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(72) Inventeurs/Inventors:
SAITO, TAKANORI, JP;
OKANO, FUMIYOSHI, JP;
IDO, TAKAYOSHI, JP
(73) Propriétaire/Owner:
TORAY INDUSTRIES, INC., JP
(74) Agent: SMART & BIGGAR LLP

(54) Titre : COMPOSITION PHARMACEUTIQUE RENFERMANT DES ANTICORPS ANTI-CAPRINS-1 DESTINEE AU TRAITEMENT DU CANCER DE LA VESICULE BILIAIRE
(54) Title: PHARMACEUTICAL COMPOSITION COMPRISING ANTI-CAPRIN-1 ANTIBODIES FOR THE TREATMENT OF GALLBLADDER CANCER

(57) **Abrégé/Abstract:**

Provided is an antibody effective for treatment and/or prevention of gallbladder cancer. A pharmaceutical composition for treatment and/or prevention of gallbladder cancer contains as an active ingredient an antibody or a fragment thereof having immunological reactivity with a CAPRIN-1 protein or a fragment thereof comprising at least seven consecutive amino acid residues of the amino acid sequence of the protein.

ABSTRACT

Provided is an antibody effective for treatment and/or prevention of gallbladder cancer. A pharmaceutical composition for treatment and/or prevention of gallbladder cancer contains as an active ingredient an antibody or a fragment thereof having immunological reactivity with a CAPRIN-1 protein or a fragment thereof comprising at least seven consecutive amino acid residues of the amino acid sequence of the protein.

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DESCRIPTION

Title of Invention: PHARMACEUTICAL COMPOSITION COMPRISING ANTI-CAPRIN-1 ANTIBODIES FOR THE TREATMENT OF GALLBLADDER CANCER

Technical Field

[0001]

The present invention relates to a medicinal use of an antibody against a CAPRIN-1 protein or a fragment thereof, for example, as an agent for treating and/or preventing gallbladder cancer.

Background Art

[0002]

Recently, various antibody drugs for treating cancers by targeting antigen proteins on cancer cells have become up in the world. The antibody drugs show certain beneficial effects as cancer-specific therapeutic agents and have received attention. However, most of the target antigen proteins are expressed also on normal cells, and administration of such an antibody impairs not only cancer cells but also normal cells expressing the antigen, resulting in a problem of side effects therefrom. Accordingly, if a cancer antigen being specifically expressed on cancer cell surface is identified and an antibody targeting the antigen can be used as a pharmaceutical agent, treatment with an antibody drug with less side effects can be expected.

[0003]

It is known to those skilled in the art as general technical knowledge that gallbladder cancer, among various cancers, is very difficult to be detected at an early stage because of its lack of symptoms and early symptoms; advanced gallbladder cancer such as lymph node metastasis, liver metastasis, lung metastasis, bone metastasis, or peritoneal metastasis is very difficult to treat, leading to a five-year survival rate of almost 0% for patients with gallbladder

cancer not amenable to surgery; and gallbladder cancer is very difficult to treat and effective therapies for the cancer have not been developed.

[0004]

Cytoplasmic- and proliferation-associated protein 1 (CAPRIN-1) has been known as an intracellular protein that is expressed in activation of normal cells in the resting phase or in occurrence of cell division and is involved in control of transport and translation of mRNA through formation of intracellular stress granules with RNA in cells. It was found that CAPRIN-1 is specifically expressed on the surface of cancer cells such as breast cancer cells, and CAPRIN-1 has been studied as a target of antibody drugs for cancer therapy (Patent Literature 1). However, in Patent Literature 1, expression of CAPRIN-1 protein on gallbladder cancer cells is not recognized, and it is not described or suggested that CAPRIN-1 protein can be an antigen protein of gallbladder cancer.

Citation List

Patent Literature

[0005]

Patent Literature 1: WO2010/016526

Summary of Invention

Technical Problem

[0006]

It is an object of the present invention to identify a cancer antigen protein being expressed on the surface of gallbladder cancer cells and to provide a use of an antibody targeting the protein as an agent for treating and/or preventing gallbladder cancer.

Solution to Problem

[0007]

The present inventors have diligently studied and, as a result, have found that a part of CAPRIN-1 protein is expressed on the cell surface of gallbladder cancer cells and also have

found that an antibody against the CAPRIN-1 protein impairs the gallbladder cancer cells expressing the CAPRIN-1 protein, and have accomplished the present invention.

[0008]

Accordingly, the present invention has the following characteristics.

[0009]

The present invention provides a pharmaceutical composition for treating and/or preventing gallbladder cancer, comprising, as an active ingredient, an antibody or a fragment thereof having immunological reactivity with a CAPRIN-1 protein comprising an amino acid sequence set forth in any of even sequence numbers from SEQ ID NOs: 2 to 30 or an amino acid sequence having a sequence identity of 80% or more, preferably 85% or more, more preferably 90% or more, and most preferably 95% or more to the amino acid sequence, or a fragment of the CAPRIN-1 protein comprising at least seven consecutive amino acid residues of the amino acid sequence of the protein.

[0009A]

In particular, the present invention relates to a pharmaceutical composition for use in treating gallbladder cancer, wherein the composition comprises an antibody or a fragment thereof and a pharmacologically acceptable carrier or medium, wherein the antibody and fragment thereof bind specifically to: a CAPRIN-1 protein that is expressed on the cell surface of the gallbladder cancer and that comprises an amino acid sequence set forth in any one of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30, or a CAPRIN-1 protein that is expressed on the cell surface of the gallbladder cancer and that comprises an amino acid sequence having a sequence identity of 80% or more to the full length of the amino acid sequence set forth in any one of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30, or a CAPRIN-1 protein fragment comprising at least seven consecutive amino acid residues of the amino acid sequence set forth in any one of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30, or in an amino acid sequence having a sequence identity of 80% or more to the full length of the amino acid sequence set forth in any one of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30, wherein the at least seven consecutive amino acid residues are expressed on the cell surface of the gallbladder cancer.

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[0010]

In another embodiment, the antibody described above is a monoclonal antibody or a polyclonal antibody.

[0011]

In another embodiment, the antibody is a human antibody, a humanized antibody, a chimeric antibody, a single-chain antibody, or a multispecific antibody.

[0012]

In another embodiment, the antibody is an antibody having immunological reactivity with a peptide comprising an amino acid sequence set forth in SEQ ID NO: 271, SEQ ID NO: 273, SEQ ID NO: 266, SEQ ID NO: 270, SEQ ID NO: 272, or SEQ ID NO: 269 or an amino acid sequence having a sequence identity of 80% or more, preferably 85% or more, more preferably 90% or more, and most preferably 95% or more to the amino acid sequence or a fragment of the peptide.

[0013]

In another embodiment, the antibody is any one of the following antibodies (a) to (ao) having immunological reactivity with the CAPRIN-1 protein, or a pharmaceutical composition

for treating and/or preventing gallbladder cancer, comprising the antibody as an active ingredient.

[0014]

(a) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 37, 38, and 39, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 41, 42, and 43, respectively.

[0015]

(b) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 47, 48, and 49, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 51, 52, and 53, respectively.

[0016]

(c) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 57, 58, and 59, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 61, 62, and 63, respectively.

[0017]

(d) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 67, 68, and 69, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 71, 72, and 73, respectively.

[0018]

(e) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid

sequences set forth in SEQ ID NOs: 77, 78, and 79, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 81, 82, and 83, respectively.

[0019]

(f) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 87, 88, and 89, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 91, 92, and 93, respectively.

[0020]

(g) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 97, 98, and 99, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 101, 102, and 103, respectively.

[0021]

(h) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 107, 108, and 109, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 111, 112, and 113, respectively.

[0022]

(i) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 117, 118, and 119, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 121, 122, and 123, respectively.

[0023]

(j) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 127, 128, and 129, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 121, 122, and 123, respectively.

[0024]

(k) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 132, 133, and 134, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 136, 137, and 138, respectively.

[0025]

(l) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 142, 143, and 144, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 146, 147, and 148, respectively.

[0026]

(m) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 142, 143, and 144, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 152, 153, and 154, respectively.

[0027]

(n) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 157, 158, and 159, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 161, 162, and 163, respectively.

[0028]

(o) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 167, 168, and 169, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 171, 172, and 173, respectively.

[0029]

(p) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 167, 168, and 169, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 177, 178, and 179, respectively.

[0030]

(q) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 167, 168, and 169, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 182, 183, and 184, respectively.

[0031]

(r) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 167, 168, and 169, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 187, 188, and 189, respectively.

[0032]

(s) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 167, 168, and 169, respectively and a light chain variable

domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 192, 193, and 194, respectively.

[0033]

(t) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 197, 198, and 199, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 201, 202, and 203, respectively.

[0034]

(u) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 207, 208, and 209, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 211, 212, and 213, respectively.

[0035]

(v) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 217, 218, and 219, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 221, 222, and 223, respectively.

[0036]

(w) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 227, 228, and 229, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 231, 232, and 233, respectively.

[0037]

(x) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid

sequences set forth in SEQ ID NOs: 237, 238, and 239, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 241, 242, and 243, respectively.

[0038]

(y) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 247, 248, and 249, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 251, 252, and 253, respectively.

[0039]

(z) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 276, 277, and 278, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 280, 281, and 282, respectively.

[0040]

(aa) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 276, 277, and 278, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 286, 287, and 288, respectively.

[0041]

(ab) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 291, 292, and 293, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 295, 296, and 297, respectively.

[0042]

(ac) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 301, 302, and 303, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 305, 306, and 307, respectively.

[0043]

(ad) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 311, 312, and 313, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 315, 316, and 317, respectively.

[0044]

(ae) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 321, 322, and 323, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 325, 326, and 327, respectively.

[0045]

(af) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 331, 332, and 333, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 335, 336, and 337, respectively.

[0046]

(ag) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 341, 342, and 343, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 345, 346, and 347, respectively.

[0047]

(ah) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 351, 352, and 353, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 354, 355, and 356, respectively.

[0048]

(ai) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 351, 352, and 357, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 354, 355, and 356, respectively.

[0049]

(aj) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 373, 374, and 375, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 377, 378, and 379, respectively.

[0050]

(ak) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 383, 384, and 385, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 387, 388, and 389, respectively.

[0051]

(al) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 393, 394, and 395, respectively and a light chain variable

domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 387, 388, and 389, respectively.

[0052]

(am) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 398, 399, and 400, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 402, 403, and 404, respectively.

[0053]

(an) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 408, 409, and 410, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 412, 413, and 414, respectively.

[0054]

(ao) An antibody comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 418, 419, and 420, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 422, 423, and 424, respectively.

[0055]

In another embodiment, the antibody or a fragment thereof of the present invention is conjugated to an antitumor agent.

[0056]

The present invention further provides a combination pharmaceutical agent comprising combination of the pharmaceutical composition of the present invention and a pharmaceutical composition containing an antitumor agent.

[0057]

The present invention further provides use of the pharmaceutical composition or the combination pharmaceutical agent of the present invention for treating a gallbladder cancer that has CAPRIN-1 protein expressed on its cell surface.

[0058]

The present specification encompasses the contents in the specification and/or the drawings of Japanese Patent Application No. 2012-080780 based on which the present application claims priority.

Advantageous Effects of Invention

[0059]

The antibody against the CAPRIN-1 protein used in the present invention (hereinafter, often referred to as "anti-CAPRIN-1 antibody") impairs gallbladder cancer cells. Accordingly, the antibody against the CAPRIN-1 protein is useful for treatment and prevention of gallbladder cancer.

Description of Embodiments

[0060]

The antitumor activity of an antibody used in the present invention against a polypeptide consisting of an amino acid sequence set forth in any of even sequence numbers from SEQ ID NOs: 2 to 30 can be evaluated by investigating the *in vivo* suppression of tumor growth in a tumor-bearing animal or investigating, as described below, whether or not a cytotoxicity through immune cells or a complement is observed on tumor cells expressing the polypeptide *in vitro*.

[0061]

The nucleotide sequences of polynucleotides encoding proteins consisting of the amino acid sequences set forth in even sequence numbers from SEQ ID NOs: 2 to 30 (i.e., SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, and 30) are set forth in odd sequence numbers from SEQ ID NOs: 1 to 29 (i.e., SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29).

[0062]

The amino acid sequences set forth in SEQ ID NOs: 6, 8, 10, 12, and 14 in sequence listing are amino acid sequences of a CAPRIN-1 protein isolated as polypeptides that bind to an antibody specifically present in serum derived from tumor-bearing dogs by a SEREX method using a dog testis tissue-derived cDNA library and serum of a breast cancer dog; the amino acid sequences set forth in SEQ ID NOs: 2 and 4 are amino acid sequences isolated as human homologous factors (homologs or orthologs) of the polypeptides; the amino acid sequence set forth in SEQ ID NO: 16 is an amino acid sequence isolated as a bovine homologous factor thereof; the amino acid sequence set forth in SEQ ID NO: 18 is an amino acid sequence isolated as a horse homologous factor thereof; the amino acid sequences set forth in SEQ ID NOs: 20 to 28 are amino acid sequences isolated as mouse homologous factors thereof; and the amino acid sequence set forth in SEQ ID NO: 30 is an amino acid sequence isolated as a chicken homologous factor (see Example 1 described below) thereof. The CAPRIN-1 protein is known to be expressed in activation of normal cells in the resting phase or in occurrence of cell division.

[0063]

The investigation revealed that the CAPRIN-1 protein is expressed on the cell surface of gallbladder cancer cells. In the present invention, an antibody that binds to a part of the CAPRIN-1 protein expressed on the cell surface of gallbladder cancer cells is preferably used. Examples of the partial peptide (fragment) of the CAPRIN-1 protein expressed on the cell surface of gallbladder cancer cells include peptides comprising at least seven consecutive amino acid residues in the region of amino acid residue positions (aa) 233 to (aa) 343, amino acid residue positions (aa) 512 to the C-terminal, or amino acid residue positions (aa) 50 to (aa) 98 of the amino acid sequences set forth in even sequence numbers from SEQ ID NOs: 2 to 30, excluding SEQ ID NOs: 6 and 18, in the sequence listing. Specifically, for example, the partial peptide (fragment) is a peptide comprising at least seven consecutive amino acid residues in an amino acid sequence set forth in SEQ ID NO: 429, SEQ ID NO: 428, SEQ ID NO: 273 (in the amino acid sequence set forth in SEQ ID NO: 273, the region of the amino acid sequence set forth in SEQ ID NO: 274 or SEQ ID NO: 275 is preferred), SEQ ID NO:

266 (in the amino acid sequence set forth in SEQ ID NO: 266, the region of the amino acid sequence set forth in SEQ ID NO: 267 or SEQ ID NO: 268 is preferred), SEQ ID NO: 270, SEQ ID NO: 272, SEQ ID NO: 269, SEQ ID NO: 430, SEQ ID NO: 431, or SEQ ID NO: 432, or in an amino acid sequence having a sequence identity of 80% or more, preferably 85% or more, more preferably 90% or more, and most preferably 95% or more, such as 96% or more, 97% or more, 98% or more, or 99% or more, to the amino acid sequence mentioned above. Examples of the antibody used in the present invention include all antibodies that bind to any of these peptides and show antitumor activity.

[0064]

The anti-CAPRIN-1 antibody used in the present invention may be any type of antibody that shows antitumor activity, and examples thereof include monoclonal antibodies; polyclonal antibodies; recombinant antibodies such as synthetic antibodies, multispecific antibodies (e.g., diabodies and triabodies), humanized antibodies, chimeric antibodies, and single-chain antibodies (scFv); human antibodies; and antibody fragments thereof such as Fab, F(ab')₂, and Fv. These antibodies and fragments thereof can be prepared by those skilled in the art through a known method. In the present invention, an antibody capable of specifically binding to a CAPRIN-1 protein is desirable, and preferred is a monoclonal antibody. However, the antibody may be a polyclonal antibody that is homogeneous and can be stably produced. When the subject is a human being, a human antibody or a humanized antibody is desirable for inhibiting or suppressing rejection reaction.

[0065]

Here, the term "specifically binding to a CAPRIN-1 protein" refers to binding specific to a CAPRIN-1 protein and substantially not binding other proteins.

[0066]

The antitumor activity of the antibody that can be used in the present invention can be evaluated by, as described below, investigating the *in vivo* suppression of tumor growth in a tumor-bearing animal or investigating whether or not a cytotoxicity through immune cells or a complement is observed on tumor cells expressing the polypeptide *in vitro*.

[0067]

The subject as an object of the treatment and/or prevention of gallbladder cancer in the present invention is a mammal such as a human being, a pet animal, a domestic animal, or an animal for competitive use; and is preferably a human being.

[0068]

The production of an antigen, the production of an antibody, and a pharmaceutical composition according to the present invention will now be described.

[0069]

<Production of antigen for producing antibody>

The protein or a fragment thereof to be used as a sensitizing antigen for preparing an anti-CAPRIN-1 antibody used in the present invention may be derived from any animal species, such as a human being, dog, bovine, horse, mouse, rat, or chicken, and is preferably selected with consideration for compatibility with the parent cells used for cell fusion. In general, the protein is preferably a protein derived from a mammal, in particular, a human being. For example, when the CAPRIN-1 protein is a human CAPRIN-1 protein, a human CAPRIN-1 protein, a partial peptide thereof, or cells expressing a human CAPRIN-1 protein can be used.

[0070]

The nucleotide sequences and the amino acid sequences of a human CAPRIN-1 protein and a homolog thereof can be obtained by, for example, accessing the GenBank (NCBI, USA) and using algorithm such as BLAST or FASTA (Karlin and Altschul, Proc. Natl. Acad. Sci. USA, 90: 5873-5877, 1993; Altschul et al., Nucleic Acids Res., 25: 3389-3402, 1997).

[0071]

In the present invention, the target is a nucleic acid or protein consisting of a sequence having a sequence identity of 70% to 100%, preferably 80% to 100%, more preferably 90% to 100%, and most preferably 95% to 100%, such as 97% to 100%, 98% to 100%, 99% to 100%, or 99.5% to 100%, to the nucleotide sequence or the amino acid sequence of the ORF or the mature part of the human CAPRIN-1 gene when the nucleotide sequence and the amino acid sequence thereof are based on the sequences set forth in SEQ ID NO: 1 or 3 and SEQ ID NO: 2 or 4, respectively. Here, the term "% sequence identity" between two amino acid (or

nucleotide) sequences refers to the percentage (%) of the number of amino acids (or nucleotides) in one sequence coinciding with those in the other sequence to the total number when the two sequences are aligned (alignment) with a maximum degree of similarity or coincidence by introducing a gap or not.

[0072]

A fragment of the CAPRIN-1 protein has a length of from an amino acid length of an epitope (antigen determinant), which is a minimum unit recognized by an antibody, to an amino acid length shorter than the total length of the protein. The epitope refers to a polypeptide fragment having antigenicity or immunogenicity in a mammal, preferably in a human being, and its minimum unit consists of about 7 to 12 amino acids, such as 8 to 11 amino acids. Examples of the epitope include the amino acid sequences set forth in SEQ ID NO: 273, SEQ ID NO: 266, SEQ ID NO: 270, SEQ ID NO: 272, and SEQ ID NO: 269; and an amino acid sequence having a sequence identity of 80% or more, preferably 85% or more, more preferably 90% or more, and most preferably 95% or more to any of the amino acid sequences.

[0073]

The polypeptide comprising a human CAPRIN-1 protein or a partial peptide thereof can be synthesized, for example, in accordance with a chemical synthesis such as a fluorenylmethyloxycarbonyl (Fmoc) method or a t-butyloxycarbonyl method (tBoc) method (Seikagaku Jikken Koza (Course of Biochemical Experiments) 1, Tanpakushitsu no Kagaku (Protein Chemistry) IV, Kagaku shushoku to peputido gosei (Chemical modification and peptide synthesis), edited by the Japanese Biochemical Society, Tokyo Kagaku Dojin (Japan), 1981). Alternatively, the peptide can be synthesized by a usual method using various commercially available peptide synthesizers. Furthermore, a target peptide can be obtained by preparing a DNA encoding the polypeptide by a known genetic engineering procedure (e.g., Sambrook, et al., Molecular Cloning, 2nd edition, Current Protocols in Molecular Biology (1989), Cold Spring Harbor Laboratory Press; Ausubel, et al., Short Protocols in Molecular Biology, 3rd edition, A compendium of Methods from Current Protocols in Molecular Biology

(1995), John Wiley & Sons), incorporating the DNA into an expression vector and introducing it into a host cell, and allowing the production of the peptide in the host cell.

[0074]

The DNA encoding the polypeptide can be readily prepared by a known genetic engineering procedure or by a usual method with a commercially available peptide synthesizer. For example, a DNA comprising the nucleotide sequence set forth in SEQ ID NO: 1 can be prepared by PCR using a pair of primers designed such that the nucleotide sequence set forth in SEQ ID NO: 1 can be amplified using a human chromosome or cDNA library as a template. The reaction conditions for the PCR can be appropriately determined, and non-limiting examples thereof include conditions in which a PCR buffer containing a heat stable DNA polymerase (e.g., Taq polymerase) and Mg^{2+} is used, and the amplification is performed by repeating, for example, 30 cycles of a process consisting of reactions at 94°C for 30 seconds (denaturation), at 55°C for 30 seconds to 1 minute (annealing), and at 72°C for 2 minutes (extension) and then performing a reaction at 72°C for 7 minutes. The procedure, conditions, and other factors of PCR are described in, for example, Ausubel, et al., Short Protocols in Molecular Biology, 3rd edition, A compendium of Methods from Current Protocols in Molecular Biology, (1995), John Wiley & Sons (in particular, the 15th chapter).

[0075]

A desired DNA can be isolated by preparing appropriate probes and primers based on the information of the nucleotide sequences and the amino acid sequences set forth in SEQ ID NOs: 1 to 30 of the sequence listing in the specification and screening, for example, a human cDNA library using the resulting probes and primers. The cDNA library is preferably constructed from cells, an organ, or tissue expressing the proteins set forth in any of even sequence numbers from SEQ ID NOs: 2 to 30. Examples of the cells and tissue include those derived from testis and cancer or tumor cells, such as leukemia, breast cancer, lymphoma, brain tumor, lung cancer, colon cancer, and gallbladder cancer. The above-described procedures such as preparation of probes or primers, construction of a cDNA library, screening of the cDNA library, and cloning of a target gene are known to those skilled in the art and can be performed, for example, in accordance with the method described in, for

example, Sambrook, et al., Molecular Cloning, 2nd edition, Current Protocols in Molecular Biology, (1989) or Ausbel, et al. (above). A DNA encoding a human CAPRIN-1 protein or a partial peptide thereof can be prepared from the thus-prepared DNA.

[0076]

The host cell may be any cell that can express the above-mentioned peptide, and examples thereof include, but not limited to, prokaryotic cells such as *E. coli* cells and eukaryotic cells such as mammalian cells, e.g., monkey kidney COS1 cells and Chinese hamster ovary CHO cells, human embryonic kidney cell line HEK293, mouse embryonic fibroblast cell line NIH3T3, yeast cells, e.g., budding yeasts and fission yeast cells, silkworm cells, and *Xenopus* egg cells.

[0077]

When prokaryotic cells are used as host cells, an expression vector having an origin of replicable in the prokaryotic cell, a promoter, a ribosome binding site, a multicloning site, a terminator, a drug resistance gene, an auxotrophic complementary gene, etc. is used. Examples of the expression vector for *E. coli* include pUC vector, pBluescriptII, pET expression system, and pGEX expression system. The polypeptide described above can be expressed in prokaryotic host cells by incorporating a DNA encoding the polypeptide into such an expression vector, transforming the prokaryotic host cells with the vector, and then culturing the resulting transformant. On this occasion, the polypeptide can also be expressed as a fusion protein with another protein.

[0078]

When eukaryotic cells are used as host cells, an expression vector for eukaryotic cells having a promoter, a splicing region, a poly(A) addition site, etc. is used. Examples of the expression vector include pKA1, pCDM8, pSVK3, pMSG, pSVL, pBK-CMV, pBK-RSV, EBV vector, pRS, pcDNA3, and pYES2. The polypeptide described above can be expressed in eukaryotic host cells as in above by incorporating a DNA encoding the polypeptide into such an expression vector, transforming the eukaryotic host cells with the vector, and then culturing the resulting transformant. When the expression vector is, for example, pIND/V5-

His, pFLAG-CMV-2, pEGFP-N1, or pEGFP-C1, the polypeptide can be expressed as a fusion protein with a tag such as His tag (e.g., (His)₆ to (His)₁₀), FLAG tag, myc tag, HA tag, or GFP.

[0079]

Introduction of an expression vector into host cells can be performed by a known method such as electroporation, calcium phosphate transfection, a liposome method, a DEAE-dextran method, micro-injection, virus infection, lipofection, or binding to a cell-penetrating peptide.

[0080]

A target polypeptide can be isolated and purified from the host cells by combining known separating procedures. Examples of the separating procedures include, but not limited to, treatment with a denaturant such as urea or a surfactant, ultrasonication, enzymatic digestion, salting out, solvent fractional precipitation, dialysis, centrifugation, ultrafiltration, gel filtration, SDS-PAGE, isoelectric focusing phoresis, ion exchange chromatography, hydrophobic chromatography, affinity chromatography, and reverse phase chromatography.

[0081]

<Structure of antibody>

An antibody is usually a heteromultimeric glycoprotein at least comprising two heavy chains and two light chains. Except for IgM, an antibody is a heterotetrameric glycoprotein of about 150 kDa comprising the same two light (L) chains and the same two heavy (H) chains. Typically, a light chain is linked to a heavy chain through a disulfide covalent bond, and the number of disulfide bonds between the heavy chains varies depending on the isotype of immunoglobulin. The heavy chains and the light chains each have an intrachain disulfide bond. Each heavy chain has a variable domain (VH domain) at one end, followed by several constant domains. Each light chain has a variable domain (VL domain) and a constant domain at its other end. The constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. The variable domains of an antibody confer binding specificity on the antibody with specific regions displaying particular variability called complementarity determining regions (CDRs). The relatively conserved portions of the

variable domains are called framework regions (FRs). The variable domains of intact heavy and light chains each comprises four FRs connected by three CDRs. The three CDRs in the heavy chain are called CDRH1, CDRH2, and CDRH3 in this order from the N-terminal side. Similarly, in the light chain, the CDRs are called CDRL1, CDRL2, and CDRL3. CDRH3 is most important in the binding specificity of an antibody to an antigen. The CDRs of each chain are held together in a contiguous state by the FRs and contribute together with CDRs of another chain to formation of the antigen binding site of the antibody. The constant domains are not directly involved in the binding of the antibody to the antigen, but exhibit various effector functions such as participation in antibody dependent cell-mediated cytotoxicity (ADCC), phagocytosis via binding to Fc γ receptor, half-life/clearance rate via neonatal Fc receptor (FcRn), and complement dependent cytotoxicity (CDC) via the C1q component of the complement cascade.

