Description

Title of Invention: NOVEL ANTI-MESOTHELIN ANTIBODY AND COMPOSITION COMPRISING THE SAME

Technical Field

[1] The present invention relates to an antibody specifically bound to mesothelin (MSLN), a nucleic acid encoding the antibody, a vector and a host cell including the nucleic acid, a method for producing the antibody, and a pharmaceutical composition for treating cancer or tumor including the antibody as an active ingredient.

[2]

Background Art

[3] An antibody is highly effective for treating various cancers or tumors including solid tumors. For example, Herceptin has been used successfully in the treatment of breast cancer, and Avastin has been used successfully in the treatment of colon cancer. The core of the development of cancer or tumor treatment with the antibody is to develop an antibody against a membrane surface protein predominantly expressed (over-expression) in tumor cells.

[4] Mesothelin (MSLN) is a glycoprotein as 69 to 71kDa precursor polypeptide, and is expressed as a precursor form of glycophosphatidylinositol (GPI)-bound protein on a cell surface. The precursor is separated from a furin site (RPRFRR) in the precursor, and forms a 32kDa megakaryocyte potentiating factor (MPF) that is N-terminal polypeptide released from the cell with a GPI-bound mesothelin membrane protein that is 40kDa C-terminal polypeptide (Hassan R. *et al.,* Clin. Cancer Res., 10(12 Pt 1):3937-3942, 2004; Chang, K. *et al., Proc. Natl. Acad. Sci. USA,* 93(1):136-40, 1996).

[5] The mesothelin was named a megakaryocyte potentiating factor (MPF) since it had been purified from a human pancreatic cell line HPC-Y5, and observed to have a megakaryocyte-potentiating activity (Yamaguchi N. *et al., J.* Biol. Chem. 269:805-808, 1994).

[6] Function of the mesothelin has not been clearly found yet. Moreover, fatal re-productive, hematological or anatomical abnormality has not been observed when producing a mesothelin gene expression-deficient mouse (Bera, T.K. *et al., Mol. Cell. Biol.* 20(8):2902-2906, 2000).

[7] The mesothelin is a glycoprotein present on a cell surface of a mesothelial lining of peritoneal, pleural and pericardial coeloms. The mesothelin is predominantly expressed (over-expressed) in mesothelioma which is cancer/tumor cell, ovarian cancer, pancreatic cancer, stomach cancer, lung cancer and endometrial cancer. On the contrary, the expression thereof is limited in a normal cell, for example, a mesothelial

cell, which may be an ideal target of tumor treatment (Argani, P. *et al.,* Clin. Cancer Res., 7(12):3862-8, 2001; Hassan, R., *et al.,* Clin. Cancer Res., 10(12 Pt 1):3937, 2004).

[8] Further, the mesothelin specifically reacts (interacts) to CA125 (MUC-16) that is a mucin-like glycoprotein present on a surface of the tumor cell confirmed as an antigen of ovarian cancer. Specifically, it appears that the binding of CA125 to the membrane bound mesothelin is able to mediate heterotype cell adhesion and metastasis, and the CA125 and the mesothelin are co-expressed in an advanced ovarian adenocarcinoma (Rump, A. *et al., J.* Biol. Chem., 279(10):9190-8, 2004). The expression of the mesothelin in an endothelium of a peritoneal cavity is correlated with a preferred part for forming metastasis of the ovarian cancer, and the mesothelin-CA125 binding facilitates peritoneal metastasis of ovarian tumor (Gubbels, J.A. *et al., Mol. Cancer*, 5(1):50, 2006).

[9] In recent years, an antibody-based targeted treatment targeting the lung cancer, the ovarian cancer, and the pancreatic cancer that express the mesothelin has been developed. As an example, mAb K1 produced by immunization of the mouse has been developed as a primary antibody against a membrane-bound mesothelin polypeptide (Chang, K., *et al.,* Int. J. Cancer, 50(3):373, 1992). However, due to low affinity of the mAb K1 antibody and poor internalization rate, an immunotoxin consisting of mAb K1 linked to a truncation type of chemically modified Pseudomonas exotoxin A is not suitable for clinical development (Hassan, R., *et al., J.* Immunother., 23(4):473, 2000; Hassan, R., *et al.,* Clin. Cancer Res., 10(12 Pt 1):3937, 2004). Then, single-chain antibodies having a higher affinity including SS1-(dsFv)-PE38 have been developed, which have an ability to kill tumor cells in vitro (Hassan, R., *et al.,* Clin. Cancer Res., 8(11):3520, 2002), and an efficacy in rodent models of human mesothelin-expression tumors (Fan, D., *et al.,* Mol. Cancer Ther., 1(8):595, 2002). It may be appreciated from the above results that the mesothelin is a target appropriate for immunotherapy of multiple cancers. However, it was observed that the SS1-(dsFv)-PE38 has immunogenicity in clinical trials, such that a second administration thereof has been discontinued in most patients, and the SS1-(dsFv)-PE38 tends to be rapidly removed from the blood, and thus, there is an attempt to induce pegylation of the SS1-(dsFv)-PE38 into a form of fusion protein, thereby increasing antibody persistence in vivo (Filpula, D., *et al.,* Bioconjugate Chem., 18(3):773, 2007).

[10] The clinical trial of the immunotoxin cancer therapy having xenograft rodent as a cancer model is often limited by deficiency of cross-reactivity between a treatment antibody and a rodent homologue thereof. In addition, a neutralizing anti-mouse Fv antibody formed from a patient treated with a rodent-derived antibody or chimeric antibody may cause dose limiting toxicity or may reduce therapeutic efficacy.

Therefore, in order to increase efficacy of the cancer treatment, a targeting antibody combined with increased affinity, a reduced dissociation rate, and rodent cross-reactivity with regard to a mesothelin antigen is required.

[11] In addition, as an additional property of the novel anti-mesothelin (MSLN) antibody, it needs to maintain affinity with regard to the mesothelin expressed on the cell surface of different cancer or tumor cells. The mesothelin is a highly variable protein, and is subjected to glycosylation as well as proteolysis after translation in multiple parts (Hassan, R., *et al., Clin Cancer Res.,* 10(12 Pt 1):3937, 2004). It appears that a transcript variant 1 (Genbank NM_005823) represents the major species shown in tumor cell lines tested to date, but since three different splicing variants were detected, variability is extended to a transcription level (Muminova, Z.E., *et al., BMC Cancer,* 4:19, 2004; Hellstrom, I., *et al., Cancer* Epidemiol. Biomarkers Prev. 15(5):1014, 2006). Accordingly, an effective anti-endothelin antibody includes variability in the glycosylation pattern that expresses different forms of mesothelins, but it is required to be unchangeably bound to a mesothelin epitope expressed on cancer or tumor cell surfaces derived from different patients, which is independent from individual variability.

[12] Therefore, the present inventors made an effort to produce a novel antibody specifically bound to MSLN, and as a result, invented the novel antibody having high affinity with regard to the MSLN over-expressed in cancer cells, and found a potential of the antibody according to the present invention as an effective anti-cancer therapeutic agent, and completed the present invention.

[13]

Disclosure of Invention

Technical Problem

[14] An object of the present invention is to provide a novel antibody specifically bound to mesothelin (MSLN), a nucleic acid encoding the antibody, a vector and a host cell including the nucleic acid, a method for producing the same, and a pharmaceutical composition for treating cancer or tumor including the antibody as an active ingredient.

[15] Another object of the present invention is to provide a novel antibody specifically bound to the mesothelin (MSLN).

[16]

Solution to Problem

[17] In order to achieve the foregoing objects, the present invention provides a mesothelin-specific antibody including: a heavy chain variable region including a heavy chain CDR1 having an amino acid sequence of SEQ ID NO: 9, 15, 21, 27, or 59; a heavy chain CDR2 having an amino acid sequence of SEQ ID NO: 10, 16, 22, 28,

60, 65, 71, 75, 80, 84, 121, 122, 123 or 125; a heavy chain CDR3 having an amino acid sequence of SEQ ID NO: 11, 17, 23, 29, 61, 66, 72, 76, 81, 85, 124 or 126.

[18] In addition, the present invention provides a mesothelin-specific antibody including: a light chain variable region including a light chain CDR1 having an amino acid sequence of SEQ ID NO: 12, 18, 24, 30, 62, 67, 70, 77, 86 or 117; a light chain CDR2 having an amino acid sequence of SEQ ID NO: 13, 19, 25, 63, 68, 73, 78 or 82; a light chain CDR3 having an amino acid sequence of SEQ ID NO: 14, 20, 26, 64, 69, 74, 79, 83, 87, 118, 119 or 120.

[19] Further, the present invention provides a mesothelin-specific antibody including: a heavy chain variable region including an amino acid sequence of SEQ ID NO: 1, 3, 5, 7, 46, 48, 51, 53, 55, 57, 112, 113, 114, 115 or 116 and a light chain variable region including an amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 47, 52, 54, 56, 58, 109, 110 or 111.

[20] In addition, the present invention provides a nucleic acid encoding the MSLN-specific antibody; and a vector containing the nucleic acid; and a cell into which the vector is introduced.

[21] Further, the present invention provides a pharmaceutical composition for treating cancer or tumor including the anti-MSLN antibody as an active ingredient.

[22]

Brief Description of Drawings

[23] FIG. 1 illustrates prediction structures of a light chain variable region (VL) and a heavy chain variable region (VH) of clone MS502 among anti-mesothelin (MSLN) antibodies.

[24] FIG. 2 illustrates relative comparison of light chain variable region mutants in view of binding force.

[25] FIG. 3 illustrates relative comparison of heavy chain variable region mutants in view of binding force.

[26] FIG. 4 illustrates a vector for expressing the MSLN in a cell line.

[27] FIG. 5 illustrates a precursor form and a mature form of the MSLN on SDS-PAGE.

[28] FIG. 6 illustrates analysis of an MSLN expression amount of cell lines and tumor cell lines expressing the MSLN.

[29] FIG. 7 illustrates selective binding analysis with regard to the cell lines expressing the MSLN by using an anti-mesothelin (MSLN) antibody of the present invention.

[30] FIG. 8 illustrates MFI values obtained by confirming whether the anti-MSLN antibody is bound to a cell membrane of mesothelioma (H226, H2052) and pancreatic cancers (AsPC-1) through FACS.

[31] FIG. 9 illustrates results obtained by confirming whether the anti-MSLN antibody of

the present invention is selectively bound to tumor cells expressing the MSLN.

[32]

Best Mode for Carrying out the Invention

[33] As used herein, term “mesothelin” or “MSLN” refers to any variants, isoforms and species homologs of human MSLN that is naturally expressed by cells.

[34] Term “human mesothelin" refers to a human sequence mesothelin such as a complete amino acid sequence of human mesothelin having Genbank Accession No. NP_005814.

[35] In an embodiment of the present invention, monoclonal antibodies that are structurally characterized to be specifically bound to the mesothelin represented by SEQ ID NO: 127 and separated, such as "clone MI323", "clone MI329", "clone MI403" and "clone MI407", and "clone MS501", "clone MS502", "clone MS503", "clone MS504", "clone MS505" and "clone MS506", and "clone C2G1", "clone C2G4", "clone C3C8", "clone 54", "clone 56", "clone 2-30", "clone 2-73" and "clone 2-78", and "clone 56-C2G4", "clone 2-30-C2G4", "clone 2-73-C2G4" and "clone 2-78-C2G4" were produced.

[36] Amino acid sequences with regard to a heavy chain CDR and a light chain CDR of each antibody are shown in Tables 2, 5, 12, and 16 below. As shown in Tables 1, 4, 11, and 15, an anti-MSLN antibody may include an amino acid sequence of a heavy chain variable region and a light chain variable region or a sequence having homology thereto.

[37] In another embodiment of the present invention, individual antibody clones of purified antibodies, i.e., “clone MI323", "clone MI329", "clone MI403", "clone MS502", and "clone C2G1", "clone C2G4", "clone C3C8", and "clone 56-C2G4", "clone 2-30-C2G4", "clone 2-73-C2G4" or "clone 2-78-C2G4" with regard to a re-combinant human MSLN, were selected by using an enzyme linked immunosorbent assay (ELISA) (data not shown), and quantitative binding force was measured by using a Biacore T-200 (GE Healthcare, U.S.A.) biosensor (Example 2-5, Example 3-11, Examples 3-14). As a result, as shown in Tables 8, 14, and 18 below, all of the produced clone antibodies have affinity to the mesothelin even though there is a slight difference.

[38] In another embodiment of the present invention, in order to evaluate whether the anti-MSLN antibody derived from the immune and synthetic library is selectively bound to a MSLN-expressing cell, an expression amount of the MSLN is measured in a cancer cell line, and an antibody binding to each cell is confirmed by FACS test. As a result, as illustrated in FIG. 5, it was confirmed that H28, MiaPaCa-2, BxPC-3, Capan-1 cell lines are MSLN-negative, and H226, H2452(H2052), AsPC-1 are MSLN-

positive by measuring whether there are the MSLN having 70kDa precursor form and 40 to 50kDa mature form from each cancer cell line.

[39] In addition, as a result obtained by performing selective binding analysis of anti-MSLN candidate antibodies with regard to the MSLN-expressing cell lines (H226, H2452(H2052), AsPC-1), as illustrated in FIG. 8 and Table 19, all of the MI323, MI329, MI403, MS502 candidate antibodies with regard to the MSLN of mesothelioma and pancreatic cancer cell lines have significant binding force even though there is a slight difference in binding degree. In particular, the MI323 candidate antibody has an excellent binding aspect.

[40] Further, as a result obtained by evaluating whether the MI323 candidate antibody having the excellent binding aspect with regard to the MSLN, MS502 candidate antibody having a different pattern of Biacore KD(Koff/Kon) value, and a heavy chain variable region mutation 2-78-C2G4 candidate antibody produced from the MS502 candidate antibody are selectively bound to MSLN-expressing tumor cells, in MiaPaCa-MSLN #2 cell that over-expresses the MSLN and MiaPaCa-2 that does not over-express the MSLN, as illustrated in FIG. 9, the MI323, MS502, and 2-78-C2G4 candidate antibodies have excellent binding aspect in the MiaPaCa-MSLN #2 cell that over-expresses the MSLN as compared to the MiaPaCa-2.

[41] Therefore, the present invention relates to an antibody specifically bound to mesothelin (MSLN), preferably, an antibody specifically bound to mesothelin represented by SEQ ID NO: 127.

[42] The antibody specifically bound to the mesothelin according to the present invention is characterized by containing a heavy chain variable region including a heavy chain CDR1 having an amino acid sequence of SEQ ID NO: 9, 15, 21, 27, or 59; a heavy chain CDR2 having an amino acid sequence of SEQ ID NO: 10, 16, 22, 28, 60, 65, 71, 75, 80, 84, 121, 122, 123 or 125; a heavy chain CDR3 having an amino acid sequence of SEQ ID NO: 11, 17, 23, 29, 61, 66, 72, 76, 81, 85, 124 or 126.

[43] The antibody specifically bound to the mesothelin according to the present invention is characterized by containing a light chain variable region including a light chain CDR1 having an amino acid sequence of SEQ ID NO: 12, 18, 24, 30, 62, 67, 70, 77, 86 or 117; a light chain CDR2 having an amino acid sequence of SEQ ID NO: 13, 19, 25, 63, 68, 73, 78 or 82; a light chain CDR3 having an amino acid sequence of SEQ ID NO: 14, 20, 26, 64, 69, 74, 79, 83, 87, 118, 119 or 120.

[44] In the present invention, the antibody specifically bound to the mesothelin may contain a heavy chain variable region including a sequence having at least 80% homology, preferably, at least 90% homology, and more preferably, 100% homology to the amino acid sequence of SEQ ID NO: 1, 3, 5, 7, 46, 48, 51, 53, 55, 57, 112, 113, 114, 115 or 116, and the antibody may contain a light chain variable region including a

sequence having at least 80% homology, preferably, at least 90% homology, and more preferably, 100% homology to the amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 47, 52, 54, 56, 58, 109, 110 or 111.

[45] In the present invention, the antibody specifically bound to the mesothelin is characterized by containing the heavy chain variable region including the amino acid sequence of SEQ ID NO: 1, 3, 5, 7, 46, 48, 51, 53, 55, 57, 112, 113, 114, 115 or 116, and the light chain variable region including the amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 47, 52, 54, 56, 58, 109, 110 or 111, and the antibody may be a human monoclonal antibody, but is not limited thereto.

[46] The amino acid sequence of the antibody may be substituted by conservative substitution. The "conservative substitution" refers to modification of polypeptide including substitution of at least one amino acid with an amino acid having similar biochemical properties to corresponding polypeptide without causing loss of biological or biochemical function. “Conservative amino acid substitution" refers to a substitution in which an amino acid residue is replaced with an amino acid residue having similar side chains. Classes of the amino acid residues having similar side chains are defined in the art. These classes include amino acids having basic side chains (e.g., lysine, arginine, histidine), amino acids having acidic side chains (e.g., aspartic acid, glutamic acid), amino acids having uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), amino acids having non-polar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), amino acids having beta-branched side chains (e.g., threonine, valine, isoleucine), and amino acids having aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). It is anticipated that the antibody of the present invention is able to still retain an activity while having the conservative amino acid substitution.

[47] Term "substantial homology" refers that when two kinds of nucleic acids or two kinds of polypeptides or designated sequences thereof are optimally aligned and compared, the nucleic acids and polypeptides having appropriate nucleotide or amino acid insertion or deletion have at least about 80% identity to the nucleotide or the amino acid, generally, have at least about 85%, preferably about 90%, 91%, 92%, 93%, 94% or 95%, and more preferably at least about 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4% or 99.5% to the nucleotide or the amino acid. Alternatively, when a fragment is hybridized with a complementary strand thereof under selective hybridization conditions, there is substantial homology to the nucleic acid. The present invention includes a nucleic acid sequence and a polypeptide sequence having substantial homology with regard to the above-described specific nucleic acid sequence and amino acid sequence.

[48] In the antibody according to the present invention, for example, the heavy chain (V_H)

CDR1, 2 and 3 sequences and the light chain (V_L) CDR1, 2 and 3 sequences shown in Table 2, Table 5, Table 12, and Table 16 may be formed by mixing structurally similar heavy chain (V_H) and light chain (V_L) sequences to be arranged in the CDR1, 2 and 3 of the heavy chain (V_H) / light chain (V_L) pairs.

