or a functional fragment of the antibody, or a pharmacologically acceptable salt of the antibody-drug conjugate: an antibody having a heavy chain and a light chain in any one combination selected from the group consisting of the following combinations (a) to (y), or a functional fragment of the antibody:

- (a) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 48 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 58,
- (b) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 48 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 60,
- (c) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 48 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 62,
- (d) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 48 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 64,

- (e) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 50 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 58,
- (f) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 50 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 60,
- (g) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 50 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 62,
- (h) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 50 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 64,
- (i) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 52 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 58,
- (j) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 52 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 60,
- (k) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 52 and a

light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 62,

(l) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 52 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 64,

(m) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 54 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 58,

(n) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 54 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 60,

(o) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 54 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 62,

(p) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 54 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 64,

(q) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 56 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 58,

(r) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 56 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 60,

(s) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 56 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 62,

(t) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 56 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 64,

(u) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 44 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 58,

(v) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 44 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 60,

(w) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 44 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 62,

(x) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 44 and a

light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 64, and (y) an antibody having any one combination selected from the group consisting of (a) to (x), wherein the heavy chain or the light chain comprises one or two or more modifications selected from the group consisting of N-linked glycosylation, O-linked glycosylation, N-terminal processing, C-terminal processing, deamidation, isomerization of aspartic acid, oxidation of methionine, addition of a methionine residue to the N-terminus, amidation of a proline residue, and a deletion of one or two amino acids at the carboxyl terminus.

[0191] 4. Medicament

Since the anti-GPR20 antibody of the present invention or the functional fragment of the antibody described in the above section "2. Production of anti-GPR20 antibody" and the Examples binds to GPR20 on the surface of tumor cells and has internalization activity, it can be used as a medicament, and in particular, as a therapeutic agent for cancer such as gastrointestinal stromal tumor, either alone or in combination with an additional drug.

[0192] Furthermore, the anti-GPR20 antibody of the present invention or the functional fragment of the antibody can be used in the detection of cells expressing GPR20.

[0193] Moreover, since the anti-GPR20 antibody of the present invention or the functional fragment of the antibody has internalization activity, it can be used as the antibody in an antibody-drug conjugate.

[0194] The anti-GPR20 antibody-drug conjugate of the present invention described in the above section "3. Anti-GPR20 antibody-drug conjugate" and the Examples, in which a drug having antitumor activity such as cytotoxic activity is used as the drug, is a conjugate of the anti-GPR20 antibody and/or the functional fragment of the antibody having internalization activity, and the drug having antitumor activity such as cytotoxic activity. Since this anti-GPR20 antibody-drug conjugate exhibits antitumor activity against cancer cells expressing GPR20, it can be used as a medicament, and in particular, as a therapeutic agent and/or a prophylactic agent for cancer.

[0195] The anti-GPR20 antibody-drug conjugate of the present invention may absorb moisture or have adsorption water, for example, to turn into a hydrate when it is left in air or subjected to recrystallization or purification procedures. Such a compound or a pharmacologically acceptable salt containing water is also included in the present invention.

[0196] When the anti-GPR20 antibody-drug conjugate of the present invention has a basic group such as an amino group, it can form a pharmacologically acceptable acid-addition salt, if desired. Examples of such an acid-

addition salt can include: hydrohalides such as hydrofluoride, hydrochloride, hydrobromide, and hydroiodide; inorganic acid salts such as nitrate, perchlorate, sulfate, and phosphate; lower alkanesulfonates such as methanesulfonate, trifluoromethanesulfonate, and ethanesulfonate; arylsulfonates such as benzenesulfonate and p-toluenesulfonate; organic acid salts such as formate, acetate, trifluoroacetate, malate, fumarate, succinate, citrate, tartrate, oxalate, and maleate; and amino acid salts such as an ornithine salt, glutamate, and aspartate.

[0197] When the anti-GPR20 antibody-drug conjugate of the present invention has an acidic group such as a carboxy group, it can form a pharmacologically acceptable base-addition salt, if desired. Examples of such a base-addition salt can include: alkali metal salts such as a sodium salt, a potassium salt, and a lithium salt; alkaline earth metal salts such as a calcium salt and a magnesium salt; inorganic salts such as an ammonium salt; and organic amine salts such as a dibenzylamine salt, a morpholine salt, a phenylglycine alkyl ester salt, an ethylenediamine salt, an N-methylglucamine salt, a diethylamine salt, a triethylamine salt, a cyclohexylamine salt, a dicyclohexylamine salt, an N,N'-dibenzylethylenediamine salt, a diethanolamine salt, an N-benzyl-N-(2-phenylethoxy)amine salt, a piperazine salt,

a tetramethylammonium salt, and a tris(hydroxymethyl)aminomethane salt.

[0198] The present invention can also include an anti-GPR20 antibody-drug conjugate in which one or more atoms constituting the antibody-drug conjugate are replaced with isotopes of the atoms. There exist two types of isotopes: radioisotopes and stable isotopes. Examples of the isotope can include isotopes of hydrogen (^2H and ^3H), isotopes of carbon (^{11}C , ^{13}C and ^{14}C), isotopes of nitrogen (^{13}N and ^{15}N), isotopes of oxygen (^{15}O , ^{17}O and ^{18}O), and isotopes of fluorine (^{18}F). A composition comprising the antibody-drug conjugate labeled with such an isotope is useful as, for example, a therapeutic agent, a prophylactic agent, a research reagent, an assay reagent, a diagnostic agent, and an *in vivo* diagnostic imaging agent. Each of every antibody-drug conjugate labeled with an isotope, and mixtures of antibody-drug conjugates labeled with an isotope at any given ratio are included in the present invention. The antibody-drug conjugate labeled with an isotope can be produced, for example, by using a starting material labeled with an isotope instead of a starting material for the production method of the present invention mentioned later according to a method known in the art.

[0199] *In vitro* cytotoxicity can be measured based on the activity of suppressing the proliferative responses of cells, for example. For example, a cancer cell line

overexpressing GPR20 is cultured, and the anti-GPR20 antibody-drug conjugate is added at different concentrations to the culture system. Thereafter, its suppressive activity against focus formation, colony formation and spheroid growth can be measured. In this context, by using a gastrointestinal stromal tumor (GIST)-derived cancer cell line, cell proliferation-suppressive activity against gastrointestinal stromal tumor can be examined.

In vivo therapeutic effects on cancer in an experimental animal can be measured, for example, by administering the anti-GPR20 antibody-drug conjugate to a nude mouse into which a tumor cell line highly expressing GPR20 has been transplanted, and then measuring a change in the cancer cells. In this context, by using an animal model derived from an immunodeficient mouse by the transplantation of gastrointestinal stromal tumor-derived cells, therapeutic effects on gastrointestinal stromal tumor can be measured.

[0200] The type of cancer to which the anti-GPR20 antibody-drug conjugate of the present invention is applied is not particularly limited as long as the cancer expresses GPR20 in cancer cells to be treated. Examples thereof can include cancer in digestive organs such as the esophagus, the stomach, the small intestine, and the large intestine, though the cancer is not limited thereto as long as the cancer expresses GPR20. More preferred

examples of the cancer can include gastrointestinal stromal tumor (GIST).

The anti-GPR20 antibody-drug conjugate of the present invention can preferably be administered to a mammal, and more preferably to a human.

[0201] A substance used in a pharmaceutical composition comprising the anti-GPR20 antibody-drug conjugate of the present invention can be appropriately selected from and used like pharmaceutical additives and the like usually used in this field, in terms of the administered dose or the administered concentration.

[0202] The anti-GPR20 antibody-drug conjugate of the present invention can be administered as a pharmaceutical composition comprising one or more pharmaceutically compatible components. For example, the pharmaceutical composition typically comprises one or more pharmaceutical carriers (e.g., sterilized liquids (e.g., water and oil (including petroleum oil and oil of animal origin, plant origin, or synthetic origin (e.g., peanut oil, soybean oil, mineral oil, and sesame oil)))). Water is a more typical carrier when the pharmaceutical composition is intravenously administered. An aqueous saline solution, an aqueous dextrose solution, and an aqueous glycerol solution can also be used as a liquid carrier, in particular, for an injection solution. Suitable pharmaceutical vehicles are known in the art. If desired, the composition may also comprise a trace

amount of a moisturizing agent, an emulsifying agent, or a pH buffering agent. Examples of suitable pharmaceutical carriers are disclosed in "Remington's Pharmaceutical Sciences" by E. W. Martin. The prescription corresponds to an administration mode.

[0203] Various delivery systems are known, and they can be used for administering the anti-GPR20 antibody-drug conjugate of the present invention. Examples of the administration route can include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, and subcutaneous routes. Administration can be performed by injection or bolus injection, for example. According to a specific preferred embodiment, the administration of the above-described antibody-drug conjugate is performed by injection. Parenteral administration is a preferred administration route.

[0204] According to a representative embodiment, the pharmaceutical composition is prescribed, as a pharmaceutical composition suitable for intravenous administration to a human, according to conventional procedures. The composition for intravenous administration is typically a solution in a sterile and isotonic aqueous buffer solution. If necessary, the medicament may also contain a solubilizing agent and a local anesthetic to alleviate pain at an injection area (e.g., lignocaine). In general, the above-described ingredients are provided, either separately or together

in a mixture in a unit dosage form, as a freeze-dried powder or an anhydrous concentrate contained in a container which is obtained by sealing in, for example, an ampoule or a sachet indicating the amount of the active agent. When the medicament is to be administered by injection, it may be administered using, for example, an injection bottle containing water or saline of sterile pharmaceutical grade. When the medicament is to be administered by injection, an ampoule of sterile water or saline for injection may be provided such that the above-described ingredients are admixed with one another before administration.

[0205] The pharmaceutical composition of the present invention may be a pharmaceutical composition comprising only the anti-GPR20 antibody-drug conjugate of the present application, or may be a pharmaceutical composition comprising the anti-GPR20 antibody-drug conjugate and at least one other therapeutic agent for cancer. The anti-GPR20 antibody-drug conjugate of the present invention can also be administered together with an additional therapeutic agent for cancer, and can thereby enhance an anticancer effect. The additional anticancer agent used for such a purpose may be administered to an individual, simultaneously, separately, or continuously, together with the antibody-drug conjugate. Otherwise, the additional anticancer agent and the anti-GPR20 antibody-drug conjugate may each be

administered to the subject at different administration intervals. Examples of such a therapeutic agent for cancer can include tyrosine kinase inhibitors including imatinib, sunitinib, and regorafenib, CDK4/6 inhibitors including palbociclib, HSP90 inhibitors including TAS-116, MEK inhibitors including MEK162, and immune checkpoint inhibitors including nivolumab, pembrolizumab, and ipilimumab, though the therapeutic agent for cancer is not limited thereto as long as the drug has antitumor activity.

[0206] Such a pharmaceutical composition can be prepared as a formulation having a selected composition and a necessary purity in the form of a freeze-dried formulation or a liquid formulation. The pharmaceutical composition prepared as a freeze-dried formulation may be a formulation containing an appropriate pharmaceutical additive used in this field. Likewise, the liquid formulation can be prepared such that the liquid formulation contains various pharmaceutical additives used in this field.

[0207] The composition and concentration of the pharmaceutical composition also vary depending on the administration method. With regard to the affinity of the anti-GPR20 antibody-drug conjugate comprised in the pharmaceutical composition of the present invention for the antigen, i.e., the dissociation constant (K_d value) of the anti-GPR20 antibody-drug conjugate to the antigen,

as the affinity increases (i.e., the K_d value is low), the pharmaceutical composition can exert medicinal effects, even if the applied dose thereof is decreased. Accordingly, the applied dose of the antibody-drug conjugate can also be determined by setting the applied dose based on the status of the affinity of the antibody-drug conjugate for the antigen. When the antibody-drug conjugate of the present invention is administered to a human, it may be administered at a dose of, for example, from approximately 0.001 to 100 mg/kg once or a plurality of times at intervals of 1 to 180 days. It can be administered preferably at a dose of from 0.1 to 50 mg/kg and more preferably 1 to 15 mg/kg a plurality of times at intervals of 2 to 3 weeks.

Examples

[0208] Hereinafter, the present invention will be specifically described in the following examples. However, these examples are not intended to limit the scope of the present invention. Furthermore, these examples should not be construed in a limited manner by any means. It is to be noted that, in the following examples, unless otherwise specified, individual operations regarding genetic manipulation have been carried out according to the method described in "Molecular Cloning" (Sambrook, J., Fritsch, E. F. and Maniatis, T., published by Cold Spring Harbor Laboratory

Press in 1989) or other methods described in experimental manuals used by persons skilled in the art, or when commercially available reagents or kits have been used, the examples have been carried out in accordance with the instructions included in the commercially available products. In the present description, reagents, solvents and starting materials are readily available from commercially available sources, unless otherwise specified.

[0209] Example 1: Production of rat anti-human GPR20 antibody having internalization activity

1)-1 Construction of human GPR20 expression vector

Using human brain-derived cDNA as a template, cDNA encoding human GPR20 protein (NP_005284) was amplified by PCR according to a method known to a person skilled in the art, and the amplification product was incorporated into a vector for mammalian expression to produce human GPR20 expression vector pcDNA3.1-hGPR20. The amino acid sequence of the human GPR20 is shown in SEQ ID NO: 1 in the sequence listing. EndoFree Plasmid Giga Kit (Qiagen N.V.) was used in the large scale preparation of pcDNA3.1-hGPR20 plasmid DNA.

[0210] 1)-2 Immunization of rats

For immunization, 6-week-old WKY/Izm female rats (Japan SLC, Inc.) were used. First, the lower legs of each rat were pre-treated with Hyaluronidase (Sigma-Aldrich Co. LLC), and thereafter, the human GPR20

expression vector pcDNA3.1-hGPR20 was intramuscularly injected into the same sites. Subsequently, employing ECM830 (BTX), *in vivo* electroporation was carried out on the same sites using a two-needle electrode. Once every two weeks, the same *in vivo* electroporation was repeated. On the 79th day, lymph nodes were collected from the rat, and then used in hybridoma preparation.

[0211] 1)-3 Hybridoma preparation

The lymph node cells were electrically fused with mouse myeloma SP2/0-ag14 cells using the Hybrimmune Hybridoma Production System (Cyto Pulse Sciences, Inc.), and the cells were then suspended and diluted with ClonaCell-HY Selection Medium D (StemCell Technologies Inc.), and then cultured under conditions of 37°C and 5% CO₂. Individual hybridoma colonies that appeared in the culture were collected as monoclonal hybridomas, then suspended in ClonaCell-HY Selection Medium E (StemCell Technologies Inc.), and then cultured under conditions of 37°C and 5% CO₂. After moderate proliferation of cells, frozen stocks of individual hybridoma cells were produced, while a culture supernatant was collected from each hybridoma, and used to screen for anti-GPR20 antibody-producing hybridomas.

[0212] 1)-4 Antibody-producing hybridoma screening by Cell-ELISA method

1)-4-1 Preparation of antigen gene-expressing cells for use in Cell-ELISA

293 α cells (a stable expression cell line derived from HEK293 cells expressing integrin α v and integrin β 3) were prepared at 5×10^5 cells/10 mL in DMEM medium supplemented with 10% FBS. In accordance with transfection procedures for using Lipofectamine 2000 (Invitrogen Corp.), DNA of pcDNA3.1-hGPR20 or pcDNA3.1 as a negative control was introduced into the 293 α cells, and the cells were dispensed in an amount of 100 μ L/well to a 96-well plate (Corning Inc.). Thereafter, the cells were cultured under conditions of 37°C and 5% CO₂ in DMEM medium supplemented with 10% FBS for 24 to 27 hours. The obtained transfected cells were used for Cell-ELISA in an adhesive state.

[0213] 1)-4-2 Cell-ELISA

The culture supernatant of the 293 α cells transfected with the expression vector prepared in Example 1)-4-1 was removed, and the culture supernatant from each hybridoma was then added to the 293 α cells transfected either with pcDNA3.1-hGPR20 or pcDNA3.1. The cells were left standing at 4°C for 1 hour. The cells in the wells were washed once with PBS (+) supplemented with 5% FBS, and thereafter, Anti-Rat IgG-Peroxidase antibody produced in rabbit (Sigma-Aldrich Co. LLC) that had been 500-fold diluted with PBS (+) supplemented with 5% FBS was added to the wells. The cells were left standing at 4°C for 1 hour. The cells in the wells were washed three times with PBS (+) supplemented with 5% FBS, and

thereafter, OPD chromogenic solution (which had been prepared by dissolving o-phenylenediamine dihydrochloride (Wako Pure Chemical Industries, Ltd.) and H₂O₂ in an OPD solution (0.05 M trisodium citrate, 0.1 M disodium hydrogen phosphate 12-water; pH 4.5), so that the substances became 0.4 mg/ml and 0.6% (v/v), respectively) was added in an amount of 100 µL/well to the wells. A coloring reaction was carried out with occasional stirring. Thereafter, 1 M HCl was added to the plate (100 µL/well) to terminate the coloring reaction, followed by measurement of the absorbance at 490 nm using a plate reader (ENVISION: PerkinElmer, Inc.). In order to select hybridomas that produce an antibody specifically binding to human GPR20 expressed on the cell membrane surface, hybridomas that produced a culture supernatant exhibiting higher absorbance in the 293α cells transfected with the pcDNA3.1-hGPR20 expression vector than that in the 293α cells transfected with the control pcDNA3.1 were selected.

[0214] 1)-5 Human GPR20-binding antibody screening by flow cytometry

1)-5-1 Preparation of antigen gene-expressing cells for use in flow cytometry analysis

293T cells were seeded in a 225-cm² flask (Sumitomo Bakelite Co., Ltd.) at 5 × 10⁴ cells/cm², and the cells were then cultured overnight under conditions of 37°C and 5% CO₂ in DMEM medium supplemented with 10% FBS.

pcDNA3.1-hGPR20 or pcDNA3.1 as a negative control was introduced into the 293T cells using Lipofectamine 2000, and the cells were further cultured overnight under conditions of 37°C and 5% CO₂. The 293T cells transfected with each expression vector were treated with TrypLE Express (Life Technologies Corp.), and the cells were washed with DMEM supplemented with 10% FBS, and then suspended in PBS supplemented with 5% FBS. The obtained cell suspension was used in flow cytometry analysis.

[0215] 1)-5-2 Flow cytometry analysis

The binding specificity to human GPR20 of an antibody produced from hybridomas that had been determined to be positive by Cell-ELISA in Example 1)-4-2 was further confirmed by flow cytometry. The suspension of the transiently expressing 293T cells prepared in Example 1)-5-1 was centrifuged, and a supernatant was then removed. Thereafter, the cells were suspended by the addition of the culture supernatant from each hybridoma. The cells were left standing at 4°C for 1 hour. The cells were washed twice with PBS supplemented with 5% FBS, and thereafter, the cells were suspended by the addition of Anti-Rat IgG FITC conjugate (Sigma-Aldrich Co. LLC) that had been 500-fold diluted with PBS supplemented with 5% FBS. The cells were left standing at 4°C for 1 hour. The cells were washed twice with PBS supplemented with 5% FBS, and then re-suspended in PBS supplemented with 5% FBS and 2 µg/ml 7-aminoactinomycin D

(Molecular Probes, Inc.), followed by detection using a flow cytometer (FC500; Beckman Coulter, Inc.). The data was analyzed using Flowjo (Tree Star, Inc.). After dead cells were removed from analysis by gating out 7-aminoactinomycin D-positive cells, a histogram of the FITC fluorescence intensity of live cells was generated. Hybridomas producing human GPR20-binding antibodies (178 clones) were selected based on results where the histogram for the antibody shifted to the strong fluorescence intensity side in the 293T cells transfected with pcDNA3.1-hGPR20 compared with the 293T cells transfected with the control pcDNA3.1. Figure 18 shows results for clone Nos. 04-002, 04-006, 04-013, 04-014, 04-020, 04-021, 04-037, 04-046, 04-047, 04-060, 04-067, 04-068, 04-079, 04-084, 04-114, 04-115, 04-117, 04-125, 04-126, 04-127, 04-133, 04-139, 04-143, 04-145, 04-151 and 04-163, and the control (w/o 1st Ab) as examples of antibodies specifically binding to human GPR20. The abscissa of Figure 18 depicts clone No., and the ordinate thereof depicts the amount of the antibody bound based on MFI (mean fluorescence intensity).

[0216] 1)-6 Screening for hybridoma producing anti-GPR20 antibody having internalization activity

The internalization activity of the anti-GPR20 antibodies was evaluated using an anti-rat IgG antibody reagent Rat-ZAP (Advanced Targeting Systems) conjugated with a toxin (saporin) inhibiting protein synthesis.

Specifically, 293 α cells caused to transiently express human GPR20 were seeded at 3×10^3 cells/well over a 96-well plate, and then cultured overnight under conditions of 37°C and 5% CO₂. After the plate was cooled on ice, 20 μ L of the culture supernatant of each anti-GPR20 antibody-producing hybridoma was added to each well, and the plate was left standing at 4°C for 1 hour. After removal of the culture supernatant by suction, DMEM supplemented with 10% FBS and 500 ng/mL Rat-ZAP was added to the plate, and the cells were cultured under conditions of 37°C and 5% CO₂ for 3 days. The number of live cells was measured by the quantification of ATP using CellTiter-Glo (registered trademark) Luminescent Cell Viability Assay. In this screening, Rat-ZAP is taken up into cells in a manner dependent on the internalization activity of the rat anti-GPR20 antibody, so that saporin inhibiting protein synthesis is released into the cells, so as to suppress cell proliferation. As a result of making a selection using a cell proliferation suppression rate of 60% or more as an indicator, 19 hybridomas (clone Nos: 04-002, 04-006, 04-013, 04-014, 04-021, 04-037, 04-046, 04-047, 04-067, 04-068, 04-079, 04-114, 04-115, 04-125, 04-126, 04-127, 04-133, 04-139 and 04-163) producing anti-GPR20 antibodies having internalization activity were selected.

[0217] 1)-7 Determination of subclass and type of rat monoclonal antibody

The heavy chain subclasses and light chain types of the rat anti-human GPR20 monoclonal antibodies were determined using a RAT MONOCLONAL ANTIBODY ISOTYPING TEST KIT (DS Pharma Biomedical Co., Ltd.). As a result, it was confirmed that clone Nos. 04-047 and 04-068 had IgG2a and κ chains, and that clone Nos. 04-002, 04-006, 04-013, 04-014, 04-021, 04-037, 04-046, 04-067, 04-079, 04-114, 04-115, 04-125, 04-126, 04-127, 04-133, 04-139 and 04-163 had IgG2b and κ chains.

[0218] 1)-8 Preparation of rat anti-human GPR20 antibody

The rat anti-human GPR20 monoclonal antibodies were purified from the hybridoma culture supernatants.

[0219] First, the volume of each rat anti-GPR20 monoclonal antibody-producing hybridoma was sufficiently increased with ClonaCell-HY Selection Medium E (StemCell Technologies Inc.), and thereafter, the medium was exchanged with Hybridoma SFM (Life Technologies Corp.) supplemented with 20% of Ultra Low IgG FBS (Life Technologies Corp.). Thereafter, the hybridoma was cultured for 4 to 5 days. The resulting culture supernatant was harvested, and insoluble matter was removed therefrom by passing through a 0.8- μ m filter, and through a 0.2- μ m filter.

[0220] An antibody was purified from the hybridoma supernatant by Protein G affinity chromatography (4 to 6°C) in one step. A buffer replacement step after the Protein G affinity chromatography purification was

carried out at 4 to 6°C. First, the culture supernatant of the hybridoma was applied to a column that had been packed with Protein G (GE Healthcare Biosciences Corp.) equilibrated with PBS. After the entire culture solution had entered the column, the column was washed with PBS in an amount of two or more times the volume of the column. Subsequently, the antibody was eluted with a 0.1 M glycine/HCl aqueous solution (pH 2.7), so that a fraction containing an antibody was collected. Immediately, the pH of the collected fraction was adjusted to 7.0 to 7.5 by the addition of 1 M Tris-HCl (pH 9.0). Thereafter, using Centrifugal UF Filter Device VIVASPIN20 (molecular weight cutoff: UF30K, Sartorius Inc., 4 to 6°C), the buffer was replaced with PBS, while the antibody was concentrated, so that the concentration of the antibody was adjusted to 0.2 mg/mL or more. Finally, the antibody was filtrated through Minisart-Plus filter (Sartorius Inc.) to obtain a purified antibody sample.

[0221] Example 2: *In vitro* evaluation of rat anti-GPR20 antibody

2)-1 Evaluation of binding ability of rat anti-GPR20 antibody by flow cytometry

In order to evaluate GPR20-binding ability, the suspension of the 293T cells transfected with pcDNA3.1-hGPR20, which had been produced by the method shown in 1)-5-1, was centrifuged, and a supernatant was then removed. Thereafter, the cells were suspended by the

addition of each of the 19 rat anti-human GPR20 monoclonal antibodies (clone Nos: 04-002, 04-006, 04-013, 04-014, 04-021, 04-037, 04-046, 04-047, 04-067, 04-068, 04-079, 04-114, 04-115, 04-125, 04-126, 04-127, 04-133, 04-139 and 04-163) having internalization activity, and the 2 rat anti-human GPR20 monoclonal antibodies (13-024 and 13-048), which had been prepared in 1)-8, or rat IgG control (R&D Systems, Inc.). The cells were left standing at 4°C for 1 hour. The cells were washed twice with PBS supplemented with 5% FBS, and then suspended by the addition of Goat Anti-Rat IgG (H+L), PE conjugate (Beckman Coulter, Inc.) that had been 320-fold diluted with PBS supplemented with 5% FBS. The cells were left standing at 4°C for 1 hour. The cells were washed twice with PBS supplemented with 5% FBS, followed by detection using a flow cytometer (FC500; Beckman Coulter, Inc.). The results are shown in Figure 19. In Figure 19, the abscissa depicts an antibody concentration (nM), and the ordinate depicts the amount of the antibody bound based on MFI (mean fluorescence intensity). As shown in Figure 19, all the amounts of the rat anti-human GPR20 antibodies bound to the 293T cells transfected with pcDNA3.1-hGPR20 were increased in a concentration-dependent manner. On the other hand, rat IgG2a and IgG2b isotype control antibodies did not exhibit GPR20-binding activity.

[0222] 2)-2 Internalization activity of rat anti-GPR20 antibody

The internalization activity of the purified rat anti-GPR20 antibodies was evaluated using an anti-rat IgG antibody reagent Rat-ZAP (Advanced Targeting Systems) conjugated with a toxin (saporin) inhibiting protein synthesis, in the same manner as that applied in 1)-6. Specifically, HEK293 cells stably expressing GPR20-EGFP protein comprising human GPR20 linked at its C-terminus to EGFP were seeded at 2.5×10^3 cells/well, and then cultured overnight under conditions of 37°C and 5% CO₂. Thereafter, each rat anti-GPR20 antibody (final concentration: 0.012 to 1 µg/mL) and Rat-ZAP (final concentration: 0.5 µg/mL) were added to the culture. The cells were cultured for 5 days, and thereafter, the number of live cells was measured by the quantification of ATP using CellTiter-Glo (registered trademark) Luminescent Cell Viability Assay. A cell proliferation-suppressive effect brought about by the addition of each anti-GPR20 antibody was indicated by relative activity to the number of live cells without antibody addition defined as 100%. Each anti-GPR20 antibody exhibited the maximum suppression of cell proliferation, when added at a final concentration of 0.11 or 0.33 µg/mL. Figure 20 shows a cell survival rate at the concentration at which each antibody exhibited the maximum suppression of cell proliferation. It is believed that antibodies having

strong internalization activity in this experiment exhibit a low cell survival rate. Rat IgG2a and IgG2b isotype control antibodies (R&D Systems, Inc.) were used as negative control antibodies recognizing an antigen unrelated to GPR20.

[0223] Example 3: Determination of nucleotide sequence of cDNA encoding variable region of rat anti-GPR20 antibody

The nucleotide sequence of cDNA encoding each of the variable regions of the 21 rat anti-GPR20 antibodies (04-002, 04-006, 04-013, 04-014, 04-021, 04-037, 04-046, 04-047, 04-067, 04-068, 04-079, 04-114, 04-115, 04-125, 04-126, 04-127, 04-133, 04-139, 04-163, 13-024, and 13-048) evaluated for their internalization activity in Example 2 was determined by the following method.

[0224] 3)-1 cDNA synthesis

A cell lysate of each anti-GPR20 antibody-producing hybridoma (50 mM Tris-HCl (pH 7.5), 250 mM LiCl, 5 mM EDTA (pH 8), 0.5% lithium dodecyl sulfate (LiDS), 2.5 mM dithiothreitol (DTT)) was mixed with oligo dT25-conjugated magnetic beads (Dynabeads mRNA DIRECT Kit, Life Technologies Corp.), so that mRNA was bound to the magnetic beads. Subsequently, the magnetic beads were washed once each with mRNA washing solution A (10 mM Tris-HCl (pH 7.5), 0.15 M LiCl, 1 mM EDTA, 0.1% LiDS, 0.1% Triton X-100) and with a solution for cDNA synthesis (50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl₂, 5 mM DTT, 0.5 mM dNTP, 0.2% Triton X-100, 1.2 units of RNase

inhibitor (Life Technologies Corp.)), followed by cDNA synthesis in a solution for cDNA synthesis to which 12 units of SuperScript III Reverse Transcriptase (Life Technologies Corp.) had been added. Subsequently, the magnetic beads were washed with a 3' tailing reaction solution (50 mM potassium phosphate, 4 mM MgCl₂, 0.5 mM dGTP, 0.2% Triton X-100, 1.2 units of RNase inhibitor (Life Technologies Corp.)), and thereafter, a 3' tailing reaction was carried out using a reaction solution to which 48 units of Terminal Transferase, recombinant (F. Hoffmann-La Roche, Ltd.) had been added.