[0082]

<Production of antibody>

The anti-CAPRIN-1 antibody in the present invention is an antibody having an immunological reactivity with the full length or a fragment of a CAPRIN-1 protein.

[0083]

Here, the term "immunological reactivity" refers to a property that an antibody and a CAPRIN-1 antigen bind to each other *in vivo*, and a function of impairing tumor (for example, death, suppression, or regression) is exhibited through such binding. That is, the antibody used in the present invention may be any antibody that binds to a CAPRIN-1 protein and thereby can impair gallbladder cancer.

[0084]

Examples of the antibody include monoclonal antibodies, polyclonal antibodies, synthetic antibodies, multispecific antibodies, human antibodies, humanized antibodies, chimeric antibodies, single-chain antibodies, and antibody fragments (e.g., Fab, F(ab')₂, and Fv). The antibody is an appropriate class of immunoglobulin molecule, such as IgG, IgE, IgM, IgA, IgD, or IgY, or an appropriate subclass, such as IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, or IgA₂.

[0085]

The antibody may be further modified by, for example, acetylation, formylation, amidation, phosphorylation, or polyethylene glycolation (PEGylation), as well as glycosylation.

[0086]

Examples of production of various antibodies will now be described.

[0087]

In a monoclonal antibody, for example, a mouse is immunized with a CAPRIN-1 protein, gallbladder cancer cells expressing the CAPRIN-1 protein, or a cell line (e.g., TGBC14TKB) thereof; the spleen is extracted from the mouse; the spleen cells are separated and are fused with mouse myeloma cells; and clones producing antibodies having cancer cell growth-inhibitory activity are selected from the resulting fused cells (hybridomas). A hybridoma producing a monoclonal antibody having cancer cell growth-inhibitory activity is isolated and is cultured, and the antibody is purified from the culture supernatant by usual affinity purification to prepare a monoclonal antibody.

[0088]

The hybridoma producing a monoclonal antibody can also be produced by, for example, as follows. First, an animal is immunized with a sensitizing antigen in accordance with a known method. In general, the sensitizing antigen is intraperitoneally or subcutaneously injected to a mammal. Specifically, the sensitizing antigen is appropriately diluted with, for example, phosphate-buffered saline (PBS) or physiological saline. The resulting suspension is optionally mixed with an appropriate amount of a normal adjuvant, such as complete Freund's adjuvant, and emulsified, and is then administered to the mammal several times at 4 to 21 days intervals. In addition, an appropriate carrier can be used in immunization with the sensitizing antigen.

[0089]

After the immunization of the mammal and confirmation of an increase in the level of a desired antibody in serum, the immune cells are collected from the mammal and are subjected to cell fusion. The immune cells are preferably spleen cells.

[0090]

Myeloma cells of a mammal are used as the other parent cells to be fused with the immune cells. As the myeloma cells, various known cell lines, for example, P3U1 (P3-X63Ag8U1), P3 (P3x63Ag8.653) (J. Immunol., (1979), 123, 1548-1550), P3x63Ag8U.1 (Current Topics in Microbiology and Immunology, (1978), 81, 1-7), NS-1 (Kohler, G. and Milstein, C., Eur. J. Immunol., (1976), 6, 511-519), MPC-11 (Margulies, D.H., et al., Cell, (1976), 8, 405-415), SP2/0 (Shulman, M. et al., Nature, (1978), 276, 269-270), FO (deSt. Groth, S.F., et al., J. Immunol. Methods, (1980), 35, 1-21), S194 (Trowbridge, I.S., J. Exp. Med., (1978), 148, 313-323), or R210 (Galfre, G. et al., Nature, (1979), 277, 131-133), can be suitably used.

[0091]

The immune cells and the myeloma cells can be fundamentally fused by a known method, for example, in accordance with the method of Kohler and Milstein (Kohler, G. and Milstein, C., Methods Enzymol., (1981), 73, 3-46).

[0092]

More specifically, the cell fusion is performed, for example, in a normal nutrient culture solution in the presence of a cell fusion accelerator. Examples of the cell fusion accelerator include polyethylene glycol (PEG) and Sendai virus (HVJ). Furthermore, an auxiliary agent such as dimethyl sulfoxide can be optionally used for increasing the fusion efficiency.

[0093]

The number ratio between the immune cells and the myeloma cells can be arbitrarily determined. For example, the ratio of the number of the immune cells to the number of myeloma cells is preferably 1 to 10. The culture solution used in the cell fusion can be, for example, a RPMI1640 culture solution or MEM culture solution suitable for growth of the myeloma cell line or a normal culture solution that is used for such cell culture. In addition, a serum replacement such as fetal calf serum (FCS) can be added to the culture solution.

[0094]

The cell fusion is performed by sufficiently mixing predetermined amounts of the immune cells and the myeloma cells in the culture solution, adding a PEG solution (average molecular weight: e.g., about 1000 to 6000) previously heated to about 37°C to the mixture, usually, at a concentration of 30% to 60% (w/v), and mixing them to form a desired hybridoma. Successively, an appropriate culture solution is added to the mixture, and the supernatant is removed by centrifugation. This procedure is repeated to remove the components, such as the fusion promoter, that are undesirable for the growth of hybridomas.

[0095]

The thus-prepared hybridoma can be selected by culturing in a usual selection culture solution, for example, a HAT culture solution (culture solution containing hypoxanthine, aminopterin, and thymidine). The culturing in the HAT culture solution is continued for a sufficient period of time (usually, several days to several weeks) for killing the cells (non-fused cells) other than the target hybridomas. Subsequently, a usual limiting dilution method is performed for screening and single cloning of the hybridoma that produces the target antibody.

[0096]

Instead of the method of obtaining a hybridoma by immunizing a non-human animal with an antigen, a hybridoma that produces a human antibody having desired activity (e.g., cell growth-inhibitory activity) can be obtained by sensitizing human lymphocytes, for example, human lymphocytes infected with EB virus, with a protein, cells expressing the protein, or a lysate thereof *in vitro*, and fusing the sensitized lymphocytes with myeloma cells derived from a human being and having permanent division ability, for example, U266 (Registration No. TIB196).

[0097]

The thus-prepared hybridoma that produces a monoclonal antibody can be passaged in a usual culture solution and can be stored in a liquid nitrogen for a long time.

[0098]

That is, a desired antigen or a cell expressing the desired antigen is used as the sensitizing antigen and is immunized in accordance with a usual method; the resulting immune

cells are fused with known parent cells by a usual cell fusion; and a monoclonal antibody-producing cell (hybridoma) is screened by a usual screening method. Thus, a hybridoma can be produced.

[0099]

Another example of the antibody that can be used in the present invention is a polyclonal antibody. The polyclonal antibody can be prepared, for example, as follows.

[0100]

Serum is prepared by immunizing a small animal, such as a mouse, a human antibody-producing mouse, or a rabbit, with a native CAPRIN-1 protein, a recombinant CAPRIN-1 protein expressed in microorganisms, such as *E. coli*, as a fusion protein with, for example, GST, or a partial peptide thereof. The serum is purified by, for example, ammonium sulfate precipitation, protein A or protein G column chromatography, DEAE ion exchange chromatography, or an affinity column chromatography coupled with a CAPRIN-1 protein or a synthetic peptide. In examples described below, a rabbit polyclonal antibody against the CAPRIN-1 protein was produced, and an antitumor effect was confirmed.

[0101]

Here, as the human antibody-producing mouse, a KM mouse (Kirin Pharma Company, Limited/Medarex Inc.) and a Xeno mouse (Amgen Inc.) are known (for example, International Publication Nos. WO02/43478 and WO02/092812). Immunization of such a mouse with a CAPRIN-1 protein or a fragment thereof can provide a complete human polyclonal antibody in the blood. Alternatively, a human-type monoclonal antibody can be produced by extracting the spleen cells from the immunized mouse and fusing the spleen cells with myeloma cells.

[0102]

The antigen can be prepared in accordance with, for example, a method using animal cells (JP Patent Publication (Kohyo) No. 2007-530068) or a method using baculovirus (e.g., International Publication No. WO98/46777). An antigen having low immunogenicity may be immunized as a conjugate with a macromolecule having immunogenicity, such as albumin.

[0103]

Furthermore, a transgenic antibody generated by gene recombination technology by cloning an antibody gene from a hybridoma, incorporating the gene into an appropriate vector, and introducing the vector into a host, can be used (e.g., see Carl, A.K. Borrebaeck, James, W. Larrick, THERAPEUTIC MONOCLONAL ANTIBODIES, Published in the United Kingdom by MACMILLAN PUBLISHERS LTD, 1990). Specifically, a cDNA of the variable domain (V domain) of an antibody is synthesized using a reverse transcriptase from the mRNA of a hybridoma. If a DNA encoding the V domain of a target antibody is prepared, the DNA is linked to a DNA encoding a desired antibody constant domain (C domain), followed by incorporation into an expression vector. Alternatively, a DNA encoding the V domain of an antibody may be incorporated into an expression vector containing the DNA of the antibody C domain. The DNA is incorporated into the expression vector such that the DNA is expressed under the control of an expression-controlling domain, for example, an enhancer and a promoter. Subsequently, the host cell is transformed with the expression vector to express the antibody.

[0104]

The anti-CAPRIN-1 antibody used in the present invention is preferably a monoclonal antibody, but may be a polyclonal antibody or a genetically altered antibody (e.g., chimeric antibody or humanized antibody).

[0105]

Examples of the monoclonal antibody include human monoclonal antibodies, non-human animal monoclonal antibodies (e.g., mouse monoclonal antibodies, rat monoclonal antibodies, rabbit monoclonal antibodies, and chicken monoclonal antibodies). The monoclonal antibody can be produced by culturing hybridomas prepared by fusing myeloma cells with the spleen cells derived from a non-human mammal (e.g., mouse or human antibody-producing mouse) immunized with a CAPRIN-1 protein. In examples described below, monoclonal antibodies were produced, and the antitumor effects thereof were confirmed. These monoclonal antibodies each comprise a heavy-chain variable (VH) domain comprising the amino acid sequence set forth in SEQ ID NO: 40, SEQ ID NO: 50, SEQ ID NO: 60, SEQ ID NO: 70, SEQ ID NO: 80, SEQ ID NO: 90, SEQ ID NO: 100, SEQ ID NO:

110, SEQ ID NO: 120, SEQ ID NO: 130, SEQ ID NO: 135, SEQ ID NO: 145, SEQ ID NO: 160, SEQ ID NO: 170, SEQ ID NO: 200, SEQ ID NO: 210, SEQ ID NO: 220, SEQ ID NO: 230, SEQ ID NO: 240, SEQ ID NO: 250, SEQ ID NO: 279, SEQ ID NO: 294, SEQ ID NO: 304, SEQ ID NO: 314, SEQ ID NO: 324, SEQ ID NO: 334, SEQ ID NO: 344, SEQ ID NO: 359, SEQ ID NO: 363, SEQ ID NO: 368, SEQ ID NO: 372, SEQ ID NO: 376, SEQ ID NO: 386, SEQ ID NO: 396, SEQ ID NO: 401, SEQ ID NO: 411, or SEQ ID NO: 421 and a light-chain variable (VL) domain comprising the amino acid sequence set forth in SEQ ID NO: 44, SEQ ID NO: 54, SEQ ID NO: 64, SEQ ID NO: 74, SEQ ID NO: 84, SEQ ID NO: 94, SEQ ID NO: 104, SEQ ID NO: 114, SEQ ID NO: 124, SEQ ID NO: 139, SEQ ID NO: 149, SEQ ID NO: 155, SEQ ID NO: 164, SEQ ID NO: 174, SEQ ID NO: 180, SEQ ID NO: 185, SEQ ID NO: 190, SEQ ID NO: 195, SEQ ID NO: 204, SEQ ID NO: 214, SEQ ID NO: 224, SEQ ID NO: 234, SEQ ID NO: 244, SEQ ID NO: 254, SEQ ID NO: 283, SEQ ID NO: 289, SEQ ID NO: 298, SEQ ID NO: 308, SEQ ID NO: 318, SEQ ID NO: 328, SEQ ID NO: 338, SEQ ID NO: 348, SEQ ID NO: 361, SEQ ID NO: 365, SEQ ID NO: 370, SEQ ID NO: 380, SEQ ID NO: 390, SEQ ID NO: 405, SEQ ID NO: 415, or SEQ ID NO: 425. The VH domain comprises CDR1 represented by the amino acid sequence set forth in SEQ ID NO: 37, SEQ ID NO: 47, SEQ ID NO: 57, SEQ ID NO: 67, SEQ ID NO: 77, SEQ ID NO: 87, SEQ ID NO: 97, SEQ ID NO: 107, SEQ ID NO: 117, SEQ ID NO: 127, SEQ ID NO: 132, SEQ ID NO: 142, SEQ ID NO: 157, SEQ ID NO: 167, SEQ ID NO: 197, SEQ ID NO: 207, SEQ ID NO: 217, SEQ ID NO: 227, SEQ ID NO: 237, SEQ ID NO: 247, SEQ ID NO: 276, SEQ ID NO: 291, SEQ ID NO: 301, SEQ ID NO: 311, SEQ ID NO: 321, SEQ ID NO: 331, SEQ ID NO: 341, SEQ ID NO: 351, SEQ ID NO: 373, SEQ ID NO: 383, SEQ ID NO: 393, SEQ ID NO: 398, SEQ ID NO: 408, or SEQ ID NO: 418, CDR2 represented by the amino acid sequence set forth in SEQ ID NO: 38, SEQ ID NO: 48, SEQ ID NO: 58, SEQ ID NO: 68, SEQ ID NO: 78, SEQ ID NO: 88, SEQ ID NO: 98, SEQ ID NO: 108, SEQ ID NO: 118, SEQ ID NO: 128, SEQ ID NO: 133, SEQ ID NO: 143, SEQ ID NO: 158, SEQ ID NO: 168, SEQ ID NO: 198, SEQ ID NO: 208, SEQ ID NO: 218, SEQ ID NO: 228, SEQ ID NO: 238, SEQ ID NO: 248, SEQ ID NO: 277, SEQ ID NO: 292, SEQ ID NO: 302, SEQ ID NO: 312, SEQ ID NO: 322, SEQ ID NO: 332, SEQ ID NO: 342, SEQ ID NO: 352, SEQ ID NO: 374, SEQ ID NO: 384, SEQ

ID NO: 394, SEQ ID NO: 399, SEQ ID NO: 409, or SEQ ID NO: 419, and CDR3 represented by the amino acid sequence set forth in SEQ ID NO: 39, SEQ ID NO: 49, SEQ ID NO: 59, SEQ ID NO: 69, SEQ ID NO: 79, SEQ ID NO: 89, SEQ ID NO: 99, SEQ ID NO: 109, SEQ ID NO: 119, SEQ ID NO: 129, SEQ ID NO: 134, SEQ ID NO: 144, SEQ ID NO: 159, SEQ ID NO: 169, SEQ ID NO: 199, SEQ ID NO: 209, SEQ ID NO: 219, SEQ ID NO: 229, SEQ ID NO: 239, SEQ ID NO: 249, SEQ ID NO: 278, SEQ ID NO: 293, SEQ ID NO: 303, SEQ ID NO: 313, SEQ ID NO: 323, SEQ ID NO: 333, SEQ ID NO: 343, SEQ ID NO: 353, SEQ ID NO: 357, SEQ ID NO: 375, SEQ ID NO: 385, SEQ ID NO: 395, SEQ ID NO: 400, SEQ ID NO: 410, SEQ ID NO: 420. The VL domain comprises CDR1 represented by the amino acid sequence set forth in SEQ ID NO: 41, SEQ ID NO: 51, SEQ ID NO: 61, SEQ ID NO: 71, SEQ ID NO: 81, SEQ ID NO: 91, SEQ ID NO: 101, SEQ ID NO: 111, SEQ ID NO: 121, SEQ ID NO: 136, SEQ ID NO: 146, SEQ ID NO: 152, SEQ ID NO: 161, SEQ ID NO: 171, SEQ ID NO: 177, SEQ ID NO: 182, SEQ ID NO: 187, SEQ ID NO: 192, SEQ ID NO: 201, SEQ ID NO: 211, SEQ ID NO: 221, SEQ ID NO: 231, SEQ ID NO: 241, SEQ ID NO: 251, SEQ ID NO: 280, SEQ ID NO: 286, SEQ ID NO: 295, SEQ ID NO: 305, SEQ ID NO: 315, SEQ ID NO: 325, SEQ ID NO: 335, SEQ ID NO: 345, SEQ ID NO: 354, SEQ ID NO: 377, SEQ ID NO: 387, SEQ ID NO: 402, SEQ ID NO: 412, or SEQ ID NO: 422, CDR2 represented by the amino acid sequence set forth in SEQ ID NO: 42, SEQ ID NO: 52, SEQ ID NO: 62, SEQ ID NO: 72, SEQ ID NO: 82, SEQ ID NO: 92, SEQ ID NO: 102, SEQ ID NO: 112, SEQ ID NO: 122, SEQ ID NO: 137, SEQ ID NO: 147, SEQ ID NO: 153, SEQ ID NO: 162, SEQ ID NO: 172, SEQ ID NO: 178, SEQ ID NO: 183, SEQ ID NO: 188, SEQ ID NO: 193, SEQ ID NO: 202, SEQ ID NO: 212, SEQ ID NO: 222, SEQ ID NO: 232, SEQ ID NO: 242, SEQ ID NO: 252, SEQ ID NO: 281, SEQ ID NO: 287, SEQ ID NO: 296, SEQ ID NO: 306, SEQ ID NO: 316, SEQ ID NO: 326, SEQ ID NO: 336, SEQ ID NO: 346, SEQ ID NO: 355, SEQ ID NO: 378, SEQ ID NO: 388, SEQ ID NO: 403, SEQ ID NO: 413, or SEQ ID NO: 423, and CDR3 represented by the amino acid sequence set forth in SEQ ID NO: 43, SEQ ID NO: 53, SEQ ID NO: 63, SEQ ID NO: 73, SEQ ID NO: 83, SEQ ID NO: 93, SEQ ID NO: 103, SEQ ID NO: 113, SEQ ID NO: 123, SEQ ID NO: 138, SEQ ID NO: 148, SEQ ID NO: 154, SEQ ID NO: 163, SEQ ID NO: 173, SEQ ID NO: 179, SEQ ID NO: 184, SEQ ID NO: 189, SEQ

ID NO: 194, SEQ ID NO: 203, SEQ ID NO: 213, SEQ ID NO: 223, SEQ ID NO: 233, SEQ ID NO: 243, SEQ ID NO: 253, SEQ ID NO: 282, SEQ ID NO: 288, SEQ ID NO: 297, SEQ ID NO: 307, SEQ ID NO: 317, SEQ ID NO: 327, SEQ ID NO: 337, SEQ ID NO: 347, SEQ ID NO: 356, SEQ ID NO: 379, SEQ ID NO: 389, SEQ ID NO: 404, SEQ ID NO: 414, or SEQ ID NO: 424.

[0106]

A chimeric antibody is produced by combining sequences derived from different animals and is, for example, an antibody consisting of the heavy and light chain variable domains of a mouse antibody and the heavy and light chain constant domains of a human antibody. The chimeric antibody can be produced by a known method, for example, by linking a DNA encoding an antibody V domain and a DNA encoding a human antibody C domain, incorporating it into an expression vector, and introducing the expression vector into a host.

[0107]

Examples of the polyclonal antibody include antibodies prepared by immunizing a human antibody-producing animal (e.g., mouse) with a CAPRIN-1 protein.

[0108]

The humanized antibody is an altered antibody also called reshaped human antibody. The humanized antibody is constructed by transplanting CDRs of an antibody derived from an immune animal into the complementarity determining region of a human antibody. A method by a general gene recombination technology is also known.

[0109]

Specifically, a DNA sequence designed for linking the CDRs of a mouse antibody and the framework regions (FRs; including FR1 to FR4) of a human antibody in the order: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, from the N-terminal side is synthesized by PCR from several oligonucleotides produced so as to have overlapping portions at the end regions. The resulting DNA is linked to a DNA encoding the constant domain of a human antibody and is incorporated into an expression vector, and the expression vector is introduced into a host to produce a humanized antibody (see EP Patent Application No. EP239400 and International

Publication No. WO96/02576). The FRs of a human antibody linked via CDRs are selected such that the complementarity determining region forms a satisfactory antigen binding site. As needed, an amino acid in the framework region in the variable domain of the antibody may be substituted such that the complementarity determining region of the reshaped human antibody forms an appropriate antigen binding site (Sato K. et al., Cancer Research, 1993, 53: 851-856). The framework region may be substituted by a framework region derived from various human antibodies (see International Publication No. WO99/51743).

[0110]

The resulting chimeric antibody or humanized antibody may be further subjected to, for example, substitution of an amino acid in the variable domain (e.g., FR) or constant domain by another amino acid.

[0111]

In the amino acid substitution, for example, less than 15, less than 10, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less of amino acids, preferably one to five amino acids, and more preferably one or two amino acids are substituted. The substituted antibody should be functionally equivalent to the non-substituted antibody. The substitution is desirably conservative amino acid substitution, which is substitution between amino acids having similar properties such as charge, side chain, polarity, and aromaticity. The amino acids having similar properties can be classified into, for example, basic amino acids (arginine, lysine, and histidine), acidic amino acids (aspartic acid and glutamic acid), non-charged polar amino acids (glycine, asparagine, glutamine, serine, threonine, cysteine, and tyrosine), non-polar amino acids (leucine, isoleucine, alanine, valine, proline, phenylalanine, tryptophan, and methionine), branched chain amino acids (leucine, valine, and isoleucine), or aromatic amino acids (phenylalanine, tyrosine, tryptophan, and histidine).

[0112]

Examples of modified antibodies include antibodies bound to various molecules such as polyethylene glycol (PEG). In the modified antibody used in the present invention, the antibody may be bound to any material. These modified antibodies can be prepared by

chemically modifying a prepared antibody. The method for the modification has been already established in this field.

[0113]

Here, the term "functionally equivalent" refers to that the objective antibody has biological or biochemical activity similar to that of an antibody used in the present invention, specifically, for example, that the objective antibody has a function of impairing tumor and does not substantially cause rejection reaction in application to a human being. Such activity is, for example, cell growth-inhibitory activity or avidity.

[0114]

The method well known to those skilled in the art for preparing a polypeptide functionally equivalent to a certain polypeptide is a method of introducing a variation into the polypeptide. For example, a person skilled in the art can prepare an antibody functionally equivalent to an antibody used in the present invention by introducing an appropriate variation into the antibody through, for example, site-directed mutagenesis (Hashimoto-Gotoh, T. et al., (1995), *Gene*, 152, 271-275; Zoller, M.J., and Smith, M., (1983), *Methods Enzymol.*, 100, 468-500; Kramer, W. et al., (1984), *Nucleic Acids Res.*, 12, 9441-9456; Kramer, W. and Fritz, H.J., (1987), *Methods Enzymol.*, 154, 350-367; Kunkel, T.A., (1985), *Proc. Natl. Acad. Sci. USA.*, 82, 488-492; Kunkel, (1988), *Methods Enzymol.*, 85, 2763-2766).

[0115]

An antibody recognizing the epitope of a CAPRIN-1 protein that is recognized by the anti-CAPRIN-1 antibody can be prepared by a method known to those skilled in the art. The antibody can be prepared by, for example, a method of producing an antibody by determining an epitope of the CAPRIN-1 protein recognized by an anti-CAPRIN-1 antibody through a usual method (e.g., epitope mapping) and using a polypeptide comprising the amino acid sequence of the epitope as the immunogen or a method of selecting an antibody having the same epitope as that of an anti-CAPRIN-1 antibody from antibodies having various epitopes produced by a usual method.

[0116]

The antibody used in the present invention preferably has an affinity constant K_a (k_{on}/k_{off}) of $10^7 M^{-1}$ or more, $10^8 M^{-1}$ or more, $5 \times 10^8 M^{-1}$ or more, $10^9 M^{-1}$ or more, $5 \times 10^9 M^{-1}$ or more, $10^{10} M^{-1}$ or more, $5 \times 10^{10} M^{-1}$ or more, $10^{11} M^{-1}$ or more, $5 \times 10^{11} M^{-1}$ or more, $10^{12} M^{-1}$ or more, or $10^{13} M^{-1}$ or more.

[0117]

The antibody used in the present invention can conjugate with an antitumor agent. The antibody and the antitumor agent can be bound to each other via a spacer having a reactive group, such as an amino group, a carboxyl group, a hydroxyl group, or a thiol group (for example, a succinic imidyl group, a formyl group, a 2-pyridyldithio group, a maleimidyl group, an alkoxycarbonyl group, or a hydroxy group).

[0118]

Examples of the antitumor agent include the following antitumor agents publicly known through documents or other items, i.e., paclitaxel, doxorubicin, daunorubicin, cyclophosphamide, methotrexate, 5-fluorouracil, thiotepa, busulfan, improsulfan, piposulfan, benzodopa, carboquone, meturedopa, uredopa, altretamine, triethylenemelamine, triethylenephosphoramidate, triethylenethiophosphoramidate, trimethylolomelamine, bullatacin, bullatacinone, camptothecin, bryostatin, callistatin, cryptophycin 1, cryptophycin 8, dolastatin, duocarmycin, eleutherobin, pancratistatin, sarcodictyin, spongistatin, chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard, carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine, calicheamicin, dynemicin, clodronate, esperamicin, aclacinomycin, actinomycin, authramycin, azaserine, bleomycin, cactinomycin, carabycin, carminomycin, carzinophilin, chromomycin, dactinomycin, detorbicin, 6-diazo-5-oxo-L-norleucine, adriamycin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozoxin, tubercidin, ubenimex, zinostatin, zorubicin, denopterin, pteropterin, trimetrexate, fludarabine, 6-mercaptopurine, thiamiprine, thioguanine, ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifuridine, enocitabine, floxuridine, androgens such

as calusterone, drostanolone propionate, epitiostanol, mepitiothane, and testolactone, aminoglutethimide, mitotane, trilostane, frolinic acid, aceglatone, aldophosphamideglycoside, aminolaevulinic acid, eniluracil, amsacrine, bestrabucil, bisantrene, edatraxate, defofamine, demecolcine, diaziquone, elfornithine, elliptinium acetate, epothilone, etoglucid, lenthinan, lonidamine, maytansine, ansamitocine, mitoguazone, mitoxantrone, mopidanmol, nitraerine, pentostatin, phenamet, pirarubicin, losoxantrone, podophyllinic acid, 2-ethylhydrazide, procarbazine, razoxane, rhizoxin, schizophyllan, spirogermanium, tenuazonic acid, triaziquone, roridine A, anguidine, urethane, vindesine, dacarbazine, mannomustine, mitobronitol, mitolactol, pipobroman, gacytosine, docetaxel, chlorambucil, gemcitabine, 6-thioguanine, mercaptopurine, cisplatin, oxaliplatin, carboplatin, vinblastine, etoposide, ifosfamide, mitoxanthrone, vincristine, vinorelbine, novantrone, teniposide, edatrexate, daunomycin, aminopterin, xeloda, ibandronate, irinotecan, topoisomerase inhibitors, difluoromethylolnitrine (DMFO), retinoic acid, capecitabine, and pharmaceutically acceptable (known) salts or (known) derivatives thereof.