[49] As used herein, term "antibody" or "antibody composition" refers to a preparation of antibody molecules having single molecule composition. Here, a monoclonal antibody composition represents single binding specificity and affinity for a specific epitope. Accordingly, term "human monoclonal antibody" refers to an antibody having a variable region and a constant region derived from a human wiring immunoglobulin sequence, and representing single binding specificity. A human antibody of the present invention may include amino acid residue that is not encoded by the human wiring immunoglobulin sequence (for example, mutants introduced by in vitro random or site-specific mutagenesis, or by in vivo somatic mutation).

[50] The "antibody" used herein is an immunoglobulin molecule which is immunologically reactive to a specific antigen, and means a protein molecule acting as a receptor that specifically recognizes an antigen, and may include all of a polyclonal antibody, a monoclonal antibody (single clone antibody), a whole antibody, and an antibody fragment. Further, the antibody may include a chimeric antibody (e.g., humanized murine antibody) and a bivalent or bispecific molecule (e.g., bispecific antibody), a diabody, a triabody, and a tetrabody.

[51] The whole antibody has a structure having two full length light chains and two full length heavy chains, and each light chain may be linked to a heavy chain via a disulfide bond. The whole antibody includes IgA, IgD, IgE, IgM, and IgG, and the IgG is a subtype, and includes IgG1, IgG2, IgG3, and IgG4. The antibody fragment means a fragment retaining an antigen-binding function, and includes Fab, Fab', F(ab')2, scFv, and Fv, etc.

[52] The Fab has a structure of variable regions of a light chain and a heavy chain and a constant region of the light chain and a first constant region (CH1 domain) of the heavy chain, and has one antigen-binding site. The Fab' is different from the Fab in that the Fab' has a hinge region including one or more cysteine residues at C terminal of a heavy chain CH1 domain. The F(ab')2 antibody is produced by achieving the disulfide bonding of the cysteine residue in the hinge region of the Fab'.

[53] The Fv (variable fragment) refers to the minimum antibody fragment only having the heavy chain variable region and the light chain variable region. In double-stranded Fv(dsFv), the heavy chain variable region and the light chain variable region are linked by the disulfide bond. In the single chain Fv(scFv), the heavy chain variable region and the light chain variable region generally are linked by a covalent bond using a peptide linker. These antibody fragment may be obtained by using a proteolytic enzyme (for

example, the Fab may be obtained by restriction-cutting the whole antibody with papain, and F(ab')2 fragment may be obtained by cutting with pepsin), and may be constructed by a recombinant DNA technology (for example, amplification by PCR (Polymerase Chain Reaction) method using DNA encoding the heavy chain of the antibody or the variable region thereof and DNA encoding the light chain or the variable region thereof as a template and using a primer pair, and amplification with combination of the DNA encoding the peptide linker of the primer pair allowing both ends thereof to link to the heavy chain or the variable region thereof and the light chain or the variable region thereof, respectively).

[54] The immunoglobulin has heavy chains and light chains, wherein the respective heavy chains and light chains include a constant region and a variable region (these regions are also known as domain). The light chain variable region and the heavy chain variable region include 3 multi-available regions called complementarity-determining region (hereinafter, referred to as "CDR"), and four framework regions. The CDR mainly acts to bind to an epitope of the antigen. The CDRs of the respective chains are sequentially called CDR1, CDR2, and CDR3 generally starting from N-terminal, and also identified by the chains in which specific CDRs are located.

[55] The monoclonal antibody (single clone antibody) used herein means an antibody molecule of single molecular composition substantially obtained in the same antibody population, and may have single binding specificity and affinity for a specific epitope.

[56] The monoclonal antibody (single clone antibody) used herein is a molecule derived from a human immunoglobulin, and all of the amino acid sequences configuring the antibody including a complementarity-determining region, a structure region are configured of human immunoglobulin amino acid sequences. The human antibody is typically used in the treatment of human diseases, which is advantageous in that i) it more favorably interacts with the human immune system, which more effectively destroys target cells by complement-dependent cytotoxicity (CDC) or antibody-dependent cell mediated cytotoxicity (ADCC), ii) the human immune system does not recognize the antibody as a foreign material, and iii) even when a smaller amount of drug is administered less frequently, a half-life in a human circulatory system is similar to that of a naturally occurring antibody.

[57] Terms "clone MI323", "clone MI329", "clone MI403" and "clone MI407" and "clone MS501", "clone MS502", "clone MS503", "clone MS504", "clone MS505" and "clone MS506" and "clone C2G1", "clone C2G4", "clone C3C8", "clone 54", "clone 56", "clone 2-30", "clone 2-73" and "clone 2-78" and "clone 56-C2G4", "clone 2-30-C2G4", "clone 2-73-C2G4" and "clone 2-78-C2G4" that are antibodies specifically bound to MSLN used herein mean antibodies bound to the MSLN and causing inhibition of biological activity of the MSLN, and may be used by mixing an

anti-MSLN antibody.

[58] Here, the "clone MI323", "clone MI329", "clone MI403" and "clone MI407" are antibodies obtained by immunizing a mouse with recombinant human MSLN, and the clone MS501", "clone MS502", "clone MS503", "clone MS504", "clone MS505" and "clone MS506" are antibodies obtained from a phage display from a scFV library, and "clone C2G1", "clone C2G4", "clone C3C8", "clone 54", "clone 56", "clone 2-30", "clone 2-73" and "clone 2-78" are antibodies obtained by introducing mutation into the "clone MS502" as shown in Table 9, and the "clone 56-C2G4", "clone 2-30-C2G4", "clone 2-73-C2G4" and "clone 2-78-C2G4" are antibodies produced by combination between the introduced mutation antibodies.

[59] K_D (equilibrium dissociation constant) of the antibody to the MSLN may be exemplified as follows.

[60] (1) the clone MI323 may have an equilibrium dissociation constant (K_D) of 1.8×10^{-8} M or less, preferably, 1.8×10^{-9}M or less, and more preferably, 1.8×10^{-10}M or less,

[61] (2) the clone MI329 may have an equilibrium dissociation constant (K_D) of 3.5×10^{-9} M or less, preferably, 3.5×10^{-10}M or less, and more preferably, 3.5×10^{-11}M or less,

[62] (3) the clone MI403 may have an equilibrium dissociation constant (K_D) of 4.5×10^{-8} M or less, preferably, 4.5×10^{-9}M or less, and more preferably, 4.5×10^{-10}M or less,

[63] (4) the clone MS502 may have an equilibrium dissociation constant (K_D) of 2.3×10^{-8} M or less, preferably, 2.3×10^{-9}M or less, and more preferably, 2.3×10^{-10}M or less (see Table 8),

[64] (5) the clone C2G1 may have an equilibrium dissociation constant (K_D) of 9.39×10^{-9} M or less, preferably, 9.39×10^{-10}M or less, and more preferably, 9.39×10^{-11}M or less,

[65] (6) the clone C2G4 may have an equilibrium dissociation constant (K_D) of 4.32×10^{-9} M or less, preferably, 4.32×10^{-10}M or less, and more preferably, 4.32×10^{-11}M or less,

[66] (7) the clone C3C8 may have an equilibrium dissociation constant (KD) of 1.22×10^{-8}M or less, preferably, 1.22×10^{-9}M or less, and more preferably, 1.22×10^{-10}M or less (see Table 14),

[67] (8) the clone 56 may have an equilibrium dissociation constant (K_D) of 1.25×10^{-8}M or less, preferably, 1.25×10^{-9}M or less, and more preferably, 1.25×10^{-10}M or less,

[68] (9) the clone 2-30 may have an equilibrium dissociation constant (K_D) of 1.66×10^{-8} M or less, preferably, 1.66×10^{-9}M or less, and more preferably, 1.66×10^{-10}M or less,

[69] (10) the clone 2-78 may have an equilibrium dissociation constant (K_D) of 1.63×10^{-9} M or less, preferably, 1.63×10^{-10}M or less, and more preferably, 1.63×10^{-11}M or less,

[70] (11) the clone 56-C2G4 may have an equilibrium dissociation constant (K_D) of 1.63×10^{-8}M or less, preferably, 1.63×10^{-9}M or less, and more preferably, 1.63×10^{-10}M or less,

[71] (12) the clone 2-30-C2G4 may have an equilibrium dissociation constant (K_D) of

2.34×10^{-8}M or less, preferably, 2.34 x 10-9M or less, and more preferably, 2.34×10^{-10}M or less,

[72] (13) the clone 2-73-C2G4 may have an equilibrium dissociation constant (K_D) of 1.65×10^{-8}M or less, preferably, 1.65×10^{-9}M or less, and more preferably, 1.65×10^{-10} M or less, and

[73] (14) the clone 2-78-C2G4 may have an equilibrium dissociation constant (K_D) of 3.72×10^{-9}M or less, preferably, 3.72×10^{-10}M or less, and more preferably, 3.72×10^{-11}M or less (see Table 18).

[74] In another embodiment of the present invention, genes of the heavy chain variable region and the light chain variable region of a mouse derived from an immune library bound to human MSLN are identified, the heavy chain variable region gene is linked to a human immunoglobulin type 1 of heavy chain constant region (IgG1 heavy chain constant region) gene, and the light chain variable region gene is linked to a human kappa light chain constant region, and these genes are inserted into protein expression vectors for animal cell, respectively, to produce vectors, followed by transfection in the Expi293F™ cell lines and culturing to produce the antibody, and the produced antibody is purified by protein A to produce the antibody (Example 1-3).

[75] In still another embodiment of the present invention, genes of the heavy chain variable region and the light chain variable region derived from a synthetic scFV library bound to human MSLN are identified, the heavy chain variable region gene is linked to a human immunoglobulin type 1 of heavy chain constant region (IgG1 heavy chain constant region) gene, and the light chain variable region gene is linked to a human kappa light chain constant region, and these genes are inserted into protein expression vectors for animal cell, respectively, to produce vectors, followed by transfection in the Expi293F™ cell lines and culturing to produce the antibody, and the antibody is purified by protein A to produce the antibody (Examples 2-4, 3-10, 3-12, and 3-13).

[76] Therefore, in another aspect of the present invention, the present invention provides a nucleic acid encoding the antibody. The nucleic acid used herein may be present in a cell, a cell lysate, or may also be present in a partially purified form or a substantially pure form. The nucleic acid is “isolated” or “is substantially pure” when it is purified from other cell components or other contaminants, for example, other cell nucleic acid or protein by standard techniques including alkaline/SDS treatment, CsCl banding, column chromatography, agarose gel electrophoresis, and other techniques well-known in the art. The nucleic acid of the present invention may be, for example, DNA or RNA, and may include an intron sequence, or may not include the intron sequence.

[77] In still another aspect of the present invention, the present invention provides a vector including the nucleic acid. For expression of the antibody or antibody fragments

thereof, DNA encoding the light chain and the heavy chain having a partial length or a full length may be obtained by standard molecular biology techniques (for example, PCR amplification or cDNA cloning using a hybridoma that expresses a target antibody), and the DNA may be "operably bound" to transcription and translation control sequences to be inserted into the expression vector.

[78] Term "operably bound" used herein may indicate that an antibody gene is ligated into the vector so that the transcription and translation control sequences in the vector have an intended function to control transcription and translation of the antibody gene. The expression vector and an expression control sequence are selected so as to have compatibility with a host cell for expression to be used. The light chain gene of the antibody and the heavy chain gene of the antibody are inserted into a separate vector, or both genes are inserted into the same expression vector. The antibody is inserted into the expression vector by a standard method (for example, ligation of an antibody gene fragment and a complementary restriction enzyme site on a vector or when the restriction enzyme site is not present at all, blunt end ligation). In some cases, the recombinant expression vector may encode a signal peptide that facilitates secretion of the antibody chain from the host cell. The antibody chain gene may be cloned into the vector so that the signal peptide is bound to an amino terminal of the antibody chain genes according to a frame. The signal peptide may be an immunoglobulin signal peptide or a heterologous signal peptide (i.e. signal peptide derived from proteins except for immunoglobulin). In addition, the recombinant expression vector has a regulatory sequence that controls the expression of the antibody chain genes in the host cell. The "regulatory sequence" may include a promoter, an enhancer and other expression control element (for example, polyadenylation signal) controlling the transcription or translation of the antibody chain gene. Those skilled in the art is able to recognize that design of the expression vector may vary by changing the regulatory sequences according to factors such as selection of the host cell to be transformed, an expression level of the protein, etc.

[79] In still another aspect of the present invention, the present invention provides a host cell including the nucleic acid or the vector. The nucleic acid or the vector is transfected into the host cell. For the "transfection", various kinds of generally used techniques such as electrophoresis, calcium phosphate precipitation, DEAE-dextran transfection, lipofection, etc., may be used to introduce an exogenous nucleic acid (DNA or RNA) into a prokaryotic host cell or an eukaryotic host cell. The antibody according to the present invention may be expressed in an eukaryotic cell, preferably, in a mammalian host cell, in consideration of applicability into a mammalian cell. The mammalian host cells suitable for expression of the antibody may include a Chinese hamster ovary (CHO) cell (for example, including a dhfr- CHO cell used together with

a DHFR selection marker), an NSO myeloma cell, a COS cell, or a SP2 cell, etc., as examples.

[80] In still another aspect of the present invention, the present invention provides a method for producing an antibody, including culturing a host cell to express the antibody. When the recombinant expression vector encoding the antibody gene is introduced into the mammalian host cell, the antibody may be produced by culturing the host cell for a sufficient period of time so that the antibody is expressed in the host cell, or more preferably, for a sufficient period of time so that the antibody is secreted into a culture medium in which the host cell is cultured.

[81] In some cases, the expressed antibody may be separated from the host cell and purified for uniformity. The separation or the purification of the antibody may be performed by a separation method, a purification method generally used for protein, for example, chromatography. The chromatography may include, for example, affinity chromatography, ion exchange chromatography or hydrophobic chromatography including protein A column and protein G column. In addition to the chromatography, the antibody may be separated and purified by additionally combining with filtration, ultrafiltration, salting out, dialysis, etc.

[82] In still another aspect of the present invention, the present invention provides a pharmaceutical composition for treating cancer or tumor including the antibody as an active ingredient.

[83] Term "cancer" or "tumor" typically refers to or describes a physiological condition of mammals characterized by cell growth/proliferation that is not controlled. Examples of the cancer include carcinoma, lymphoma (e.g., Hodgkin's and non-Hodgkin's lymphoma), blastoma, sarcoma and leukemia, but are not limited thereto. More specific examples of the cancer include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, lung adenocarcinoma, lung squamous cell carcinoma, peritoneal cancer, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatocellular cancer, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulvar cancer, thyroid cancer, liver carcinoma, leukemia and other lymphoproliferative disorders, and various types of head and neck cancer. The cancer in the present invention is preferably mesothelin-positive cancer, and is selected from the group consisting of pancreatic cancer, ovarian cancer, lung cancer, stomach cancer, endometrial cancer, and mesothelioma.

[84] The present invention provides a pharmaceutical composition including a therapeutically effective amount of anti-MSLN antibody and a pharmaceutically acceptable carrier. The “pharmaceutically acceptable carrier" is a material that is able to be added

in the active ingredient to help formulation or stabilization of the preparation, and it does not cause significant adverse toxicological effects to patients.

[85] The carrier refers to a carrier or diluent that does not inhibit biological activity and properties of an administered compound without stimulating the patients. The pharmaceutically acceptable carrier in the composition to be formulated as a liquid solution is sterilized and is suitable for a living body. Saline, sterile water, Ringer's solution, buffered saline, albumin injection solution, dextrose solution, maltodextrin solution, glycerol, ethanol may be used as the carrier, or at least one component thereof may be mixed to be used, and other conventional additives such as an antioxidant, buffer, a bacteriostatic agent, etc., may be added as needed. In addition, the composition may be prepared into formulations for injection, such as an aqueous solution, suspension, emulsion, etc., pill, a capsule, a granule or a tablet by further adding diluent, dispersant, surfactant, binder and lubricant thereto. Other carriers are described in, for example, [Remington's Pharmaceutical Sciences (E. W. Martin)]. The composition may contain the therapeutically effective amount of at least one anti-MSLN antibody.

[86] The pharmaceutically acceptable carrier includes sterile aqueous solution or dispersion and sterile powder for preparing extemporaneous sterile injectable solution or dispersion. The use of such media and agents for pharmaceutical active materials is known in the art. The composition is preferably formulated for parenteral injection. The composition may be formulated as a solution, a micro-emulsion, a liposome, or other ordered structures suitable for high drug concentration. The carrier may be, for example, a solvent or dispersion medium containing water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, etc.,) and suitable mixtures thereof. In some cases, the composition may include, isotonic agent, for example, sugar, polyalcohols such as mannitol, sorbitol, or sodium chloride. The sterile injectable solution may be prepared by incorporating a required amount of active compound into an appropriate solvent with one kind of the above-described components or a combination thereof, followed by sterile micro filtration as needed. In general, the dispersion is prepared by incorporating the active compound into a sterile vehicle containing basic dispersion medium and other required components from the above-described components. The sterile powder for preparing the sterile injectable solution is obtained by vacuum drying and freeze-drying (lyophilization) active ingredient powder and any additional desirable component powder from previously sterile-filtered solution.

[87] The pharmaceutical composition may be administered orally or parenterally in the dosage and frequency that may vary depending on severity of suffering patients. The composition may be administered to a patient as a bolus or by continuous infusion as needed. For example, the bolus administration of the antibody of the present invention

which is presented as a Fab fragment may have an amount of 0.0025 to 100mg/kg body weight, 0.025 to 0.25mg/kg, 0.010 to 0.10mg/kg or 0.10 to 0.50mg/kg. For the continuous infusion, the antibody of the present invention which is presented as the Fab fragment may be administered at 0.001 to 100 mg/kg kg/min, 0.0125 to 1.25mg/kg/min, 0.010 to 0.75mg/kg/min, 0.010 to 1.0mg/kg/min or 0.10 to 0.50mg/kg/min for 1 to 24 hours, 1 to 12 hours, 2 to 12 hours, 6 to 12 hours, 2 to 8 hours, or 1 to 2 hours. When the antibody of the present invention which is presented as a full-length antibody (having a complete constant region is administered, an administration amount may be about 1 to 10 mg/kg body weight, 2 to 8 mg/kg, or 5 to 6 mg/kg. The full-length antibody is typically administered via injection that lasts for 30 minutes to 35 minutes. An administration frequency depends on the severity of the condition. The frequency may be 3 times every week to once in a week or in two weeks.