[0225] 3)-2 Amplification and sequencing of rat immunoglobulin heavy and light chain variable region gene fragments

The magnetic beads were washed with a TE solution (10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.1% Triton X-100), and thereafter, the rat immunoglobulin heavy chain and light chain genes were amplified by 5'-RACE PCR. Specifically, the magnetic beads were transferred to a PCR reaction solution (0.2 μ M each primer, 0.2 mM each dNTP, 0.25 units of PrimeSTAR HS DNA Polymerase (Takara Bio Inc.)), and the reaction was carried out at 35 cycles each involving 94°C for 30 seconds and 68°C for 90 seconds. The primer sets used were as described below. In the primer sequences, D represents a mixed base consisting of A, G, or T, and N represents a mixed base of A, C, G, or T.

[0226] PCR primer set (for heavy chain)

5'-GCTAGCGCTACCGGACTCAGATCCCCCCCCCCCCDN-3' (Nhe-polyC-S)
(SEQ ID NO: 66)

5'-TCACTGAGCTGGTGAGAGTGTAGAGCCC-3' (rIg γ -AS1) (SEQ ID NO:
67)

5'-TCACCGAGCTGCTGAGGGTGTAGAGCCC-3' (rIg γ -AS2) (SEQ ID NO:
68)

PCR primer set (for light chain)

5'-GCTAGCGCTACCGGACTCAGATCCCCCCCCCCCCDN-3' (Nhe-polyC-S)
(SEQ ID NO: 66) (which was the same as that for the heavy
chain)

5'-TCAGTAACACTGTCCAGGACACCATCTC-3' (rIg κ -AS) (SEQ ID NO:
69)

[0227] The fragments amplified by the above-described PCR
reaction were sequenced to analyze their nucleotide
sequences. Oligonucleotides having the nucleotide
sequences 5'-CTGGCTCAGGGAAATAGCC-3' (rIg γ -seq) (SEQ ID
NO: 70) and 5'-TCCAGTTGCTAACTGTTCC-3' (rIg κ -seq) (SEQ ID
NO: 71) were used as a sequencing primer for the heavy
chain and a sequencing primer for the light chain,
respectively.

The sequencing was carried out using a gene sequence
analysis apparatus ("ABI PRISM 3700 DNA Analyzer; Applied
Biosystems, Inc." or "Applied Biosystems 3730xl Analyzer;
Applied Biosystems, Inc."). In the sequencing reaction,
Dye Terminator Cycle Sequencing System with AmpliTaq DNA

polymerase (Life Technologies Corp.) and GeneAmp 9700 (Applied Biosystems, Inc.) were used.

[0228] As a result of comparing, with one another, amino acid sequences predicted from the determined nucleotide sequences encoding the heavy chain and light chain variable regions of the 22 rat anti-GPR20 antibodies, the antibodies were classified into the following 3 groups: group A (04-002, 04-006, 04-013, 04-014, 04-037, 04-046, 04-047, 04-067, 04-068, 04-079, 04-114, 04-125, 04-126, 04-127, 04-133, 04-139, and 04-163), group B (04-021 and 04-115), and group C (13-024 and 13-048).

The full-length sequences of the heavy chain and light chain of each antibody were determined by linking them to known constant region sequences. The nucleotide sequence and amino acid sequence of the constant region of rat heavy chain IgG2b were used with reference to the nucleotide sequence and the amino acid sequence of AABR03048905 (IGHG2B*01) disclosed in IMGT, the international ImMunoGeneTics information system (registered trademark). The nucleotide sequence and the amino acid sequence of the constant region of rat light chain IgK were used with reference to the nucleotide sequence and amino acid sequence of V01241 (IGKC*01) also disclosed in this system.

[0229] The heavy chain of the 04-046 antibody has the amino acid sequence shown in SEQ ID NO: 2 in the sequence listing. In the heavy chain amino acid sequence shown in

SEQ ID NO: 2 in the sequence listing, the amino acid sequence consisting of the amino acid residues at positions 1 to 19 is a signal sequence, the amino acid sequence consisting of the amino acid residues at positions 20 to 142 is a variable region, and the amino acid sequence consisting of the amino acid residues at positions 143 to 475 is a constant region. The aforementioned variable region has CDRH1 consisting of the amino acid sequence at positions 45 to 54, CDRH2 consisting of the amino acid sequence at positions 69 to 78, and CDRH3 consisting of the amino acid sequence at positions 118 to 131, in SEQ ID NO: 2 in the sequence listing. The heavy chain variable region of the 04-046 antibody has the amino acid sequence shown in SEQ ID NO: 3 in the sequence listing. The CDRH1 of the 04-046 antibody has the amino acid sequence shown in SEQ ID NO: 4 in the sequence listing, the amino acid sequence of the CDRH2 has the amino acid sequence shown in SEQ ID NO: 5 in the sequence listing, and the amino acid sequence of the CDRH3 has the amino acid sequence shown in SEQ ID NO: 6 in the sequence listing. Furthermore, the sequence of the heavy chain of the 04-046 antibody is shown in Figure 1.

[0230] The light chain of the 04-046 antibody has the amino acid sequence shown in SEQ ID NO: 7 in the sequence listing. In the light chain amino acid sequence shown in SEQ ID NO: 7 in the sequence listing, the amino acid

sequence consisting of the amino acid residues at positions 1 to 21 is a signal sequence, the amino acid sequence consisting of the amino acid residues at positions 21 to 128 is a variable region, and the amino acid sequence consisting of the amino acid residues at positions 129 to 233 is a constant region. The aforementioned variable region has CDRL1 consisting of the amino acid sequence at positions 43 to 53, CDRL2 consisting of the amino acid sequence at positions 69 to 75, and CDRL3 consisting of the amino acid sequence at positions 108 to 116, in SEQ ID NO: 7 in the sequence listing. The light chain variable region of the 04-046 antibody has the amino acid sequence shown in SEQ ID NO: 8 in the sequence listing. The CDRL1 of the 04-046 antibody has the amino acid sequence shown in SEQ ID NO: 9 in the sequence listing, the amino acid sequence of the CDRL2 has the amino acid sequence shown in SEQ ID NO: 10 in the sequence listing, and the amino acid sequence of the CDRL3 has the amino acid sequence shown in SEQ ID NO: 11 in the sequence listing. Furthermore, the sequence of the light chain of the 04-046 antibody is shown in Figure 2.

[0231] The heavy chain of the 04-079 antibody has the amino acid sequence shown in SEQ ID NO: 12 in the sequence listing. In the heavy chain amino acid sequence shown in SEQ ID NO: 12 in the sequence listing, the amino acid sequence consisting of the amino acid residues at

positions 1 to 19 is a signal sequence, the amino acid sequence consisting of the amino acid residues at positions 20 to 142 is a variable region, and the amino acid sequence consisting of the amino acid residues at positions 143 to 475 is a constant region. The aforementioned variable region has CDRH1 consisting of the amino acid sequence at positions 45 to 54, CDRH2 consisting of the amino acid sequence at positions 69 to 78, and CDRH3 consisting of the amino acid sequence at positions 118 to 131, in SEQ ID NO: 12 in the sequence listing. The heavy chain variable region of the 04-079 antibody has the amino acid sequence shown in SEQ ID NO: 13 in the sequence listing. The CDRH1 of the 04-079 antibody has the amino acid sequence shown in SEQ ID NO: 14 in the sequence listing, the amino acid sequence of the CDRH2 has the amino acid sequence shown in SEQ ID NO: 15 in the sequence listing, and the amino acid sequence of the CDRH3 has the amino acid sequence shown in SEQ ID NO: 16 in the sequence listing. Furthermore, the sequence of the heavy chain of the 04-079 antibody is shown in Figure 3.

[0232] The light chain of the 04-079 antibody has the amino acid sequence shown in SEQ ID NO: 17 in the sequence listing. In the light chain amino acid sequence shown in SEQ ID NO: 17 in the sequence listing, the amino acid sequence consisting of the amino acid residues at positions 1 to 20 is a signal sequence, the amino acid

sequence consisting of the amino acid residues at positions 21 to 128 is a variable region, and the amino acid sequence consisting of the amino acid residues at positions 129 to 233 is a constant region. The aforementioned variable region has CDRL1 consisting of the amino acid sequence at positions 43 to 53, CDRL2 consisting of the amino acid sequence at positions 69 to 75, and CDRL3 consisting of the amino acid sequence at positions 108 to 116, in SEQ ID NO: 17 in the sequence listing. The light chain variable region of the 04-079 antibody has the amino acid sequence shown in SEQ ID NO: 18 in the sequence listing. The CDRL1 of the 04-079 antibody has the amino acid sequence shown in SEQ ID NO: 19 in the sequence listing, the amino acid sequence of the CDRL2 has the amino acid sequence shown in SEQ ID NO: 20 in the sequence listing, and the amino acid sequence of the CDRL3 has the amino acid sequence shown in SEQ ID NO: 21 in the sequence listing. Furthermore, the sequence of the light chain of the 04-079 antibody is shown in Figure 4.

[0233] The heavy chain of the 04-126 antibody has the amino acid sequence shown in SEQ ID NO: 22 in the sequence listing. In the heavy chain amino acid sequence shown in SEQ ID NO: 22 in the sequence listing, the amino acid sequence consisting of the amino acid residues at positions 1 to 19 is a signal sequence, the amino acid sequence consisting of the amino acid residues at

positions 20 to 142 is a variable region, and the amino acid sequence consisting of the amino acid residues at positions 143 to 475 is a constant region. The aforementioned variable region has CDRH1 consisting of the amino acid sequence at positions 45 to 54, CDRH2 consisting of the amino acid sequence at positions 69 to 78, and CDRH3 consisting of the amino acid sequence at positions 118 to 131, in SEQ ID NO: 22 in the sequence listing. The heavy chain variable region of the 04-126 antibody has the amino acid sequence shown in SEQ ID NO: 23 in the sequence listing. The CDRH1 of the 04-126 antibody has the amino acid sequence shown in SEQ ID NO: 24 in the sequence listing, the amino acid sequence of the CDRH2 has the amino acid sequence shown in SEQ ID NO: 25 in the sequence listing, and the amino acid sequence of the CDRH3 has the amino acid sequence shown in SEQ ID NO: 26 in the sequence listing. Furthermore, the sequence of the heavy chain of the 04-126 antibody is shown in Figure 5.

[0234] The light chain of the 04-126 antibody has the amino acid sequence shown in SEQ ID NO: 27 in the sequence listing. In the light chain amino acid sequence shown in SEQ ID NO: 27 in the sequence listing, the amino acid sequence consisting of the amino acid residues at positions 1 to 20 is a signal sequence, the amino acid sequence consisting of the amino acid residues at positions 21 to 128 is a variable region, and the amino

acid sequence consisting of the amino acid residues at positions 129 to 233 is a constant region. The aforementioned variable region has CDRL1 consisting of the amino acid sequence at positions 43 to 53, CDRL2 consisting of the amino acid sequence at positions 69 to 75, and CDRL3 consisting of the amino acid sequence at positions 108 to 116, in SEQ ID NO: 27 in the sequence listing. The light chain variable region of the 04-126 antibody has the amino acid sequence shown in SEQ ID NO: 28 in the sequence listing. The CDRL1 of the 04-126 antibody has the amino acid sequence shown in SEQ ID NO: 29 in the sequence listing, the amino acid sequence of the CDRL2 has the amino acid sequence shown in SEQ ID NO: 30 in the sequence listing, and the amino acid sequence of the CDRL3 has the amino acid sequence shown in SEQ ID NO: 31 in the sequence listing. Furthermore, the sequence of the light chain of the 04-126 antibody is shown in Figure 6.

[0235] The heavy chain amino acid sequence of the 04-046 antibody is encoded by the nucleotide sequence shown in SEQ ID NO: 32 in the sequence listing. In the nucleotide sequence shown in SEQ ID NO: 32 in the sequence listing, the nucleotide sequence consisting of the nucleotides at positions 58 to 426 encodes the heavy chain variable region of the 04-046 antibody, and the nucleotide sequence consisting of the nucleotides at positions 427 to 1425 encodes the heavy chain constant region of the

04-046 antibody. The nucleotide sequence encoding the aforementioned variable region has a polynucleotide consisting of the nucleotide sequence at nucleotide positions 133 to 162 encoding CDRH1, a polynucleotide consisting of the nucleotide sequence at nucleotide positions 205 to 234 encoding CDRH2, and a polynucleotide consisting of the nucleotide sequence at nucleotide positions 352 to 393 encoding CDRH3, in SEQ ID NO: 32. The nucleotide sequence of the heavy chain variable region of the 04-046 antibody is also shown in SEQ ID NO: 33 in the sequence listing. Furthermore, the sequence of SEQ ID NO: 32 is shown in Figure 1.

[0236] The light chain amino acid sequence of the 04-046 antibody is encoded by the nucleotide sequence shown in SEQ ID NO: 34 in the sequence listing. In the nucleotide sequence shown in SEQ ID NO: 34 in the sequence listing, the nucleotide sequence consisting of the nucleotides at positions 61 to 384 encodes the light chain variable region of the 04-046 antibody, and the nucleotide sequence consisting of the nucleotides at positions 385 to 699 encodes the light chain constant region of the 04-046 antibody. The nucleotide sequence encoding the aforementioned variable region has a polynucleotide consisting of the nucleotide sequence at nucleotide positions 127 to 159 encoding CDRL1, a polynucleotide consisting of the nucleotide sequence at nucleotide positions 205 to 225 encoding CDRL2, and a polynucleotide

consisting of the nucleotide sequence at nucleotide positions 322 to 348 encoding CDRL3, in SEQ ID NO: 34.

The nucleotide sequence of the light chain variable region of the 04-046 antibody is also shown in SEQ ID NO: 35 in the sequence listing. Furthermore, the sequence of SEQ ID NO: 34 is shown in Figure 2.

[0237] The heavy chain amino acid sequence of the 04-079 antibody is encoded by the nucleotide sequence shown in SEQ ID NO: 36 in the sequence listing. In the nucleotide sequence shown in SEQ ID NO: 36 in the sequence listing, the nucleotide sequence consisting of the nucleotides at positions 58 to 426 encodes the heavy chain variable region of the 04-079 antibody, and the nucleotide sequence consisting of the nucleotides at positions 427 to 1425 encodes the heavy chain constant region of the 04-079 antibody. The nucleotide sequence encoding the aforementioned variable region has a polynucleotide consisting of the nucleotide sequence at nucleotide positions 133 to 162 encoding CDRH1, a polynucleotide consisting of the nucleotide sequence at nucleotide positions 205 to 234 encoding CDRH2, and a polynucleotide consisting of the nucleotide sequence at nucleotide positions 352 to 393 encoding CDRH3, in SEQ ID NO: 36. The nucleotide sequence of the heavy chain variable region of the 04-079 antibody is also shown in SEQ ID NO: 37 in the sequence listing. Furthermore, the sequence of SEQ ID NO: 37 is shown in Figure 10.

[0238] The light chain amino acid sequence of the 04-079 antibody is encoded by the nucleotide sequence shown in SEQ ID NO: 38 in the sequence listing. In the nucleotide sequence shown in SEQ ID NO: 38 in the sequence listing, the nucleotide sequence consisting of the nucleotides at positions 61 to 384 encodes the light chain variable region of the 04-079 antibody, and the nucleotide sequence consisting of the nucleotides at positions 385 to 699 encodes the light chain constant region of the 04-079 antibody. The nucleotide sequence encoding the aforementioned variable region has a polynucleotide consisting of the nucleotide sequence at nucleotide positions 127 to 159 encoding CDRL1, a polynucleotide consisting of the nucleotide sequence at nucleotide positions 205 to 225 encoding CDRL2, and a polynucleotide consisting of the nucleotide sequence at nucleotide positions 322 to 348 encoding CDRL3, in SEQ ID NO: 38. The nucleotide sequence of the light chain variable region of the 04-079 antibody is also shown in SEQ ID NO: 39 in the sequence listing. Furthermore, the sequence of SEQ ID NO: 38 is shown in Figure 4.

[0239] The heavy chain amino acid sequence of the 04-126 antibody is encoded by the nucleotide sequence shown in SEQ ID NO: 40 in the sequence listing. In the nucleotide sequence shown in SEQ ID NO: 40 in the sequence listing, the nucleotide sequence consisting of the nucleotides at positions 58 to 426 encodes the heavy chain variable

region of the 04-126 antibody, and the nucleotide sequence consisting of the nucleotides at positions 427 to 1425 encodes the heavy chain constant region of the 04-126 antibody. The nucleotide sequence encoding the aforementioned variable region has a polynucleotide consisting of the nucleotide sequence at nucleotide positions 133 to 162 encoding CDRH1, a polynucleotide consisting of the nucleotide sequence at nucleotide positions 205 to 234 encoding CDRH2, and a polynucleotide consisting of the nucleotide sequence at nucleotide positions 352 to 393 encoding CDRH3, in SEQ ID NO: 40. The nucleotide sequence of the heavy chain variable region of the 04-126 antibody is also shown in SEQ ID NO: 41 in the sequence listing. Furthermore, the sequence of SEQ ID NO: 40 is shown in Figure 5.

[0240] The light chain amino acid sequence of the 04-126 antibody is encoded by the nucleotide sequence shown in SEQ ID NO: 42 in the sequence listing. In the nucleotide sequence shown in SEQ ID NO: 42 in the sequence listing, the nucleotide sequence consisting of the nucleotides at positions 61 to 384 encodes the light chain variable region of the 04-126 antibody, and the nucleotide sequence consisting of the nucleotides at positions 385 to 699 encodes the light chain constant region of the 04-126 antibody. The nucleotide sequence encoding the aforementioned variable region has a polynucleotide consisting of the nucleotide sequence at nucleotide

positions 127 to 159 encoding CDRL1, a polynucleotide consisting of the nucleotide sequence at nucleotide positions 205 to 225 encoding CDRL2, and a polynucleotide consisting of the nucleotide sequence at nucleotide positions 322 to 348 encoding CDRL3, in SEQ ID NO: 42. The nucleotide sequence of the light chain variable region of the 04-1216 antibody is also shown in SEQ ID NO: 43 in the sequence listing. Furthermore, the sequence of SEQ ID NO: 42 is shown in Figure 6.

[0241] Example 4: Analysis of GPR20-binding site of anti-GPR20 monoclonal antibody

Binding sites of anti-human GPR20 antibodies were classified by measuring their binding activity against mouse GPR20, N-terminally FLAG-tagged human GPR20, and human/mouse chimeric GPR20 in which each of four extracellular regions (EC1, EC2, EC3, and EC4) of human GPR20 was substituted with a mouse GPR20-derived sequence, respectively, by Cell-ELISA. Figure 21 is a diagram of the comparison between the amino acid sequences of human GPR20 (NP_005284) and mouse GPR20 (NP_775541). The four extracellular regions EC1 to EC4 are represented by positions in the amino acid sequence of human GPR20. EC1 corresponds to positions 1 to 48, EC2 corresponds to positions 108 to 125, EC3 corresponds to positions 190 to 196, and EC4 corresponds to positions 260 to 275.

[0242] 4)-1 Construction of mouse GPR20 expression vector

According to a method known to a person skilled in the art, cDNA was artificially synthesized based on Ref Seq sequence NM_173365 of mouse GPR20, and then cloned into pcDNA-DEST40 expression vector (Invitrogen Corp.) to construct mouse GPR20 expression vector pcDNA-mGPR20.

[0243] 4)-2 Construction of N-terminally FLAG-tagged human GPR20 and human/mouse chimeric GPR20 expression vectors

4)-2-1

In the construction of expression vectors given below, using the full-length human GPR20 expression vector pcDNA3.1-hGPR20 produced in Example 1 as a template, each PCR reaction was carried out with the primer set given below. In this reaction, KOD FX DNA polymerase (Toyobo Co., Ltd.) was used, and the reaction was carried out at 15 cycles or 10 cycles each involving 98°C for 10 seconds, 58°C for 30 seconds, and 68°C for 7 minutes. Thereafter, the obtained PCR product was treated with the restriction enzyme DpnI. *Escherichia coli* TOP10 (Invitrogen Corp.) was transformed with this DpnI-digested DNA to construct expression vectors for N-terminally FLAG-tagged human GPR20 and human GPR20 with EC2 or EC3 or EC4 substituted with a mouse GPR20-derived sequence. The primer set used in each PCR reaction was as follows.

[0244] PCR primer set (for N-terminally FLAG-tagged human GPR20)

5'-GACTACAAAGACGATGACGACAAGCCCTCTGTGTCTCCAGC-3' (NFLAG-1;

SEQ ID NO: 72)

5'-CTTGTCGTCATCGTCTTTGTAGTCCATGGTGGAGCCTGC-3' (NFLAG-2;

SEQ ID NO: 73)

PCR primer set (for human GPR20 with EC2 substituted with a mouse GPR20-derived sequence)

5'-ACGCGCTTCGCTGTGTTCTACGGCGCCAG-3' (mEC2-1; SEQ ID NO:

74)

5'-CTGGCGCCGTAGAACACAGCGAAGCGCGT-3' (mEC2-2; SEQ ID NO:

75)

PCR primer set (for human GPR20 with EC3 substituted with a mouse GPR20-derived sequence)

5'-TGTCGGTGCTGGGCGTGAAGTCGGGTGGACGATCATGCTGCCGTGTCTT-3'

(mEC3-1; SEQ ID NO: 76)

5'-AAGACACGGCAGCATGATCGTCCACCCGACTTCACGCCCAGCACCGACA-3'

(mEC3-2; SEQ ID NO: 77)

PCR primer set (for human GPR20 with EC4 substituted with a mouse GPR20-derived sequence)

5'-TGGCGCTGTGGCCCAACGTACCTAAGCACACGAGCCTCGTGGT-3' (mEC4-

1; SEQ ID NO: 78)

5'-ACCACGAGGCTCGTGTGCTTAGGTACGTTGGGCCACAGCGCCA-3' (mEC4-

2; SEQ ID NO: 79)

[0245] 4)-2-2

An expression vector for human GPR20 with EC1 substituted with a mouse GPR20-derived sequence was constructed using an In-Fusion (registered trademark) HD Cloning Kit (Clontech Laboratories, Inc.). Specifically,

using the full-length human GPR20 expression vector pcDNA3.1-hGPR20 produced in Example 1 as a template, a PCR reaction was carried out with the primer set given below and KOD FX DNA polymerase (Toyobo Co., Ltd.) at 10 cycles each involving 98°C for 10 seconds, 58°C for 30 seconds, and 68°C for 7 minutes to amplify a DNA fragment comprising the vector sequence.

PCR primer set (for human GPR20 with EC1 substituted with a mouse GPR20-derived sequence - 1)

5'-GCGCTGATGGCGGTGCACGGAGCCATCT-3' (mEC1-1; SEQ ID NO: 80)

5'-AGAGGGCATGGTGGAGCCTGCTTT-3' (mEC1-2; SEQ ID NO: 81)

[0246] Also, using the full-length mouse GPR20 expression vector pcDNA-mGPR20 constructed in 4)-1 as a template, a PCR reaction was carried out with the primer set given below at 20 cycles in the same manner as above to amplify a DNA fragment encoding the EC1 region of mouse GPR20.

PCR primer set (for human GPR20 with EC1 substituted with a mouse GPR20-derived sequence - 2)

5'-AGGCTCCACCATGCCCTCTGCGTTGTCTATGA-3' (mEC1-3; SEQ ID NO: 82)

5'-CACCGCCATCAGCGCTTGCCACAGGCTGGGGAAGGTGGCTTGCA-3' (mEC1-4; SEQ ID NO: 83)

The above-described two DNA fragments were subjected to agarose gel electrophoresis according to the standard method, and DNA fragments having the size of interest were isolated using QIAquick Gel Extraction Kit (Promega

Corp.). These two DNA fragments were annealed together through the reaction of an In-Fusion HD enzyme (Clontech Laboratories, Inc.), and *Escherichia coli* TOP10 (Invitrogen Corp.) was transformed with the ligation product to construct an expression vector for human GPR20 with ECD1 substituted with a mouse GPR20-derived sequence. [0247] 4)-3 Evaluation of binding properties of anti-human GPR20 antibody by Cell-ELISA

The binding activity of the anti-GPR20 antibodies 04-046, 04-079, 04-126, 04-021, 13-024, and 13-048 against 293 α cells into which different GPR20 expression vectors were transiently introduced was examined by the same method as the Cell-ELISA method shown in Example 1. The measurement results are shown in Figures 22(a) and 22(b). On the abscissa of Figure 22, EV depicts a control, human GPR20 depicts cells expressing human full-length GPR20, FLAG-huGPR20 depicts cells expressing N-terminally FLAG-tagged human GPR20, mouse GPR20 depicts cells expressing mouse GPR20, hGPR20_mECD1 depicts cells expressing chimeric GPR20 in which ECD1 of human GPR20 was substituted with ECD1 of mouse GPR20, hGPR20_mECD2 depicts cells expressing chimeric GPR20 in which ECD2 of human GPR20 was substituted with ECD2 of mouse GPR20, hGPR20_mECD3 depicts cells expressing chimeric GPR20 in which ECD3 of human GPR20 was substituted with ECD3 of mouse GPR20, and hGPR20_mECD4 depicts cells expressing

chimeric GPR20 in which ECD4 of human GPR20 was substituted with ECD4 of mouse GPR20.

[0248] Among the three classifications based on antibody sequence similarity shown in Example 3, 04-046, 04-079 and 04-126 in group A did not bind to mouse GPR20, and lost binding activity as a result of substituting EC1 (also referred to as ECD1) or EC2 (also referred to as ECD2) of human GPR20 with that of mouse-derived GPR20. This indicates that 04-046, 04-079 and 04-126 recognize a conformation consisting of EC1 and EC2 of GPR20.

[0249] 04-021 in group B exhibited weak binding activity against mouse GPR20, and the binding activity was attenuated to the same level as the binding activity against mouse GPR20 as a result of substituting EC1 of human GPR20 with that of mouse-derived GPR20.

Furthermore, 04-021 did not exhibit binding activity against N-terminally FLAG-tagged human GPR20, indicating that this antibody recognizes the N-terminus or its neighborhood of GPR20 EC1.

[0250] 13-024 and 13-048 in group C did not bind to mouse GPR20, and the binding activity was remarkably decreased as a result of substituting EC1 of human GPR20 with that of mouse-derived GPR20, indicating that these antibodies bind to EC1.

[0251] From the above-described experiment, Table 1 shows the correspondence between the GPR20 extracellular region recognized by each antibody and the internalization

activity (the internalization activity is stronger as the cell survival rate is lower) of the antibody shown in Figure 20.

[0252]

[Table 1]

Group classification based on amino acid sequence of antibody	Antibody No.	Subclass	Cell survival rate (%) in internalization activity evaluation	GPR20 extracellular region recognized
A	04-126	IgG2b	54	EC1, EC2
A	04-114	IgG2b	57	EC1, EC2
A	04-046	IgG2b	57	EC1, EC2
A	04-067	IgG2b	58	EC1, EC2
A	04-002	IgG2b	60	EC1, EC2
A	04-014	IgG2b	60	EC1, EC2
A	04-163	IgG2b	61	EC1, EC2
A	04-139	IgG2b	61	EC1, EC2
A	04-079	IgG2b	62	EC1, EC2
A	04-006	IgG2b	63	EC1, EC2
A	04-127	IgG2b	63	EC1, EC2
A	04-125	IgG2b	64	EC1, EC2
A	04-013	IgG2b	67	EC1, EC2
A	04-133	IgG2b	67	EC1, EC2
A	04-047	IgG2a	69	EC1, EC2
B	04-021	IgG2b	70	EC1 N-terminus
A	04-037	IgG2b	71	EC1, EC2
C	13-048	IgG2b	74	EC1
A	04-068	IgG2a	76	EC1, EC2
C	13-024	IgG2b	77	EC1
B	04-115	IgG2b	86	EC1 N-terminus

[0253] When the "GPR20 extracellular region recognized" in the table is indicated by the range of the amino acid sequence of human GPR20, EC1 corresponds to positions 1 to 48, EC2 corresponds to positions 108 to 125, EC3 corresponds to positions 190 to 196, and EC4 corresponds to positions 260 to 275. As a result of studying the relationship between the internalization activity (the

internalization activity is higher as the cell survival rate is lower) of each antibody and the region recognized by the anti-GPR20 antibody, it was found that all the antibodies that exhibited a cell survival rate of less than 70% and thus seemed to have high internalization activity were antibodies derived from group A. Therefore, it was demonstrated that antibodies that exhibit high internalization activity are concentrated in antibodies of group A which recognize a conformation consisting of EC1 and EC2 of human GPR20. EC2 of human GPR20 differed only in the tyrosine residue at position 113 from that (phenylalanine) of mouse GPR20, suggesting that antibodies of group A which recognize a conformation consisting of EC1 and EC2 recognize this tyrosine residue.