[0119]

Whether or not a conjugate of an antibody and an antitumor agent shows antitumor activity can be evaluated by, for example, if the antibody is an anti-CAPRIN-1 antibody derived from a mouse, evaluating the antitumor effect on human cancer cells *in vitro* through simultaneous reaction of a conjugate of a secondary antibody that binds to a mouse antibody and a drug. For example, evaluation can be performed using an anti-human IgG antibody conjugated with saporin (Hum-ZAP (Advanced Targeting Systems, Inc.)).

[0120]

In addition, combination administration of the antibody used in the present invention and an antitumor agent can provide a higher therapeutic effect. This method can be applied to a cancer patient expressing a CAPRIN-1 protein at either before or after surgery. In particular, after surgery, higher prevention of cancer recurrence and longer survival period can be obtained in a cancer expressing a CAPRIN-1 protein conventionally treated with an antitumor agent alone.

[0121]

Examples of the antitumor agent used in the combination administration include the above-mentioned antitumor agents publicly known through documents or other items, and pharmaceutically acceptable (known) salts or (known) derivatives thereof. Among these agents, in particular, preferably used are cyclophosphamide, paclitaxel, docetaxel, vinorelbine, etc.

[0122]

Alternatively, the antibody used in the present invention can be labeled with a radioisotope publicly known through documents or other items, such as ^{211}At , ^{131}I , ^{125}I , ^{90}Y , ^{186}Re , ^{188}Re , ^{153}Sm , ^{212}Bi , ^{32}P , ^{175}Lu , or ^{176}Lu . The isotope is desirably one effective for therapy or diagnosis of tumor.

[0123]

The antibody used in the present invention is an antibody having immunological reactivity with a CAPRIN-1 protein or specifically binding to a CAPRIN-1 protein and exhibiting cytotoxicity or tumor growth-inhibitory activity against gallbladder cancer. The antibody should have a structure that can almost or completely avoid rejection reaction in the objective animal to which the antibody is administered. Examples of such antibodies include, when the objective animal is a human being, human antibodies, humanized antibodies, chimeric antibodies (e.g., human-mouse chimeric antibodies), single-chain antibodies, and multispecific antibodies (e.g., diabodies and triabodies). Such an antibody is a recombinant antibody in which the variable domains of the heavy and light chains are derived from a human antibody, or in which the variable domains of the heavy and light chains consist of complementarity determining regions (CDR1, CDR2, and CDR3) derived from a non-human animal antibody and a framework region derived from a human antibody, or in which the variable domains of the heavy and light chains are derived from a non-human animal antibody and the constant domains of the heavy and light chains are derived from a human antibody. The former antibodies are preferred.

[0124]

These recombinant antibodies can be produced as follows. A DNA encoding an anti-human CAPRIN-1 monoclonal antibody (e.g., human monoclonal antibody, mouse

monoclonal antibody, rat monoclonal antibody, rabbit monoclonal antibody, or chicken monoclonal antibody) is cloned from antibody-producing cells such as hybridomas; a DNA encoding the light chain variable domain and the heavy chain variable domain of the antibody is produced using the resulting DNA as a template by, for example, RT-PCR; and the sequence of each variable domain of the light and heavy chains or the sequence of each of CDR1, CDR2, and CDR3 is determined based on the Kabat EU numbering system (Kabat, et al., Sequences of Proteins of Immunological Interest, 5th Ed., Public Health Service, National Institute of Health, Bethesda, Md. (1991)).

[0125]

Furthermore, DNAs encoding the variable domains or DNAs encoding the CDRs are produced by gene recombination technology (Sambrook, et al., Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory Press, (1989)) or with a DNA synthesizer. Here, the human monoclonal antibody-producing hybridoma can be produced by immunizing a human antibody-producing animal (e.g., mouse) with a human CAPRIN-1 protein and then fusing the spleen cells excised from the immune animal with myeloma cells. Separately, as necessary, a DNA encoding the variable domain and the constant domain of a light or heavy chain derived from a human antibody is produced by gene recombination technology or with a DNA synthesizer.

[0126]

In the case of a humanized antibody, the CDR-coding sequences in the DNAs encoding the variable domains of the light chain or the heavy chain derived from a human antibody are substituted with the corresponding CDR-coding sequences of an antibody derived from an animal (e.g., mouse, rat, or chicken) other than human beings to produce DNAs. The resulting DNAs are each linked to DNAs encoding the constant domains of the light chain or the heavy chain derived from a human antibody to produce a DNA encoding a humanized antibody.

[0127]

In the case of a chimeric antibody, DNAs encoding the variable domains of the light chain or the heavy chain of an antibody derived from an animal (e.g., mouse, rat, or chicken)

other than human beings are each linked to DNAs encoding the constant domains of the light chain or the heavy chain derived from a human antibody to produce a DNA encoding a chimeric antibody.

[0128]

In the case of a single-chain antibody, the antibody is composed of a heavy chain variable domain and a light chain variable domain linearly linked to each other via a linker, and a DNA encoding the single-chain antibody can be produced by binding a DNA encoding the heavy chain variable domain, a DNA encoding the linker, and a DNA encoding the light chain variable domain. Here, the heavy chain variable domain and the light chain variable domain are both derived from a human antibody or derived from a human antibody in which only the CDR is substituted with the CDR of an antibody derived from an animal (e.g., mouse, rat, chicken) other than human beings. The linker is composed of 12 to 19 amino acids, and examples thereof include (G₄S)₃ of 15 amino acids (G. -B. Kim, et al., Protein Engineering Design and Selection, 2007, 20(9): 425-432).

[0129]

In the case of a bispecific antibody (diabody), the antibody can specifically bind to two different epitopes, and a DNA encoding the bispecific antibody can be produced by, for example, binding a DNA encoding the heavy chain variable domain A, a DNA encoding the light chain variable domain B, a DNA encoding the heavy chain variable domain B, and a DNA encoding the light chain variable domain A in this order (provided that the DNA encoding the light chain variable domain B and the DNA encoding the heavy chain variable domain B are bound to each other via a DNA encoding a linker as described above). Here, the heavy chain variable domain and the light chain variable domain are both derived from a human antibody or derived from a human antibody in which only the CDR is substituted with the CDR of an antibody derived from an animal (e.g., mouse, rat, chicken) other than human beings.

[0130]

A recombinant antibody can be produced by incorporating the thus-produced recombinant DNA into one or more appropriate vectors and introducing the vector or vectors

into host cells (e.g., mammal cells, yeast cells, or insect cells) to (co)express the DNA (P.J. Delves., ANTIBODY PRODUCTION ESSENTIAL TECHNIQUES, 1997; WILEY, P. Shepherd, and C. Dean., Monoclonal Antibodies, 2000, OXFORD UNIVERSITY PRESS; J.W. Goding., Monoclonal Antibodies: principles and practice, 1993, ACADEMIC PRESS).

[0131]

Examples of the antibodies of the present invention produced by the above-described methods include the following antibodies (a) to (ao).

[0132]

(a) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 37, 38, and 39, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 41, 42, and 43, respectively, described in WO2011/096528, (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 40 and a light chain variable domain set forth in SEQ ID NO: 44).

[0133]

Antibodies (b) each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 47, 48, and 49, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 51, 52, and 53, respectively, described in WO2011/096519, (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 50 and a light chain variable domain set forth in SEQ ID NO: 54).

[0134]

(c) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 57, 58, and 59, respectively and a light chain variable

domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 61, 62, and 63, respectively, described in WO2011/096517, (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 60 and a light chain variable domain set forth in SEQ ID NO: 64).

[0135]

(d) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 67, 68, and 69, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 71, 72, and 73, respectively, described in WO2011/096528, (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 70 and a light chain variable domain set forth in SEQ ID NO: 74).

[0136]

(e) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 77, 78, and 79, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 81, 82, and 83, respectively, described in WO2011/096528, (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 80 and a light chain variable domain set forth in SEQ ID NO: 84).

[0137]

(f) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 87, 88, and 89, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 91, 92, and 93, respectively,

described in WO2011/096528, (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 90 and a light chain variable domain set forth in SEQ ID NO: 94).

[0138]

(g) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 97, 98, and 99, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 101, 102, and 103, respectively, described in WO2011/096528, (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 100 and a light chain variable domain set forth in SEQ ID NO: 104).

[0139]

(h) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 107, 108, and 109, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 111, 112, and 113, respectively, described in WO2011/096528, (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 110 and a light chain variable domain set forth in SEQ ID NO: 114).

[0140]

(i) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 117, 118, and 119, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 121, 122, and 123, respectively, described in WO2011/096533, (for example, an antibody comprising a heavy chain variable

domain set forth in SEQ ID NO: 120 and a light chain variable domain set forth in SEQ ID NO: 124).

[0141]

(j) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 127, 128, and 129, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 121, 122, and 123, respectively, described in WO2011/096533, (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 130 and a light chain variable domain set forth in SEQ ID NO: 124).

[0142]

(k) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 132, 133, and 134, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 136, 137, and 138, respectively, described in WO2011/096533, (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 135 and a light chain variable domain set forth in SEQ ID NO: 139).

[0143]

(l) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 142, 143, and 144, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 146, 147, and 148, respectively, described in WO2011/096534, (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 145 and a light chain variable domain set forth in SEQ ID NO: 149).

[0144]

(m) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 142, 143, and 144, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 152, 153, and 154, respectively, described in WO2011/096534, (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 145 and a light chain variable domain set forth in SEQ ID NO: 155).

[0145]

(n) Antibodies each comprising a heavy chain variable domain comprising CDRs of SEQ ID NOs: 157, 158, and 159 and a light chain variable domain comprising CDRs of SEQ ID NOs: 161, 162, and 163, described in WO2011/096534, (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 160 and a light chain variable domain set forth in SEQ ID NO: 164).

[0146]

(o) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 167, 168, and 169, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 171, 172, and 173, respectively, described in WO2011/096534, (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 170 and a light chain variable domain set forth in SEQ ID NO: 174).

[0147]

(p) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 167, 168, and 169, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3)

consisting of amino acid sequences set forth in SEQ ID NOs: 177, 178, and 179, respectively, described in WO2010/016526, (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 170 and a light chain variable domain set forth in SEQ ID NO: 180).

[0148]

(q) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 167, 168, and 169, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 182, 183, and 184, respectively, described in WO2010/016526, (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 170 and a light chain variable domain set forth in SEQ ID NO: 185).

[0149]

(r) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 167, 168, and 169, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 187, 188, and 189, respectively, described in WO2010/016526, (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 170 and a light chain variable domain set forth in SEQ ID NO: 190).

[0150]

(s) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 167, 168, and 169, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 192, 193, and 194, respectively, described in WO2010/016526, (for example, an antibody comprising a heavy chain variable

domain set forth in SEQ ID NO: 170 and a light chain variable domain set forth in SEQ ID NO: 195).

[0151]

(t) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 197, 198, and 199, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 201, 202, and 203, respectively, described in WO2010/016526, (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 200 and a light chain variable domain set forth in SEQ ID NO: 204).

[0152]

(u) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 207, 208, and 209, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 211, 212, and 213, respectively, described in WO2010/016526, (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 210 and a light chain variable domain set forth in SEQ ID NO: 214).

[0153]

(v) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 217, 218, and 219, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 221, 222, and 223, respectively, described in WO2010/016526, (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 220 and a light chain variable domain set forth in SEQ ID NO: 224).

[0154]

(w) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 227, 228, and 229, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 231, 232, and 233, respectively, described in WO2010/016526, (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 230 and a light chain variable domain set forth in SEQ ID NO: 234).

[0155]

(x) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 237, 238, and 239, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 241, 242, and 243, respectively, described in WO2010/016526, (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 240 and a light chain variable domain set forth in SEQ ID NO: 244).

[0156]

(y) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 247, 248, and 249, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 251, 252, and 253, respectively, described in WO2010/016526, (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 250 and a light chain variable domain set forth in SEQ ID NO: 254).

[0157]

(z) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 276, 277, and 278, respectively and complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 280, 281, and 282, respectively, described in WO2013/018894 (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 279 and a light chain variable domain set forth in SEQ ID NO: 283).

[0158]

(aa) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 276, 277, and 278, respectively and complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 286, 287, and 288, respectively, described in WO2013/018894 (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 279 and a light chain variable domain set forth in SEQ ID NO: 289).

[0159]

(ab) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 291, 292, and 293, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 295, 296, and 297, respectively, described in WO2013/018894, (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 294 and a light chain variable domain set forth in SEQ ID NO: 298).

[0160]

(ac) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 301, 302, and 303, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3)

consisting of amino acid sequences set forth in SEQ ID NOs: 305, 306, and 307, respectively, described in WO2013/018892, (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 304 and a light chain variable domain set forth in SEQ ID NO: 308).

[0161]

(ad) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 311, 312, and 313, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 315, 316, and 317, respectively, described in WO2013/018891, (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 314 and a light chain variable domain set forth in SEQ ID NO: 318).

[0162]

(ae) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 321, 322, and 323, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 325, 326, and 327, respectively, described in WO2013/018889, (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 324 and a light chain variable domain set forth in SEQ ID NO: 328).

[0163]

(af) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 331, 332, and 333, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 335, 336, and 337, respectively, described in WO2013/018883, (for example, an antibody comprising a heavy chain variable

domain set forth in SEQ ID NO: 334 and a light chain variable domain set forth in SEQ ID NO: 338).

[0164]

(ag) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 341, 342, and 343, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 345, 346, and 347, respectively, or fragments of the antibodies (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 344 and a light chain variable domain set forth in SEQ ID NO: 348).

[0165]

(ah) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 351, 352, and 353, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 354, 355, and 356, respectively, or fragments of the antibodies (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 359 and a light chain variable domain set forth in SEQ ID NO: 361, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 368 and a light chain variable domain set forth in SEQ ID NO: 370, and an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 372 and a light chain variable domain set forth in SEQ ID NO: 370).

[0166]

(ai) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 351, 352, and 357, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 354, 355, and 356, respectively,

or fragments of the antibodies (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 363 and a light chain variable domain set forth in SEQ ID NO: 365).

[0167]

(aj) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 373, 374, and 375, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 377, 378, and 379, respectively, or fragments of the antibodies (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 376 and a light chain variable domain set forth in SEQ ID NO: 380).

[0168]

(ak) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 383, 384, and 385, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 387, 388, and 389, respectively, or fragments of the antibodies (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 386 and a light chain variable domain set forth in SEQ ID NO: 390).

[0169]

(al) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 393, 394, and 395, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 387, 388, and 389, respectively, or fragments of the antibodies (for example, an antibody comprising a heavy chain variable

domain set forth in SEQ ID NO: 396 and a light chain variable domain set forth in SEQ ID NO: 390).

[0170]

(am) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 398, 399, and 400, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 402, 403, and 404, respectively, or fragments of the antibodies (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 401 and a light chain variable domain set forth in SEQ ID NO: 405).

[0171]

(an) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) set forth in SEQ ID NOs: 408, 409, and 410, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) set forth in SEQ ID NOs: 412, 413, and 414, respectively, or fragments of the antibodies (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 411 and a light chain variable domain set forth in SEQ ID NO: 415).

[0172]

(ao) Antibodies each comprising a heavy chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) set forth in SEQ ID NOs: 418, 419, and 420, respectively and a light chain variable domain comprising complementarity determining regions (CDR1, CDR2, and CDR3) set forth in SEQ ID NOs: 422, 423, and 424, respectively, or fragments of the antibodies (for example, an antibody comprising a heavy chain variable domain set forth in SEQ ID NO: 421 and a light chain variable domain set forth in SEQ ID NO: 425).

[0173]

Here, the amino acid sequences set forth in SEQ ID NOs: 67, 68, and 69 are CDR1, CDR2, and CDR3, respectively of the heavy chain variable domain of a mouse antibody, similarly, sets of amino acid sequences set forth in SEQ ID NOs: 77, 78, and 79, SEQ ID NO: 87, 88, and 89, SEQ ID NO: 97, 98, and 99, SEQ ID NO: 107, 108, and 109, SEQ ID NO: 117, 118, and 119, SEQ ID NO: 127, 128, and 129, SEQ ID NO: 132, 133, and 134, SEQ ID NO: 142, 143, and 144, SEQ ID NO: 157, 158, and 159, SEQ ID NO: 167, 168, and 169, SEQ ID NO: 197, 198, and 199, SEQ ID NO: 207, 208, and 209, SEQ ID NO: 217, 218, and 219, SEQ ID NO: 227, 228, and 229, SEQ ID NO: 237, 238, and 239, SEQ ID NOs: 247, 248, and 249, SEQ ID NOs: 276, 277, and 278; 291, 292, and 293; 301, 302, and 303; 311, 312, and 313; 321, 322, and 323; 331, 332, and 333; 341, 342, and 343; 373, 374, and 375; 383, 384, and 385; 393, 394, and 395; 398, 399, and 400; 408, 409, and 410; and 418, 419, and 420 are each a set of CDR1, CDR2, and CDR3 of the heavy chain variable domain of a mouse antibody. Similarly, sets of amino acid sequences set forth in SEQ ID NO: 71, 72, and 73, SEQ ID NO: 81, 82, and 83, SEQ ID NO: 91, 92, and 93, SEQ ID NO: 101, 102, and 103, SEQ ID NO: 111, 112, and 113, SEQ ID NO: 121, 122, and 123, SEQ ID NO: 136, 137, and 138, SEQ ID NO: 146, 147, and 148, SEQ ID NO: 152, 153, and 154, SEQ ID NO: 161, 162, and 163, SEQ ID NO: 171, 172, and 173, SEQ ID NO: 177, 178, and 179, SEQ ID NO: 182, 183, and 184, SEQ ID NO: 187, 188, and 189, SEQ ID NO: 192, 193, and 194, SEQ ID NO: 201, 202, and 203, SEQ ID NO: 211, 212, and 213, SEQ ID NO: 221, 222, and 223, SEQ ID NO: 231, 232, and 233, SEQ ID NO: 241, 242, and 243, SEQ ID NO: 251, 252, and 253, SEQ ID NO: 280, 281, and 282, SEQ ID NO: 286, 287, and 288, SEQ ID NO: 295, 296, and 297, SEQ ID NO: 305, 306, and 307, SEQ ID NO: 315, 316, and 317, SEQ ID NO: 325, 326, and 327, SEQ ID NO: 335, 336, and 337, SEQ ID NO: 345, 346, and 347, SEQ ID NO: 377, 378, and 379, SEQ ID NO: 387, 388, and 389, SEQ ID NO: 402, 403, and 404, SEQ ID NO: 412, 413, and 414, SEQ ID NO: 422, 423, and 424 are each a set of CDR1, CDR2, and CDR3 of the light chain variable domain of a mouse antibody.

[0174]

Similarly, the amino acid sequences set forth in SEQ ID NOs: 37, 38, and 39, SEQ ID NOs: 47, 48, and 49, or SEQ ID NOs: 57, 58, and 59 are each CDR1, CDR2, and CDR3,

respectively of the heavy chain variable domain of a chicken antibody; and the amino acid sequences set forth in SEQ ID NOs: 41, 42, and 43, SEQ ID NOs: 51, 52, and 53, or SEQ ID NOs: 61, 62, and 63 are each CDR1, CDR2, and CDR3, respectively of the light chain variable domain of a chicken antibody.

[0175]

Similarly, the amino acid sequences set forth in SEQ ID NOs: 351, 352, and 353 are CDR1, CDR2, and CDR3, respectively of the heavy chain variable domain of a rabbit antibody; and the amino acid sequences set forth in SEQ ID NOs: 354, 355, and 356 are CDR1, CDR2, and CDR3, respectively of the light chain variable domain of a rabbit antibody.

[0176]

Examples of the humanized antibody, chimeric antibody, single-chain antibody, and multispecific antibody used in the present invention include the following antibodies (those exemplified as antibodies (ah)).

[0177]

(i) Antibodies each comprising a heavy chain variable domain comprising the amino acid sequences set forth in SEQ ID NOs: 351, 352, and 353 and the amino acid sequence of the framework region derived from a human antibody; and a light chain variable domain comprising the amino acid sequences set forth in SEQ ID NOs: 354, 355, and 356 and the amino acid sequence of the framework region derived from a human antibody.

[0178]

(ii) Antibodies each comprising a heavy chain variable domain comprising the amino acid sequences set forth in SEQ ID NOs: 351, 352, and 353 and the amino acid sequence of the framework region derived from a human antibody; a heavy chain constant domain comprising the amino acid sequence derived from a human antibody; a light chain variable domain comprising the amino acid sequences set forth in SEQ ID NOs: 354, 355, and 356 and the amino acid sequence of the framework region derived from a human antibody; and a light chain constant domain comprising the amino acid sequence derived from a human antibody.

[0179]

(iii) Antibodies each comprising a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 368, a heavy chain constant domain comprising the amino acid sequence derived from a human antibody, a light chain variable domain comprising the amino acid sequences set forth in SEQ ID NO: 370, and a light chain constant domain comprising the amino acid sequence derived from a human antibody.

[0180]

The sequences of the constant domains and the variable domains of human antibody heavy and light chains are available from, for example, NCBI (e.g., GenBank or UniGene, USA). For example, the sequence of the human IgG₁ heavy chain constant domain can be referred to as Registration No. J00228, the sequence of the human IgG₂ heavy chain constant domain can be referred to as Registration No. J00230, the sequence of the human IgG₃ heavy chain constant domain can be referred to as Registration No. X03604, the sequence of the human IgG₄ heavy chain constant domain can be referred to as Registration No. K01316, the sequence of the human light chain κ constant domain can be referred to as, for example, Registration No. V00557, X64135, or X64133, and the sequence of the human light chain λ constant domain can be referred to as, for example, Registration No. X64132 or X64134.

[0181]

Examples of the humanized antibodies exemplified as the antibodies (ah) include the antibodies (ai), antibodies comprising the heavy chain variable domain set forth in SEQ ID NO: 368 and the light chain variable domain set forth in SEQ ID NO: 370, and antibodies comprising the heavy chain variable domain set forth in SEQ ID NO: 372 and the light chain variable domain set forth in SEQ ID NO: 370.

[0182]

These antibodies preferably have cytotoxicity and can thereby show antitumor effects.

[0183]

It is obvious that the specific sequences of the variable domains and CDRs of heavy chains and light chains of the above-mentioned antibodies are intended to merely show examples and are not limited to specific sequences. A hybridoma producing another human antibody or a non-human animal antibody (e.g., mouse antibody) against a human CAPRIN-1

protein is produced, and the monoclonal antibody produced by the hybridoma is collected and is determined whether or not the antibody is a target antibody using the immunological affinity to the human CAPRIN-1 protein and cytotoxicity as indices. After the identification of the hybridoma producing a target monoclonal antibody, the DNA encoding the variable domains of the heavy and light chains of the target antibody is produced from the hybridoma as described above, and the DNA is sequenced. The DNA is used for producing another antibody.

[0184]

Furthermore, the antibody used in the present invention may have substitution, deletion, or addition of one to several (preferably one or two) amino acids of each of the antibodies (i) to (iv), in particular, in the sequence of the framework region and/or the sequence constant domain, as long as the specificity, i.e., the specific recognition of the CAPRIN-1 protein, is maintained. Herein, the term "several" refers to two to five, preferably two or three.

[0185]

The antitumor effect by the anti-CAPRIN-1 antibody used in the present invention on gallbladder cancer cells expressing CAPRIN-1 protein is believed to be caused by the following mechanism.

[0186]

The mechanism involves the effector-cell antibody-dependent cellular cytotoxicity (ADCC) of CAPRIN-1-protein-expressing cells and complement-dependent cellular cytotoxicity (CDC) of CAPRIN-1-protein-expressing cells.

[0187]

Accordingly, the activity of the anti-CAPRIN-1 antibody used in the present invention can be evaluated by measuring the ADCC activity or CDC activity on gallbladder cancer cells expressing the CAPRIN-1 protein *in vitro*, as specifically shown in the following examples.

[0188]

The anti-CAPRIN-1 antibody used in the present invention binds to CAPRIN-1 protein on gallbladder cancer cells and shows antitumor action by the above-mentioned activity, and it is therefore believed that the antibody is useful for therapy or prevention of gallbladder cancer.

That is, the present invention provides a pharmaceutical composition, of which the active ingredient is the anti-CAPRIN-1 antibody, for treating and/or preventing gallbladder cancer. In the case of administering the anti-CAPRIN-1 antibody to a human being (antibody therapy), the antibody is preferably human antibody or a humanized antibody for reducing immunogenicity.

[0189]

A higher binding affinity of the anti-CAPRIN-1 antibody to the CAPRIN-1 protein on gallbladder cancer cell surface provides stronger antitumor activity by the anti-CAPRIN-1 antibody. Accordingly, an anti-CAPRIN-1 antibody having high binding affinity to a CAPRIN-1 protein is expected to show a stronger antitumor effect and can be applied to a pharmaceutical composition for treating and/or preventing gallbladder cancer. As the high binding affinity, as described above, the binding constant (affinity constant) K_a (k_{on}/k_{off}) is preferably 10^7 M^{-1} or more, 10^8 M^{-1} or more, $5 \times 10^8 \text{ M}^{-1}$ or more, 10^9 M^{-1} or more, $5 \times 10^9 \text{ M}^{-1}$ or more, 10^{10} M^{-1} or more, $5 \times 10^{10} \text{ M}^{-1}$ or more, 10^{11} M^{-1} or more, $5 \times 10^{11} \text{ M}^{-1}$ or more, 10^{12} M^{-1} or more, or 10^{13} M^{-1} or more.

[0190]

<Binding to antigen-expressing cell>

The ability of an antibody to bind to a CAPRIN-1 protein can be specified through binding assay by, for example, ELISA, Western blotting, immunofluorescence, or flow cytometry, as described in Examples.

[0191]

<Immunohistochemical staining>

The antibody recognizing a CAPRIN-1 protein can be tested for reactivity with the CAPRIN-1 protein by an immunohistochemical method well known to those skilled in the art using paraformaldehyde or acetone fixed frozen sections or paraformaldehyde fixed paraffin-embedded tissue sections from tissue derived from a patient during surgery or tissue derived from an animal carrying heterotransplant inoculated with a cell line expressing a CAPRIN-1 protein naturally or after transfection.

[0192]

An antibody reactive with a CAPRIN-1 protein can be stained by various methods for immunohistochemical staining. For example, the antibody can be visualized by reacting a horseradish peroxidase-conjugated goat anti-mouse or anti-rabbit antibody.

[0193]

<Pharmaceutical composition>

The target of the pharmaceutical composition for treating and/or preventing gallbladder cancer of the present invention may be any gallbladder cancer (cells) expressing a CAPRIN-1 gene.

[0194]

The terms "tumor" and "cancer" used throughout the present specification refer to a malignant neoplasm and are used interchangeably.