[88] In addition, the composition may be administered to a patient via a subcutaneous injection. For example, the anti-MSLN antibody having an administration amount of 10 to 100 mg may be weekly, biweekly, or monthly administered to a patient through subcutaneous injection.

[89] As used herein, the "therapeutically effective amount" means an amount sufficient to treat diseases at a reasonable benefit/risk ratio applicable for medical treatment, and an amount of a combination of the anti-MSLN antibody. The exact amount may vary depending on a number of factors that include components and physical characteristics of a therapeutic composition, intended patient population, individual patient considerations, etc., but are not limited thereto, and may be easily determined by those skilled in the art. When completely considering these factors, it is important to administer the minimum amount sufficient to obtain the maximum effect without the side effect, and this dosage may be easily determined by an expert in the field.

[90] The dosage of the pharmaceutical composition of the present invention is not specifically limited, but is changed according to various factors including a health state and weight, severity of the disease of a patient, and a drug type, an administration route, and administration time. The composition may be administered in routes that are typically allowed in mammals including rat, mouse, cattle, human, etc., for example, orally, rectally, intravenously, subcutaneously, intrauterinely or intracerebrovascularly in a single dose amount or multidose per day.

[91]

[92] Example

[93] Hereinafter, the present invention will be described in more detail with reference to the following Examples. However, the following examples are only for exemplifying the present invention, and it will be obvious to those skilled in the art that the scope of

the present invention is not construed to be limited to these examples.

[94]

[95] Example 1: Method for producing anti-MSLN antibody from immune library

[96] It is intended to produce an anti-cancer antibody therapeutic agent using an antibody against mesothelin (MSLN) over-expressed on a cancer cell surface.

[97] 1-1: Selection of anti-MSLN antibody

[98] A mouse was immunized with a recombinant human MSLN and a spleen was removed to extract B lymphocyte. Total RNA was separated from the B lymphocyte and cDNA was synthesized. Various antibody genes of the mouse were cloned from the synthesized cDNA using polymerase chain reaction (PCR), and inserted into pComb3X phagemid to produce an antibody library displaying antibody fragments of various sequences. In order to find the antibody specifically bound to human MSLN from the antibody library, magnetic beads having the MSLN fixed thereto were mixed with the antibody library, and clones bound to a target antigen were separated and cultured. Then the clones (MI323, MI329, MI403, and MI407) specifically bound to the target antigen (human MSLN) were individually identified through an enzyme-linked immunosorbent assay (ELISA), and antibody gene sequences and amino acid sequence thereof were identified through base sequence analysis.

[99] As a result, as shown in Table 1, the clones specifically bound to human MSLN could be selected, and amino acid sequences thereof were identified.

[100] Table 2 shows CDR amino acid sequences of the clone antibodies of Table 1 on the basis of Kabat numbering.

[101]

[102] [Table 1]

Clone	Variable Region	Amino Acid Sequence	SEQ ID NO:
MI323	heavy chain	EVQLQQSGPELVKPGTSVKISCKASGYSFTS YFIQWVKQRPGQGLEWIGWIFPGSGNTKYN EMFKGKATLAADTSSSTAYMQLSSLTSEDS AVYFCARSGGYQYYFDYWGQGTSVTVSS	1
	light chain	DIVMTQSHKFMSTSVGDRVSITCKASQDVS TAVAWYQQKPGQSPKLLIYSASYRYPGVPD RFTGSGSGTDFTFTISSVQAEDLALYYCQQH YSTPWTFGGGTKLEIKR	2
MI329	heavy chain	EVMLVESGGDLVKPGGSLKLSCAASGFTFS SYAMSWVRRTPEKRLEWVATINSDGSYTF YPDSVKGRFTISRDNAKNTLYLQMNSLRSE DTAMYYCARWGENWYFDVWGAGTTVTV SS	3
	light chain	DVVMTQTPLSLPVSLGDQASISCRSSQSLVH SNGNTYLHWYLQKPGQSPKLLIYKVSNRFS GVPDRFSGSGSGTDFTLKISRVEAEDLGIYF CSQSTHFPRTFGGATKLELKR	4
MI403	heavy chain	EVQVVESGGGLVKPGGSLKLSCAASGFAFS SYDMSWVRQTPEKRLEWVAYISSGGGSTY YPDTVKGRFTISRDNAKNTLYLQMNSLKSE DTAMYYCARQGTAVKNYWYFDVWGAGT SVTVSS	5
	light chain	DIVMTQSPASLAVSLGQRATISCRASQSVST SSSSYVHWYQQRPGQPPKLLIKYASNLESG VPARFSGSGSGTDFTLNIHPVEEEDTGTYYC QHSWEIPFTFGSGTKLEIKR	6
MI407	heavy chain	EVKLVESGGGLVKPGGSLKLSCAASGFPFS NYDMSWVRQTPEKRLEWVAYISSGGGNTY YPDTVKGRFTISRDNAKNTLYLQMSSLKSE DTALYFCVRQGTSVESYWYFDVWGAGTTV TVSS	7
	light chain	DIVLTQSPASLAVSLGQRATISCRASQSVSTS SSSYIHWYQQKPGQPPKLLIKYASNLESGVP	8

		ARFSGSGSGTDFTLNIHPVEEDDTATYYCQ HSWEIPFTFGSGTELEIKR	

[103]

[104] [Table 2]

Clone	Variable Region	CDR1	CDR2	CDR3
MI323	heavy chain	SYFIQ(SEQ ID NO: 9)	WIFPGSGNTKY NEMFKG(SEQ ID NO: 10)	SGGYQYYFDY(SE Q ID NO: 11)
	light chain	KASQDVSTA VA(SEQ ID NO: 12)	SASYRYP(SEQ ID NO: 13)	QQHYSTPWT(SEQ ID NO: 14)
MI329	heavy chain	SYAMS(SEQ ID NO: 15)	TINSDGSYTFYP DSVKG(SEQ ID NO: 16)	WGENWYFDV(SEQ ID NO: 17)
	light chain	RSSQSLVHSN GNTYLH(SEQ ID NO: 18)	KVSNRFS(SEQ ID NO: 19)	SQSTHFPRT(SEQ ID NO: 20)
MI403	heavy chain	SYDMS(SEQ ID NO: 21)	YISSGGGSTYYP DTVKG(SEQ ID NO: 22)	QGTAVKNYWYFD V(SEQ ID NO: 23)
	light chain	RASQSVSTSS SSYVH(SEQ ID NO: 24)	YASNLES(SEQ ID NO: 25)	QHSWEIPFT(SEQ ID NO: 26)
MI407	heavy chain	NYDMS(SEQ ID NO: 27)	YISSGGGNTYY PDTVKG(SEQ ID NO: 28)	QGTSVESYWYFDV (SEQ ID NO: 29)
	light chain	SVSTSSSSYIH (SEQ ID NO: 30)	YASNLES(SEQ ID NO: 25)	QHSWEIPFT(SEQ ID NO: 26)

[105]

[106] 1-2: IgG gene cloning of MI323, MI329, MI403, and MI407 monoclonal antibodies

[107] The pComb3X phagemid containing the genes encoding the heavy chain variable regions of the MI323, MI329, MI403, and MI407 clone antibodies was extracted, and

used as a template for PCR with a forward primer containing NotI (Table 3: SEQ ID NOs: 31 to 34) and a reverse primer containing ApaI (Table 3: SEQ ID NO: 35) by using Accupower Pfu PCR premix (Bioneer). The PCR was performed by repeating exposure at 94℃ for 10 minutes, and then exposure at 94℃ for 15 seconds, at 56℃ for 30 seconds, and at 72℃ for 90 seconds 30 times, and reacting at 72℃ for 10 minutes. In the amplified genes, DNA bands having an expected size were confirmed on 1% agarose gel, and were separated using a gel extraction kit, respectively. Then, the separated genes reacted with NotI, ApaI restriction enzymes at 37℃ for 12 hours or more, and the genes reacted with the restriction enzymes were separated on 1% agarose gel again. A pcIW plasmid vector containing human immunoglobulin type 1 of heavy chain constant region (IgG1 heavy chain constant region) gene was also cut by the same method as above and separated on agarose gel. The separated MI323, MI329, MI403, and MI407 heavy chain variable region genes were inserted into NotI, ApaI sites of a linear pcIW vector containing the human heavy chain constant region by using a T4 DNA ligase (Cat.No.M0203S, New England BioLabs (NEB)). The ligation reaction materials were transformed into XL1-Blue bacteria (Electroporation-Competent Cells; Cat.No.200228, Stratagene), plated on an LB plate (Cat.No.LN004CA, NaraeBiotech) containing carbenicillin, and cultured at 37℃ for 12 hours or more. Then single colonies were chosen and cultured, and plasmids were separated by using a plasmid mini kit (Cat.No.27405, QIAGEN), and confirmed by DNA sequencing.

[108] The pComb3X phagemid containing the genes encoding the light chain variable regions of the MI323, MI329, MI403, and MI407 clone antibodies was extracted, and used as a template for PCR of the light chain variable regions of the MI323, MI329, MI403, and MI407 clone antibodies, wherein the PCR was performed with a forward primer containing NotI (Table 3: SEQ ID NO: 36, 38, 40, 42) and a reverse primer (Table 3: SEQ ID NO: 37, 39, 41, 43) by using Accupower Pfu PCR premix. Further, the human antibody kappa light chain constant region was subjected to PCR with a forward primer (Table 3: SEQ ID NO: 44) and a reverse primer containing HindIII (Table 3: SEQ ID NO: 45). The PCR was performed by repeating exposure at 94℃ for 10 minutes, and then exposure at 94℃ for 15 seconds, at 56℃ for 30 seconds, and at 72℃ for 90 seconds 30 times, and reacting at 72℃ for 10 minutes. In the amplified genes, DNA bands having a predicted size were confirmed on 1% agarose gel, and were separated using a gel extraction kit, respectively. Then, the respective light chain variable regions and light chain constant regions were mixed, followed by overlapping PCR, such that the genes expressing the light chain region were cloned. The PCR was performed by repeating exposure at 94℃ for 10 minutes, and then exposure at 94℃ for 15 seconds, at 56℃ for 30 seconds, and at 72℃ for 90 seconds 30 times, and reacting

at 72℃ for 10 minutes. In the amplified genes, DNA bands having an expected size were confirmed on 1% agarose gel, and were separated using a gel extraction kit, respectively. Then, the separated genes reacted with NotI, HindIII restriction enzymes at 37℃ for 12 hours or more, and the genes reacted with the restriction enzymes were separated on 1% agarose gel again. The pcIW plasmid vector was also cut by the same method as above and separated on agarose gel. The separated MI323, MI329, MI403, and MI407 light chain region genes were inserted into NotI, HindIII sites of a linear pcIW vector by using a T4 DNA ligase (Cat.No.M0203S, New England BioLabs (NEB)). The ligation reaction materials were transformed into XL1-Blue bacteria (Electroporation-Competent Cells; Cat.No.200228, Stratagene), plated on an LB plate (Cat.No.LN004CA, NaraeBiotech) containing carbenicillin, and cultured at 37℃ for 12 hours or more. Then single colonies were chosen and cultured, and plasmids were separated by using a plasmid mini kit (Cat.No.27405, QIAGEN), and confirmed by DNA sequencing.

[109]

[110] [Table 3]

Name	DNA nucleotide sequence	SEQ ID NO:
MI323VH-F	GCGGCCGCCATGTACTTGGGACTGAACTATGTATTC ATAGTTTTTCTCTTAAATGGTGTCCAGAGTGAGGTC CAGCTGCAGCAGTCT	31
MI329VH-F	GCGGCCGCCATGTACTTGGGACTGAACTATGTATTC ATAGTTTTTCTCTTAAATGGTGTCCAGAGTGAGGTG ATGCTGGTGGAGTCT	32
MI403VH-F	GCGGCCGCCATGTACTTGGGACTGAACTATGTATTC ATAGTTTTTCTCTTAAATGGTGTCCAGAGTGAGGTG CAGGTGGTGGAGTCT	33
MI407VH-F	GCGGCCGCCATGTACTTGGGACTGAACTATGTATTC ATAGTTTTTCTCTTAAATGGTGTCCAGAGTGAGGTG AAGTTGGTGGAGTCT	34
VHApaI-R	ACCGATGGGCCCTTGGTGGA	35
MI323VL-F	GCGGCCGCCATGGATAGCCAGGCTCAGGTGCTGATG CTGCTGCTGCTGTGGGTGTCAGGGACTTGCGGGGAC ATTGTGATGACCCAGTCTCACAAA	36
MI323VLCL-R	ACACTAGGAGCGGCCACGGTTCGTTTGATTTCCAGT TTGGTCCCT	37
MI329VL-F	GCGGCCGCCATGGATAGCCAGGCTCAGGTGCTGATG CTGCTGCTGCTGTGGGTGTCAGGGACTTGCGGGGAC GTTGTGATGACCCAGACTCCACTC	38
MI329VLCL-R	ACACTAGGAGCGGCCACGGTTCGTTTCAGCTCCAGC TTGGTC	39
MI403VL-F	GCGGCCGCCATGGATAGCCAGGCTCAGGTGCTGATG CTGCTGCTGCTGTGGGTGTCAGGGACTTGCGGGGAT ATTGTGATGACCCAGTCTCCTGCT	40
MI403VLCL-R	ACACTAGGAGCGGCCACGGTTCGTTTTATTTCCAAC TTTGTCCCCGA	41
MI407VL-F	GCGGCCGCCATGGATAGCCAGGCTCAGGTGCTGATG CTGCTGCTGCTGTGGGTGTCAGGGACTTGCGGGGAT ATTGTGTTGACACAGTCTCCTGCT	42

MI407VLCL-R	ACACTAGGAGCGGCCACGGTTCGTTTTATTTCCAACTCTGTCCCCG	43
Ck-F	ACCGTGGCCGCTCCTAGTGT	44
CkSHB-R	NNNNGGATCCAAGCTTACTAGCACTCCCC	45

[111]

[112] 1-3: Production and purification of IgG of MI323, MI329, MI403, and MI407 clone antibodies

[113] In order to produce and purify the anti-MSLN antibody MI323, MI329, MI403, and MI407 clones obtained by a mouse immune response, Expi293F™ cells were inoculated at a concentration of 2.0×10^6 cell/mL the day before transfection. After incubation (37℃, 8% CO_2, 125 rpm) for 24 hours, Expi293™ expression medium (Cat.No.A1435101, Gibco) was added to prepare a product of 30 mL having a concentration of 2.5×10^6 cell/mL (viability = 95%). 30 μg of DNA (pcIW-anti-MSLN heavy chain: 15 μg, pcIW-anti-MSLN light chain: 15 μg) was diluted in an OptiPro™ SEM medium (Cat.No.12309019, Gibco) so as to have a total volume of 1.5 mL, and reacted at room temperature for 5 minutes. 1.5 mL of the OptiProTM SEM medium (Cat.No.12309019, Gibco) was mixed with 80 μL of an ExpiFectamine™ 293 reagent (Cat.No.A14524, Gibco) so that a total volume is 1.5mL, and reacted at room temperature for 5 minutes. After the reaction for 5 minutes, 1.5 mL of diluted DNA and 1.5 mL of diluted ExpiFectamineTM 293 reagent were well-mixed with each other, and reacted at room temperature for 20 to 30 minutes. 3 mL of the mixture of DNA and ExpiFectamineTM 293 reagent was treated in the Expi293F™ cells. After suspension-culture (37 ℃, 8% CO_2, 125 rpm) for 16 to 18 hours, 150 μL of ExpiFectamineTM 293 Enhancer 1 (Cat.No.A14524, Gibco) and 1.5 mL of ExpiFectamineTM 293 Enhancer 2 (Cat.No.A14524, Gibco) were added thereto, followed by suspension-culturing for 5 days. After the culturing, cell debris was removed by centrifugation at 4000rpm for 20 minutes, and the supernatant passed through 0.22 μm filter to be prepared. MabSelect Xtra (Cat.No.17-5269-02, GE Healthcare) which is protein A resin having 100 μL was prepared for each 30 mL of the culture fluid, followed by centrifugation at 1000 rpm for 2 minutes to remove a storage solution, and the obtained product was washed with 400 μL of protein A binding buffer (Cat.No.21007, Pierce) 3 times. The protein A resin was added to the prepared culture fluid and rotation-reacted at room temperature for 30 minutes. The mixture of the culture fluid and the resin was put into a pierce spin column snap-cap (Cat.No.69725, Thermo), and then, only the resin was left in the column using QIAvac 24 Plus (Cat.No.19413, QIAGEN) vacuum manifold. 5 mL of protein A binding buffer was added to wash the resin, 200 μL of a protein A elution buffer (Cat.No.21009, Pierce) was added thereto. The resultant

material was reacted by resuspension at room temperature for 2 minutes, and centrifuged at 1000 rpm for 1 minute, and eluted. Each eluate was neutralized by adding 2.5 μL of 1.5M Tris-HCl (pH 9.0). The elution was performed 4 to 6 times, and each fraction was quantified by using Nanodrop 200C (Thermo Scientific). The fractions in which protein is detected were collected, and exchanged with a PBS (Phosphate-Buffered Saline) buffer using Zeba Spin Desalting Columns, 7K MWCO, 5 mL (Cat.No.0089892, Pierce). Then, protein electrophoresis (SDS-PAGE) was performed under reduction and non-reduction condition to finally verify the concentration quantification and the antibody state, and the antibody was kept at 4℃.