[0254] Example 5: Preparation of human chimeric anti-GPR20 antibody 04-046Ch

5)-1 Construction of human chimeric anti-GPR20 antibody expression vector

5)-1-1 Construction of chimeric and humanized antibody light chain expression vector pCMA-LK

An approx. 5.4-kb fragment, which had been obtained by digesting plasmid pcDNA3.3-TOPO/LacZ (Invitrogen Corp.) with the restriction enzymes XbaI and PmeI, was ligated with a DNA fragment comprising a DNA sequence (SEQ ID NO: 84) encoding a human κ chain secretion signal and a human κ chain constant region, using an In-Fusion

Advantage PCR cloning kit (Clontech Laboratories, Inc.), to prepare pCDNA3.3/LK.

[0255] Using pCDNA3.3/LK as a template, PCR was carried out with the primer set given below, and the obtained approx. 3.8-kb fragment was phosphorylated and then self-ligated to construct the chimeric and humanized antibody light chain expression vector pCMA-LK having a signal sequence, a cloning site, and the DNA sequence of the human κ chain constant region downstream of a CMV promoter.

Primer set

5'-TATACCGTCGACCTCTAGCTAGAGCTTGGC-3' (3.3-F1; SEQ ID NO: 85)

5'-GCTATGGCAGGGCCTGCCGCCCCGACGTTG-3' (3.3-R1; SEQ ID NO: 86)

[0256] 5)-1-2 Construction of chimeric and humanized antibody IgG1 type heavy chain expression vector pCMA-G1

A DNA fragment, which had been obtained by digesting pCMA-LK with XbaI and PmeI to remove the DNA sequence encoding the κ chain secretion signal and the human κ chain constant region therefrom, was ligated to a DNA fragment comprising a DNA sequence (SEQ ID NO: 87) encoding a human heavy chain signal sequence and the amino acids in a human IgG1 constant region, using an In-Fusion Advantage PCR cloning kit (Clontech Laboratories, Inc.), to construct a chimeric and humanized antibody IgG1 type heavy chain expression vector pCMA-G1 having a

signal sequence, a cloning site, and the DNA sequence of the human IgG1 heavy chain constant region downstream of CMV promoter.

[0257] 5)-1-3 Construction of human chimeric anti-GPR20 antibody heavy chain expression vector

A human chimeric antibody heavy chain expression vector was constructed based on the amino acid sequence (SEQ ID NO: 3) of the heavy chain variable region of the rat anti-GPR20 antibody 04-046 determined in Example 3)-2. A DNA fragment corresponding to nucleotide positions 36 to 443 including a DNA sequence encoding the variable region in the nucleotide sequence (SEQ ID NO: 46) of the heavy chain of the human chimeric anti-GPR20 antibody 04-046Ch was synthesized (GENEART, artificial gene synthesis service). Using the synthesized DNA fragment as a template, a DNA fragment comprising a DNA sequence encoding the heavy chain variable region of the human chimeric anti-GPR20 antibody was amplified with KOD - Plus- (Toyobo Co., Ltd.) and the primer set given below. Using an In-Fusion HD PCR cloning kit (Clontech Laboratories, Inc.), the amplified DNA fragment was inserted into a site of the chimeric and humanized antibody IgG1 type heavy chain expression vector pCMA-G1 that had been cleaved with the restriction enzyme BspI, so as to construct a human chimeric anti-GPR20 antibody 04-046Ch heavy chain expression vector. The obtained expression vector was named "pCMA/04-046Ch-H". The amino

acid sequence of the heavy chain of the human chimeric anti-GPR20 antibody 04-046Ch is shown in SEQ ID NO: 44. The nucleotide sequence of SEQ ID NO: 46 and the amino acid sequence of SEQ ID NO: 44 are also shown in Figure 7.

Primer set

5'-AGCTCCCAGATGGGTGCTGAGC-3' (EG-Inf-F; SEQ ID NO: 88)

5'-GGGCCCTTGGTGGAGGCTGAGC-3' (EG1-Inf-R; SEQ ID NO: 89)

[0258] 5)-1-4 Construction of human chimeric anti-GPR20 antibody light chain expression vector

A human chimeric antibody light chain expression vector was constructed based on the amino acid sequence (SEQ ID NO: 8) of the light chain variable region of the rat anti-GPR20 antibody 04-046 determined in Example 3)-2. A DNA fragment corresponding to nucleotide positions 38 to 399 including a DNA sequence encoding the variable region in the nucleotide sequence (SEQ ID NO: 47) of the light chain of the human chimeric anti-GPR20 antibody 04-046Ch was synthesized (GENEART, artificial gene synthesis service). Using the synthesized DNA fragment as a template, a DNA fragment comprising a DNA sequence encoding the light chain variable region of the human chimeric anti-GPR20 antibody was amplified with KOD - Plus- (Toyobo Co., Ltd.) and the primer set given below. Using an In-Fusion HD PCR cloning kit (Clontech Laboratories, Inc.), the amplified DNA fragment was inserted into a site of the chimeric and humanized antibody light chain expression vector pCMA-LK that had

been cleaved with the restriction enzyme BsiWI, so as to construct a human chimeric anti-GPR20 antibody 04-046Ch light chain expression vector. The obtained expression vector was named "pCMA/04-046Ch-L". The amino acid sequence of the light chain of the human chimeric anti-GPR20 antibody 04-046Ch is shown in SEQ ID NO: 45. The nucleotide sequence of SEQ ID NO: 47 and the amino acid sequence of SEQ ID NO: 45 are also shown in Figure 8.

Primer set

5'-CTGTGGATCTCCGGCGGTACGGC-3' (CM-LKF; SEQ ID NO: 90)
5'-GGAGGGGGCGGCCACGGCTCTCTTCAGTTC-3' (046L-R; SEQ ID NO: 91)

5)-2 Expression and purification of human chimeric anti-GPR20 antibody

5)-2-1 Expression of human chimeric anti-GPR20 antibody

In accordance with the manual, FreeStyle 293F cells (Invitrogen Corp.) were cultured and passaged. 1.2×10^9 FreeStyle 293F cells (Invitrogen Corp.) in the logarithmic growth phase were seeded on 3-L Fernbach Erlenmeyer Flask (Corning Inc.), then diluted with FreeStyle 293 expression medium (Invitrogen Corp.) at 2.0×10^6 cells/mL, and shake-cultured at 90 rpm in an 8% CO₂ incubator at 37°C for 1 hour. 1.8 mg of Polyethyleneimine (Polyscience #24765) was dissolved in 20 mL of Opti-Pro SFM medium (Invitrogen Corp.). Meanwhile, the heavy chain expression vector (0.24 mg)

and the light chain expression vector (0.36 mg) prepared using NucleoBond Xtra (Takara Bio Inc.) were added to 20 mL of Opti-Pro SFM medium (Invitrogen Corp.). To 20 mL of the Polyethyleneimine/Opti-Pro SFM mixed solution, 20 mL of the expression vector/Opti-Pro SFM mixed solution was added, and the obtained mixture was gently stirred. After incubation for 5 minutes, the mixture was added to the FreeStyle 293F cells. The cells were shake-cultured at 90 rpm in an 8% CO₂ incubator at 37°C for 4 hours, and thereafter, 600 mL of EX-CELL VPRO medium (SAFC Biosciences Inc.), 18 mL of GlutaMAX I (GIBCO), and 30 mL of Yeastolate Ultrafiltrate (GIBCO) were added to the culture. The cells were further shake-cultured at 90 rpm in an 8% CO₂ incubator at 37°C for 7 days. The obtained culture supernatant was filtrated through a Disposable Capsule Filter (Advantec #CCS-045-E1H).

[0259] A human chimeric anti-GPR20 antibody obtained by the combination of pCMA/04-046Ch-H and pCMA/04-046Ch-L was named "04-046Ch".

[0260] 5)-2-2 Purification of 04-046Ch by two-step process

The antibody was purified from the culture supernatant obtained in Example 5)-2-1, by a two-step process, namely, by rProtein A affinity chromatography (at 4 to 6°C) and ceramic hydroxyapatite (at room temperature). Buffer replacement after the rProtein A affinity chromatography purification and after the

ceramic hydroxyapatite purification was carried out at 4 to 6°C. The culture supernatant was applied to MabSelectSuRe (GE Healthcare Biosciences Corp., HiTrap column) that had been equilibrated with PBS. After the entire culture solution had entered the column, the column was washed with PBS in an amount of two or more times the volume of the column. Subsequently, elution was carried out using a 2 M arginine hydrochloride solution (pH 4.0), so that a fraction containing an antibody was collected. This fraction was dialyzed (Thermo Fisher Scientific Inc., Slide-A-Lyzer Dialysis Cassette), so that the buffer was replaced with PBS. Thereafter, an antibody solution that had been 5-fold diluted with a buffer of 5 mM sodium phosphate/50 mM MES/pH 7.0 was applied to a ceramic hydroxyapatite column (Bio-Rad Laboratories, Inc., Bio-Scale CHT Type-1 Hydroxyapatite Column) that had been equilibrated with a buffer of 5 mM NaPi/50 mM MES/30 mM NaCl/pH 7.0. Elution was carried out on a linear concentration gradient of sodium chloride, so that a fraction containing an antibody was collected. This fraction was dialyzed (Thermo Fisher Scientific Inc., Slide-A-Lyzer Dialysis Cassette), so that the buffer was replaced with HBSor (25 mM histidine/5% sorbitol, pH 6.0). The antibody was concentrated with Centrifugal UF Filter Device VIVASPIN20 (molecular weight cutoff: UF10K, Sartorius Inc., 4°C), thereby adjusting the IgG concentration to 20 mg/mL or

more. Finally, the antibody was filtrated through a Minisart-Plus filter (Sartorius Inc.) to obtain a purified sample.

[0261] 5)-3 Evaluation of binding activity of human chimeric anti-GPR20 antibody

The GPR20-binding activity of the produced human chimeric anti-GPR20 antibody 04-046Ch was confirmed by flow cytometry. Using Lipofectamine 2000, pcDNA3.1-hGPR20 or pcDNA3.1 was transiently introduced into 293T cells by the same method as that applied in Example 1)-5-1. The cells were cultured overnight under conditions of 37°C and 5% CO₂, and thereafter, a cell suspension was prepared. To the obtained cell suspension, the human chimeric anti-GPR20 antibody 04-046Ch or human IgG as a negative control was added, and the obtained mixture was left standing at 4°C for 1 hour. Thereafter, the cells were washed twice with PBS supplemented with 5% FBS, and then suspended by the addition of PE-labeled F(ab')₂ Fragment anti-human IgG, Fcγ antibody (Jackson ImmunoResearch Inc.) that had been 320-fold diluted with PBS supplemented with 5% FBS. The cells were left standing at 4°C for 1 hour. The cells were washed twice with PBS supplemented with 5% FBS, and then re-suspended in PBS supplemented with 5% FBS, followed by detection using a flow cytometer (FC500; Beckman Coulter, Inc.). The data was analyzed using Flowjo (Tree Star, Inc.). 04-046Ch bound to the 293T cells transfected with

pcDNA3.1-hGPR20 in an antibody concentration-dependent manner (Figure 23(a)), without binding to the 293T cells transfected with the negative control pcDNA3.1 (Figure 23(b)). The abscissa in Figure 23 depicts an antibody concentration, and the ordinate depicts mean fluorescent intensity indicating the amount of the antibody bound.

[0262] Example 6: Production of humanized anti-GPR20 antibody

6)-1 Design of humanized form of anti-GPR20 antibody
04-046

6)-1-1 Molecular modeling of 04-046 variable region

The molecular modeling of the variable regions of 04-046 was carried out according to a method known as homology modeling (Methods in Enzymology, 203, 121-153, (1991)). The primary sequences of the variable regions of human immunoglobulins registered in Protein Data Bank (Nuc. Acid Res. 35, D301-D303 (2007)) (three-dimensional structures inferred from X-ray crystal structures are available) were compared with the variable regions of 04-046 determined in Example 3)-2. 1SY6 and 1LK3 were selected as sequences having the highest sequence identity to the heavy chain and light chain variable regions of 04-046. The three-dimensional structures of framework regions were produced by combining, with one another, the coordinates of 1SY6 and 1LK3 corresponding to the heavy chain and light chain of 04-046, so as to obtain a "framework model". After that, the

representative conformation of each CDR was incorporated into the framework model. Finally, in order to obtain a molecular model with possible variable regions of 046, an energy calculation for eliminating atomic contact that was disadvantageous in terms of energy was carried out. The above-described procedures were carried out using a commercially available protein three-dimensional structure analysis program Discovery Studio (Accelrys Inc.).

[0263] 6)-1-2 Design of amino acid sequence of humanized h046

A humanized antibody of 04-046 (hereinafter, referred to as "humanized h046") was constructed according to a method generally known as CDR grafting (Proc. Natl. Acad. Sci. USA 86, 10029-10033 (1989)). An acceptor antibody was selected based on amino acid identity in the framework regions.

The sequences of the framework regions of 04-046 were compared with the framework regions of human subgroup consensus sequences determined by KABAT et al. (Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service National Institutes of Health, Bethesda, MD. (1991)). The consensus sequences of human γ chain subgroup 1 and human κ chain subgroup 1 had high sequence identity in their framework regions as to the heavy chain and light chain, respectively, and based on this, they were selected as acceptors. With regard to

the acceptors, the amino acid residues in the framework regions were aligned with the amino acid residues of 04-046, so that the positions, at which different amino acids were used, were identified. The positions of these residues were analyzed using the three-dimensional model of 04-046 constructed above, and donor residues to be grafted onto the acceptors were selected based on the criteria given by Queen et al. (Proc. Natl. Acad. Sci. USA 86, 10029-10033 (1989)). The thus-selected several donor residues were introduced into an acceptor antibody, so as to construct the sequence of humanized h046 as in the manner described in the following examples.

[0264] 6)-2 Humanization of 04-046 heavy chain

6)-2-1 Humanized h046-H4b type heavy chain

A humanized h046 heavy chain designed by substituting glutamine at amino acid position 24 with valine, leucine at amino acid position 30 with valine, alanine at amino acid position 31 with lysine, serine at amino acid position 35 with alanine, isoleucine at amino acid position 39 with valine, lysine at amino acid position 57 with arginine, threonine at amino acid positions 40 and 59 with alanine, threonine at amino acid position 60 with proline, isoleucine at amino acid position 67 with methionine, lysine at amino acid position 86 with arginine, alanine at amino acid position 87 with valine, leucine at amino acid position 89 with isoleucine, valine at amino acid position 91 with alanine,

phenylalanine at amino acid position 99 with threonine, glutamine at amino acid position 101 with glutamic acid, threonine at amino acid position 106 with arginine, proline at amino acid position 107 with serine, aspartic acid at amino acid position 108 with glutamic acid, serine at amino acid position 120 with threonine, isoleucine at amino acid position 122 with valine, valine at amino acid position 136 with threonine, and methionine at amino acid position 137 with leucine in SEQ ID NO: 44 as to the variable region moiety (amino acid sequence consisting of the amino acid residues at positions 20 to 142 in the amino acid sequence shown in SEQ ID NO: 44) in the human chimeric antibody 04-046Ch heavy chain, was named "humanized h046-H4b type heavy chain" (also referred to as "h046-H4b").

[0265] The amino acid sequence of the humanized h046-H4b type heavy chain is shown in SEQ ID NO: 48 in the sequence listing. In the amino acid sequence shown in SEQ ID NO: 48, the sequence consisting of the amino acid residues at positions 1 to 19 corresponds to a signal sequence, the sequence consisting of the amino acid residues at positions 20 to 142 corresponds to a heavy chain variable region, and the sequence consisting of the amino acid residues at positions 143 to 472 corresponds to a heavy chain constant region. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 48 is shown in SEQ ID NO: 49 in the sequence listing. In

the nucleotide sequence of SEQ ID NO: 49, the sequence consisting of the nucleotides at positions 1 to 57 encodes a signal sequence, the sequence consisting of the nucleotides at positions 58 to 426 corresponds to a sequence encoding the heavy chain variable region, and the sequence consisting of the nucleotides at positions 427 to 1416 corresponds to a sequence encoding the heavy chain constant region. The amino acid sequence of SEQ ID NO: 48 is also shown in Figure 9.

[0266] 6)-2-2 Humanized h046-H4e type heavy chain

A humanized h046 heavy chain designed by substituting glutamine at amino acid position 20 with glutamic acid, glutamine at amino acid position 24 with valine, leucine at amino acid position 30 with valine, alanine at amino acid position 31 with lysine, serine at amino acid position 35 with alanine, isoleucine at amino acid position 39 with valine, lysine at amino acid position 57 with arginine, threonine at amino acid position 59 with alanine, threonine at amino acid position 60 with proline, isoleucine at amino acid position 67 with methionine, lysine at amino acid position 86 with arginine, alanine at amino acid positions 68 and 87 with valine, leucine at amino acid position 89 with isoleucine, valine at amino acid position 91 with alanine, phenylalanine at amino acid position 99 with threonine, glutamine at amino acid position 101 with glutamic acid, threonine at amino acid

position 106 with arginine, proline at amino acid position 107 with serine, aspartic acid at amino acid position 108 with glutamic acid, serine at amino acid position 120 with threonine, isoleucine at amino acid position 122 with valine, valine at amino acid position 136 with threonine, and methionine at amino acid position 137 with leucine in SEQ ID NO: 44 as to the variable region moiety in the human chimeric antibody 04-046Ch heavy chain, was named "humanized h046-H4e type heavy chain" (also referred to as "h046-H4e").

The amino acid sequence of the humanized h046-H4e type heavy chain is shown in SEQ ID NO: 50 in the sequence listing. In the amino acid sequence of SEQ ID NO: 50, the sequence consisting of the amino acid residues at positions 1 to 19 corresponds to a signal sequence, the sequence consisting of the amino acid residues at positions 20 to 142 corresponds to a heavy chain variable region, and the sequence consisting of the amino acid residues at positions 143 to 472 corresponds to a heavy chain constant region. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 50 is shown in SEQ ID NO: 51 in the sequence listing. In the nucleotide sequence of SEQ ID NO: 51, the sequence consisting of the nucleotides at positions 1 to 57 encodes a signal sequence, the sequence consisting of the nucleotides at positions 58 to 426 corresponds to a sequence encoding the heavy chain variable region, and

the sequence consisting of the nucleotides at positions 427 to 1416 corresponds to a sequence encoding the heavy chain constant region. The amino acid sequence of SEQ ID NO: 50 is also shown in Figure 10.

[0267] 6)-2-3 Humanized h046-H5b type heavy chain

A humanized h046 heavy chain designed by substituting glutamine at amino acid position 24 with valine, leucine at amino acid position 30 with valine, alanine at amino acid position 31 with lysine, serine at amino acid position 35 with alanine, isoleucine at amino acid position 39 with valine, lysine at amino acid position 57 with arginine, threonine at amino acid position 59 with alanine, threonine at amino acid position 60 with proline, isoleucine at amino acid position 67 with methionine, lysine at amino acid position 86 with arginine, alanine at amino acid position 87 with valine, leucine at amino acid position 89 with isoleucine, valine at amino acid position 91 with alanine, phenylalanine at amino acid position 99 with asparagine, glutamine at amino acid position 101 with glutamic acid, threonine at amino acid position 106 with arginine, proline at amino acid position 107 with serine, aspartic acid at amino acid position 108 with glutamic acid, serine at amino acid position 120 with threonine, isoleucine at amino acid position 122 with valine, valine at amino acid position 136 with threonine, and methionine at amino acid position 137 with leucine in SEQ ID NO: 44

as to the variable region moiety in the human chimeric antibody 04-046Ch heavy chain, was named "humanized h046-H5b type heavy chain" (also referred to as "h046-H5b").

The amino acid sequence of the humanized h046-H5b type heavy chain is shown in SEQ ID NO: 52 in the sequence listing. In the amino acid sequence of SEQ ID NO: 52, the sequence consisting of the amino acid residues at positions 1 to 19 corresponds to a signal sequence, the sequence consisting of the amino acid residues at positions 20 to 142 corresponds to a heavy chain variable region, and the sequence consisting of the amino acid residues at positions 143 to 472 corresponds to a heavy chain constant region. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 52 is shown in SEQ ID NO: 53 in the sequence listing. In the nucleotide sequence of SEQ ID NO: 53, the sequence consisting of the nucleotides at positions 1 to 57 encodes a signal sequence, the sequence consisting of the nucleotides at positions 58 to 426 corresponds to a sequence encoding the heavy chain variable region, and the sequence consisting of the nucleotides at positions 427 to 1416 corresponds to a sequence encoding the heavy chain constant region. The amino acid sequence of SEQ ID NO: 52 is also shown in Figure 11.

[0268] 6)-2-4 Humanized h046-H8 type heavy chain

A humanized h046 heavy chain designed by substituting phenylalanine at amino acid position 80 with

asparagine in relation to the variable region moiety in the human chimeric antibody 04-046Ch heavy chain shown in SEQ ID NO: 44, was named "humanized h046-H8 type heavy chain" (also referred to as "h046-H8").

The amino acid sequence of the humanized h046-H8 type heavy chain is shown in SEQ ID NO: 54 in the sequence listing. In the amino acid sequence of SEQ ID NO: 54, the sequence consisting of the amino acid residues at positions 1 to 19, the sequence consisting of the amino acid residues at positions 20 to 142, and the sequence consisting of the amino acid residues at positions 143 to 472 correspond to a signal sequence, a heavy chain variable region, and a heavy chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 54 is shown in SEQ ID NO: 55 in the sequence listing. In the nucleotide sequence of SEQ ID NO: 55, the sequence consisting of the nucleotides at positions 1 to 57, the sequence consisting of the nucleotides at positions 58 to 426, and the sequence consisting of the nucleotides at positions 427 to 1416 encode the signal sequence, the heavy chain variable region sequence, and the heavy chain constant region sequence, respectively. The amino acid sequence of SEQ ID NO: 54 is also shown in Figure 12.

[0269] 6)-2-5 Humanized h046-H10 type heavy chain

A humanized h046 heavy chain designed by substituting isoleucine at amino acid position 39 with

valine and phenylalanine at amino acid position 99 with asparagine in SEQ ID NO: 44 in relation to the variable region moiety in the human chimeric antibody 04-046Ch heavy chain shown in SEQ ID NO: 44, was named "humanized h046-H10 type heavy chain" (also referred to as "h046-H10").

[0270] The amino acid sequence of the humanized h046-H10 type heavy chain is shown in SEQ ID NO: 56 in the sequence listing. In the amino acid sequence of SEQ ID NO: 56, the sequence consisting of the amino acid residues at positions 1 to 19 corresponds to a signal sequence, the sequence consisting of the amino acid residues at positions 20 to 142 corresponds to a heavy chain variable region, and the sequence consisting of the amino acid residues at positions 143 to 472 corresponds to a heavy chain constant region. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 56 is shown in SEQ ID NO: 57 in the sequence listing. In the nucleotide sequence of SEQ ID NO: 57, the sequence consisting of the nucleotides at positions 1 to 57 encodes a signal sequence, the sequence consisting of the nucleotides at positions 58 to 426 encodes the heavy chain variable region sequence, and the sequence consisting of the nucleotides at positions 427 to 1416 encodes the heavy chain constant region sequence. The amino acid sequence of SEQ ID NO: 56 is also shown in Figure 13.

[0271] 6)-3 Humanization of 04-046 light chain

6)-3-1 Humanized h046-L1 type light chain

A humanized h046 light chain designed by substituting threonine at amino acid position 22 with isoleucine, valine at amino acid position 23 with glutamine, leucine at amino acid position 24 with methionine, alanine at amino acid position 29 with serine, alanine at amino acid position 31 with serine, valine at amino acid position 32 with alanine, leucine at amino acid position 34 with valine, glutamine at amino acid position 36 with aspartic acid, serine at amino acid position 41 with threonine, arginine at amino acid position 58 with lysine, serine at amino acid position 59 with proline, glutamine at amino acid position 61 with lysine, glutamine at amino acid position 62 with alanine, aspartic acid at amino acid position 95 with serine, proline at amino acid position 96 with serine, valine at amino acid position 97 with leucine, glutamic acid at amino acid position 98 with glutamine, aspartic acid at amino acid position 100 with glutamic acid, isoleucine at amino acid position 102 with phenylalanine, asparagine at amino acid position 104 with threonine, alanine at amino acid position 119 with glutamine, leucine at amino acid position 123 with valine, leucine at amino acid position 125 with isoleucine, and alanine at amino acid position 128 with threonine, and inserting serine between amino acid positions 29 and 30, in SEQ ID NO: 45 as to the

variable region moiety in the human chimeric antibody 04-046Ch light chain, was named "humanized h046-L1 type light chain" (also referred to as "h046-L1").

The amino acid sequence of the humanized h046-L1 type light chain is shown in SEQ ID NO: 58 in the sequence listing. In the amino acid sequence of SEQ ID NO: 58, the sequence consisting of the amino acid residues at positions 1 to 20 corresponds to a signal sequence, the sequence consisting of the amino acid residues at positions 21 to 129 corresponds to a light chain variable region, and the sequence consisting of the amino acid residues at positions 130 to 234 corresponds to a light chain constant region. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 58 is shown in SEQ ID NO: 59 in the sequence listing. In the nucleotide sequence of SEQ ID NO: 58, the sequence consisting of the nucleotides at positions 1 to 60 encodes a signal sequence, the sequence consisting of the nucleotides at positions 61 to 387 encodes the light chain variable region sequence, and the sequence consisting of the nucleotides at positions 388 to 702 encodes the light chain constant region sequence. The amino acid sequence of SEQ ID NO: 58 is also shown in Figure 14.

[0272] 6)-3-2 Humanized h046-L2 type light chain

A humanized h046 light chain designed by substituting threonine at amino acid position 22 with

isoleucine, valine at amino acid position 23 with glutamine, leucine at amino acid position 24 with methionine, alanine at amino acid position 29 with serine, alanine at amino acid position 21 with serine, valine at amino acid position 32 with alanine, leucine at amino acid position 34 with valine, glutamine at amino acid position 36 with aspartic acid, serine at amino acid position 41 with threonine, arginine at amino acid position 58 with lysine, serine at amino acid position 59 with proline, glutamine at amino acid position 61 with lysine, aspartic acid at amino acid position 95 with serine, proline at amino acid position 96 with serine, valine at amino acid position 97 with leucine, glutamic acid at amino acid position 98 with glutamine, aspartic acid at amino acid position 100 with glutamic acid, isoleucine at amino acid position 102 with phenylalanine, asparagine at amino acid position 104 with threonine, alanine at amino acid position 119 with glutamine, leucine at amino acid position 123 with valine, leucine at amino acid position 125 with isoleucine, and alanine at amino acid position 128 with threonine, and inserting serine between amino acid positions 29 and 30, in SEQ ID NO: 45 as to the variable region moiety in the human chimeric antibody 04-046Ch light chain, was named "humanized h046-L2 type light chain" (also referred to as "h046-L2").

The amino acid sequence of the humanized h046-L2 type light chain is shown in SEQ ID NO: 60 in the sequence listing. In the amino acid sequence of SEQ ID NO: 60, the sequence consisting of the amino acid residues at positions 1 to 20 corresponds to a signal sequence, the sequence consisting of the amino acid residues at positions 21 to 129 corresponds to a light chain variable region, and the sequence consisting of the amino acid residues at positions 130 to 234 corresponds to a light chain constant region. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 60 is shown in SEQ ID NO: 61 in the sequence listing. In the nucleotide sequence of SEQ ID NO: 61, the sequence consisting of the nucleotides at positions 1 to 60 encodes a signal sequence, the sequence consisting of the nucleotides at positions 61 to 387 encodes the light chain variable region sequence, and the sequence consisting of the nucleotides at positions 388 to 702 encodes the light chain constant region sequence. The amino acid sequence of SEQ ID NO: 60 is also shown in Figure 15.

[0273] 6)-3-3 Humanized h046-L6 type light chain

A humanized h046 light chain designed by substituting valine at amino acid position 23 with glutamine, alanine at amino acid position 29 with serine, alanine at amino acid position 31 with serine, valine at amino acid position 32 with alanine, leucine at amino

acid position 34 with valine, glutamine at amino acid position 36 with aspartic acid, serine at amino acid position 41 with threonine, arginine at amino acid position 58 with lysine, serine at amino acid position 59 with proline, glutamine at amino acid position 61 with lysine, asparagine at amino acid position 72 with aspartic acid, leucine at amino acid position 73 with arginine, aspartic acid at amino acid position 95 with serine, proline at amino acid position 96 with serine, valine at amino acid position 97 with leucine, glutamic acid at amino acid position 98 with glutamine, aspartic acid at amino acid position 100 with glutamic acid, isoleucine at amino acid position 102 with phenylalanine, alanine at amino acid position 119 with glutamine, leucine at amino acid position 123 with valine, leucine at amino acid position 125 with isoleucine, and alanine at amino acid position 128 with threonine, and inserting serine between amino acid positions 29 and 30, in SEQ ID NO: 45 as to the variable region moiety in the human chimeric antibody 04-046Ch light chain, was named "humanized h046-L6 type light chain" (also referred to as "h046-L6").

