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(71) Applicant *(for all designated States except US)*: **GILEAD BIOLOGICS, INC.** [US/US]; 333 Lakeside Drive, Foster City, CA 94404 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **SMITH, Victoria** [AU/US]; 333 Lakeside Drive, Foster City, CA 94404 (US). **MCCAULEY, Scott** [US/US]; 333 Lakeside Drive, Foster City, CA 94404 (US).

(74) Agents: **MILLS, Army A.** et al.; Morrison & Foerster LLP, 12531 High Bluff Drive, Suite 100, San Diego, CA 92130-2040 (US).

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(54) Title: ANTIBODIES TO MATRIX METALLOPROTEINASE 9

FIGURE 1

Anti-MMP9 humanized heavy chains

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AB0041    QVQLRESGPG LVAPSQSLSI TCTVSGFSLL SYGVHWVRQP PGKGLEWLGV
VH1       QVQLQESGPG LVKPSETLSL TCTVSGFSLL SYGVHWVRQP PGKGLEWLGV
VH2       QVQLQESGPG LVKPSETLSL TCTVSGFSLL SYGVHWVRQP PGKGLEWLGV
VH3       QVQLQESGPG LVKPSETLSL TCTVSGFSLL SYGVHWVRQP PGKGLEWLGV
VH4       QVQLQESGPG LVKPSETLSL TCTVSGFSLL SYGVHWVRQP PGKGLEWLGV

AB0041    IWTGGTTNYN SALMSRLSIS KDDSKSQVFL KMNSLQTDDT AIYYCARYYY
VH1       IWTGGTTNYN SALMSRLTIS KDDSKSTVYL KMNSLKTEDT AIYYCARYYY
VH2       IWTGGTTNYN SALMSRLTIS KDDSKNTVYL KMNSLKTEDT AIYYCARYYY
VH3       IWTGGTTNYN SALMSRFTIS KDDSKNTVYL KMNSLKTEDT AIYYCARYYY
VH4       IWTGGTTNYN SALMSRFTIS KDDSKNTLYL KMNSLKTEDT AIYYCARYYY

AB0041    GMDYWGQGTS VTVSS  (SEQ ID NO:3)
VH1       GMDYWGQGTS VTVSS  (SEQ ID NO:5)
VH2       GMDYWGQGTL VTVSS  (SEQ ID NO:6)
VH3       GMDYWGQGTL VTVSS  (SEQ ID NO:7)
VH4       GMDYWGQGTL VTVSS  (SEQ ID NO:8)
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(57) Abstract: The present disclosure provides compositions and methods of use involving binding proteins, e.g., antibodies and antigen-binding fragments thereof, that bind to the matrix metalloproteinase-9 (MMP9) protein (MMP9 is also known as gelatinase-B), such as where the binding proteins comprise an immunoglobulin (Ig) heavy chain (or functional fragment thereof) and an Ig light chain (or functional fragment thereof).

ANTIBODIES TO MATRIX METALLOPROTEINASE 9

REFERENCE TO SEQUENCE LISTING SUBMITTED VIA EFS-WEB

[0001] The entire content of the following electronic submission of the sequence listing via the USPTO EFS-WEB server, as authorized and set forth in MPEP §1730 II.B.2(a)(C), is incorporated herein by reference in its entirety for all purposes. The sequence listing is identified on the electronically filed text file as follows:

File Name	Date of Creation	Size (bytes)
246102008540Seqlist	February 29, 2012	65,102 bytes

FIELD

[0002] This disclosure is in the field of extracellular enzymes, extracellular matrix enzymes, proteases and immunology.

BACKGROUND

[0003] Matrix metalloproteinases (MMPs) belong to a family of extracellular enzymes involved in forming and remodeling the extracellular matrix. These enzymes contain a conserved catalytic domain in which a zinc atom is coordinated by three histidine residues. Over 20 members of this family are known, organized into a number of groups including collagenases, gelatinases, stromelysins, matrilysins, enamelysins and membrane MMPs.

[0004] MMP2 and MMP9 belong to the gelatinase group of matrix metalloproteinases. Besides containing signal peptide, propeptide, catalytic, zinc-binding and heamopexin-like domains common to most MMPs, the gelatinases also contain a plurality of fibronectin-like domains and an O-glycosylated domain.

[0005] MMPs are associated with a number of diseases. However, available inhibitors of MMPs have been unsuccessful, in part due to toxicity and lack of efficacy. Therefore, there is a need for specific and effective MMP inhibitors.

SUMMARY

[0006] The present disclosure provides compositions and methods of use involving binding proteins, e.g., antibodies and antigen-binding fragments thereof, that bind to matrix metalloproteinase-9 (MMP9) protein (also known as gelatinase-B). The binding proteins typically are antibodies or fragments (e.g., antigen-binding fragments) thereof and typically contain an immunoglobulin (Ig) heavy chain (or functional fragment thereof) and an Ig light chain (or functional fragment thereof). The heavy chain is typically an IgG, typically a human

IgG, such as an IgG1 or IgG4, or other IgG such as an IgG2, or modified version thereof. The light chain typically is a kappa chain.

