Cripto-1.

[0009]

The present invention was completed on the basis of this finding and broadly includes the subject matter in the following embodiments.

[0010]

I. Antibody

I-1. A human-derived anti-human Cripto-1 monoclonal antibody that specifically recognizes the extracellular domain of human Cripto-1, the antibody comprising at least one CDR selected from the group consisting of a heavy-chain CDR 1, a heavy-chain CDR 2, a light-chain CDR 1, and a light-chain CDR 2,

wherein

the heavy-chain CDR 1 contains any one of the following amino acid sequences:

(A) the amino acid sequence represented by SEQ ID NO: 1,

(B) an amino acid sequence formed by substituting, deleting, or adding 1 to 3 amino acid residues in the amino acid sequence (A),

(C) the amino acid sequence represented by SEQ ID NO: 2, and

(D) an amino acid sequence formed by substituting, deleting, or adding 1 to 3 amino acid residues in the amino acid sequence (C),

the heavy-chain CDR 2 contains any one of the following amino acid sequences:

(E) the amino acid sequence represented by SEQ ID NO: 3, or

(F) an amino acid sequence formed by substituting, deleting, or adding 1 to 3 amino acid residues in the amino acid sequence (E),

(G) the amino acid sequence represented by SEQ ID NO: 4, and

(H) an amino acid sequence formed by substituting, deleting, or adding 1 to 3 amino acid residues in the amino acid sequence (G),

the light-chain CDR 1 contains

(I) the amino acid sequence represented by SEQ ID NO: 7, or

(J) an amino acid sequence formed by substituting, deleting, or adding 1 to 3 amino acid residues in the amino acid sequence (I), and

the light-chain CDR 2 contains

(K) the amino acid sequence represented by SEQ ID NO: 8, or

(L) an amino acid sequence formed by substituting, deleting, or adding 1 to 3 amino acid residues in the amino acid sequence (K).

[0011]

I-2. The antibody according to I-1, comprising the heavy-chain CDR 1 and the heavy-chain CDR 2 and/or the light-chain CDR 1 and the light-chain CDR 2.

[0012]

I-3. The antibody according to I-1 or I-2, comprising

a heavy-chain CDR 3 containing

(M) the amino acid sequence represented by SEQ ID NO: 5, or

(N) an amino acid sequence formed by substituting, deleting, or adding 1 to 3 amino acid residues in the amino acid sequence (~~N~~M); and/or

a light-chain CDR 3 containing

(O) the amino acid sequence represented by SEQ ID NO: 9, or

(P) an amino acid sequence formed by substituting, deleting, or adding 1 to 3 amino acid residues in the amino acid sequence (O).

[0013]

I-4. The antibody according to any one of I-1 to I-3, comprising

a heavy-chain variable region containing

(Q) the amino acid sequence represented by SEQ ID NO: 6, or

(R) an amino acid sequence formed by substituting, deleting, or adding 1 to 50 amino acid residues in the amino acid sequence (Q); and/or

a light-chain variable region containing

(S) the amino acid sequence represented by SEQ ID NO: 10, or

(T) an amino acid sequence formed by substituting, deleting, or adding 1 to 50 amino acid residues in the amino acid sequence (S).

[0014]

I-5. The antibody according to any one of I-1 to I-4, having a structure of Fv, scfv, diabody, triabody, tetrabody, or a combination thereof.

[0015]

I-6. The antibody according to any one of I-1 to I-5, comprising a constant region.

[0016]

I-7. The antibody according to any one of I-1 to I-6, having a structure of an immunoglobulin, Fab, $F(ab')_2$, minibody, scfv-Fc, or a combination thereof.

[0017]

I-8. The antibody according to any one of I-1 to I-7, having cytotoxic activity.

[0018]

I-9. The antibody according to I-8, wherein the cytotoxic activity is ADCC ~~activity~~, CDC ~~activity~~, or ADCP activity.

[0019]

I-10. The antibody according to any one of I-1 to I-9, which is a multispecific antibody.

[0020]

I-11. The antibody according to any one of I-1 to I-10, to which a cytotoxin is bindable.

[0021]

II. Polynucleotide

II-1. A polynucleotide encoding the antibody of any one of I-1 to I-11.

[0022]

III. Cell

III-1. A cell producing the antibody of any one of I-1 to I-11.

[0023]

III-2. A cell retaining the polynucleotide of II-1.

[0024]

IV. Food or Beverage Composition

IV-1. A food or beverage composition comprising the antibody of any one of I-1 to I-11.