[0195]

The gallbladder cancer as a target in the present invention expresses a gene encoding an amino acid sequence set forth in any of even sequence numbers from SEQ ID NOs: 2 to 30, an amino acid sequence having a sequence identity of 80% or more, preferably 90% or more, more preferably 95% or more, and most preferably 97% or more to the amino acid sequence, or a partial sequence comprising at least seven, preferably at least eight consecutive amino acid residues of any of these amino acid sequences. Examples of the gallbladder cancer include, but not limited to, carcinoma arising in gallbladder (primary cancer) and metastatic cancer.

[0196]

The objective animal is mammals such as a primate, a pet animal, a domestic animal, and an animal for competitive use, and is preferably a human being, a dog, or a cat.

[0197]

The pharmaceutical composition of the antibody used in the present invention can be readily formulated by a method known to those skilled in the art. The pharmaceutical composition can be used, for example, parenterally in a form of an aseptic solution with water or another pharmaceutically acceptable liquid or an injection of a suspension preparation. For example, it is proposed to formulate by appropriately combining the pharmaceutical

composition with a pharmacologically acceptable carrier or medium, specifically, sterilized water, physiological saline, vegetable oil, an emulsifier, a suspending agent, a surfactant, a stabilizer, a flavoring agent, an excipient, a vehicle, an antiseptic, or a binder, and mixing them at a unit dosage form desired in enforcement of generally recognized drug manufacture. The amount of the active ingredient in such a drug is controlled so as to provide an appropriate dose within an indicated range.

[0198]

The aseptic composition for injection can be prescribed in accordance with the enforcement of usual pharmaceutical preparation using a vehicle such as distilled water for injection.

[0199]

Examples of aqueous solutions for injection include physiological saline and isotonic solutions containing glucose or other adjuvants, such as D-sorbitol, D-mannose, D-mannitol, or sodium chloride. The aqueous solution may be used together with an appropriate solubilizer, for example, alcohol, specifically, ethanol or polyalcohol; propylene glycol, polyethylene glycol, or a nonionic detergent; or polysorbate 80(TM) or HCO-60.

[0200]

Examples of oily liquids include sesame oil and soybean oil, and the oily liquid may be used together with benzyl benzoate or benzyl alcohol as a solubilizer. In addition, a buffer such as a phosphate buffer or a sodium acetate buffer, a soothing agent such as procaine hydrochloride, a stabilizer such as benzyl alcohol or phenol, or an antioxidant may be blended. The prepared injection is usually packed in an appropriate ampoule.

[0201]

The administration is oral or parenteral and is preferably parenteral, and examples thereof include injection, transnasal, pulmonary, and transdermal dosage forms. In the injection dosage form, for example, systemic or local administration can be performed by intravenous, intramuscular, intraperitoneal, or subcutaneous injection.

[0202]

The administration method can be appropriately selected based on the age, weight, sex, symptoms, etc. of a patient. The dose of the pharmaceutical composition containing an antibody or a polynucleotide encoding the antibody can be selected, for example, within a range of 0.0001 to 1000 mg/kg body weight per once or, for example, within a range of 0.001 to 100000 mg/body per patient. These numerical values are not necessarily restrictive. The dose and administration method vary depending on the weight, age, sex, symptoms, etc. of a patient, but can be appropriately selected by those skilled in the art.

[0203]

Gallbladder cancer can be treated and/or prevented by administering the pharmaceutical composition of the present invention to a subject.

[0204]

The present invention further encompasses a method of treating and/or preventing gallbladder cancer comprising administering the pharmaceutical composition of the present invention together with an antitumor agent as exemplified above or a pharmaceutical composition containing such an antitumor agent to a subject. The antibody or a fragment thereof of the present invention and the antitumor agent can be simultaneously or separately administered to a subject. In the case of separate administration, either pharmaceutical composition may be administered earlier or later, and the administration interval, doses, administration routes, and the frequency of administration thereof can be appropriately selected by a medical specialist. Examples of the other medicinal dosage form to be simultaneously administered also include pharmaceutical compositions prepared by mixing the antibody or a fragment thereof of the present invention and an antitumor agent in a pharmacologically acceptable carrier (or medium) and formulating the mixture. The description for the prescription, formulation, administration route, dose, cancer, etc. relating to the pharmaceutical composition containing the antibody of the present invention and the dosage form can be applied to any of the pharmaceutical compositions containing antitumor agents and the dosage forms. Therefore, the present invention also provides a combination pharmaceutical agent (also referred to as "pharmaceutical kit") for treatment and/or prevention

of gallbladder cancer, comprising the pharmaceutical composition of the present invention and a pharmaceutical composition containing an antitumor agent as exemplified above.

[0205]

The present invention also provides a pharmaceutical composition for treating and/or preventing gallbladder cancer, comprising the antibody or a fragment thereof of the present invention and an antitumor agent together with a pharmacologically acceptable carrier.

[0206]

Alternatively, the antitumor agent may be conjugated with the antibody or a fragment thereof of the present invention. The conjugate can be mixed with a pharmacologically acceptable carrier (or medium) and formulated into a pharmaceutical composition as in above.

Examples

[0207]

The present invention will now be more specifically described based on examples, but the scope of the present invention is not limited by these examples.

[0208]

[Example 1] Identification of cancer antigen protein by SEREX method

(1) Preparation of cDNA library

Total RNA was extracted from the testis tissue of a healthy dog by an acid guanidium-phenol-chloroform method, and poly(A) RNA was purified using Oligotex-dT30 mRNA purification Kit (manufactured by Takara Shuzo Co., Ltd.) in accordance with the protocol attached to the kit.

[0209]

A cDNA phage library derived from dog testis was synthesized using the obtained mRNA (5 µg). The cDNA phage library was prepared using cDNA Synthesis Kit, ZAP-cDNA Synthesis Kit, and ZAP-cDNA Gigapack III Gold Cloning Kit (manufactured by Stratagene Corporation) in accordance with the protocols attached to the kits. The size of the produced cDNA phage library was 7.73×10^5 pfu/mL.

[0210]

(2) Screening of cDNA library with serum

The cDNA phage library derived from dog testis was used for immunoscreening. Specifically, host *E. coli* (XL1-Blue MRF⁺) cells were infected with the library such that 2210 clones were formed on a Φ 90×15 mm NZY agarose plate. Then, the host *E. coli* cells were cultured at 42°C for 3 to 4 hours to produce plaques. The plate was covered with a nitrocellulose membrane (Hybond C Extra: manufactured by GE Healthcare Bio-Sciences), impregnated with isopropyl- β -D-thiogalactoside (IPTG), at 37°C for 4 hours to introduce and express proteins, and the proteins were transferred to the membrane. Subsequently, the membrane was collected and immersed in TBS (10 mM Tris-HCl, 150 mM NaCl, pH 7.5) containing 0.5% skimmed milk and was shaken overnight at 4°C to prevent non-specific reaction. This filter was reacted with 500-fold diluted ill dog serum at room temperature for 2 to 3 hours.

[0211]

The ill dog serum collected from breast cancer dogs was used. The serum was stored at -80°C and was pretreated immediately before the use. The pretreatment of the serum was performed as follows: host *E. coli* (XL1-Blue MRF⁺) cells were infected with λ ZAP Express phage into which no foreign genes were inserted and were cultured on NZY plate medium at 37°C overnight. Subsequently, a 0.2 M NaHCO₃ buffer, pH 8.3, containing 0.5 M NaCl was added to the plate, followed by being left to stand at 4°C for 15 hours. Then, the supernatant was collected as an *E. coli*/phage extract. Subsequently, the collected *E. coli*/phage extract was allowed to pass through NHS-column (manufactured by GE Healthcare Bio-Sciences) to immobilize the proteins derived from the *E. coli* and the phage. Serum from the ill dogs was allowed to pass through the protein-immobilized column for removing, from the serum, antibodies adsorbing to *E. coli* or the phage. The serum fraction passed through the column was 500-fold diluted with TBS containing 0.5% skimmed milk to provide an immunoscreening material.

[0212]

The membrane blotted with the thus-treated serum and the fusion protein was washed with TBS-T (0.05% Tween 20/TBS) four times and was then subjected to reaction with a

secondary antibody, goat anti-dog IgG diluted by 5000-fold with TBS containing 0.5% skimmed milk (Goat anti Dog IgG-h+I HRP conjugated: manufactured by BETHYL Laboratories, Inc.), at room temperature for 1 hour. Detection by enzymatic color reaction using a NBT/BCIP reaction solution (manufactured by Roche Diagnostics K.K.) was performed, and colonies corresponding to the color reaction positive positions on the Φ 90×15 mm NZY agarose plate were collected and were each dissolved in 500 μ L of an SM buffer (100 mM NaCl, 10 mM MgClSO₄, 50 mM Tris-HCl, 0.01% gelatin, pH 7.5). Second, third, or more screenings were performed by the same procedure as above until the color reaction positive colony was unified. As a result, five positive clones were isolated by screening of 30940 phage clones that react with IgG in serum.

[0213]

(3) Homology search of isolated antigen gene

In order to use the five positive clones isolated by the above-described method for nucleotide sequence analysis, the phage vector was converted to a plasmid vector. Specifically, 200 μ L of a solution of host E. coli (XL1-Blue MRF') adjusted to an absorbance OD600 of 1.0 was mixed with 250 μ L of purified phage solution and 1 μ L of ExAssist helper phage (manufactured by Stratagene Corporation). After reaction at 37°C for 15 minutes, 3 mL of an LB medium was added to the mixture, followed by culturing at 37°C for 2.5 to 3 hours. Immediately after the culturing, the culture medium was warmed in a water bath of 70°C for 20 minutes, followed by centrifugation at 4°C, 1000×g, for 15 minutes. The supernatant was collected as a phagemid solution. Subsequently, 200 μ L of a solution of phagemid host E. coli (SOLR) adjusted to an absorbance OD600 of 1.0 was mixed with 10 μ L of a purified phage solution. After reaction at 37°C for 15 minutes, 50 μ L of the reaction mixture was seeded on an LB agar medium containing ampicillin (final concentration: 50 μ g/mL), followed by culturing at 37°C overnight. Single colonies of transformed SOLR were collected and were cultured in an LB medium containing ampicillin (final concentration: 50 μ g/mL) at 37°C, and a plasmid DNA having an intended insertion was purified with QIAGEN plasmid Miniprep Kit (manufactured by Qiagen).

[0214]

The purified plasmid was subjected to analysis of the full-length insert sequence by a primer walking method using the T3 primer set forth in SEQ ID NO: 31 and the T7 primer set forth in SEQ ID NO: 32. As a result of this sequence analysis, gene sequences set forth in SEQ ID NOs: 5, 7, 9, 11, and 13 were obtained. Homology search of the nucleotide sequences and the amino acid sequences (SEQ ID NOs: 6, 8, 10, 12, and 14) of the genes for known genes using a homology search program, BLAST search (<http://www.ncbi.nlm.nih.gov/BLAST/>) revealed that all of the resulting five genes each encode a CAPRIN-1 protein. The sequence identity among the five genes was 100% in nucleotide sequence, in the region to be translated into a protein, and 99% in amino acid sequence. The sequence identity of the genes with a gene encoding a human homologous factor was 94% in nucleotide sequence, in the region to be translated into a protein, and 98% in amino acid sequence. The nucleotide sequences of the human homologous factor are set forth in SEQ ID NOs: 1 and 3, and the amino acid sequences are set forth in SEQ ID NOs: 2 and 4. The sequence identity of the obtained dog genes with a gene encoding a bovine homologous factor was 94% in nucleotide sequence, in the region to be translated into a protein, and 97% in amino acid sequence. The nucleotide sequence of the bovine homologous factor is set forth in SEQ ID NO: 15, and the amino acid sequence is set forth in SEQ ID NO: 16. The sequence identity between the gene encoding the human homologous factor and the gene encoding the bovine homologous factor was 94% in nucleotide sequence, in the region to be translated into a protein, and 93% to 97% in amino acid sequence. The sequence identity of the obtained dog genes with a gene encoding a horse homologous factor was 93% in nucleotide sequence, in the region to be translated into a protein, and 97% in amino acid sequence. The nucleotide sequence of the horse homologous factor is set forth in SEQ ID NO: 17, and the amino acid sequence is set forth in SEQ ID NO: 18. The sequence identity between the gene encoding the human homologous factor and the gene encoding the horse homologous factor was 93% in nucleotide sequence, in the region to be translated into a protein, and 96% in amino acid sequence. The sequence identity of the obtained dog genes with a gene encoding a mouse homologous factor was 87% to 89% in nucleotide sequence, in the region to be translated into a protein, and 95% to 97% in amino acid sequence. The

nucleotide sequences of the mouse homologous factor are set forth in SEQ ID NOs: 19, 21, 23, 25, and 27, and the amino acid sequences are set forth in SEQ ID NOs: 20, 22, 24, 26, and 28. The sequence identity between the gene encoding the human homologous factor and the gene encoding the mouse homologous factor was 89% to 91% in nucleotide sequence, in the region to be translated into a protein, and 95% to 96% in amino acid sequence. The sequence identity of the obtained dog genes with a gene encoding a chicken homologous factor was 82% in nucleotide sequence, in the region to be translated into a protein, and 87% in amino acid sequence. The nucleotide sequence of the chicken homologous factor is set forth in SEQ ID NO: 29, and the amino acid sequence is set forth in SEQ ID NO: 30. The sequence identity between the gene encoding the human homologous factor and the gene encoding the chicken homologous factor was 81% to 82% in nucleotide sequence, in the region to be translated into a protein, and 86% in amino acid sequence.

[0215]

(4) CAPRIN-1 gene expression analysis with human gallbladder cancer cells

The genes obtained by the above-described method were investigated for expression in a cell line of human gallbladder cancer TGBC14TKB (obtained from The Institute of Physical and Chemical Research) by RT-PCR. Reverse transcription was performed as follows: Total RNA was extracted from 50 to 100 mg of each tissue and 5 to 10×10^6 cells of each cell line with TRIZOL reagent (manufactured by invitrogen) in accordance with the protocol attached to the reagent. Using this total RNA, cDNA was synthesized with Superscript First-Strand Synthesis System for RT-PCR (manufactured by invitrogen) in accordance with the protocol attached to the system. The PCR was performed using primers (set forth in SEQ ID NOs: 33 and 34) specific to the resulting gene as follows: The total volume of a mixture containing 0.25 μ L of the sample prepared by the reverse transcription, 2 μ M of each of the primers, 0.2 mM of each dNTP, and 0.65 U of ExTaq polymerase (manufactured by Takara Shuzo Co., Ltd.) was adjusted to 25 μ L with the buffer attached to the reagent, and a process consisting of reactions at 94°C for 30 seconds, at 60°C for 30 seconds, and at 72°C for 30 seconds was repeated 30 cycles with Thermal Cycler (manufactured by BIO-RAD Laboratories, Inc.). The gene-specific primers amplified the region of 698 to 1124 nucleotides of the nucleotide

sequence (human CAPRIN-1 gene) set forth in SEQ ID NO: 1. For comparison, GAPDH-specific primers (set forth in SEQ ID NOs: 35 and 36) were simultaneously used. As a result, expression was observed in the cell line TGBC14TKB.

[0216]

[Example 2] Production of anti-human CAPRIN-1 polyclonal antibody

A mixture of 1 mg of a human CAPRIN-1 recombinant protein produced in accordance with example 3 of WO2010/016526 and an equivalent volume of an incomplete Freund's adjuvant (IFA) solution was subcutaneously injected to a rabbit four times with 2 weeks intervals. The blood was then collected to obtain antiserum containing a polyclonal antibody. The antiserum was further purified using a protein G carrier (manufactured by GE Healthcare Bioscience) to obtain an anti-CAPRIN-1 polyclonal antibody. Serum of a rabbit not administered with the antigen was similarly purified with a protein G carrier and was used as a control antibody.

[0217]

[Example 3] Expression analysis of CAPRIN-1 protein in human gallbladder cancer

(1) Expression analysis of CAPRIN-1 protein on human gallbladder cancer cells

A human gallbladder cancer cell line TGBC14TKB, which was confirmed to express the CAPRIN-1 gene, was investigated for whether or not a CAPRIN-1 protein is expressed on the cell surface. 1×10^6 cells of TGBC14TKB of which gene expression was confirmed in above were centrifuged with a 1.5-mL micro centrifugation tube. To the cells was added 2 μ g (5 μ L) of the anti-CAPRIN-1 polyclonal antibody prepared in Example 2. The mixture was suspended in 95 μ L of PBS containing 0.1% fetal bovine serum, and the suspension was left to stand on ice for 1 hour. After washing with PBS, the cells were suspended in 5 μ L of a FITC-labeled goat anti-rabbit IgG antibody (manufactured by Santa Cruz Biotechnology, Inc.) and 95 μ L of PBS containing 0.1% fetal bovine serum (FBS), and the suspension was left to stand on ice for 1 hour. After washing with PBS, the fluorescence intensity was measured with FACS Calibur available from Becton, Dickinson and Company. Separately, as a control, the same procedure as in above was performed using the control antibody prepared in Example 2, instead of the anti-CAPRIN-1 polyclonal antibody. As a result, the fluorescence

intensity in TGBC14TKB to which the anti-human CAPRIN-1 polyclonal antibody was added was 20% or more higher than that in the control in every case. This demonstrates that the CAPRIN-1 protein was expressed on the cell surface of the human gallbladder cancer cell line. The rate of increase in the fluorescence intensity is represented by the rate of increase in the mean fluorescence intensity (MFI value) in each cell and is calculated by the following calculation formula:

[0218]

Rate of increase in mean fluorescence intensity (rate of increase in fluorescence intensity) (%) = ((MFI value of cells reacted with anti-human CAPRIN-1 antibody) - (MFI value of control))/(MFI value of control) × 100.

[0219]

(2) Expression analysis of CAPRIN-1 protein in human gallbladder cancer tissue

Twenty-six gallbladder cancer tissue samples of a paraffin embedded human gallbladder cancer tissue array (manufactured by BIOMAX, Inc.) were subjected to immunohistochemical staining. The human gallbladder cancer tissue array was treated at 60°C for 3 hours and was then put in a staining bottle filled with xylene. The xylene in the bottle was replaced by fresh one three times at every 5 minutes. Subsequently, the same procedure was performed using ethanol and PBS-T instead of xylene. The human gallbladder cancer tissue array was put in a staining bottle filled with a 10 mM citric acid buffer (pH 6.0) containing 0.05% Tween 20 and was treated at 125°C for 5 minutes, followed by being left to stand at room temperature for 40 minutes or more. Excess water around the section was wiped away with a Kimwipe, the section was encircled with Dako pen, and an appropriate amount of Peroxidase Block (manufactured by DAKO) was dropwise added thereto. After being left to stand at room temperature for 5 minutes, the section was put in a staining bottle filled with PBS-T, and PBS-T was replaced by fresh one three times at every 5 minutes. As a blocking solution, a PBS-T solution containing 10% FBS was placed onto the section, followed by being left to stand in a moisture chamber at room temperature for 1 hour. A solution in which the concentration of the anti-CAPRIN-1 polyclonal antibody prepared in Example 2 was adjusted to 10 µg/mL with a PBS-T solution containing 5% FBS was further

placed onto the section, and the section was left to stand in a moist chamber at 4°C overnight and was then washed in PBS-T for 10 minutes three times. An appropriate amount of Peroxidase Labelled Polymer Conjugate (manufactured by DAKO) was dropwise placed onto the section, followed by being left to stand in a moisture chamber at room temperature for 30 minutes. After washing in PBS-T for 10 minutes three times, DAB color-developing solution (manufactured by DAKO) was placed onto the section, followed by being left to stand at room temperature for about 10 minutes. The color-developing solution was removed, and the section was washed in PBS-T for 10 minutes three times, was then rinsed with distilled water, was put in 70%, 80%, 90%, 95%, and 100% ethanol solutions in this order for 1 minute in each ethanol solution, and was left to stand in xylene overnight. The slide glass was taken out and was enclosed in Glycergel Mounting Medium (manufactured by DAKO) for observation. The results demonstrated that the CAPRIN-1 protein was highly expressed in 19 samples (73%) of 26 samples of gallbladder cancer tissue in total.

[0220]

[Example 4] Antitumor effect (ADCC activity) of anti-CAPRIN-1 polyclonal antibody on gallbladder cancer cells

Whether or not an anti-CAPRIN-1 antibody can impair the gallbladder cancer cells expressing a CAPRIN-1 protein was investigated. Evaluation was performed using the anti-human CAPRIN-1 polyclonal antibody prepared in Example 2. 1×10^6 cells of a human gallbladder cancer cell line TGBC14TKB, which were confirmed to express the CAPRIN-1 protein, were collected in a 50-mL centrifugation tube, and 100 μCi of $^{51}\text{chromium}$ was added thereto, followed by incubation at 37°C for 2 hours. Subsequently, the cells were washed with RPMI1640 medium containing 10% fetal calf serum three times and were then added to a 96-well V-bottom plate at 1×10^3 cells per well. To each well was added 1 μg of the anti-human CAPRIN-1 polyclonal antibody and further added 2×10^5 lymphocytes isolated from human peripheral blood, followed by culturing at 37°C in 5% CO_2 for 4 hours. After the culturing, the amount of $^{51}\text{chromium}$ (Cr) secreted from the impaired tumor cells into the culture supernatant was measured to calculate the ADCC activity on the gallbladder cancer cells by the anti-human CAPRIN-1 polyclonal antibody. The results demonstrated that in the

case of the anti-human CAPRIN-1 polyclonal antibody, the ADCC activity on TGBC14TKB was 14% or more, whereas the ADCC activity on TGBC14TKB was less than 5% in the case of using the control antibody prepared from the peripheral blood of a rabbit not immunized with the antigen and was also less than 5% in the case of not using any antibodies. Accordingly, it was revealed that the ADCC activity in the use of an anti-CAPRIN-1 antibody can impair gallbladder cancer cells expressing the CAPRIN-1 protein. The cytotoxicity is the results, as described above, when the anti-CAPRIN-1 antibody used in the present invention, lymphocytes, and 1×10^3 tumor cells with 51 chromium were mixed and cultured for 4 hours, and is shown as the cytotoxicity on the tumor cells calculated by the following calculation formula * by measuring the amount of 51 chromium released into the medium after the culturing.

[0221]

Formula *: cytotoxicity (%) = (amount of 51 chromium released from tumor cells in the presence of anti-CAPRIN-1 antibody and lymphocytes)/(amount of 51 chromium released from tumor cells in the presence of 1 N hydrochloric acid) $\times$ 100.

[0222]

[Example 5] Production of anti-CAPRIN-1 mouse and chicken monoclonal antibodies

The human CAPRIN-1 recombinant protein (100 μ g) produced in Example 2 was mixed with the same quantity of MPL/TDM adjuvant (manufactured by Sigma-Aldrich Co., LLC.), and the mixture was used as an antigen solution for one mouse. The antigen solution was intraperitoneally administered to 6-week old Balb/c mice (manufactured by Japan SLC, Inc.) and further administered three times or 24 times with one week intervals to complete the immunization. The spleen was extracted on the third day from the last immunization and was ground between sterilized two slide glasses and was washed with PBS (-) (manufactured by Nissui Pharmaceutical Co., Ltd.), followed by centrifugation at 1500 rpm for 10 minutes to remove the supernatant. This procedure was repeated three times to obtain spleen cells. The resulting spleen cells and mouse myeloma cells SP2/0 (purchased from ATCC) were mixed at a ratio of 10:1, and a PEG solution prepared by mixing 200 μ L of RPMI1640 medium containing 10% FBS and 800 μ L of PEG 1500 (manufactured by Boehringer

Ingelheim GmbH) and heated to 37°C was added to the resulting mixture, followed by being left to stand for 5 minutes for cell fusion. Centrifugation at 1700 rpm for 5 minutes was performed, and the supernatant was removed. The cells were suspended in a mixture of 150 mL of RPMI1640 medium (HAT selection medium) containing 15% FBS and 2% equivalents of a HAT solution manufactured by Gibco, and the suspension was seeded to 15 plates, which were 96-well plates (manufactured by Nunc), at 100 µL per well. Culturing at 37°C in 5% CO₂ for 7 days gave hybridomas of the spleen cells and the myeloma cells.

[0223]

Hybridomas were selected using, as an index the binding affinity of the antibodies produced by the hybridomas to a CAPRIN-1 protein. A 1 µg/mL solution of CAPRIN-1 protein prepared in Example 2 was added to a 96-well plate at 100 µL per well, followed by being left to stand at 4°C for 18 hours. Each well was washed with PBS-T three times, and 400 µL of a 0.5% bovine serum albumin (BSA) solution (manufactured by Sigma-Aldrich Co., LLC.) was added to each well, followed by being left to stand at room temperature for 3 hours. The solution was removed, and each well was washed with 400 µL of PBS-T three times, and 100 µL of the hybridoma culture supernatant prepared above was added to each well, followed by being left to stand at room temperature for 2 hours. Each well was washed with PBS-T three times, and 100 µL of a HRP-labeled anti-mouse IgG (H+L) antibody (manufactured by life technologies) diluted by 5000-fold with PBS was added to each well, followed by being left to stand at room temperature for 1 hour. Each well was washed with PBS-T three times, and 100 µL of a TMB substrate solution (manufactured by Thermo Fisher Scientific K.K.) was added to each well, followed by being left to stand for 15 to 30 minutes for color reaction. After the coloring, 100 µL of 1 N sulfuric acid was added to each well to stop the reaction. The absorbance was measured at 450 nm and 595 nm with an absorption spectrometer. As a result, several hybridomas producing antibodies showing high absorbance values were selected.

[0224]

The selected hybridomas were seeded to a 96-well plate at 0.5 cells per well and were cultured. After one week, hybridomas forming single colonies were observed in the wells.

The cells in the wells were further cultured, and hybridomas were selected using, as an index, the binding affinity of the antibodies produced by the cloned hybridomas to a CAPRIN-1 protein. A 1 µg/mL solution of CAPRIN-1 protein prepared in Example 2 was added to a 96-well plate at 100 µL per well, followed by being left to stand at 4°C for 18 hours. Each well was washed with PBS-T three times, and 400 µL of a 0.5% BSA solution was added to each well, followed by being left to stand at room temperature for 3 hours. The solution was removed, and each well was washed with 400 µL of PBS-T three times, and 100 µL of the hybridoma culture supernatant prepared above was added to each well, followed by being left to stand at room temperature for 2 hours. Each well was washed with PBS-T three times, and 100 µL of a HRP-labeled anti-mouse IgG (H+L) antibody (manufactured by life technologies) diluted by 5000-fold with PBS was added to each well, followed by being left to stand at room temperature for 1 hour. Each well was washed with PBS-T three times, and 100 µL of a TMB substrate solution (manufactured by Thermo Fisher Scientific K.K.) was added to each well, followed by being left to stand for 15 to 30 minutes for color reaction. After the coloring, 100 µL of 1 N sulfuric acid was added to each well to stop the reaction. The absorbance was measured at 450 nm and 595 nm with an absorption spectrometer. As a result, 150 hybridoma strains producing mouse monoclonal antibodies reactive to a CAPRIN-1 protein were obtained.