[0007] Among the MMP9 binding proteins, e.g., antibodies, are those that bind specifically to MMP9 and not to other matrix metalloproteinases. Such MMP9 binding proteins find use in applications in which it is necessary or desirable to obtain specific modulation (*e.g.*, inhibition) of MMP9, e.g., without directly affecting the activity of other matrix metalloproteinases. Thus, in certain embodiments of the present disclosure an anti-MMP9 antibody or fragment thereof is a specific inhibitor of the activity of MMP9. In some aspects, the MMP9 binding proteins disclosed herein will be useful for inhibition of MMP9 while allowing normal function of other, related matrix metalloproteinases.

[0008] The antibodies and fragments can be described with reference to their amino acid sequences or portions thereof, and/or various functions such as binding specificity to MMP9 or particular epitopes thereof or the ability to compete for binding to epitopes on MMP9 with particular antibodies, and/or activity, such as the ability to inhibit MMP9, e.g., non-competitively.

[0009] The antibodies and fragments include those having a heavy chain variable (VH) region having a heavy chain complementary determining region (CDR) with an amino acid sequence of SEQ ID NO: 13, SEQ ID NO: 14, or SEQ ID NO: 15; those having a light chain variable (VL) region having a light chain complementary determining region (CDR) with an amino acid sequence of SEQ ID NO: 16, SEQ ID NO: 17, or SEQ ID NO: 18. Exemplary antibodies and fragments include those having a heavy chain CDR1 with the amino acid sequence of SEQ ID NO: 13, a heavy chain CDR2 with the amino acid sequence of SEQ ID NO: 14, and a heavy chain CDR3 with the amino acid sequence of SEQ ID NO: 15, and those having a heavy chain CDR3 of SEQ ID NO: 15. Exemplary antibodies and fragments further include those with a light chain CDR1 with the amino acid sequence of SEQ ID NO: 16, a light chain CDR2 with the amino acid sequence of SEQ ID NO: 17, and a light chain CDR3 with the amino acid sequence of SEQ ID NO: 18, and those having a light chain CDR3 with the amino acid sequence of SEQ ID NO: 18, as well as those having heavy chain CDRs of SEQ ID NOs: 13, 14, and 15, and light chain CDRs of SEQ ID NOs: 16, 17, and 18.

[0010] Exemplary antibodies and fragments further include those having a heavy chain CDR1 with the amino acid sequence of SEQ ID NO: 34, a heavy chain CDR2 with the amino acid sequence of SEQ ID NO: 35, and a heavy chain CDR3 with the amino acid sequence of SEQ ID NO: 36, those with a heavy chain CDR3 with the amino acid sequence of SEQ ID NO: 36, those with a light chain CDR1 with the amino acid sequence of SEQ ID NO: 37, a light chain CDR2 with the amino acid sequence of SEQ ID NO: 38, and a light chain CDR3 with the

amino acid sequence of SEQ ID NO: 39, those with a light chain CDR3 with the amino acid sequence of SEQ ID NO: 39, as well as those having heavy chain CDRs of SEQ ID NOs: 34, 35, and 36, and light chain CDRs of SEQ ID NOs: 37, 38, and 39.

[0011] Exemplary antibodies and fragments further include those having a light chain CDR1 with the amino acid sequence of SEQ ID NO: 42, a light chain CDR2 with the amino acid sequence of SEQ ID NO: 43, and a light chain CDR3 with the amino acid sequence of SEQ ID NO: 44, and those with a light chain CDR3 with the amino acid sequence of SEQ ID NO: 44.

[0012] The antibodies and fragments further include those having a VH region with an amino acid sequence set forth in SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 8, and those having a VL region with an amino acid sequence set forth in SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, or SEQ ID NO: 12, as well as antibodies and fragments having a VH region with an amino acid sequence set forth in SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 8 and a VL region with an amino acid sequence set forth in SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, or SEQ ID NO: 12. In a particular example, the antibodies or fragments have a VH region of SEQ ID NO: 7 and a VL region of SEQ ID NO: 12, or at least at or about 75 %, 80 %, 85 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % or more sequence identity with such sequences. They further include those having a VH region with an amino acid sequence set forth in SEQ ID NO: 32 or 47, and those with a VL region with an amino acid sequence set forth in SEQ ID NO: 33 or in SEQ ID NO: 41 or in SEQ ID NO: 48, and combinations thereof, and sequence having at least at or about 75 %, 80 %, 85 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % or more sequence identity with such sequences.