[0025]

IV-2. The food or beverage composition according to IV-1, which is for use in inhibiting the proliferation of a cancer stem cell.

[0026]

[0045]

· Light-chain CDR 1

The light-chain CDR 1 contained in the antibody according to the present invention contains the following amino acid sequence (I) or (J).

[0046]

(I) The amino acid sequence represented by SEQ ID NO: 7.

(J) An amino acid sequence formed by substituting, deleting, or adding 1 to 3 amino acid residues in the amino acid sequence (I).

[0047]

The number of amino acid residues deleted, substituted, or added in the amino acid sequence (J) is not particularly limited as long as the antibody can specifically recognize the extracellular domain of human Cripto-1. The “substitution” is preferably a conservative substitution.

[0048]

· Light-chain CDR 2

The light-chain CDR 2 contained in the antibody according to the present invention contains the following amino acid sequence (K) or (L).

[0049]

(K) The amino acid sequence represented by SEQ ID NO: 8.

(L) An amino acid sequence formed by substituting, deleting, or adding 1 to 3 amino acid residues in the amino acid sequence (~~L~~K).

[0050]

The number of amino acid residues deleted, substituted, or added in the amino acid sequence (L) is not particularly limited as long as the antibody can specifically recognize the extracellular domain of human Cripto-1. The “substitution” is preferably a conservative substitution.

[0051]

In a more preferable embodiment, the antibody according to the present invention further contains a heavy-chain CDR 3 or a light-chain CDR 3, or both the heavy-chain CDR 3 and the light-chain CDR 3. The heavy-chain CDR 3 contains the following amino

(Q).

[0059]

The number of amino acid residues deleted, substituted, or added in the amino acid sequence (R) is not particularly limited as long as the antibody can specifically recognize the extracellular domain of human Cripto-1. For example, the number of amino acid residues deleted, substituted, or added in the amino acid sequence (R) is preferably 1 to 45, more preferably 1 to 40, 1 to 35, 1 to 30, 1 to 25, 1 to 20, 1 to 19, 1 to 18, 1 to 17, 1 to 16, 1 to 15, 1 to 14, 1 to 13, 1 to 12, 1 to 11, 1 to 10, 1 to 9, 1 to 8, 1 to 7, 1 to 6, 1 to 5, 1 to 4, or 1 to 3, and most preferably 1 to 2.

[0060]

The amino acid residues deleted, substituted, or added in the heavy-chain variable region are preferably the amino acid residues of FR. The “substitution” is preferably a conservative substitution.

[0061]

The light-chain variable region contains the following amino acid sequence (S) or (T).

[0062]

(S) The amino acid sequence represented by SEQ ID NO: 10.

(T) An amino acid sequence formed by substituting, deleting, or adding 1 to 50 amino acid residues in the amino acid sequence (~~P~~S).

[0063]

The number of amino acid residues deleted, substituted, or added in the amino acid sequence (~~P~~S) is not particularly limited as long as the antibody can specifically recognize the extracellular domain of human Cripto-1. For example, the number of amino acid residues deleted, substituted, or added is preferably 1 to 45, more preferably 1 to 40, 1 to 35, 1 to 30, 1 to 25, 1 to 20, 1 to 19, 1 to 18, 1 to 17, 1 to 16, 1 to 15, 1 to 14, 1 to 13, 1 to 12, 1 to 11, 1 to 10, 1 to 9, 1 to 8, 1 to 7, 1 to 6, 1 to 5, 1 to 4, or 1 to 3, and most preferably 1 to 2.

based on this numerical range taking the target body weight etc. into consideration.

[0100]

The interval at which the pharmaceutical composition ~~food or beverage composition~~ according to the present invention is administered can also be set as appropriate, for example, according to the gender and age of the target, the application form of the pharmaceutical composition~~food or beverage composition~~, and the extent of the effects desired, and is not particularly limited. For example, the pharmaceutical composition ~~food or beverage composition~~ according to the present invention may be administered once a day, or multiple times (e.g., two to three times) at intervals a day. The composition can also be administered at intervals of a few days or a few weeks.

[0101]

6. Method for Prevention and/or Treatment

The prevention method according to the present invention is a method for preventing cancer including administering a human-derived anti-human monoclonal antibody that specifically recognizes the extracellular domain of human Cripto-1 to a living organism that desires the prevention of developing cancer.

[0102]

The treatment method according to the present invention is a method for treating cancer including administering a human-derived anti-human monoclonal antibody that specifically recognizes the extracellular domain of human Cripto-1 to a living organism that has developed cancer.