[0225]

Subsequently, from these mouse monoclonal antibodies, antibodies reactive to the cell surface of cancer cells expressing the CAPRIN-1 protein were selected. Specifically, 1×10^6 cells of human breast cancer cell line MDA-MB-231V were centrifuged with a 1.5-mL micro centrifugation tube. To the cells was added 100 µL of the culture supernatant of the above-described hybridomas, followed by being left to stand on ice for 1 hour. After washing with PBS, to the cells was added a FITC-labeled goat anti-mouse IgG antibody (manufactured by life technologies) diluted by 500-fold with PBS containing 0.1% FBS, followed by being left to stand on ice for 1 hour. After washing with PBS, the fluorescence intensity was measured with FACS Calibur available from Becton, Dickinson and Company. Separately, as a control, the same procedure as in above was performed using non-treated serum of a 6-week old Balb/c

mouse diluted by 500-fold with a hybridoma culturing medium, instead of the antibody. As a result, 22 mouse monoclonal antibodies (mouse monoclonal antibodies #1 to #22) that showed higher fluorescence intensities compared to the control, i.e., reacted with the cell surface of breast cancer cells were selected.

[0226]

In order to produce a chicken monoclonal antibody, 300 µg of an antigen protein (human CAPRIN-1 protein) set forth in SEQ ID NO: 2 prepared in Example 2 was mixed with the same quantity of complete Freund's adjuvant, and the mixture was used as an antigen solution for one chicken. The antigen solution was intraperitoneally administered to 7-week old chickens and further administered seven times with four weeks intervals to complete the immunization. The spleen was extracted on the fourth day from the last immunization and was ground between sterilized two slide glasses and was washed with PBS (-) (manufactured by Nissui Pharmaceutical Co., Ltd.), followed by centrifugation at 1500 rpm for 10 minutes to remove the supernatant. This procedure was repeated three times to obtain spleen cells. The resulting spleen cells and light chain-deficient chicken myeloma cells established by transformation from chicken using bird reticuloendotheliosis virus were mixed at a ratio of 5:1, and a PEG solution prepared by mixing 200 µL of IMDM medium containing 10% FBS and 800 µL of PEG 1500 (manufactured by Boehringer Ingelheim GmbH) and heated to 37°C was added to the resulting mixture, followed by being left to stand for 5 minutes for cell fusion. Centrifugation at 1700 rpm for 5 minutes was performed, and the supernatant was removed. The cells were suspended in a mixture of 300 mL of IMDM medium (HAT selection medium) containing 10% FBS and 2% equivalents of a HAT solution manufactured by Gibco, and the suspension was seeded to 30 plates, which were 96-well plates (manufactured by Nunc), at 100 L per well. Culturing at 37°C in 5% CO₂ for 7 days gave hybridomas by fusion of the spleen cells and the myeloma cells.

[0227]

Hybridomas were selected using, as an index, the binding affinity of the antibodies produced by the hybridomas to a CAPRIN-1 protein. A 1 µg/mL solution of CAPRIN-1 protein prepared in Example 2 was added to a 96-well plate at 100 µL per well, followed by

being left to stand at 4°C for 18 hours. Each well was washed with PBS-T three times, and 400 µL of a 0.5% bovine serum albumin (BSA) solution (manufactured by Sigma-Aldrich Co., LLC.) was added to each well, followed by being left to stand at room temperature for 3 hours. The solution was removed, and each well was washed with 400 µL of PBS-T three times, and 100 µL of the hybridoma culture supernatant prepared above was added to each well, followed by being left to stand at room temperature for 2 hours. Each well was washed with PBS-T three times, and 100 µL of a HRP-labeled anti-chicken IgY antibody (manufactured by Sigma-Aldrich Co., LLC.) diluted by 5000-fold with PBS was added to each well, followed by being left to stand at room temperature for 1 hour. Each well was washed with PBS-T three times, and 100 µL of a TMB substrate solution (manufactured by Thermo Fisher Scientific K.K.) was added to each well, followed by being left to stand for 15 to 30 minutes for color reaction. After the coloring, 100 µL of 1 N sulfuric acid was added to each well to stop the reaction. The absorbance was measured at 450 nm and 595 nm with an absorption spectrometer. As a result, several hybridomas producing antibodies showing high absorbance values were selected.

[0228]

The selected hybridomas were seeded to a 96-well plate at 0.5 cells per well and were cultured. After one week, hybridomas forming single colonies were observed in the wells. The cells in the wells were further cultured, and hybridomas were selected using, as an index, the binding affinity of the antibodies produced by the cloned hybridomas to a CAPRIN-1 protein. A 1 µg/mL solution of human CAPRIN-1 protein was added to a 96-well plate at 100 µL per well, followed by being left to stand at 4°C for 18 hours. Each well was washed with PBS-T three times, and 400 µL of a 0.5% BSA solution was added to each well, followed by being left to stand at room temperature for 3 hours. The solution was removed, and each well was washed with 400 µL of PBS-T three times, and 100 µL of the hybridoma culture supernatant prepared above was added to each well, followed by being left to stand at room temperature for 2 hours. Each well was washed with PBS-T three times, and 100 µL of a HRP-labeled anti-chicken IgY antibody (manufactured by Sigma-Aldrich Co., LLC.) diluted by 5000-fold with PBS was added to each well, followed by being left to stand at room

temperature for 1 hour. Each well was washed with PBS-T three times, and 100 μ L of a TMB substrate solution (manufactured by Thermo Fisher Scientific K.K.) was added to each well, followed by being left to stand for 15 to 30 minutes for color reaction. After the coloring, 100 μ L of 1 N sulfuric acid was added to each well to stop the reaction. The absorbance was measured at 450 nm and 595 nm with an absorption spectrometer. As a result, several hybridoma strains producing monoclonal antibodies showing reactivity with the CAPRIN-1 protein were obtained.

[0229]

Subsequently, from these monoclonal antibodies, antibodies reactive to the cell surface of cancer cells expressing the CAPRIN-1 protein were selected. Specifically, 5×10^5 cells of human breast cancer cell line MDA-MB-231V were centrifuged with a 1.5-mL micro centrifugation tube. To the cells was added 100 μ L of the culture supernatant of the above-described hybridomas, followed by being left to stand on ice for 1 hour. After washing with PBS, to the cells was added a FITC-labeled goat anti-chicken IgG (H+L) antibody (manufactured by SouthernBiotech) diluted by 30-fold with PBS containing 0.1% FBS, followed by being left to stand on ice for 1 hour. After washing with PBS, the fluorescence intensity was measured with FACS Calibur available from Becton, Dickinson and Company. Separately, the same procedure as in above was performed using a hybridoma culturing medium to prepare a control sample. As a result, three monoclonal antibodies (chicken monoclonal antibodies #1, #2, and #3) that showed higher fluorescence intensities compared to the control, i.e., reacted with the cell surface of breast cancer cells expressing the CAPRIN-1 protein were selected.

[0230]

[Example 6] Characterization of selected antibody

(1) Cloning of gene of variable domain of anti-CAPRIN-1 monoclonal antibody

mRNA was extracted from each of hybridoma strains producing 22 mouse monoclonal antibodies and 3 chicken monoclonal antibodies selected in Example 5. Genes of the heavy-chain variable (VH) domains and the light-chain variable (VL) domains of all anti-CAPRIN-1 monoclonal antibodies were prepared by RT-PCR using primers specific for mouse FR1-

derived sequence and mouse FR4-derived sequence in the hybridomas producing mouse monoclonal antibodies and primers specific for chicken FR1-derived sequence and chicken FR4-derived sequence in the hybridomas producing chicken monoclonal antibodies. These genes were cloned into a pCR2.1 vector (manufactured by life technologies) for sequencing.

[0231]

(1)-1 RT-PCR

mRNA was prepared from 1×10^6 hybridomas of each strain producing a mouse monoclonal antibody with mRNA micro purification kit (manufactured by GE Healthcare Bio-Sciences), and the resulting mRNA was reverse-transcribed with SuperScriptII 1st strand synthesis kit (manufactured by life technologies) to synthesize cDNA. These procedures were performed in accordance with the protocol attached to each kit. The gene of the antibody was amplified by PCR using the resulting cDNA. In order to obtain a gene of the VH domain, a primer (SEQ ID NO: 257) specific to the mouse heavy chain FR1 sequence and a primer (SEQ ID NO: 258) specific to the mouse heavy chain FR4 sequence were used. In order to obtain a gene of the VL domain, a primer (SEQ ID NO: 259) specific to the mouse light chain FR1 sequence and a primer (SEQ ID NO: 260) specific to the mouse light chain FR4 were used. These primers were designed by referring to Jones, S.T. and Bending, M.M., Bio/Technology, 9, 88-89 (1991). In the PCR, Ex-taq (manufactured by Takara Bio Inc.) was used. A cDNA sample was added to 5 μ L of 10 $\times$ EX Taq Buffer, 4 μ L (2.5 mM) of dNTP Mixture, 2 μ L (1.0 μ M) of each primer, and 0.25 μ L (5 U/ μ L) of Ex Taq, and the total volume was adjusted to 50 μ L with sterilized water. After treatment at 94°C for 2 minutes, a cycle consisting of denaturation at 94°C for 1 minute, annealing at 58°C for 30 seconds, and extension at 72°C for 1 minute was repeated for 30 cycles.

[0232]

Total RNA was extracted from 1×10^6 hybridomas of each strain producing a chicken monoclonal antibody using High Pure RNA Isolation Kit (manufactured by Roche Diagnostics K.K.), and cDNA was synthesized using PrimeScript II 1st strand cDNA Synthesis Kit (manufactured by Takara Bio Inc.). These procedures were performed in accordance with the protocol attached to each kit. The chicken antibody heavy chain variable domain gene

and the chicken antibody light chain variable domain gene were each amplified using the synthesized cDNA as a template and KOD-Plus-DNA Polymerase (manufactured by Toyobo Co., Ltd.) by PCR according to a usual method. In order to obtain a gene of the VH domain of a chicken antibody, a primer specific to the chicken heavy chain FR1 sequence and a primer specific to the chicken heavy chain FR4 sequence were used. In order to obtain a gene of the VL domain, a primer specific to the chicken light chain FR1 sequence and a primer specific to the chicken light chain FR4 were used.

[0233]

(1)-2 Cloning

Each PCR product prepared above was subjected to agarose gel electrophoresis, and the DNA bands of the VH domain and the VL domain were cut out. DNA fragments were purified with QIAquick Gel purification kit (manufactured by Qiagen) in accordance with the protocol attached to the kit. Each purified DNA was cloned into a pCR2.1 vector using a TA cloning kit (manufactured by life technologies). The linked vector was transformed into DH5a competent cells (manufactured by Toyobo Co., Ltd.) in accordance with a usual method. Ten clones of each transformant was cultured in a medium (100 µg/mL ampicillin) at 37°C overnight, and each plasmid DNA was purified using Qiaspin Miniprep kit (manufactured by Qiagen).

[0234]

(1)-3 Sequencing

The VH domain and VL domain genes in each of the plasmids prepared above were sequenced using M13 forward primer (SEQ ID NO: 261) and M13 reverse primer (SEQ ID NO: 262) with a fluorescence sequencer (DNA sequencer 3130XL, manufactured by ABI) using BigDye terminator Ver 3.1 cycle sequencing kit manufactured by ABI in accordance with the protocol attached to the kit. As a result, each gene sequence and amino acid sequence were determined.

[0235]

That is, these monoclonal antibodies each comprise a heavy-chain variable (VH) domain (sequence number of the gene sequence is shown in parentheses) comprising the

amino acid sequence set forth in SEQ ID NO: 40 (SEQ ID NO: 45), SEQ ID NO: 50 (SEQ ID NO: 55), SEQ ID NO: 60 (SEQ ID NO: 65), SEQ ID NO: 70 (SEQ ID NO: 75), SEQ ID NO: 80 (SEQ ID NO: 85), SEQ ID NO: 90 (SEQ ID NO: 95), SEQ ID NO: 100 (SEQ ID NO: 105), SEQ ID NO: 110 (SEQ ID NO: 115), SEQ ID NO: 120 (SEQ ID NO: 125), SEQ ID NO: 130 (SEQ ID NO: 131), SEQ ID NO: 135 (SEQ ID NO: 140), SEQ ID NO: 145 (SEQ ID NO: 150), SEQ ID NO: 160 (SEQ ID NO: 165), SEQ ID NO: 170 (SEQ ID NO: 175), SEQ ID NO: 200 (SEQ ID NO: 205), SEQ ID NO: 210 (SEQ ID NO: 215), SEQ ID NO: 220 (SEQ ID NO: 225), SEQ ID NO: 230 (SEQ ID NO: 235), SEQ ID NO: 240 (SEQ ID NO: 245), or SEQ ID NO: 250 (SEQ ID NO: 255) and a light-chain variable (VL) domain (sequence number of the gene sequence is shown in parentheses) comprising the amino acid sequence set forth in SEQ ID NO: 44 (SEQ ID NO: 46), SEQ ID NO: 54 (SEQ ID NO: 56), SEQ ID NO: 64 (SEQ ID NO: 66), SEQ ID NO: 74 (SEQ ID NO: 76), SEQ ID NO: 84 (SEQ ID NO: 86), SEQ ID NO: 94 (SEQ ID NO: 96), SEQ ID NO: 104 (SEQ ID NO: 106), SEQ ID NO: 114 (SEQ ID NO: 116), SEQ ID NO: 124 (SEQ ID NO: 126), SEQ ID NO: 139 (SEQ ID NO: 141), SEQ ID NO: 149 (SEQ ID NO: 151), SEQ ID NO: 155 (SEQ ID NO: 156), SEQ ID NO: 164 (SEQ ID NO: 166), SEQ ID NO: 174 (SEQ ID NO: 176), SEQ ID NO: 180 (SEQ ID NO: 181), SEQ ID NO: 185 (SEQ ID NO: 186), SEQ ID NO: 190 (SEQ ID NO: 191), SEQ ID NO: 195 (SEQ ID NO: 196), SEQ ID NO: 204 (SEQ ID NO: 206), SEQ ID NO: 214 (SEQ ID NO: 216), SEQ ID NO: 224 (SEQ ID NO: 226), SEQ ID NO: 234 (SEQ ID NO: 236), SEQ ID NO: 244 (SEQ ID NO: 246), or SEQ ID NO: 254 (SEQ ID NO: 256). The VH domain comprises CDR1 represented by the amino acid sequence set forth in SEQ ID NO: 37, SEQ ID NO: 47, SEQ ID NO: 57, SEQ ID NO: 67, SEQ ID NO: 77, SEQ ID NO: 87, SEQ ID NO: 97, SEQ ID NO: 107, SEQ ID NO: 117, SEQ ID NO: 127, SEQ ID NO: 132, SEQ ID NO: 142, SEQ ID NO: 157, SEQ ID NO: 167, SEQ ID NO: 197, SEQ ID NO: 207, SEQ ID NO: 217, SEQ ID NO: 227, SEQ ID NO: 237, or SEQ ID NO: 247, CDR2 represented by the amino acid sequence set forth in SEQ ID NO: 38, SEQ ID NO: 48, SEQ ID NO: 58, SEQ ID NO: 68, SEQ ID NO: 78, SEQ ID NO: 88, SEQ ID NO: 98, SEQ ID NO: 108, SEQ ID NO: 118, SEQ ID NO: 128, SEQ ID NO: 133, SEQ ID NO: 143, SEQ ID NO: 158, SEQ ID NO: 168, SEQ ID NO: 198, SEQ ID NO: 208, SEQ ID NO: 218, SEQ ID NO: 228, SEQ ID NO: 238, or SEQ ID NO: 248, and CDR3

represented by the amino acid sequence set forth in SEQ ID NO: 39, SEQ ID NO: 49, SEQ ID NO: 59, SEQ ID NO: 69, SEQ ID NO: 79, SEQ ID NO: 89, SEQ ID NO: 99, SEQ ID NO: 109, SEQ ID NO: 119, SEQ ID NO: 129, SEQ ID NO: 134, SEQ ID NO: 144, SEQ ID NO: 159, SEQ ID NO: 169, SEQ ID NO: 199, SEQ ID NO: 209, SEQ ID NO: 219, SEQ ID NO: 229, SEQ ID NO: 239, or SEQ ID NO: 249. The VL domain comprises CDR1 represented by the amino acid sequence set forth in SEQ ID NO: 41, SEQ ID NO: 51, SEQ ID NO: 61, SEQ ID NO: 71, SEQ ID NO: 81, SEQ ID NO: 91, SEQ ID NO: 101, SEQ ID NO: 111, SEQ ID NO: 121, SEQ ID NO: 136, SEQ ID NO: 146, SEQ ID NO: 152, SEQ ID NO: 161, SEQ ID NO: 171, SEQ ID NO: 177, SEQ ID NO: 182, SEQ ID NO: 187, SEQ ID NO: 192, SEQ ID NO: 201, SEQ ID NO: 211, SEQ ID NO: 221, SEQ ID NO: 231, SEQ ID NO: 241, or SEQ ID NO: 251, CDR2 represented by the amino acid sequence set forth in SEQ ID NO: 42, SEQ ID NO: 52, SEQ ID NO: 62, SEQ ID NO: 72, SEQ ID NO: 82, SEQ ID NO: 92, SEQ ID NO: 102, SEQ ID NO: 112, SEQ ID NO: 122, SEQ ID NO: 137, SEQ ID NO: 147, SEQ ID NO: 153, SEQ ID NO: 162, SEQ ID NO: 172, SEQ ID NO: 178, SEQ ID NO: 183, SEQ ID NO: 188, SEQ ID NO: 193, SEQ ID NO: 202, SEQ ID NO: 212, SEQ ID NO: 222, SEQ ID NO: 232, SEQ ID NO: 242, or SEQ ID NO: 252, and CDR3 represented by the amino acid sequence set forth in SEQ ID NO: 43, SEQ ID NO: 53, SEQ ID NO: 63, SEQ ID NO: 73, SEQ ID NO: 83, SEQ ID NO: 93, SEQ ID NO: 103, SEQ ID NO: 113, SEQ ID NO: 123, SEQ ID NO: 138, SEQ ID NO: 148, SEQ ID NO: 154, SEQ ID NO: 163, SEQ ID NO: 173, SEQ ID NO: 179, SEQ ID NO: 184, SEQ ID NO: 189, SEQ ID NO: 194, SEQ ID NO: 203, SEQ ID NO: 213, SEQ ID NO: 223, SEQ ID NO: 233, SEQ ID NO: 243, or SEQ ID NO: 253.

[0236]

The amino acid sequence of the heavy chain variable domain of each of the resulting monoclonal antibodies is set forth in SEQ ID NO: 40, SEQ ID NO: 50, SEQ ID NO: 60, SEQ ID NO: 70, SEQ ID NO: 80, SEQ ID NO: 90, SEQ ID NO: 100, SEQ ID NO: 110, SEQ ID NO: 120, SEQ ID NO: 130, SEQ ID NO: 135, SEQ ID NO: 145, SEQ ID NO: 160, SEQ ID NO: 170, SEQ ID NO: 200, SEQ ID NO: 210, SEQ ID NO: 220, SEQ ID NO: 230, SEQ ID NO: 240, and SEQ ID NO: 250, and the amino acid sequence of the light chain variable domain is set forth in SEQ ID NO: 44, SEQ ID NO: 54, SEQ ID NO: 64, SEQ ID NO: 74,

SEQ ID NO: 84, SEQ ID NO: 94, SEQ ID NO: 104, SEQ ID NO: 114, SEQ ID NO: 124, SEQ ID NO: 139, SEQ ID NO: 149, SEQ ID NO: 155, SEQ ID NO: 164, SEQ ID NO: 174, SEQ ID NO: 180, SEQ ID NO: 185, SEQ ID NO: 190, SEQ ID NO: 195, SEQ ID NO: 204, SEQ ID NO: 214, SEQ ID NO: 224, SEQ ID NO: 234, SEQ ID NO: 244, and SEQ ID NO: 254.

[0237]

That is, mouse monoclonal antibody #1 comprises a heavy chain variable domain set forth in SEQ ID NO: 70 and a light chain variable domain set forth in SEQ ID NO: 74; mouse monoclonal antibody #2 comprises a heavy chain variable domain set forth in SEQ ID NO: 80 and a light chain variable domain set forth in SEQ ID NO: 84; mouse monoclonal antibody #3 comprises a heavy chain variable domain set forth in SEQ ID NO: 90 and a light chain variable domain set forth in SEQ ID NO: 94; mouse monoclonal antibody #4 comprises a heavy chain variable domain set forth in SEQ ID NO: 100 and a light chain variable domain set forth in SEQ ID NO: 104; mouse monoclonal antibody #5 comprises a heavy chain variable domain set forth in SEQ ID NO: 110 and a light chain variable domain set forth in SEQ ID NO: 114; mouse monoclonal antibody #6 comprises a heavy chain variable domain set forth in SEQ ID NO: 120 and a light chain variable domain set forth in SEQ ID NO: 124; mouse monoclonal antibody #7 comprises a heavy chain variable domain set forth in SEQ ID NO: 130 and a light chain variable domain set forth in SEQ ID NO: 124; mouse monoclonal antibody #8 comprises a heavy chain variable domain set forth in SEQ ID NO: 135 and a light chain variable domain set forth in SEQ ID NO: 139; mouse monoclonal antibody #9 comprises a heavy chain variable domain set forth in SEQ ID NO: 145 and a light chain variable domain set forth in SEQ ID NO: 149; mouse monoclonal antibody #10 comprises a heavy chain variable domain set forth in SEQ ID NO: 145 and a light chain variable domain set forth in SEQ ID NO: 155; mouse monoclonal antibody #11 comprises a heavy chain variable domain set forth in SEQ ID NO: 160 and a light chain variable domain set forth in SEQ ID NO: 164; mouse monoclonal antibody #12 comprises a heavy chain variable domain set forth in SEQ ID NO: 170 and a light chain variable domain set forth in SEQ ID NO: 174; mouse monoclonal antibody #13 comprises a heavy chain variable domain set forth in SEQ ID NO: 170 and a light chain variable domain set forth in SEQ ID NO: 180; mouse monoclonal

antibody #14 comprises a heavy chain variable domain set forth in SEQ ID NO: 170 and a light chain variable domain set forth in SEQ ID NO: 185; mouse monoclonal antibody #15 comprises a heavy chain variable domain set forth in SEQ ID NO: 170 and a light chain variable domain set forth in SEQ ID NO: 190; mouse monoclonal antibody #16 comprises a heavy chain variable domain set forth in SEQ ID NO: 170 and a light chain variable domain set forth in SEQ ID NO: 195; mouse monoclonal antibody #17 comprises a heavy chain variable domain set forth in SEQ ID NO: 200 and a light chain variable domain set forth in SEQ ID NO: 204; mouse monoclonal antibody #18 comprises a heavy chain variable domain set forth in SEQ ID NO: 210 and a light chain variable domain set forth in SEQ ID NO: 214; mouse monoclonal antibody #19 comprises a heavy chain variable domain set forth in SEQ ID NO: 220 and a light chain variable domain set forth in SEQ ID NO: 224; mouse monoclonal antibody #20 comprises a heavy chain variable domain set forth in SEQ ID NO: 230 and a light chain variable domain set forth in SEQ ID NO: 234; mouse monoclonal antibody #21 comprises a heavy chain variable domain set forth in SEQ ID NO: 240 and a light chain variable domain set forth in SEQ ID NO: 244; and mouse monoclonal antibody #22 comprises a heavy chain variable domain set forth in SEQ ID NO: 250 and a light chain variable domain set forth in SEQ ID NO: 254..

[0238]

The amino acid sequences of the heavy chain variable domains of the resulting chicken monoclonal antibodies are set forth in SEQ ID NOS: 40, 50, and 60; and the amino acid sequences of the light chain variable domains are set forth in SEQ ID NOS: 44, 54, and 64.

[0239]

That is, the chicken monoclonal antibody #1 comprises a heavy chain variable domain set forth in SEQ ID NO: 40 and a light chain variable domain set forth in SEQ ID NO: 44, wherein the CDRs 1 to 3 in the heavy chain variable domain consist of the amino acid sequences set forth in SEQ ID NOS: 37, 38, and 39, respectively; and the CDRs 1 to 3 in the light chain variable domain consist of the amino acid sequences set forth in SEQ ID NOS: 41, 42, and 43, respectively. The chicken monoclonal antibody #2 comprises a heavy chain variable domain set forth in SEQ ID NO: 50 and a light chain variable domain set forth in SEQ

ID NO: 54, wherein the CDRs 1 to 3 in the heavy chain variable domain consist of the amino acid sequences set forth in SEQ ID NOs: 47, 48, and 49, respectively; and the CDRs 1 to 3 in the light chain variable domain consist of the amino acid sequences set forth in SEQ ID NOs: 51, 52, and 53, respectively. The chicken monoclonal antibody #3 comprises a heavy chain variable domain set forth in SEQ ID NO: 60 and a light chain variable domain set forth in SEQ ID NO: 64, wherein the CDRs 1 to 3 in the heavy chain variable domain consist of the amino acid sequences set forth in SEQ ID NOs: 57, 58, and 59, respectively; and the CDRs 1 to 3 in the light chain variable domain consist of the amino acid sequences set forth in SEQ ID NOs: 61, 62, and 63, respectively.

[0240]

(2) Production of human-chicken chimeric recombinant antibody and mouse-chicken chimeric antibody

Both terminals of an amplified fragment of the gene of the heavy chain variable domain set forth in SEQ ID NO: 40 of the chicken monoclonal antibody #1 prepared in the above (1) were treated with restriction enzymes, and the fragment was purified and was inserted into pcDNA4/myc-His vector (manufactured by life technologies) containing a leader sequence derived from a chicken antibody comprising the sequence set forth in SEQ ID NO: 263 and the H-chain constant domain of human IgG₁ comprising the sequence set forth in SEQ ID NO: 264 in accordance with a usual method. Separately, both terminals of an amplified fragment of the gene of the light chain variable domain set forth in SEQ ID NO: 44 of the chicken monoclonal antibody #1 were treated with restriction enzymes, and the fragment was purified and was inserted into pcDNA3.1/myc-His vector (manufactured by life technologies) containing a leader sequence derived from a chicken antibody comprising the sequence set forth in SEQ ID NO: 263 and the L-chain constant domain of human IgG₁ comprising the sequence set forth in SEQ ID NO: 265 in accordance with a usual method.