[114]

[115] Example 2: Method for producing anti-MSLN antibody from phage display synthetic scFv library

[116] 2-1: Selection of anti-human MSLN scFv antibody using phage display

[117] For a primary panning, 1 mL of 1013 or more library stock was reacted in a solid phase polystyrene tube (Cat.No.444202, Nunc) coated with MSLN at 37 ℃ for 2 hours. At the same time, 10 μL of XL1-Blue bacteria (electroporation-competent cells; Cat.No.200228, Stratagene) were inoculated with 10 μL of SB 10ml/tetracycline and grown to an OD_{600} of 0.8 to 1.0. The library stock obtained after reaction at 37℃ for 2 hours was washed with 5ml of 0.05% Tween 20/PBS four times, and from a secondary panning, the number of times of washing with 0.05% Tween 20/PBS increased according to an increase in the number of times of panning. Then, the resultant material was cultured with 1%BSA/0.1M Glycine pH 2.0 at room temperature for 10 minutes to purify the phagemid. The purified phagemid was transferred to a 50 mL tube and neutralized with 70 μL of 2M Tris. 9 mL of XL1-Blue bacteria (electroporation-competent cells; Cat.No.200228, Stratagene) were treated, and 1 mL of the bacteria were treated in a washed tube. The bacteria were infected at room temperature for 30 minutes, and 10 mL of SB, 20 μL of tetracycline, 10 μL of carbenicillin were added thereto, followed by suspension-culturing at 37℃ and 220 rpm for 1 hour. Then, the bacteria were treated with 1 mL of VCS M13 helper phage (10^{11} pfu), and suspension-cultured at 37℃ and at 220 rpm for 1 hour, and treated with 80 mL of SB, 100 μL of kanamycin, and 100 μL of carbenicillin, and cultured at 37℃ and at 220 rpm for 12 hours or more. The bacteria cultured over 12 hours were centrifuged at 3500 rpm and at 4℃ for 10 minutes, and the supernatant was transferred to a new tube. 20 mL of 20% PEG/15% NaCl was added thereto, well-mixed, and reacted in ice for 30 minutes. Then, the supernatant was discarded, and pellets were collected and resuspended with 2 mL of 1% BSA/PBS at 8000 rpm, and at 4℃ for 30 minutes, and centrifuged at 15000 rpm and 4℃ for 10 minutes. Here, the collected pellets were discarded and 1 mL of the supernatant (2 mL) was stored at -20℃ and the remainder (1

mL) was used in the following order panning.

[118]

[119] 2-2: Securing individual clones according to ELISA

[120] Single colonies of a phage display synthetic scFv library final amplified population were collected, and cultured with 1.5 mL of SB/carbenicillin to an OD_{600} of 0.8 to 1.0 at 37℃ and at 220 rpm, and then cultured with 1 mM IPTG at 30℃ and at 200 rpm for 12 hours or more. The reaction materials were centrifuged at 5500 rpm for 5 minutes, and only each supernatant was added to ELISA plates containing underlying MSLN antigen, and reacted at room temperature for 2 hours. Then, the resultant materials were washed with PBST (1XPBS, 0.05% tween 20) four times, and HRP/ Anti-hFab-HRP conjugate diluted by 1/5000 with 1% BSA/1XPBS was added thereto, and reacted at room temperature for 1 hour, and washed with PBST (1XPBS, 0.05% tween 20) 4 times. Then, a TMB solution was added thereto and reacted for 5 to 10 minutes, and a TMB stop solution was added thereto. Next, O.D values were measured at a measurement wavelength of 450 nm using a TECAN sunrise, and clones having high O.D. value were secured as individual clones.

[121] As a result, as shown in Table 4, the clones specifically bound to the human MSLN were able to be selected, and amino acid sequences thereof were identified.

[122] Table 5 shows CDR amino acid sequences of the clone antibodies of Table 4 on the basis of Kabat numbering.

[123]

[124] [Table 4]

Clone	variable region	amino acid sequence	SEQ ID NO:
MS501	heavy chain	EVQLLESGGGLVQPGGSLRLSCAASGFT FSNYAMSWVRQAPGKGLEWVSGIYPDS GSTYYADSVKGRFTISRDNSKNTLYLQM NSLRAEDTAVYYCARNIYTFDYWGQGT LVTVSS	46
	light chain	QSVLTQPPSASGTPGQRVTISCSGSSSNIG SNAVSWYQQLPGTAPKLLIYYNNQRPSG VPDRFSGSKSGTSASLAISGLRSEDEADY YCGSWDSSLSGYVFGGGTKLTVLG	47
MS502	heavy chain	EVQLLESGGGLVQPGGSLRLSCAASGFT FSNYAMSWVRQAPGKGLEWVSGIPPDS GSKYYADSVRGRFTVSRDNSKNTLYLQ MNSLRAEDTAVYYCAKNMLSFDYWGQ GTLVTVSS	48
	light chain	QSVLTQPPSASGTPGQRVTISCTGSSSNIG SNAVSWYQQLPGTAPKLLIYYNSKRPSG VPDRFSGSKSGTSASLAISGLRSEDEADY YCGSWDSSLNGYVFGGGTKVTVLG	49
MS502-1	heavy chain	EVQLLESGGGLVQPGGSLRLSCAASGFT FSNYAMSWVRQAPGKGLEWVSGIPPDS GSKYYADSVRGRFTVSRDNSKNTLYLQ MNSLRAEDTAVYYCAKNMLSFDYWGQ GTLVTVSS	48
	light chain	QSVLTQPPSASGTPGQRVTISCICSSSNIG SNAVSWYQQLPGTAPKLLIYYNSKRPSG VPDRFSGSKSGTSASLAISGLRSEDEADY YCGSWDSSLNGYVFGGGTKLTVLG	50
MS503	heavy chain	EVQLLESGGGLVQPGGSLRLSCAASGFT FSNYAMSWVRQAPGKGLEWVSSIYPGD GSTYYADSVKGRFTISRDNSKNTLYLQM NSLRAEDTAVYYCAKNAFTFDYWGQGT LVTVSS	51
	light chain	QSVLTQPPSASGTPGQRVTISCSGSSSNIG	52

		SNAVSWYQQLPGTAPKLLIYYNSHRPSG VPDRFSGSKSGTSASLAISGLRSEDEADY YCGTWDSSLSGYVFGGGTKLTVLG	
MS504	heavy chain	EVQLLESGGGLVQPGGSLRLSCAASGFT FSNYAMSWVRQAPGKGLEWVSSIYPNG SSKYYADSVKGRFTISRDNSKNTLYLQM NSLRAEDMAVYYCAKNLLTFDYWGQG TLVTVSS	53
	light chain	QSVLTQPPSASGPPGQRVTISCTGSSSNIG NNSVSWYQQLPGTAPKLLIYYDSHRPSG VPDRFSGSKSGTSASLAIGGLRSEDEADY YCGAWDDSLNAYVFGGGTKLTVLG	54
MS505	heavy chain	EVQLLESGGGLVQPGGSLRLSCAASGFT FSNYAMSWVRQAPGKGLEWVSAIYPDG SNKYYADSVKGRFTVSRDNSKNTLYLQ MNSLRAEDTAVYYCARNAYTFDYWGQ GTLVTVSS	55
	light chain	QSVLTQPPSASGTPGRRVTISCSGSSSNIG SNAVSWYQQLPGTAPKLLIYYNSQRPSG VPDRFSGSKSGTSASLAISGLRSEDEADY YCGSWDSSLNGYVFGGGTKLTVLG	56
MS506	heavy chain	EVQLLESGGGLVQPGGSLRLSCAASGFT FSNYAMSWVRQAPGKGLEWVSSIYPGS GSTYYADSVKGRFTISRDNSKNTLYLQM NSLRAEDTAVYYCARNLYTFDYWGQGT LVTVSS	57
	light chain	QSVLTQPPSASGTPGQRVTISCTGSSSNIG SNAVTWYQQLPGTAPKLLIYYDSHRPSG VPDRFSGSKSGTSASLAISGPRSEDEADY YCGAWDSSLSAYVFGGGTKLTVLG	58

[125]

[126] [Table 5]

Clone	variable region	CDR1	CDR2	CDR3
MS501	heavy chain	NYAMS(SEQ ID NO: 59)	GIYPDSGSTYYA DSVKG(SEQ ID NO: 60)	NIYTFDY(SEQ ID NO: 61)
	light chain	SGSSSNIGSNA VS(SEQ ID NO: 62)	YNNQRPS(SEQ ID NO: 63)	GSWDSSLSGYV(SEQ ID NO: 64)
MS502	heavy chain	NYAMS(SEQ ID NO: 59)	GIPPDSGSKYYA DSVRG(SEQ ID NO: 65)	NMLSFDY(SEQ ID NO: 66)
	light chain	TGSSSNIGSNA VS(SEQ ID NO: 67)	YNSKRPS(SEQ ID NO: 68)	GSWDSSLNGYV(SEQ ID NO: 69)
MS502-1	heavy chain	NYAMS(SEQ ID NO: 59)	GIPPDSGSKYYA DSVRG(SEQ ID NO: 65)	NMLSFDY(SEQ ID NO: 66)
	light chain	ICSSSNIGSNA VS(SEQ ID NO: 70)	YNSKRPS(SEQ ID NO: 68)	GSWDSSLNGYV(SEQ ID NO: 69)
MS503	heavy chain	NYAMS(SEQ ID NO: 59)	SIYPGDGSTYYA DSVKG(SEQ ID NO: 71)	NAFTFDY(SEQ ID NO: 72)
	light chain	SGSSSNIGSNA VS(SEQ ID NO: 62)	YNSHRPS(SEQ ID NO: 73)	GTWDSSLSGYV(SEQ ID NO: 74)
MS504	heavy chain	NYAMS(SEQ ID NO: 59)	SIYPNGSSKYYA DSVKG(SEQ ID NO: 75)	NLLTFDY(SEQ ID NO: 76)
	light chain	TGSSSNIGNNS VS(SEQ ID NO: 77)	YDSHRPS(SEQ ID NO: 78)	GAWDDSLNAYV (SEQ ID NO: 79)
MS505	heavy chain	NYAMS(SEQ ID NO: 59)	AIYPDGSNKYYA DSVKG(SEQ ID NO: 80)	NAYTFDY(SEQ ID NO: 81)

	light chain	SGSSSNIGSNAVS(SEQ ID NO: 62)	YNSQRPS(SEQ ID NO: 82)	GSWDSSLNGYV(SEQ ID NO: 83)
MS506	heavy chain	NYAMS(SEQ ID NO: 59)	SIYPGSGSTYYADSVKG(SEQ ID NO: 84)	NLYTFDY(SEQ ID NO: 85)
	light chain	TGSSSNIGSNAVT(SEQ ID NO: 86)	YDSHRPS(SEQ ID NO: 78)	GAWDSSLSAYV(SEQ ID NO: 87)

[127]

[128] 2-3: IgG gene cloning of anti-mesothelin antibody

[129] The pComb3X phagemid containing the genes encoding the light chain variable regions of the secured MS501, MS502, MS503, MS504, MS505, and MS506 clone antibodies was extracted, and used as a template for PCR of the light chain variable regions of the MS501, MS502, MS503, MS504, MS505, and MS506 clone antibodies, wherein the PCR was performed with a forward primer containing NotI (SEQ ID NO: 86) and a reverse primer (SEQ ID NO: 87) by using Accupower Pfu PCR premix. Further, human antibody kappa light chain constant region was subjected to PCR with a forward primer (Table 6: SEQ ID NO: 88) and a reverse primer (Table 6: SEQ ID NO: 89). The PCR was performed by repeating exposure at 94℃ for 10 minutes, and then exposure at 94℃ for 15 seconds, at 56℃ for 30 seconds, and at 72℃ for 90 seconds 30 times, and reacting at 72℃ for 10 minutes. In the amplified genes, DNA bands having an expected size were confirmed on 1% agarose gel, and were separated using a gel extraction kit, respectively. Then, the respective light chain variable regions and light chain constant regions were mixed, followed by overlapping PCR, such that the genes expressing the light chain region were cloned. The PCR was performed by repeating exposure at 94℃ for 10 minutes, and then exposure at 94℃ for 15 seconds, at 56℃ for 30 seconds, and at 72℃ for 90 seconds 30 times, and reacting at 72℃ for 10 minutes. In the amplified genes, DNA bands having an expected size were confirmed on 1% agarose gel, and were separated using a gel extraction kit, respectively. Then, the separated genes reacted with NotI, HindIII restriction enzymes at 37℃ for 12 hours or more, and the genes reacted with the restriction enzymes were separated on 1% agarose gel again. The pcIW plasmid vector was also cut by the same method as above and separated on agarose gel. The separated MS501, MS502, MS503, MS504, MS505, MS506 light chain region genes were inserted into NotI, HindIII sites of the linear pcIW vector by using a T4 DNA ligase (Cat.No.M0203S, New England BioLabs (NEB)). The ligation reaction materials were transformed into XL1-Blue

bacteria (Electroporation-Competent Cells; Cat.No.200228, Stratagene), plated on an LB plate (Cat.No.LN004CA, NaraeBiotech) containing carbenicillin, and cultured at 37℃ for 12 hours or more. Then single colonies were chosen and cultured, and plasmids were separated by using a plasmid mini kit (Cat.No.27405, QIAGEN), and confirmed by DNA sequencing.

[130] The pComb3X phagemid containing the genes encoding the heavy chain variable regions of the MS501, MS502, MS503, MS504, MS505, and MS506 clone antibodies was extracted, and used as a template for PCR with a forward primer containing NotI (Table 6: SEQ ID NO: 90) and a reverse primer containing ApaI (Table 6: SEQ ID NO: 91) by using Accupower Pfu PCR premix (Bioneer). The PCR was performed by repeating exposure at 94℃ for 10 minutes, and then exposure at 94℃ for 15 seconds, at 56℃ for 30 seconds, and at 72℃ for 90 seconds 30 times, and reacting at 72℃ for 10 minutes. In the amplified genes, DNA bands having an expected size were confirmed on 1% agarose gel, and were separated using a gel extraction kit, respectively. Then, the separated genes reacted with NotI, ApaI restriction enzymes at 37℃ for 12 hours or more, and the genes reacted with the restriction enzymes were separated on 1% agarose gel again. A pcIW plasmid vector containing human immunoglobulin type 1 of heavy chain constant region (IgG1 heavy chain constant region) gene was also cut by the same method as above and separated on agarose gel. The separated MS501, MS502, MS503, MS504, MS505, and MS506 heavy chain variable region genes were inserted into NotI, ApaI sites of the linear pcIW vector containing the human heavy chain constant region by using a T4 DNA ligase (Cat.No.M0203S, New England BioLabs (NEB)). The ligation reaction materials were transformed into XL1-Blue bacteria (Electroporation-Competent Cells; Cat.No.200228, Stratagene), plated on an LB plate (Cat.No.LN004CA, NaraeBiotech) containing carbenicillin, and cultured at 37℃ for 12 hours or more. Then single colonies were chosen and cultured, and plasmids were separated by using a plasmid mini kit (Cat.No.27405, QIAGEN), and confirmed by DNA sequencing.

[131]

[132] [Table 6]

Name	Nucleotide sequence	SEQ ID NO:
NotI-Leader-VL-F	NNNNGCGGCCGCCATGGATAGCCAGGCTCAGGTGCTGATGCTGCTGCTGCTGTGGGTGTCAGGGACTTG CGGGCAGTCTGTGCTGACTCAGCCA	88
VL-R	GGGGTTGGCCTTGGGCTGGCCTAGGACCGTCAGCTTGGT	89
VL-CL-F	CAGCCCAAGGCCAACCCC	90
HindIII-VL-R	NNNNGGATCCAAGCTTACTAACATTCTGTAGGGGCCACTGTC	91
HD-Heavy-F	GGTGTCCAGGCGGCCGCCATGTACTTGGGACTGAACTATGTATTCATAGTTTTTCTCTTAAATGGTGTCCAGAGTGAGGTGCAGCTGTTGGAGTCTG	92
HD-Heavy-R	GGGGGAAGACCGATGGGCCCTTGGTGGAGGCTGAGCTCACGGTGACCAGTGT	93

[133]

[134] 2-4: Production and purification of IgG of MS501, MS502, MS503, MS504, MS505, MS506 clone antibodies

[135] In order to produce and purify the MS501, MS502, MS503, MS504, MS505, MS506 clone antibodies obtained from the phage display scFv library, Expi293F™ cells were inoculated at a concentration of 2.5×10^6 cell/mL the day before transfection. After incubation (37℃, 8% CO_2, 125 rpm) for 24 hours, Expi293™ expression medium (Cat.No.A1435101, Gibco) was added to prepare a product of 30 mL having a concentration of 2.5×10^6 cell/mL (viability = 95%). 30μg of DNA (pcIw-MS502 heavy chain variable region: 15 μg, pcIw-anti-Mesothelin light chain variable region: 15 μg) was diluted in an OptiPro™ SEM medium (Cat.No.12309019, Gibco) so as to have a total volume of 1.5 mL, and reacted at room temperature for 5 minutes. 1.5 mL of the OptiPro™ SEM medium (Cat.No.12309019, Gibco) was mixed with 80 μL of an ExpiFectamine™ 293 reagent (Cat.No.A14524, Gibco) so that a total volume is 1.5 mL, and reacted at room temperature for 5 minutes. After the reaction for 5 minutes, 1.5 mL of diluted DNA and 1.5 mL of diluted ExpiFectamine™ 293 reagent were well-mixed with each other, and reacted at room temperature for 20 to 30 minutes. 3 mL of the mixture of DNA and ExpiFectamine™ 293 reagent was treated in the Expi293F™

cells. After suspension-culture (37 ℃, 8% CO_2, 125 rpm) for 16 to 18 hours, 150 μL of ExpiFectamine™ 293 Enhancer 1(Cat.No.A14524, Gibco) and 1.5 mL of Expi-Fectamine™ 293 Enhancer2 (Cat.No.A14524, Gibco) were added thereto, followed by suspension-culturing for 5 days. After the culturing, cell debris was removed by centrifugation at 4000rpm for 20 minutes, and the supernatant passed through 0.22 μm filter to be prepared. MabSelect Xtra (Cat.No.17-5269-02, GE Healthcare) which is protein A resin having 100 μL was prepared for each 30 mL of the culture fluid, followed by centrifugation at 1000 rpm for 2 minutes to remove a storage solution, and the product was washed with 400 μL of protein A binding buffer (Cat.No.21007, Pierce) 3 times. The protein A resin was added to the prepared culture fluid and rotation-reacted at room temperature for 30 minutes. The mixture of the culture fluid and the resin was put into a pierce spin column snap-cap (Cat.No.69725, Thermo), and then, only the resin was left in the column using QIAvac 24 Plus (Cat.No.19413, QIAGEN) vacuum manifold. 5 mL of protein A binding buffer was added to wash the resin, 200 μL of a protein A elution buffer (Cat.No.21009, Pierce) was added thereto. The resultant material was reacted by resuspension at room temperature for 2 minutes, and centrifuged at 1000 rpm for 1 minute, and eluted. Each eluate was neutralized by adding 2.5 μL of 1.5M Tris-HCl (pH 9.0). The elution was performed 4 to 6 times, and each fraction was quantified by using Nanodrop 200C (Thermo scientific). The fractions in which protein is detected were collected, and exchanged with a PBS (Phosphate-Buffered Saline) buffer using Zeba Spin Desalting Columns, 7K MWCO, 5 mL (Cat.No.0089892, Pierce). Then, protein electrophoresis (SDS-PAGE) was performed under reduction and non-reduction condition to finally verify the concentration quantification and the antibody state, and the antibody was kept at 4℃.