[0013] The antibodies and fragments further include those having a VH region with an amino acid sequence set forth in SEQ ID NO: 1, and/or having a VL region with an amino acid sequence set forth in SEQ ID NO: 2, and/or a VH or VL region having at least at or about 75 %, 80 %, 85 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % or more sequence identity with such sequences.

[0014] In some cases, the heavy chain is encoded by a polynucleotide having a nucleotide sequence selected from the group consisting of SEQ ID NOs: 19-22 and the light chain is encoded by a polynucleotide having a nucleotide sequence selected from the group consisting of SEQ ID NOs: 23-26.

[0015] In some embodiments, the antibodies or fragments thereof inhibit the enzymatic activity of MMP9, such as by non-competitive inhibition.

[0016] The antibodies and fragments further include those that specifically bind to a given epitope of MMP9. In some cases, the epitope is an epitope specifically bound by any of the above-described antibodies. In one example, the epitope contains an amino acid residue (i.e., one or more amino acid residue(s)) outside of cysteine-switch active pocket of SEQ ID NO: 27. In certain examples, the epitope includes an amino acid residue (i.e., one or more amino acid residue(s)) within a given region of MMP9, for example, where the region is residues 104-202 of SEQ ID NO: 27. In some examples, the epitope includes an amino acid residue (i.e., one or more amino acid residue(s)) within a given region of MMP9, for example, where the region is residues 104-119, residues 159-166, or residues 191-202 of SEQ ID NO: 27. In one example, the epitope includes an amino acid residue (i.e., one or more amino acid residue) within a region of MMP9 that is residues 104-119 of SEQ ID NO: 27, an amino acid residue within a region of MMP9 that is residues 159-166 of SEQ ID NO: 27, and an amino acid residue within a region of MMP9 that is residues 191-202 of SEQ ID NO: 27. In some cases, the epitope includes E111, D113, R162, or I198 of SEQ ID NO: 27. In some cases, it includes R162 of SEQ ID NO: 27. In some cases, it includes E111, D113, R162, and I198 of SEQ ID NO: 27.

[0017] In some cases, the antibody or fragment is human or is humanized.

[0018] In some examples, the antibodies and fragments specifically bind to human MMP9 with a dissociation constant (K_d) equal to or lower than 100 nM, optionally lower than 10 nM, optionally lower than 1 nM, optionally lower than 0.5 nM, optionally lower than 0.1 nM, optionally lower than 0.01 nM, or optionally lower than 0.005 nM, in certain examples, between 0.1 and 0.2 nM, or between 0.1 and 10 pM, e.g., between 0.4 and 9 pm, such as between 0.4 and 8.8 pm, in the form of monoclonal antibody, scFv, Fab, or other form of antibody measured at a temperature of about 4°C, 25°C, 37°C or 42°C.

[0019] Also among the provided antibodies and fragments are those having at least at or about 75 %, 80 %, 85 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % or more sequence identity with any of the above-described antibodies or containing various portions with at least at or about 75 %, 80 %, 85 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % or more sequence identity with the respective portions of the antibodies described above, such as having a VH region with such identity with SEQ ID NO: 7 and a VL region with such identity with SEQ ID NO: 12. Also provided are antibodies that compete for binding to MMP9 with any of the above-described antibodies, such as those that compete for binding to MMP9 with an antibody having a VH region with the amino acid sequence set forth in SEQ ID NO: 7 and a VL region with the amino acid sequence set forth in SEQ ID NO: 12.

[0020] Also provided are isolated nucleic acids encoding the antibodies and fragments, such as nucleic acids including a coding sequence for any of the above-described antibodies and

fragments. Among the provided nucleic acids are those containing a nucleotide sequence encoding a heavy chain polypeptide comprising CDRs with the amino acid sequences set forth in SEQ ID NOs: 13-15, and/or a light chain polypeptide comprising CDRs with the amino acid sequences set forth in SEQ ID NOs: 16-18. In one example, the nucleotide sequence encodes the heavy chain polypeptide, which has an amino acid sequence selected from the group consisting of SEQ ID NOS: 1, 3, and 5-8. In another example, the nucleotide sequence encodes the light chain polypeptide, which has an amino acid sequence selected from the group consisting of SEQ ID NOS: 2, 4, and 9-12. In one example, the nucleotide sequence includes a sequence selected from the group consisting of SEQ ID NOs: 19-26, such as SEQ ID NO: 21, SEQ ID NO: 26, or SEQ ID NOs: 21 and 26. Also provided are vectors containing such nucleic acids and cells including the same, such as host cells.

[0021] Also provided are pharmaceutical compositions including the antibodies, fragments, nucleic acids, vectors, and cells. In some examples, the pharmaceutical compositions further include a carrier or excipient, such as a pharmaceutically acceptable or biologically acceptable carrier or excipient. In some cases, the pharmaceutical compositions are used in the provided therapeutic methods and uses.

