Title: ANTIBODY SPECIFICALLY BINDING TO EPITOPE IN SEMA DOMAIN OF C-MET

Abstract: An antibody or antigen binding fragment thereof that specifically binds to an epitope in a SEMA domain of C-MET protein, and pharmaceutical compositions, methods, kits, nucleic acids, and cells related thereto.

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Description

Title of Invention: ANTIBODY SPECIFICALLY BINDING TO EPITOPE IN SEMA DOMAIN OF C-MET

Technical Field

The invention relates to antibody or antigen binding fragment thereof that specifically binds to an epitope in a SEMA domain of c-Met protein, and to pharmaceutical compositions, methods, kits, nucleic acids, and cells related thereto.

Background Art

Hepatocyte growth factor (HGF) is a mesenchyme-derived pleitrophic cytokine that binds the extracellular region of the tyrosine kinase receptor, c-Met, to induce mitogenesis, movement, morphogenesis, and angiogenesis in various normal cells and tumor cells. Regulation of the HGF/c-Met signaling pathway is implicated in various mechanisms related to cancer, such as tumor progression, metastasis, migration, invasion, and angiogenesis. In addition, c-Met amplification or mutation is thought to drive ligand-independent tumorigenesis. Thus, c-Met has recently emerged as a new target for anti-cancer therapy.

In particular, c-Met is known to be involved in induction of resistance to commonly used anti-cancer drugs, and thus, is regarded as an important player in personalized treatments. Representative anti-cancer drugs targeting epidermal growth factor receptor (EGFR) (ERBB1), such as Erbitux and Tarceva, work by blocking signal transduction related to cancer development. Herceptin, which is a well-known breast cancer drug, targets ERBB2 (HER2) and works by blocking signal transduction necessary for cell proliferation. However, recent findings have indicated that among patients resistant to the drugs described above, anti-cancer drugs do not work due to overexpression of c-Met and activation of other types of signal transduction that leads to cell proliferation. Thus, many pharmaceutical firms are developing anti-cancer drugs to inhibit c-Met.

The related art discloses therapeutic antibody drugs that inhibit the function of c-Met. In this related art, however, antibodies having an original structure induce dimerization of c-Met molecules, thereby causing cancer.

In another related art, which discloses therapeutic antibody drugs that inhibit the function of c-Met, the antibody is capable of inhibiting the binding of c-Met to HGF c-Met, which is a c-Met ligand, but the binding of the antibody to c-Met induces the dimerization of c-Met, independent from the ligand. As a result, the antibody acts as an agonist that induces the transduction of cancer-causing signals.

Another related art discloses, to prevent the dimerization of c-Met, a one-armed antagonistic antibody with respect to c-Met, which is prepared by modifying an agonist, a
two-armed antibody, using a genetic recombinant method, and product development in clinical trials is currently under way. However, even in this related art, the antibody works only when the treatment is performed together with chemical therapy, and when the antibody is independently treated, anti-cancer therapeutic effects are proven to be low. Therefore, research into the target on c-Met is needed to develop a novel pharmaceutical composition for preventing or treating cancer that inhibits the function of c-Met.

**Disclosure of Invention**

**Technical Problem**

[7] Provided is an antibody or antigen binding fragment thereof that specifically binds to an epitope in a SEMA domain of c-Met protein.

[8] Also provided are pharmaceutical compositions for preventing or treating cancer, methods of treating cancer, methods of screening for a c-Met antagonist, kits for diagnosing cancer, nucleic acids encoding the antibody or antigen binding fragment, cells comprising the nucleic acids, and methods for preparing the antibody or antigen binding fragment.

**Solution to Problem**

[9] Reference will now be made in detail to embodiments, examples of which are illustrated in the accompanying drawings, wherein like reference numerals refer to like elements throughout. In this regard, the present embodiments may have different forms and should not be construed as being limited to the descriptions set forth herein. Accordingly, the embodiments are merely described below, by referring to the figures, to explain aspects of the present description. As used herein, the term "and/or" includes any and all combinations of one or more of the associated listed items.

[10] According to an embodiment of the present invention, there is provided an antibody or antigen binding fragment thereof that specifically binds to an epitope in a SEMA domain of c-Met protein, wherein the epitope has the amino acid sequence of SEQ ID NO: 1 or a portion thereof.

[11] The term "c-Met" or "c-Met protein" refers to a receptor tyrosine kinase that binds hepatocyte growth factor (HGF). The c-Met protein includes polypeptides encoded by nucleotide sequences identified as GenBank Accession Number NM_000245, proteins encoded by polypeptide sequences identified as GenBank Accession Number NM_000236, or extracellular regions thereof. The receptor tyrosine kinase c-Met participates in various mechanisms, such as cancer development, metastasis, migration, invasion, and angiogenesis.

[12] The HGF receptor, c-Met, has three regions: extracellular, transmembrane, and intracellular. The extracellular region consists of a SEMA domain, which is a HGF-binding
domain, with a structure in which a α-subunit is linked by a disulfide bond to a β-subunit, a plexin-semaphorins-integrin (PSI) homology domain, and an immunoglobulin-like fold shared by plexins and transcriptional factors (IPT) domain. In other words, the SEMA domain of c-Met protein exists in the extracellular region of c-Met and corresponds to a HGF-binding region. In particular, the epitope having an amino acid sequence of SEQ ID NO: 1 or a portion thereof corresponds to a loop region between second and third propeller domains among epitopes in the SEMA domain of c-Met protein.

[13] The term "epitope" used herein indicates an antigenic determinant and is interpreted to mean a site on an antigen recognized by an antibody. The epitope may be a polypeptide having an amino acid sequence of SEQ ID NO: 2 or 3. The polypeptide may also be an epitope existing in the SEMA domain of c-Met protein.

[14] The epitope having an amino acid sequence of SEQ ID NO: 2 corresponds to an outermost region of a loop region between second and third propeller domains in the SEMA domain of c-Met protein, and the epitope having an amino acid sequence of SEQ ID NO: 3 refers to a site to which an antibody or an antigen binding fragment thereof most specifically binds.

[15] The antibody or the antigen binding fragment thereof may include a heavy chain variable region including at least one heavy chain complementarity determining region amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 5, and SEQ ID NO: 6, and a light chain variable region including at least one light chain complementarity determining region amino acid sequence selected from the group consisting of SEQ ID NO: 7, SEQ ID NO: 8, and SEQ ID NO: 9.

[16] The heavy chain variable region may have an amino acid sequence of SEQ ID NO: 10, and the light chain variable region may have an amino acid sequence of SEQ ID NO: 11.

[17] The antibody or the antigen binding fragment thereof may be an antigen binding fragment selected from the group consisting of monoclonal antibody, bispecific antibody, multispecific antibody, or antigen binding fragment selected from the group consisting of scFv, (scFv)_2, Fab, Fab', and F(ab')_2.

[18] A naturally occurring intact antibody, or immunoglobulin, includes four polypeptides: two full-length light chains and two full-length heavy chains, in which each light chain is linked to a heavy chain by disulfide bonds. Each heavy chain has a constant region and a variable region. Similarly, each light chain has a constant region and a variable region. There are five heavy chain classes (isotypes): gamma (γ), mu (μ), alpha (α), delta (δ), or epsilon (ε), and additionally several subclasses gamma 1 (γ1), gamma 2(γ2), gamma 3(γ3), gamma 4(γ4), alpha 1(al), and alpha 2(a2). The light chain constant region can be either kappa (κ) or lambda (λ) type. The variable
regions differ in sequence among antibodies and are used in the binding and specificity of a given antibody to its particular antigen.

The term "heavy chain" used herein is understood to include a full-length heavy chain including a variable region \( (V_H) \) having amino acid sequences that determine specificity for antigens and a constant region having three constant domains \( (C_{H1}, C_{H2}, \text{ and } C_{H3}) \), and fragments thereof. In addition, the term "light chain" used herein is understood to include a full-length light chain including a variable region \( (V_L) \) having amino acid sequences that determine specificity for antigens and a constant region \( (C_L) \), and fragments thereof.

The term "complementarity determining region (CDR)" used herein refers to an amino acid sequence found in the variable region of a heavy chain or a light chain of an immunoglobulin. The CDRs determine the specificity of an antibody and may provide a contact residue for binding to a specific epitope of an antigen. The heavy chain and the light chain may respectively include three CDRs \( (CDRH1, CDRH2, \text{ and } CDRH3) \), and \( (CDRL1, CDRL2, \text{ and } CDRL3) \). Four framework regions, which have more highly conserved amino acid sequences than the CDRs, separate the CDR regions in the \( V_H \) or \( V_L \).

The term "antigen binding fragment" used herein refers to fragments of an intact immunoglobulin, and any part of a polypeptide including antigen binding regions having the ability to specifically bind to the antigen. For example, the antigen binding fragment may be a \( F(ab')_2 \) fragment, a Fab' fragment, a Fab fragment, a Fv fragment, or a scFv fragment, but is not limited thereto. A Fab fragment has one antigen binding site and contains the variable regions of a light chain and a heavy chain, the constant region of the light chain, and the first constant region \( C_{H1} \) of the heavy chain. A Fab' fragment is different from the Fab fragment in that the Fab' fragment additionally includes the hinge region of the heavy chain, including at least one cysteine residue at the C-terminal of the heavy chain \( C_{H1} \) region. The \( F(ab')_2 \) fragment is produced whereby cysteine residues of the Fab' fragment are joined by a disulfide bond at the hinge region. A Fv fragment is the minimal antibody fragment having only heavy chain variable regions and light chain variable regions, and a recombinant technique for producing the Fv fragment is well known in the art. Two-chain Fv fragments may have a structure in which heavy chain variable regions are linked to light chain variable regions by a non-covalent bond. Single-chain Fv fragments generally may have a dimer structure as in the two-chain Fv fragments in which heavy chain variable regions are covalently bound to light chain variable regions via a peptide linker or heavy and light chain variable regions are directly linked to each other at the C-terminal thereof. The antigen binding fragment may be obtained using a protease (for example, a whole antibody is digested with papain to obtain Fab fragments, and is digested with pepsin
to obtain F(ab')$_2$ fragments), and may be prepared by a genetic recombinant technique.