[0274] The amino acid sequence of the humanized h046-L6 type light chain is shown in SEQ ID NO: 62 in the sequence listing. In the amino acid sequence of SEQ ID NO: 62, the sequence consisting of the amino acid residues at positions 1 to 20 corresponds to a signal

sequence, the sequence consisting of the amino acid residues at positions 21 to 129 corresponds to a light chain variable region, and the sequence consisting of the amino acid residues at positions 130 to 234 corresponds to a light chain constant region. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 62 is shown in SEQ ID NO: 63 in the sequence listing. In the nucleotide sequence of SEQ ID NO: 63, the sequence consisting of the nucleotides at positions 1 to 60 encodes a signal sequence, the sequence consisting of the nucleotides at positions 61 to 387 encodes the light chain variable region sequence, and the sequence consisting of the nucleotides at positions 388 to 702 encodes the light chain constant region sequence. The amino acid sequence of CDRL2 (SASDRES) is shown in SEQ ID NO: 92 in the sequence listing.

The amino acid sequence of SEQ ID NO: 62 is also shown in Figure 16.

6)-3-4 Humanized h046-L7 type light chain

A humanized h046 light chain designed by substituting valine at amino acid position 23 with glutamine, alanine at amino acid position 29 with serine, alanine at amino acid position 31 with serine, valine at amino acid position 32 with alanine, leucine at amino acid position 34 with valine, glutamine at amino acid position 36 with aspartic acid, serine at amino acid position 41 with threonine, arginine at amino acid

position 58 with lysine, serine at amino acid position 59 with proline, glutamine at amino acid position 61 with lysine, serine at amino acid position 71 with glycine, aspartic acid at amino acid position 95 with serine, proline at amino acid position 96 with serine, valine at amino acid position 97 with leucine, glutamic acid at amino acid position 98 with glutamine, aspartic acid at amino acid position 100 with glutamic acid, isoleucine at amino acid position 102 with phenylalanine, alanine at amino acid position 119 with glutamine, leucine at amino acid position 123 with valine, leucine at amino acid position 125 with isoleucine, and alanine at amino acid position 128 with threonine, and inserting serine between amino acid positions 29 and 30, as to the variable region moiety in the human chimeric antibody 04-046Ch light chain shown in SEQ ID NO: 45, was named "humanized h046-L7 type light chain" (also referred to as "h046-L7").

[0275] The amino acid sequence of the humanized h046-L7 type light chain is shown in SEQ ID NO: 64 in the sequence listing. In the amino acid sequence of SEQ ID NO: 64, the sequence consisting of the amino acid residues at positions 1 to 20 corresponds to a signal sequence, the sequence consisting of the amino acid residues at positions 21 to 129 corresponds to a light chain variable region, and the sequence consisting of the amino acid residues at positions 130 to 234 corresponds to a light chain constant region. The nucleotide

sequence encoding the amino acid sequence of SEQ ID NO: 64 is shown in SEQ ID NO: 65 in the sequence listing. In the nucleotide sequence of SEQ ID NO: 65, the sequence consisting of the nucleotides at positions 1 to 60 encodes a signal sequence, the sequence consisting of the nucleotides at positions 61 to 387 encodes the light chain variable region sequence, and the sequence consisting of the nucleotides at positions 388 to 702 encodes the light chain constant region sequence. The amino acid sequence of CDRL2 (SAGNLES) is shown in SEQ ID NO: 93 in the sequence listing.

The amino acid sequence of SEQ ID NO: 64 is also shown in Figure 17.

[0276] 6)-4 Design of humanized h046 by combination of heavy chain and light chain

An antibody consisting of the humanized h046-H4e type heavy chain and the humanized h046-L1 type light chain was designed and named "humanized h046-H4e/L1" (also referred to as "h046-H4e/L1"). An antibody consisting of the humanized h046-H4e type heavy chain and the humanized h046-L2 type light chain was designed and named "humanized h046-H4e/L2" (also referred to as "h046-H4e/L2"). An antibody consisting of the humanized h046-H4e type heavy chain and the humanized h046-L6 type light chain was designed and named "humanized h046-H4e/L6" (also referred to as "h046-H4e/L6"). An antibody consisting of the humanized h046-H4e type heavy chain and

the humanized h046-L7 type light chain was designed and named "humanized h046-H4e/L7" (also referred to as "h046-H4e/L7"). An antibody consisting of the humanized h046-H4b type heavy chain and the humanized h046-L1 type light chain was designed and named "humanized h046-H4b/L1" (also referred to as "h046-H4b/L1"). An antibody consisting of the humanized h046-H4b type heavy chain and the humanized h046-L2 type light chain was designed and named "humanized h046-H4b/L2" (also referred to as "h046-H4b/L2"). An antibody consisting of the humanized h046-H4b type heavy chain and the humanized h046-L6 type light chain was designed and named "humanized h046-H4b/L6" (also referred to as "h046-H4b/L6"). An antibody consisting of the humanized h046-H4b type heavy chain and the humanized h046-L7 type light chain was designed and named "humanized h046-H4b/L7" (also referred to as "h046-H4b/L7"). An antibody consisting of the humanized h046-H5b type heavy chain and the humanized h046-L1 type light chain was designed and named "humanized h046-H5b/L1" (also referred to as "h046-H5b/L1"). An antibody consisting of the humanized h046-H5b type heavy chain and the humanized h046-L2 type light chain was designed and named "humanized h046-H5b/L2" (also referred to as "h046-H5b/L2"). An antibody consisting of the humanized h046-H5b type heavy chain and the humanized h046-L6 type light chain was designed and named "humanized h046-H5b/L6" (also referred to as "h046-H5b/L6"). An antibody

consisting of the humanized h046-H5b type heavy chain and the humanized h046-L7 type light chain was designed and named "humanized h046-H5b/L7" (also referred to as "h046-H5b/L7"). An antibody consisting of the humanized h046-H8 type heavy chain and the humanized h046-L1 type light chain was designed and named "humanized h046-H8/L1" (also referred to as "h046-H8/L1"). An antibody consisting of the humanized h046-H8 type heavy chain and the humanized h046-L2 type light chain was designed and named "humanized h046-H8/L2" (also referred to as "h046-H8/L2"). An antibody consisting of the humanized h046-H8 type heavy chain and the humanized h046-L6 type light chain was designed and named "humanized h046-H8/L6" (also referred to as "h046-H8/L6"). An antibody consisting of the humanized h046-H8 type heavy chain and the humanized h046-L7 type light chain was designed and named "humanized h046-H8/L7" (also referred to as "h046-H8/L7"). An antibody consisting of the humanized h046-H10 type heavy chain and the humanized h046-L1 type light chain was designed and named "humanized h046-H10/L1" (also referred to as "h046-H10/L1"). An antibody consisting of the humanized h046-H10 type heavy chain and the humanized h046-L2 type light chain was designed and named "humanized h046-H10/L2" (also referred to as "h046-H10/L2"). An antibody consisting of the humanized h046-H10 type heavy chain and the humanized h046-L6 type light chain was designed and named "humanized h046-H10/L6"

(also referred to as "h046-H10/L6"). An antibody consisting of the humanized h046-H10 type heavy chain and the humanized h046-L7 type light chain was designed and named "humanized h046-H10/L7" (also referred to as "h046-H10/L7"). An antibody consisting of the human chimeric c046 heavy chain and the humanized h046-L1 type light chain was designed and named "human chimeric c046/L1" (also referred to as "h046-Hwt/L1"). An antibody consisting of the human chimeric c046 heavy chain and the humanized h046-L2 type light chain was designed and named "human chimeric c046/L2" (also referred to as "h046-Hwt/L2"). An antibody consisting of the human chimeric c046 heavy chain and the humanized h046-L6 type light chain was designed and named "human chimeric c046/L6" (also referred to as "h046-Hwt/L6"). An antibody consisting of the human chimeric c046 heavy chain and the humanized h046-L7 type light chain was designed and named "human chimeric c046/L7" (also referred to as "h046-Hwt/L7"). The antibodies thus designed can be produced in accordance with Examples 6)-5 and 6)-7, and can be evaluated in accordance with Examples 6)-6, 8), and 9).

[0277] 6)-5 Expression of humanized anti-GPR20 antibody - (1)

6)-5-1 Construction of humanized h046 heavy chain expression vector

6)-5-1-1 Construction of humanized h046-H4b type heavy chain expression vector

The DNA fragment A shown in SEQ ID NO: 94 was synthesized (GENEART, artificial gene synthesis service). The synthesized DNA fragment was inserted into a site of the chimeric and humanized antibody IgG1 type heavy chain expression vector pCMA-G1 that had been cleaved with the restriction enzyme BspI, by the same method as that applied in Example 5)-1-3, so as to construct plasmid A. A humanized h046-H4b type heavy chain expression vector was constructed by introducing a mutation by using KOD - Plus- Mutagenesis Kit (Toyobo Co., Ltd.) with plasmid A as a template and with the primer set given below. The constructed expression vector was named "pCMA/h046-H4b". The nucleotide sequence of the humanized h046-H4b type heavy chain is shown in SEQ ID NO: 49, and the amino acid sequence thereof is shown in SEQ ID NO: 48.

Primer set:

5'-ATCAACCCTGGCAGCGGCCACACCAACTAC-3' (Hb-F; SEQ ID NO: 95)

5'-GAAGCCCATGTACTTCAGGCCCTGTCCAGGGG-3' (Hb-R; SEQ ID NO: 96)

6)-5-1-2 Construction of humanized h046-H5b type heavy chain expression vector

The DNA fragment B shown in SEQ ID NO: 97 was synthesized (GENEART, artificial gene synthesis service). The synthesized DNA fragment was inserted into a site of the chimeric and humanized antibody IgG1 type heavy chain expression vector pCMA-G1 that had been cleaved with the

restriction enzyme BspI, by the same method as that applied in Example 5)-1-3, so as to construct plasmid B. A mutation was introduced with plasmid B as a template by the same method as that applied in 6)-5-1-1, so as to construct a humanized h046-H5b type heavy chain expression vector. The constructed expression vector was named "pCMA/h046-H5b". The nucleotide sequence of the humanized h046-H5b type heavy chain is shown in SEQ ID NO: 53, and the amino acid sequence thereof is shown in SEQ ID NO: 52.

[0278] 6)-5-1-3 Construction of humanized h046-H8 type heavy chain expression vector

A mutation was introduced with pCMA/04-046Ch-H constructed in Example 5)-1-3 as a template using the primer set given below and KOD -Plus- Mutagenesis Kit (Toyobo Co., Ltd.), so as to construct a humanized h046-H8 type heavy chain expression vector. The constructed expression vector was named "pCMA/h046-H8". The nucleotide sequence of the humanized h046-H8 type heavy chain is shown in SEQ ID NO: 55, and the amino acid sequence thereof is shown in SEQ ID NO: 54.

Primer set:

5'-AACATGCAGCTGTCCAGCCTGACCCCCGACGAC-3' (H08-F; SEQ ID NO: 98)

5'-GGCGGTGCTGCTGCTCTTGTCCACGGTCAG-3' (H08-R; SEQ ID NO: 99)

6)-5-1-4 Construction of humanized h046-H10 type heavy chain expression vector

A mutation was introduced with pCMA/h046-H8 constructed in Example 6)-5-1-3 as a template using the primer set given below and KOD -Plus- Mutagenesis Kit (Toyobo Co., Ltd.), so as to construct a humanized h046-H10 type heavy chain expression vector. The constructed expression vector was named "pCMA/h046-H10". The nucleotide sequence of the humanized h046-H10 type heavy chain is shown in SEQ ID NO: 57, and the amino acid sequence thereof is shown in SEQ ID NO: 56.

5'-GTGAGCTGCAAGGCCAGCGGCTACACCTTCACC-3' (H10-F; SEQ ID NO: 100)

5'-CTTCACGCTGCTGCCAGGCTTGGCCAGTTC-3' (H10-R; SEQ ID NO: 101)

[0279] 6)-5-2 Construction of humanized h046 light chain expression vector

6)-5-2-1 Construction of humanized h046-L1 type light chain expression vector

A DNA fragment corresponding to nucleotide positions 37 to 402 including a DNA sequence encoding the variable region in the humanized h046-L1 nucleotide sequence shown in SEQ ID NO: 59 was synthesized (GENEART, artificial gene synthesis service). Using the synthesized DNA fragment as a template, a DNA fragment comprising a DNA sequence encoding the humanized h046-L1 variable region was amplified with KOD -Plus- (Toyobo Co., Ltd.) and the

primer set given below. Using In-Fusion HD PCR cloning kit (Clontech Laboratories, Inc.), the amplified DNA fragment was inserted into a site of the chimeric and humanized antibody light chain expression vector pCMA-LK constructed in Example 5)-1-1 that had been cleaved with the restriction enzyme BsiWI, so as to construct a humanized h046-L1 expression vector. The obtained expression vector was named "pCMA/h046-L1". The amino acid sequence of the humanized h046-L1 is shown in SEQ ID NO: 58.

Primer set

5'-CTGTGGATCTCCGGCGCGTACGGC-3' (CM-LKF; SEQ ID NO: 90)

5'-GGAGGGGGCGGCCACCGTACG-3' (KCL-Inf-R; SEQ ID NO: 102)

[0280] 6)-5-2-2 Construction of humanized h046-L2 type light chain expression vector

A DNA fragment comprising a DNA sequence encoding the humanized h046-L2 variable region corresponding to nucleotide positions 37 to 402 in the humanized h046-L2 nucleotide sequence shown in SEQ ID NO: 61 was synthesized (GENEART, artificial gene synthesis service). A humanized h046-L2 expression vector was constructed by the same method as that applied in Example 6)-5-2-1. The obtained expression vector was named "pCMA/h046-L2". The amino acid sequence of the humanized h046-L2 is shown in SEQ ID NO: 60.

[0281] 6)-5-2-3 Construction of humanized h046-L6 type light chain expression vector

A DNA fragment comprising a DNA sequence encoding the humanized h046-L6 variable region corresponding to nucleotide positions 37 to 402 in the humanized h046-L6 nucleotide sequence shown in SEQ ID NO: 63 was synthesized (GENEART, artificial gene synthesis service). A humanized h046-L6 expression vector was constructed by the same method as that applied in Example 6)-5-2-1. The obtained expression vector was named "pCMA/h046-L6". The amino acid sequence of the humanized h046-L6 is shown in SEQ ID NO: 62.

[0282] 6)-5-2-4 Construction of humanized h046-L7 type light chain expression vector

A DNA fragment comprising a DNA sequence encoding the humanized h046-L2 variable region corresponding to nucleotide positions 37 to 402 in the humanized h046-L7 nucleotide sequence shown in SEQ ID NO: 65 was synthesized (GENEART, artificial gene synthesis service). A humanized h046-L7 expression vector was constructed by the same method as that applied in Example 6)-5-2-1. The obtained expression vector was named "pCMA/h046-L7". The amino acid sequence of the humanized h046-L7 is shown in SEQ ID NO: 64.

[0283] 6)-5-3 Small-scale production of humanized h046 antibody

In accordance with the manual, FreeStyle 293F cells (Invitrogen Corp.) were cultured and passaged. 1×10^7 FreeStyle 293F cells (Invitrogen Corp.) in the

logarithmic growth phase were diluted into 9.6 mL with FreeStyle 293 expression medium (Invitrogen Corp.), then seeded in 30-mL Square Storage Bottle (Nalgene), and shake-cultured at 90 rpm in an 8% CO₂ incubator at 37°C for 1 hour. 30 µg of Polyethyleneimine (Polyscience #24765) was dissolved in 200 µL of Opti-Pro SFM (Invitrogen Corp.). Meanwhile, the light chain expression vector (6 µg) and the heavy chain expression vector (4 µg) prepared using NucleoBond Xtra (Takara Bio Inc.) were added to 200 µL of Opti-Pro SFM (Invitrogen Corp.). To 200 µL of the Polyethyleneimine/Opti-Pro SFM mixed solution, 200 µL of the expression vector/Opti-Pro SFM mixed solution was added, and the obtained mixture was gently stirred. After incubation for 5 minutes, the mixture was added to the FreeStyle 293F cells. The cells were shake-cultured at 90 rpm in an 8% CO₂ incubator at 37°C for 7 days. The obtained culture supernatant was filtrated through Minisart-Plus filter (Sartorius Inc.) to obtain a sample for evaluation.

[0284] Humanized h046-H4b/L7 was obtained by the combination of pCMA/h046-H4b and pCMA/h046-L7. Humanized h046-H5b/L2 was obtained by the combination of pCMA/h046-H5b and pCMA/h046-L2. Humanized h046-Hwt/L6 was obtained by the combination of pCMA/04-046Ch-H and pCMA/h046-L6. Humanized h046-H8/L1 was obtained by the combination of pCMA/h046-H8 and pCMA/h046-L1. Humanized h046-H10/L1 was obtained by the combination of pCMA/h046-H10 and

pCMA/h046-L1. Humanized h046-H10/L6 was obtained by the combination of pCMA/h046-H10 and pCMA/h046-L6.

[0285] 6)-6 *In vitro* evaluation of humanized anti-GPR20 antibody

6)-6-1 Evaluation of binding activity of humanized anti-GPR20 antibody

The GPR20-binding activity of the humanized anti-GPR20 antibodies produced in Example 6)-5-3 was confirmed by flow cytometry. Using Lipofectamine 2000, pcDNA3.1-hGPR20 was transiently introduced into 293T cells by the same method as that applied in Example 1)-5-1. The cells were cultured overnight under conditions of 37°C and 5% CO₂, and thereafter, a cell suspension was prepared. To the obtained cell suspension, each humanized anti-GPR20 antibody was added, and the obtained mixture was left standing at 4°C for 1 hour. Thereafter, the cells were washed twice with PBS supplemented with 5% FBS, and then suspended by the addition of PE-labeled F(ab')₂ Fragment anti-human IgG, Fcγ antibody (Jackson ImmunoResearch Inc.) that had been 320-fold diluted with PBS supplemented with 5% FBS. The cells were left standing at 4°C for 1 hour. The cells were washed twice with PBS supplemented with 5% FBS, and then re-suspended in PBS supplemented with 5% FBS, followed by detection using a flow cytometer (BD FACSCant II; BD Biosciences). The data was analyzed using Flowjo (Tree Star, Inc.). As a result, it was confirmed that the produced humanized

anti-GPR20 antibodies bound to the 293T cells transfected with pcDNA3.1-hGPR20. Figure 24 shows the results for the humanized anti-GPR20 antibodies specifically bound to human GPR20. In the histograms of Figure 24, the abscissa depicts the fluorescence intensity of PE indicating the amount of the antibody bound, and the ordinate depicts a cell count. The shaded histogram shows a negative control that was not reacted with an anti-GPR20 antibody, and the open histogram shows that, when each anti-GPR20 antibody was reacted, fluorescence intensity was enhanced by the binding of the antibody to GPR20 on the cell surface.

[0286] 6)-6-2 Evaluation of internalization activity of humanized anti-GPR20 antibody

The internalization activity of the humanized anti-GPR20 antibodies produced in Example 6)-5-3 was evaluated using an anti-human IgG antibody reagent Hum-ZAP (Advanced Targeting Systems) conjugated with a toxin (saporin) inhibiting protein synthesis, by the same method as that applied in 1)-6. Specifically, HEK293 cells stably expressing GPR20-EGFP protein comprising human GPR20 linked at its C-terminus to EGFP were seeded at 2.5×10^3 cells/well, and then cultured overnight under conditions of 37°C and 5% CO₂. Each humanized anti-GPR20 antibody (final concentration: 0.015 to 1.27 µg/mL) and Hum-ZAP (final concentration: 1 µg/mL) was added to the culture. The cells were cultured for 4 days, and

thereafter, the number of live cells was measured by the quantification of ATP using CellTiter-Glo (registered trademark) Luminescent Cell Viability Assay. As a result, the humanized anti-GPR20 antibodies, humanized h046-H4b/L7, humanized h046-H5b/L2, humanized h046-Hwt/L6, humanized h046-H8/L1, humanized h046-H10/L1 and humanized h046-H10/L6, produced in Example 6)-5-3 exhibited a cell proliferation-suppressive effect by antibody internalization.

[0287] 6)-7 Large scale production of humanized anti-GPR20 antibody - (2)

6)-7-1 Construction of humanized h046 heavy chain expression vector

6)-7-1-1 Construction of humanized h046-H4b type heavy chain expression vector

A DNA fragment comprising a sequence encoding the humanized h046_H4b type heavy chain corresponding to nucleotide positions 58 to 1416 of the nucleotide sequence (2) of the humanized h046_H4b type heavy chain shown in SEQ ID NO: 103 in the sequence listing was synthesized (GENEART, artificial gene synthesis service). Using the synthesized DNA fragment, in accordance with the protocols of GS Gene Expression System (registered trademark) by Lonza, a humanized h046_H4b type heavy chain expression vector was constructed. The constructed expression vector was named "GSV-h046_H4b".

[0288] 6)-7-1-2 Construction of humanized h046-H5b type heavy chain expression vector

A DNA fragment comprising a sequence encoding the humanized h046_H5b type heavy chain corresponding to nucleotide positions 58 to 1416 of the nucleotide sequence (2) of the humanized h046_H5b type heavy chain shown in SEQ ID NO: 104 in the sequence listing was synthesized (GENEART, artificial gene synthesis service). Using the synthesized DNA fragment, in accordance with the protocols of GS Gene Expression System (registered trademark) by Lonza, a humanized h046_H5b type heavy chain expression vector was constructed. The constructed expression vector was named "GSV-h046_H5b".

[0289] 6)-7-1-3 Construction of humanized h046-Hwt type heavy chain expression vector

A DNA fragment comprising a sequence encoding the humanized h046_Hwt type heavy chain corresponding to nucleotide positions 58 to 1416 of the nucleotide sequence (2) of the humanized h046_Hwt type heavy chain shown in SEQ ID NO: 105 in the sequence listing was synthesized (GENEART, artificial gene synthesis service). Using the synthesized DNA fragment, in accordance with the protocols of GS Gene Expression System (registered trademark) by Lonza, a humanized h046_Hwt type heavy chain expression vector was constructed. The constructed expression vector was named "GSV-h046_Hwt".

[0290] 6)-7-1-4 Construction of humanized h046-H8 type heavy chain expression vector

A DNA fragment comprising a sequence encoding the humanized h046_H8 type heavy chain corresponding to nucleotide positions 58 to 1416 of the nucleotide sequence (2) of the humanized h046_H8 type heavy chain shown in SEQ ID NO: 106 in the sequence listing was synthesized (GENEART, artificial gene synthesis service). Using the synthesized DNA fragment, in accordance with the protocols of GS Gene Expression System (registered trademark) by Lonza, a humanized h046_H8 type heavy chain expression vector was constructed. The constructed expression vector was named "GSV-h046_H8".

[0291] 6)-7-1-5 Construction of humanized h046-H10 type heavy chain expression vector

A DNA fragment comprising a sequence encoding the humanized h046_H10 type heavy chain corresponding to nucleotide positions 58 to 1416 of the nucleotide sequence (2) of the humanized h046_H10 type heavy chain shown in SEQ ID NO: 107 in the sequence listing was synthesized (GENEART, artificial gene synthesis service). Using the synthesized DNA fragment, in accordance with the protocols of GS Gene Expression System (registered trademark) by Lonza, a humanized h046_H10 type heavy chain expression vector was constructed. The constructed expression vector was named "GSV-h046_H10".

[0292] 6)-7-1-6 Construction of humanized h046-H4e type heavy chain expression vector

A DNA fragment comprising a sequence encoding the humanized h046_H4e type heavy chain corresponding to nucleotide positions 58 to 1416 of the nucleotide sequence of the humanized h046_H4e type heavy chain shown in SEQ ID NO: 51 in the sequence listing was synthesized (GENEART, artificial gene synthesis service). Using the synthesized DNA fragment, in accordance with the protocols of GS Gene Expression System (registered trademark) by Lonza, a humanized h046_H4e type heavy chain expression vector was constructed. The constructed expression vector was named "GSV-h046_H4e".

[0293] 6)-7-2 Construction of humanized h046 light chain expression vector

6)-7-2-1 Construction of humanized h046-L1 type light chain expression vector

A DNA fragment comprising a sequence encoding the humanized h046_L1 type light chain corresponding to nucleotide positions 61 to 702 of the nucleotide sequence (2) of the humanized h046_L1 type light chain shown in SEQ ID NO: 108 in the sequence listing was synthesized (GENEART, artificial gene synthesis service). Using the synthesized DNA fragment, in accordance with the protocols of GS Gene Expression System (registered trademark) by Lonza, a humanized h046_L1 type light chain

expression vector was constructed. The constructed expression vector was named "GSV-h046_L1".

[0294] 6)-7-2-2 Construction of humanized h046-L2 type light chain expression vector

A DNA fragment comprising a sequence encoding the humanized h046_L2 type light chain corresponding to nucleotide positions 61 to 702 of the nucleotide sequence (2) of the humanized h046_L2 type light chain shown in SEQ ID NO: 109 in the sequence listing was synthesized (GENEART, artificial gene synthesis service). Using the synthesized DNA fragment, in accordance with the protocols of GS Gene Expression System (registered trademark) by Lonza, a humanized h046_L2 type light chain expression vector was constructed. The constructed expression vector was named "GSV-h046_L2".

[0295] 6)-7-2-3 Construction of humanized h046-L6 type light chain expression vector

A DNA fragment comprising a sequence encoding the humanized h046_L6 type light chain corresponding to nucleotide positions 61 to 702 of the nucleotide sequence (2) of the humanized h046_L6 type light chain shown in SEQ ID NO: 110 in the sequence listing was synthesized (GENEART, artificial gene synthesis service). Using the synthesized DNA fragment, in accordance with the protocols of GS Gene Expression System (registered trademark) by Lonza, a humanized h046_L6 type light chain

expression vector was constructed. The constructed expression vector was named "GSV-h046_L6".

[0296] 6)-7-2-4 Construction of humanized h046-L7 type light chain expression vector

A DNA fragment comprising a sequence encoding the humanized h046_L7 type light chain corresponding to nucleotide positions 61 to 702 of the nucleotide sequence of the humanized h046_L7 type light chain shown in SEQ ID NO: 111 in the sequence listing was synthesized (GENEART, artificial gene synthesis service). Using the synthesized DNA fragment, in accordance with the protocols of GS Gene Expression System (registered trademark) by Lonza, a humanized h046_L7 type light chain expression vector was constructed. The constructed expression vector was named "GSV-h046_L7".

[0297] 6)-7-3 Construction of humanized anti-GPR20 antibody expression vector

6)-7-3-1 Construction of humanized h046-H4b/L7 expression vector

In accordance with the protocols of GS Gene Expression System (registered trademark) by Lonza, a humanized h046-H4b/L7 expression vector was constructed from the constructed expression vectors "GSV-h046_H4b" and "GSV-h046_L7". The obtained expression vector was named "DGV-h046_H4bL7-GS".

[0298] 6)-7-3-2 Construction of humanized h046-H5b/L2 expression vector

In accordance with the protocols of GS Gene Expression System (registered trademark) by Lonza, a humanized h046-H5b/L2 expression vector was constructed from the constructed expression vectors "GSV-h046_H5b" and "GSV-h046_L2". The obtained expression vector was named "DGV-h046_H5bL2-GS".

[0299] 6)-7-3-3 Construction of humanized h046-Hwt/L6 expression vector

In accordance with the protocols of GS Gene Expression System (registered trademark) by Lonza, a humanized h046-Hwt/L6 expression vector was constructed from the constructed expression vectors "GSV-h046_Hwt" and "GSV-h046_L6". The obtained expression vector was named "DGV-h046_HwtL6-GS".

[0300] 6)-7-3-4 Construction of humanized h046-H8/L1 expression vector

In accordance with the protocols of GS Gene Expression System (registered trademark) by Lonza, a humanized h046-H8/L1 expression vector was constructed from the constructed expression vectors "GSV-h046_H8" and "GSV-h046_L1". The obtained expression vector was named "DGV-h046_H8L1-GS".

[0301] 6)-7-3-5 Construction of humanized h046-H10/L1 expression vector

In accordance with the protocols of GS Gene Expression System (registered trademark) by Lonza, a humanized h046-H10/L1 expression vector was constructed

from the constructed expression vectors "GSV-h046_H10" and "GSV-h046_L1". The obtained expression vector was named "DGV-h046_H10L1-GS".

[0302] 6)-7-3-6 Construction of humanized h046-H10/L6 expression vector

In accordance with the protocols of GS Gene Expression System (registered trademark) by Lonza, a humanized h046-H10/L6 expression vector was constructed from the constructed expression vectors "GSV-h046_H10" and "GSV-h046_L6". The obtained expression vector was named "DGV-h046_H10L6-GS".

[0303] 6)-7-3-7 Construction of humanized h046-H4e/L7 expression vector

In accordance with the protocols of GS Gene Expression System (registered trademark) by Lonza, a humanized h046-H4e/L7 expression vector was constructed from the constructed expression vectors "GSV-h046_H4e" and "GSV-h046_L7". The obtained expression vector was named "DGV-h046_H4eL7-GS".

[0304] 6)-7-4 Preparation of cells that produce humanized anti-GPR20 antibody

6)-7-4-1 Preparation of cells that produce humanized h046-H4b/L7

CHOK1SV cells (Lonza) were transfected with the humanized h046-H4b/L7 expression vector DGV-h046_H4bL7-GS, which had been constructed in Example 6)-7-3-1 in accordance with the protocols of GS Gene Expression

System (registered trademark) by Lonza, so as to construct a cell line producing the humanized h046-H4b/L7. The obtained producing cell line was named "GPR1-12".