[0022] Also provided are methods and uses of the antibodies, fragments, nucleic acids, vectors, cells, and compositions, for example in therapeutics, such as inhibiting MMP9 in a subject, and diagnostics, such as for detecting MMP9 in the subject.

[0023] For example, provided are diagnostic and prognostic methods involving detection of MMP9, and agents (such as any of the above-described anti-MMP9 antibodies and other MMP9 binding proteins) for use in such methods. In some cases, the diagnostic method detects MMP9 expression in a test sample from a subject. Such methods can be carried out, for example, by contacting the test sample with an antibody or fragment as described herein (such as any of the above-described antibodies or fragments) and detecting binding of the antibody or fragment to protein in the sample, thereby detecting the presence of MMP9. In some cases, a sample is first obtained or provided. In some examples, the methods include comparing the amount or level of MMP9 detected to a control level or amount, such as by comparing the amount of binding detected in the test sample with an amount of binding of the antibody or fragment to a control sample. In some cases, the methods involve simply comparing a test level and a control level of MMP9. In some cases, a higher test level (as compared to the control level) is indicative of the disease or condition.

[0024] In some cases, the MMP9 detected by the method indicates the presence of a disease or condition in the subject, such as an MMP9-associated disease or condition. In some cases, the methods further include treating the subject or adjusting (i.e., altering or discontinuing)

treatment of the subject based on the results of the method, e.g., based on the levels of MMP9 detected in the sample. Among the biological samples are tissue, cells isolated from such tissues, and the like. In some cases, the methods are performed on liquid samples, such as blood, plasma, serum, whole blood, saliva, urine, or semen. Tissue samples include, for example, formalin-fixed or frozen tissue sections.

[0025] Also provided are methods of inhibiting MMP9 activity in a subject and/or treating a disease or condition in the subject, for example, using an agent that non-competitively inhibits MMP9, and agents (such as any of the above-described anti-MMP9 antibodies and other MMP9 binding proteins) for use in such methods. The methods generally are carried out by administering to the subject an MMP9 binding protein, such as an MMP9-binding antibody or fragment thereof as provided herein, e.g., in an effective amount. The antibody or fragment generally specifically binds to and non-competitively inhibits MMP9, for example, such that MMP9 activity is inhibited in the subject. In some cases, the antibody or fragment is one that binds MMP9 outside of the cysteine-switch active pocket, such as in one of the epitopes described above. In some cases, the antibody or fragment does not substantially bind to an MMP protein other than MMP9 and/or does not substantially bind to MMP2.

[0026] The subject generally is one with a disease or condition, typically one associated with increased or decreased MMP9 expression and/or activity. In certain cases, the subject with a disease or condition associated with increased MMP9 expression and/or activity. In other cases, the subject with a disease or condition associated with decreased MMP9 expression and/or activity.

[0027] Also provided are MMP9 polypeptides, including mutant MMP9 polypeptides, such as those containing residues 111-198 of SEQ ID NO: 27, and those having an amino acid sequence containing residues 111-198 of SEQ ID NO: 27 with an amino acid substitution at residue 111, 113, 162, or 198 of SEQ ID NO 27, or with an amino acid substitution at all such residues.

[0028] Also provided are uses of any of the above-described antibodies, nucleic acids, vectors, cells, and compositions, in the therapeutic and diagnostic methods described above.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] **Figure 1** shows the amino acid sequence of the heavy chain variable region of a mouse monoclonal anti-MMP9 antibody (AB0041), along with the amino acid sequences of humanized variants of heavy chain (VH1-VH4), aligned to show differences in framework amino acid sequence resulting from humanization. CDRs are shown in italics, and amino acids

that are different in the humanized variants, compared to the parent mouse monoclonal, are underlined.

[0030] **Figure 2** shows the amino acid sequence of the light chain variable region of a mouse monoclonal anti-MMP9 antibody (AB0041), along with the amino acid sequences of humanized variants of this light chain (VH1-VH4), aligned to show differences in framework amino acid sequence resulting from humanization. CDRs are shown in italics, and amino acids that are different in the humanized variants, compared to the parent mouse monoclonal, are underlined.

[0031] **Figure 3** shows a schematic diagram of the MMP9 protein.

[0032] **Figure 4** shows a comparison between the amino acid sequences of the heavy and light chains of antibodies designated AB0041, M4, and M12.