[22] The c-Met may be derived from c-Met selected from the group consisting of a human c-Met, a monkey c-Met, a mouse c-Met, and a rat c-Met.

[23] According to another embodiment of the present invention, there is provided a pharmaceutical composition for preventing or treating cancer, including a therapeutically effective amount of an antibody or antigen binding fragment thereof that specifically binds to an epitope in a SEMA domain of c-Met protein, wherein the epitope has the amino acid sequence of SEQ ID NO: 1 or a portion thereof, and a pharmaceutically acceptable carrier, a diluent, or an excipient.

[24] The cancer may be squamous cell carcinoma, small-cell lung cancer, non-small-cell lung cancer, adenocarcinoma of the lung, squamous cell carcinoma of the lung, peritoneal carcinoma, skin cancer, melanoma in the skin or eyeball, rectal cancer, cancer near the anus, esophagus cancer, small intestinal tumor, endocrine gland cancer, parathyroid cancer, adrenal cancer, soft-tissue sarcoma, urethral cancer, chronic or acute leukemia, lymphocytic lymphoma, hepatoma, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatocellular adenoma, breast cancer, colon cancer, large intestine cancer, endometrial carcinoma or uterine carcinoma, salivary gland tumor, kidney cancer, prostate cancer, vulvar cancer, thyroid cancer, or head or neck cancers.

[25] The epitope may be a polypeptide having an amino acid sequence of SEQ ID NO: 2 or 3.

[26] The pharmaceutical composition for preventing or treating cancer may include a pharmaceutically acceptable carrier. The pharmaceutically acceptable carrier may be lactose, dextrose, sucrose, sorbitol, mannitol, starch, gum acacia, calcium phosphate, alginates, gelatin, calcium silicate, micro-crystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrup, methyl cellulose, methylhydroxy benzoate, propylhydroxy benzoate, talc, magnesium stearate, and/or mineral oil, but is not limited thereto. The pharmaceutical composition may further include a lubricant, a wetting agent, a sweetener, a flavor enhancer, an emulsifying agent, a suspension agent, and/or a preservative.

[27] The pharmaceutical composition for preventing or treating cancer may be administered orally or parenterally. The parenteral administration may include intravenous injection, subcutaneous injection, muscular injection, intraperitoneal injection, endothelial administration, local administration, intranasal administration, intrapulmonary administration, and rectal administration. Since oral administration leads to digestions of protein or peptide, an active ingredient may be coated or formulated in the pharmaceutical composition to prevent digestion. In addition, the pharmaceutical composition may be equipped with a targeting ability to home in on specific cells upon
A suitable dosage of the pharmaceutical composition for preventing or treating cancer may depend on many factors, such as formulation methods, administration methods, ages of patients, body weight, gender, pathologic conditions, diets, administration time, administration route, excretion speed, and reaction sensitivity. A desirable dosage of the pharmaceutical composition may be in the range of about 0.001 to 100 mg/kg for an adult. The term "therapeutically effective amount" used herein refers to a sufficient amount used in preventing or treating cancer or angiogenesis-related diseases.

The pharmaceutical composition may be formulated with a pharmaceutically acceptable carrier and/or an excipient into a unit or a multiple dosage form by a well-known method in the art. In this regard, the formulation may be a solution in oil or an aqueous medium, a suspension, syrup, an emulsifying solution, an extract, powder, granules, a tablet, or a capsule, and may further include a dispersing or a stabilizing agent. In addition, the pharmaceutical composition may be administered as an individual drug, or together with other drugs, and may be administered sequentially or simultaneously with pre-existing drugs. The pharmaceutical composition includes the antibody or the antigen binding fragment thereof, and thus, may be formulated as an immunoliposome. The liposome containing the antibody may be prepared using a well-known method in the art. The immunoliposome is a lipid composition including phosphatidylcholine, cholesterol, and polyethyleneglycol-derived phosphatidylethanolamine, and may be prepared by a reverse phase evaporation method. For example, Fab' fragments may be adhered to the liposome through thiol-disulfide exchange. A chemical drug, such as doxorubicin, may also be included in the liposome.

The antibody or antigen binding fragment may be an antagonist of c-Met protein.

The term "antagonist" is used in the broadest sense herein, and is understood to include all molecules that partially or entirely block, inhibit, and/or neutralize at least one biological activity of a target (for example, c-Met). For example, the term antagonist antibody refers to an antibody that inhibits or decreases the biological activity of an antigen, for example c-Met, that the antibody binds. The antagonist may reduce receptor phosphorylation, or inactivate or kill cells that have been activated by a ligand, by binding of a receptor with respect to a ligand. In addition, the antagonist may completely block the interaction between a receptor and a ligand or substantially decrease the interaction therebetween by changing a tertiary structure of the receptor or down-regulating.

In one embodiment, the antibody or the antigen binding fragment thereof may include a heavy chain variable region including at least one heavy chain comple-
mentarity determining region amino acid sequence selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 7, and SEQ ID NO: 8 and a light chain variable region including at least one light chain complementarity determining region amino acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, and SEQ ID NO: 11. In addition, the heavy chain variable region may have an amino acid sequence of SEQ ID NO: 12, and the light chain variable region may have an amino acid sequence of SEQ ID NO: 13.

According to another embodiment of the present invention, there is provided a method of treating cancer, the method including administering to a subject a pharmaceutical composition including a therapeutically effective amount of an antibody or antigen binding fragment thereof that specifically binds to an epitope in a SEMA domain of c-Met protein, wherein the epitope has the amino acid sequence of SEQ ID NO: 1 or a portion thereof, and a pharmaceutically acceptable carrier, a diluent, or an excipient.

The pharmaceutical composition for preventing or treating cancer and the administration method are described above.

The subjects to which the pharmaceutical composition for preventing or treating cancer is administered may include animals. For example, the animals may be humans, dogs, cats, or mice.

According to another embodiment of the present invention, there is provided a method of screening a c-Met antagonist, the method including: contacting an epitope in a SEMA domain with a sample to be analyzed, wherein the epitope has the amino acid sequence of SEQ ID NO: 1 or a portion thereof; detecting the binding of the epitope to the sample, wherein, if the epitope and the sample exhibit a binding affinity ranging from about 1 pM to about 10 nM, the sample is a candidate c-Met antagonist.

In the screening method, first, the epitope in the SEMA domain of c-Met protein that has the amino acid sequence of SEQ ID NO: 1 or a portion thereof is contacted with the sample to be analyzed. The c-Met protein may be derived from, but is not limited to, c-Met selected from the group consisting of a human, a monkey, a mouse, and a rat. The term sample used herein refers to a certain material used in a screening method to confirm whether the sample binds with the epitope in the SEMA domain of c-Met protein that has the amino acid sequence of SEQ ID NO: 1 or a portion thereof.

Examples of the sample include, but are not limited to, polypeptides such as antibodies and antigen binding fragments thereof, chemicals, polynucleotides, antisense-RNA, short hairpin RNA (shRNA), small interference RNA (siRNA), and natural extracts.

Subsequently, a binding affinity between the sample to be analyzed and the epitope in the SEMA domain of c-Met protein that has the amino acid sequence of SEQ ID NO: 1 or a portion thereof is measured. The measurement of binding affinity may be
performed using various methods known in the art. For example, the binding affinity may be measured using a Biacore device. In general, a range of the binding affinity that is allowable as a therapeutic drug may be defined such that a binding constant $K_D$ is 10 nM or less. That is, for example, if the binding affinity range is from about 1 pM to about 10 nM, from about 10 pM to about 10 nM, or from about 100 pM to about 10 nM when the binding affinity between the epitope in the SEMA domain of c-Met protein that has the amino acid sequence of SEQ ID NO: 1 or a portion thereof and the sample to be analyzed (e.g., antibody) is measured using a Biacore device by surface plasmon resonance, the sample (e.g., antibody) may be determined as a candidate material for diagnosing, preventing, or treating cancer.

The epitope may be a polypeptide having an amino acid sequence of SEQ ID NO: 2 or 3. In other words, even when the polypeptide having an amino acid sequence of SEQ ID NO: 2 or 3 is used in the screening method instead of the epitope in the SEMA domain of c-Met protein that has the amino acid sequence of SEQ ID NO: 1 or a portion thereof, the same screening results may be obtained.

According to another embodiment of the present invention, there is provided a kit for diagnosing cancer, including the antibody or the antigen binding fragment thereof and other biotechnical tools for various applications using epitope binding of antibodies, antibody fragments, and proteins.

The cancer may be, but is not limited to, lung cancer or ovarian cancer. In some patients with lung cancer or ovarian cancer, it is known that 168th amino acid, that is, Glu in the amino acid sequence of SEQ ID NO: 3 of the epitope in the SEMA domain of c-Met protein is substituted with Asp (M.S&tt\eret&\,Ther.Adv.Med.Oncol., 3(4):171-184(2011)).

An antibody or antigen binding fragment that specifically binds to an epitope in a SEMA domain of c-Met protein that has the amino acid sequence of SEQ ID NO: 1, the amino acid sequence of SEQ ID NO: 2, or the amino acid sequence of SEQ ID NO: 3 may be included in a biological sample. For example, the biological sample may be, but is not limited to, a tissue, cell, or whole blood of a suspected cancer patient.