[0305] 6)-7-4-2 Preparation of cells that produce humanized h046-H5b/L2

CHOK1SV cells (Lonza) were transfected with the humanized h046-H5b/L2 expression vector DGV-h046_H5bL2-GS, which had been constructed in Example 6)-7-3-2 in accordance with the protocols of GS Gene Expression System (registered trademark) by Lonza, so as to construct a cell line producing the humanized h046-H5b/L2. The obtained producing cell line was named "GPR2-15".

[0306] 6)-7-4-3 Preparation of cells that produce humanized h046-Hwt/L6

CHOK1SV cells (Lonza) were transfected with the humanized h046-Hwt/L6 expression vector DGV-h046_H4wtL6-GS, which had been constructed in Example 6)-7-3-3 in accordance with the protocols of GS Gene Expression System (registered trademark) by Lonza, so as to construct a cell line producing the humanized h046-Hwt/L6. The obtained producing cell line was named "GPR3-2".

[0307] 6)-7-4-4 Preparation of cells that produce humanized h046-H8/L1

CHOK1SV cells (Lonza) were transfected with the humanized h046-H8/L1 expression vector DGV-h046_H8L1-GS, which had been constructed in Example 6)-7-3-4 in accordance with the protocols of GS Gene Expression

System (registered trademark) by Lonza, so as to construct a cell line producing the humanized h046-H8/L1. The obtained producing cell line was named "GPR4-1".

[0308] 6)-7-4-5 Preparation of cells that produce humanized h046-H10/L1

CHOK1SV cells (Lonza) were transfected with the humanized h046-H10/L1 expression vector DGV-h046_H10L1-GS, which had been constructed in Example 6)-7-3-5 in accordance with the protocols of GS Gene Expression System (registered trademark) by Lonza, so as to construct a cell line producing the humanized h046-H10/L1. The obtained producing cell line was named "GPR5-10".

[0309] 6)-7-4-6 Preparation of cells that produce humanized h046-H10/L6

CHOK1SV cells (Lonza) were transfected with the humanized h046-H10/L6 expression vector DGV-h046_H10L6-GS, which had been constructed in Example 6)-7-3-6 in accordance with the protocols of GS Gene Expression System (registered trademark) by Lonza, so as to construct a cell line producing the humanized h046-H10/L6. The obtained producing cell line was named "GPR6-7".

[0310] 6)-7-4-7 Preparation of cells that produce humanized h046-H4e/L7

CHOK1SV cells (Lonza) were transfected with the humanized h046-H4e/L7 expression vector DGV-h046_H4eL7-GS, which had been constructed in Example 6)-7-3-7 in accordance with the protocols of GS Gene Expression

System (registered trademark) by Lonza, so as to construct a cell line producing the humanized h046-H4e/L7. The obtained producing cell line was named "GPE-23".
[0311] 6)-7-5 Culture of cells that produce humanized anti-GPR20 antibody

6)-7-5-1 Culture of cells that produce humanized h046-H4b/L7

The humanized h046-H4b/L7-producing cell line "GPR1-12" prepared in Example 6)-7-4-1 was cultured using a culture apparatus Wave reactor (GE Healthcare Japan Corp.). The producing cell line "GPR1-12" was thawed in C36 (JX Energy) medium, and then cultured at 120 rpm in a 5% CO₂ incubator at 37°C. The obtained culture solution was diluted with C36 medium, and then expansively cultured at 120 rpm in a 5% CO₂ incubator at 37°C. The obtained culture solution was diluted with C36 medium at a cell density of 30×10^4 cells/mL, and then transferred into a WAVE CELLBAG (GE Healthcare Biosciences Corp.), followed by culture at 37°C in 5% CO₂, at an air-supplying rate of 0.3 L/min, at a rotation rate of 18 to 24 rpm, at an angle of 6 to 8°, for 10 days. From the 3rd day after initiation of the culture, FM4Ae2 medium (self-prepared) was added every day to the culture in an amount of 6% of the initial culture volume per day. The obtained culture solution was roughly filtrated through a depth filter Millistak MCOHC054H1 (Merck Millipore), and then filtrated through a 0.22-μm filter (Sartorius Inc.)

attached to Flexboy Bags. This filtrate was named "h046-H4b/L7 culture supernatant".

[0312] 6)-7-5-2 Culture of cells that produce humanized h046-H5b/L2

In the same manner as that applied in Example 6)-7-5-1, the humanized h046-H5b/L2-producing cell line "GPR2-15" prepared in Example 6)-7-4-2 was cultured and expanded, and thereafter, the cells were subjected to fed-batch culture using a culture apparatus Wave reactor (GE Healthcare Japan Corp.). The obtained culture was diluted with C36 medium at a cell density of 30×10^4 cells/mL, and then transferred into a WAVE CELLBAG (GE Healthcare Bioscience), followed by culture for 11 days. The obtained culture solution was filtrated, and the obtained filtrate was named "h046-H5b/L2 culture supernatant".

[0313] 6)-7-5-3 Culture of cells that produce humanized h046-Hwt/L6

In the same manner as that applied in Example 6)-7-5-1, the humanized h046-Hwt/L6-producing cell line "GPR3-2" prepared in Example 6)-7-4-3 was cultured and expanded, and thereafter, the cells were subjected to fed-batch culture using a culture apparatus Wave reactor (GE Healthcare Japan Corp.). The obtained culture was diluted with C36 medium at a cell density of 30×10^4 cells/mL, and then transferred into a WAVE CELLBAG (GE Healthcare Bioscience), followed by culture for 14 days.

The obtained culture solution was filtrated, and the obtained filtrate was named "h046-Hwt/L6 culture supernatant".

[0314] 6)-7-5-4 Culture of cells that produce humanized h046-H8/L1

In the same manner as that applied in Example 6)-7-5-1, the humanized h046-H8/L1-producing cell line "GPR4-1" prepared in Example 6)-7-4-4 was cultured and expanded, and thereafter, the cells were subjected to fed-batch culture using a culture apparatus Wave reactor (GE Healthcare Japan Corp.). The obtained culture was diluted with C36 medium at a cell density of 30×10^4 cells/mL, and then transferred into a WAVE CELLBAG (GE Healthcare Bioscience), followed by culture for 11 days. The obtained culture solution was filtrated, and the obtained filtrate was named "h046-H8/L1 culture supernatant".

[0315] 6)-7-5-5 Culture of cells that produce humanized h046-H10/L1

In the same manner as that applied in Example 6)-7-5-1, the humanized h046-H10/L1-producing cell line "GPR5-10" prepared in Example 6)-7-4-5 was cultured and expanded, and thereafter, the cells were subjected to fed-batch culture using a culture apparatus Wave reactor (GE Healthcare Japan Corp.). The obtained culture was diluted with C36 medium at a cell density of 30×10^4 cells/mL, and then transferred into a WAVE CELLBAG (GE

Healthcare Bioscience), followed by culture for 10 days. The obtained culture solution was filtrated, and the obtained filtrate was named "h046-H10/L1 culture supernatant".

[0316] 6)-7-5-6 Culture of cells that produce humanized h046-H10/L6

In the same manner as that applied in Example 6)-7-5-1, the humanized h046-H10/L6-producing cell line "GPR6-7" prepared in Example 6)-7-4-6 was cultured and expanded, and thereafter, the cells were subjected to fed-batch culture using a culture apparatus Wave reactor (GE Healthcare Japan Corp.). The obtained culture was diluted with C36 medium at a cell density of 30×10^4 cells/mL, and then transferred into a WAVE CELLBAG (GE Healthcare Bioscience), followed by culture for 12 days. The obtained culture solution was filtrated, and the obtained filtrate was named "h046-H10/L6 culture supernatant".

[0317] 6)-7-5-7 Culture of cells that produce humanized h046-H4e/L7

In the same manner as that applied in Example 6)-7-5-1, the humanized h046-H4e/L7-producing cell line "GPE-23" prepared in Example 6)-7-4-7 was cultured and expanded, and thereafter, the cells were subjected to fed-batch culture using a culture apparatus Wave reactor (GE Healthcare Japan Corp.). The obtained culture was diluted with C36 medium at a cell density of 30×10^4

cells/mL, and then transferred into a WAVE CELLBAG (GE Healthcare Bioscience), followed by culture for 11 days. The obtained culture solution was filtrated, and the obtained filtrate was named "h046-H4e/L7 culture supernatant".

[0318] 6)-7-6 Purification of humanized anti-GPR20 antibody

6)-7-6-1 Purification of humanized h046-H4b/L7

The "h046-H4b/L7 culture supernatant" obtained in Example 6)-7-5-1 was purified by a three-step process, namely, by rProtein A affinity chromatography, anion exchange chromatography, and cation exchange chromatography. First, the culture supernatant was applied to rProtein A affinity chromatographic resin that had been equilibrated with PBS. After the entire culture solution had entered the column, the column was washed with PBS, a buffer solution containing arginine, and PBS. Subsequently, the remaining substance in the column was eluted with an acetate buffer, and an absorption peak at 280 nm was then collected. The collected solution was neutralized with a Tris buffer, and then roughly filtrated through a glass fiber filter AP20 (Merck Millipore). Thereafter, the solution was filtrated through Stericup-GV (Merck Millipore) that was a 0.22- μ m filter, and the resultant filtrate was defined as an rProtein A purified pool.

[0319] Subsequently, the rProtein A purified pool was applied to an anion exchange chromatographic resin that had been equilibrated with PBS. After the entire applied solution had entered the column, PBS was supplied. A flow-through fraction and the absorption peak at 280 nm at the time of the supply of PBS were collected. The pH of the collected solution was adjusted with acetic acid. The solution was filtrated through Stericup-GV (Merck Millipore) that was a 0.22- μ m filter, and the resultant filtrate was defined as an AEX purified pool.

Subsequently, the AEX purified pool was applied to a cation exchange chromatographic resin that had been equilibrated with an acetate buffer. After the entire applied solution had entered the column, the column was washed with an acetate buffer. Thereafter, elution was carried out using an acetate buffer containing a high concentration of NaCl, and the absorption peak at 280 nm was collected. The collected solution was filtrated through Stericup-GV (Merck Millipore) that was a 0.22- μ m filter, and the resultant filtrate was defined as a CEX purified pool.

The CEX purified pool was concentrated to an antibody concentration of 20 mg/mL with Pellicon 3 Cassette 30 kDa (Merck Millipore), and the buffer was then replaced with a histidine buffer (25 mM histidine, 5% sorbitol, pH 6.0). Finally, the solution was filtrated through Stericup-GV (Merck Millipore) that was

a 0.22- μ m filter, so as to obtain a purified sample.

This purified sample was named "h046_H4b/L7".

[0320] 6)-7-6-2 Purification of humanized h046-H5b/L2

The "h046-H5b/L2 culture supernatant" obtained in Example 6)-7-5-2 was purified by a three-step process, namely, by rProtein A affinity chromatography, anion exchange chromatography, and cation exchange chromatography. First, the culture supernatant was applied to rProtein A affinity chromatographic resin that had been equilibrated with PBS. After the entire culture solution had entered the column, the column was washed with PBS, a buffer solution containing arginine, and PBS. Subsequently, the remaining substance in the column was eluted with an acetate buffer, and an absorption peak at 280 nm was then collected. The collected solution was neutralized with a Tris buffer, and then roughly filtrated through a glass fiber filter AP20 (Merck Millipore). Thereafter, the solution was filtrated through Stericup-GV (Merck Millipore) that was a 0.22- μ m filter, and the resultant filtrate was defined as an rProtein A purified pool.

[0321] Subsequently, the rProtein A purified pool was applied to an anion exchange chromatographic resin that had been equilibrated with PBS. After the entire applied solution had entered the column, PBS was supplied. A flow-through fraction and the absorption peak at 280 nm at the time of the supply of PBS were collected. The pH

of the collected solution was adjusted with acetic acid. The solution was filtrated through Stericup-GV (Merck Millipore) that was a 0.22- μ m filter, and the resultant filtrate was defined as an AEX purified pool.

[0322] Subsequently, the AEX purified pool was applied to a cation exchange chromatographic resin that had been equilibrated with an acetate buffer. After the entire applied solution had entered the column, the column was washed with an acetate buffer. Thereafter, elution was carried out using an acetate buffer containing a high concentration of NaCl, and the absorption peak at 280 nm was collected. The collected solution was filtrated through Stericup-GV (Merck Millipore) that was a 0.22- μ m filter, and the resultant filtrate was defined as a CEX purified pool.

The CEX purified pool was concentrated to an antibody concentration of 40 mg/mL with Pellicon 3 Cassette 30 kDa (Merck Millipore), and the buffer was then replaced with a histidine buffer (25 mM histidine, 5% sorbitol, pH 6.0). Finally, the solution was filtrated through Stericup-GV (Merck Millipore) that was a 0.22- μ m filter, so as to obtain a purified sample. This purified sample was named "h046_H5b/L2".

[0323] 6)-7-6-3 Purification of humanized h046-Hwt/L6

The "h046-Hwt/L6 culture supernatant" obtained in Example 6)-7-5-3 was purified by a three-step process, namely, by rProtein A affinity chromatography, anion

exchange chromatography, and cation exchange chromatography. First, the culture supernatant was applied to rProtein A affinity chromatographic resin that had been equilibrated with PBS. After the entire culture solution had entered the column, the column was washed with PBS, a buffer solution containing arginine, and PBS. Subsequently, the remaining substance in the column was eluted with an acetate buffer, and an absorption peak at 280 nm was then collected. The collected solution was neutralized with a Tris buffer. The solution was filtrated through Millipore Express SHC (Merck Millipore) that was a 0.5/0.2- μ m filter, and the resultant filtrate was defined as an rProtein A purified pool.

[0324] Subsequently, the rProtein A purified pool was applied to an anion exchange chromatographic resin that had been equilibrated with PBS. After the entire applied solution had entered the column, PBS was supplied. A flow-through fraction and the absorption peak at 280 nm at the time of the supply of PBS were collected. The pH of the collected solution was adjusted with acetic acid. The solution was filtrated through Stericup-GV (Merck Millipore) that was a 0.22- μ m filter, and the resultant filtrate was defined as an AEX purified pool.

Subsequently, the AEX purified pool was applied to a cation exchange chromatographic resin that had been equilibrated with an acetate buffer. After the entire applied solution had entered the column, the column was

washed with an acetate buffer. Thereafter, elution was carried out using an acetate buffer containing a high concentration of NaCl, and the absorption peak at 280 nm was collected. The collected solution was filtrated through Stericup-GV (Merck Millipore) that was a 0.22- μ m filter, and the resultant filtrate was defined as a CEX purified pool.

The CEX purified pool was concentrated to an antibody concentration of 40 mg/mL with Pellicon 3 Cassette 30 kDa (Merck Millipore), and the buffer was then replaced with a histidine buffer (25 mM histidine, 5% sorbitol, pH 6.0). Finally, the solution was filtrated through Stericup-GV (Merck Millipore) that was a 0.22- μ m filter, so as to obtain a purified sample. This purified sample was named "h046-Hwt/L6".

[0325] 6)-7-6-4 Purification of humanized h046-H8/L1

The "h046-H8/L1 culture supernatant" obtained in Example 6)-7-5-4 was purified by a three-step process, namely, by rProtein A affinity chromatography, anion exchange chromatography, and cation exchange chromatography. First, the culture supernatant was applied to rProtein A affinity chromatographic resin that had been equilibrated with PBS. After the entire culture solution had entered the column, the column was washed with PBS, a buffer solution containing arginine, and PBS. Subsequently, the remaining substance in the column was eluted with an acetate buffer, and an absorption peak at

280 nm was then collected. The collected solution was neutralized with a Tris buffer. The solution was filtrated through Stericup-GV (Merck Millipore) that was a 0.22- μ m filter, and the resultant filtrate was defined as an rProtein A purified pool.

[0326] Subsequently, the rProtein A purified pool was applied to an anion exchange chromatographic resin that had been equilibrated with PBS. After the entire applied solution had entered the column, PBS was supplied. A flow-through fraction and the absorption peak at 280 nm at the time of the supply of PBS were collected. The pH of the collected solution was adjusted with acetic acid. The solution was filtrated through Stericup-GV (Merck Millipore) that was a 0.22- μ m filter, and the resultant filtrate was defined as an AEX purified pool.

Subsequently, the AEX purified pool was applied to a cation exchange chromatographic resin that had been equilibrated with an acetate buffer. After the entire applied solution had entered the column, the column was washed with an acetate buffer. Thereafter, elution was carried out using an acetate buffer containing a high concentration of NaCl, and the absorption peak at 280 nm was collected. The collected solution was filtrated through Stericup-GV (Merck Millipore) that was a 0.22- μ m filter, and the resultant filtrate was defined as a CEX purified pool.

The CEX purified pool was concentrated to an antibody concentration of 40 mg/mL with Pellicon 3 Cassette 30 kDa (Merck Millipore), and the buffer was then replaced with a histidine buffer (25 mM histidine, 5% sorbitol, pH 6.0). Finally, the solution was filtrated through Stericup-GV (Merck Millipore) that was a 0.22- μ m filter, so as to obtain a purified sample. This purified sample was named "h046-H8/L1".

[0327] 6)-7-6-5 Purification of humanized h046-H10/L1

The "h046-H10/L1 culture supernatant" obtained in Example 6)-7-5-5 was purified by a three-step process, namely, by rProtein A affinity chromatography, anion exchange chromatography, and cation exchange chromatography. First, the culture supernatant was applied to rProtein A affinity chromatographic resin that had been equilibrated with PBS. After the entire culture solution had entered the column, the column was washed with PBS, a buffer solution containing arginine, and PBS. Subsequently, the remaining substance in the column was eluted with an acetate buffer, and an absorption peak at 280 nm was then collected. The collected solution was neutralized with a Tris buffer, and then roughly filtrated through a glass fiber filter AP20 (Merck Millipore). Thereafter, the solution was filtrated through Stericup-GV (Merck Millipore) that was a 0.22- μ m filter, and the resultant filtrate was defined as an rProtein A purified pool.

[0328] Subsequently, the rProtein A purified pool was applied to an anion exchange chromatographic resin that had been equilibrated with PBS. After the entire applied solution had entered the column, PBS was supplied. A flow-through fraction and the absorption peak at 280 nm at the time of the supply of PBS were collected. The pH of the collected solution was adjusted with acetic acid. The solution was filtrated through Stericup-GV (Merck Millipore) that was a 0.22- μ m filter, and the resultant filtrate was defined as an AEX purified pool.

Subsequently, the AEX purified pool was applied to a cation exchange chromatographic resin that had been equilibrated with an acetate buffer. After the entire applied solution had entered the column, the column was washed with an acetate buffer. Thereafter, elution was carried out using an acetate buffer containing a high concentration of NaCl, and the absorption peak at 280 nm was collected. The collected solution was filtrated through Stericup-GV (Merck Millipore) that was a 0.22- μ m filter, and the resultant filtrate was defined as a CEX purified pool.

The CEX purified pool was concentrated to an antibody concentration of 40 mg/mL with Pellicon 3 Cassette 30 kDa (Merck Millipore), and the buffer was then replaced with a histidine buffer (25 mM histidine, 5% sorbitol, pH 6.0). Finally, the solution was filtrated through Stericup-GV (Merck Millipore) that was

a 0.22- μ m filter, so as to obtain a purified sample.

This purified sample was named "h046-H10/L1".

[0329] 6)-7-6-6 Purification of humanized h046-H10/L6

The "h046-H10/L6 culture supernatant" obtained in Example 6)-7-5-6 was purified by a three-step process, namely, by rProtein A affinity chromatography, anion exchange chromatography, and cation exchange chromatography. First, the culture supernatant was applied to rProtein A affinity chromatographic resin that had been equilibrated with PBS. After the entire culture solution had entered the column, the column was washed with PBS, a buffer solution containing arginine, and PBS. Subsequently, the remaining substance in the column was eluted with an acetate buffer, and an absorption peak at 280 nm was then collected. The collected solution was neutralized with a Tris buffer. The solution was filtrated through Stericup-GV (Merck Millipore) that was a 0.22- μ m filter, and the resultant filtrate was defined as an rProtein A purified pool.

[0330] Subsequently, the rProtein A purified pool was applied to an anion exchange chromatographic resin that had been equilibrated with PBS. After the entire applied solution had entered the column, PBS was supplied. A flow-through fraction and the absorption peak at 280 nm at the time of the supply of PBS were collected. The pH of the collected solution was adjusted with acetic acid. The solution was filtrated through Stericup-GV (Merck

Millipore) that was a 0.22- μ m filter, and the resultant filtrate was defined as an AEX purified pool.

Subsequently, the AEX purified pool was applied to a cation exchange chromatographic resin that had been equilibrated with an acetate buffer. After the entire applied solution had entered the column, the column was washed with an acetate buffer. Thereafter, elution was carried out using an acetate buffer containing a high concentration of NaCl, and the absorption peak at 280 nm was collected. The collected solution was filtrated through Stericup-GV (Merck Millipore) that was a 0.22- μ m filter, and the resultant filtrate was defined as a CEX purified pool.

The CEX purified pool was concentrated to an antibody concentration of 40 mg/mL with Pellicon 3 Cassette 30 kDa (Merck Millipore), and the buffer was then replaced with a histidine buffer (25 mM histidine, 5% sorbitol, pH 6.0). Finally, the solution was filtrated through Stericup-GV (Merck Millipore) that was a 0.22- μ m filter, so as to obtain a purified sample. This purified sample was named "h046-H10/L6".

[0331] 6)-7-6-7 Purification of humanized h046-H4e/L7

The "h046-H4e/L7 culture supernatant" obtained in Example 6)-7-5-7 was purified by a three-step process, namely, by rProtein A affinity chromatography, anion exchange chromatography, and cation exchange chromatography. First, the culture supernatant was

applied to rProtein A affinity chromatographic resin that had been equilibrated with PBS. After the entire culture solution had entered the column, the column was washed with PBS, a buffer solution containing arginine, and PBS. Subsequently, the remaining substance in the column was eluted with an acetate buffer, and an absorption peak at 280 nm was then collected. The collected solution was neutralized with a Tris buffer. The solution was filtrated through Millipore Express SHC (Merck Millipore) that was a 0.5/0.2- μ m filter, and the resultant filtrate was defined as an rProtein A purified pool.

[0332] Subsequently, the rProtein A purified pool was applied to an anion exchange chromatographic resin that had been equilibrated with PBS. After the entire applied solution had entered the column, PBS was supplied. A flow-through fraction and the absorption peak at 280 nm at the time of the supply of PBS were collected. The pH of the collected solution was adjusted with acetic acid. The solution was filtrated through Stericup-GV (Merck Millipore) that was a 0.22- μ m filter, and the resultant filtrate was defined as an AEX purified pool.

Subsequently, the AEX purified pool was applied to a cation exchange chromatographic resin that had been equilibrated with an acetate buffer. After the entire applied solution had entered the column, the column was washed with an acetate buffer. Thereafter, elution was carried out using an acetate buffer containing a high

concentration of NaCl, and the absorption peak at 280 nm was collected. The collected solution was filtrated through Stericup-GV (Merck Millipore) that was a 0.22- μ m filter, and the resultant filtrate was defined as a CEX purified pool.

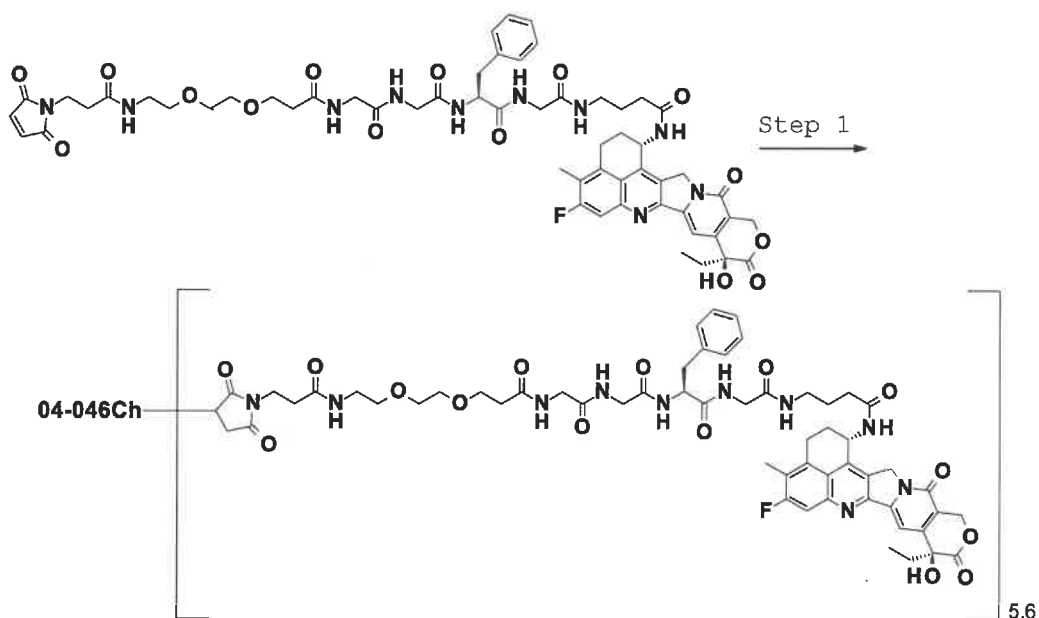
The CEX purified pool was concentrated to an antibody concentration of 40 mg/mL with Pellicon 3 Cassette 30 kDa (Merck Millipore), and the buffer was then replaced with a histidine buffer (25 mM histidine, 5% sorbitol, pH 6.0). Finally, the solution was filtrated through Stericup-GV (Merck Millipore) that was a 0.22- μ m filter, so as to obtain a purified sample. This purified sample was named "h046-H4e/L7".

[0333] Example 7: Production of anti-GPR20 antibody-drug conjugate

7)-1 Production of antibody-drug conjugate - (1)

[0334]

[Formula 14]



[0335] Step 1: Antibody-drug conjugate (1)

Reduction of antibody: 04-046Ch produced in Example 5)-2 was adjusted to 10 mg/mL with PBS6.0/EDTA by using common procedures B (using $1.47 \text{ mLmg}^{-1}\text{cm}^{-1}$ as 280 nm absorption coefficient) and C described in production method 1. To this solution (3.40 mL), an aqueous solution of 9.4 mM TCEP (Tokyo Chemical Industry Co., Ltd.) (0.104 mL; 5.0 equivalents per antibody molecule) and a 1 M aqueous dipotassium hydrogen phosphate solution (Nacalai Tesque, Inc.; 0.170 mL) were added. After confirming that the solution had a pH within 7.4 ± 0.1 , the interchain disulfide bond in the antibody was reduced by incubating the solution at 37°C for 1 hour.

[0336] Conjugation between antibody and drug linker: The above-described solution was incubated at 15°C for 10

minutes. Subsequently, a 10 mM solution of N-{3-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]propanoyl}glycylglycyl-L-phenylalanyl-N-(4-{[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino}-4-oxobutyl)glycinamide in dimethyl sulfoxide (0.189 mL; 8.6 equivalents per antibody molecule) was added thereto, and the obtained mixture was incubated at 15°C for 1 hour to conjugate the drug linker to the antibody. Subsequently, an aqueous solution of 100 mM NAC (Sigma-Aldrich Co. LLC) (0.0284 mL; 12.9 equivalents per antibody molecule) was added thereto, and the obtained mixture was further stirred at room temperature for 20 minutes to terminate the reaction of the drug linker.

[0337] Purification: The above-described solution was purified by common procedure D described in production method 1 to obtain 14.0 mL of a solution containing the antibody-drug conjugate "046Ch-ADC2".

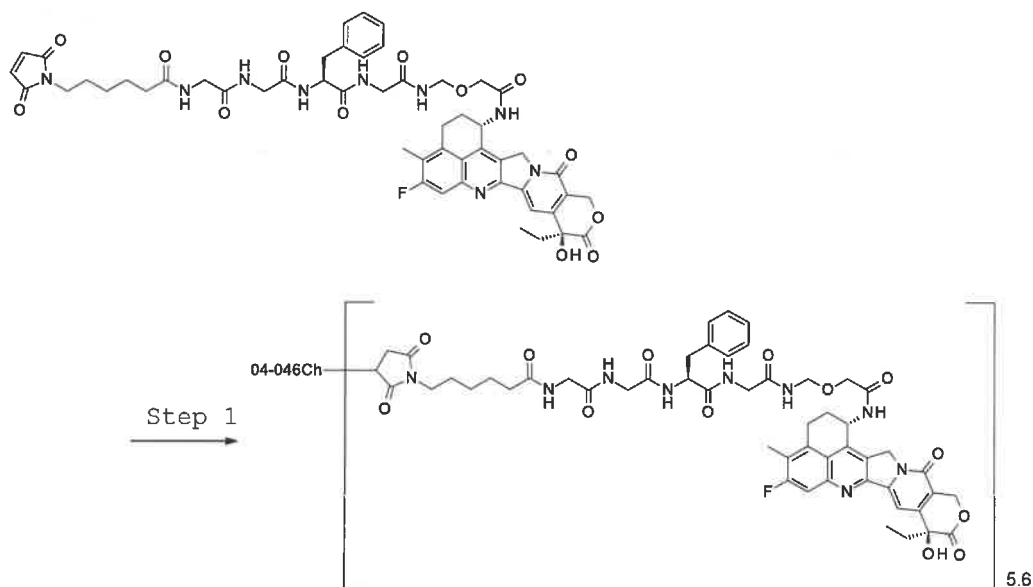
[0338] Characterization: Using common procedure E (using $\epsilon_{D,280} = 4964$ and $\epsilon_{D,370} = 18982$) described in production method 1, the following characteristic values were obtained.