DETAILED DESCRIPTION

[0033] Practice of the present disclosure employs, unless otherwise indicated, standard methods and conventional techniques in the fields of cell biology, toxicology, molecular biology, biochemistry, cell culture, immunology, oncology, recombinant DNA and related fields as are within the skill of the art. Such techniques are described in the literature and thereby available to those of skill in the art. See, for example, Alberts, B. *et al.*, "Molecular Biology of the Cell," 5th edition, Garland Science, New York, NY, 2008; Voet, D. *et al.* "Fundamentals of Biochemistry: Life at the Molecular Level," 3rd edition, John Wiley & Sons, Hoboken, NJ, 2008; Sambrook, J. *et al.*, "Molecular Cloning: A Laboratory Manual," 3rd edition, Cold Spring Harbor Laboratory Press, 2001; Ausubel, F. *et al.*, "Current Protocols in Molecular Biology," John Wiley & Sons, New York, 1987 and periodic updates; Freshney, R.I., "Culture of Animal Cells: A Manual of Basic Technique," 4th edition, John Wiley & Sons, Somerset, NJ, 2000; and the series "Methods in Enzymology," Academic Press, San Diego, CA. See also, for example, "Current Protocols in Immunology," (R. Coico, series editor), Wiley, last updated August 2010.

[0034] Certain MMPs play roles in tumor growth, metastasis, inflammation, autoimmunity, and vascular disease. *See*, for example, Hu *et al.* (2007) *Nature Reviews: Drug Discovery* **6:**480-498. Thus, it is desirable to inhibit the activity of one or more particular MMPs in certain therapeutic settings. While sharing significant homology at a sequence level, the expression and functional roles of the two gelatinases MMP9 and MMP2 vary significantly. MMP9 expression is induced by a number of disease associated cytokines and growth factors. Also, the MMP9 knockout mouse is protected in a variety of disease models, whereas MMP2 is more constitutively expressed and the MMP2 knockout animals tend toward little protection. Some studies have shown that MMP2 knockout mouse exhibited worse disease in challenge models. For some diseases or disorders, the activity of more than one MMPs is inhibited. In clinical

studies, the inhibitors to more than one MMPs have caused adverse effects, such as toxicity or lack of efficacy, that are not desired. It has been shown that the activity of certain MMPs, e.g., MMP2, is often required for normal tissue homeostasis and/or is protective against disease. Certain available MMP inhibitors have caused side effects.

[0035] Among the provided embodiments are agents, including therapeutic reagents, such as antibodies and antigen-binding fragments thereof, that specifically inhibit the catalytic activity of a single MMP or a select plurality of MMPs, such as MMP9 and that do not react with or inhibit certain other MMPs or any other MMPs. Also among the provided embodiments are methods and uses of the same for treatment of various diseases.

MMP9 Binding Proteins

[0036] The present disclosure provides binding proteins, e.g., antibodies and fragments (e.g., antigen-binding fragments) thereof, that bind to the matrix metalloproteinase-9 (MMP9) protein (MMP9 is also known as gelatinase-B), e.g., human MMP9, such as the human MMP9 having an amino acid sequence set forth in SEQ ID NO: 27 or SEQ ID NO: 28. The binding proteins of the present disclosure generally comprise an immunoglobulin (Ig) heavy chain (or functional fragment thereof) and an Ig light chain (or functional fragment thereof).

[0037] The disclosure further provides MMP9 binding proteins that bind specifically to MMP9 and not to other matrix metalloproteinases such as MMP1, MMP2, MMP3, MMP7, MMP9, MMP10, MMP12, and MMP13. Such specific MMP9 binding proteins are thus generally not significantly or detectably crossreactive with non-MMP9 matrix metalloproteinases. MMP9 binding proteins that specifically bind MMP9 find use in applications in which it is necessary or desirable to obtain specific modulation (*e.g.*, inhibition) of MMP9, e.g., without directly affecting the activity of other matrix metalloproteinases.

[0038] In certain embodiments of the present disclosure, an anti-MMP9 antibody is an inhibitor of the activity of MMP9, and can be a specific inhibitor of MMP9. In one embodiment, the MMP9 binding proteins disclosed herein is useful for inhibition of MMP9 while not affecting other matrix metalloproteinases. "An inhibitor of MMP" or "inhibitor of MMP9 activity" can be an antibody or an antigen binding fragment thereof that directly or indirectly inhibits activity of MMP9, including but not limited to enzymatic processing, inhibiting action of MMP9 on it substrate (e.g., by inhibiting substrate binding, substrate cleavage, and the like), and the like.

[0039] The present disclosure also provides MMP9 binding proteins that specifically bind to non-mouse MMP9, such as human MMP9, Cynomolgus monkey MMP9, and rat MMP9.

[0040] The present disclosure also provides MMP9 binding proteins (e.g., anti-MMP9 antibodies and functional fragments thereof) that act as non-competitive inhibitors. A "non-competitive inhibitor" refers to an inhibitor binds at site away from substrate binding site of an enzyme, and thus can bind the enzyme and effect inhibitory activity regardless of whether or not the enzyme is bound to its substrate. The non-competitive or allosteric inhibition is generally independent of substrate association or concentration. Such non-competitive inhibitors can, for example, provide for a level of inhibition that can be substantially independent of substrate concentration.