[0241]

Subsequently, the recombinant vector containing the heavy chain variable domain set forth in SEQ ID NO: 40 of the chicken monoclonal antibody #1 and the recombinant vector containing the light chain variable domain set forth in SEQ ID NO: 44 of the chicken

monoclonal antibody #1 were introduced into CHO-K1 cells (obtained from Riken Cell Bank). Specifically, 2×10^5 CHO-K1 cells cultured in each well, of a 12-well culture plate, containing 1 mL of Ham's F12 medium (manufactured by life technologies) containing 10% FBS were washed with PBS (-). To each well were added 1 mL of fresh Ham's F12 medium containing 10% FBS and a mixture of 30 μ L of OptiMEM (manufactured by life technologies) containing 250 ng of each of the above-mentioned vectors and 30 μ L of Polyfect transfection reagent (manufactured by Qiagen). The CHO-K1 cells introduced with the recombinant vectors were cultured in Ham's F12 medium containing 10% FBS, 200 μ g/mL Zeocin (manufactured by life technologies), and 200 μ g/mL Geneticin (manufactured by Roche) and were then seeded to a 96-well plate at 0.5 cells per well to produce a cell line stably producing human-chicken chimeric antibody #1 (#1) comprising the variable domain of the chicken monoclonal antibody #1. Similarly, cell lines stably producing human-chicken chimeric antibody #2 (#2) and human-chicken chimeric antibody #3 (#3) were produced from chicken monoclonal antibodies #2 and #3, respectively.

[0242]

The produced cell lines were each cultured at 5×10^5 cells/mL in a 150-cm² flask containing 30 mL of serum-free OptiCHO medium (manufactured by life technologies) for 5 days to obtain a culture supernatant containing #1, #2, or #3.

[0243]

Similarly, both terminals of an amplified fragment of the gene of the heavy chain variable domain set forth in SEQ ID NO: 40 of the chicken monoclonal antibody #1 were treated with restriction enzymes, and the fragment was purified and was inserted into pcDNA4/myc-His vector (manufactured by life technologies) containing a leader sequence derived from a chicken antibody and the H-chain constant domain of mouse IgG₁ in accordance with a usual method. Separately, both terminals of an amplified fragment of the gene of the light chain variable domain set forth in SEQ ID NO: 44 of the chicken monoclonal antibody #1 were treated with restriction enzymes, and the fragment was purified and was inserted into pcDNA3.1/myc-His vector (manufactured by life technologies) containing a leader sequence derived from a chicken antibody and the L-chain constant domain of mouse

IgG₁ in accordance with a usual method. These vectors were introduced into CHO-K1 cells as in above to produce a cell line stably producing mouse-chicken chimeric antibody #1 comprising the variable domain of chicken monoclonal antibody #1. Similarly, cell lines stably producing mouse-chicken chimeric antibody #2 (#2) and mouse-chicken chimeric antibody #3 (#3) were produced from chicken monoclonal antibodies #2 and #3, respectively.

[0244]

The produced cell lines were each cultured at 5×10^5 cells/mL in a 150-cm² flask containing 30 mL of serum-free OptiCHO medium (manufactured by life technologies) for 5 days to obtain a culture supernatant containing mouse-chicken chimeric antibody #1, mouse-chicken chimeric antibody #2, and mouse-chicken chimeric antibody #3.

[0245]

(3) Expression of CAPRIN-1 protein on gallbladder cancer cell surface using prepared monoclonal antibody

Subsequently, a gallbladder cell line TGBC14TKB, which was confirmed to express a CAPRIN-1 gene, was investigated for whether or not a CAPRIN-1 protein is expressed on the surfaces of these cells. 1×10^6 cells of TGBC14TKB were centrifuged with a 1.5-mL micro centrifugation tube. To the cells was added the culture supernatant (100 μ L) containing any of anti-CAPRIN-1 mouse monoclonal antibodies #1 to #22 reacting to cancer cell surface produced in Example 4 and anti-CAPRIN-1 mouse-chicken chimeric antibodies #1 to #3 produced in the above (2), followed by being left to stand on ice for 1 hour. After washing with PBS, the cells were suspended in a FITC-labeled goat anti-mouse IgG antibody (manufactured by life technologies) diluted by 500-fold with PBS containing 0.1% FBS, followed by being left to stand on ice for 1 hour. After washing with PBS, the fluorescence intensity was measured with FACS Calibur available from Becton, Dickinson and Company. Separately, as a control, the same procedure as in above was performed using an iso-type control antibody, instead of the culture supernatants containing the anti-CAPRIN-1 mouse monoclonal antibodies #1 to #22 and the mouse-chicken chimeric antibodies #1 to #3. As a result, the fluorescence intensity in the cells to which any of the monoclonal antibodies #1 to #22 and the mouse-chicken chimeric antibodies #1 to #3 was added was 20% or more higher

than that in the control in every case. Specifically, in the case of using mouse-chicken chimeric antibody #1, the fluorescence intensity was enhanced by 200% or more. This demonstrates that the CAPRIN-1 protein was expressed on the cell surface of the human gallbladder cancer cell lines. The rate of increase in the fluorescence intensity is represented by the rate of increase in the mean fluorescence intensity (MFI value) in each cell and is calculated by the following calculation formula:

[0246]

Rate of increase in mean fluorescence intensity (rate of increase in fluorescence intensity) (%) = ((MFI value of cells reacted with anti-human CAPRIN-1 antibody) - (MFI value of control))/(MFI value of control) $\times$ 100.

(4) Antitumor effect (ADCC activity) of anti-CAPRIN-1 antibody on human gallbladder cancer cells

Among the antibodies prepared above, human-chicken chimeric antibody #1 was used for evaluation of cytotoxicity (ADCC activity) on human gallbladder cancer cells. Human-chicken chimeric antibody #1 contained in the culture supernatant prepared in the above (2) was purified using Hitrap Protein A Sepharose FF (manufactured by GE Healthcare Bio-Sciences), substituted with PBS (-), and filtered through a filter of 0.22 μ m (manufactured by Millipore Corporation), and was used as the antibody for measuring activity. 1×10^6 cells of a human gallbladder cancer cell line TGBC14TKB were collected in a 50-mL centrifugation tube, and 100 μ Ci of 51 chromium was added thereto, followed by incubation at 37°C for 2 hours. Subsequently, the cells were washed with RPMI1640 medium containing 10% FBS three times and were then added to a 96-well V-bottom plate at 2×10^3 cells per well as target cells. To each well was added 1.2 μ g of the antibody purified above. Separately, a cell population containing human NK cells was isolated from human peripheral blood lymphocytes by the following procedure: Human peripheral mononuclear cells were subjected to reaction with FITC fluorescent dye-labeled antibodies (anti-human CD3 antibody, anti-human CD20 antibody, anti-human CD19 antibody, anti-human CD11c antibody, and anti-HLA-DR antibody (Becton, Dickinson and Company)), and a cell population containing NK cells not stained with these antibodies was isolated using a cell sorter (FACS Vantage SE (Becton,

Dickinson and Company)) or a human NK cell separation kit (NK Cell Isolation Kit (manufactured by Miltenyi Biotec GmbH)). The cell population containing NK cells were further added to the plate at 2×10^5 cells per well, followed by culturing at 37°C in 5% CO_2 for 4 hours. After the culturing, the amount of $^{51}\text{chromium}$ secreted from the impaired tumor cells into the culture supernatant was measured to calculate the ADCC activity on the gallbladder cancer cells by the anti-CAPRIN-1 antibody. As a result, the cytotoxicities of human-chicken chimeric antibody #1 on TGBC14TKB were 20%, whereas the cytotoxicities on TGBC14TKB were both less than 5% in the case of using the monoclonal antibody that reacts with the CAPRIN-1 protein itself but does not react with the cell surface of cancer cells and in the case of not using antibodies. Similarly, the cytotoxicities on TGBC14TKB of anti-CAPRIN-1 mouse monoclonal antibodies #1 to #22, human-chicken chimeric antibodies #2 and #3 were also investigated and were all 15% or more, whereas the cytotoxicities were less than 5% in the case of using the monoclonal antibody that reacts with the CAPRIN-1 protein itself but does not react with the cell surface of cancer cells and in the case of not using antibodies. The results above demonstrated that the prepared anti-CAPRIN-1 monoclonal antibodies impair cancer cells expressing the CAPRIN-1 protein through ADCC activity. The cytotoxicity is the results, as described above, when the anti-CAPRIN-1 antibody used in the present invention, the cell population containing human NK cells, and 2×10^3 tumor cells with $^{51}\text{chromium}$ were mixed and cultured for 4 hours, and is shown as the cytotoxicity on the tumor cells calculated by the following calculation formula * by measuring the amount of $^{51}\text{chromium}$ released into the medium after the culturing.

[0247]

Formula *: cytotoxicity (%) = (amount of $^{51}\text{chromium}$ released from tumor cells in the presence of cell population containing anti-CAPRIN-1 antibody and NK cells)/(amount of $^{51}\text{chromium}$ released from tumor cells in the presence of 1 N hydrochloric acid) $\times$ 100.

[0248]

[Example 7] Identification of peptide of CAPRIN-1 protein binding to anti-CAPRIN-1 antibody reacting to cell surface of cancer cells

Partial sequences of the CAPRIN-1 protein recognized by anti-CAPRIN-1 antibodies were identified using anti-CAPRIN-1 monoclonal antibodies #12 to #22, which react to cell surface of cancer cells, prepared above.

[0249]

First, DTT (manufactured by Fluka) was added at a final concentration of 10 mM to 100 μ L of a 1 μ g/ μ L solution of recombinant CAPRIN-1 protein in PBS, followed by reaction at 95°C for 5 minutes to reduce the disulfide bond in the CAPRIN-1 protein. Next, iodoacetamide (manufactured by Wako Pure Chemical Industries, Ltd.) was added thereto at a final concentration of 20 mM, followed by alkylation of the thiol group at 37°C under a light-shielded condition for 30 minutes. To 40 μ g of the resulting reduced alkylated CAPRIN-1 protein was added 50 μ g of any of anti-CAPRIN-1 monoclonal antibodies #12 to #22. The total amount of each mixture was adjusted to 1 mL with a 20 mM phosphate buffer (pH 7.0), followed by reaction at 4°C overnight with stirring.

[0250]

Subsequently, trypsin (manufactured by Promega K.K.) was added at a final concentration of 0.2 μ g to each reaction mixture, followed by reaction at 37°C for 1, 2, 4, or 12 hours. The reaction mixture was mixed with protein A-glass beads (manufactured by GE Healthcare Bio-Sciences) blocked with PBS containing 1% BSA (manufactured by Sigma-Aldrich Co., LLC.) and washed with PBS in advance and 1 mM calcium carbonate in a NP-40 buffer (20 mM phosphate buffer (pH 7.4), 5 mM EDTA, 150 mM NaCl, 1% NP-40), followed by reaction for 30 minutes.

[0251]

Each reaction solution was washed with a 25 mM ammonium carbonate buffer (pH 8.0), followed by elution of antigen-antibody complexes with 100 μ L of 0.1% formic acid. The eluate was analyzed by LC-MS using Q-TOF Premier (manufactured by Waters-MicroMass) in accordance with the protocol attached to the instrument.

[0252]

As a result, a polypeptide set forth in SEQ ID NO: 273 was identified as a partial sequence of the CAPRIN-1 protein recognized by all of anti-CAPRIN-1 monoclonal

antibodies #12 to #22. Furthermore, a peptide set forth in SEQ ID NO: 274 was identified as a partial sequence of the polypeptide set forth in above SEQ ID NO: 273 recognized by the monoclonal antibodies #13 to #16, #17 to #19, and #21; and a partial sequence peptide set forth in SEQ ID NO: 275 was found to be recognized by the monoclonal antibodies #13 to #16. [0253]

Epitope peptides in the CAPRIN-1 protein recognized by antibodies were identified using human-chicken chimeric monoclonal antibody #1, human-chicken chimeric monoclonal antibody #3, and mouse monoclonal antibodies #1 to #11. Candidate peptides (93 peptides) each consisting of 12 to 16 amino acids of the amino acid sequence of the human CAPRIN-1 protein were synthesized and were each dissolved at a concentration of 1 mg/mL in DMSO. [0254]

Each peptide was dissolved at a concentration of 30 µg/mL in a 0.1 M sodium carbonate buffer (pH 9.6). The solution was added to a 96-well plate (manufactured by Nunc, Product No. 436006) at 100 µL per well, followed by being left to stand at 4°C overnight. The solution was removed, and 200 µL of 10 mM ethanolamine/0.1 M sodium carbonate buffer (pH 9.6) was added to each well, followed by being left to stand at room temperature for 1 hour. The solution was removed, and each well was washed with PBS containing 0.5% Tween 20 (PBST) twice to prepare a peptide-immobilized plate. [0255]

The cell culture supernatant containing human-chicken chimeric monoclonal antibody #1 (#1), human-chicken chimeric monoclonal antibody #3 (#3), or a mouse monoclonal antibody (#1, #2, #3, #4, #5, #6, #7, #8, #9, #10, or #11) was added to each plate at an amount of 50 µL per well, followed by shaking at room temperature for 1 hour. The solution was removed, and each well was washed with PBST three times. Subsequently, a secondary antibody solution containing a HRP-labeled anti-human IgG antibody (manufactured by life technologies) diluted by 3000- to 4000-fold with PBST was added to the human-chicken chimeric monoclonal antibody wells at 50 µL per well, while a secondary antibody solution containing a HRP-labeled anti-mouse IgG antibody (manufactured by life technologies) diluted by 3000- to 4000-fold with PBST was added to the mouse monoclonal antibody wells

at a 50 μ L per well. The solution was removed, and each well was washed with PBST six times.

[0256]

Color reaction was performed by adding 100 μ L of a TMB substrate solution (manufactured by Thermo Fisher Scientific K.K.) to each well and leaving the mixture to stand for 15 to 30 minutes. After the coloring, 100 μ L of 1 N sulfuric acid was added to each well to stop the reaction. The absorbance was measured at 450 nm and 595 nm with an absorption spectrometer. As a result, a polypeptide set forth in SEQ ID NO: 266 was identified as a partial sequence of the CAPRIN-1 recognized by all of the anti-CAPRIN-1 antibodies: human-chicken chimeric monoclonal antibody #1 and anti-CAPRIN-1 monoclonal antibodies #1 to #5. In addition, a peptide set forth in SEQ ID NO: 267 was identified as a partial peptide of the polypeptide set forth in SEQ ID NO: 266 recognized by human-chicken chimeric monoclonal antibody #1 and mouse monoclonal antibodies #3 and #4; and a peptide set forth in SEQ ID NO: 268 was identified as a partial peptide of the polypeptide set forth in SEQ ID NO: 266 recognized by mouse monoclonal antibodies #1, #2, and #5. It was therefore demonstrated that the polypeptide set forth in SEQ ID NO: 266 contains an epitope region for the anti-CAPRIN-1 antibodies: human-chicken chimeric monoclonal antibody #1 and mouse monoclonal antibodies #1 to #5. Furthermore, a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 270 was identified as a partial sequence of the CAPRIN-1 protein recognized by all of anti-CAPRIN-1 monoclonal antibodies #6, #7, and #8. It was therefore demonstrated that the polypeptide set forth in SEQ ID NO: 270 contains an epitope region for anti-CAPRIN-1 antibodies #6, #7, and #8. In addition, a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 272 was identified as a partial sequence of the CAPRIN-1 protein recognized by all of anti-CAPRIN-1 monoclonal antibodies #9, #10, and #11. It was therefore demonstrated that the polypeptide set forth in SEQ ID NO: 272 contains an epitope region for anti-CAPRIN-1 antibodies #9, #10, and #11. In addition, a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 269 was identified as a partial sequence of the CAPRIN-1 protein recognized by human-chicken chimeric

monoclonal antibody #3. It was therefore demonstrated that the polypeptide set forth in SEQ ID NO: 269 contains an epitope region for human-chicken chimeric monoclonal antibody #3.
[0257]

[Example 8] Production of mouse monoclonal antibodies #30 and #34 to #36 against CAPRIN-1 protein

(1) Production of mouse anti-CAPRIN-1 monoclonal antibodies #30 and #34 to #36

A mixture of 100 µg of a human CAPRIN-1 protein comprising the amino acid sequence set forth in SEQ ID NO: 2 prepared in accordance with example 3 of WO2010/016526 mixed with the same quantity of MPL+TDM adjuvant (manufactured by Sigma-Aldrich Co., LLC.) was used as an antigen solution for one mouse. The antigen solution was intraperitoneally administered to 6-week old Balb/c mice (manufactured by Japan SLC, Inc.) and further administered seven times with one week intervals to complete the immunization. The spleen was extracted on the third day from the last immunization and was ground between sterilized two slide glasses and was washed with PBS (-) (manufactured by Nissui Pharmaceutical Co., Ltd.), followed by centrifugation at 1500 rpm for 10 minutes to remove the supernatant. This procedure was repeated three times to obtain spleen cells. The resulting spleen cells and mouse myeloma cells SP2/0 (purchased from ATCC) were mixed at a ratio of 10:1, and a PEG solution prepared by mixing 200 µL of RPMI1640 medium containing 10% FBS and 800 µL of PEG 1500 (manufactured by Boehringer Ingelheim GmbH) and heated to 37°C was added to the resulting mixture, followed by being left to stand for 5 minutes for cell fusion. Centrifugation at 1700 rpm for 5 minutes was performed, and the supernatant was removed. The cells were suspended in a mixture of 150 mL of RPMI1640 medium (HAT selection medium) containing 15% FBS and 2% equivalents of a HAT solution manufactured by Gibco, and the suspension was seeded to 15 plates, which were 96-well plates (manufactured by Nunc), at 100 µL per well. Culturing at 37°C in 5% CO₂ for 7 days gave hybridomas by fusion of the spleen cells and the myeloma cells.

[0258]

Hybridomas were selected using, as an index, the binding affinity of the antibodies produced by the hybridomas to a CAPRIN-1 protein. A 1 µg/mL solution of CAPRIN-1

protein prepared by the method described in example 3 of WO2010/016526 was added to a 96-well plate at 100 μ L per well, followed by being left to stand at 4°C for 18 hours. Each well was washed with PBS-T three times, and 400 μ L of a 0.5% bovine serum albumin (BSA) solution (manufactured by Sigma-Aldrich Co., LLC.) was added to each well, followed by being left to stand at room temperature for 3 hours. The solution was removed, and each well was washed with 400 μ L of PBS-T three times, and 100 μ L of the hybridoma culture supernatant prepared above was added to each well, followed by being left to stand at room temperature for 2 hours. Each well was washed with PBS-T three times, and 100 μ L of a HRP-labeled anti-mouse IgG (H+L) antibody (manufactured by life technologies) diluted by 5000-fold with PBS was added to each well, followed by being left to stand at room temperature for 1 hour. Each well was washed with PBS-T three times, and 100 μ L of a TMB substrate solution (manufactured by Thermo Fisher Scientific K.K.) was added to each well, followed by being left to stand for 15 to 30 minutes for color reaction. After the coloring, 100 μ L of 1 N sulfuric acid was added to each well to stop the reaction. The absorbance was measured at 450 nm and 595 nm with an absorption spectrometer. As a result, several hybridomas producing antibodies showing high absorbance values were selected.

[0259]

The selected hybridomas were seeded to a 96-well plate at 0.5 cells per well and were cultured. After one week, hybridomas forming single colonies were observed in the wells. The cells in the wells were further cultured, and hybridomas were selected using, as an index, the binding affinity of the antibodies produced by the cloned hybridomas to a CAPRIN-1 protein. A 1 μ g/mL solution of CAPRIN-1 protein prepared by the method described in example 3 of WO2010/016526 was added to a 96-well plate at 100 μ L per well, followed by being left to stand at 4°C for 18 hours. Each well was washed with PBS-T three times, and 400 μ L of a 0.5% BSA solution was added to each well, followed by being left to stand at room temperature for 3 hours. The solution was removed, and each well was washed with 400 μ L of PBS-T three times, and 100 μ L of the hybridoma culture supernatant prepared above was added to each well, followed by being left to stand at room temperature for 2 hours.

Each well was washed with PBS-T three times, and 100 μ L of a HRP-labeled anti-mouse IgG (H+L) antibody (manufactured by life technologies) diluted by 5000-fold with PBS was added to each well, followed by being left to stand at room temperature for 1 hour. Each well was washed with PBS-T three times, and 100 μ L of a TMB substrate solution (manufactured by Thermo Fisher Scientific K.K.) was added to each well, followed by being left to stand for 15 to 30 minutes for color reaction. After the coloring, 100 μ L of 1 N sulfuric acid was added to each well to stop the reaction. The absorbance was measured at 450 nm and 595 nm with an absorption spectrometer. As a result, several hybridoma cell lines producing monoclonal antibodies reactive to the CAPRIN-1 protein were obtained.

[0260]

Subsequently, from the resulting monoclonal antibodies, antibodies reactive to the cell surface of breast cancer cells expressing the CAPRIN-1 were selected. Specifically, 1×10^6 cells of human breast cancer cell line MDA-MB-231V were centrifuged with a 1.5-mL micro centrifugation tube. To the cells was added 100 μ L of the culture supernatant of the above-described hybridomas, followed by being left to stand on ice for 1 hour. After washing with PBS, to the cells was added a FITC-labeled goat anti-mouse IgG antibody (manufactured by life technologies) diluted by 500-fold with PBS containing 0.1% FBS, followed by being left to stand on ice for 1 hour. After washing with PBS, the fluorescence intensity was measured with FACS Calibur available from Becton, Dickinson and Company. Separately, the same procedure as in above was performed as a control using non-treated serum of a 6-week old Balb/c mouse diluted by 500-fold with a hybridoma culturing medium, instead of the antibody. As a result, four monoclonal antibodies (mouse anti-CAPRIN-1 antibodies #30 and #34 to #36) that showed higher fluorescence intensities compared to the control, i.e., reacted with the cell surface of breast cancer cells were selected.

[0261]

(2) Identification of CAPRIN-1 epitope recognized by each mouse anti-CAPRIN-1 monoclonal antibody

The CAPRIN-1 epitope regions recognized by the resulting four monoclonal antibodies were identified. Candidate peptides (93 peptides) each consisting of 12 to 16 amino acids of

the amino acid sequence of the human CAPRIN-1 protein were synthesized and were each dissolved at a concentration of 1 mg/mL in DMSO.

[0262]

Each peptide was dissolved at a concentration of 30 $\mu\text{g/mL}$ in a 0.1 M sodium carbonate buffer (pH 9.6). The solution was added to a 96-well plate (manufactured by Nunc, Product No. 436006) at 100 μL per well, followed by being left to stand at 4°C overnight. The solution was removed, and 200 μL of 10 mM ethanolamine/0.1 M sodium carbonate buffer (pH 9.6) was added to each well, followed by being left to stand at room temperature for 1 hour. The solution was removed, and each well was washed with PBS containing 0.5% Tween 20 (PBST) twice to prepare a peptide-immobilized plate.

[0263]

The cell culture supernatant containing anti-CAPRIN-1 antibody #1 was added to the plate at an amount of 50 μL per well, followed by shaking at room temperature for 1 hour. The solution was removed, and each well was washed with PBST three times. Subsequently, 50 μL of a secondary antibody solution containing a HRP-labeled anti-mouse IgG antibody (manufactured by life technologies) diluted by 3000- to 4000-fold with PBST was added to each well. The solution was removed, and each well was washed with PBST six times.

[0264]

Color reaction was performed by adding 100 μL of a TMB substrate solution (manufactured by Thermo Fisher Scientific K.K.) to each well and leaving the mixture to stand for 15 to 30 minutes. After the coloring, 100 μL of 1 N sulfuric acid was added to each well to stop the reaction. The absorbance was measured at 450 nm and 595 nm with an absorption spectrometer.

[0265]

As a result, a polypeptide set forth in SEQ ID NO: 429 was identified as a partial sequence of CAPRIN-1 recognized by mouse anti-CAPRIN-1 antibody #30; a polypeptide set forth in SEQ ID NO: 431 was identified as a partial sequence of CAPRIN-1 recognized by mouse anti-CAPRIN-1 antibody #34; and a polypeptide set forth in SEQ ID NO: 432 was

identified as a partial sequence of CAPRIN-1 recognized by mouse anti-CAPRIN-1 antibodies #35 and #36.

[0266]

(3) Cloning of gene of variable domain of each mouse anti-CAPRIN-1 monoclonal antibody

The resulting monoclonal antibodies were analyzed for the gene sequence encoding the variable domains and their amino acid sequences in accordance with the method described in example 5 of WO2010/016526.

[0267]

The results demonstrated that mouse anti-CAPRIN-1 antibody #30 comprises a heavy chain variable domain consisting of the amino acid sequence set forth in SEQ ID NO: 344 and a light chain variable domain consisting of the amino acid sequence set forth in SEQ ID NO: 348. The gene sequence encoding the heavy chain variable domain is set forth in SEQ ID NO: 349; and the gene sequence encoding the light chain variable domain is set forth in SEQ ID NO: 350. CDRs 1 to 3 in the heavy chain variable domain consist of the amino acid sequences set forth in SEQ ID NOS: 341, 342, and 343, respectively. CDRs 1 to 3 in the light chain variable domain consist of the amino acid sequences set forth in SEQ ID NOS: 345, 346, and 347, respectively.

[0268]

Furthermore, the results demonstrated that mouse anti-CAPRIN-1 antibody #34 comprises a heavy chain variable domain consisting of the amino acid sequence set forth in SEQ ID NO: 401 and a light chain variable domain consisting of the amino acid sequence set forth in SEQ ID NO: 405. The gene sequence encoding the heavy chain variable domain is set forth in SEQ ID NO: 406; and the gene sequence encoding the light chain variable domain is set forth in SEQ ID NO: 407. CDRs 1 to 3 in the heavy chain variable domain consist of the amino acid sequences set forth in SEQ ID NOS: 398, 399, and 400, respectively. CDRs 1 to 3 in the light chain variable domain consist of the amino acid sequences set forth in SEQ ID NOS: 402, 403, and 404, respectively.

[0269]

The results demonstrated that mouse anti-CAPRN-1 antibody #35 comprises a heavy chain variable domain consisting of the amino acid sequence set forth in SEQ ID NO: 411 and a light chain variable domain consisting of the amino acid sequence set forth in SEQ ID NO: 415. The gene sequence encoding the heavy chain variable domain is set forth in SEQ ID NO: 416; and the gene sequence encoding the light chain variable domain is set forth in SEQ ID NO: 417. CDRs 1 to 3 in the heavy chain variable domain consist of the amino acid sequences set forth in SEQ ID NOs: 408, 409, and 410, respectively. CDRs 1 to 3 in the light chain variable domain consist of the amino acid sequences set forth in SEQ ID NOs: 412, 413, and 414, respectively.

[0270]

The results demonstrated that mouse anti-CAPRN-1 antibody #36 comprises a heavy chain variable domain consisting of the amino acid sequence set forth in SEQ ID NO: 421 and a light chain variable domain consisting of the amino acid sequence set forth in SEQ ID NO: 425. The gene sequence encoding the heavy chain variable domain is set forth in SEQ ID NO: 426; and the gene sequence encoding the light chain variable domain is set forth in SEQ ID NO: 427. CDRs 1 to 3 in the heavy chain variable domain consist of the amino acid sequences set forth in SEQ ID NOs: 418, 419, and 420, respectively. CDRs 1 to 3 in the light chain variable domain consist of the amino acid sequences set forth in SEQ ID NOs: 422, 423, and 424, respectively.