The antibody or antigen binding fragment thereof that specifically binds to an epitope in a SEMA domain of c-Met protein that has the amino acid sequence of SEQ ID NO: 1 or a portion thereof, the amino acid sequence of SEQ ID NO: 2, or the amino acid sequence of SEQ ID NO: 3 has a high binding affinity with the epitope having an amino acid sequence of SEQ ID NO: 3 and a low binding affinity with an epitope (SEQ ID NO: 70) of c-Met protein having the above-described variation. Thus, if a biological sample derived from a suspected cancer patient forms an antigen-antibody complex when contacted with the epitope having an amino acid sequence of SEQ ID NO: 3, but not when contacted with the epitope having an amino acid sequence of SEQ
The formation of the antigen-antibody complex may be confirmed using various detection methods, such as a colorimetric method, an electrochemical method, a fluorimetric method, luminometry, a particle counting method, a visual assessment method, or a scintillation counting method.

The term "detection" used herein refers to a process, which is used to detect an antigen-antibody complex, performed using various markers. Examples of the markers include, but are not limited to, an enzyme, a fluorescent material, a ligand, a 1u-minescent material, nanoparticles, and a radioactive isotope.

Examples of the enzyme include acetylcholinesterase, alkaline phosphatase, β-D-galactosidase, horseradish peroxidase, and β-lactamase. Examples of the fluorescent material include fluorescein, Eu⁴⁺, a Eu³⁺ chelate, and cryptatep. The ligand may be biotin derivatives or the like. The luminescent material may be acridinium ester, isoluminol derivatives, or the like. Examples of the nanoparticles include colloid gold nanoparticles and colored latex nanoparticles. Examples of the radioactive isotope include ⁵⁷Co, ³H, ¹²⁵I and ¹²⁵I-Bonto Hunter reagents.

For example, the antigen-antibody complex may be detected using an enzyme-linked immunosorbsent assay (ELISA) method. Examples of the ELISA method include direct ELISA using a labeled antibody recognizing an antigen immobilized on a solid support, indirect ELISA using a labeled secondary antibody recognizing a capture antibody in a complex of an antibody recognizing an antigen immobilized on a solid support, direct sandwich ELISA using another labeled antibody recognizing an antigen in an antigen-antibody complex immobilized on a solid support, and indirect sandwich ELISA in which another labeled antibody recognizing an antigen in an antigen-antibody complex immobilized on a solid support is reacted, and then a labeled secondary antibody recognizing the other labeled antibody is used. The antibody or the antigen binding fragment thereof may have a detectable marker. If the antibody or the antigen binding fragment thereof does not have a detectable marker, it may be treated with another antibody capable of capturing the antibody or the antigen binding fragment thereof and having a detectable marker.

According to another embodiment of the present invention, there is provided a nucleic acid encoding an antibody or antigen binding fragment thereof that specifically binds to an epitope in a SEMA domain of c-Met protein, wherein the epitope has the amino acid sequence of SEQ ID NO: 1 or a portion thereof. The nucleic acid encoding the antibody or antigen binding fragment thereof may be, for example, DNA or RNA and may optionally be incorporated in a vector.

According to another embodiment of the present invention, there is provided a cell comprising a nucleic acid encoding an antibody or antigen binding fragment thereof...
that specifically binds to an epitope in a SEMA domain of c-Met protein, wherein the epitope has the amino acid sequence of SEQ ID NO: 1 or a portion thereof.

According to another embodiment of the present invention, there is provided a method of preparing an antibody or antigen binding fragment thereof that specifically binds to an epitope in a SEMA domain of c-Met protein, wherein the epitope has the amino acid sequence of SEQ ID NO: 1 or a portion thereof, comprising expressing a nucleic acid encoding the antibody or antigen binding fragment thereof in a cell.

**Brief Description of Drawings**

These and/or other aspects will become apparent and more readily appreciated from the following description of the embodiments, taken in conjunction with the accompanying drawings of which:

FIG. 1 is a diagram showing the use of overlap extension PCR to obtain an scFv library gene of an huAbF46 antibody in which a desired CDR is mutated;

FIG. 2 is an image showing results of confirming recognition of mouse antibody AbF46 with respect to full-length c-Met, according to an embodiment;

FIG. 3 is a set of images showing results of confirming recognition of mouse antibody AbF46 with respect to a SEMA domain, according to an embodiment;

FIG. 4 is a set of graphs showing enzyme-linked immunosorbent assay (ELISA) results for epitope mapping of huAbF46, according to an embodiment;

FIGS. 5a and 5b are images confirming a position of an epitope of huAbF46 on a SEMA domain, according to an embodiment;

FIGS. 6a and 6b are graphs showing results of confirming a degree of agonism of humanized antibody huAbF46 by BrdU assay, according to an embodiment;

FIG. 7 is a graph illustrating results of in vitro cell viability of huAbF46-H4-Al, huAbF46-H4-A2, huAbF46-H4-A3, and huAbF46-H4-A5 antibodies according to an embodiment;

FIGS. 8a and 8b are graphs showing results of confirming a degree of agonism of humanized antibody huAbF46 by Akt phosphorylation, according to an embodiment;

FIG. 9 is a graph illustrating anti-cancer effects of huAbF46-H4-Al, huAbF46-H4-A2, huAbF46-H4-A3, and huAbF46-H4-A5 antibodies according to an embodiment by measuring degrees of degradation of c-Met;

FIG. 10 is a graph showing in vitro cell viability analysis results of humanized antibody huAbF46, according to an embodiment;

FIGS. 11a to 11c are graphs showing results of analyzing in vivo anti-cancer effects of mouse antibody AbF46 and chimeric antibody chAbF46 by using a mouse brain cancer xenograft model or stomach cancer xenograft model, according to an embodiment; and
FIG. 12 is a graph showing results of analyzing in vivo anti-cancer effects of mouse antibody AbF46 and humanized antibody huAbF46 by using a mouse lung cancer xenograft model, according to an embodiment.

Mode for the Invention

One or more embodiments of the present invention will now be described in further detail with reference to the following Examples. However, these examples are for illustrative purposes only and are not intended to limit the scope of the invention.

Example 1: Production of mouse antibody AbF46 against c-Met

(1) Immunization of mice

To obtain immunized mice necessary for developing hybridoma cell lines, 100 μg of human c-Met/Fc fusion protein (R&D Systems) and a complete Freund's adjuvant in the same amount were mixed, and the mixture was administered via an intraperitoneal injection to each of five 4 to 6-week-old BALB/c mice (Japan SLC, Inc.). After two weeks, the antigen (half the previously injected amount) was mixed with an incomplete Freund's adjuvant using the same method as described above, and the mixture was administered to each mouse via an intraperitoneal injection. After one week, final boosting was performed, and blood was collected from the tail of each mouse after three days to obtain serum. Then, serum was diluted at 1/1000 with PBS, and an ELISA was performed to analyze whether the titer of the antibody recognizing c-Met increased. Afterwards, mice in which a sufficient amount of the antibody was obtained were selected, and a cell fusion process was performed on the selected mice.

(2) Cell fusion and preparation of the hybridoma cells

Three days before a cell fusion experiment, a mixture of 50 μg of PBS and human c-Met/Fc fusion protein was administered via an intraperitoneal injection to each mouse. Each immunized mouse was anesthetized, and its spleen located on the left side of the body was then extracted and ground with a mesh to isolate cells, which were mixed with a culture medium (DMEM) to prepare a spleen cell suspension. The suspension was centrifuged to collect a cell layer. The obtained 1x10⁸ of spleen cells were mixed with 10⁸ of myeloma cells (Sp2/0), and the mixture was centrifuged to precipitate the cells. The precipitate was slowly dispersed, treated with 1 ml of 45% polyethylene glycol (PEG) in DMEM, and maintained at 37°C for one minute before adding 1 ml of DMEM. After introducing additional 10 ml of DMEM for 1 minute, the resultant was maintained in a water bath at 37°C for 5 minutes. The total amount thereof was made to reach 50 ml, and the resultant was centrifuged. The resulting cell precipitate was re-suspended in an isolation medium (HAT medium) at concentration of 1-2x10⁵ cells/ml. Then, the resultant was distributed to a 96-well plate (0.1 ml per well), which was placed in a carbon dioxide incubator at 37°C to prepare the hybridoma cells.
(3) Selection of the hybridoma cells that produce monoclonal antibodies against c-Met protein

To select the hybridoma cells that specifically bind to c-Met from the hybridoma cells prepared in (2), the prepared hybridoma cells were screened by an ELISA using as an antigen human c-Met/Fc fusion protein and human Fc protein.

50 μl (2 μg/ml) of human c-Met/Fc fusion protein was coated on each well of a microtiter plate, and unreacted antigens were removed by washing. To exclude antibodies binding to Fc, but not to c-Met, the human Fc protein was coated on each well of a different microtiter plate using the same method as above. Next, 50 μl of hybridoma cell suspension was added to each well of the microtiter plates to react for 1 hour. Then, the microwell plates were washed with phosphate buffer-tween 20 (TBST) solution so as to remove unreacted culture. Goat anti-mouse IgG-horseradish peroxidase (IgG-HRP) was added thereto, and a reaction was allowed to occur at room temperature for 1 hour, and washing was performed with the TBST solution. Subsequently, substrate solution (OPD) of peroxidase was added to each well, and the reaction degree was evaluated by measuring the absorption at 450 nm using an ELISA reader. Through this method, hybridoma cell lines that produce antibodies highly specifically binding to the human c-Met protein and not to the human Fc protein were repeatedly selected. A limiting dilution was performed on the obtained hybridoma cell lines to obtain a single clone of hybridoma cell lines producing monoclonal antibodies. The selected hybridoma cell line producing the monoclonal antibody was registered in the Korean Cell Line Bank with accession number KCLRF-BP-00220.