[136]

[137] 2-5: Measurement of quantitative binding force of anti-MSLN antibody with regard to antigen

[138] Quantitative binding force (affinity) of the purified anti-MSLN antibodies, i.e., MI323, MI329, MI403, MS502 clone antibodies with regard to recombinant human mesothelin (MSLN) was measured by using a Biacore T-200 (GE Healthcare, U.S.A.) biosensor. The MSLN (Cat.No.3265-MS, R&D systems) purified from the HEK293 cells was fixed to a CM5 chip (GE Healthcare, U.S.A.) so as to satisfy 200 Rmax by using an amine-carboxylic reaction. Then, the clone C2G1 antibody, the clone C2G4 antibody or the clone C3C8 antibody serially diluted with HBS-EP buffer (10mM HEPES, pH7.4, 150mM NaCl, 3mM EDTA, 0.005% surfactant P20) was allowed to flow at a concentration range of 0.078nM to 5nM and at a flow rate of 30μL/min for association of 120 seconds and dissociation of 1800 seconds. The dissociation of the antibody bound to the MSLN was induced by flowing 10 mM Glycine-HCl pH 1.5 at a

flow rate of 30 μL/min for 30 seconds (Table 7). The affinity was obtained as movement speed constants (K_{on} and K_{off}) and an equilibrium dissociation constant (K_D) by using a Biacore T-200 evaluation software (Table 8).

[139]

[140] [Table 7]

SPR	Biacore T200
Chip	CM5
Running Buffer	HBS-EP pH7.4
Flow rate	30μL/min
Association / dissociation time	120 sec / 600 sec
IgG Conc.	0.078~5nM, 1/2 serial dilution
Regeneration	10mM Glycine-HCl pH1.5, 30 sec

[141]

[142] [Table 8]

	K_{on}	K_{off}	K_D
MI323	2.7×10^{5}	4.8×10^{-5}	1.8×10^{-10}
MI329	1.4×10^{6}	5.1×10^{-5}	3.5×10^{-11}
MI403	8.9×10^{4}	4.0×10^{-5}	4.5×10^{-10}
MS502	1.9×10^{7}	4.3×10^{-3}	2.3×10^{-10}

[143]

[144] Example 3: Method for producing MS502 clone affinity maturation antibody

[145] 3-1: Design of MS502 clone affinity maturation library

[146] The amino acid sequence of the anti-mesothelin MS502 clone was entered to the Swiss model homepage (http://swissmodel.expasy.org/) to find a template sequence. 50 sequences were found on the basis of homologous sequences, and modeling was performed by designating each sequence as the template. 50 sequences were listed by priority on the basis of QMEAN4 values (Cβ, all atom, solvation, torsion), and the template was selected in consideration of sequence identity and resolution in addition to the QMEAN4 values. 3g6a.1.B was selected for the heavy chain variable region, and 3qhz.1.B (LCDR1,2) and 4o5l.1.A (LCDR3) were selected for the light chain variable region. The MS502 structure obtained on the basis of the selected template was analyzed by pymol program, and paratope was selected on the basis of the protruding amino acid of the CDR (see FIG. 1). Among them, VL CDR2 Y49, Y50, K53 were excluded from the mutation candidate amino acids even though it was an-

ticipated that they are paratopes since Tyr and Lys are amino acids generally having a positive effect on bonding. The introduction of mutation was designed by 50% preserving the existing sequence, increasing Tyr, Ser, Gly rates, and controlling the nucleotide sequence using a handmix primer (IDT, U.S.A.) so that hydrophilic, hydrophobic, positive charge, and negative charge rates were uniformly included (Table 9). The heavy chain variable region was divided into each of three fragments since the mutation was introduced into all of CDR1, 2, and 3, and they were subjected to fragment PCR and overlapping PCR to construct a library, and the light chain variable region was divided into each of two fragments since the mutation was introduced into CDR1 and 3 only, and they were subjected to fragment PCR and overlapping PCR to construct a library. A theoretical size of the library according to the introduction of the mutation in the case of the heavy chain variable region was 5.83×10^{10}, and the theoretical size of the library according to the introduction of the mutation in the case of the light chain variable region was 2.16×10^{4}.

[147]

[148] [Table 9]

HCDR1											
N31				Y32				A33			
Tyr	7	THR	7	Tyr	49	THR	1	Tyr	0.5	THR	7
Ser	8	Gln	0	Ser	8	Gln	0	Ser	7.5	Gln	0.5
Gly	1	Asn	49	Gly	1	Asn	7	Gly	7	Asn	0.5
Arg	1	Ala	1	Arg	1	Ala	1	Arg	1.5	Ala	49
Lys	0	Val	1	Lys	0	Val	1	Lys	0.5	Val	7
His	7	Leu	1	His	7	Leu	1	His	0.5	Leu	1.5
Glu	0	Met	0	Glu	0	Met	0	Glu	3.5	Met	0.25
Asp	7	Ile	7	Asp	7	Ile	1	Asp	3.5	Ile	0.75
Phe	1	Pro	1	Phe	7	Pro	1	Phe	0.5	Pro	7
TRP	0	Cys	1	TRP	0	Cys	7	TRP	0.25	Cys	0.5
		STOP	0			STOP	0			STOP	0.75

HCDR2											
D53				G55				S56			
Tyr	7	THR	1	Tyr	0.5	THR	1	Tyr	17.5	THR	7
Ser	2	Gln	0	Ser	4.5	Gln	0.5	Ser	49	Gln	0
Gly	7	Asn	7	Gly	49	Asn	0.5	Gly	0	Asn	2.5
Arg	1	Ala	7	Arg	10.5	Ala	7	Arg	0	Ala	7
Lys	0	Val	7	Lys	0.5	Val	7	Lys	0	Val	0.5
His	7	Leu	1	His	0.5	Leu	1.5	His	2.5	Leu	0.5
Glu	0	Met	0	Glu	3.5	Met	0.25	Glu	0	Met	0
Asp	49	Ile	1	Asp	3.5	Ile	0.75	Asp	2.5	Ile	0.5
Phe	1	Pro	1	Phe	0.5	Pro	1	Phe	3.5	Pro	7
TRP	0	Cys	1	TRP	1.75	Cys	3.5	TRP	0	Cys	0
		STOP	0			STOP	2.25			STOP	0

HCDR3											
L98				S99				F100			
Tyr	0.5	THR	1	Tyr	14	THR	7	Tyr	10.5	THR	1.5
Ser	1.5	Gln	3.5	Ser	49	Gln	0	Ser	7.75	Gln	0
Gly	1	Asn	0.5	Gly	0	Asn	2	Gly	0.25	Asn	2.25
Arg	7.5	Ala	1	Arg	0	Ala	7	Arg	0.5	Ala	0.5
Lys	0.5	Val	7	Lys	0	Val	1	Lys	0	Val	3.5
His	3.5	Leu	52.5	His	2	Leu	1	His	1.5	Leu	7
Glu	0.5	Met	1.75	Glu	0	Met	0	Glu	0	Met	0
Asp	0.5	Ile	5.25	Asp	2	Ile	1	Asp	0.75	Ile	10.5
Phe	3.5	Pro	7	Phe	7	Pro	7	Phe	49	Pro	1
TRP	0.25	Cys	0.5	TRP	0	Cys	0	TRP	0	Cys	3.5
		STOP	0.75			STOP	0			STOP	0

[149]

LCDR1			
S30			
Tyr	18.8	THR	7
Ser	52.5	Gln	0
Gly	0	Asn	2.5
Arg	0	Ala	7
Lys	0	Val	0.5
His	2.5	Leu	0.5
Glu	0	Met	0
Asp	2.5	Ile	0.5
Phe	3.75	Pro	7
TRP	0	Cys	0
		STOP	0

LCDR3											
W92				S94				N95b			
Tyr	0	THR	2	Tyr	14	THR	7	Tyr	7	THR	7
Ser	14	Gln	0	Ser	49	Gln	0	Ser	8	Gln	0
Gly	6	Asn	0	Gly	0	Asn	2	Gly	1	Asn	49
Arg	12	Ala	2	Arg	0	Ala	7	Arg	1	Ala	1
Lys	0	Val	2	Lys	0	Val	1	Lys	0	Val	1
His	0	Leu	16	His	2	Leu	1	His	7	Leu	1
Glu	0	Met	2	Glu	0	Met	0	Glu	0	Met	0
Asp	0	Ile	0	Asp	2	Ile	1	Asp	7	Ile	7
Phe	0	Pro	2	Phe	7	Pro	7	Phe	1	Pro	1
TRP	42	Cys	0	TRP	0	Cys	0	TRP	0	Cys	1
		STOP	0			STOP	0			STOP	0

[150]

[151] 3-2: Construction of light chain variable region library

[152] In order to introduce the mutation into the library, first, the light chain variable region was divided into 2 parts, and they were subjected to fragment PCR. The light chain variable region fragment No. 1 had a light chain variable region gene sequence of anti-mesothelin MS502 clone as the template, and a forward primer (SEQ ID NO: 93) and a reverse primer (SEQ ID NO: 94) were added thereto, and the light chain variable region fragment No. 2 had the light chain variable region gene sequence of anti-mesothelin MS502 clone as the template, and a forward primer (Table 10: SEQ ID NO: 95) and a reverse primer (Table 10: SEQ ID NO: 96) were added thereto, and then, each fragment was subjected to PCR using a Primestar polymerase premix (Takara). The PCR was performed by repeating exposure at 98℃ for 2 minutes, and then exposure at 98℃ for 10 seconds, at 60℃ for 15 seconds, and at 72℃ for 20 seconds 30 times, and reacting at 72℃ for 10 minutes. In the amplified genes, DNA bands having an expected size were confirmed on 1% agarose gel, and were separated using a gel extraction kit (QIAquick Gel Extraction Kit, CAT. NO. 28706, QIAGEN), respectively. The light chain constant region had a light chain lambda region as a template, and a forward primer (Table 10: SEQ ID NO: 97), and a reverse primer (Table 10: SEQ ID NO: 98) were added thereto, and each fragment was subjected to PCR using a Primestar polymerase premix. The PCR was performed by repeating exposure at 98℃ for 2 minutes, and then exposure at 98℃ for 10 seconds, at 60℃ for

15 seconds, and at 72℃ for 30 seconds 30 times, and reacting at 72℃ for 10 minutes. In the amplified genes, DNA bands having an expected size were confirmed on 1% agarose gel, and were separated using a gel extraction kit, respectively. The secured light chain variable region fragments 1 and 2, and light chain constant region at a molar ratio of 1:1:1 were used as a template, and a forward primer (SEQ ID NO: 92) and a reverse primer (SEQ ID NO: 98) were added thereto, and each fragment was subjected to PCR using a Primestar polymerase premix. The PCR was performed by repeating exposure at 98℃ for 2 minutes, and then exposure at 98℃ for 20 seconds, at 60℃ for 30 seconds, and at 72℃ for 60 seconds 30 times, and reacting at 72℃ for 10 minutes. In the amplified genes, DNA bands having an expected size were confirmed on 1% agarose gel, and were separated using a gel extraction kit to secure light chain variable-constant region affinity maturation gene. The secured gene was reacted with NruI and XbaI (NEB) restriction enzyme at 37℃ for 4 hours. The genes reacted with the restriction enzyme were separated on 1% Agarose gel again. The separated gene was inserted into the NruI, XbaI site of the linear pComb3x vector containing the MS502 heavy chain variable-constant region by using a T4 DNA ligase (Cat.No.M0203S, NEB). The ligation reaction material was transformed into XL1-Blue bacteria (Electroporation-competent cells; Cat.No.200228, Stratagene), and cultured in 300 mL of LB medium at 37℃ and at 220 rpm for 1 hour. Then, the resultant material was treated with 150 μL of carbenicillin and 300 μL of tetracycline, and was cultured with shaking at 37℃ and at 220 rpm for 1 hour. The resultant material was treated with VCS M13 helper phage 4.5 mL (10^{11}pfu) and cultured with shaking at 37℃ and at 220 rpm for 1 hour, and treated with 300 μL of kanamycin and 300 μL of carbenicillin, and cultured overnight at 37℃ and at 220 rpm. The next day, the cultured cells were centrifuged at 4000 rpm for 20 minutes, and the supernatant was transferred to a new vessel. In order to precipitate the phage, 5X PEG/NaCl was used to add 1X PEG/NaCl to the supernatant, and the obtained product allowed to stand on ice over 30 minutes. The precipitate phage was centrifuged at 8000 rpm for 30 minutes. The supernatant was discarded and the precipitated phage was re-suspended with 10 mL of PBS. In order to remove cell debris, the phage dissolved in 10 mL of PBS was centrifuged at 14,000 rpm for 10 minutes to separate the supernatant, and stored at 4℃. The library size was confirmed by taking 100 μL of culture fluid after 1 hour of the transformation, plating the culture fluid on the LB plate (Cat.No.LN004CA, NaraeBiotech) containing carbenicillin in serial dilution manner, culturing at 37℃ for 12 hours or more, and counting the colonies.

[153]

[154] 3-3: Construction of heavy chain variable region library

[155] In order to introduce the mutation into the library, first, the heavy chain variable

region was divided into 3 parts, and they were subjected to fragment PCR. The heavy chain variable region fragment No. 1 had a heavy chain variable region gene sequence of anti-mesothelin MS502 clone as the template, and a forward primer (Table 10: SEQ ID NO: 99) and a reverse primer (Table 10: SEQ ID NO: 100) were added thereto, and the heavy chain variable region fragment No. 2 had the heavy chain variable region gene sequence of anti-mesothelin MS502 clone as the template, and a forward primer (Table 10: SEQ ID NO: 101) and a reverse primer (Table 10: SEQ ID NO: 102) were added thereto, and the heavy chain variable region fragment No. 3 had the heavy chain variable region gene sequence of anti-mesothelin MS502 clone as the template, and a forward primer (Table 10: SEQ ID NO: 103) and a reverse primer (Table 10: SEQ ID NO: 104) were added thereto, and then, each fragment was subjected to PCR using a Primestar polymerase premix (Takara). The PCR was performed by repeating exposure at 98℃ for 2 minutes, and then exposure at 98℃ for 10 seconds, at 60℃ for 15 seconds, and at 72℃ for 20 seconds 30 times, and reacting at 72℃ for 10 minutes. In the amplified genes, DNA bands having an expected size were confirmed on 1% agarose gel, and were separated using a gel extraction kit, respectively. The secured heavy chain variable region fragments 1, 2, and 3 at a molar ratio of 1:1:1 were used as a template, and a forward primer (Table 10: SEQ ID NO: 99) and a reverse primer (Table 10: SEQ ID NO: 106) were added thereto, and each fragment was subjected to PCR using a Primestar polymerase premix. The PCR was performed by repeating exposure at 98℃ for 2 minutes, and then exposure at 98℃ for 20 seconds, at 60℃ for 30 seconds, and at 72℃ for 60 seconds 30 times, and reacting at 72℃ for 10 minutes. In the amplified genes, DNA bands having an expected size were confirmed on 1% agarose gel, and were separated using a gel extraction kit to secure heavy chain variable region affinity maturation gene. The secured gene was reacted with XhoI and ApaI (NEB) restriction enzyme at 37℃ for 4 hours. The genes reacted with the restriction enzyme were separated on 1% Agarose gel again. The separated gene was inserted into the XhoI, ApaI site of the linear pComb3x vector containing the MS502 light chain variable-constant region by using a T4 DNA ligase (Cat.No.M0203S, NEB). The ligation reaction material was transformed into XL1-Blue bacteria (Electroporation-competent cells; Cat.No.200228, Stratagene), and cultured in 300 mL of LB medium at 37℃ and at 220 rpm for 1 hour. Then, the resultant material was treated with 150 μL of carbenicillin and 300 μL of tetracycline, and was suspension-cultured at 37℃ and at 220 rpm for 1 hour. The resultant material was treated with VCS M13 helper phage 4.5mL (1011pfu) and cultured with shaking at 37℃ and at 220 rpm for 1 hour, and treated with 300 μL of kanamycin and 300 μL of carbenicillin, and cultured overnight at 37℃ and at 220 rpm. Next day, the cultured cells were centrifuged at 4000 rpm for 20 minutes, and the supernatant was transferred to a new

vessel. In order to precipitate the phage, 5X PEG/NaCl was used to add 1X PEG/NaCl to the supernatant, and the obtained product allowed to stand on ice over 30 minutes. The precipitated phage was centrifuged at 8000 rpm for 30 minutes. The supernatant was discarded and the precipitated phage was re-suspended with 10 mL of PBS. In order to remove cell debris, the phage dissolved in 10 mL of PBS was centrifuged at 14,000 rpm for 10 minutes to separate the supernatant, and stored at 4℃. The library size was confirmed by taking 100 μL of culture fluid after 1 hour of the transformation, plating the culture fluid on the LB plate (Cat.No.LN004CA, NaraeBiotech) containing carbenicillin in serial dilution manner, culturing at 37℃ for 12 hours or more, and counting the colonies.