[0103]

For the human-derived anti-human monoclonal antibody that specifically recognizes the extracellular domain of human Cripto-1, the “1. Antibody” section above can be referred to.

[0104]

For specific prevention or treatment methods according to the present invention, the “1. Antibody,” “4. Food or Beverage Composition,” and “5. Pharmaceutical Composition” sections above

<Table 1 : Clone #35>	
VH-CDR1	GNSVSSNSAA (SEQ ID NO: 1) SVSSNSAAWN (SEQ ID NO: 2)
VH-CDR2	TYYRSKWYN (SEQ ID NO: 3) YRSKWYN (SEQ ID NO: 4)
VH-CDR3	ARGFCSGGRCYSFDY (SEQ.ID:No. 5)
VH (SEQ ID NO: 6)	QVQLQQSGPGLVKPSQTLSLTCAISGNSVSSNSAAWNWIRRSPSRGLEWLGR TYYRSKWYNDYAVSVKSRITIKPDTSKNQFSLQLNSVTPEDTAMYYCARGFC SGGRCYSFDYWGPGTLVTVSSGSASAPTAS
VL-CDR1	DSDVGAYGS (SEQ ID NO: 7)
VL-CDR2	DVT (SEQ ID NO: 8)
VL-CDR3	QAWDSGIHVVF (SEQ ID NO: 9)
VL (SEQ ID NO: 10)	QSVLTQPASVSGSPGQSITISCTGTDSDVGAYGSASWYRHSPGKAPQLIIYD VTNRPSGVSNRFYGSKSGNTASLTISGLQAEDEADYYCQAWDSGIHVVFGGG TQLTVLG

[0109]

To obtain this antibody, the base sequences encoding the heavy-chain variable region and light-chain variable region of #35 clone were incorporated into vectors for antibody expression, thereby preparing a pHIgH5zeo vector, a pHIgL5hyg vector, and a pTX7z-HigG1L-#35 vector (Figs. 1 and 2). These vectors were transiently introduced into CHO cells to allow the cells to secrete #35 clone antibody. Finally, the antibody was purified from the culture supernatant of CHO cells according to a standard method by using a Protein A column, and the solvent was replaced with Dulbecco’s physiological phosphate buffer.

[0110]

Example 2: Evaluation of Antigen Recognition Ability by ELISA

The culture supernatant of the #35 clone antibody was collected and diluted 25-fold with a phosphate buffer, further followed by performing continuous dilution every 2-fold. The diluted culture supernatant was added to a 96-well plate, and IgG molecules (human-derived anti-human Cripto-1 antibody) adsorbed on each well were detected with an HRP-labeled anti-human IgG goat antibody (produced by Abcam). Fig. ~~2~~ 3 shows the results.

[0111]

As shown in Fig. ~~1~~3, the coloring due to HRP is decreased with the degree of the dilution of the culture supernatant containing the #35 clone antibody. This indicates

that the #35 clone antibody specifically adsorbed on its antigen, human Cripto-1.

[0112]

Example 3: Evaluation of Specificity to (Human) Cancer Tissue Using Tissue Array

Immunostaining was performed on a human cancer tissue array (BCN801, US Biomax Inc.). The #35 clone was used as a primary antibody. ALEXA568-labeled anti-human IgG goat antibody (Invitrogen) was used as a secondary antibody to detect human Cripto-1 in each cancer tissue (pancreas cancer tissue, thyroid gland cancer tissue, prostate cancer tissue, stomach cancer tissue, cervical cancer tissue, colorectal cancer tissue, and breast cancer tissue). These cancer tissues contained cancer stem cells as well as cancer cells, and Cripto-1 was thought to be bound to the surface of the cell membrane of the cancer stem cells by GPI-anchors. In a negative control experiment, the signal background was confirmed by using only the secondary antibody. Figs. ~~3~~ 4 to ~~5~~ 6 show the results.

[0113]

As shown in Figs. ~~3~~ 4 to ~~5~~6, staining images by the #35 clone antibody were confirmed in all of the cancer tissues.

[0114]

Example 4: Effect of Antibody on Cancer Stem Cell

The effect on the proliferation of cancer stem cells was evaluated by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). The U251MG SC1 cells used as cancer stem cells were prepared by the method described below.

[0115]

U251MG cells (brain tumor cell) obtained from the ECACC (European Collection of Authenticated Cell Cultures) were subcutaneously transplanted into nude mice (BALB/c-nu/nu, female) to form a tumor. Subsequently, the tumor was harvested and cultured according to a standard method. The tumor cells were screened with cells that grow hyaluronic-acid-dependently as a reference, thereby obtaining U251MG SC1 cells.