Antibody concentration: 2.26 mg/mL, antibody yield: 31.6 mg (93%), and average number of conjugated drug molecules (n) per antibody molecule: 5.6.

7)-2 Production of antibody-drug conjugate (2)

[0339]

[Formula 15]



[0340] Step 1: Antibody-drug conjugate (2)

Reduction of antibody: 04-046Ch produced in Example 5)-2 was adjusted to 10 mg/mL with PBS6.0/EDTA by using common procedures B (using $1.47 \text{ mLmg}^{-1}\text{cm}^{-1}$ as 280 nm absorption coefficient) and C described in production method 1. To this solution (3.40 mL), an aqueous solution of 9.4 mM TCEP (Tokyo Chemical Industry Co., Ltd.) (0.104 mL; 5.0 equivalents per antibody molecule) and a 1 M aqueous dipotassium hydrogen phosphate solution (Nacalai Tesque, Inc.; 0.0509 mL) were added. After confirming that the solution had a pH within 7.0 ± 0.1 , the interchain disulfide bond in the antibody was reduced by incubating the solution at 37°C for 1 hour.

[0341] Conjugation between antibody and drug linker: The above-described solution was incubated at 15°C for 10 minutes. Subsequently, a 9.3 M solution of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]glycylglycyl-L-phenylalanyl-N-[(2-[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino)-2-oxoethoxy)methyl]glycinamide in dimethyl sulfoxide (0.176 mL; 8.6 equivalents per antibody molecule) was added thereto, and the obtained mixture was incubated at 15°C for 1 hour to conjugate the drug linker to the antibody. Subsequently, an aqueous solution of 100 mM NAC (Sigma-Aldrich Co. LLC) (0.0284 mL; 12.9 equivalents per antibody molecule) was added thereto, and the obtained mixture was further stirred at room temperature for 20 minutes to terminate the reaction of the drug linker.

[0342] Purification: The above-described solution was purified by common procedure D described in production method 1 to obtain 14.0 mL of a solution containing the antibody-drug conjugate "046Ch-ADC1".

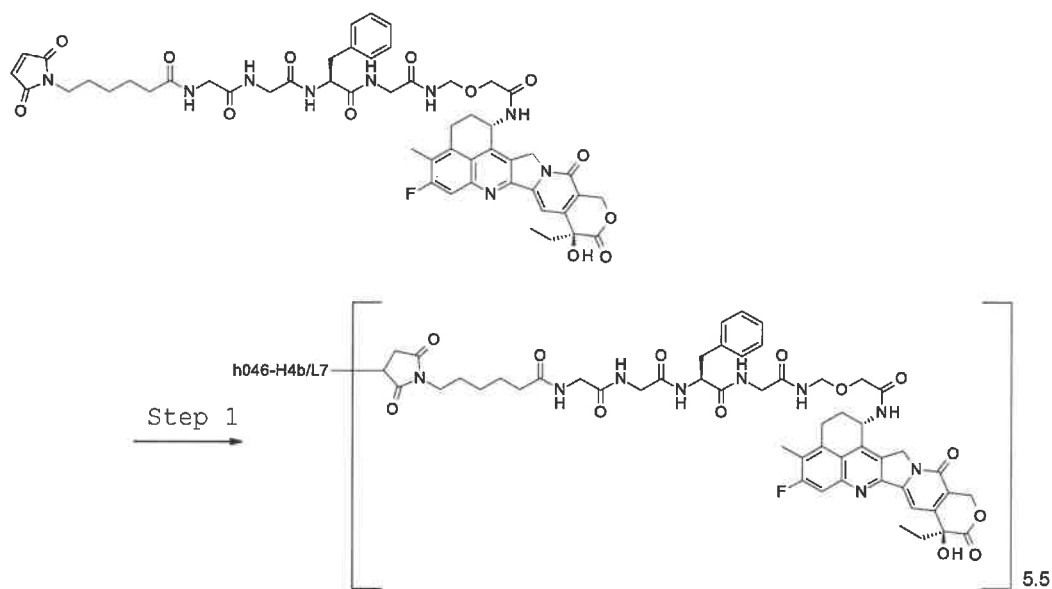
[0343] Characterization: Using common procedure E (using $\epsilon_{D,280} = 5178$ and $\epsilon_{D,370} = 20217$) described in production method 1, the following characteristic values were obtained.

Antibody concentration: 2.23 mg/mL, antibody yield: 31.2 mg (92%), and average number of conjugated drug molecules (n) per antibody molecule: 5.6.

7)-3 Production of antibody-drug conjugate (3)

[0344]

[Formula 16]



[0345] Step 1: Antibody-drug conjugate (3)

Reduction of antibody: h046-H4b/L7 produced in Example 6)-7-6-1 was adjusted to 10 mg/mL with PBS6.0/EDTA by using common procedures B (using 1.31 mLmg⁻¹cm⁻¹ as 280 nm absorption coefficient) and C described in production method 1. To this solution (6.25 mL), an aqueous solution of 10 mM TCEP (Tokyo Chemical Industry Co., Ltd.) (0.237 mL; 5.5 equivalents per antibody molecule) and a 1 M aqueous dipotassium hydrogen phosphate solution (Nacalai Tesque, Inc.; 0.0940 mL) were added. After confirming that the solution had a pH

within 7.0 ± 0.1 , the interchain disulfide bond in the antibody was reduced by incubating the solution at 37°C for 1 hour.

[0346] Conjugation between antibody and drug linker: The above-described solution was incubated at 15°C for 10 minutes. Subsequently, a 10 mM solution of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]glycylglycyl-L-phenylalanyl-N-[(2-[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino)-2-oxoethoxy)methyl]glycinamide in dimethyl sulfoxide (0.388 mL; 9.0 equivalents per antibody molecule) was added thereto, and the obtained mixture was incubated at 15°C for 1 hour to conjugate the drug linker to the antibody. Subsequently, an aqueous solution of 100 mM NAC (Sigma-Aldrich Co. LLC) (0.0390 mL; 9.0 equivalents per antibody molecule) was added thereto, and the obtained mixture was further stirred at room temperature for 20 minutes to terminate the reaction of the drug linker.

[0347] Purification: The above-described solution was purified by common procedure D described in production method 1 to obtain 30.0 mL of a solution containing the antibody-drug conjugate "h046-H4b/L7-ADC1".

Characterization: Using common procedures E and F ((using $\epsilon_{D,280} = 5178$ and $\epsilon_{D,370} = 20217$) described in

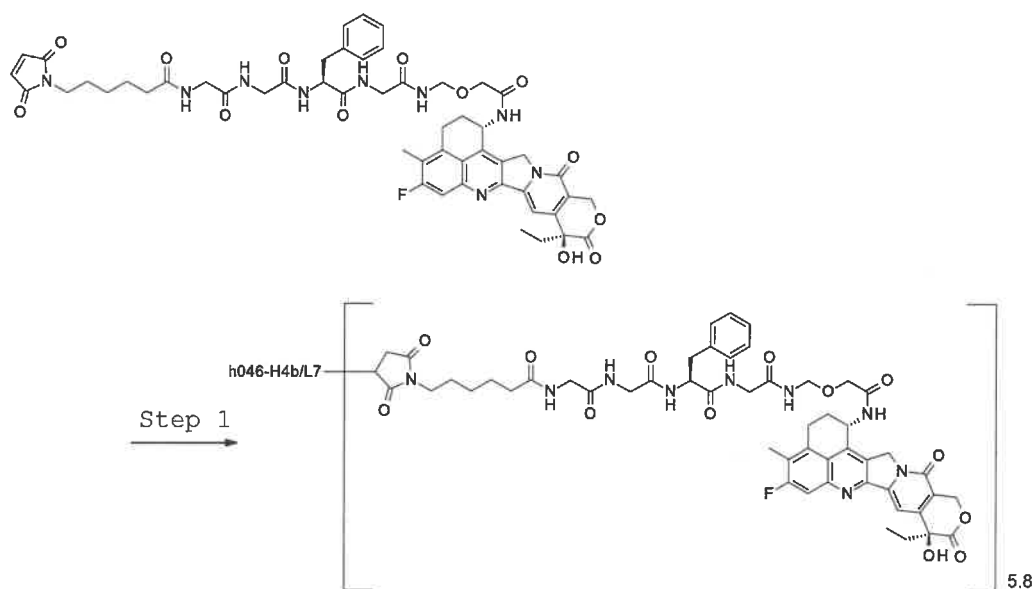
production method 1, the following characteristic values were obtained.

Antibody concentration: 2.03 mg/mL, antibody yield: 61.0 mg (97%), average number of conjugated drug molecules (n) per antibody molecule measured by common procedure E: 5.5, and average number of conjugated drug molecules (n) per antibody molecule measured by common procedure F: 7.8.

[0348] 7)-4 Production of antibody-drug conjugate (4)

[0349]

[Formula 17]



[0350] Step 1: Antibody-drug conjugate (4)

Reduction of antibody: h046-H4b/L7 produced in Example 6)-7-6-1 was adjusted to 10 mg/mL with PBS6.0/EDTA by using common procedures B (using 1.31 mLmg⁻¹cm⁻¹ as 280 nm absorption coefficient) and C described in production method 1. This solution (300 mL)

was placed in a 1000-mL polycarbonate Erlenmeyer flask, and then charged with a 1 M aqueous dipotassium hydrogen phosphate solution (4.80 mL) and thereafter with a 10 mM aqueous TCEP solution (11.3 mL; 5.5 equivalents per antibody molecule) at room temperature with stirring using a magnetic stirrer. After confirming that the solution had a pH within 7.0 ± 0.1 , the stirring was terminated, and the interchain disulfide bond in the antibody was reduced by incubating the solution at 37°C for 2 hours.

[0351] Conjugation between antibody and drug linker: The above-described solution was cooled to 15°C. Thereafter, a DMSO solution containing 10 mM N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]glycylglycyl-L-phenylalanyl-N-[(2-[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino}-2-oxoethoxy)methyl]glycinamide (18.5 mL; 9.0 equivalents per antibody molecule) was gradually added dropwise thereto with stirring. The obtained mixture was stirred at 15°C for 30 minutes to conjugate the drug linker to the antibody. Subsequently, a 100 mM aqueous NAC solution (1.85 mL; 9.0 equivalents per antibody molecule) was added thereto with stirring, and the obtained mixture was further stirred at room temperature for 20 minutes to terminate the reaction of unreacted drug linker.

[0352] Purification: The obtained solution was purified by ultrafiltration using an ultrafiltration apparatus constituted by an ultrafiltration membrane (Merck Japan, Ltd., Pellicon XL Cassette, Ultracell 30 KDa), a tube pump (Cole-Parmer International, USA, MasterFlex pump model 77521-40, pump head model 7518-00), and a tube (Cole-Parmer International, USA, MasterFlex tube L/S16). Specifically, while ABS was added dropwise (a total of 3.00 L) as a buffer solution for purification to the reaction solution, ultrafiltration purification was carried out, so as to replace the buffer with ABS and further concentrate the solution while removing non-conjugated drug linker and other low-molecular-weight reagents. The obtained purified solution was subjected to microfiltration (0.22 μm , PVDF membrane, twice) to obtain 83.0 mL of a solution containing the antibody-drug conjugate "h046-H4b/L7-ADC1".

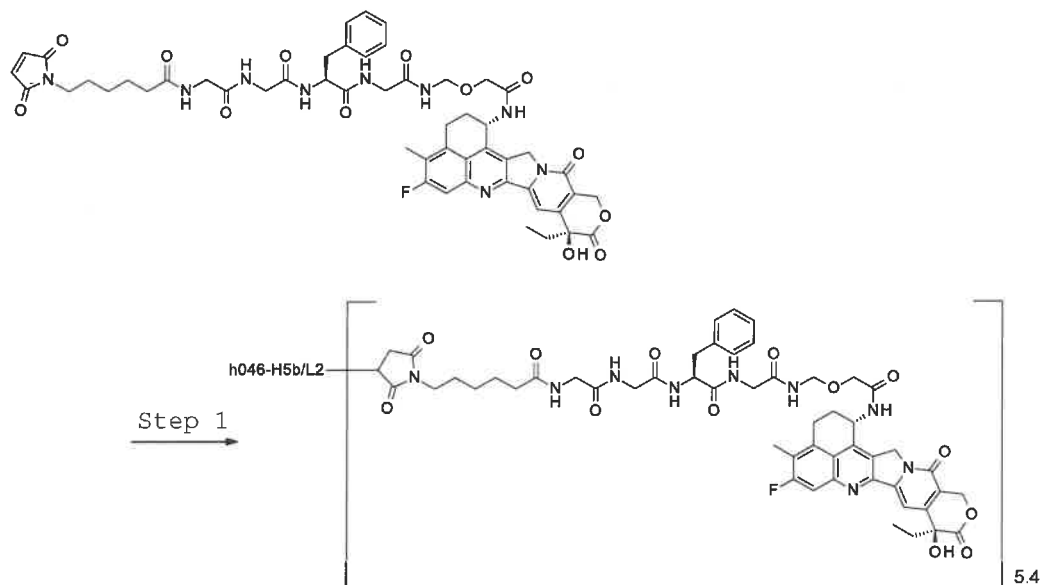
[0353] Characterization: Using common procedures E and F (using $\epsilon_{\text{D},280} = 5178$ and $\epsilon_{\text{D},370} = 20217$), the following characteristic values were obtained.

[0354] Antibody concentration: 23.1 mg/mL, antibody yield: 2.94 g (98%), average number of conjugated drug molecules (n) per antibody molecule measured by common procedure E: 5.8, and average number of conjugated drug molecules (n) per antibody molecule measured by common procedure F: 7.4.

[0355] 7)-5 Production of antibody-drug conjugate (5)

[0356]

[Formula 18]



[0357] Step 1: Antibody-drug conjugate (5)

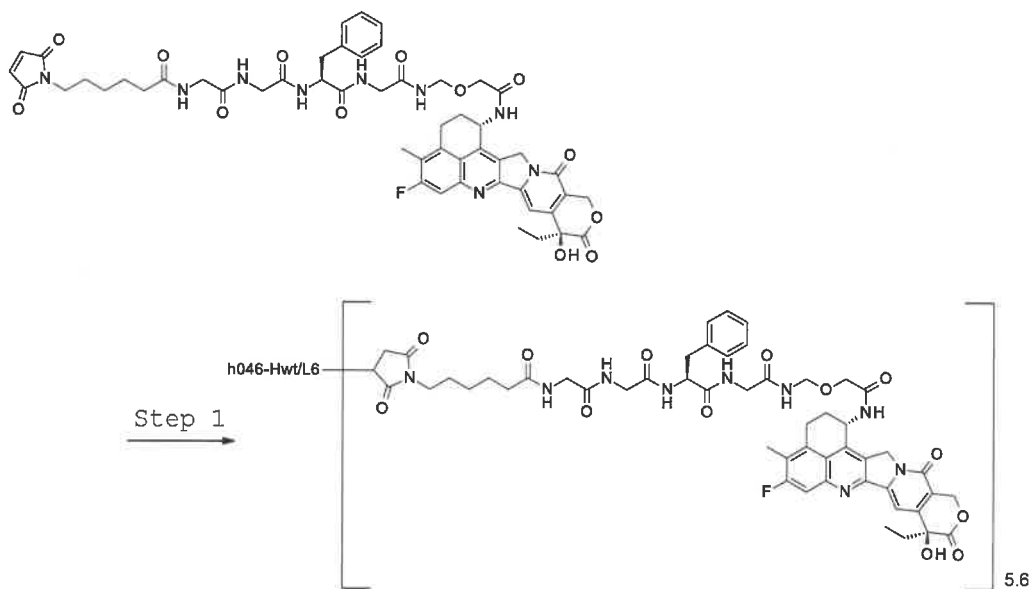
Using h046-H5b/L2 (using $1.31 \text{ mLmg}^{-1}\text{cm}^{-1}$ as 280 nm absorption coefficient) produced in Example 6)-7-6-2, the antibody-drug conjugate "h046-H5b/L2-ADC1" was obtained by the same method as that applied in step 1 of Example 7-3.

[0358] Antibody concentration: 2.04 mg/mL , antibody yield: 61.3 mg (98%), average number of conjugated drug molecules (n) per antibody molecule measured by common procedure E: 5.4, and average number of conjugated drug molecules (n) per antibody molecule measured by common procedure F: 7.9.

[0359] 7)-6 Production of antibody-drug conjugate (6)

[0360]

[Formula 19]



[0361] Step 1: Antibody-drug conjugate (6)

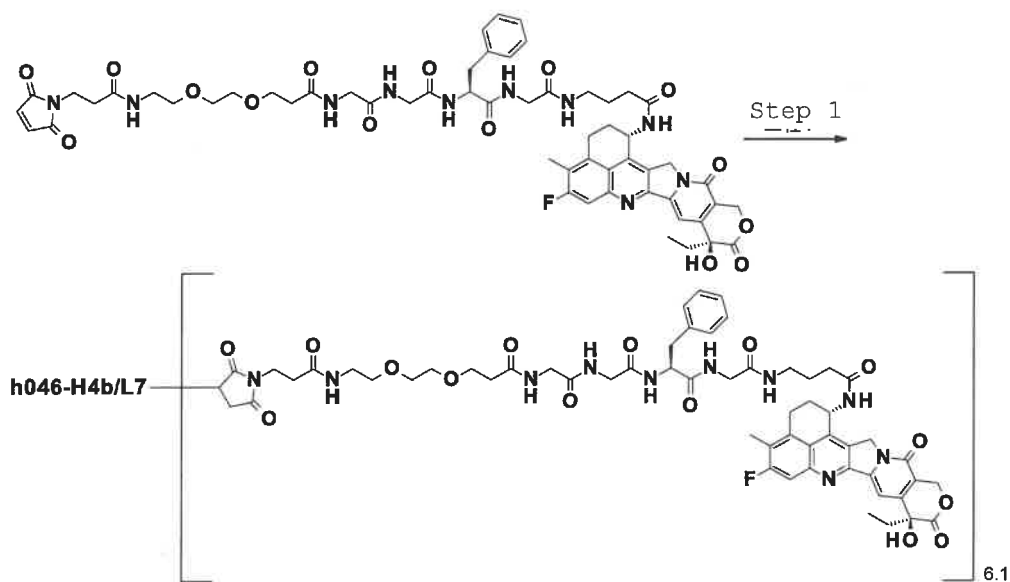
Using h046-Hwt/L6 (using $1.31 \text{ mLmg}^{-1}\text{cm}^{-1}$ as 280 nm absorption coefficient) produced in Example 6)-7-6-3, the antibody-drug conjugate "h046-Hwt/L6-ADC1" was obtained by the same method as that applied in step 1 of Example 7-3.

[0362] Antibody concentration: 2.03 mg/mL , antibody yield: 60.9 mg (95%), average number of conjugated drug molecules (n) per antibody molecule measured by common procedure E: 5.6, and average number of conjugated drug molecules (n) per antibody molecule measured by common procedure F: 7.8.

[0363] 7)-7 Production of antibody-drug conjugate (7)

[0364]

[Formula 20]



[0365] Step 1: Antibody-drug conjugate (7)

Reduction of antibody: h046-H4b/L7 produced in Example 6)-7-6-1 was adjusted to 10 mg/mL with PBS6.0/EDTA by using common procedures B (using 1.31 mLmg⁻¹cm⁻¹ as 280 nm absorption coefficient) and C described in production method 1. To this solution (5.00 mL), an aqueous solution of 10 mM TCEP (Tokyo Chemical Industry Co., Ltd.) (0.193 mL; 5.5 equivalents per antibody molecule) and a 1 M aqueous dipotassium hydrogen phosphate solution (Nacalai Tesque, Inc.; 0.0752 mL) were added. After confirming that the solution had a pH within 7.0 ± 0.1, the interchain disulfide bond in the antibody was reduced by incubating the solution at 37°C for 1 hour.

[0366] Conjugation between antibody and drug linker: The above-described solution was incubated at 15°C for 10

minutes. Subsequently, a 10 mM solution of N-{3-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]propanoyl}glycylglycyl-L-phenylalanyl-N-(4-{[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino}-4-oxobutyl)glycinamide in dimethyl sulfoxide (0.315 mL; 9.0 equivalents per antibody molecule) was added thereto, and the obtained mixture was incubated at 15°C for 1 hour to conjugate the drug linker to the antibody. Subsequently, an aqueous solution of 100 mM NAC (Sigma-Aldrich Co. LLC) (0.0316 mL; 9.0 equivalents per antibody molecule) was added thereto, and the obtained mixture was further stirred at room temperature for 20 minutes to terminate the reaction of the drug linker.

[0367] Purification: The above-described solution was purified by common procedure D described in production method 1 to obtain 24.0 mL of a solution containing the antibody-drug conjugate "h046-H4b/L7-ADC2".

[0368] Characterization: Using common procedures E and F (using $\epsilon_{D,280} = 4964$ and $\epsilon_{D,370} = 18982$) described in production method 1, the following characteristic values were obtained.

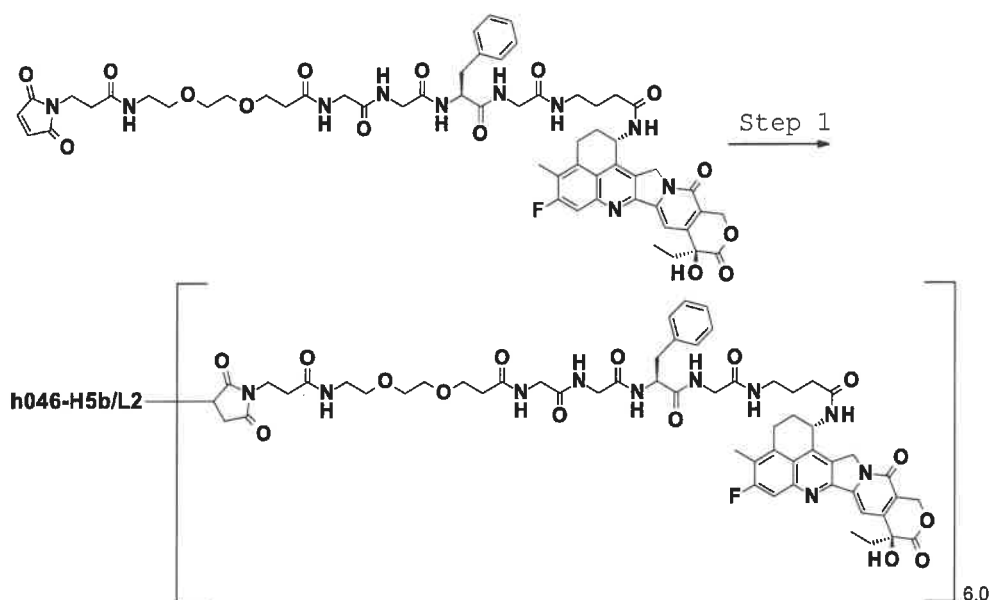
Antibody concentration: 2.07 mg/mL, antibody yield: 49.6 mg (99%), average number of conjugated drug molecules (n) per antibody molecule measured by common

procedure E: 6.1, and average number of conjugated drug molecules (n) per antibody molecule measured by common procedure F: 7.6.

[0369] 7)-8 Production of antibody-drug conjugate (8)

[0370]

[Formula 21]



[0371] Step 1: Antibody-drug conjugate (8)

Reduction of antibody: h046-H5b/L2 produced in Example 6)-7-6-2 was adjusted to 10 mg/mL with PBS6.0/EDTA by using common procedures B (using 1.31 mLmg⁻¹cm⁻¹ as 280 nm absorption coefficient) and C described in production method 1. To this solution (6.25 mL), an aqueous solution of 10 mM TCEP (Tokyo Chemical Industry Co., Ltd.) (0.237 mL; 5.5 equivalents per antibody molecule) and a 1 M aqueous dipotassium hydrogen phosphate solution (Nacalai Tesque, Inc.; 0.0940 mL) were added. After confirming that the solution had a pH

within 7.0 ± 0.1 , the interchain disulfide bond in the antibody was reduced by incubating the solution at 37°C for 1 hour.

[0372] Conjugation between antibody and drug linker: The above-described solution was incubated at 15°C for 10 minutes. Subsequently, a 10 mM solution of N-{3-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]propanoyl}glycylglycyl-L-phenylalanyl-N-(4-{{(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino}-4-oxobutyl)glycinamide in dimethyl sulfoxide (0.388 mL; 9.0 equivalents per antibody molecule) was added thereto, and the obtained mixture was incubated at 15°C for 1 hour to conjugate the drug linker to the antibody. Subsequently, an aqueous solution of 100 mM NAC (Sigma-Aldrich Co. LLC) (0.0390 mL; 9.0 equivalents per antibody molecule) was added thereto, and the obtained mixture was further stirred at room temperature for 20 minutes to terminate the reaction of the drug linker.

[0373] Purification: The above-described solution was purified by common procedure D described in production method 1 to obtain 30.0 mL of a solution containing the antibody-drug conjugate "h046-H5b/L2-ADC2".

Characterization: Using common procedures E and F (using $\epsilon_{D,280} = 4964$ and $\epsilon_{D,370} = 18982$) described in

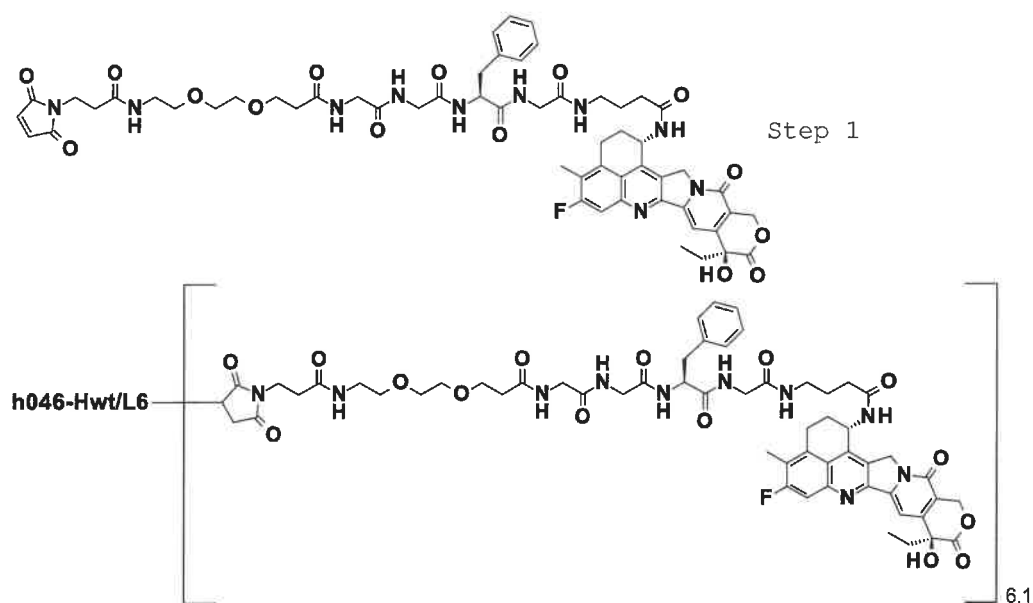
production method 1, the following characteristic values were obtained.

Antibody concentration: 2.07 mg/mL, antibody yield: 62.2 mg (99%), average number of conjugated drug molecules (n) per antibody molecule measured by common procedure E: 6.0, and average number of conjugated drug molecules (n) per antibody molecule measured by common procedure F: 7.7.

[0374] 7)-9 Production of antibody-drug conjugate (9)

[0375]

[Formula 22]



[0376] Step 1: Antibody-drug conjugate (9)

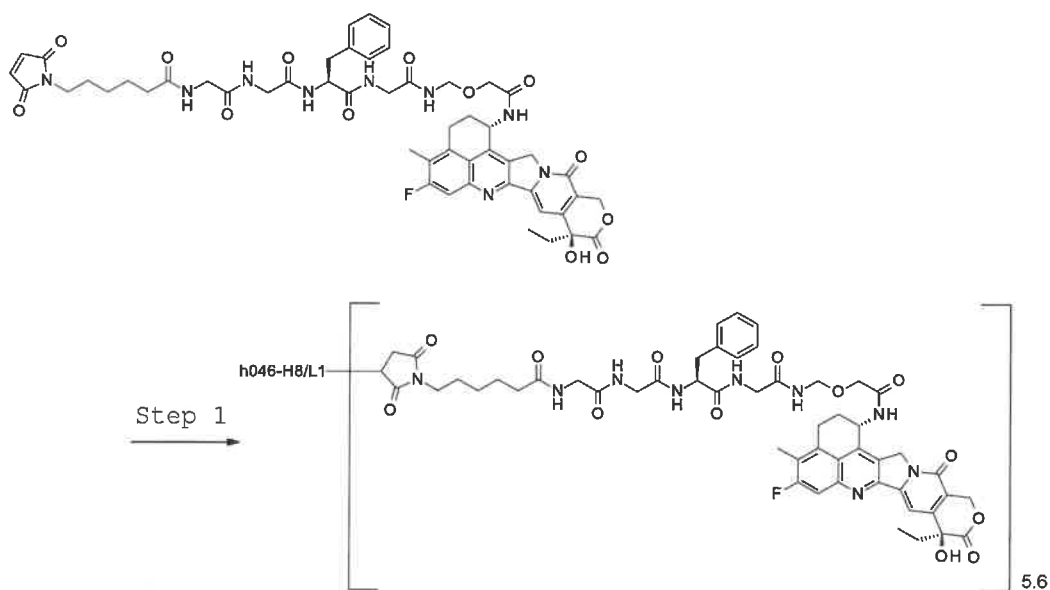
Using h046-Hwt/L6 (using $1.31 \text{ mLmg}^{-1}\text{cm}^{-1}$ as 280 nm absorption coefficient) produced in Example 6)-7-6-3, the antibody-drug conjugate "h046-Hwt/L6-ADC2" was obtained by the same method as that applied in step 1 of Example 7)-8.