[0271]

(4) Expression analysis of CAPRN-1 protein on gallbladder cancer cell surface using each mouse anti-CAPRN-1 monoclonal antibody

A human gallbladder cancer cell line TGBC14TKB was investigated whether or not a CAPRN-1 protein is expressed on the cell surface. 5×10^5 cells of the gallbladder cancer cell line were centrifuged with a 1.5-mL micro centrifugation tube. The cells were subjected to reaction with each mouse anti-CAPRN-1 antibody at a final concentration of 20 $\mu\text{g/mL}$, followed by being left to stand on ice for 1 hour. After washing with PBS, the cells were reacted with a 100-fold diluted Alexa488-labeled goat anti-mouse IgG antibody (manufactured by life technologies), followed by being left to stand on ice for 30 hours. After washing with

PBS, the fluorescence intensity was measured with FACS Calibur available from Becton, Dickinson and Company. As a negative control, only the secondary antibody was used in the reaction. As a result, the fluorescence intensities in the cells to which the anti-CAPRIN-1 antibody was added were 35% or more higher than that in the control in the gallbladder cancer cell line. This demonstrates that the CAPRIN-1 protein is expressed on the cell surface of the gallbladder cancer cell line.

[0272]

[Example 9] Production of mouse monoclonal antibodies #31 to #33 against CAPRIN-1 protein

(1) Production of mouse anti-CAPRIN-1 antibody #31

A mixture of 100 µg of a human CAPRIN-1 protein comprising the amino acid sequence set forth in SEQ ID NO: 2 prepared in accordance with example 3 of WO2010/016526 mixed with the same quantity of MPL+TDM adjuvant (manufactured by Sigma-Aldrich Co., LLC.) was used as an antigen solution for one mouse. The antigen solution was intraperitoneally administered to 6-week old Balb/c mice (manufactured by Japan SLC, Inc.) and further administered seven times with one week intervals to complete the immunization. The spleen was extracted on the third day from the last immunization and was ground between sterilized two slide glasses and was washed with PBS (-) (manufactured by Nissui Pharmaceutical Co., Ltd.), followed by centrifugation at 1500 rpm for 10 minutes to remove the supernatant. This procedure was repeated three times to obtain spleen cells. The resulting spleen cells and mouse myeloma cells SP2/0 (purchased from ATCC) were mixed at a ratio of 10:1, and a PEG solution prepared by mixing 200 µL of RPMI1640 medium containing 10% FBS and 800 µL of PEG 1500 (manufactured by Boehringer Ingelheim GmbH) and heated to 37°C was added to the resulting mixture, followed by being left to stand for 5 minutes for cell fusion. Centrifugation at 1700 rpm for 5 minutes was performed, and the supernatant was removed. The cells were suspended in a mixture of 150 mL of RPMI1640 medium (HAT selection medium) containing 15% FBS and 2% equivalents of a HAT solution manufactured by Gibco, and the suspension was seeded to 15 plates, which

were 96-well plates (manufactured by Nunc), at 100 μ L per well. Culturing at 37°C in 5% CO₂ for 7 days gave hybridomas by fusion of the spleen cells and the myeloma cells.

[0273]

Hybridomas were selected using, as an index, the binding affinity of the antibodies produced by the hybridomas to a CAPRIN-1 protein. A 1 μ g/mL solution of CAPRIN-1 protein prepared by the method described in example 3 of WO2010/016526 was added to a 96-well plate at 100 μ L per well, followed by being left to stand at 4°C for 18 hours. Each well was washed with PBS-T three times, and 400 μ L of a 0.5% bovine serum albumin (BSA) solution (manufactured by Sigma-Aldrich Co., LLC.) was added to each well, followed by being left to stand at room temperature for 3 hours. The solution was removed, and each well was washed with 400 μ L of PBS-T three times, and 100 μ L of the hybridoma culture supernatant prepared above was added to each well, followed by being left to stand at room temperature for 2 hours. Each well was washed with PBS-T three times, and 100 μ L of a HRP-labeled anti-mouse IgG (H+L) antibody (manufactured by life technologies) diluted by 5000-fold with PBS was added to each well, followed by being left to stand at room temperature for 1 hour. Each well was washed with PBS-T three times, and 100 μ L of a TMB substrate solution (manufactured by Thermo Fisher Scientific K.K.) was added to each well, followed by being left to stand for 15 to 30 minutes for color reaction. After the coloring, 100 μ L of 1 N sulfuric acid was added to each well to stop the reaction. The absorbance was measured at 450 nm and 595 nm with an absorption spectrometer. As a result, several hybridomas producing antibodies showing high absorbance values were selected.

[0274]

The selected hybridomas were seeded to a 96-well plate at 0.5 cells per well and were cultured. After one week, hybridomas forming single colonies were observed in the wells. The cells in the wells were further cultured, and hybridomas were selected using, as an index, the binding affinity of the antibodies produced by the cloned hybridomas to a CAPRIN-1 protein. A 1 μ g/mL solution of the CAPRIN-1 protein prepared by the method described in example 3 of WO2010/016526 was added to a 96-well plate at 100 μ L per well, followed by

being left to stand at 4°C for 18 hours. Each well was washed with PBS-T three times, and 400 µL of a 0.5% BSA solution was added to each well, followed by being left to stand at room temperature for 3 hours. The solution was removed, and each well was washed with 400 µL of PBS-T three times, and 100 µL of the hybridoma culture supernatant prepared above was added to each well, followed by being left to stand at room temperature for 2 hours. Each well was washed with PBS-T three times, and 100 µL of a HRP-labeled anti-mouse IgG (H+L) antibody (manufactured by life technologies) diluted by 5000-fold with PBS was added to each well, followed by being left to stand at room temperature for 1 hour. Each well was washed with PBS-T three times, and 100 µL of a TMB substrate solution (manufactured by Thermo Fisher Scientific K.K.) was added to each well, followed by being left to stand for 15 to 30 minutes for color reaction. After the coloring, 100 µL of 1 N sulfuric acid was added to each well to stop the reaction. The absorbance was measured at 450 nm and 595 nm with an absorption spectrometer. As a result, 61 hybridomas producing monoclonal antibodies reactive to the CAPRIN-1 protein were obtained.

[0275]

Subsequently, from the resulting monoclonal antibodies, antibodies reactive to the cell surface of breast cancer cells expressing CAPRIN-1 were selected. Specifically, 1×10^6 cells of human breast cancer cell line MDA-MB-231V were centrifuged with a 1.5-mL micro centrifugation tube. To the cells was added 100 µL of the culture supernatant of the above-described hybridomas, followed by being left to stand on ice for 1 hour. After washing with PBS, to the cells was added a FITC-labeled goat anti-mouse IgG antibody (manufactured by life technologies) diluted by 500-fold with PBS containing 0.1% FBS, followed by being left to stand on ice for 1 hour. After washing with PBS, the fluorescence intensity was measured with FACS Calibur available from Becton, Dickinson and Company. Separately, the same procedure as in above was performed as a control using non-treated serum of a 6-week old Balb/c mouse diluted by 500-fold with a hybridoma culturing medium, instead of the antibody. As a result, one mouse monoclonal antibody (mouse anti-CAPRIN-1 antibody #31) showing higher fluorescence intensity compared to the control, i.e., reacting with the cell surface of breast cancer cells was selected.

[0276]

(2) Identification of CAPRIN-1 epitope recognized by mouse anti-CAPRIN-1 antibody #31

The CAPRIN-1 epitope region recognized was identified using monoclonal antibody (mouse anti-CAPRIN-1 antibody #31) against CAPRIN-1 reactive to the cell surface of cancer cells obtained in the above (1). Candidate peptides (93 peptides) each consisting of 12 to 16 amino acids of the amino acid sequence of the human CAPRIN-1 protein were synthesized and were each dissolved at a concentration of 1 mg/mL in DMSO.

[0277]

Each peptide was dissolved at a concentration of 30 $\mu\text{g/mL}$ in a 0.1 M sodium carbonate buffer (pH 9.6). The solution was added to a 96-well plate (manufactured by Nunc, Product No. 436006) at 100 μL per well, followed by being left to stand at 4°C overnight. The solution was removed, and 200 μL of 10 mM ethanolamine/0.1 M sodium carbonate buffer (pH 9.6) was added to each well, followed by being left to stand at room temperature for 1 hour. The solution was removed, and each well was washed with PBS containing 0.5% Tween 20 (PBST) twice to prepare a peptide-immobilized plate.

[0278]

The cell culture supernatant containing anti-CAPRIN-1 antibody #31 was added to the plate at an amount of 50 μL per well, followed by shaking at room temperature for 1 hour. The solution was removed, and each well was washed with PBST three times. Subsequently, 50 μL of a secondary antibody solution containing a HRP-labeled anti-mouse IgG antibody (manufactured by life technologies) diluted by 3000- to 4000-fold with PBST was added to each well. The solution was removed, and each well was washed with PBST six times.

[0279]

Color reaction was performed by adding 100 μL of a TMB substrate solution (manufactured by Thermo Fisher Scientific K.K.) to each well and leaving the mixture to stand for 15 to 30 minutes. After the coloring, 100 μL of 1 N sulfuric acid was added to each well to stop the reaction. The absorbance was measured at 450 nm and 595 nm with an absorption spectrometer.

[0280]

As a result, a polypeptide set forth in SEQ ID NO: 430 was identified as a partial sequence of CAPRIN-1 recognized by mouse anti-CAPRIN-1 antibody #31 prepared in the above (1).

[0281]

(3) Production of mouse anti-CAPRIN-1 antibodies #32 and #33

As in the method of the above (1), a fusion protein of a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 430 identified in the above (2) and a carrier protein, Keyhole limpet haemocyanin (KLH), was used as an immunogen; the immunogen was mixed with the same quantity of an adjuvant, TiterMax Gold (registered trademark) (CytRx Corp.); and the mixture was intraperitoneally administered to each mouse four times at 7 days intervals at 100 µg per once. The spleen cells were extracted on the third day from the last immunization. As in the method of the above (1), the spleen cells were fused with mouse myeloma cells to produce hybridomas. Subsequently, antibodies were selected using, as an index, the reactivity of the antibodies contained in the culture supernatants of the resulting hybridomas with a 1 µg/mL solution of CAPRIN-1 protein prepared in example 3 of WO2010/016526 and with a fusion protein of the amino acid sequence set forth in SEQ ID NO: 5 used as the immunogen and a carrier protein, BSA. Specifically, 100 µL of a 1 µg/mL solution of CAPRIN-1 protein prepared in example 3 of WO2010/016526 and 100 µL of a 30 µg/mL solution of the fusion protein of the amino acid sequence set forth in SEQ ID NO: 5 and the carrier protein, BSA, were added to each well of a 96-well plate, followed by being left to stand at 4°C for 18 hours. Each well was washed with PBS-T, and 400 µL of a solution of Block Ace (DS Pharma Biomedical Co., Ltd.) was added to each well, followed by being left to stand at room temperature for 3 hours. The solution was removed, and each well was washed with PBS-T, and 100 µL of the hybridoma culture supernatant prepared above was added to each well, followed by being left to stand at room temperature for 2 hours. Each well was washed with PBS-T, and 100 µL of a HRP-labeled anti-mouse IgG (H+L) antibody (manufactured by life technologies) diluted by 5000-fold with PBS was added to each well, followed by being left to stand at room temperature for 1 hour. Each well was

washed with PBS-T, and 100 μ L of a TMB substrate solution (manufactured by Thermo Fisher Scientific K.K.) was added to each well, followed by being left to stand for 5 to 30 minutes for color reaction. After the coloring, 100 μ L of 1 N sulfuric acid was added to each well to stop the reaction. The absorbance was measured at 450 nm and 595 nm with an absorption spectrometer. As a result, hybridomas producing antibodies showing high absorbance values were selected.

[0282]

The selected hybridomas were seeded to a 96-well plate at 0.3 cells per well and were cultured. After one week, hybridomas forming single colonies were observed in the wells. The cells in the wells were further cultured, and hybridomas producing antibodies against a partial sequence of the CAPRIN-1 protein, the amino acid sequence set forth in SEQ ID NO: 430, were selected using, as an index, the binding affinity of the antibodies produced by the cloned hybridomas to the amino acid sequence set forth in SEQ ID NO: 430.

[0283]

Monoclonal antibodies reactive to the cell surface of breast cancer cells expressing CAPRIN-1 were selected from the monoclonal antibodies produced by the resulting hybridomas. Specifically, 1×10^6 cells of human breast cancer cell line MDA-MB-231V were centrifuged with a 1.5-mL micro centrifugation tube. To the cells was added 100 μ L of the culture supernatant of the above-described hybridomas, followed by being left to stand on ice for 1 hour. After washing with PBS, to the cells was added a FITC-labeled goat anti-mouse IgG antibody (manufactured by life technologies) diluted by 500-fold with PBS containing 0.1% FBS, followed by being left to stand on ice for 1 hour. After washing with PBS, the fluorescence intensity was measured with FACS Calibur available from Becton, Dickinson and Company. Separately, the same procedure as in above was performed as a negative control using non-treated serum of a 6-week old Balb/c mouse diluted by 500-fold with a hybridoma culturing medium instead of the antibody and using only the secondary antibody in the reaction. As a result, two mouse monoclonal antibodies (mouse anti-CAPRIN-1 antibody #32 and mouse anti-CAPRIN-1 antibody #33) showing higher fluorescence intensity

compared to the negative control, i.e., reacting with the cell surface of breast cancer cells were obtained.

[0284]

It was investigated whether or not the resulting mouse anti-CAPRIN-1 antibodies #32 and #33 specifically react with a polypeptide having the amino acid sequence set forth in SEQ ID NO: 430, a partial sequence of CAPRIN-1, used as the immunogen. A solution of 30 $\mu\text{g/mL}$ of the amino acid sequence set forth in SEQ ID NO: 430 or of a partial sequence other than the amino acid sequence set forth in SEQ ID NO: 430 of CAPRIN-1 in an aqueous solution of 0.1 M sodium carbonate was added to a 96-well plate for ELISA, Immobilizer Amino (Nunc), at an amount of 100 $\mu\text{g/mL}$, followed by reaction at 4°C overnight to immobilize the peptide to the well. An aqueous solution of 0.1 M sodium carbonate containing 10 mM ethanolamine was added to the peptide-immobilized wells, followed by being left to stand at room temperature for 1 hour. The solution in the wells was removed. After washing with PBS-T, 400 μL of a Block Ace solution was added to each well, followed by being left to stand at room temperature for 3 hours. The solution in the wells was removed. After washing with PBS-T, 50 μL of the culture supernatant containing mouse anti-CAPRIN-31 #32 or #33 was added to each well, followed by reaction at room temperature for 1 hour. After washing with PBS-T, 50 μL of a HRP-labeled anti-mouse IgG (H+L) antibody (manufactured by life technologies) diluted by 5000-fold with the Block Ace solution was added to each well, followed by being left to stand at room temperature for 1 hour. Each well was sufficiently washed with PBS-T, and 100 μL of a TMB substrate solution (manufactured by Thermo Fisher Scientific K.K.) was added to each well, followed by being left to stand for 5 to 30 minutes for color reaction. After the coloring, 100 μL of 1 N sulfuric acid was added to each well to stop the reaction. The absorbance was measured at 450 nm and 595 nm with an absorption spectrometer. As a result, mouse anti-CAPRIN-1 antibodies #32 and #33 did not react with the partial sequence of CAPRIN-1 not containing the amino acid sequence set forth in SEQ ID NO: 430 and specifically reacted with only the polypeptides comprising the amino acid sequence set forth in SEQ ID NO: 430. This

therefore demonstrated that the polypeptide set forth in SEQ ID NO: 430 comprises an epitope region for the mouse monoclonal antibodies #32 and #33.

[0285]

(4) Characterization of mouse anti-CAPRIN-1 antibodies #31 to #33

From the mouse anti-CAPRIN-1 antibodies #31 to #33 prepared in the above (1) and (3), amplified fragments of the genes encoding variable domains were obtained in accordance with the method described in example 5 of WO2010/016526, and the gene sequences and the amino acid sequences were analyzed. The resulting gene sequence encoding the heavy chain variable domain of the mouse anti-CAPRIN-1 antibody #31 is set forth in SEQ ID NO: 381, and its amino acid sequence is set forth in SEQ ID NO: 376. The gene sequence encoding the light chain variable domain is set forth in SEQ ID NO: 382, and its amino acid sequence is set forth in SEQ ID NO: 380. Similarly, the resulting gene sequence encoding the heavy chain variable domain of the mouse anti-CAPRIN-1 antibody #32 is set forth in SEQ ID NO: 391, and its amino acid sequence is set forth in SEQ ID NO: 386. The gene sequence encoding the light chain variable domain is set forth in SEQ ID NO: 392, and its amino acid sequence is set forth in SEQ ID NO: 390. The resulting gene sequence encoding the heavy chain variable domain of the mouse anti-CAPRIN-1 antibody #33 is set forth in SEQ ID NO: 397, and its amino acid sequence is set forth in SEQ ID NO: 396. The gene sequence encoding the light chain variable domain is set forth in SEQ ID NO: 392, and its amino acid sequence is set forth in SEQ ID NO: 390.

[0286]

In addition, it was confirmed that CDRs 1 to 3 in the heavy chain variable domain of mouse anti-CAPRIN-1 antibody #31 consist of the amino acid sequences set forth in SEQ ID NOs: 373, 374, and 375, respectively, and that CDRs 1 to 3 in the light chain variable domain consist of the amino acid sequences set forth in SEQ ID NOs: 377, 378, and 379, respectively. Similarly, it was confirmed that CDRs 1 to 3 in the heavy chain variable domain of mouse anti-CAPRIN-1 antibody #32 consist of the amino acid sequences set forth in SEQ ID NOs: 383, 384, and 385, respectively, and that CDRs 1 to 3 in the light chain variable domain consist of the amino acid sequences set forth in SEQ ID NOs: 387, 388, and 389, respectively.

It was also confirmed that CDRs 1 to 3 in the heavy chain variable domain of mouse anti-CAPRIN-1 antibody #33 consist of the amino acid sequences set forth in SEQ ID NOs: 393, 394, and 395, respectively, and that CDRs 1 to 3 in the light chain variable domain consist of the amino acid sequences set forth in SEQ ID NOs: 387, 388, and 389, respectively.

[0287]

[Example 10] Expression analysis of CAPRIN-1 protein on gallbladder cancer cell surface using mouse anti-CAPRIN-1 monoclonal antibodies #30 to #36

A human gallbladder cancer cell line TGBC14TKB was investigated whether or not a CAPRIN-1 protein is expressed on the cell surface using mouse anti-CAPRIN-1 monoclonal antibodies #30 to #36. 5×10^5 cells of TGBC14TKB were centrifuged with a 1.5-mL micro centrifugation tube. The cells were subjected to reaction with each of mouse anti-CAPRIN-1 antibodies #30 to #36 at a final concentration of 20 $\mu\text{g/mL}$, followed by being left to stand on ice for 1 hour. After washing with PBS, the cells were reacted with a 100-fold diluted Alexa488-labeled goat anti-mouse IgG antibody (manufactured by life technologies), followed by being left to stand on ice for 30 hours. After washing with PBS, the fluorescence intensity was measured with FACS Calibur available from Becton, Dickinson and Company. Separately, the cells were subjected to reaction with only the secondary antibody as a negative control. As a result, the fluorescence intensities in TGBC14TKB to which mouse anti-CAPRIN-1 monoclonal antibodies #30 to #36 were added were 35% or more higher than that in the control. This demonstrates that the CAPRIN-1 protein is expressed on the cell membrane surface of the gallbladder cancer cell line.

[0288]

[Example 11] Production of human-mouse chimeric anti-CAPRIN-1 antibody

Both terminals of an amplified fragment of the gene comprising the heavy chain variable domain of each of mouse anti-CAPRIN-1 antibodies #30 to #36 were treated with restriction enzymes, and the fragment was purified and was inserted into pcDNA4/myc-His vector (manufactured by life technologies) containing a leader sequence derived from a mouse antibody and the H-chain constant domain of human IgG₁ comprising the amino acid sequence set forth in SEQ ID NO: 264, in accordance with a usual method. Both terminals of an

amplified fragment of the gene comprising the light chain variable domain of each of mouse anti-CAPRIN-1 antibodies #30 to #36 were treated with restriction enzymes, and the fragment was purified and was inserted into pcDNA4/myc-His vector (manufactured by life technologies) containing a leader sequence derived from a mouse antibody and the L-chain constant domain of human IgG₁ comprising the amino acid sequence set forth in SEQ ID NO: 265, in accordance with a usual method.

[0289]

Subsequently, the recombinant vector containing the heavy chain variable domain of any of mouse anti-CAPRIN-1 antibodies #30 to #36 and the recombinant vector containing the light chain variable domain of the mouse anti-CAPRIN-1 antibody were introduced into CHO-K1 cells (obtained from Riken Cell Bank). Specifically, 2×10^5 CHO-K1 cells cultured in each well, of a 12-well culture plate, containing 1 mL of Ham's F12 medium (manufactured by life technologies) containing 10% FBS were washed with PBS (-). To each well were added 1 mL of fresh Ham's F12 medium containing 10% FBS and a mixture of 30 μ L of OptiMEM (manufactured by life technologies) containing 250 ng of each of the above-mentioned vectors and 30 μ L of Polyfect transfection reagent (manufactured by Qiagen). The CHO-K1 cells introduced with the recombinant vectors were cultured in Ham's F12 medium containing 10% FBS, 200 μ g/mL Zeocin (manufactured by life technologies), and 200 μ g/mL Geneticin (manufactured by Roche) and were then seeded to a 96-well plate at 0.5 cells per well to produce cell lines stably producing human-mouse chimeric anti-CAPRIN-1 antibodies #30 to #36 comprising the variable domains of the mouse anti-CAPRIN-1 antibodies #30 to #36.

[0290]

The produced cell lines were each cultured at 5×10^5 cells/mL in a 150-cm² flask containing 30 mL of serum-free OptiCHO medium (manufactured by life technologies) for 5 days to obtain culture supernatants containing human-mouse chimeric anti-CAPRIN-1 antibody #30 to #36, respectively.

[0291]

[Example 12] Antitumor activity (ADCC activity) of anti-CAPRIN-1 antibodies on gallbladder cancer cells

In order to evaluate the intensity of the cytotoxicity, on gallbladder cancer cells expressing CAPRIN-1, of antibodies against peptides derived from CAPRIN-1 set forth in SEQ ID NOs: 429 to 432, ADCC activity was measured using human-mouse chimeric anti-CAPRIN-1 antibodies #30 to #36. 1×10^5 cells of each of the gallbladder cancer cell line TGBC14TKB were collected in a 50-mL micro centrifugation tube and were incubated with 100 μ Ci of 51 chromium at 37°C for 2 hours. Subsequently, the cells were washed with RPMI1640 medium containing 10% fetal bovine serum three times. Separately, any of human-mouse chimeric anti-CAPRIN-1 antibodies #30 to #36 was added to each well of a 96-well V-bottom plate at a final concentration of 5 μ g/mL, and 2×10^5 human NK cells separated from human peripheral blood lymphocytes as effector cells by a usual method were added to each well. To each well were added 2×10^3 gallbladder cancer cells with 51 chromium prepared above, and the mixture was cultured for 4 hours. The amount of 51 chromium released into the medium after the culturing, and the cytotoxicity on gallbladder cancer cells was calculated by the following calculation formula *:

[0292]

Formula *: cytotoxicity (%) = (amount of 51 chromium released from target cells in the presence of antibody against CAPRIN-1 and lymphocytes)/(amount of 51 chromium released from target cells in the presence of 1 N hydrochloric acid) $\times$ 100.

[0293]

As a result, every human-mouse chimeric anti-CAPRIN-1 antibody showed 20% or more activity on gallbladder cancer cells, whereas the activity of a human IgG₁ antibody used as the negative control was less than 7% on gallbladder cancer cells.

[0294]

[Example 13] Production of anti-CAPRIN-1 monoclonal antibody using rabbit

(1) Production of rabbit anti-CAPRIN-1 monoclonal antibody #1

A mixture of 300 μ g of an antigen protein (human CAPRIN-1) mixed with the same quantity of a complete Freund's adjuvant was used as an antigen solution for one rabbit. In the second and subsequent immunization, a mixture with an incomplete Freund's adjuvant was used. The antigen solution was intraperitoneally administered to 7-week old rabbits and

further administered seven times with four weeks intervals to complete the immunization. The spleen was extracted on the fourth day from the last immunization and was ground between sterilized two slide glasses and was washed with PBS (-) (manufactured by Nissui Pharmaceutical Co., Ltd.), followed by centrifugation at 1500 rpm for 10 minutes to remove the supernatant. This procedure was repeated three times to obtain spleen cells. The resulting spleen cells and rabbit myeloma cells were mixed at a ratio of 5:1, and a PEG solution prepared by mixing 200 μ L of IMDM medium containing 10% FBS and 800 μ L of PEG 1500 (manufactured by Boehringer Ingelheim GmbH) and heated to 37°C was added to the resulting mixture, followed by being left to stand for 5 minutes for cell fusion. Centrifugation at 1700 rpm for 5 minutes was performed, and the supernatant was removed. The cells were suspended in a mixture of 300 mL of IMDM medium (HAT selection medium) containing 10% FBS and 2% equivalents of a HAT solution manufactured by Gibco, and the suspension was seeded to 30 plates, which were 96-well plates (manufactured by Nunc), at 100 μ L per well. Culturing at 37°C in 5% CO₂ for 7 days gave hybridomas of the spleen cells and the rabbit myeloma cells.

[0295]

Hybridomas were selected using, as an index, the reactivity of the antibodies produced by the hybridomas to a CAPRIN-1 protein. A 1 μ g/mL solution of CAPRIN-1 protein was added to a 96-well plate at 100 μ L per well, followed by being left to stand at 4°C for 18 hours. Each well was washed with PBS-T three times, and 400 μ L of a 0.5% bovine serum albumin (BSA) solution (manufactured by Sigma-Aldrich Co., LLC.) was added to each well, followed by being left to stand at room temperature for 3 hours. The solution was removed, and each well was washed with 400 μ L of PBS-T three times, and 100 μ L of the hybridoma culture supernatant prepared above was added to each well, followed by being left to stand at room temperature for 2 hours. Each well was washed with PBS-T three times, and 100 μ L of a HRP-labeled anti-rabbit antibody diluted by 5000-fold with PBS was added to each well, followed by being left to stand at room temperature for 1 hour. Each well was washed with PBS-T three times, and 100 μ L of a TMB substrate solution (manufactured by Thermo Fisher Scientific K.K.) was added to each well, followed by being left to stand for 15 to 30 minutes

for color reaction. After the coloring, 100 μ L of 1 N sulfuric acid was added to each well to stop the reaction. The absorbance was measured at 450 nm and 595 nm with an absorption spectrometer. As a result, several hybridomas producing antibodies showing high absorbance values were selected.