(4) Production and purification of the monoclonal antibody

The hybridoma cells obtained in (3) above were cultured in a serum free medium to produce and purify the monoclonal antibodies from the culture.

First, AbF46 hybridoma cells cultured in 50 ml of culture medium (DMEM) with 10% FBS were centrifuged to obtain cell precipitate, which was washed with 20 ml of PBS more than twice to remove FBS. Then, 50 ml of DMEM was introduced to resuspend the cell precipitate, and the resultant was incubated in a carbon dioxide incubator at 37°C for 3 days. After centrifugation to remove antibody-producing cells, cell culture including antibodies was isolated and stored at 4°C, or was used directly. Antibodies were purified from 50 to 300 ml of the culture using a AKTA purification device (GE Health) equipped with an affinity column (protein G agarose column; Pharmacia, USA), and the purified antibodies were stored by replacing the supernatant with PBS using a filter for protein aggregation (Amicon).

Example 2: Preparation of chimeric antibody chAbF46 against c-Met

In general, mouse antibodies are likely to provoke an immune rejection response when administered to humans for the purpose of treatment. To address this problem,
from the mouse antibody AbF46 prepared according to Example 1, a chimeric antibody chAbF46, in which a constant region rather than a variable region involved in antigen binding is substituted with a sequence of a human antibody IgGl, was prepared.

A gene having a base sequence corresponding to a heavy chain of 'EcoRI-signal sequence-VH-Nhel-CH-TGA-Xhol' (SEQ ID NO: 12) was synthesized and a gene having a base sequence corresponding to a light chain of 'EcoRI-signal sequence-VL-BsiWI-CL-TGA-Xhol' (SEQ ID NO: 13) was synthesized. Afterwards, a fragment of DNA having the base sequence corresponding to a heavy chain (SEQ ID NO: 12) was cloned into pOptiVECT™-TOPO TA Cloning Kit included in OptiCHO™ Antibody Express Kit (Cat no. 12762-019) manufactured by Invitrogen by using a restriction enzyme EcoRI (NEB, R0101S), and a fragment of DNA having the base sequence corresponding to a light chain (SEQ ID NO: 13) was cloned into pcDNA™3.3-TOPO TA Cloning Kit (Cat no. 8300-01) included in OptiCHO™ Antibody Express Kit (Cat no. 12762-019) manufactured by Invitrogen by using a restriction enzyme Xhol (NEB, R0146S), thereby completing construction of vectors for the expression of a chimeric antibody.

Each of the constructed vectors was amplified using Qiagen Maxiprep kit (Cat no. 12662). The vector including the DNA fragment having the heavy chain base sequence and the vector including the DNA fragment having the light chain base sequence were transfected at a ratio of 4:1 (80 ug:20 ug) into 2.5xl0^7 of 293T cells to which 360 ml of 2M CaCl₂ was added. Thereafter, the transfected cells were cultured in a DMEM medium including 10% FBS at 37°C in 5% CO₂ for 5 hours, and then cultured in a FBS-free DMEM medium at 37°C in 5% CO₂ for 48 hours.

The cultured cells were centrifuged to obtain 100 ml of a supernatant and the supernatant was purified using AKTA Prime (GE healthcare). A Protein A column (GE healthcare, 17-0405-03) was installed in AKTA Prime, and the culture was flowed therethrough at a flow rate of 5 ml/min and was eluted with IgG elution buffer (Thermo Scientific, 21004). The buffer was exchanged with a PBS buffer, thereby obtaining a finally purified chimeric antibody AbF46 (hereinafter, referred to as chAbF46).

Example 3: Preparation of humanized antibody huAbF46 from chimeric antibody chAbF46

(1) Heavy chain humanization

To design HI-heavy chain and H3-heavy chain, first, a human germline gene that is most homologous to a VH gene of mouse antibody AbF46 was analyzed through NCBI Ig Blast. As a result, VH3-71 was confirmed to have 83% homology at an amino acid level, CDR-H1, CDR-H2, and CDR-H3 of mouse antibody AbF46 were defined
by Kabat numbering, and the CDRs of mouse antibody AbF46 were introduced into a framework of the VH3-71 gene. In this regard, 30th, 48th, 73rd and 78th amino acids were back-mutated to the original amino acid sequences of mouse antibody AbF46 (i.e., (S→T), (V→L), (D→N), and (T→L), respectively). Afterwards, 83rd and 84th amino acids were further mutated (i.e., (R→K) and (A→T), respectively), thereby completing construction of HI-heavy chain (SEQ ID NO: 14) and H3-heavy chain (SEQ ID NO: 15).

To design a H4-heavy chain, a sequence of a human antibody framework was searched. As a result, CDR-H1, CDR-H2, and CDR-H3 of mouse antibody AbF46 having sequences that are closely homologous to a framework sequence of mouse antibody AbF46 and defined by Kabat numbering using an pre-existing VH3 subtype known to be most stable were found and used to construct H4-heavy chain (SEQ ID NO: 16).

(2) Light chain humanization

To design HI-light chain (SEQ ID NO: 17) and H2-light chain (SEQ ID NO: 18), first, a human germline gene that is most homologous to a VL gene of mouse antibody AbF46 was analyzed through NCBI Ig Blast. As a result, VK4-1 was confirmed to have 75% homology at an amino acid level, CDR-L1, CDR-L2, and CDR-L3 of mouse antibody AbF46 were defined by Kabat numbering, and the CDRs of mouse antibody AbF46 were introduced into a framework of the VK4-1 gene. In this regard, in the HI-light chain, 36th, 46th, and 49th amino acids were back-mutated to the original amino acid sequences of mouse antibody AbF46 (i.e., (Y→H), (L→M), and (Y→I), respectively), and, in the H2-light chain, only a 49th amino acid was back-mutated (i.e., (Y→I)), thereby completing construction of a HI-light chain and a H2-light chain.

To design H3-light chain (SEQ ID NO: 19), a human germline gene that is most homologous to a VL gene of mouse antibody AbF46 was analyzed through NCBI Blast. As a result, VK2-40 as well as VK4-1 was found. VK2-40 was confirmed to have 61% homology with mouse antibody AbF46 VL at an amino acid level, CDR-L1, CDR-L2, and CDR-L3 of mouse antibody AbF46 were defined by Kabat numbering, and the CDR regions of mouse antibody AbF46 were introduced to a VK4-1 framework. In the H3-light chain, 36th, 46th and 49th amino acids were back-mutated (i.e., (Y→H, L→M, and Y→I, respectively).

To design the H4-light chain (SEQ ID NO: 20), sequences of a human antibody framework were searched. As a result, CDR-L1, CDR-L2, and CDR-L3 of mouse antibody AbF46 defined by Kabat number using a pre-existing Vk1 subtype known to be the most stable were introduced. In this regard, the H4-light chain was constructed such that 36th, 46th and 49th amino acids were further back-mutated (i.e., (Y→H, L→M,
and \( Y \rightarrow I \), respectively).

Thereafter, a DNA fragment having base sequences corresponding to the heavy chains (\( H^\text{I-heavy} \): SEQ ID NO: 21, \( H^\text{3-heavy} \): SEQ ID NO: 22, \( H^\text{4-heavy} \): SEQ ID NO: 23) was cloned into pOptiVEC\textsuperscript{TM}-TOPO TA Cloning Kit included in OptiCHO\textsuperscript{TM} Antibody Express Kit (Catno. 12762-019) manufactured by Invitrogen by using a restriction enzyme EcoRI (NEB,R0101S), and a DNA fragment having base sequences corresponding to the light chains was cloned into pcDNA\textsuperscript{TM}3.3-TOPO TA Cloning Kit included in OptiCHO\textsuperscript{TM} Antibody Express Kit (Catno. 12762-019) manufactured by Invitrogen by using a restriction enzyme Xhol (NEB,R0146S), thereby completing construction of vectors for the expression of a humanized antibody.

Each of the constructed vectors was amplified using Qiagen Maxiprep kit (Cat no. 12662). The vector including the DNA fragment having the heavy chain base sequences and the vector including the DNA fragment having the light chain base sequences were transfected at a ratio of 4:1 (80 ug:20 ug) into 2.5x10\(^7\) of 293T cells to which 360 \( \mu \)l of 2M CaCl\(_2\) was added. Thereafter, the transfected cells were cultured in a DMEM medium including 10% FBS at 37\(^\circ\)C in 5% \( \text{CO}_2\) for 5 hours, and then cultured in a FBS-free DMEM medium at 37\(^\circ\)C in 5% \( \text{CO}_2\) for 48 hours.

The cultured cells were centrifuged to obtain 100 ml of a supernatant and the supernatant was purified using AKTA Prime (GE healthcare). A Protein A column (GE healthcare, 17-0405-03) was installed in AKTA Prime, and the culture was made to flow therethrough at a flow rate of 5 ml/min and was eluted with IgG elution buffer (Thermo Scientific, 21004). The buffer was exchanged with a PBS buffer, thereby obtaining a finally purified humanized antibody AbF46 (hereinafter, referred to as huAbF46). In this regard, the humanized antibody AbF46 used in subsequent Examples included H4-heavy chain and H4-light chain. The variable region of heavy chain (\( \text{VH} \)) for huAbF46-H4 has an amino acid sequence of 'EVQLVESGGGLVQPSG-SLRLSCAAASGFTFTDYYMSWVRQAPKGKGLEWLGFRIRNKANGYTEYSASVK GRFTISRDFNS KNTLYQMNLSRAEDT AVYYCARDNWF AYWGGTGLVT VSS' (SEQ ID NO: 83) and the variable region of light chain (\( \text{VL} \)) for huAbF46-H4 has an amino acid sequence of 'DIQMTQSPSLASVGDRTITCCKSSQSLASGNQNY-LAWHQQKPGAPKMLIIWASTRVSGVPSRFSGSGSTDDFTTLTISSLQPEDFATY YCQQYSAPLTGQQGTKVEIKR' (SEQ ID NO: 84).