[0116]

To confirm the cancer stem cell property of the thus-obtained U251MG SC1 cell lines, an experiment to confirm the expression of SOX2, c-Myc, OCT3/4, KLF4, and NANOG genes was performed by quantification PCR using probes that recognize individual genes in accordance with a standard method. Table 2 below shows the base sequences of the probes that recognize individual genes used in quantitative PCR. GAPDH was used as an internal standard. Fig. 7 shows the results.

[0117]

Table 2

~~遺伝子名~~ GENE NAME		~~塩基配列（5'→3'）~~ Nucleotide Sequence (5' → 3')
GAPDH	F	CAACGACCACTTTGTCAAGCTC ~~(配列番号 13)~~ (Seq ID No.: 13)
	R	GGTCTACATGGCAACTGTGAGG ~~(配列番号 14)~~ (Seq ID No.: 14)
SOX2	F	GGGAAATGGGAGGGGTGCAAAAGAGG ~~(配列番号 15)~~ (Seq ID No.: 15)
	R	TTGCGTGAGTGTGGATGGGATTGGTG ~~(配列番号 16)~~ (Seq ID No.: 16)
c-Myc	F	GCGTCCTGGGAAGGGAGATCCGGAGC ~~(配列番号 17)~~ (Seq ID No.: 17)
	R	TTGAGGGCATCGTCGCGCGGGAGGCTG ~~(配列番号 18)~~ (Seq ID No.: 18)
OCT3/4	F	GACAGGGGGAGGGGAGGAGCTAGG ~~(配列番号 19)~~ (Seq ID No.: 19)
	R	CTTCCCTCCAACCAGTTGCCCCAAAC ~~(配列番号 20)~~ (Seq ID No.: 20)
KLF4	F	ATGCTCACCCCACCTTCTTC ~~(配列番号 21)~~ (Seq ID No.: 21)
	R	TTCTCACCTGTGTGGGTTCG ~~(配列番号 22)~~ (Seq ID No.: 22)
NANOG	F	CAGCCCCGATTCTTCCACCAGTCCC ~~(配列番号 23)~~ (Seq ID No.: 23)
	R	CGGAAGATTCCCAGTCGGGTTCACC ~~(配列番号 24)~~ (Seq ID No.: 24)

[0118]

As shown in Fig. 7, whereas the expression of OCT3/4 and NANOG was not detected in U251MG cells before screening, the expression of all of the SOX2, c-Myc, OCT3/4, KLF4, and NANOG genes was confirmed in U251MG SC1 cells obtained after screening. This clarified that U251MG SC1 cells obtained by screening U251MG cells were cell lines having a cancer stem cell property.

[0119]

Subsequently, an MTT assay was performed to confirm the inhibitory effect of the #35 clone antibody on self-renewal

potential (proliferation) of the U251MG SC1 cells obtained by screening.

[0120]

First, cells suspended in DMEM or RPMI1640 containing 10% fetal bovine serum were seeded into a 96-well plate at 5×10^3 cells/well. After culturing for 24 hours in 5% CO_2 at 37°C, a solution of the #35 clone antibody subjected to serial dilution was added to each well, and the culturing was continued for another 72 hours. Then, MTT dissolved in PBS at 5 mg/mL was added to give a final concentration of 1 mg/ml. After 4 hours of culturing, the formed formazan was dissolved in 0.02N HCl, and 10% w/v SDS was added, followed by dissolution at 37°C for a day and night. The absorbance of this solution at ~~540~~ 570 nm was measured to evaluate the cell viability. Fig. 8 shows the results.

[0121]

As shown in Fig. 8, the results revealed that the growth of U251MG SC1 cell lines obtained as cancer stem cells by screening U251MG was inhibited in the presence of the #35 clone antibody, and that the growth was inhibited greater with the increase in concentration of the #35 clone antibody. This clarified that the #35 clone antibody exhibits an effect in inhibiting the proliferation of cancer stem cells.

[0122]

Cancer stem cells are known to form spheres when cultured in a non-adhesive environment, such as in a low-adsorption dish. Thus, in order to confirm the effect of the #35 clone antibody in inhibiting the proliferation of cancer stem cells, an experiment to measure the number of spheres of cancer stem cells was performed with reference to the method disclosed in Fig. 4A of NPL 4. The specifically used cancer stem cells were NTERA2 clone D1 (EACC strain number: 01071221). The experiment to measure the number of spheres by using these cells also clarified that as shown in Fig. 8, the #35 clone antibody exhibited an effect in inhibiting the proliferation of cancer stem cells (data not shown).