[0041] MMP9 binding proteins (e.g., antibodies and functional fragments thereof) of the present disclosure include those that bind MMP9, particularly human MMP9, and having a heavy chain polypeptide (or functional fragment thereof) that has at least about 80%, 85%, 90%, 95% or more amino acid sequence identity to a heavy chain polypeptide disclosed herein.

[0042] MMP9 binding proteins (e.g., antibodies and functional fragments thereof) of the present disclosure include those that bind MMP9, particularly human MMP9, and having a light polypeptide (or functional fragment thereof) that has at least about 80%, 85%, 90%, 95% or more amino acid sequence identity to a heavy chain polypeptide disclosed herein.

[0043] MMP9 binding proteins (e.g., antibodies and functional fragments thereof) of the present disclosure include those that bind MMP9, particularly human MMP9, and have a heavy chain polypeptide (or functional fragment thereof) having the complementarity determining regions ("CDRs") of heavy chain polypeptide and the CDRs of a light chain polypeptide (or functional fragment thereof) as disclosed herein.

[0044] "Homology" or "identity" or "similarity" as used herein in the context of nucleic acids and polypeptides refers to the relationship between two polypeptides or two nucleic acid molecules based on an alignment of the amino acid sequences or nucleic acid sequences, respectively. Homology and identity can each be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When an equivalent position in the compared sequences is occupied by the same base or amino acid, then the molecules are identical at that position; when the equivalent site occupied by the same or a similar amino acid residue (e.g., similar in steric and/or electronic nature), then the molecules can be referred to as homologous (similar) at that position. Expression as a percentage of homology/similarity or identity refers to a function of the number of identical or similar amino acids at positions shared by the compared sequences. In comparing two sequences, the absence of residues (amino acids or nucleic acids) or presence of extra residues also decreases the identity and homology/similarity.

[0045] As used herein, "identity" means the percentage of identical nucleotide or amino acid residues at corresponding positions in two or more sequences when the sequences are aligned to maximize sequence matching, i.e., taking into account gaps and insertions. Sequences are generally aligned for maximum correspondence over a designated region, e.g., a region at least about 20, 25, 30, 35, 40, 45, 50, 55, 60, 65 or more amino acids or nucleotides in length, and can be up to the full-length of the reference amino acid or nucleotide. For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer program, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

[0046] Examples of algorithms that are suitable for determining percent sequence identity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al. (1990) J. Mol. Biol. 215: 403-410 and Altschul et al. (1977) Nucleic Acids Res. 25: 3389-3402, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov). Further exemplary algorithms include ClustalW (Higgins D., et al. (1994) Nucleic Acids Res 22: 4673-4680), available at www.ebi.ac.uk/Tools/clustalw/index.html.

[0047] Residue positions which are not identical can differ by conservative amino acid substitutions. Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine.

[0048] Sequence identity between two nucleic acids can also be described in terms of hybridization of two molecules to each other under stringent conditions. The hybridization conditions are selected following standard methods in the art (see, for example, Sambrook, et al., Molecular Cloning: A Laboratory Manual, Second Edition, (1989) Cold Spring Harbor, N.Y.). An example of stringent hybridization conditions is hybridization at 50°C or higher and 0.1 × SSC (15 mM sodium chloride/1.5 mM sodium citrate). Another example of stringent hybridization conditions is overnight incubation at 42 °C in a solution: 50 % formamide, 5 ×

SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5 × Denhardt's solution, 10% dextran sulfate, and 20 mg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1 × SSC at about 65 °C. Stringent hybridization conditions are hybridization conditions that are at least as stringent as the above representative conditions, where conditions are considered to be at least as stringent if they are at least about 80% as stringent, typically at least 90% as stringent as the above specific stringent conditions.

[0049] Accordingly, the present disclosure provides, for example, antibodies or antigen binding fragments thereof, comprising a heavy chain variable region polypeptide having at least 80%, 85%, 90%, 95%, or greater amino acid sequence identity to an amino acid sequence of a heavy chain variable region described herein (e.g., SEQ ID NOS:1 or 5-8), and a variable light chain polypeptide having at least 80%, 85%, 90%, 95%, or greater amino acid sequence identity to an amino acid sequence of a light chain polypeptide as set forth herein (e.g., SEQ ID NOS:2 or 9-12).

[0050] Examples of anti-MMP9 antibodies of the present disclosure are described in more detail below.