**Example 4: Selection of affinity maturated Ab from huAbF46 antibody and identification of binding affinity thereof**

1. **Preparation of sc\( \text{Fv} \) library of the huAbF46 antibody**

Genes for preparing sc\( \text{Fv} \) of the huAbF46 antibody were designed by using the heavy chain variable region and light chain variable region of the huAbF46 antibody. Each of the heavy chain variable region and light chain variable region was designed to have a
'VH-linker-VL' form, in which the linker was designed to have an amino acid sequence of 'GLGGLGGGSGGGGSGGSSGVGS' (SEQ ID NO: 28). A polynucleotide (SEQ ID NO: 29) encoding scFv of huAbF46 antibody designed as described above was synthesized (Bioneer, Inc.), and a vector for expressing the polynucleotide was represented as SEQ ID NO: 30. Then, resultants expressed by the vector were analyzed, and c-Met specific binding was identified.

(2) Preparation of gene library for affinity maturation

1) Selection of target CDR and preparation of primer

For affinity maturation of the huAbF46 antibody, 6 complementarity determining regions (CDRs) were defined by 'Kabat numbering' from the prepared mouse antibody AbF46. CDRs are shown in Table 1.

<table>
<thead>
<tr>
<th>CDR1</th>
<th>CDR2</th>
<th>CDR3</th>
</tr>
</thead>
<tbody>
<tr>
<td>AbF46 heavy chain amino acid sequence</td>
<td>DYYMS (SEQ ID NO: 4)</td>
<td>DNWFAY (SEQ ID NO: 6)</td>
</tr>
<tr>
<td>AbF46 light chain amino acid sequence</td>
<td>KSSQSLASGNNQNNYLA (SEQ ID NO: 7)</td>
<td>QQSYSAPLT (SEQ ID NO: 9)</td>
</tr>
</tbody>
</table>

Primers were prepared as follows in order to randomly introduce sequences of target CDR. According to existing methods of randomly introducing sequences, N codon was used such that any base could be introduced into sites to be mutated at the same rate (25% A, 25% G, 25% C, and 25% T). However, according to the current embodiment, in order to randomly introduce bases into CDRs of the huAbF46 antibody, 85% of the first and second nucleotides were preserved among three wild-type nucleotides coding amino acids of each CDR, and 5% of each of the other three bases was introduced. In addition, the primer was designed such that the three bases could be introduced into the third nucleotide (33% G, 33% C, and 33% T).

2) Preparation of huAbF46 antibody libraries and identification of binding force to c-Met

The construction of an antibody gene library was performed using the primers prepared in operation (1) described above. A polynucleotide encoding scFv of the huAbF46 antibody was used as a template. Two PCR fragments were prepared as shown in FIG. 1 and libraries for each of the 6 CDRs were constructed by using an overlap extension PCR.
The binding forces of the wild-type antibody (scFv of huAbF46) and library antibodies to c-Met were identified. While the binding force of most library antibodies to c-Met was lower than that of the wild-type antibody, mutants in which the binding force to c-Met was not reduced were identified.

(3) Selection of antibodies with improved affinity from the library

Library antibodies having an improved c-Met binding force were sequenced. The obtained sequences are shown in Table 2 below and were transformed into IgG. Among the clones below, 4 types of antibodies produced from L3-1, L3-2, L3-3, and L3-5 were selected and subsequent experiments were performed using these antibodies. The variable region of light chain (VL) for antibody produced from L3-1 has an amino acid sequence of ‘DIQMTQSPSSLSASVGDRVTITCKSSQQLASGNQN-NYLAWHQKPGKAPKMLIIWASRVSQPSRGSGITDFTLTISSLQPEDFA TYYCQQSYRPTFGQGTKVEIKR’ (SEQ ID NO: 85).

Table 2
<table>
<thead>
<tr>
<th>Name of clone</th>
<th>Library</th>
<th>CDR sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>H11-4</td>
<td>CDR-H1</td>
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<tr>
<td>YC151</td>
<td>CDR-H1</td>
<td>PDYYMS(SEQ ID NO: 32)</td>
</tr>
<tr>
<td>YC193</td>
<td>CDR-H1</td>
<td>SDYYMS(SEQ ID NO: 33)</td>
</tr>
<tr>
<td>YC244</td>
<td>CDR-H2</td>
<td>RNNANGNT(SEQ ID NO: 34)</td>
</tr>
<tr>
<td>YC321</td>
<td>CDR-H2</td>
<td>RNKVNGYT(SEQ ID NO: 35)</td>
</tr>
<tr>
<td>YC354</td>
<td>CDR-H3</td>
<td>DNWLSY(SEQ ID NO: 36)</td>
</tr>
<tr>
<td>YC374</td>
<td>CDR-H3</td>
<td>DNWLTY(SEQ ID NO: 37)</td>
</tr>
<tr>
<td>L1-1</td>
<td>CDR-L1</td>
<td>KSSHSLLASGNQNNYLA(SEQ ID NO: 38)</td>
</tr>
<tr>
<td>L1-3</td>
<td>CDR-L1</td>
<td>KSSRSLSSGHNKLYLA(SEQ ID NO: 39)</td>
</tr>
<tr>
<td>L1-4</td>
<td>CDR-L1</td>
<td>KSSKSLLASGNQNNYLA(SEQ ID NO: 40)</td>
</tr>
<tr>
<td>L1-12</td>
<td>CDR-L1</td>
<td>KSSRSLLASGNQNNYLA(SEQ ID NO: 41)</td>
</tr>
<tr>
<td>L1-22</td>
<td>CDR-L1</td>
<td>KSSHSLLASGNQNNYLA(SEQ ID NO: 42)</td>
</tr>
<tr>
<td>L2-9</td>
<td>CDR-L2</td>
<td>WASKRVN(SEQ ID NO: 43)</td>
</tr>
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<td>CDR-L2</td>
<td>WGSTRVSN(SEQ ID NO: 44)</td>
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<tr>
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<td>CDR-L2</td>
<td>WGSTRVPN(SEQ ID NO: 45)</td>
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</tr>
<tr>
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<td>CDR-L3</td>
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</tr>
<tr>
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<td>CDR-L3</td>
<td>AQSYSHPFS(SEQ ID NO: 48)</td>
</tr>
<tr>
<td>L3-5</td>
<td>CDR-L3</td>
<td>QQSYSRPF(SEQ ID NO: 49)</td>
</tr>
<tr>
<td>L3-32</td>
<td>CDR-L3</td>
<td>QQSYSKFTP(SEQ ID NO: 50)</td>
</tr>
</tbody>
</table>

(4) Transformation of selected antibodies to IgG

A polynucleotide encoding the heavy chain of the selected 4 types of antibodies (L3-1, L3-2, L3-3, and L3-5) consisted of 'EcoRI-signal sequence-VH-Nhel-CH-Xhol' (SEQ ID NO: 51), and amino acids of the heavy chain were not modified after affinity maturation, and thus the heavy chain of the huAbF46 antibody was used. However, the hinge region was replaced with a U6-HC7 hinge region (SEQ ID NO: 52), not with the hinge region of human IgGl. A gene of the light chain was designed to have 'EcoRI-signal sequence-VL-BsiWI-CL-Xhol', and polynucleotides (SEQ ID NOs: 53 to 56) encoding light chain variable regions of the selected 4 types of antibodies after affinity maturation were synthesized by Bioneer, Inc. Then, vectors for expression of an-
tibodies having improved affinity were constructed by cloning a DNA fragment (SEQ ID NO: 51) having the sequence corresponding to the heavy chain in a pOptiVEC™-TOPO TA Cloning Kit included in an OptiCHO™ Antibody Express Kit (Cat No. 12762-019) manufactured by Invitrogen and DNA fragments (aDNA fragment including L3-1-derived CDR-L3 (SEQ ID NO: 53), a DNA fragment including L3-2-derived CDR-L3 (SEQ ID NO: 54), a DNA fragment including L3-3-derived CDR-L3 (SEQ ID NO: 55), and a DNA fragment including L3-5-derived CDR-L3 (SEQ ID NO: 56)) corresponding to the light chain in a pcDNA™3.3-TOPO TA Cloning Kit (Cat No. 8300-01) by using a restriction enzyme, EcoRI (NEB, R0101S) and XhoI (NEB, R0146S), respectively.

The constructed vectors were amplified using a Qiagen Maxiprep kit (Cat No. 12662), and vectors including the heavy chain and vectors including the light chain were added to 293T cells (2.5x10^7) at a ratio of about 4:1 (about 80 ug:20 ug) with 360 μl of 2M CaCl_2 and were transfected. Next, the mixture was cultured in a DMEM medium with 10% FBS at 37°C in 5% CO_2 conditions for 5 hours, and then cultured in a DMEM medium without FBS at 37°C in 5% CO_2 conditions for 48 hours.