[0377] Antibody concentration: 2.10 mg/mL, antibody yield: 62.9 mg (99%), average number of conjugated drug molecules (n) per antibody molecule measured by common procedure E: 6.1, and average number of conjugated drug molecules (n) per antibody molecule measured by common procedure F: 7.4.

[0378] 7)-10 Production of antibody-drug conjugate (10)

[0379]

[Formula 23]



[0380] Step 1: Antibody-drug conjugate (10)

Using h046-H8/L1 (using $1.31 \text{ mLmg}^{-1}\text{cm}^{-1}$ as 280 nm absorption coefficient) produced in Example 6)-7-6-4, the antibody-drug conjugate "h046-H8/L1-ADC1" was obtained by the same method as that applied in step 1 of Example 7)-3.

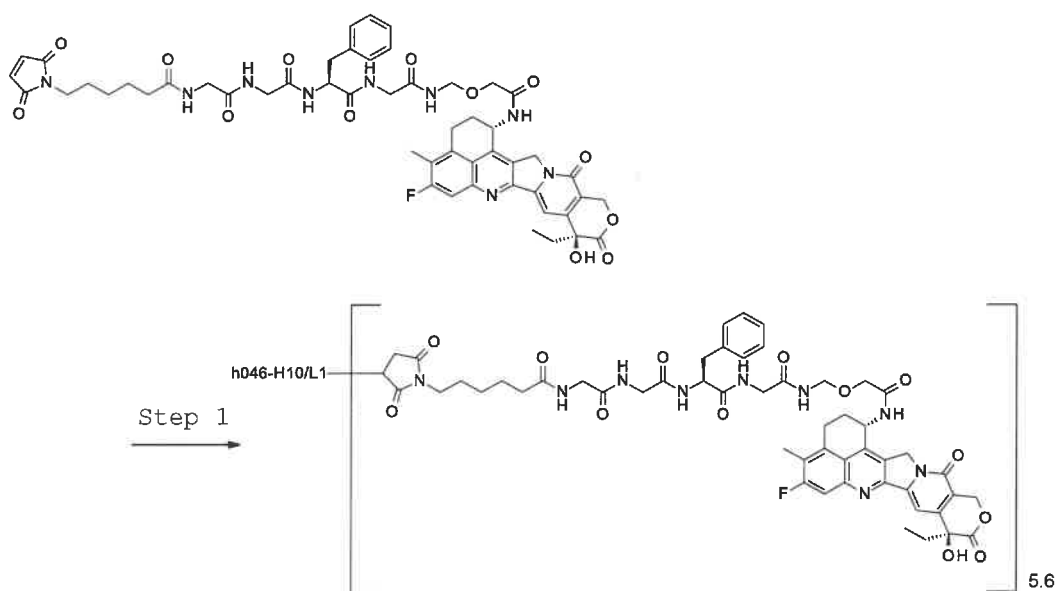
[0381] Antibody concentration: 1.75 mg/mL, antibody yield: 52.6 mg (86%), average number of conjugated drug molecules (n) per antibody molecule measured by common

procedure E: 5.6, and average number of conjugated drug molecules (n) per antibody molecule measured by common procedure F: 7.9.

[0382] 7)-11 Production of antibody-drug conjugate (11)

[0383]

[Formula 24]



[0384] Step 1: Antibody-drug conjugate (11)

Using h046-H10/L1 (using $1.31 \text{ mLmg}^{-1}\text{cm}^{-1}$ as 280 nm absorption coefficient) produced in Example 6)-7-6-5, the antibody-drug conjugate "h046-H10/L1-ADC1" was obtained by the same method as that applied in step 1 of Example 7)-3.

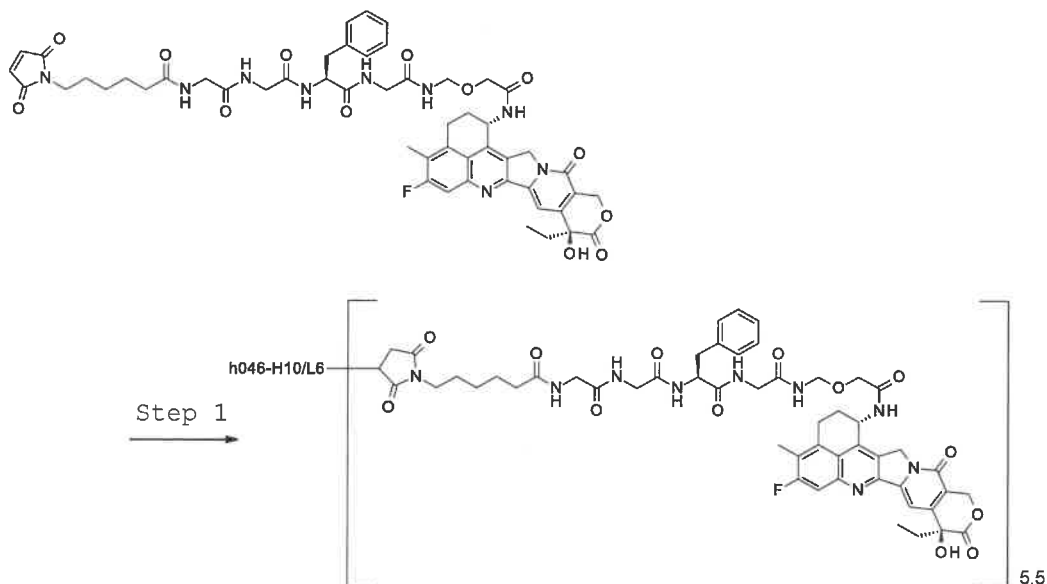
[0385] Antibody concentration: 1.85 mg/mL, antibody yield: 55.6 mg (90%), average number of conjugated drug molecules (n) per antibody molecule measured by common procedure E: 5.6, and average number of conjugated drug

molecules (n) per antibody molecule measured by common procedure F: 7.9.

[0386] 7)-12 Production of antibody-drug conjugate (12)

[0387]

[Formula 25]



[0388] Step 1: Antibody-drug conjugate (12)

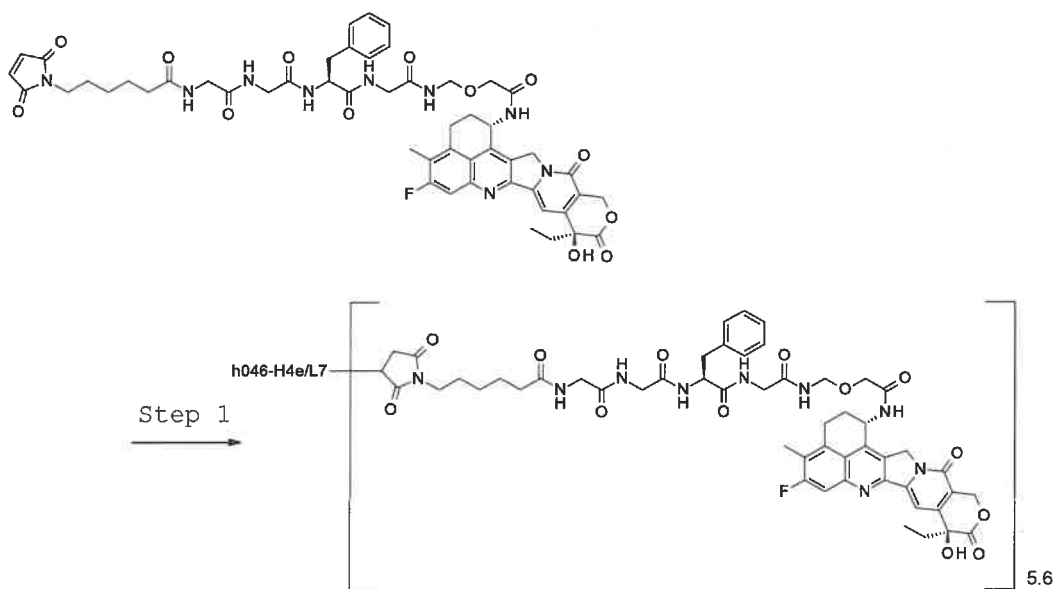
Using h046-H10/L6 (using $1.31 \text{ mLmg}^{-1}\text{cm}^{-1}$ as 280 nm absorption coefficient) produced in Example 6)-7-6-6, the antibody-drug conjugate "h046-H10/L6-ADC1" was obtained by the same method as that applied in step 1 of Example 7)-3.

[0389] Antibody concentration: 1.83 mg/mL , antibody yield: 55.0 mg (87%), average number of conjugated drug molecules (n) per antibody molecule measured by common procedure E: 5.5, and average number of conjugated drug molecules (n) per antibody molecule measured by common procedure F: 8.0.

[0390] 7)-13 Production of antibody-drug conjugate (13)

[0391]

[Formula 26]



[0392] Step 1: Antibody-drug conjugate (13)

Reduction of antibody: h046-H4e/L7 produced in Example 6)-7-6-7 was adjusted to 10 mg/mL with PBS6.0/EDTA by using common procedures B (using $1.31 \text{ mLmg}^{-1}\text{cm}^{-1}$ as 280 nm absorption coefficient) and C described in production method 1. To this solution (11.0 mL), an aqueous solution of 10 mM TCEP (Tokyo Chemical Industry Co., Ltd.) (0.412 mL; 5.5 equivalents per antibody molecule) and a 1 M aqueous dipotassium hydrogen phosphate solution (Nacalai Tesque, Inc.; 0.165 mL) were added. After confirming that the solution had a pH within 7.0 ± 0.1 , the interchain disulfide bond in the antibody was reduced by incubating the solution at 37°C for 1 hour.

[0393] Conjugation between antibody and drug linker: The above-described solution was incubated at 15°C for 10 minutes. Subsequently, a 10 mM solution of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]glycylglycyl-L-phenylalanyl-N-[(2-[[[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino}-2-oxoethoxy)methyl]glycinamide in dimethyl sulfoxide (0.674 mL; 9.0 equivalents per antibody molecule) was added thereto, and the obtained mixture was incubated at 15°C for 1 hour to conjugate the drug linker to the antibody. Subsequently, an aqueous solution of 100 mM NAC (Sigma-Aldrich Co. LLC) (0.0674 mL; 9.0 equivalents per antibody molecule) was added thereto, and the obtained mixture was further stirred at room temperature for 20 minutes to terminate the reaction of the drug linker.

[0394] Purification: The above-described solution was purified by common procedure D described in production method 1 to obtain 34.5 mL of a solution containing the antibody-drug conjugate "h046-H4e/L7-ADC1".

Characterization: Using common procedures E and F (using $\epsilon_{D,280} = 5178$ and $\epsilon_{D,370} = 20217$) described in production method 1, the following characteristic values were obtained.

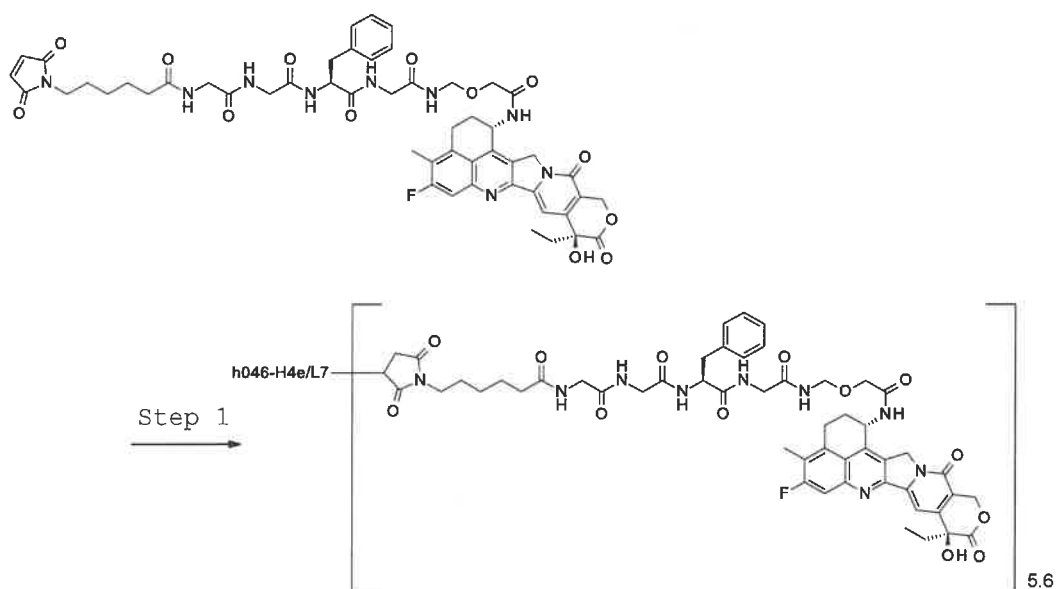
Antibody concentration: 3.01 mg/mL, antibody yield: 104 mg (95%), average number of conjugated drug molecules

(n) per antibody molecule measured by common procedure E: 5.6, and average number of conjugated drug molecules (n) per antibody molecule measured by common procedure F: 7.7.

[0395] 7)-14 Production of antibody-drug conjugate (14)

[0396]

[Formula 27]



[0397] Step 1: Antibody-drug conjugate (14)

Using h046-H4e/L7 (using $1.31 \text{ mLmg}^{-1}\text{cm}^{-1}$ as 280 nm absorption coefficient) produced in Example 6)-7-6-7, the antibody-drug conjugate "h046-H4e/L7-ADC1" was obtained by the same method as that applied in step 1 of Example 7-13.

[0398] Antibody concentration: 3.14 mg/mL, antibody yield: 108 mg (99%), average number of conjugated drug molecules (n) per antibody molecule measured by common procedure E: 5.6, and average number of conjugated drug

molecules (n) per antibody molecule measured by common procedure F: 7.6.

[0399] Example 8: Evaluation of *in vitro* activity of antibody-drug conjugates

8)-1-1 Evaluation of binding activity of humanized anti-GPR20 antibody and antibody-drug conjugate

The GPR20-binding activity of the humanized anti-GPR20 antibodies produced in Example 6)-7-6 and the antibody-drug conjugates produced in Example 7 was evaluated by flow cytometry. As a result of analyzing, using a flow cytometer (BD FACSCant II; BD Biosciences), their binding to 293T cells transiently transfected with pcDNA3.1-hGPR20 by the same method as that applied in Example 6)-6-1, antibody concentration-dependent binding activity was confirmed. Representative reaction examples thereof are shown in Figure 25. In Figure 25, the abscissa depicts an antibody concentration (nM), and the ordinate depicts the amount of the antibody bound based on MFI (mean fluorescence intensity). As shown in Figure 25, the amounts of the humanized anti-GPR20 antibodies h046-H4b/L7 and h046-H4e/L7, and the antibody-drug conjugates (4) and (13) bound to the 293T cells transfected with pcDNA3.1-hGPR20 were increased in a concentration-dependent manner.

[0400] 8)-2 Generation of a stably-expressing cell line, GIST-T1/GPR20

A stable cell line, GIST-T1/GPR20 was produced by infecting GIST-T1 cells (available from Cosmo Bio Co., Ltd.) with a recombinant retrovirus for human GPR20 expression.

[0401] 8)-2-1 Preparation of human GPR20 expression retrovirus vector

A human GPR20 expression retrovirus vector was prepared using an In-Fusion HD Cloning Kit (Clontech Laboratories, Inc.). Specifically, using the human GPR20 expression vector pCDNA3.1-hGPR20 produced in Example 1 as a template, a PCR reaction was carried out with the primer set given below. In this reaction, KOD FX DNA polymerase (Toyobo Co., Ltd.) was used, and the reaction was carried out at 30 cycles each involving 98°C for 10 seconds, 58°C for 30 seconds, and 68°C for 2 minutes. Thereafter, the obtained PCR product comprising a GPR20 cDNA fragment was subjected to agarose gel electrophoresis, and DNA having the size of interest was extracted using a QIAquick Gel Extraction Kit (Qiagen N.V.). Using an In-Fusion HD Enzyme premix, this DNA fragment was mixed with retrovirus vector pLPCX (Clontech Laboratories, Inc.) that had been digested with the restriction enzymes EcoRI and NotI. After a ligation reaction, *Escherichia coli* TOP10 (Invitrogen Corp.) was transformed with the ligation product to construct human GPR20 expression retrovirus vector pLPCX-GPR20. The primer set used in PCR was as follows.

[0402] PCR primer set (for In-Fusion cloning of pLPCX and GPR20 cDNA fragment)

5'-CTCAAGCTTCGAATTCACCATGCCCTCTGTGTCTCCA-3' (LPCX-1; SEQ ID NO: 112)

5'-TTGGCCGAGGCGGCCTCCTAAGCCTCGGGCCCATTAG-3' (LPCX-1; SEQ ID NO: 113)

8)-2-2 Establishment of stably expressing cell line GIST-T1/GPR20

Using Lipofectamine 2000 (Invitrogen Corp.), pLPCX-GPR20 was transiently introduced into retrovirus packaging cells 293-10A1. 72 hours later, a culture supernatant containing recombinant retrovirus was collected, and then added to the GIST-T1 cell culture system, so that the cells were infected with the virus. 3 days after the infection, the GIST-T1 cells were seeded at 1 cell/well over a 96-well plate, and then cultured under conditions of 37°C and 5% CO₂ for a long period in a medium to which 0.3 to 1 µg/mL puromycin had been added, so as to establish cell line GIST-T1/GPR20 stably expressing GPR20.

[0403] 8)-3 Evaluation of *in vitro* cell proliferation-suppressive activity of ADC against GIST-T1/GPR20 cells

The GIST-T1/GPR20 cells were seeded over a 96-well plate at 2.5×10^3 cells/100 µL/well in DMEM medium supplemented with 10% FBS, and the cells were then cultured overnight under conditions of 37°C and 5% CO₂. Each antibody-drug conjugate produced in Example 7 was

added to the cells such that the final concentrations were from 0.032 to 100 nM. After culture for 7 to 11 days, the number of live cells was measured by the quantification of ATP using CellTiter-Glo (registered trademark) Luminescent Cell Viability Assay. Figure 26 shows concentration-dependent cell proliferation-suppressive activity when the antibody-drug conjugate (1) produced from the human chimeric antibody was added to the cells, and Figure 27 shows concentration-dependent cell proliferation-suppressive activity when each of the antibody-drug conjugates (7), (8) and (9) produced from the humanized antibodies was added to the cells. hIgG-ADC2 in this experiment was an antibody-drug conjugate produced from human IgG recognizing an antigen unrelated to GPR20, and was used as a negative control.

[0404] Example 9: *In vivo* antitumor effect of antibody-drug conjugate

The antitumor effects of the antibody-drug conjugates were evaluated using animal models derived from immunodeficient mice by the transplantation of human gastrointestinal stromal tumor-derived cells. Five- to 6-week-old female BALB/c nude mice (CAnN.Cg-Foxnlnu/CrlCrlj, Charles River Laboratories Japan Inc.) were acclimatized for 4 to 7 days under SPF conditions before use in the experiment. The mice were fed with a sterilized solid diet (FR-2, Funabashi Farms Co., Ltd) and given sterilized tap water (which had been prepared

by adding a 5 to 15 ppm sodium hypochlorite solution to tap water). The longest diameter and the shortest diameter of the transplanted tumor were measured once or twice a week using electronic digital calipers (CD-15CX, Mitutoyo Corp.), and the volume of the tumor was then calculated according to the following equation.

$$\text{Tumor volume (mm}^3\text{)} = 1/2 \times \text{Longest diameter (mm)} \times [\text{Shortest diameter (mm)}]^2$$

Each antibody-drug conjugate was diluted with ABS buffer (10 mM acetate buffer, 5% sorbitol, pH 5.5) (Nacalai Tesque, Inc.), and the dilution was intravenously administered at a volume of 10 mL/kg to the tail of each mouse. ABS buffer was administered in the same manner as above to a control group (vehicle group). Five or six mice per group were used in the experiment.

[0405] 9)-1 Antitumor effect - (1)

The GIST-T1/GPR20 cells prepared in 8)-1-2 were suspended in Matrigel (Corning Inc.), and the cell suspension was subcutaneously transplanted at a dose of 5×10^6 cells to the right flank region of each female nude mouse (Day 0). On Day 14, the mice were randomly grouped. On Day 14, the antibody-drug conjugate (1) or (2) was intravenously administered at a dose of 10 mg/kg to the tail of each mouse. As a negative control, an antibody-drug conjugate produced using human IgG was administered at a dose of 10 mg/kg in the same manner as above. The administration of the antibody-drug conjugate (1) or (2)

remarkably decreased tumor volume, and both the antibody-drug conjugates were effective for tumor regression (Figure 28). It is to be noted that, in the drawing, the abscissa depicts the number of days, and the ordinate depicts tumor volume. hIgG-ADC1 and hIgG-ADC2 were antibody-drug conjugates produced from human IgG recognizing an antigen unrelated to GPR20, and were used as negative controls.

[0406] 9)-2 Antitumor effect - (2)

2×10^7 cells of human gastrointestinal stromal tumor cell line GIST430 (obtained from Brigham Women's Hospital) were subcutaneously transplanted to the right flank region of each female nude mouse (Day 0). On Day 29, the mice were randomly grouped. On Days 29, 36, and 43, the antibody-drug conjugate (1) or (2) was intravenously administered at a dose of 10 mg/kg to the tail of each mouse. The administration of the antibody-drug conjugate (1) or (2) remarkably decreased tumor volume compared with that in the control group, and both the antibody-drug conjugates exerted a tumor growth-suppressive effect (Figure 29).

[0407] 9)-3 Antitumor effect - (3)

In the same manner as that applied in Example 9)-1, the GIST-T1/GPR20 cells were subcutaneously transplanted to female nude mice (Day 0). On Day 24, the mice were randomly grouped. On Day 24, the antibody-drug conjugate (6) or (9) was intravenously administered at a dose of 10

mg/kg to the tail of each mouse. The administration of the antibody-drug conjugate (6) or (9) remarkably decreased tumor volume, and both the antibody-drug conjugates were effective for tumor regression (Figure 30).

[0408] 9)-4 Antitumor effect - (4)

In the same manner as that applied in Example 9)-1, the GIST-T1/GPR20 cells were subcutaneously transplanted to female nude mice (Day 0). On Day 17, the mice were randomly grouped. On Day 17, the antibody-drug conjugate (3), (5), (6), (10), (11), or (12) was intravenously administered at a dose of 1 mg/kg to the tail of each mouse. The administration of the antibody-drug conjugate (3), (5), (6), (10), (11), or (12) remarkably decreased tumor volume, and all the antibody-drug conjugates exerted a tumor growth-suppressive effect (Figure 31).

[0409] 9)-5 Antitumor effect - (5)

In the same manner as that applied in Example 9)-1, the GIST-T1/GPR20 cells were subcutaneously transplanted to female nude mice (Day 0). On Day 17, the mice were randomly grouped. On Day 17, the antibody-drug conjugate (13) was intravenously administered at a dose of 0.3, 1, or 3 mg/kg to the tail of each mouse. The administration of the antibody-drug conjugate (13) decreased tumor volume in a dose-dependent manner, and the antibody-drug conjugate was effective for tumor regression at a dose of 1 mg/kg or more (Figure 32).

[0410] 9)-6 Antitumor effect - (6)

GIST020 (obtained from National Institutes of Biomedical Innovation, Health and Nutrition), which had been passaged and maintained by subcutaneously transplanting a tumor block excised from a patient with gastrointestinal stromal tumor in the small intestine to immunodeficient mice, was subcutaneously transplanted to the right flank regions of female nude mice (Day 0). On Day 55, the mice were randomly grouped. On Days 55 and 75, the antibody-drug conjugate (4) or (13) was intravenously administered at a dose of 10 mg/kg to the tail of each mouse. The administration of the antibody-drug conjugate (4) or (13) remarkably decreased tumor volume compared with that in the control group, and both the antibody-drug conjugates exerted a tumor growth-suppressive effect (Figure 33).

[0411] 9)-7 Antitumor effect - (7)

GIST1 (obtained from University of Toyama), which had been passaged and maintained by subcutaneously transplanting a tumor block excised from a patient with gastrointestinal stromal tumor in the esophagus to immunodeficient mice, was subcutaneously transplanted to the right flank regions of female nude mice (Day 0). On Day 38, the mice were randomly grouped. On Days 38 and 59, the antibody-drug conjugate (13) was intravenously administered at a dose of 3 or 10 mg/kg to the tail of each mouse. The administration of the antibody-drug

conjugate (13) remarkably decreased tumor volume compared with that in the control group, and the antibody-drug conjugate exerted a tumor growth-suppressive effect (Figure 34).

[0412] 9)-8 Antitumor effect - (8)

1×10^7 cells of stomach cancer cell line NCI-N87 strongly expressing a HER2 molecule but expressing no GPR20 were subcutaneously transplanted to the right flank regions of female nude mice (Day 0). On Day 6, the mice were randomly grouped. On Day 6, the antibody-drug conjugate (14) was intravenously administered at a dose of 3 or 10 mg/kg to the tail of each mouse. However, the antibody-drug conjugate (14) exhibited no significant tumor growth-suppressive effect. On the other hand, the administration of a drug conjugate produced from an anti-HER2 antibody remarkably decreased tumor volume compared with that in the control group, and the antibody-drug conjugate exerted a tumor growth-suppressive effect (Figure 35).

9)-9 Antitumor effect - (9)

GIST074 (obtained from National Institutes of Biomedical Innovation, Health and Nutrition), which had been passaged and maintained by subcutaneously transplanting a tumor block derived from a patient with gastrointestinal stromal tumor in the stomach unresponsive to regorafenib treatment to immunodeficient mice, was subcutaneously transplanted to the right flank

regions of female nude mice (Day 0). On Day 29, the mice were randomly grouped. On Days 29 and 50, the antibody-drug conjugate (14) was intravenously administered at a dose of 10 mg/kg to the tail of each mouse. The administration of the antibody-drug conjugate (14) completely suppressed tumor growth (Figure 36). On the other hand, when imatinib, sunitinib, and regorafenib were orally administered once a day at doses of 90, 30, and 4 mg/kg, respectively, on the days indicated by the triangle marks in Figure 36, tumor growth was not completely suppressed. As is evident from these results, the antibody-drug conjugate (14) exerted an antitumor effect even on gastrointestinal stromal tumor that exhibited resistance to the 3 tyrosine kinase inhibitors serving as standard therapeutic drugs.

[0413] 9)-10 Antitumor effect - (10)

The effect of combined use with sunitinib was evaluated in models with human gastrointestinal stromal tumor cell line GIST430/654 (obtained from Brigham Women's Hospital) having imatinib resistance mutation V654A in the KIT gene. 2×10^7 cells of GIST430/654 were subcutaneously transplanted to the right flank region of each female nude mouse (Day 0). On Day 21, the mice were randomly grouped. As shown in Figure 37, on Day 21, the antibody-drug conjugate (3) was intravenously administered at a single dose of 10 mg/kg to the tail of each mouse. Imatinib and sunitinib were orally

administered once a day at doses of 150 and 40 mg/kg, respectively, on the days indicated by the triangle marks. In the GIST430/654 models, imatinib did not suppress tumor growth, whereas the suppression of tumor growth was observed in the group to which the antibody-drug conjugate (3) or sunitinib had been administered. In the combined use group of the antibody-drug conjugate (3) and sunitinib, stronger medicinal effects than those brought about by single agents were observed, and tumor regression was found.

Industrial Applicability

[0414] The present invention provides an anti-GPR20 antibody having internalization activity and an antibody-drug conjugate comprising the antibody. The antibody-drug conjugate can be used as a therapeutic drug for a gastrointestinal stromal tumor, and the like.