[156]

[157] [Table 10]

Name	Nucleotide sequence	SEQ ID NO:
MS502 VL FR1 Fo NruI	GC Tcgcgattgcagtggcactggctggtttcgctaccgtggc-ccaggcggcc CAG TCT GTG CTG ACT CAG CCA CCC TCA	94
MS502 VL FR1 Fo	CAG TCT GTG CTG ACT CAG CCA CCC TCA	95
MS502 VL FR1 Re	GAG CTG CTG GTA CCA GGA GAC AGC ATT RX5X4 GCC AAT ATT AGA TGA AGA GCC AGT ACA AGA	96
MS502 VL FR2 Fo	GCC AAT ATT AGA TGA AGA GCC AGT ACA AGA	97
MS502 VL FR2 Re	ACC TAG GAC GGT CAC CTT GGT GCC TCC GCC GAA GAC ATA ACC RX3X3 CAG GCT RX7X4 ATC CX8X4 AGA ACC ACA GTA ATA ATC AGC CTC ATC CTC GGA	98
MS502 CL Fo	GGC ACC AAG GTG ACC GTC CTA GGT CAG CCC AAG GCC AAC CCC ACT GTC	99
MS502 CL Re	GCT CTA GAA CAT TCT GTA GGG GCC ACT GTC TTC TC	100
MS502 VH FR1 Fo NcoI	gcccatggcc GAG GTG CAG CTG TTG GAG TCT GGG	101
MS502 VH FR1 Re	AGC CTG GCG GAC CCA GCT CAT NX1X2 RX3X4 RX3X3 GCT AAA GGT GAA TCC AGA GGC CGC ACA	102
MS502 VH FR2 Fo	atgagctgggtccgccaggct	103
MS502 VH FR2 Re	GGT GAA CCG ACC TCT TAC AGA ATC AGC GTA ATA TTT RX5X4 NX2X2 ACT RX3X2 AGG AGG GAT CCC TGA GAC CCA CTC	104
MS502 VH FR3 Fo	AAA TAT TAC GCT GAT TCT GTA AGA GGT CGG TTC ACC	105
MS502 VH	TGA GCT CAC CGT GAC CAG TGT ACC CTG GCC	106

FR3 Re	CCA GTA GTC RX6X6 RX7X4 NX4X1 CAT ATT TTT CGC ACA GTA ATA CAC GGC CGT	
MS502 VH Re	TGA GCT CAC CGT GAC CAG TGT ACC CTG	107
MS502 VH Re ApaI	GCG GGC CCT TGG TGG AGG CTG AGC TCA CCG TGA CCA GTG TAC CCT G	108

[158]

[159] In Table 10, X codon is a degenerative codon in which each ACGT is controlled at a specific rate, and is able to control a rate of the amino acids to be translated. As an example, for the NX1X2 codon of SEQ ID NO: 102, N is encoded by A, C, G, T with a random ratio, X1 is encoded at 10% of A, 10% of C, 70% of G, and 10% of T, and X2 is encoded at 10% of A, 70% of C, 10% of G, and 10% of T. This is designed for the reverse primer, and thus, if it is converted to the forward direction, it is the X2X1N codon, wherein X2 is encoded at 10% of A, 70% of C, 10% of G, and 10% of T, and X1 is encoded at 10% of A, 10% of C, 70% of G, and 10% of T. As a result, Kabat No. 31 of the heavy chain variable region CDR1 is translated into amino acids, Tyr 7%, Ser 8%, Gly 1%, His 7%, Asp 7%, Phe 1%, Thr 7%, Asn 49%, Ala 1%, Val 1%, Leu 1%, Ile 7%, Pro 1%, Cys 1%.

[160]

[161] 3-4: Selection of light chain variable region mutation antibody

[162] 1 mL of recombinant human protein MSLN having a concentration of 1 μg/mL was put in a solid phase polystyrene tube (Cat.No.444202, Nunc), and the tube coated at 4℃ for 12 hours or more was washed with 5 mL of 0.05% PBST three times. 5 mL of 1%BSA/PBS was put in the MSLN-coated immuno tube, followed by blocking at room temperature for 2 hours. A blocking buffer was removed from the immuno tube, and then, the light chain variable region phage library was treated in the tube and reacted at room temperature for 2 hours. Then, the obtained product was washed with 5 mL of PBST four times. The immuno tube was treated with 1 mL of glycine (pH 2.0) elution buffer, and reacted at room temperature for 10 minutes to obtain the supernatant. After elution, 100 μL 0of 1.5M Tris-Cl (pH 8.8) was added to the phage and neutralized. 10 mL of XL1-Blue bacteria (electroporation-competent cells; Cat.No.200228, Stratagene) cultured for about 2 hours (OD_{600} = 0.8 to 1.0) were treated with the neutralized phage. After infection at room temperature for 30 minutes, 10 mL of SB, 20 μL of tetracycline (50 mg/ml), and 10 μL of carbenicillin (100 mg/mL) were added to 10 mL of the infected XL1-Blue bacteria (electroporation-competent cells; Cat.No.200228, Stratagene), and cultured with shaking (200rpm) at 37℃ for 1 hour. The bacteria were treated with 1 mL of VCSM13

helper phage (> 10^{11} pfu/ml), and cultured with shaking (200 rpm) at 37°C for 1 hour. After 1 hour incubation, the bacteria were treated with 80 mL of SB, 100 μL of kanamycin, and 100 μL of carbenicillin (100 mg/mL), and cultured overnight (200 rpm) at 37°C. The overnight cultured library was centrifuged at 4000 rpm for 15 minutes to separate the supernatant only, and 5X PEG/NaCl was used to add 1X PEG/NaCl to the supernatant, and the obtained product allowed to stand on ice over 30 minutes. The supernatant was removed by centrifugation at 8000 rpm for 30 minutes, and pellets were re-suspended with 2 ml of 1% BSA/PBS and centrifuged at 12000 rpm for 10 minutes. Then, only the supernatant was taken and used in the following order panning. This process was repeated four times.

[163]

[164] 3-5: Selection of heavy chain variable region mutation antibody

[165] The supernatant was removed with 50 μL of streptavidin microbead (Miltenyl biotec 130-048-101), and 1 mL of PBS was added thereto to perform washing three times. 1 mL of PBS containing 1% BSA was added to the bead, and the bead was rotated at room temperature for 2 hours, followed by blocking. 50 nM MSLN was put in 500 μL of PBS, and mixed with 500 μL of MS502 VH rational library phage and rotation-reacted at room temperature for 1 hour. The blocking buffer was removed from the bead, and the bead was treated with a solution containing the MSLN and the phage, and reacted at room temperature for 15 minutes. Then, the obtained product was washed with 1 mL of PBST six times. The bead was treated with 1 mL of glycine (pH 2.0) elution buffer, and reacted at room temperature for 10 minutes to obtain the supernatant. After elution, 100 μL of 1.5M Tris-Cl (pH 8.8) was added to the phage and neutralized. 10 mL of XL1-Blue bacteria (electroporation-competent cells; Cat.No.200228, Stratagene) cultured for about 2 to 2.5 hours (OD_{600} = 0.8 to 1.0) were treated with the neutralized phage. After infection at room temperature for 30 minutes, 10 mL of SB, 20 μL of tetracycline (50 mg/ml), and 10 μL of carbenicillin (100 mg/mL) were added to 10 mL of the infected XL1-Blue bacteria (electroporation-competent cells; Cat.No.200228, Stratagene), and cultured with shaking (200rpm) at 37°C for 1 hour. The bacteria were treated with 1 mL of VCSM13 helper phage(> 10^{11} pfu/ml), and suspension-cultured (200 rpm) at 37°C for 1 hour. After 1 hour incubation, the bacteria were treated with 80 mL of SB, 100 μL of kanamycin, and 100 μL of carbenicillin (100 mg/mL), and cultured overnight (200 rpm) at 37°C. The overnight cultured library was centrifuged at 4000 rpm for 15 minutes to separate the supernatant only, and 5X PEG/NaCl buffer was used to add 1X PEG/NaCl to the supernatant, and the obtained product allowed to stand on ice over 30 minutes. The supernatant was removed by centrifugation at 8000 rpm for 30 minutes, and pellets were re-suspended with 2m of 1% BSA/PBS and centrifuged at 12000 rpm

for 10 minutes. Then, only the supernatant was taken and used in the following order panning. A total of primary, secondary, and tertiary bead panning with regard to the MSLN were performed by using MS502 V_H rational library phage. As a result of the secondary and tertiary pannings, it was confirmed that output titers were increased, which showed that the antibodies against the MSLN were amplified.

[166]

[167] 3-6: Secure of individual clones according to ELISA

[168] Single colonies of a final amplified population of each library of light chain/heavy chain variable regions were collected, and cultured with 1.5 mL of SB/carbenicillin up to an OD_{600} of 0.8 to 1.0 at 37℃ and at 220 rpm, and then cultured with 1 mM IPTG at 30℃ and at 200 rpm for 12 hours or more. The reaction materials were centrifuged at 5500 rpm for 5 minutes, and only each supernatant was added to ELISA plates containing underlying MSLN antigen, and reacted at room temperature for 2 hours. Then, the resultant materials were washed with PBST (1XPBS, 0.05% tween 20) four times, and HRP/Anti-hFab-HRP conjugate diluted by 1/5000 with 1% BSA/1XPBS was added thereto, and reacted at room temperature for 1 hour, and washed with PBST (1XPBS, 0.05% tween 20) 4 times. Then, a TMB solution was added and allowed to stand for 5 to 10 minutes, and a TMB stop solution was added thereto. Next, O.D values were measured at a measurement wavelength of 450 nm using a TECAN sunrise, and clones having high O.D value were secured as individual clones.

[169] As a result, as shown in Table 11, the clones specifically bound to the human MSLN were able to be selected, and amino acid sequences thereof were identified.

[170] Table 12 shows CDR amino acid sequences of the clone antibodies of Table 11 on the basis of Kabat numbering.

[171]

[172] [Table 11]

clone	variable region	amino acid sequence	SEQ ID NO:
C2G1	heavy chain	EVQLLESGGGLVQPGGSLRLSCAAS GFTFSNYAMSWVRQAPGKGLEWVS GIPPDSGSKYYADSVRGRFTVSRDNS KNTLYLQMNSLRAEDTAVYYCAKN MLSFDYWGQGTLVTVSS	49
	light chain	QSVLTQPPSASGTPGQRVTISCTGSSS NIGPNAVSWYQQLPGTAPKLLIYYNS KRPSGVPDRFSGSKSGTSASLAISGLR SEDEADYYCGSWDSSLSGYVFGGGT KVTVLG	109
C2G4	heavy chain	EVQLLESGGGLVQPGGSLRLSCAAS GFTFSNYAMSWVRQAPGKGLEWVS GIPPDSGSKYYADSVRGRFTVSRDNS KNTLYLQMNSLRAEDTAVYYCAKN MLSFDYWGQGTLVTVSS	48
	light chain	QSVLTQPPSASGTPGQRVTISCTGSSS NIGSNAVSWYQQLPGTAPKLLIYYNS KRPSGVPDRFSGSKSGTSASLAISGLR SEDEADYYCGSWDPSLNGYVFGGGT KVTVLG	110
C3C8	heavy chain	EVQLLESGGGLVQPGGSLRLSCAAS GFTFSNYAMSWVRQAPGKGLEWVS GIPPDSGSKYYADSVRGRFTVSRDNS KNTLYLQMNSLRAEDTAVYYCAKN MLSFDYWGQGTLVTVSS	48
	light chain	QSVLTQPPSASGTPGQRVTISCTGSSS NIGPNAVSWYQQLPGTAPKLLIYYNS KRPSGVPDRFSGSKSGTSASLAISGLR SEDEADYYCGSWDSDLRGYVFGGG TKVTVLG	111
54	heavy chain	EVQLLESGGGLVQPGGSLRLSCAAS GFTFSNYAMSWVRQAPGKGLEWVS GIYPDSSSTYYADSVKGRFTISRDNS	112

		KNTLYLQMNSLRAEDTAVYYCARNI YTFDYWGQGTLVTVSS	
	light chain	QSVLTQPPSASGTPGQRVTISCTGSSS NIGSNAVSWYQQLPGTAPKLLIYYNS KRPSGVPDRFSGSKSGTSASLAISGLR SEDEADYYCGSWDSSLNGYVFGGGT KVTVLG	49
56	heavy chain	EVQLLESGGGLVQPGGSLRLSCAAS GFTFSNYAMSWVRQAPGKGLEWVS GIPPDSASKYYADSVRGRFTVSRDNS KNTLYLQMNSLRAEDTAVYYCAKN MLSFDYWGQGTLVTVSS	113
	light chain	QSVLTQPPSASGTPGQRVTISCTGSSS NIGSNAVSWYQQLPGTAPKLLIYYNS KRPSGVPDRFSGSKSGTSASLAISGLR SEDEADYYCGSWDSSLNGYVFGGGT KVTVLG	49
2-30	heavy chain	EVQLLESGGGLVQPGGSLRLSCAAS GFTFSNYAMSWVRQAPGKGLEWVS GIPPDSNSKYYADSVRGRFTVSRDNS KNTLYLQMNSLRAEDTAVYYCAKN MRTFDYWGQGTLVTVSS	114
	light chain	QSVLTQPPSASGTPGQRVTISCTGSSS NIGSNAVSWYQQLPGTAPKLLIYYNS KRPSGVPDRFSGSKSGTSASLAISGLR SEDEADYYCGSWDSSLNGYVFGGGT KVTVLG	49
2-73	heavy chain	EVQLLESGGGLVQPGGSLRLSCAAS GFTFSNYAMSWVRQAPGKGLEWVS GIPPNSDSKYYADSVRGRFTVSRDNS KNTLYLQMNSLRAEDTAVYYCAKN MLSFDYWGQGTLVTVSS	115
	light chain	QSVLTQPPSASGPPGQRVTISCTGSSS NIGNNSVSWYQQLPGTAPKLLIYYDS HRPSGVPDRFSGSKSGTSASLAIGGL RSEDEADYYCGAWDDSLNAYVFGG	49

		GTKLTVLG	
2-78	heavy chain	EVQLLESGGGLVQPGGSLRLSCAAS GFTFSNYAMSWVRQAPGKGLEWVS GIPPDSGSKYYADSVRGRFTVSRDNS KNTLYLQMNSLRAEDTAVYYCAKN MFSFDYWGQGTLVTVSS	116
	light chain	QSVLTQPPSASGTPGQRVTISCTGSSS NIGSNAVSWYQQLPGTAPKLLIYYNS KRPSGVPDRFSGSKSGTSASLAISGLR SEDEADYYCGSWDSSLNGYVFGGGT KVTVLG	49

[173]

[174] [Table 12]

clone	variable region	CDR1	CDR2	CDR3
C2G1	heavy chain	NYAMS(SEQ ID NO: 59)	GIPPDSGSKYYADSVRG(SEQ ID NO: 65)	NMLSFDY(SEQ ID NO: 66)
	light chain	TGSSSNIGPNAVS(SEQ ID NO: 117)	YNSKRPS(SEQ ID NO: 68)	GSWDSSLSGYV(SEQ ID NO: 118)
C2G4	heavy chain	NYAMS(SEQ ID NO: 59)	GIPPDSGSKYYADSVRG(SEQ ID NO: 65)	NMLSFDY(SEQ ID NO: 66)
	light chain	TGSSSNIGSNAVS(SEQ ID NO: 67)	YNSKRPS(SEQ ID NO: 68)	GSWDPSLNGYV(SEQ ID NO: 119)
C3C8	heavy chain	NYAMS(SEQ ID NO: 59)	GIPPDSGSKYYADSVRG(SEQ ID NO: 65)	NMLSFDY(SEQ ID NO: 66)
	light chain	TGSSSNIGPNAVS(SEQ ID NO: 117)	YNSKRPS(SEQ ID NO: 68)	GSWDSDLRGYV(SEQ ID NO: 120)
54	heavy chain	NYAMS(SEQ ID NO: 59)	GIPPDSSSKYYADSVRG(SEQ ID NO: 121)	NMLSFDY(SEQ ID NO: 66)
	light chain	TGSSSNIGSNAVS(SEQ ID NO: 67)	YNSKRPS(SEQ ID NO: 68)	GSWDSSLNGYV(SEQ ID NO: 69)
56	heavy chain	NYAMS(SEQ ID NO: 59)	GIPPDSASKYYADSVRG(SEQ ID NO: 122)	NMLSFDY(SEQ ID NO: 66)
	light chain	TGSSSNIGSNAVS(SEQ ID NO: 67)	YNSKRPS(SEQ ID NO: 68)	GSWDSSLNGYV(SEQ ID NO: 69)
2-30	heavy chain	NYAMS(SEQ ID NO: 59)	GIPPDSNSKYYADSVRG(SEQ ID NO:	NMRTFDY(SEQ ID NO: 124)

			123)	
	light chain	TGSSSNIGSNAVS(SEQ ID NO: 67)	YNSKRPS(SEQ ID NO: 68)	GSWDSSLNGYV (SEQ ID NO: 69)
2-73	heavy chain	NYAMS(SEQ ID NO: 59)	GIPPNSDSKYYADSVRG(SEQ ID NO: 125)	NMLSFDY(SEQ ID NO: 66)
	light chain	TGSSSNIGSNAVS(SEQ ID NO: 67)	YNSKRPS(SEQ ID NO: 68)	GSWDSSLNGYV (SEQ ID NO: 69)
2-78	heavy chain	NYAMS(SEQ ID NO: 59)	GIPPDSGSKYYADSVRG(SEQ ID NO: 65)	NMFSFDY(SEQ ID NO: 126)
	light chain	TGSSSNIGSNAVS(SEQ ID NO: 67)	YNSKRPS(SEQ ID NO: 68)	GSWDSSLNGYV (SEQ ID NO: 69)

[175]

[176] 3-7: Relative comparison and selection in binding force of individual clones using SPR

[177] In order to compare and measure the binding force with culture fluid of individual clones secured from confirmation of the individual clones according to ELISA method conducted in Example 3-3, first, the human MSLN on the biacore series S CM5 chip (GE healthcare) was dissolved in pH 4.0 acetate buffer to be 1 μg/ml, and allowed to flow at a flow rate of 10 μL/min and fixed to 1000 Ru. The Fab supernatant expressed in Example 3-6 was diluted by 1/10 with pH 7.4 HBS-EP buffer, and was allowed to flow at a flow rate of 30 μL/min for association of 120 seconds, and dissociation of 180 seconds to conduct binding analysis. The regeneration was performed with 10 mM glycine-HCl pH 1.5 buffer for 30 seconds. The ELISA and the SPR binding data were compared and analyzed to select the final clones of the light chain variable region and the heavy chain variable region.

[178] As a result, as illustrated in FIGS. 2 and 3, the binding force of the light chain variable region mutations and the heavy chain variable region mutations were relatively compared, and C2G1, C2G4, and C3C8 were selected for the final clones of the light chain variable region and 56, 2-30, 2-58, 2-73, and 2-78 were selected for the final clones of the heavy chain variable region.