The cultured cells were centrifuged, and 100 ml of each supernatant was purified using AKTA Prime (GE healthcare). Protein A column (GE healthcare, 17-0405-03) was placed in the AKTA Prime, and the cultured solution was flowed at a flow rate of 5 ml/min and was eluted with IgG elution buffer (Thermo Scientific, 21004). The buffer was exchanged with a PBS buffer, and thus 4 types of antibodies having improved affinity (hereinafter, huAbF46-H4-A1, huAbF46-H4-A2, huAbF46-H4-A3, and huAbF46-H4-A5) were purified.

(5) Analysis of binding affinity of selected antibodies

Affinities of the 4 types of antibodies against c-Met antigen were measured by using a Biacore (GE healthcare). About 80 to 110 RU of each antibody was immobilized on a CM5 chip, and 9 different concentrations ranging from 0.39 nM to 100 nM of human c-Met protein, as an antigen, were injected at a rate of 30 μl/min to obtain k_{on} values and k_{off} values as shown in Table 3. Then, K_D values were calculated based thereon. A binding force of huAbF46 to c-Met antigen was about 2.19 nM, and binding forces of the four types of antibodies having improved affinity were in a range of 0.06 nM to 0.50 nM (Table 3). This indicates that affinities of the antibodies, which were improved in the form of scFv, were further improved by about 5 times to about 37 times after being transformed to IgG.

Table 3
Example 5: Confirm the ability of mouse antibody AbF46 to recognize c-Met
(1) Confirm the ability of mouse antibody AbF46 to recognize full length c-Met

To confirm the ability of mouse antibody AbF46 to recognize an extracellular domain of c-Met, a polynucleotide (SEQ ID NO: 57) encoding c-Met was cloned into a pcDNA5 vector (Invitrogen), and the resultant vector was expressed in a 293T cell (Korea Cell Line Bank) using an in vitro transcription and translation kit (TnT kit, Promega, Madison, USA). Afterwards, mouse antibody AbF46 was mixed with protein G-conjugated agarose beads (Invitrogen), a 293T cell lysate including synthesized c-Met protein or c-Met produced by reaction from the in vitro transcription and translation kit was added to the mixture, and immunoprecipitation was then performed on the resultant mixture. The immunoprecipitated resultant was subjected to electrophoresis through SDS-PAGE and then analyzed by Western blotting.

As illustrated in FIG. 2, it was confirmed that mouse antibody AbF46 accurately recognized a full-length c-Met antigen.

(2) Confirm the ability of mouse antibody AbF46 to recognize a SEMA domain

To confirm which region of the extracellular domain of c-Met mouse antibody AbF46 binds to, first, the extracellular domain of c-Met was divided into three regions, and a DNA fragment encoding each region was then cloned into a pcDNA5 vector. In this regard, the three regions were a SEMA domain (SEQ ID NO: 58), a PSI-IPT domain (SEQ ID NO: 59), and a TyrKc domain (SEQ ID NO: 60), and the DNA fragments encoding the three regions cloned into the pcDNA5 vector were represented by SEQ ID NO: 61, SEQ ID NO: 62, and SEQ ID NO: 63, respectively.

After each DNA fragment was cloned into the vector, each vector was expressed in a 293T cell (Korea Cell Line Bank) using an in vitro transcription and translation kit (TnT kit, Promega, Madison, USA). Afterwards, mouse antibody AbF46 was mixed with protein G-conjugated agarose beads (Invitrogen), a 293T cell lysate including synthesized c-Met protein or c-Met produced by reaction from the in vitro transcription and translation kit was added to the mixture, and immunoprecipitation was then performed on the resultant mixture. The immunoprecipitated resultant was subjected to

<table>
<thead>
<tr>
<th>Antibody</th>
<th>$k_{on}(1/Ms)$</th>
<th>$k_{off}(1/s)$</th>
<th>$K_D$(nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>huAbF46</td>
<td>$3.29 \times 10^5$</td>
<td>$7.23 \times 10^4$</td>
<td>2.19</td>
</tr>
<tr>
<td>huAbF46-H4-A1</td>
<td>$7.39 \times 10^5$</td>
<td>$4.53 \times 10^5$</td>
<td>0.06</td>
</tr>
<tr>
<td>huAbF46-H4-A2</td>
<td>$5.02 \times 10^5$</td>
<td>$2.53 \times 10^4$</td>
<td>0.50</td>
</tr>
<tr>
<td>huAbF46-H4-A3</td>
<td>$4.19 \times 10^5$</td>
<td>$1.43 \times 10^4$</td>
<td>0.34</td>
</tr>
<tr>
<td>huAbF46-H4-A5</td>
<td>$5.72 \times 10^5$</td>
<td>$2.40 \times 10^4$</td>
<td>0.42</td>
</tr>
</tbody>
</table>
electrophoresis through SDS-PAGE and then analyzed by Western blotting.

As illustrated in FIG. 3, it was confirmed that mouse antibody AbF46 was bound to the SEMA domain of c-Met. Mouse IgG was used as a negative control, and a 5D5 antibody (isolated from a hybridoma cell with ATCC Cat. # HB 11895 and purified) was used as a positive control. In FIG. 3, "input" refers to all resulting materials synthesized without immunoprecipitation that were loaded on a gel. From the results, it is confirmed that all the synthesized c-Met proteins are intact regardless of whether they bind to the antibody.

Example 6: Analysis for epitope of huAbF46

1) Preparation of peptide for epitope mapping of huAbF46

543 amino acid sequences, including the SEMA domain of c-Met and structures thereof, are represented in PDB (Protein Database) ID: 1UZY, and 6,063 other sequences capable of producing a conformational epitope and a discontinuous epitope were designed and synthesized based on the 543 amino acid sequences by using a Chemically Linked Peptides on Scaffolds (CLIPS) technology (Timmerman et al., Functional reconstruction and synthetic mimicry of a conformational epitope using CLIPS™ technology J.Mol.Recognit. 20:283-300(2007)). The peptide array fabrication will now be described in more detail. The CLIPS technology developed by PepScan is used to prepare peptides having an intrinsic structure called CLIPS rather than linear peptides having a length of about 15 amino acids, prepared using a known typical method. The binding affinity of huAbF46 with the linear peptides and the CLIPS peptides was measured. Among the CLIPS peptides, T2 CLIPS peptides are prepared such that two cysteines are linked together to form a loop so that the peptides have an artificial structure, and T3 CLIPS peptides are prepared such that three cysteines are linked together to form a loop so that the peptides have an artificial structure. In addition, binding-type peptides such as T2T3 or T2T2 CLIPS peptides may be prepared.

A total number of 6,063 peptides were prepared for epitope mapping (peptide array design was applied to PepScan). In this regard, 1st through 529th peptides, which are typical linear peptides, were prepared such that the peptides had a length of 15 amino acids and an overlapped region between certain regions. 530th through 1,058th peptides were prepared by introducing 1st through 529th peptides to T2 CLIPS peptides. 1,059th through 2,014th peptides, i.e., a total number of 956 peptides, were prepared by linking two peptides each having 15 amino acids to T3 CLIPS peptides. 2,015th through 6,063rd, i.e., a total number of 4,048 peptides, were prepared as peptides for searching epitopes having conformational and discontinuous structures through binding between peptide groups having 8 to 35 amino acid residues.
For example, a peptide array including T2 CLIPS peptides was prepared as follows. 0.5 mM of a 1,3-bis(bromomethyl)benzene solution including T2 CLIPS peptides was dissolved in ammonium bicarbonate (20 mM, pH 7.9)/acetonitrile (1:1)(v/v), and the resultant solution was added to a peptide array. The T2 CLIPS peptides as a template were bound to two cysteine side chains existing in a solid-phase bound peptide of the peptide array (455-well plate having 3 µl of wells). The peptide array was slowly shaken in the solution for 30 to 60 minutes. Lastly, the peptide array was sufficiently washed with a large amount of water, was ultrasonically fragmented in a lysate-buffer containing 1% SDS/0.1% beta-mercaptoethanol in PBS (pH 7.2) at 70°C for 30 minutes, and further ultrasonically fragmented in water for 45 minutes. T3 CLIPS peptides were prepared using the same method as described above, except that the T3 CLIPS peptides as a template were bound to three cysteine side chains.

As a result of performing epitope mapping by using the peptides by ELISA, a core epitope of huAbF46 was confirmed to be EEPSQ (SEQ ID NO: 3) a peptide consisting of the 168th through 171st amino acids of Met protein.

2) ELISA for epitope mapping of huAbF46

For epitope mapping, PEPSAN-based ELISA was performed using a total number of 529 linear and CLIPS peptides. The peptides were maintained at room temperature for 30 minutes by using a 5% blocking solution to provoke a reaction (4% ovalbumin, 5% horse serum, and 1% Tween 80). Then, 1 to 100 ug/ml of huAbF46 antibody, maintained in PBS containing 1% Tween 80 at 4°C overnight, was reacted with the peptides and the resultant product was then washed. Thereafter, the resultant product was treated with rabbit-anti-sheep antibody (SIGMA) and washed with PBS, and the washed product was then treated with peroxidase-attached swine-anti-rabbit antibody (SIGMA) and washed with PBS. Then, the resultant product was treated with 2 id/ml of peroxidase 2,2'-azino-di-3-ethylbenzthiazoline sulfonate (ABTS)(SIGMA) in 3% H2O2, and a color reaction was measured after 1 hour.

As a result, as illustrated in FIG. 4, only the peptides including EEPSQ (SEQ ID NO: 3) of both the linear peptides and the CLIPS peptides exhibited a specific ELISA positive reaction, and thus the huAbF46 antibody was confirmed to recognize the linear and conformational epitopes of c-Met.

In addition, an ELISA was performed in the same manner as described above by using polypeptides with E168D mutation, which is a representative SEMA domain mutation of c-Met known to be found in some patients with lung cancer or ovarian cancer, among the epitopes including the peptides including EEPSQ (SEQ ID NO: 3). The results are shown in Table 4 below.