Antibodies

[0051] The MMP9 binding proteins include antibodies and functional fragments thereof, such as those that specifically bind to MMP9. As used herein, the term "antibody" means an isolated or recombinant polypeptide binding agent that comprises peptide sequences (*e.g.*, variable region sequences) that specifically bind an antigenic epitope. The term is used in its broadest sense and specifically covers monoclonal antibodies (including full-length monoclonal antibodies), polyclonal antibodies, human antibodies, humanized antibodies, chimeric antibodies, nanobodies, diabodies, multispecific antibodies (*e.g.*, bispecific antibodies), and antibody fragments including but not limited to Fv, scFv, Fab, Fab' $F(ab')_2$ and Fab_2, so long as they exhibit the desired biological activity. The term "human antibody" refers to antibodies containing sequences of human origin, except for possible non-human CDR regions, and does not imply that the full structure of an immunoglobulin molecule be present, only that the antibody has minimal immunogenic effect in a human (*i.e.*, does not induce the production of antibodies to itself).

[0052] An "antibody fragment" comprises a portion of a full-length antibody, for example, the antigen binding or variable region of a full-length antibody. Such antibody fragments may also be referred to herein as "functional fragments: or "antigen-binding fragments". Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies (Zapata *et al.* (1995) *Protein Eng.* **8(10):**1057-1062); single-chain antibody molecules; and

multispecific antibodies formed from antibody fragments. Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen combining sites and is still capable of cross-linking antigen.

[0053] "Fv" is a minimum antibody fragment containing a complete antigen-recognition and -binding site. This region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the three complementarity-determining regions (CDRs) of each variable domain interact to define an antigen-binding site on the surface of the V_H-V_L dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or an isolated V_H or V_L region comprising only three of the six CDRs specific for an antigen) has the ability to recognize and bind antigen, although generally at a lower affinity than does the entire F_v fragment.

[0054] The "F_{ab}" fragment also contains, in addition to heavy and light chain variable regions, the constant domain of the light chain and the first constant domain (CH_1) of the heavy chain. Fab fragments were originally observed following papain digestion of an antibody. Fab' fragments differ from Fab fragments in that F(ab') fragments contain several additional residues at the carboxy terminus of the heavy chain CH_1 domain, including one or more cysteines from the antibody hinge region. $F(ab')_2$ fragments contain two Fab fragments joined, near the hinge region, by disulfide bonds, and were originally observed following pepsin digestion of an antibody. Fab'-SH is the designation herein for Fab' fragments in which the cysteine residue(s) of the constant domains bear a free thiol group. Other chemical couplings of antibody fragments are also known.

[0055] The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to five major classes: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2.

[0056] "Single-chain Fv" or "sFv" or "scFv" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. In some embodiments, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains, which enables the sFv to form the desired structure for antigen binding. For a review of sFv, see Pluckthun, in *The Pharmacology of Monoclonal Antibodies,* vol. 113 (Rosenburg and Moore eds.) Springer-Verlag, New York, pp. 269-315 (1994).

[0057] The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) in the same polypeptide chain (V_H-V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain, thereby creating two antigen-binding sites. Diabodies are additionally described, for example, in EP 404,097; WO 93/11161 and Hollinger *et al.* (1993) *Proc. Natl. Acad. Sci. USA* **90:**6444-6448.

[0058] An "isolated" antibody is one that has been identified and separated and/or recovered from a component of its natural environment. Components of its natural environment may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In some embodiments, an isolated antibody is purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, for example, more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence, *e.g.*, by use of a spinning cup sequenator, or (3) to homogeneity by gel electrophoresis (*e.g.*, SDS-PAGE) under reducing or nonreducing conditions, with detection by Coomassie blue or silver stain. The term "isolated antibody" includes an antibody *in situ* within recombinant cells, since at least one component of the antibody's natural environment will not be present. In certain embodiments, isolated antibody is prepared by at least one purification step.

[0059] As used herein, “immunoreactive” refers to antibodies or fragments thereof that are specific to a sequence of amino acid residues (“binding site” or “epitope”), yet if are cross-reactive to other peptides/proteins, are not toxic at the levels at which they are formulated for administration to human use. "Epitope" refers to that portion of an antigen capable of forming a binding interaction with an antibody or antigen binding fragment thereof. An epitope can be a linear peptide sequence (i.e., “continuous”) or can be composed of noncontiguous amino acid sequences (i.e., “conformational” or “discontinuous”). The term “preferentially binds” means that the binding agent binds to the binding site with greater affinity than it binds unrelated amino acid sequences.

[0060] Anti-MMP9 antibodies can be described in terms of the CDRs of the heavy and light chains. As used herein, the term "CDR" or "complementarity determining region" is intended to mean the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. These particular regions have been described by Kabat et al., J. Biol. Chem. 252:6609-6616 (1977); Kabat et al., U.S. Dept. of Health and Human Services, "Sequences of proteins of immunological interest" (1991); by Chothia et al., J. Mol. Biol. 196:901-917 (1987); and MacCallum et al., J. Mol. Biol. 262:732-745 (1996), where the definitions include overlapping or subsets of amino acid residues when compared against each

other. Nevertheless, application of either definition to refer to a CDR of an antibody or grafted antibodies or variants thereof is intended to be within the scope of the term as defined and used herein. The amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth below in Table 1 as a comparison.