[179]

[180] 3-8: IgG gene cloning of clone MS502 light chain variable region mutation antibody

[181] Each of the secured light chain variable region C2G1, C2G4, and C3C8 genes as the template was subjected to PCR by using PrimeSTAR HS DNA polymerase (Cat.No.R010B; Takara) and the forward primer containing NotI (Table 6: SEQ ID NO: 86) and the reverse primer (Table 6: SEQ ID NO: 87). Further, human antibody kappa light chain constant region was subjected to PCR with the forward primer (Table 6: SEQ ID NO: 88) and the reverse primer (Table 6: SEQ ID NO: 89). The PCR was performed by repeating exposure at 94℃ for 10 minutes, and then exposure at 94℃ for 15 seconds, at 56℃ for 30 seconds, and at 72℃ for 90 seconds 30 times, and reacting at 72℃ for 10 minutes. In the amplified genes, DNA bands having an expected size were confirmed on 1% agarose gel, and were separated using a gel extraction kit, respectively. Then, the respective light chain variable regions and light chain constant regions were mixed, followed by overlapping PCR, such that the genes expressing the light chain region were cloned. The PCR was performed by repeating exposure at 94℃ for 10 minutes, and then exposure at 94℃ for 15 seconds, at 56℃ for 30 seconds, and at 72℃ for 90 seconds 30 times, and reacting at 72℃ for 10 minutes. In the amplified genes, DNA bands having an expected size were confirmed on 1% agarose gel, and were separated using a gel extraction kit, respectively. Then, the separated genes reacted with NotI, HindIII restriction enzymes at 37℃ for 12 hours or more, and the genes reacted with the restriction enzymes were separated on 1% agarose gel again. The pcIW plasmid vector was also cut by the same method as above and separated on agarose gel. The separated C2G1, C2G4, and C3C8 light chain region genes were inserted into NotI, HindIII sites of the linear pcIW vector by using a T4 DNA ligase (Cat.No.M0203S, New England BioLabs (NEB)). The ligation reaction materials were transformed into XL1-Blue bacteria (Electroporation-Competent Cells; Cat.No.200228, Stratagene), plated on an LB plate (Cat.No.LN004CA, NaraeBiotech) containing carbenicillin, and cultured at 37℃ for 12 hours or more. Then single colonies were chosen and cultured, and plasmids were separated by using a plasmid mini kit (Cat.No.27405, QIAGEN), and confirmed by DNA sequencing.

[182]

[183] 3-9: IgG gene cloning of clone MS502 heavy chain variable region mutation antibody

[184] Each of the heavy chain variable region 56, 2-30, 2-58, 2-73, and 2-78 genes as the template was subjected to PCR by using PrimeSTAR HS DNA polymerase (Cat.No.R010B; Takara) and the forward primer containing NotI (Table 6: SEQ ID NO: 90) and the reverse primer containing ApaI (Table 6: SEQ ID NO: 91). The PCR was performed by repeating exposure at 98℃ for 2 minutes, and then exposure at 98℃ for 10 seconds, at 58℃ for 10 seconds, and at 72℃ for 30 seconds 30 times, and

reacting at 72°C for 5 minutes. In the amplified genes, DNA bands having an expected size were confirmed on 1% agarose gel, and were separated using a gel extraction kit, respectively. Then, the three kinds of separated genes were reacted with KpnI and ApaI restriction enzyme at 37°C for 4 hours. The genes reacted with the restriction enzyme were separated on 1% Agarose gel again. The pcIW plasmid vector was also cut by the same method as above and separated on agarose gel. The separated genes were inserted into the NotI, ApaI sites of the linear pclw vector containing the human heavy chain constant region by using a T4 DNA ligase. The ligation reaction materials were transformed into XL1-Blue bacteria (Electroporation-Competent Cells; Cat.No.200228, Stratagene), plated on an LB plate (Cat.No.LN004CA, NaraeBiotech) containing carbenicillin, and cultured at 37°C for 12 hours or more. Then single colonies were chosen and cultured, and plasmids were separated by using a plasmid mini kit (Cat.No.27405, QIAGEN), and confirmed by DNA sequencing.

[185]

[186] 3-10: Production and purification of IgG of clone MS502 light chain variable region mutation antibody

[187] In order to produce and purify the light chain variable region mutation antibodies C2G1, C2G4, and C3C8, Expi293F™ cells were inoculated at a concentration of 2.5 × 10^6 cell/mL the day before transfection. After incubation (37°C, 8% CO_2, 125 rpm) for 24 hours, Expi293™ expression medium (Cat.No.A1435101, Gibco) was added to prepare a product of 30 mL having a concentration of 2.5 × 10^6 cell/mL (viability = 95%). 30 μg of DNA (pcIw-MS502 heavy chain variable region: 15 μg, pcIw-anti-Mesothelin light chain variable region mutant: 15 μg) was diluted in an OptiPro™ SEM medium (Cat.No.12309019, Gibco) so as to have a total volume of 1.5 mL, and reacted at room temperature for 5 minutes. 1.5 mL of the OptiPro™ SEM medium (Cat.No.12309019, Gibco) was mixed with 80 μL of an ExpiFectamine™ 293 reagent (Cat.No.A14524, Gibco) so that a total volume is 1.5 mL, and reacted at room temperature for 5 minutes. After the reaction for 5 minutes, 1.5 mL of diluted DNA and 1.5 mL of diluted ExpiFectamine™ 293 reagent were well-mixed with each other, and reacted at room temperature for 20 to 30 minutes. 3 mL of the mixture of DNA and ExpiFectamine™ 293 reagent was treated in the Expi293F™ cells. After suspension-culture (37 °C, 8% CO_2, 125 rpm) for 16 to 18 hours, 150 μL of ExpiFectamine™ 293 Enhancer 1(Cat.No.A14524, Gibco) and 1.5 mL of ExpiFectamine™ 293 Enhancer2 (Cat.No.A14524, Gibco) were added thereto, followed by suspension-culturing for 5 days. After the culturing, cell debris was removed by centrifugation at 4000rpm for 20 minutes, and the supernatant passed through 0.22 μm filter to be prepared. MabSelect Xtra (Cat.No.17-5269-02, GE Healthcare) which is protein A resin having 100 μL was prepared for each 30 mL of the culture fluid, followed by centrifugation at 1000 rpm

for 2 minutes to remove a storage solution, and the obtained product was washed with 400 μL of protein A binding buffer (Cat.No.21007, Pierce) 3 times. The protein A resin was added to the prepared culture fluid and rotation-reacted at room temperature for 30 minutes. The mixture of the culture fluid and the resin was put into a pierce spin column snap-cap (Cat.No.69725, Thermo), and then, only the resin was left in the column using QIAvac 24 Plus(Cat.No.19413, QIAGEN) vacuum manifold. 5 mL of protein A binding buffer was added to wash the resin, and 200 μL of a protein A elution buffer (Cat.No.21009, Pierce) was added thereto. The resultant material was reacted by resuspension at room temperature for 2 minutes, and centrifuged at 1000 rpm for 1 minute, and eluted. Each eluate was neutralized by adding 2.5 μL of 1.5M Tris-HCl (pH 9.0). The elution was performed 4 to 6 times, and each fraction was quantified by using Nanodrop 200C (Thermo scientific). The fractions in which protein is detected were collected, and exchanged with a PBS (Phosphate-Buffered Saline) buffer using Zeba Spin Desalting Columns, 7K MWCO, 5 mL (Cat.No.0089892, Pierce). Then, protein electrophoresis (SDS-PAGE) was performed under reduction and non-reduction condition to finally verify the concentration quantification and the antibody state, and the antibody was kept at 4℃.

[188]

[189] 3-11: Measurement of quantitative binding force of MS502 light chain variable region mutation antibody with regard to MSLN antigen

[190] Quantitative binding force (affinity) of the purified anti-MSLN antibodies, i.e., the MS502 clone light chain variable region mutation antibodies C2G1, C2G4, and C3C8 with regard to the recombinant human mesothelin (MSLN) was measured by using a Biacore T-200 (GE Healthcare, U.S.A.) biosensor. The MSLN (Cat.No.3265-MS, R&D systems, U.S.A.) purified from the HEK293 cells was fixed to a CM5 chip (GE Healthcare, CAT. No. BR-1005-30) so as to satisfy 200 Rmax by using an amine-carboxylic reaction. Then, the clone C2G1 antibody, the clone C2G4 antibody or the clone C3C8 antibody serially diluted with HBS-EP buffer (10mM HEPES, pH7.4, 150mM NaCl, 3mM EDTA, 0.005% surfactant P20) was allowed to flow at a concentration range of 0.078 nM to 5 nM and at a flow rate of 30 μL/min for association of 120 seconds and dissociation of 1800 seconds. The dissociation of the antibody bound to the MSLN was induced by flowing 10 mM Glycine-HCl pH 1.5 at a flow rate of 30 μL/min for 30 seconds (Table 13). The affinity was obtained as movement speed constants (K_{on} and K_{off}) and an equilibrium dissociation constant (K_D) by using a Biacore T-200 evaluation software (Table 14).

[191]

[192] [Table 13]

SPR	Biacore T200
Chip	CM5
Running Buffer	HBS-EP pH7.4
Flow rate	30μL/min
Association / dissociation time	120 sec / 600 sec
IgG Conc.	0.078~5nM, 1/2 serial dilution
Regeneration	10mM Glycine-HCl pH1.5, 30 sec

[193]

[194] [Table 14]

	K_{on}	K_{off}	K_D
C2G1	7.20×10^{7}	6.76×10^{-3}	9.39×10^{-11}
C2G4	1.40×10^{8}	6.05×10^{-3}	4.32×10^{-11}
C3C8	5.84×10^{7}	7.11×10^{-3}	1.22×10^{-10}

[195]

[196] 3-12: Production and purification of IgG of clone MS502 heavy chain variable region mutation antibody

[197] In order to produce and purify the heavy chain variable region mutation antibodies 56, 2-30, 2-58, 2-73, 2-78, Expi293F™ cells were inoculated at a concentration of 2.5 × 10^6 cell/mL the day before transfection. After incubation (37°C, 8% CO_2, 125 rpm) for 24 hours, Expi293™ expression medium (Cat.No.A1435101, Gibco) was added to prepare a product of 30 mL having a concentration of 2.5 × 10^6 cell/mL (viability = 95%). 30 μg of DNA (pcIw-MS502 heavy chain variable region mutation: 15 μg, pcIw-MS502 light chain variable region: 15 μg) was diluted in an OptiPro™ SEM medium (Cat.No.12309019, Gibco) so as to have a total volume of 1.5 mL, and reacted at room temperature for 5 minutes. 1.5 mL of the OptiPro™ SEM medium (Cat.No.12309019, Gibco) was mixed with 80 μL of an ExpiFectamine™ 293 reagent (Cat.No.A14524, Gibco) so that a total volume is 1.5 mL, and reacted at room temperature for 5 minutes. After the reaction for 5 minutes, 1.5 mL of diluted DNA and 1.5 mL of diluted ExpiFectamine™ 293 reagent were well-mixed with each other, and reacted at room temperature for 20 to 30 minutes. 3 mL of the mixture of DNA and ExpiFectamine™ 293 reagent was treated in the Expi293F™ cells. After suspension-culture (37 °C, 8% CO_2, 125 rpm) for 16 to 18 hours, 150 μL of ExpiFectamine™ 293 Enhancer 1 (Cat.No.A14524, Gibco) and 1.5 mL of ExpiFectamine™ 293 Enhancer2

(Cat.No.A14524, Gibco) were added thereto, followed by suspension-culturing for 5 days. After the culturing, cell debris was removed by centrifugation at 4000rpm for 20 minutes, and the supernatant passed through 0.22 μm filter to be prepared. MabSelect Xtra (Cat.No.17-5269-02, GE Healthcare) which is protein A resin having 100 μL was prepared for each 30 mL of the culture fluid, followed by centrifugation at 1000 rpm for 2 minutes to remove a storage solution, and the obtained product was washed with 400 μL of protein A binding buffer (Cat.No.21007, Pierce) 3 times. The protein A resin was added to the prepared culture fluid and rotation-reacted at room temperature for 30 minutes. The mixture of the culture fluid and the resin was put into a pierce spin column snap-cap (Cat.No.69725, Thermo), and then, only the resin was left in the column using QIAvac 24 Plus(Cat.No.19413, QIAGEN) vacuum manifold. 5 mL of protein A binding buffer was added to wash the resin, 200 μL of a protein A elution buffer (Cat.No.21009, Pierce) was added thereto. The resultant material was reacted by resuspension at room temperature for 2 minutes, and centrifuged at 1000 rpm for 1 minute, and eluted. Each eluate was neutralized by adding 2.5 μL of 1.5M Tris-HCl (pH 9.0). The elution was performed 4 to 6 times, and each fraction was quantified by using Nanodrop 200C (Thermo scientific). The fractions in which protein is detected were collected, and exchanged with a PBS (Phosphate-Buffered Saline) buffer using Zeba Spin Desalting Columns, 7K MWCO, 5 mL (Cat.No.0089892, Pierce). Then, protein electrophoresis (SDS-PAGE) was performed under reduction and non-reduction condition to finally verify the concentration quantification and the antibody state, and the antibody was kept at 4°C.

[198]

[199] 3-13: Production and purification of IgG of heavy chain variable region mutation antibody combined with final light chain variable region mutation C2G4 clone of clone MS502

[200] The C2G4 clone having the most excellent value in the measurement of affinity among the light chain variable region mutations was selected and fixed as the final clone of the light chain variable region. Then, in order to combine, produce and purify the heavy chain variable region mutation antibodies 56, 2-30, 2-58, 2-73, and 2-78, Expi293F™ cells were inoculated at a concentration of 2.5×10^6 cell/mL the day before transfection. After incubation (37°C, 8% CO_2, 125 rpm) for 24 hours, Expi293™ expression medium (Cat.No.A1435101, Gibco) was added to prepare a product of 30 mL having a concentration of 2.5×10^6 cell/mL (viability = 95%). 30 μg of DNA (pcIw-MS502 heavy chain variable region mutation antibody: 15 μg, pcIw-light chain variable region mutation C2G4: 15 μg) was diluted in an OptiPro™ SEM medium (Cat.No.12309019, Gibco) so as to have a total volume of 1.5 mL, and reacted at room temperature for 5 minutes. 1.5 mL of the OptiPro™ SEM medium

(Cat.No.12309019, Gibco) was mixed with 80 μL of an ExpiFectamine™ 293 reagent (Cat.No.A14524, Gibco) so that a total volume is 1.5 mL, and reacted at room temperature for 5 minutes. After the reaction for 5 minutes, 1.5 mL of diluted DNA and 1.5 mL of diluted ExpiFectamine™ 293 reagent were well-mixed with each other, and reacted at room temperature for 20 to 30 minutes. 3 mL of the mixture of DNA and ExpiFectamine™ 293 reagent was treated in the Expi293F™ cells. After suspension-culture (37 °C, 8% CO_2, 125 rpm) for 16 to 18 hours, 150 μL of ExpiFectamine™ 293 Enhancer 1 (Cat.No.A14524, Gibco) and 1.5 mL of ExpiFectamine™ 293 Enhancer2 (Cat.No.A14524, Gibco) were added thereto, followed by suspension-culturing for 5 days. After the culturing, cell debris was removed by centrifugation at 4000rpm for 20 minutes, and the supernatant passed through 0.22 μm filter to be prepared. MabSelect Xtra (Cat.No.17-5269-02, GE Healthcare) which is protein A resin having 100 μL was prepared for each 30 mL of the culture fluid, followed by centrifugation at 1000 rpm for 2 minutes to remove a storage solution, and the obtained product was washed with 400 μL of protein A binding buffer (Cat.No.21007, Pierce) 3 times. The protein A resin was added to the prepared culture fluid and rotation-reacted at room temperature for 30 minutes. The mixture of the culture fluid and the resin was put into a pierce spin column snap-cap (Cat.No.69725, Thermo), and then, only the resin was left in the column using QIAvac 24 Plus (Cat.No.19413, QIAGEN) vacuum manifold. 5 mL of protein A binding buffer was added to wash the resin, 200 μL of a protein A elution buffer (Cat.No.21009, Pierce) was added thereto. The resultant material was reacted by resuspension at room temperature for 2 minutes, and centrifuged at 1000 rpm for 1 minute, and eluted. Each eluate was neutralized by adding 2.5 μL of 1.5M Tris-HCl (pH 9.0). The elution was performed 4 to 6 times, and each fraction was quantified by using Nanodrop 200C (Thermo scientific). The fractions in which protein is detected were collected, and exchanged with a PBS (Phosphate-Buffered Saline) buffer using Zeba Spin Desalting Columns, 7K MWCO, 5 mL (Cat.No.0089892, Pierce). Then, protein electrophoresis (SDS-PAGE) was performed under reduction and non-reduction condition to finally verify the concentration quantification and the antibody state, and the antibody was kept at 4°C.