Table 4
From the above results, it was confirmed that the huAbF46 antibodies were not able to bind to the SEMA domain of c-Met with the E168D mutation. This indicates that the antibodies may be used in a diagnosis method for providing cancer development information.

3) Analysis of epitope mapping results of huAbF46

From the results shown above, it was confirmed that the huAbF46 antibodies specifically bound to both the linear and CLIPS peptides including the EEPSQ (SEQ ID NO: 3) peptides consisting of 168th to 171st amino acids of c-Met protein without a non-specific reaction. This indicates that the huAbF46 antibodies bind to both the linear and conformational epitopes of c-Met protein. In terms of molecular structures (PyMOL 1.4.1), Cn3D 4.1 (NCBI), as illustrated in FIG. 5, it was confirmed that an epitope of huAbF46 was located at a SEMA domain. In addition, it was confirmed that a binding site of HGF was a position corresponding to a loop close to a direct binding site.

(2) Analysis of full positional scanning results

<table>
<thead>
<tr>
<th>Core peptide sequence</th>
<th>Synthesized peptide sequence</th>
<th>ELISA value (antibody binding of huAbF46)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EEPSQ (SEQ ID NO: 3)</td>
<td>FAPQIEEPSQCPDCVVSAKGVL (SEQ ID NO: 64)</td>
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<td>CQIEEPSQAPC (SEQ ID NO: 67)</td>
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<td></td>
<td>CIEDPSQAPDC (SEQ ID NO: 75)</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>CEDPSQAPDAC (SEQ ID NO: 76)</td>
<td>132</td>
</tr>
</tbody>
</table>
Each amino acid region of the EEPSQ (SEQ ID NO: 3) sequence was substituted with 20 amino acids rather than the original amino acids, and any change that occurred in the binding affinity between each peptide and huAbF46 antibody was analyzed through 7 peptide arrays.

As a result of the analysis, it was confirmed which amino acid of the amino acid sequences of the EEPSQ (SEQ ID NO: 3) sequence played a key role in binding with the antibody. In particular, it was confirmed that the EEP sequence in EEPSQ (SEQ ID NO: 3) played a very critical role in binding with the antibody.

Example 7: Analysis of binding affinity of huAbF46 antibody by SEMA domain mutation

Each amino acid region or the total number of 5 amino acids of the EEPSQ (SEQ ID NO: 3) sequence was substituted with alanine rather than the original amino acid, and a binding affinity between each peptide ('AAAAA' (SEQ ID NO: 77), 'AEPSQ' (SEQ ID NO: 78), 'EAPSQ' (SEQ ID NO: 79), 'EEASQ' (SEQ ID NO: 80), 'EEPAQ' (SEQ ID NO: 81), 'EEPMA' (SEQ ID NO: 82)) and the huAbF46 antibody was measured using Biacore (GE healthcare). About 80 to 110 RU of the huAbF46 antibody was immobilized on a CM5 chip, and 100 nM to 0.39 nM of the peptides having amino acid sequences of SEQ ID NOS: 77 through 82 were injected thereto at a rate of 30 nA/min in 9 different concentrations, thereby obtaining $k_{on}$ and $k_{off}$ values as shown in Table 5 below, and $K_D$ values were calculated therefrom. As a result, it was confirmed that the huAbF46 antibodies were not able to bind to the peptides with the substituted amino acids. From this result, the EEPSQ (SEQ ID NO: 3) sequence was confirmed to be an essential epitope of the huAbF46 antibody.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Antigen</th>
<th>$k_{off}$(1/Ms)</th>
<th>$k_{on}$(1/s)</th>
<th>$K_D$(nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>huAbF46</td>
<td>EEPSQ (SEQ ID NO: 3)</td>
<td>4.30x10^4</td>
<td>7.05x10^4</td>
<td>1.64</td>
</tr>
<tr>
<td>huAbF46</td>
<td>AAAAA (SEQ ID NO: 77)</td>
<td>Not bound</td>
<td></td>
<td></td>
</tr>
<tr>
<td>huAbF46</td>
<td>AEPSQ (SEQ ID NO: 78)</td>
<td>Not bound</td>
<td></td>
<td></td>
</tr>
<tr>
<td>huAbF46</td>
<td>EAPSQ (SEQ ID NO: 79)</td>
<td>Not bound</td>
<td></td>
<td></td>
</tr>
<tr>
<td>huAbF46</td>
<td>EEASQ (SEQ ID NO: 80)</td>
<td>Not bound</td>
<td></td>
<td></td>
</tr>
<tr>
<td>huAbF46</td>
<td>EEPAQ (SEQ ID NO: 81)</td>
<td>4.32x10^4</td>
<td>6.16x10^4</td>
<td>1.43</td>
</tr>
<tr>
<td>huAbF46</td>
<td>EEPSA (SEQ ID NO: 82)</td>
<td>Not bound</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Example 8: Comparison of agonism dysfunction degree of huAbF46 antibody

(l) BrdU assay
To compare a degree of agonism against a huAbF46 antibody, a BrdU assay was performed using NCI-H441 cells. The NCI-H441 cells, which are human lung cancer cells, were suspended in a RPMI 1640 medium (Gibco) at a concentration of 2x10^5 cells/ml and 100 μl of the suspension was distributed to each well of a 96-well tissue culture plate (Corning,Lowell,MA). The cells were cultured at 37°C in 5% CO₂ conditions for 24 hours, and a diluted RPMI 1640 medium was added to the antibodies after the medium was completely removed. The cells were cultured at 37°C in 5% CO₂ conditions for 24 hours, 5-bromo-2'-deoxyuridine (BrdU) was added thereto, the cells were further cultured for 3 hours, and a BrdU assay (Roche,Indianapolis,IN) was performed thereon. The cells were subjected to denaturation/fixation on a plate, anti-BrdU antibodies were added thereto, a substrate was added 1 hour thereafter, and a color reaction was measured using an ELISA spectraMax reader (Molecular Devices, Sunny vale,CA). In this regard, the agonism of mouse antibody AbF46 was compared with the agonism of huAbF46 antibody. Mouse IgG was used as a negative control and a 5D5 antibody known to be an agonist was used as a positive control.

As illustrated in FIG. 6a, it was confirmed that the huAbF46 antibody reduced a degree of agonism dysfunction, similar to that of the 5D5 antibody. In addition, referring to FIG. 6b, among the 4 types of antibodies having improved affinity, agonism side effects of 3 types were reduced. Thus, it was identified that safeties thereof were respectively improved by 25% (huAbF46-H4-A1), 28% (huAbF46-H4-A2), 13% (huAbF46-H4-A3), and 21% (huAbF46-H4-A5) at a concentration of 10 μg/ml.

(2) In vitro cell proliferation analysis

In order to identify anti-cancer effects of the 4 types of antibodies having improved affinity, in vitro cell proliferation analysis was performed using MKN45 gastric cancer cells on which c-Met is expressed (Japanese Cancer Research Bank, JCRB, Tokyo, Japan).

1x10^4 MKN45 cells suspended in 50 μl of 5% FBS/DMEM culture medium were introduced to each well of a 96-well plate. Then, the cells were either not treated or treated with 50 μl of the 4 types of antibodies at a concentration of 0.008, 0.04, 0.2, or 1 μg/ml. After incubating for 72 hours, the number of cells were quantified by using a CellTiter-Glo Luminescent Cell Viability Assay Kit (Promega, G7570) with a luminometer (PerkinElmer, 2104 Multilabel reader).

As shown in FIG. 7, relative cell viability of the antibody (huAbF46) in which the affinity was not improved was 77% at the lowest concentration of 0.008 μg/ml, and relative cell viabilities of antibodies having improved affinity, i.e., huAbF46-H4-A1, huAbF46-H4-A2, and huAbF46-H4-A5 were respectively 74, 73, and 72% similar to each other. The relative cell viability of huAbF46-H4-A3, as 66%, was considerably
increased. In addition, at 0.04 ug/ml where the viability are maximized, relative cell vi-
abilities of all of the 4 types of antibodies were less than 53% that is viability of the
5D5 antibody. Accordingly, it was identified that, as a result of improving affinity, ef-
iciency and safety were significantly improved compared to the control group.

(3) Akt phosphorylation

To compare a degree of agonism against a huAbF46 antibody, a phosphorylation
degree of Akt protein, which is an indicator involved in downstream signal
transduction and cell proliferation of c-Met, was confirmed using Caki-1 cells (Korea
Cell Line Bank). Mouse IgG was used as a negative control and a 5D5 antibody known
to be an agonist was used as a positive control.

2x10^5 cells/ml of the Caki-1 cell was distributed to each well of a 96-well plate, and,
after 24 hours, 5 ug/ml of an antibody was treated with the cells of each well in a
serum-free state for 30 minutes. The cells treated with the antibodies were lysed, and a
phosphorylation degree of Akt protein was measured using PathScan phospho-AKT1
(Ser473) chemiluminescent Sandwich ELISA kit (Cell Signaling, cat.no #7134S) and
analyzed.

As illustrated in FIGS. 8a and 8b, the phosphorylation degree of Akt protein in a case
in which the huAbF46 antibody was treated was confirmed to be less than 30%. From
the results, it was confirmed that the huAbF46 antibody had reduced agonism dys-
function.

(4) Identification of degree of degradation of c-Met

In order to identify anti-cancer effects of the 4 types of antibodies having improved
affinity, the degree of degradation of c-Met bound to the antibody was evaluated. A
relative total amount of c-Met was obtained by measuring the change of the total
amount of c-Met after the antibody bound to c-Met to degrade c-Met via inter-
nalization, and thus efficacy of the antibody was evaluated.