Sequence Listing Free Text

[0415]

SEQ ID NO: 44: Amino acid sequence of 04-046Ch heavy chain

SEQ ID NO: 45: Amino acid sequence of 04-046Ch light chain

SEQ ID NO: 46: Nucleotide sequence of 04-046Ch antibody heavy chain

SEQ ID NO: 47: Nucleotide sequence of 04-046Ch light chain

SEQ ID NO: 48: Amino acid sequence of h046-H4b

SEQ ID NO: 49: Nucleotide sequence (1) of h046-H4b

SEQ ID NO: 50: Amino acid sequence of h046-H4e

SEQ ID NO: 51: Nucleotide sequence of h046-H4e

SEQ ID NO: 52: Amino acid sequence of h046-H5b

SEQ ID NO: 53: Nucleotide sequence (1) of h046-H5b

SEQ ID NO: 54: Amino acid sequence of h046-H8

SEQ ID NO: 55: Nucleotide sequence (1) of h046-H8

SEQ ID NO: 56: Amino acid sequence of h046-H10

SEQ ID NO: 57: Nucleotide sequence (1) of h046-H10

SEQ ID NO: 58: Amino acid sequence of h046-L1

SEQ ID NO: 59: Nucleotide sequence (1) of h046-L1

SEQ ID NO: 60: Amino acid sequence of h046-L2

SEQ ID NO: 61: Nucleotide sequence (1) of h046-L2

SEQ ID NO: 62: Amino acid sequence of h046-L6

SEQ ID NO: 63: Nucleotide sequence (1) of h046-L6

SEQ ID NO: 64: Amino acid sequence of h046-L7

SEQ ID NO: 65: Nucleotide sequence (1) of h046-L7

SEQ ID NO: 66: PCR primer Nhe-polyC-S

SEQ ID NO: 67: PCR primer rIg γ -AS1

SEQ ID NO: 68: PCR primer rIg γ -AS2

SEQ ID NO: 69: PCR primer rIg κ -AS

SEQ ID NO: 70: PCR primer rIg γ -seq

SEQ ID NO: 71: PCR primer rIg κ -seq

SEQ ID NO: 72: PCR primer NFLAG-1

SEQ ID NO: 73: PCR primer NFLAG-2
SEQ ID NO: 74: PCR primer mEC2-1
SEQ ID NO: 75: PCR primer mEC2-2
SEQ ID NO: 76: PCR primer mEC3-1
SEQ ID NO: 77: PCR primer mEC3-2
SEQ ID NO: 78: PCR primer mEC4-1
SEQ ID NO: 79: PCR primer mEC4-2
SEQ ID NO: 80: PCR primer mEC1-1
SEQ ID NO: 81: PCR primer mEC1-2
SEQ ID NO: 82: PCR primer mEC1-3
SEQ ID NO: 83: PCR primer mEC1-4
SEQ ID NO: 85: PCR primer 3.3-F1
SEQ ID NO: 86: PCR primer 3.3-R1
SEQ ID NO: 88: PCR primer EG-Inf-F
SEQ ID NO: 89: PCR primer EG1-Inf-R
SEQ ID NO: 90: PCR primer CM-LKF
SEQ ID NO: 91: PCR primer 046L-R
SEQ ID NO: 92: Amino acid sequence of h046-L6 CDRL2
SEQ ID NO: 93: Amino acid sequence of h046-L7 CDRL2
SEQ ID NO: 94: DNA fragment A
SEQ ID NO: 95: PCR primer Hb-F
SEQ ID NO: 96: PCR primer Hb-R
SEQ ID NO: 97: DNA fragment B
SEQ ID NO: 98: H08-F
SEQ ID NO: 99: H08-R
SEQ ID NO: 100: H10-F
SEQ ID NO: 101: H10-R

SEQ ID NO: 102: PCR primer KCL-Inf-R
SEQ ID NO: 103: Nucleotide sequence (2) of h046-H4b
SEQ ID NO: 104: Nucleotide sequence (2) of h046-H5b
SEQ ID NO: 105: Nucleotide sequence (2) of h046-Hwt
SEQ ID NO: 106: Nucleotide sequence (2) of h046-H8
SEQ ID NO: 107: Nucleotide sequence (2) of h046-H10
SEQ ID NO: 108: Nucleotide sequence (2) of h046-L1
SEQ ID NO: 109: Nucleotide sequence (2) of h046-L2
SEQ ID NO: 110: Nucleotide sequence (2) of h046-L6
SEQ ID NO: 111: Nucleotide sequence (2) of h046-L7
SEQ ID NO: 112: PCR primer LPCX-1
SEQ ID NO: 113: PCR primer LPCX-2

Claims

[Claim 1]

An antibody having the following properties (a) and (b) or a functional fragment of the antibody:

- (a) specifically binding to GPR20, and
- (b) having internalization ability that permits cellular uptake after binding to GPR20.

[Claim 2]

The antibody or the functional fragment of the antibody according to claim 1, wherein the GPR20 is a molecule consisting of the amino acid sequence shown in SEQ ID NO: 1.

[Claim 3]

The antibody or the functional fragment of the antibody according to claim 1 or 2, which specifically binds to a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 1 and does not specifically bind to a polypeptide with the amino acid Y (tyrosine) at amino acid position 113 substituted with a different amino acid.

[Claim 4]

The antibody or the functional fragment of the antibody according to any one of claims 1 to 3, which specifically binds to a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 1 and does not specifically bind to a polypeptide with the amino acid Y

(tyrosine) at amino acid position 113 substituted with F (phenylalanine).

[Claim 5]

The antibody or the functional fragment of the antibody according to any one of claims 1 to 4, which specifically binds to a conformation consisting of the amino acid sequence at amino acid positions 1 to 48 and the amino acid sequence at amino acid positions 108 to 125 in SEQ ID NO: 1.

[Claim 6]

The antibody or the functional fragment of the antibody according to any one of claims 1 to 5, which specifically binds to at least one amino acid selected from the amino acid sequence at amino acid positions 1 to 48 and at least one amino acid selected from the amino acid sequence at amino acid positions 108 to 125 in SEQ ID NO: 1.

[Claim 7]

The antibody or the functional fragment of the antibody according to any one of claims 1 to 6, wherein at least one of the amino acids to which the antibody or the functional fragment specifically binds is the amino acid at amino acid position 113 of SEQ ID NO: 1.

[Claim 8]

The antibody or the functional fragment of the antibody according to any one of claims 1 to 7, which has competitive inhibitory activity, for binding to GPR20,

against any one antibody selected from the group consisting of the following antibodies (a) to (c):

(a) an antibody having a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 475 of SEQ ID NO: 2 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 233 of SEQ ID NO: 7,

(b) an antibody having a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 475 of SEQ ID NO: 12 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 233 of SEQ ID NO: 17, and

(c) an antibody having a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 475 of SEQ ID NO: 22 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 233 of SEQ ID NO: 27.

[Claim 9]

The antibody or the functional fragment of the antibody according to any one of claims 1 to 8, which comprises CDRH1, CDRH2 and CDRH3 in any one combination selected from the group consisting of the following combinations (a) to (c), and CDRL1, CDRL2 and CDRL3 in any one combination selected from the group consisting of the following combinations (d) to (h):

(a) CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 4, CDRH2 consisting of the amino acid sequence

shown in SEQ ID NO: 5, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 6,

(b) CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 14, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 15, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 16,

(c) CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 24, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 25, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 26,

(d) CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 9, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 10, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 11,

(e) CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 9, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 92, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 11,

(f) CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 9, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 93, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 11,

(g) CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 19, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 20, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 21, and

(h) CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 29, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 30, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 31.

[Claim 10]

The antibody or the functional fragment of the antibody according to any one of claims 1 to 9, which comprises CDRH1, CDRH2 and CDRH3, and CDRL1, CDRL2 and CDRL3 in any one combination selected from the group consisting of the following combinations (a) to (e):

(a) CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 4, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 5, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 6, and CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 9, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 10, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 11, .

(b) CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 4, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 5, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 6, and CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 9, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 92, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 11,

(c) CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 4, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 5, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 6, and CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 9, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 93, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 11,

(d) CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 14, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 15, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 16, and CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 19, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 20, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 21, and

(e) CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 24, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 25, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 26, and CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 29, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 30, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 31.

[Claim 11]

The antibody or the functional fragment of the antibody according to any one of claims 1 to 10, which

has any one heavy chain variable region selected from the group consisting of the following variable regions (a) to (c), and any one light chain variable region selected from the following variable regions (d) to (h):

a heavy chain variable region selected from the group consisting of

(a) a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 3,

(b) a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 13, and

(c) a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 23,

(d) a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 8,

(e) a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 62,

(f) a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 64,

(g) a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 18, and

(h) a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 28.

[Claim 12]

The antibody or the functional fragment of the antibody according to any one of claims 1 to 11, which

comprises a heavy chain variable region and a light chain variable region in any one combination selected from the group consisting of the following combinations (a) to (e):

(a) a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 3, and a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 8,

(b) a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 3, and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 62,

(c) a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 3, and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 64,

(d) a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 13, and a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 18, and

(e) a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 23, and a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 28.

[Claim 13]

The antibody or the functional fragment of the antibody according to any one of claims 1 to 12, wherein the constant region is a human-derived constant region.

[Claim 14]

The antibody or the functional fragment of the antibody according to claim 13, which comprises a heavy chain consisting of the amino acid sequence shown in SEQ ID NO: 44 and a light chain consisting of the amino acid sequence shown in SEQ ID NO: 45.

[Claim 15]

The antibody or the functional fragment of the antibody according to any one of claims 1 to 14, which is humanized.

[Claim 16]

The antibody or the functional fragment of the antibody according to claim 15, which has a heavy chain variable region consisting of any one amino acid sequence selected from the group consisting of the following amino acid sequences (a) to (h), and a light chain variable region consisting of any one amino acid sequence selected from the group consisting of the following amino acid sequences (i) to (o):

(a) the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 48,

(b) the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 50,

- (c) the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 52,
- (d) the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 54,
- (e) the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 56,
- (f) the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 44,
- (g) an amino acid sequence having a homology of at least 95% or more to the sequence of a framework region other than at each CDR sequence in the sequences of (a) to (f),
- (h) an amino acid sequence comprising a deletion, substitution or addition of one or several amino acids in the sequence of a framework region other than at each CDR sequence in the sequences of (a) to (g),
- (i) the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 58,
- (j) the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 60,
- (k) the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 62,
- (l) the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 64,
- (m) the amino acid sequence at amino acid positions 21 to 128 in SEQ ID NO: 45,
- (n) an amino acid sequence having a homology of at least 95% or more to the sequence of a framework region other

than at each CDR sequence in the sequences of (i) to (m),
and

(o) an amino acid sequence comprising a deletion,
substitution or addition of one or several amino acids in
the sequence of a framework region other than at each CDR
sequence in the sequences of (i) to (n).

[Claim 17]

The antibody or the functional fragment of the
antibody according to claim 16, which comprises a heavy
chain variable region and a light chain variable region
in any one combination selected from the group consisting
of the following combinations (a) to (t):

(a) a heavy chain variable region consisting of the amino
acid sequence at amino acid positions 20 to 142 in SEQ ID
NO: 48 and a light chain variable region consisting of
the amino acid sequence at amino acid positions 21 to 129
in SEQ ID NO: 58,

(b) a heavy chain variable region consisting of the amino
acid sequence at amino acid positions 20 to 142 in SEQ ID
NO: 48 and a light chain variable region consisting of
the amino acid sequence at amino acid positions 21 to 129
in SEQ ID NO: 60,

(c) a heavy chain variable region consisting of the amino
acid sequence at amino acid positions 20 to 142 in SEQ ID
NO: 48 and a light chain variable region consisting of
the amino acid sequence at amino acid positions 21 to 129
in SEQ ID NO: 62,

(d) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 48 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 64,

(e) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 50 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 58,

(f) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 50 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 60,

(g) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 50 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 62,

(h) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 50 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 64,

(i) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID

NO: 52 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 58,

(j) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 52 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 60,

(k) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 52 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 62,

(l) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 52 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 64,

(m) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 54 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 58,

(n) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 54 and a light chain variable region consisting of

the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 60,

(o) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 54 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 62,

(p) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 54 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 64,

(q) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 56 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 58,

(r) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 56 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 60,

(s) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 56 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 62, and

(t) a heavy chain variable region consisting of the amino acid sequence at amino acid positions 20 to 142 in SEQ ID NO: 56 and a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 64.

[Claim 18]

The antibody or the functional fragment of the antibody according to claim 16 or 17, which comprises a heavy chain and a light chain in any one combination selected from the group consisting of the following combinations (a) to (x):

(a) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 48 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 58,

(b) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 48 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 60,

(c) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 48 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 62,

(d) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 48 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 64,

- (e) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 50 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 58,
- (f) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 50 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 60,
- (g) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 50 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 62,
- (h) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 50 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 64,
- (i) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 52 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 58,
- (j) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 52 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 60,
- (k) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 52 and a

light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 62,

(l) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 52 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 64,

(m) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 54 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 58,

(n) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 54 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 60,

(o) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 54 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 62,

(p) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 54 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 64,

(q) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 56 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 58,

- (r) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 56 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 60,
- (s) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 56 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 62,
- (t) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 56 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 64,
- (u) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 44 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 58,
- (v) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 44 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 60,
- (w) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 44 and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 62; and
- (x) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 44 and a

light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 64.

[Claim 19]

The functional fragment of the antibody according to any one of claims 1 to 18, wherein the functional fragment is selected from the group consisting of Fab, F(ab)2, Fab' and Fv.

[Claim 20]

A polynucleotide encoding the antibody or the functional fragment of the antibody according to any one of claims 1 to 19.

[Claim 21]

The polynucleotide according to claim 20, which comprises polynucleotides in any one combination selected from the group consisting of the following combinations (a) to (e):

(a) a polynucleotide encoding CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 4, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 5 and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 6, and a polynucleotide encoding CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 9, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 10 and CDRL consisting of the amino acid sequence shown in SEQ ID NO: 11,

(b) a polynucleotide encoding CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 4, CDRH2

consisting of the amino acid sequence shown in SEQ ID NO: 5 and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 6, and a polynucleotide encoding CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 9, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 92 and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 11,

(c) a polynucleotide encoding CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 4, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 5 and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 6, and a polynucleotide encoding CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 9, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 93 and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 11,

(d) a polynucleotide encoding CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 14, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 15 and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 16, and a polynucleotide encoding CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 19, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 20 and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 21, and

(e) a polynucleotide encoding CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 24, CDRH2

consisting of the amino acid sequence shown in SEQ ID NO: 25 and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 26, and a polynucleotide encoding CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 29, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 30 and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 31.

[Claim 22]

The polynucleotide according to claim 20 or 21, which comprises polynucleotides in any one combination selected from the group consisting of the following combinations (a) to (e):

(a) a polynucleotide encoding a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 3, and a polynucleotide encoding a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 8,

(b) a polynucleotide encoding a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 3, and a polynucleotide encoding a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 62,

(c) a polynucleotide encoding a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 3, and a polynucleotide encoding a light chain variable region consisting of the amino acid sequence at amino acid positions 21 to 129 in SEQ ID NO: 64,

(d) a polynucleotide encoding a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 13, and a polynucleotide encoding a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 18, and

(e) a polynucleotide encoding a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 23, and a polynucleotide encoding a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 28.

[Claim 23]

An expression vector comprising the polynucleotide according to any one of claims 20 to 22.

[Claim 24]

A host cell transformed with the expression vector according to claim 23.

[Claim 25]

The host cell according to claim 23, wherein the host cell is a eukaryotic cell.

[Claim 26]

A method for producing an antibody of interest or a functional fragment of the antibody, which comprises a step of culturing the host cell according to claim 24 or 25, and a step of collecting an antibody of interest or a functional fragment of the antibody from the culture obtained by the aforementioned step.

[Claim 27]

An antibody or a functional fragment of the antibody obtained by the production method according to claim 26.

[Claim 28]

The functional fragment of the antibody according to claim 27, wherein the functional fragment is selected from the group consisting of Fab, F(ab)₂, Fab' and Fv.

[Claim 29]

The antibody or the functional fragment of the antibody according to any one of claims 1 to 19, 27 and 28, which comprises one or two or more modifications selected from the group consisting of N-linked glycosylation, O-linked glycosylation, N-terminal processing, C-terminal processing, deamidation, isomerization of aspartic acid, oxidation of methionine, addition of a methionine residue to the N-terminus, amidation of a proline residue, and a heavy chain comprising a deletion of one or two amino acids at the carboxyl terminus.

[Claim 30]

The antibody according to claim 29, wherein one or two amino acids are deleted at the carboxyl terminus of a heavy chain thereof.

[Claim 31]

The antibody according to claim 30, wherein one amino acid is deleted at each of the carboxyl termini of both of the heavy chains thereof.

[Claim 32]

The antibody according to any one of claims 29 to 32, wherein a proline residue at the carboxyl terminus of a heavy chain thereof is further amidated.

[Claim 33]

The antibody or the functional fragment of the antibody according to any one of claims 1 to 19 and 26 to 31, wherein sugar chain modification is regulated in order to enhance antibody-dependent cellular cytotoxic activity.

[Claim 34]

An antibody-drug conjugate comprising the antibody or the functional fragment of the antibody according to any one of claims 1 to 19 and 27 to 33 conjugated to a drug.

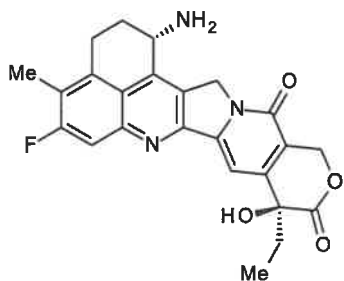
[Claim 35]

The antibody-drug conjugate according to claim 34, wherein the drug is an antitumor compound.

[Claim 36]

The antibody-drug conjugate according to claim 35, wherein the antitumor compound is an antitumor compound represented by the following formula:

[Formula 1]



[Claim 37]

The antibody-drug conjugate according to claim 35 or 36, wherein the antibody is conjugated to the antitumor compound via a linker having any structure selected from the group consisting of the following formulas (a) to (f):

(a) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-$,

(b) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-$,

(c) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2-\text{O}-\text{CH}_2-\text{C}(=\text{O})-$,

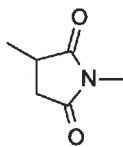
(d) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2\text{CH}_2-\text{O}-\text{CH}_2-\text{C}(=\text{O})-$,

(e) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{NH}-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-$, and

(f) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{NH}-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-$,

wherein the antibody is connected to the terminus of $-(\text{Succinimid-3-yl-N})$, the antitumor compound is connected to the carbonyl group of the $-(\text{CH}_2)_n-\text{C}(=\text{O})-$ moiety with the nitrogen atom of the amino group at position 1 as a connecting position, GGFG represents an amino acid sequence consisting of glycine-glycine-phenylalanine-glycine linked through peptide bonds, and $-(\text{Succinimid-3-yl-N})-$ has a structure represented by the following formula:

[Formula 2]



which is connected to the antibody at position 3 thereof and is connected to a methylene group in the linker structure containing this structure on the nitrogen atom at position 1.

[Claim 38]

The antibody-drug conjugate according to any one of claims 34 to 37, wherein the linker is represented by any formula selected from the group consisting of the following formulas (a) to (c):

(a) -(Succinimid-3-yl-N)-CH₂CH₂CH₂CH₂CH₂-C(=O)-GGFG-NH-CH₂-O-CH₂-C(=O)-,

(b) -(Succinimid-3-yl-N)-CH₂CH₂CH₂CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂-O-CH₂-C(=O)-, and

(c) -(Succinimid-3-yl-N)-CH₂CH₂-C(=O)-NH-CH₂CH₂O-CH₂CH₂O-CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂CH₂-C(=O)-.

[Claim 39]

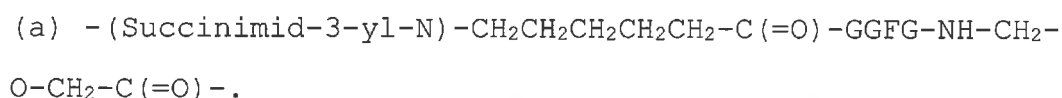
The antibody-drug conjugate according to any one of claims 34 to 38, wherein the linker is represented by the following formula (a) or (b):

(a) -(Succinimid-3-yl-N)-CH₂CH₂CH₂CH₂CH₂-C(=O)-GGFG-NH-CH₂-O-CH₂-C(=O)-, and

(b) -(Succinimid-3-yl-N)-CH₂CH₂-C(=O)-NH-CH₂CH₂O-CH₂CH₂O-CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂CH₂-C(=O)-.

[Claim 40]

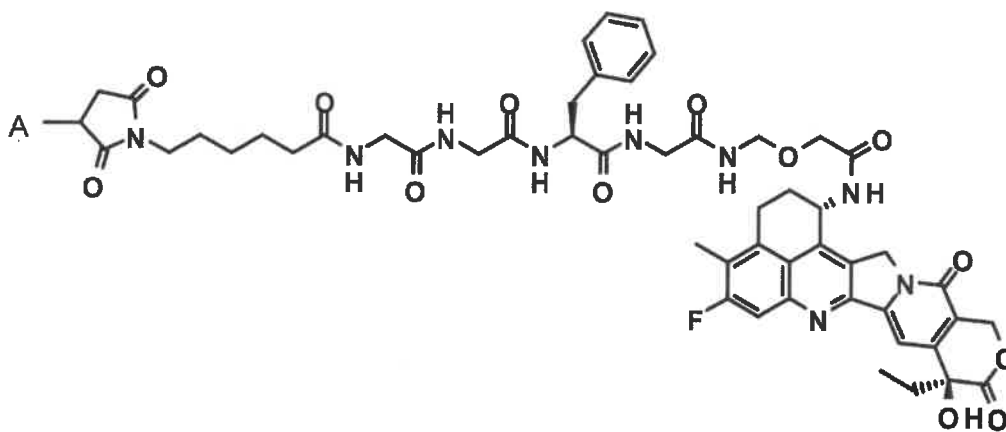
The antibody-drug conjugate according to any one of claims 34 to 39, wherein the linker is represented by the following formula (a):



[Claim 41]

The antibody-drug conjugate according to any one of claims 34 to 40, wherein the antibody is conjugated to a drug linker represented by the following formula [Formula 3] (wherein A represents a connecting position to the antibody) by a thioether bond:

[Formula 3]

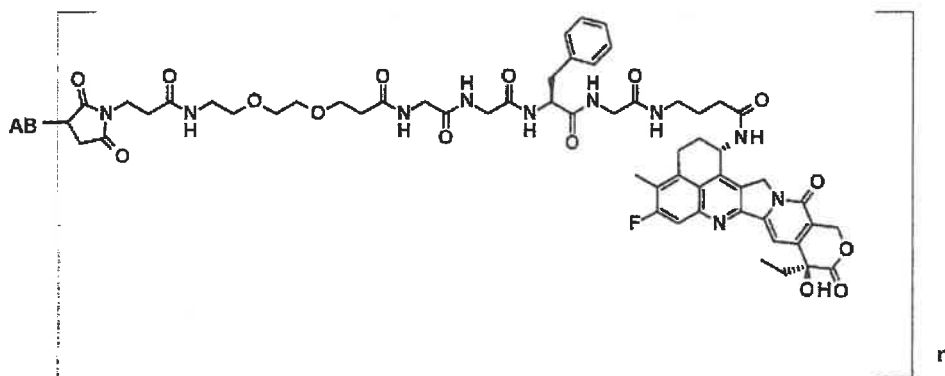


[Claim 42]

The antibody-drug conjugate according to any one of claims 34 to 40, which has a structure represented by the following formula [Formula 4], wherein AB represents the antibody or the functional fragment of the antibody, n represents an average number

of units of the drug-linker structure conjugated to the antibody per antibody, and the antibody is connected to the linker via a sulfhydryl group derived from the antibody:

[Formula 4]

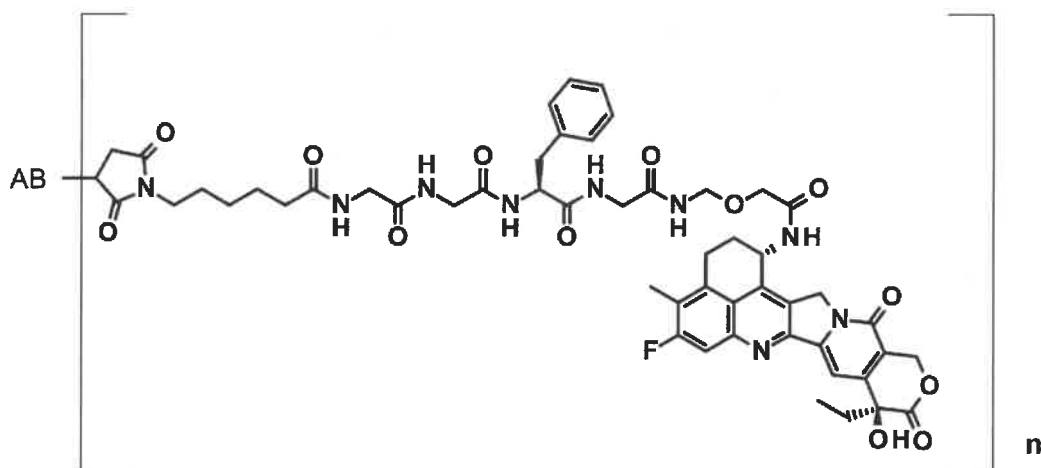


[Claim 43]

The antibody-drug conjugate according to any one of claims 34 to 41, which is represented by the following formula [Formula 5],

wherein AB represents the antibody or the functional fragment of the antibody, n represents an average number of units of the drug-linker structure conjugated to the antibody per antibody, and the antibody is connected to the linker via a sulfhydryl group derived from the antibody:

[Formula 5]



[Claim 44]

The antibody-drug conjugate according to any one of claims 41 to 43, wherein the antibody is an antibody comprising a heavy chain and a light chain in any one combination selected from the group consisting of the following combinations (a) to (x), or a functional fragment of the antibody:

- (a) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 48, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 58,
- (b) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 48, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 60,
- (c) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 48, and a

light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 62,

(d) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 48, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 64,

(e) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 50, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 58,

(f) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 50, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 60,

(g) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 50, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 62,

(h) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 50, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 64,

(i) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 52, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 58,

(j) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 52, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 60,

(k) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 52, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 62,

(l) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 52, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 64,

(m) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 54, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 58,

(n) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 54, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 60,

(o) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 54, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 62,

(p) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 54, and a

light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 64,

(q) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 56, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 58,

(r) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 56, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 60,

(s) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 56, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 62,

(t) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 474 in SEQ ID NO: 56, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 64,

(u) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 44, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 58,

(v) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 44, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 60,

(w) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 44, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 62, and

(x) a heavy chain consisting of the amino acid sequence at amino acid positions 20 to 472 in SEQ ID NO: 44, and a light chain consisting of the amino acid sequence at amino acid positions 21 to 234 in SEQ ID NO: 64.

[Claim 45]

The antibody-drug conjugate according to claim 44, wherein the heavy chain of the antibody comprises one or two or more modifications selected from the group consisting of N-linked glycosylation, O-linked glycosylation, N-terminal processing, C-terminal processing, deamidation, isomerization of aspartic acid, oxidation of methionine, addition of a methionine residue to the N-terminus, amidation of a proline residue, and a heavy chain comprising a deletion of one or two amino acids at the carboxyl terminus.

[Claim 46]

The antibody-drug conjugate according to any one of claims 34 to 45, wherein the average number of the selected drug-linker structure conjugated per antibody is in a range of from 1 to 10.

[Claim 47]

The antibody-drug conjugate according to any one of claims 34 to 46, wherein the average number of the

selected drug-linker structure conjugated per antibody is in a range of from 2 to 8.

[Claim 48]

The antibody-drug conjugate according to any one of claims 34 to 47, wherein the average number of the selected drug-linker structure conjugated per antibody is in a range of from 3 to 8.

[Claim 49]

The antibody-drug conjugate according to any one of claims 34 to 48, wherein the average number of the selected drug-linker structure conjugated per antibody is in a range of from 7 to 8.

[Claim 50]

The antibody-drug conjugate according to any one of claims 34 to 49, wherein the average number of the selected drug-linker structure conjugated per antibody is 8.

[Claim 51]

A pharmaceutical composition comprising any component selected from the antibodies or the functional fragments of the antibodies according to claims 1 to 19 and 27 to 33 and the antibody-drug conjugates according to claims 34 to 50, a salt of the component, or a hydrate of the component or the salt.

[Claim 52]

The pharmaceutical composition according to claim 51, which is an antitumor drug.

[Claim 53]

The antitumor drug according to claim 52, wherein the tumor is a tumor expressing GPR20.

[Claim 54]

The antitumor drug according to claim 50 or 51, wherein the tumor is gastrointestinal stromal tumor.

[Claim 55]

The pharmaceutical composition or the antitumor drug according to any one of claims 49 to 54, further comprising an additional antitumor drug.

[Claim 56]

A method for treating a tumor, which comprises administering any component selected from the antibodies or the functional fragments of the antibodies according to claims 1 to 19 and 27 to 33, the antibody-drug conjugates according to claims 34 to 50, salts of these components, and hydrates of these components or the salts to an individual.

[Claim 57]

The treatment method according to claim 56, wherein the tumor is gastrointestinal stromal tumor.

[Claim 58]

The treatment method according to claim 56 or 57, wherein the tumor is a tumor that exhibits resistance to a tyrosine kinase inhibitor.

[Claim 59]

A method for treating a tumor, which comprises administering a pharmaceutical composition comprising at least one component selected from the antibodies or the functional fragments of the antibodies according to claims 1 to 19 and 27 to 33, the antibody-drug conjugates according to claims 34 to 50, salts of these components, and hydrates of these components or the salts, and at least one antitumor drug to an individual, simultaneously, separately, or continuously.

[Claim 60]

The method for treating a tumor according to claim 59, wherein the antitumor drug is a tyrosine kinase inhibitor.

[Claim 61]

The method for treating a tumor according to claim 60, wherein the tyrosine kinase inhibitor is at least one selected from sunitinib, imatinib, and regorafenib.

[Claim 62]

The method for treating a tumor according to any one of claims 56 to 61, wherein the tumor is gastrointestinal stromal tumor that exhibits resistance to a tyrosine kinase inhibitor.

[Claim 63]

A method for producing an antibody-drug conjugate, which comprises a step of culturing the host cell according to claim 24 or 25, a step of collecting an antibody of interest or a functional fragment of the

antibody from the culture obtained by the aforementioned step, and a step of reacting the antibody or the functional fragment of the antibody obtained by the aforementioned step with a drug-linker intermediate compound.