CLAIMS

[Claim 1]

A human-derived anti-human Cripto-1 monoclonal antibody that specifically recognizes the extracellular domain of human Cripto-1, the antibody comprising at least one CDR selected from the group consisting of a heavy-chain CDR 1, a heavy-chain CDR 2, a light-chain CDR 1, and a light-chain CDR 2,
wherein
the heavy-chain CDR 1 contains any one of the following amino acid sequences:
(A) the amino acid sequence represented by SEQ ID NO: 1,
(B) an amino acid sequence formed by substituting, deleting, or adding 1 to 3 amino acid residues in the amino acid sequence (A),
(C) the amino acid sequence represented by SEQ ID NO: 2, and
(D) an amino acid sequence formed by substituting, deleting, or adding 1 to 3 amino acid residues in the amino acid sequence (C),
the heavy-chain CDR 2 contains any one of the following amino acid sequences:
(E) the amino acid sequence represented by SEQ ID NO: 3, or
(F) an amino acid sequence formed by substituting, deleting, or adding 1 to 3 amino acid residues in the amino acid sequence (E),
(G) the amino acid sequence represented by SEQ ID NO: 4, and
(H) an amino acid sequence formed by substituting, deleting, or adding 1 to 3 amino acid residues in the amino acid sequence (G),
the light-chain CDR 1 contains
(I) the amino acid sequence represented by SEQ ID NO: 7, or
(J) an amino acid sequence formed by substituting, deleting, or adding 1 to 3 amino acid residues in the amino acid sequence (I), and
the light-chain CDR 2 contains
(K) the amino acid sequence represented by SEQ ID NO: 8, or
(L) an amino acid sequence formed by substituting, deleting, or adding 1 to 3 amino acid residues in the amino acid sequence (K).

[Claim 2]

The antibody according to claim 1, comprising the heavy-chain CDR 1 and the heavy-chain CDR 2 and/or the light-chain CDR 1 and the light-chain CDR 2.

[Claim 3]

The antibody according to claim 1 or 2, comprising

a heavy-chain CDR 3 containing

(M) the amino acid sequence represented by SEQ ID NO: 5, or

(N) an amino acid sequence formed by substituting, deleting, or adding 1 to 3 amino acid residues in the amino acid sequence (~~N~~M); and/or

a light-chain CDR 3 containing

(O) the amino acid sequence represented by SEQ ID NO: 9, or

(P) an amino acid sequence formed by substituting, deleting, or adding 1 to 3 amino acid residues in the amino acid sequence (O).

[Claim 4]

The antibody according to any one of claims 1 to 3, comprising a heavy-chain variable region containing

(Q) the amino acid sequence represented by SEQ ID NO: 6, or

(R) an amino acid sequence formed by substituting, deleting, or adding 1 to 50 amino acid residues in the amino acid sequence (Q); and/or

a light-chain variable region containing

(S) the amino acid sequence represented by SEQ ID NO: 10, or

(T) an amino acid sequence formed by substituting, deleting, or adding 1 to 50 amino acid residues in the amino acid sequence (S).

[Claim 5]

The antibody according to any one of claims 1 to 4, having a structure of Fv, scfv, diabody, triabody, tetrabody, or a combination thereof.

[Claim 6]

The antibody according to any one of claims 1 to 5, comprising a constant region.

[Claim 7]

The antibody according to any one of claims 1 to 6, having a structure of an immunoglobulin, Fab, $F(ab')_2$, minibody, scfv-Fc, or a combination thereof.

[Claim 8]

The antibody according to any one of claims 1 to 7, having cytotoxic activity.

[Claim 9]

The antibody according to claim 8, wherein the cytotoxic activity is ADCC ~~activity~~, CDC ~~activity~~, or ADCP activity.

[Claim 10]

The antibody according to any one of claims 1 to 9, to which a cytotoxin is bindable.

[Claim 11]

A polynucleotide encoding the antibody of any one of claims 1 to 10.

[Claim 12]

A cell producing the antibody of any one of claims 1 to 10.

[Claim 13]

A cell retaining the polynucleotide of claim 11.

[Claim 14]

A food or beverage composition comprising the antibody of any one of claims 1 to 10.

[Claim 15]

A pharmaceutical composition comprising the antibody of any one of claims 1 to 10.