MKN45 cells (2 x 10^5 cells/ml) and each of the 4 types of antibodies (5 ug/ml) were
simultaneously introduced to a 96-well plate and incubated for 24 hours. Then, lysis of
the cells treated with antibodies was performed and a change of the total amount of c-
Met was measured using a Human total HGF R/c-MET ELISA KIT (R&D systems,
DYC358) and analyzed.

As a result, referring to FIG. 9, it was identified that the degree of degradation of c-
Met was improved when treated with the 4 types of antibodies having improved
affinity compared to the huAbF46 antibody. The degree of degradation of c-Met
treated with huAbF46-H4-A1 was increased by about 37% compared to huAbF46. The
degrees of degradation of c-Met treated with huAbF46-H4-A2, huAbF46-H4-A3, and
huAbF46-H4-A5 were increased by about 28% compared to huAbF46.

Example 9: Analysis of in vitro anti-cancer effect of huAbF46 antibody
To confirm anti-cancer effects of the humanized antibody huAbF46 by inhibiting proliferation of cancer cells, *in vitro* cell proliferation analysis was performed using MKN45 stomach cancer cells expressing c-Met molecules on their surfaces (Japanese Cancer Research Bank, JCRB, Tokyo, Japan).

$1 \times 10^4$ of MKN45 cells were distributed into each well of a 96-well plate together with 50 $\mu l$ of a 5% FBS/DMEM culture, and the cells were either not treated with huAbF46 antibody or were treated with 0.008, 0.04, 0.2 or 1 $\mu g/ml$ of the huAbF46 antibody. The treated cells were cultured for 72 hours, and the number of the cultured cells were counted using a luminometer (PerkinElmer, 2104 Multilabel reader) by using CellTiter-Glo® luminescent Cell Viability Assay Kit (Promega, G7570).

As illustrated in FIG. 10, it was confirmed that while the mouse IgG used as a negative control did not inhibit proliferation of cancer cells, the huAbF46 antibody did inhibit proliferation of cancer cells.

**Example 10: Confirmation of *in vivo* anti-cancer effects of mouse antibody AbF46, chAbF46 and huAbF46**

To confirm the anti-cancer effects of the mouse antibody AbF46, the chimeric antibody chAbF46, and the humanized antibody huAbF46 prepared according to the Examples above, it was evaluated whether sizes of tumors were reduced by administration of these antibodies *in vivo* using a mouse xenograft model administered with U87MG brain cancer cells (Korean Cell Line Bank), stomach cancer cell lines MKN45 Japanese Cancer Research Bank, JCRB, Tokyo, Japan) or lung cancer cell lines NCI-H441 (ATCC Cat.# HTB-174). Each of the three types of antibodies have the same complementarity determining region (CDR) and, thus, are expected to bind to the same epitope of c-Met.

The mouse xenograft model was produced such that 50 $\mu l$ of U87MG brain cancer cells, stomach cancer cells MKN45, or lung cancer cells NCI-H441 (3 x 10^6/cells/50 $\mu l$) was administered via subcutaneous injection to 6-week-old male BALB/C nude mice (available from ORIENT BIO Inc.). 12 mice per group that contracted cancer were randomly selected, and the produced model was used in the experiment. 10 mg/kg of the three antibodies was administered via intravenous injection to the mice once a week after formation of cancer cells. In addition, as a control, 10 mg/kg and 20 mg/kg of the mouse antibody AbF46 were administered via intraperitoneal injection to the mouse model twice a week.

As illustrated in FIGS. 11a to 11c, as a result of measuring sizes of tumors over time in the mouse xenograft group (U87MG brain cancer cells, stomach cancer cells MKN45), it was confirmed that the mouse antibody AbF46 and the chimeric antibody chAbF46 had significant anti-cancer effects. In this regard, the number of mice per group in an experimental group (mouse antibody AbF46 and chAbF46) and a control
(vehicle) was 12, and an average and SEM of each group were represented. In FIGS. 11a to 11c, p-values obtained by comparing the two experimental groups and the control by using repeated measures ANOVA were represented by * (p<0.05, **: p<0.01, ****: p<0.0001).

In FIG. 12, as a result of measuring sizes of tumors over time in the mouse xenograft model (lung cancer cells NCI-H441), it was confirmed that the mouse antibody AbF46 and the humanized antibody huAbF46 had significant anti-cancer effects. In this regard, the number of mice per group in an experimental group (mouse antibodies AbF46 and chAbF46) and a control (vehicle) was 15, and an average and SEM of each group were represented. In FIG. 12, p-values obtained by comparing the two experimental groups and the control by using repeated measures ANOVA were represented by * (p<0.05, ****: p<0.0001).

As described above, according to the one or more of the above embodiments of the present invention, there is provided an antibody that specifically binds to an epitope in a SEMA domain of c-Met and a pharmaceutical composition for preventing or treating cancer that includes the antibody, whereby cancer may be efficiently prevented or treated.

It should be understood that the exemplary embodiments described herein should be considered in a descriptive sense only and not for purposes of limitation. Descriptions of features or aspects within each embodiment should typically be considered as available for other similar features or aspects in other embodiments.
Claims

[Claim 1] An antibody or antigen binding fragment thereof that specifically binds to an epitope in a SEMA domain of c-Met protein, wherein the epitope has the amino acid sequence of SEQ ID NO: 1 or portion thereof.

[Claim 2] The antibody or antigen binding fragment of claim 1, wherein the epitope consists of SEQ ID NO: 2 or 3.

[Claim 3] The antibody or antigen binding fragment of claim 1 or 2, wherein the antibody or antigen binding fragment comprises a heavy chain variable region comprising at least one heavy chain complementarity determining region amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 5, and SEQ ID NO: 6, and a light chain variable region comprising at least one light chain complementarity determining region amino acid sequence selected from the group consisting of SEQ ID NO: 7, SEQ ID NO: 8, and SEQ ID NO: 9.

[Claim 4] The antibody or antigen binding fragment of claim 3, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 10, and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 11.

[Claim 5] The antibody or antigen binding fragment of any of claims 1-4, wherein the antibody or antigen binding fragment thereof is a monoclonal antibody, bispecific antibody, multispecific antibody or antigen binding fragment selected from the group consisting of scFv, (scFv)₂, Fab, Fab', and F(ab')₂.

[Claim 6] The antibody or antigen binding fragment of any of claims 1-5, wherein the c-Met protein is human c-Met, monkey c-Met, mouse c-Met, and rat c-Met.

[Claim 7] The antibody or antigen binding fragment of any of claims 1-6, wherein the antibody or antigen binding fragment is an antagonist of c-Met protein.

[Claim 8] A pharmaceutical composition for preventing or treating cancer, the pharmaceutical composition comprising a therapeutically effective amount of the antibody or antigen binding fragment of any of claims 1-7 and a pharmaceutically acceptable carrier, diluent, or excipient.

[Claim 9] The pharmaceutical composition of claim 8, wherein the cancer is squamous cell carcinoma, small-cell lung cancer, non-small-cell lung cancer, adenocarcinoma of the lung, squamous cell carcinoma of the lung, peritoneal carcinoma, skin cancer, melanoma in the skin or
eyeball, rectal cancer, cancer near the anus, esophagus cancer, small intestinal tumor, endocrine gland cancer, parathyroid cancer, adrenal cancer, soft-tissue sarcoma, urethral cancer, chronic or acute leukemia, lymphocytic lymphoma, hepatoma, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatocellular adenoma, breast cancer, colon cancer, large intestine cancer, endometrial carcinoma or uterine carcinoma, salivary gland tumor, kidney cancer, prostate cancer, vulvar cancer, thyroid cancer, or head or neck cancer.

[Claim 10] A method of treating cancer, the method comprising administering to a subject the antibody or antigen binding fragment of any of claims 1-9, or a pharmaceutical composition comprising the antibody or antigen binding fragment of any of claims 1-9 and a pharmaceutically acceptable carrier, a diluent, or an excipient.

[Claim 11] A method of screening for a c-Met antagonist, the method comprising: contacting an epitope in a SEMA domain of c-Met protein with a sample to be analyzed, wherein the epitope comprises an amino acid sequence of SEQ ID NO: 1; and detecting binding of the epitope to the sample, wherein, if the epitope and the sample exhibit a binding affinity ranging from about 1 pM to about 10 nM, the sample is a candidate c-Met antagonist.

[Claim 12] The method of claim 11, wherein the epitope consists of SEQ ID NO: 2 or 3.

[Claim 13] The method of claim 11 or 12, wherein the sample comprises an antibody.

[Claim 14] The method of claim 11, wherein the method is used to screen for candidate materials for diagnosing, preventing, or treating cancer.

[Claim 15] A kit for diagnosing cancer, the kit comprising the antibody or antigen binding fragment thereof according to any of claims 1-7.

[Claim 16] The kit of claim 15, wherein the cancer is lung cancer or ovarian cancer.

[Claim 17] A nucleic acid encoding the antibody or antigen binding fragment of any of claims 1-7, optionally in a vector.

[Claim 18] A cell comprising the nucleic acid of claim 17.

[Claim 19] A method of preparing an antibody or antigen binding fragment comprising expressing the nucleic acid of claim 17 in a cell.
**Fig. 1**

- RANDOM NUCLEOTIDE SEQUENCE
- TEMPLATE (huAbF46scFv)
- FRAGMENT PCR
- PCR FRAGMENT 1
- PCR FRAGMENT 2
- OVERLAP / EXTENSION PCR
- huAbF46scFv GENE INTRODUCED
  RANDOM NUCLEOTIDE SEQUENCE
[Fig. 3]