[201]

[202] [Table 15]

Clone	variable region	amino acid sequence	SEQ ID NO:
56-C2G4	heavy chain	EVQLLESGGGLVQPGGSLRLSCAASGFTFSN YAMSWVRQAPGKGLEWVSGIPPDSASKYY ADSVRGRFTVSRDNSKNTLYLQMNSLRAED TAVYYCAKNMLSFDYWGQGTLVTVSS	113
	light chain	QSVLTQPPSASGTPGQRVTISCTGSSSNIGSN AVSWYQQLPGTAPKLLIYYNSKRPSGVPDRF SGSKSGTSASLAISGLRSEDEADYYCGSWDP SLNGYVFGGGTKVTVLG	110
2-30-C2G4	heavy chain	EVQLLESGGGLVQPGGSLRLSCAASGFTFSN YAMSWVRQAPGKGLEWVSGIPPDSNSKYY ADSVRGRFTVSRDNSKNTLYLQMNSLRAED TAVYYCAKNMRTFDYWGQGTLVTVSS	114
	light chain	QSVLTQPPSASGTPGQRVTISCTGSSSNIGSN AVSWYQQLPGTAPKLLIYYNSKRPSGVPDRF SGSKSGTSASLAISGLRSEDEADYYCGSWDP SLNGYVFGGGTKVTVLG	110
2-73-C2G4	heavy chain	EVQLLESGGGLVQPGGSLRLSCAASGFTFSN YAMSWVRQAPGKGLEWVSGIPPNSDSKYY ADSVRGRFTVSRDNSKNTLYLQMNSLRAED TAVYYCAKNMLSFDYWGQGTLVTVSS	115
	light chain	QSVLTQPPSASGTPGQRVTISCTGSSSNIGSN AVSWYQQLPGTAPKLLIYYNSKRPSGVPDRF SGSKSGTSASLAISGLRSEDEADYYCGSWDP SLNGYVFGGGTKVTVLG	110
2-78-C2G4	heavy chain	EVQLLESGGGLVQPGGSLRLSCAASGFTFSN YAMSWVRQAPGKGLEWVSGIPPDSGSKYY ADSVRGRFTVSRDNSKNTLYLQMNSLRAED TAVYYCAKNMFSFDYWGQGTLVTVSS	116
	light chain	QSVLTQPPSASGTPGQRVTISCTGSSSNIGSN AVSWYQQLPGTAPKLLIYYNSKRPSGVPDRF SGSKSGTSASLAISGLRSEDEADYYCGSWDP SLNGYVFGGGTKVTVLG	110

[203]

[204] [Table 16]

Clone	variable region	CDR1	CDR2	CDR3
56-C2G4	heavy chain	NYAMS(SEQ ID NO: 59)	GIPPDSASKYYADSVRG(SEQ ID NO: 122)	NMLSFDY(SEQ ID NO: 66)
	light chain	TGSSSNIGSNAVS(SEQ ID NO: 67)	YNSKRPS(SEQ ID NO: 68)	GSWDPSLNGYV(SEQ ID NO: 119)
2-30-C2G4	heavy chain	NYAMS(SEQ ID NO: 59)	GIPPDSNSKYYADSVRG(SEQ ID NO: 123)	NMRTFDY(SEQ ID NO: 124)
	light chain	TGSSSNIGSNAVS(SEQ ID NO: 67)	YNSKRPS(SEQ ID NO: 68)	GSWDPSLNGYV(SEQ ID NO: 120)
2-73-C2G4	heavy chain	NYAMS(SEQ ID NO: 59)	GIPPNSDSKYYADSVRG(SEQ ID NO: 125)	NMLSFDY(SEQ ID NO: 66)
	light chain	TGSSSNIGSNAVS(SEQ ID NO: 67)	YNSKRPS(SEQ ID NO: 68)	GSWDPSLNGYV(SEQ ID NO: 119)
2-78-C2G4	heavy chain	NYAMS(SEQ ID NO: 59)	GIPPDSGSKYYADSVRG(SEQ ID NO: 65)	NMFSFDY(SEQ ID NO: 126)
	light chain	TGSSSNIGSNAVS(SEQ ID NO: 67)	YNSKRPS(SEQ ID NO: 68)	GSWDPSLNGYV(SEQ ID NO: 119)

[205]

[206] 3-14: Measurement of quantitative binding force of MS502 heavy chain variable region mutation antibody and combination of C2G4 and heavy chain variable region mutation antibody with regard to MSLN antigen

[207] The quantitative binding force (affinity) of each of the purified anti-MSLN antibodies, i.e., MS502 clone heavy chain variable region mutation antibodies 56, 2-30, 2-73, 2-78, and the combination antibodies of the C2G4 light chain variable region and

the heavy chain variable region mutation antibodies including 56-C2G4, 2-30-C2G4, 2-73-C2G4, 2-78-C2G4 with regard to recombinant human mesothelin (MSLN) was measured by using a Biacore T-200 (GE Healthcare, U.S.A.) biosensor. The MSLN (Cat.No.3265-MS, R&D systems, U.S.A.) purified from the HEK293 cells was fixed to a CM5 chip (GE Healthcare, CAT. No. BR-1005-30) so as to satisfy 200 Rmax by using an amine-carboxylic reaction. Then, the clone 56 antibody, the clone 2-30 antibody, the clone 2-73 antibody, the clone 2-78 antibody, the clone 56-C2G4 antibody, the clone 2-30-C2G4 antibody, the clone 2-73-C2G4 antibody, and the clone 2-78-C2G4 antibody serially diluted with HBS-EP buffer (10mM HEPES, pH7.4, 150mM NaCl, 3mM EDTA, 0.005% surfactant P20) were allowed to flow at a concentration range of 0.078 nM to 5 nM and at a flow rate of 30μL/min for association of 120 seconds and dissociation of 1800 seconds. The dissociation of the antibody bound to the MSLN was induced by flowing 10 mM Glycine-HCl pH 1.5 at a flow rate of 30 μL/min for 30 seconds (Table 17). The affinity was obtained as movement speed constants (K_{on} and K_{off}) and an equilibrium dissociation constant (K_D) by using a Biacore T-200 evaluation software (Table 18).

[208]

[209] [Table 17]

SPR	Biacore T200
Chip	CM5
Running Buffer	HBS-EP pH7.4
Flow rate	30μL/min
Association / dissociation time	120 sec / 600 sec
IgG Conc.	0.078~5nM, 1/2 serial dilution
Regeneration	10mM Glycine-HCl pH1.5, 30 sec

[210]

[211] [Table 18]

	K_{on}	K_{off}	K_D
56	1.26×10^{7}	1.57×10^{-3}	1.25×10^{-10}
2-30	1.92×10^{8}	3.19×10^{-2}	1.66×10^{-10}
2-78	1.50×10^{8}	2.44×10^{-3}	1.63×10^{-11}
56 - C2G4	6.61×10^{7}	1.08×10^{-2}	1.63×10^{-10}
2-30 - C2G4	1.15×10^{8}	2.70×10^{-2}	2.34×10^{-10}
2-73 - C2G4	8.98×10^{7}	1.48×10^{-2}	1.65×10^{-10}
2-78 - C2G4	2.10×10^{8}	7.82×10^{-3}	3.72×10^{-11}

[212]

[213] Example 4: FACS analysis of anti-MSLN antibody binding to MSLN-expressing cancer cell

[214] In order to evaluate whether the anti-MSLN antibody derived from the immune and synthetic library is selectively bound to the MSLN-expressing cell, an expression amount of the MSLN was measured in a cancer cell line, and the antibody binding each cell was confirmed by FACS test.

[215]

[216] 4-1: Construction of MSLN-expressing cell line

[217] The plasmid (pCMV/MSLN) containing the MSLN expressing unit and hygromycin resistant gene was delivered into MiaPaCa-2 pancreatic cancer cell confirmed as an MSLN negative cell line, by using a jetPEI(polyethyleneimine) transfection system (Polyplus, 101-40) (FIG. 4). After 48 hours, the cell culture fluid was replaced with a culture fluid containing hygromycin B (200 μg/mL). 10 colonies having resistance against the hygromycin were obtained while exchanging the culture fluid every 3 days, to confirm each MSLN expression amount by a Western blotting method. With respect to four kinds of pancreatic cancer cell lines (MiaPaCa-2, BxPC-3, Capan-1, AsPC-1) and two kinds of mesothelioma cell lines (H28, H2452), the MSLN expression amount in the cells was confirmed by using the anti-MSLN antibody (# 133489, Abcam) through the Western blotting method. The culturing cells were separated by adding a Tryple Express solution and stored in a 15 mL tube, followed by centrifugation at 2000 rpm at room temperature for 3 minutes to decant the culture fluid, and the obtained product was suspended with 100 μL 1xSDS-PAGE sample buffer (50mM Tris(pH6.8), 2% SDS, 100mM DTT (dithiothreitol), 0.1% BPB(bromophenol blue), 10% glycerol) and heated for 5 minutes. The obtained product was centrifuged to collect the supernatant, followed by electrophoresis in 4-12% SDS-PAGE, at 20 mA for about 2

hours, and a transfer unit was used to transfer the separated protein to a PVDF membrane, and then, electrophoresis was performed at 300mA with tris-glycine buffer (39mM glycine, 48mM tris, 0.037% SDS, 20% methanol) for about 90 minutes. The PVDF membrane in which the protein was transferred was subjected to blocking at room temperature for 1 hour by using a TBS blocking solution. The anti-MSLN antibody (# 133489, Abcam) as the primary antibody was diluted by 1:2,000 with 5% skim milk/1xTBST buffer, and reacted at room temperature for about 1 hour, and washed with 1xTBST buffer 6 times every 5 minutes. The anti-mouse HRP (KPL, MA, U.S.A.) as the secondary antibody was diluted by 1:20,000 with 5% skim milk/ 1xTBST buffer, and reacted for 30 minutes, and washed with 1xTBST buffer 6 times every 5 minutes. Then, the MSLN protein band was verified by color development reaction solution (ECL, Amersham, UK).

[218] As a result, as illustrated in FIG. 5, it was confirmed that H28, MiaPaCa-2, BxPC-3, Capan-1 cell lines are MSLN-negative, and H226, H2452(H2052), AsPC-1 are MSLN-positive by measuring whether there are the MSLN having 70kDa precursor form and 40 to 50kDa mature form from each cancer cell line.

[219]

[220] 4-2: MSLN expression amount analysis in MSLN-expressing cell lines and MSLN-expressing tumor cell lines

[221] FACS test was performed on the MSLN present in the cell surface with regard to MiaPaCa-MSLN which is a cell line in which the MSLN was artificially expressed. Cells to be analyzed that were grown in a culture dish were separated by adding a Tryple Express solution and stored in a 50 mL tube, followed by centrifugation at 2000 rpm at room temperature for 3 minutes to decant the culture fluid, and the obtained product was washed once with PBS. The cells were suspended with FACS buffer, and transferred to a round bottom tube and centrifuged at 2000 rpm at room temperature for 3 minutes. The supernatant was discarded and the cells were well-loosened with the FACS buffer so as to have a cell density of 4×10^5/mL. Then, 1 μg of candidate antibody was added thereto at 4°C. After 1 hour, the resultant material was washed with the FACS buffer twice, and goat-derived anti-human IgG antibody (FITC junction) was added in an amount of 1 μL for each sample to be combined at 4°C for 30 minutes. The cells were collected by centrifugation at 2000 rpm for 3 minutes, and 500 μL of fixation buffer was added to resuspend the cells, and the cells were measured by FACS calibur (FIG. 6).

[222]

[223] 4-3: Selective binding analysis of anti-MSLN antibody with regard to MSLN-expressing cell line

[224] The FACS test was performed to evaluate whether the anti-MSLN antibody was se-

lectively bound to the MSLN-overexpressing cell line (MiaPaCa-MSLN #2). The anti-MSLN candidate antibody was labeled by the method described in Example 4-2 above, and the cells were measured by FACS calibur (FIG. 7).

[225] The FACS test was performed to evaluate whether the MI323, MI329, MI403, and MS502 candidate antibodies having excellent binding force were well-bound even to the cell line MSLN of mesothelioma (H226, H2052) and pancreatic cancer (AsPC-1), and results were compared with MFI values.

[226]

[227] [Table 19]

MSLN Ab	Miapaca2	H226	ASPC-1	H2052
MSLN test	—	+	+	++
MS502	—	++	++	++
MI403	—	+	++	++
MI323	—	+++	++	++
MI329	—	+++	++	++
Morab	—	+++	++	++
BAY94-9343	—	+++	++	++

[228]

[229] As a result, as shown in FIG. 8 and Table 19, all of the MI323, MI329, MI403, and MS502 candidate antibodies with regard to the MSLN of mesothelioma and pancreatic cancer cell lines had significant binding force even though there is a slight difference in binding degree. In particular, the MI323 candidate antibody had an excellent binding aspect.

[230] Further, whether the MI323 candidate antibody having the excellent binding aspect with regard to the MSLN, MS502 candidate antibody having a different pattern of Biacore $K_D(K_{off}/K_{on})$ value, and the heavy chain variable region mutation 2-78-C2G4 candidate antibody produced from the MS502 candidate antibody were selectively bound to MSLN-expressing tumor cells, was evaluated in MiaPaCa-MSLN #2 cell that over-expresses the MSLN and MiaPaCa-2 that does not over-express the MSLN.

[231] As a result, as illustrated in FIG. 9, the MI323, MS502, and 2-78-C2G4 candidate antibodies had excellent binding aspect in the MSLN-overexpressing MiaPaCa-MSLN #2 cell as compared to the MiaPaCa-2.

[232]

Industrial Applicability

[233] The antibody specifically bound to the mesothelin according to the present invention

has high affinity and specificity to an antigen to be effectively usable for treatment or diagnosis of cancer or tumor diseases.

[234] The present invention has been described in detail based on particular features thereof, and it is obvious to those skilled in the art that these specific technologies are merely preferable embodiments and thus the scope of the present invention is not limited to the embodiments. Therefore, the substantial scope of the present invention will be defined by the accompanying claims and their equivalents.

[235]

Sequence Listing Free Text

[236] Attached electronic file.

Claims

1. An antibody binding to mesothelin comprising a heavy chain variable region comprising:a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 9, a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 10, and a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 11, a light chain variable region comprising a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 12, a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 13 and a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 14;

a heavy chain variable region comprising a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 15, a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 16, and a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 17, a light chain variable region comprising a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 18, a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 19 and a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 20;

a heavy chain variable region comprising a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 21, a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 22, and a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 23, a light chain variable region comprising a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 24, a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 25 and a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 26;

a heavy chain variable region comprising a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 27, a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 28, and a heavy chain CDR3 comprising an amino acid

sequence of SEQ ID NO: 29, a light chain variable region comprising a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 30, a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 25 and a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 26,

a heavy chain variable region comprising a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 59, a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 60, and a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 61, a light chain variable region comprising a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 62, a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 63 and a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 64,

a heavy chain variable region comprising a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 59, a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 65, and a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 66, a light chain variable region comprising a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 67, a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 68 and a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 69,

a heavy chain variable region comprising a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 59, a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 65, and a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 66, a light chain variable region comprising a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 70, a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 68 and a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 69,

a heavy chain variable region comprising a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 59, a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 65, and a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 66, a light chain variable region comprising a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 117, a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 68 and a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 118,

a heavy chain variable region comprising a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 59, a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 65, and a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 66, a light chain variable region comprising a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 67, a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 68 and a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 119,

a heavy chain variable region comprising a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 59, a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 65, and a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 66, a light chain variable region comprising a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 117, a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 68 and a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 120,

a heavy chain variable region comprising a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 59, a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 71, and a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 72, a light chain variable region comprising a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 62, a light chain CDR2

comprising an amino acid sequence of SEQ ID NO: 73 and a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 74,

a heavy chain variable region comprising a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 59, a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 75, and a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 76, a light chain variable region comprising a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 77, a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 78 and a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 79,

a heavy chain variable region comprising a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 59, a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 80, and a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 81, a light chain variable region comprising a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 62, a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 82 and a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 83,

a heavy chain variable region comprising a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 59, a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 84, and a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 85, a light chain variable region comprising a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 86, a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 78 and a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 87,

a heavy chain variable region comprising a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 59, a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 121, and a heavy chain CDR3 comprising an amino acid

sequence of SEQ ID NO: 66, a light chain variable region comprising a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 67, a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 68 and a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 69,

a heavy chain variable region comprising a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 59, a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 122, and a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 66, a light chain variable region comprising a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 67, a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 68 and a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 69,

a heavy chain variable region comprising a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 59, a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 122, and a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 66, a light chain variable region comprising a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 67, a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 68 and a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 119,

a heavy chain variable region comprising a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 59, a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 122, and a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 66, a light chain variable region comprising a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 67, a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 68 and a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 120,

a heavy chain variable region comprising a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 59, a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 123, and a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 124, a light chain variable region comprising a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 67, a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 68 and a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 69,

a heavy chain variable region comprising a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 59, a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 123, and a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 124, a light chain variable region comprising a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 67, a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 68 and a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 120,

a heavy chain variable region comprising a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 59, a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 125, and a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 66, a light chain variable region comprising a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 67, a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 68 and a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 69,

a heavy chain variable region comprising a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 59, a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 125, and a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 66, a light chain variable region comprising a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 67, a light chain CDR2

comprising an amino acid sequence of SEQ ID NO: 68 and a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 119,

a heavy chain variable region comprising a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 59, a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 65, and a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 126, a light chain variable region comprising a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 67, a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 68 and a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 69; or

a heavy chain variable region comprising a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 59, a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 65, and a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 126, a light chain variable region comprising a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 67, a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 68 and a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 119.

2. The antibody of claim 1, wherein the antibody comprises the heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 1, 3, 5, 7, 46, 48, 51, 53, 55, 57, 112, 113, 114, 115 or 116.

3. The antibody of claim 1, wherein the light chain variable region comprises an amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 47, 49, 50, 52, 54, 56, 58, 109, 110 or 111.

4. The antibody of claim 1,

a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 1, a light chain variable region comprising an amino acid sequence of SEQ ID NO: 2;

a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 3, a light chain variable region comprising an amino acid sequence of SEQ ID NO: 4;

a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 5, a light chain variable region comprising an amino acid sequence of SEQ ID NO: 6;

a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 7, a light chain variable region comprising an amino acid sequence of SEQ ID NO: 8;

a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 46, a light chain variable region comprising an amino acid sequence of SEQ ID NO: 47;

a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 48, a light chain variable region comprising an amino acid sequence of SEQ ID NO: 49;

a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 48, a light chain variable region comprising an amino acid sequence of SEQ ID NO: 50;

a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 51, a light chain variable region comprising an amino acid sequence of SEQ ID NO: 52;

a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 53, a light chain variable region comprising an amino acid sequence of SEQ ID NO: 54;

a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 55, a light chain variable region comprising an amino acid sequence of SEQ ID NO: 56;

a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 57, a light chain variable region comprising an amino acid sequence of SEQ ID NO: 58;

a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 48, a light chain variable region comprising an amino acid sequence of SEQ ID NO: 109;

a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 48, a light chain variable region comprising an amino acid sequence of SEQ ID NO: 110;

a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 48, a light chain variable region comprising an amino acid sequence of SEQ ID NO: 111;
a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 112, a light chain variable region comprising an amino acid sequence of SEQ ID NO: 49;
a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 113, a light chain variable region comprising an amino acid sequence of SEQ ID NO: 49;
a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 114, a light chain variable region comprising an amino acid sequence of SEQ ID NO: 49;
a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 115, a light chain variable region comprising an amino acid sequence of SEQ ID NO: 49;
a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 116, a light chain variable region comprising an amino acid sequence of SEQ ID NO: 49;
a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 113, a light chain variable region comprising an amino acid sequence of SEQ ID NO: 110;
a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 114, a light chain variable region comprising an amino acid sequence of SEQ ID NO: 110;
a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 115, a light chain variable region comprising an amino acid sequence of SEQ ID NO: 110; or
a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 116, a light chain variable region comprising an amino acid sequence of SEQ ID NO: 110.

5. A nucleic acid encoding the antibody according to any one of claims 1 to 4.

6. A vector comprising the nucleic acid according to claim 5.

7. A host cell comprising the vector according to claim 6.

8. A method of preparing the antibody binding to mesothelin comprising expressing the antibody by culturing host cell according to claim 7.

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