[0296]

The selected hybridomas were seeded to a 96-well plate at 0.5 cells per well and were cultured. After one week, hybridomas forming single colonies were observed in the wells. The cells in the wells were further cultured, and hybridomas were selected using, as an index, the reactivity of the antibodies produced by the cloned hybridomas to a CAPRIN-1 protein. A 1 μ g/mL solution of the CAPRIN-1 protein was added to a 96-well plate at 100 μ L per well, followed by being left to stand at 4°C for 18 hours. Each well was washed with PBS-T three times, and 400 μ L of a 0.5% BSA solution was added to each well, followed by being left to stand at room temperature for 3 hours. The solution was removed, and each well was washed with 400 μ L of PBS-T three times, and 100 μ L of the hybridoma culture supernatant prepared above was added to each well, followed by being left to stand at room temperature for 2 hours. Each well was washed with PBS-T three times, and 100 μ L of a HRP-labeled anti-rabbit IgG antibody diluted by 5000-fold with PBS was added to each well, followed by being left to stand at room temperature for 1 hour. Each well was washed with PBS-T three times, and 100 μ L of a TMB substrate solution (manufactured by Thermo Fisher Scientific K.K.) was added to each well, followed by being left to stand for 15 to 30 minutes for color reaction. After the coloring, 100 μ L of 1 N sulfuric acid was added to each well to stop the reaction. The absorbance was measured at 450 nm and 595 nm with an absorption spectrometer. As a result, several hybridomas producing monoclonal antibodies reactive to the CAPRIN-1 protein were obtained.

[0297]

Subsequently, monoclonal antibodies reactive to the cell surface of cancer cells expressing CAPRIN-1 were selected from the rabbit monoclonal antibodies reactive to the CAPRIN-1 protein. Specifically, 2×10^5 cells of human breast cancer cell line MDA-MB-231V and of human lung cancer cell line QG56 were centrifuged with a 1.5-mL micro

centrifugation tube. To the cells was added 100 μ L of the culture supernatant of the above-described hybridomas, followed by being left to stand on ice for 1 hour. After washing with PBS, to the cells was added a FITC-labeled anti-rabbit IgG (H+L) antibody or Alexa488-labeled anti-rabbit IgG (H+L) diluted by 100-fold with PBS (-) containing 0.05% FBS, followed by being left to stand on ice for 1 hour. After washing with PBS, the fluorescence intensity was measured with FACS Calibur available from Becton, Dickinson and Company. Separately, the same procedure as in above was performed using a hybridoma culturing medium to prepare a negative control sample. As a result, one rabbit anti-CAPRIN-1 monoclonal antibody (rabbit anti-CAPRIN-1 monoclonal antibody #1) showing higher fluorescence intensity compared to the negative control, i.e., reacting with the cell surface of cancer cell lines MDA-MB-231 and QG56 expressing CAPRIN-1 was selected.

[0298]

Subsequently, the CAPRIN-1 epitope recognized by the selected rabbit anti-CAPRIN-1 monoclonal antibody #1 was identified. Candidate peptides (93 peptides) each consisting of 12 to 16 amino acids of the amino acid sequence of the human CAPRIN-1 protein were synthesized and were each dissolved at a concentration of 1 mg/mL in DMSO. Each peptide was dissolved at a concentration of 30 μ g/mL in a 0.1 M sodium carbonate buffer (pH 9.6). The solution was added to a 96-well plate (manufactured by Nunc, Product No. 436006) at 100 μ L per well, followed by being left to stand at 4°C overnight. The solution was removed, and 200 μ L of 10 mM ethanolamine/0.1 M sodium carbonate buffer (pH 9.6) was added to each well, followed by being left to stand at room temperature for 1 hour. The solution was removed, and each well was washed with PBS containing 0.5% Tween 20 (PBST) twice to prepare a peptide-immobilized plate. For confirmation, this plate was also provided with a well to which the CAPRIN-1 protein was immobilized in accordance with the method described above. Rabbit anti-CAPRIN-1 monoclonal antibody #1 purified to a concentration of 0.1 μ g/mL by a usual method was added to the plate at an amount of 50 μ L per well, followed by shaking at room temperature for 1 hour. The solution was removed, and each well was washed with PBST three times. Subsequently, 50 μ L of a secondary antibody solution containing a HRP-labeled anti-rabbit IgG antibody diluted by 3000- to 4000-fold with

PBST was added to each well. The solution was removed, and each well was washed with PBST six times. Color reaction was performed by adding 100 μ L of a TMB substrate solution (manufactured by Thermo Fisher Scientific K.K.) to each well and leaving the mixture to stand for 15 to 30 minutes. After the coloring, 100 μ L of 1 N sulfuric acid was added to each well to stop the reaction. The absorbance was measured at 450 nm and 595 nm with an absorption spectrometer. As a result, rabbit anti-CAPRIN-1 monoclonal antibody, rabbit anti-CAPRIN-1 monoclonal antibody #1 was reactive to only the polypeptides comprising the amino acid sequence set forth in SEQ ID NO: 430 in 93 peptides synthesized as partial peptides of CAPRIN-1 and was not reactive to other polypeptides. In addition, rabbit anti-CAPRIN-1 monoclonal antibody #1 was specifically reactive to the CAPRIN-1 protein. This result remonstrated that an epitope for rabbit anti-CAPRIN-1 monoclonal antibody #1 is contained in the polypeptide set forth in SEQ ID NO: 430.

[0299]

Subsequently, from rabbit anti-CAPRIN-1 monoclonal antibody #1 prepared above, an amplified fragment of the gene encoding the variable domain was obtained in accordance with the method described in example 5 of WO2010/016526, and the gene sequence and the amino acid sequence were analyzed. Specifically, mRNA was extracted from the hybridoma producing rabbit anti-CAPRIN-1 monoclonal antibody #1, and the genes of the heavy-chain variable (VH) domain and the light-chain variable (VL) domain of the antibody were obtained by RT-PCR using primers specific to the rabbit variable domain sequences. In order to determine the sequence, the genes were cloned into pCR2.1 vectors (manufactured by life technologies). The gene sequences of the VH domain and the VL domain in each plasmid prepared by cloning were determined using M13 forward primer and M13 reverse primer with a fluorescence sequencer.

[0300]

The results demonstrated that the resulting rabbit anti-CAPRIN-1 monoclonal antibody #1 comprises a heavy chain variable domain set forth in SEQ ID NO: 359 in which CDRs 1 to 3 consist of amino acid sequences set forth in SEQ ID NOs: 351, 352, and 353, respectively,

and a light chain variable domain set forth in SEQ ID NO: 361 in which CDRs 1 to 3 consist of amino acid sequences set forth in SEQ ID NOs: 354, 355, and 356, respectively.

[0301]

(2) Production of human-rabbit chimeric anti-CAPRIN-1 antibody #1

The gene set forth in SEQ ID NO: 358 for expressing the heavy chain variable domain of the rabbit anti-CAPRIN-1 monoclonal antibody #1 prepared above and the gene set forth in SEQ ID NO: 360 for expressing the light chain variable domain were respectively inserted into a mammalian cell expression vector containing human IgG₁ heavy chain constant domain and a mammalian cell expression vector containing human IgG₁ light chain constant domain. A culture supernatant containing human-rabbit chimeric anti-CAPRIN-1 antibody #1 humanized by introducing the produced two recombinant expression vectors into mammalian cells in accordance with a usual method was obtained.

[0302]

(3) Antigen specificity, reactivity with cancer cells, and antitumor activity of human-rabbit chimeric anti-CAPRIN-1 antibody #1

Human-rabbit chimeric anti-CAPRIN-1 antibody #1 contained in the culture supernatant prepared in the above (2) was purified using Hitrap Protein A Sepharose FF (manufactured by GE Healthcare Bio-Sciences) in accordance with a usual method, substituted with PBS (-), and filtered through a filter of 0.22 μ m (manufactured by Millipore Corporation), and was used for investigation of the antigen specificity, reactivity with cancer cells, and antitumor effect.

[0303]

First, as in the above (1), the reaction specificity of human-rabbit chimeric anti-CAPRIN-1 antibody #1 on the CAPRIN-1 protein and a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 430, which is the epitope for rabbit anti-CAPRIN-1 monoclonal antibody #1, was investigated. The results demonstrated that human-rabbit chimeric anti-CAPRIN-1 antibody #1 had reaction specificity on the CAPRIN-1 protein and the polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 430, as in rabbit anti-CAPRIN-1 monoclonal antibody #1.

[0304]

Next, the reactivity of human-rabbit chimeric anti-CAPRIN-1 antibody #1 to the CAPRIN-1 protein on the cell surface of a gallbladder cancer cell line TGBC14TKB was investigated. 1×10^6 cells of each cell line were centrifuged with a 1.5-mL micro centrifugation tube. To the cells was added the cell culture supernatant (100 μ L) containing the antibody, followed by being left to stand on ice for 1 hour. After washing with PBS, to the cells was added an Alexa488-labeled goat anti-human IgG (H+L) antibody (manufactured by life technologies) diluted by 100-fold with PBS containing 0.1% FBS, followed by being left to stand at 4°C for 60 minutes. After washing with PBS (-), the fluorescence intensity was measured with FACS Calibur available from Becton, Dickinson and Company. Separately, the cells were reacted with only the secondary antibody as a negative control. As a result, human-rabbit chimeric anti-CAPRIN-1 antibody #1 showed higher reactivity by 30% or more higher fluorescence intensity compared to the negative control. This demonstrated that a part of the CAPRIN-1 protein set forth in SEQ ID NO: 430 was expressed on the cell surface of the human cancer cell lines. The rate of increase in the fluorescence intensity is represented by the rate of increase in the mean fluorescence intensity (MFI value) in each cell and is calculated by the following calculation formula: Rate of increase in mean fluorescence intensity (rate of increase in fluorescence intensity) (%) = ((MFI value of cells reacted with anti-human CAPRIN-1 antibody) - (MFI value of control))/(MFI value of control) $\times$ 100.

[0305]

Furthermore, the antitumor activity of human-rabbit chimeric anti-CAPRIN-1 antibody #1 on a gallbladder cancer cell line TGBC14TKB was evaluated. 1×10^6 cells of the gallbladder cancer cell line were collected in a 50-mL micro centrifugation tube and were incubated with 100 μ Ci of 51 chromium at 37°C for 2 hours. Subsequently, the cells were washed with RPMI1640 medium containing 10% FBS three times to prepare target cells. Purified human-rabbit chimeric anti-CAPRIN-1 antibody #1 was added to a 96-well V-bottom plate at a final concentration of 5 μ g/mL. Subsequently, 2×10^5 human NK cells separated from human peripheral blood lymphocytes prepared in accordance with a usual method were added to each well. 2×10^3 target cells were mixed with the antibody in each well of the 96-

well V-bottom plate, followed by culturing at 37°C in 5% CO₂ for 4 hours. After the culturing, the amount of ⁵¹chromium secreted from the impaired tumor cells into the culture supernatant was measured to calculate the cytotoxicity on the gallbladder cancer cells by the anti-CAPRIN-1 antibody. Separately, the reaction was performed using an iso-type control antibody as a negative control. As a result, human-rabbit chimeric anti-CAPRIN-1 antibody #1 showed antitumor activity of 25% or more on gallbladder cancer cells, whereas the cytotoxicity in the case of using the iso-type control antibody was less than 5% on gallbladder cancer cells. These results revealed that the antibody against the peptide derived from CAPRIN-1 set forth in SEQ ID NO: 430, human-rabbit chimeric anti-CAPRIN-1 antibody #1, shows antitumor activity on the gallbladder cancer cells expressing CAPRIN-1 through ADCC activity.

[0306]

[Example 14] Production of humanized anti-CAPRIN-1 antibodies #1 to #3

A humanized antibody of rabbit anti-CAPRIN-1 antibody was produced. Based on the information of the amino acid sequence of the heavy chain variable domain of rabbit anti-CAPRIN-1 monoclonal antibody #1, the nucleotide sequence set forth in SEQ ID NO: 362 was designed such that CDRs 1 to 3 in the heavy chain variable domain consist of the amino acid sequences set forth in SEQ ID NOs: 351, 352, and 357, respectively, and that the framework region can express a heavy chain variable domain (SEQ ID NO: 363) comprising the sequence of a human antibody. The nucleotide sequence was inserted into a mammalian cell expression vector containing the heavy chain constant domain of human IgG₁. Similarly, the nucleotide sequence set forth in SEQ ID NO: 364 was designed such that CDRs 1 to 3 in the light chain variable domain consist of the amino acid sequences set forth in SEQ ID NOs: 354, 355, and 356, respectively, and that the framework region can express a light chain variable domain (SEQ ID NO: 365) comprising the sequence of a human antibody. The nucleotide sequence was inserted into a mammalian cell expression vector containing the light chain constant domain of human IgG₁. The two recombinant expression vectors were introduced into mammalian cells in accordance with a usual method to obtain a culture supernatant containing humanized anti-CAPRIN-1 antibody #1.

[0307]

In addition, based on the information of the amino acid sequence of the heavy chain variable domain of rabbit anti-CAPRIN-1 monoclonal antibody #1, the nucleotide sequence set forth in SEQ ID NO: 367 was designed such that CDRs 1 to 3 consist of the amino acid sequences set forth in SEQ ID NOs: 351, 352, and 353, respectively, and that the framework region can express a heavy chain variable domain (SEQ ID NO: 368) comprising the sequence of a human antibody. The nucleotide sequence was inserted into a mammalian cell expression vector containing the heavy chain constant domain of human IgG₁. Similarly, the nucleotide sequence set forth in SEQ ID NO: 369 was designed such that CDRs 1 to 3 in the light chain variable domain consist of the amino acid sequences set forth in SEQ ID NOs: 354, 355, and 356, respectively, and that the framework region can express a light chain variable domain (SEQ ID NO: 370) comprising the sequence of a human antibody. The nucleotide sequence was inserted into a mammalian cell expression vector containing the light chain constant domain of human IgG₁. The two recombinant expression vectors were introduced into mammalian cells in accordance with a usual method to obtain a culture supernatant containing humanized anti-CAPRIN-1 antibody #2.

[0308]

In addition, based on the information of the amino acid sequence of the heavy chain variable domain of rabbit anti-CAPRIN-1 monoclonal antibody #1, the nucleotide sequence set forth in SEQ ID NO: 371 was designed such that CDRs 1 to 3 consist of the amino acid sequences set forth in SEQ ID NOs: 351, 352, and 353, respectively, and that the framework region can express a heavy chain variable domain (SEQ ID NO: 372) comprising the sequence of a human antibody. The nucleotide sequence was inserted into a mammalian cell expression vector containing the heavy chain constant domain of human IgG₁. Similarly, the nucleotide sequence set forth in SEQ ID NO: 369 was designed such that CDRs 1 to 3 in the light chain variable domain consist of the amino acid sequences set forth in SEQ ID NOs: 354, 355, and 356, respectively, and that the framework region can express a light chain variable domain (SEQ ID NO: 370) comprising the sequence of a human antibody. The nucleotide sequence was inserted into a mammalian cell expression vector containing the light chain

constant domain of human IgG₁. The two recombinant expression vectors were introduced into mammalian cells in accordance with a usual method to obtain a culture supernatant containing humanized anti-CAPRIN-1 antibody #3.

[0309]

Antigen specificity, reactivity with cancer cells, and antitumor activity of humanized anti-CAPRIN-1 antibody

The reactivity with CAPRIN-1 of the three humanized anti-CAPRIN-1 antibodies #1 to #3 prepared above was evaluated. As a result, the reactivity of these antibodies with the CAPRIN-1 protein, the epitope peptide set forth in SEQ ID NO: 430, and gallbladder cancer cell lines was equivalent to that of human-rabbit chimeric anti-CAPRIN-1 monoclonal antibody #1. The antitumor activity on gallbladder cancer cell lines of these three humanized anti-CAPRIN-1 antibodies was also evaluated. The results demonstrated that the antitumor activity of every antibody was equivalent to that of the human-rabbit chimeric anti-CAPRIN-1 monoclonal antibody #1.

[0310]

[Example 15] Expression analysis of CAPRIN-1 protein on gallbladder cancer cell surface using mouse anti-CAPRIN-1 monoclonal antibodies #23 to #29

As in Example 10, a human gallbladder cancer cell line TGBC14TKB was investigated whether or not a CAPRIN-1 protein is expressed on the cell surface using anti-CAPRIN-1 monoclonal antibody #23 comprising a heavy chain variable domain set forth in SEQ ID NO: 279 comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 276, 277, and 278, respectively and a light chain variable domain set forth in SEQ ID NO: 283 comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 280, 281, and 282, respectively prepared in WO/2013/018894; anti-CAPRIN-1 monoclonal antibody #24 comprising a heavy chain variable domain set forth in SEQ ID NO: 279 comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 276, 277, and 278, respectively and a light chain variable domain set forth in SEQ ID NO: 289 comprising complementarity determining

regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 286, 287, and 288, respectively; anti-CAPRIN-1 monoclonal antibody #25 comprising a heavy chain variable domain set forth in SEQ ID NO: 294 comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 291, 292, and 293, respectively and a light chain variable domain set forth in SEQ ID NO: 298 comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 295, 296, and 297, respectively; anti-CAPRIN-1 monoclonal antibody #26 comprising a heavy chain variable domain set forth in SEQ ID NO: 304 comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 301, 302, and 303, respectively and a light chain variable domain set forth in SEQ ID NO: 308 comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 305, 306, and 307, respectively prepared in WO/2013/018894; anti-CAPRIN-1 monoclonal antibody #27 comprising a heavy chain variable domain set forth in SEQ ID NO: 314 comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 311, 312, and 313, respectively and a light chain variable domain set forth in SEQ ID NO: 318 comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 315, 316, and 317, respectively prepared in WO/2013/018891; anti-CAPRIN-1 monoclonal antibody #28 comprising a heavy chain variable domain set forth in SEQ ID NO: 324 comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 321, 322, and 323, respectively and a light chain variable domain set forth in SEQ ID NO: 328 comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 325, 326, and 327, respectively prepared in WO/2013/018889; and anti-CAPRIN-1 monoclonal antibody #29 comprising a heavy chain variable domain set forth in SEQ ID NO: 334 comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 331, 332, and 333, respectively and a light chain variable domain set forth in

SEQ ID NO: 338 comprising complementarity determining regions (CDR1, CDR2, and CDR3) consisting of amino acid sequences set forth in SEQ ID NOs: 335, 336, and 337, respectively prepared in WO/2013/018883. As a result, reactivity with gallbladder cancer cell lines equivalent to those of mouse anti-CAPRIN-1 monoclonal antibodies #30 to #36 in Example 10 was observed.

[0311]

[Example 16] Antitumor activity on gallbladder cancer cells of human-mouse chimeric anti-CAPRIN-1 antibodies #23 to #29

Cell lines stably producing human-mouse chimeric anti-CAPRIN-1 antibodies #23 to #29 respectively having the variable domains of mouse anti-CAPRIN-1 antibodies #23 to #29 described in Example 15 were produced by a method similar to that in Example 11 to obtain culture supernatants containing human-mouse chimeric anti-CAPRIN-1 antibodies #23 to #29. The antibodies purified from the supernatants by a usual method were used for investigation of antitumor activity on gallbladder cancer cells. In order to evaluate the intensity of cytotoxicity on gallbladder cancer cells expressing CAPRIN-1, ADCC activity was measured using human-mouse chimeric anti-CAPRIN-1 antibodies #23 to #29. The ADCC activity on a gallbladder cancer cell line TGBC14TKB was evaluated by a method similar to that in Example 12. As a result, every human-mouse chimeric anti-CAPRIN-1 antibody showed 20% or more activity on the gallbladder cancer cell line TGBC14TKB, whereas the activity of a human IgG₁ antibody used as the negative control was less than 5% on gallbladder cancer cells.

Industrial Applicability

[0312]

The antibody of the present invention is useful for treatment and/or prevention of gallbladder cancer.

SEQUENCE LISTING IN ELECTRONIC FORM

In accordance with Section 111(1) of the Patent Rules, this description contains a sequence listing in electronic form in ASCII text format (file: 55232-52 Seq 17-09-14 v1.txt).

A copy of the sequence listing in electronic form is available from the Canadian Intellectual Property Office.

CLAIMS:

1. A pharmaceutical composition for use in treating gallbladder cancer,

wherein the composition comprises an antibody or a fragment thereof and a pharmacologically acceptable carrier or medium,

wherein the antibody and fragment thereof bind specifically to:

a CAPRIN-1 protein that is expressed on the cell surface of the gallbladder cancer and that comprises an amino acid sequence set forth in any one of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30, or

a CAPRIN-1 protein that is expressed on the cell surface of the gallbladder cancer and that comprises an amino acid sequence having a sequence identity of 80% or more to the full length of the amino acid sequence set forth in any one of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30, or

a CAPRIN-1 protein fragment comprising at least seven consecutive amino acid residues of the amino acid sequence set forth in any one of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30, or in an amino acid sequence having a sequence identity of 80% or more to the full length of the amino acid sequence set forth in any one of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30, wherein the at least seven consecutive amino acid residues are expressed on the cell surface of the gallbladder cancer.

2. The pharmaceutical composition for use according to Claim 1, wherein the CAPRIN-1 protein fragment comprises at least seven consecutive amino acid residues in the region of amino acid residue positions from 233 to 343, amino acid residue positions from 512 to the C-terminus, or amino acid residue positions from 50 to 98 of the amino acid sequence set forth in any one of SEQ ID NO: 2, 4, 8, 10, 12, 14, 16, 20, 22, 24, 26, 28, or 30.

3. The pharmaceutical composition for use according to Claim 1 or 2, wherein the CAPRIN-1 protein fragment comprises at least seven consecutive amino acid residues in an amino acid sequence set forth in SEQ ID NO: 267, SEQ ID NO: 429, SEQ ID NO: 428,

SEQ ID NO: 273, SEQ ID NO: 266, SEQ ID NO: 270, SEQ ID NO: 272, SEQ ID NO: 269, SEQ ID NO: 430, SEQ ID NO: 431, or SEQ ID NO: 432 or in an amino acid sequence having a sequence identity of 80% or more to the full length of the amino acid sequence set forth in any one of SEQ ID NO: 267, 429, 428, 273, 266, 270, 272, 269, 430, 431, or 432.

4. The pharmaceutical composition for use according to any one of Claims 1 to 3, wherein the antibody is a monoclonal antibody or a polyclonal antibody.

5. The pharmaceutical composition for use according to any one of Claims 1 to 4, wherein the antibody is a human antibody, a humanized antibody, a chimeric antibody, a single-chain antibody, or a multispecific antibody.

6. The pharmaceutical composition for use according to any one of Claims 1 to 5, wherein the antibody and fragment thereof bind specifically to the CAPRIN-1 protein or to the CAPRIN-1 protein fragment, wherein the antibody comprises a heavy chain variable domain and a light chain variable domain, and wherein the sequences of the heavy chain complementarity determining regions (CDR1, CDR2, and CDR3) and the sequences of the light chain CDR1, CDR2, and CDR3 consist of the sequences set forth in the following (a) to (ao):

(a) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 37, 38, and 39, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 41, 42, and 43, respectively;

(b) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 47, 48, and 49, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 51, 52, and 53, respectively;

(c) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 57, 58, and 59, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 61, 62, and 63, respectively;

- (d) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 67, 68, and 69, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 71, 72, and 73, respectively;
- (e) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 77, 78, and 79, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 81, 82, and 83, respectively;
- (f) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 87, 88, and 89, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 91, 92, and 93, respectively;
- (g) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 97, 98, and 99, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 101, 102, and 103, respectively;
- (h) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 107, 108, and 109, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 111, 112, and 113, respectively;
- (i) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 117, 118, and 119, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 121, 122, and 123, respectively;
- (j) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 127, 128, and 129, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 121, 122, and 123, respectively;
- (k) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 132, 133, and 134, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 136, 137, and 138, respectively;

- (l) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 142, 143, and 144, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 146, 147, and 148, respectively;
- (m) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 142, 143, and 144, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 152, 153, and 154, respectively;
- (n) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 157, 158, and 159, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 161, 162, and 163, respectively;
- (o) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 167, 168, and 169, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 171, 172, and 173, respectively;
- (p) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 167, 168, and 169, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 177, 178, and 179, respectively;
- (q) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 167, 168, and 169, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 182, 183, and 184, respectively;
- (r) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 167, 168, and 169, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 187, 188, and 189, respectively;
- (s) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 167, 168, and 169, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 192, 193, and 194, respectively;

- (t) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 197, 198, and 199, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 201, 202, and 203, respectively;
- (u) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 207, 208, and 209, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 211, 212, and 213, respectively;
- (v) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 217, 218, and 219, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 221, 222, and 223, respectively;
- (w) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 227, 228, and 229, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 231, 232, and 233, respectively;
- (x) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 237, 238, and 239, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 241, 242, and 243, respectively;
- (y) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 247, 248, and 249, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 251, 252, and 253, respectively;
- (z) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 276, 277, and 278, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 280, 281, and 282, respectively;
- (aa) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 276, 277, and 278, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 286, 287, and 288, respectively;

(ab) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 291, 292, and 293, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 295, 296, and 297, respectively;

(ac) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 301, 302, and 303, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 305, 306, and 307, respectively;

(ad) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 311, 312, and 313, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 315, 316, and 317, respectively;

(ae) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 321, 322, and 323, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 325, 326, and 327, respectively;

(af) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 331, 332, and 333, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 335, 336, and 337, respectively;

(ag) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 341, 342, and 343, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 345, 346, and 347, respectively;

(ah) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 351, 352, and 353, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 354, 355, and 356, respectively;

(ai) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 351, 352, and 357, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 354, 355, and 356, respectively;

(aj) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 373, 374, and 375, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 377, 378, and 379, respectively;

(ak) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 383, 384, and 385, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 387, 388, and 389, respectively;

(al) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 393, 394, and 395, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 387, 388, and 389, respectively;

(am) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 398, 399, and 400, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 402, 403, and 404, respectively;

(an) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 408, 409, and 410, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 412, 413, and 414, respectively; or

(ao) heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 418, 419, and 420, respectively, and light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 422, 423, and 424, respectively.

7. The pharmaceutical composition for use according to any one of Claims 1 to 6, wherein the antibody or fragment thereof is conjugated to an antitumor agent.

8. A combination pharmaceutical agent for use in treating gallbladder cancer, comprising the pharmaceutical composition as defined in any one of Claims 1 to 7 and a pharmaceutical composition comprising an antitumor agent and a pharmacologically acceptable carrier or medium, wherein the CAPRIN-1 protein is expressed on the cell surface of the gallbladder cancer.

9. Use of the pharmaceutical composition as defined in any one of Claims 1 to 7 or the combination pharmaceutical agent as defined in Claim 8 for treating gallbladder cancer, wherein the CAPRIN-1 protein is expressed on the cell surface of the gallbladder cancer.