Table 1: CDR Definitions

	Kabat[1]	Chothia[2]	MacCallum[3]
V_H CDR1	31-35	26-32	30-35
V_H CDR2	50-65	53-55	47-58
V_H CDR3	95-102	96-101	93-101
V_L CDR1	24-34	26-32	30-36
V_L CDR2	50-56	50-52	46-55
V_L CDR3	89-97	91-96	89-96

[1]Residue numbering follows the nomenclature of Kabat et al., *supra*

[2]Residue numbering follows the nomenclature of Chothia et al., *supra*

[3]Residue numbering follows the nomenclature of MacCallum et al., *supra*

[0061] As used herein, the term "framework" when used in reference to an antibody variable region is intended to mean all amino acid residues outside the CDR regions within the variable region of an antibody. A variable region framework is generally a discontinuous amino acid sequence between about 100-120 amino acids in length but is intended to reference only those amino acids outside of the CDRs. As used herein, the term "framework region" is intended to mean each domain of the framework that is separated by the CDRs.

[0062] In some embodiments, an antibody is a humanized antibody or a human antibody. Humanized antibodies include human immununoglobulins (recipient antibody) in which residues from a complementary-determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. Thus, humanized forms of non-human (*e.g.*, murine) antibodies are chimeric immunoglobulins which contain minimal sequence derived from non-human immunoglobulin. The non-human sequences are located primarily in the variable regions, particularly in the complementarity-determining regions (CDRs). In some embodiments, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies can also comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences. In certain embodiments, a humanized antibody comprises substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDRs correspond to those of a non-human immunoglobulin and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. For the purposes of the present disclosure,

humanized antibodies can also include immunoglobulin fragments, such as Fv, Fab, Fab', $F(ab')_2$ or other antigen-binding subsequences of antibodies.

[0063] The humanized antibody can also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. See, for example, Jones *et al.* (1986) *Nature* **321:**522-525; Riechmann *et al.* (1988) Nature **332:**323-329; and Presta (1992) *Curr. Op. Struct. Biol.* **2:**593-596.

[0064] Methods for humanizing non-human antibodies are known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are often referred to as "import" or "donor" residues, which are typically obtained from an "import" or "donor" variable domain. For example, humanization can be performed essentially according to the method of Winter and co-workers , by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. See, for example, Jones *et al.*, *supra*; Riechmann *et al., supra* and Verhoeyen *et al.* (1988) *Science* **239:**1534-1536. Accordingly, such "humanized" antibodies include chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In certain embodiments, humanized antibodies are human antibodies in which some CDR residues and optionally some framework region residues are substituted by residues from analogous sites in rodent antibodies (*e.g.*, murine monoclonal antibodies).

[0065] Human antibodies can also be produced, for example, by using phage display libraries. Hoogenboom *et al.* (1991) *J. Mol. Biol,* **227:**381; Marks *et al.* (1991) *J. Mol. Biol.* **222:**581. Other methods for preparing human monoclonal antibodies are described by Cole *et al.* (1985) "Monoclonal Antibodies and Cancer Therapy," Alan R. Liss, p. 77 and Boerner *et al.* (1991) *J. Immunol.* **147:**86-95.

[0066] Human antibodies can be made by introducing human immunoglobulin loci into transgenic animals (*e.g.*, mice) in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon immunological challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks *et al.* (1992) *Bio/Technology* **10:**779-783 (1992); Lonberg *et al.* (1994) *Nature* **368:** 856-859; Morrison (1994) *Nature* **368:**812-813; Fishwald *et al.* (1996) *Nature Biotechnology* **14:**845-851; Neuberger (1996) *Nature Biotechnology* **14:**826; and Lonberg *et al.* (1995) *Intern. Rev. Immunol.* **13:**65-93.

[0067] Antibodies can be affinity matured using known selection and/or mutagenesis methods as described above. In some embodiments, affinity matured antibodies have an affinity which is five times or more, ten times or more, twenty times or more, or thirty times or more than that of the starting antibody (generally murine, rabbit, chicken, humanized or human) from which the matured antibody is prepared.

[0068] An antibody can also be a bispecific antibody. Bispecific antibodies are monoclonal, and may be human or humanized antibodies that have binding specificities for at least two different antigens. In the present case, the two different binding specificities can be directed to two different MMPs, or to two different epitopes on a single MMP (*e.g.*, MMP9).

[0069] An antibody as disclosed herein can also be an immunoconjugate. Such immunoconjugates comprise an antibody (*e.g.*, to MMP9) conjugated to a second molecule, such as a reporter An immunoconjugate can also comprise an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, a toxin (*e.g.*, an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (*i.e.*, a radioconjugate).

[0070] An antibody that "specifically binds to" or is "specific for" a particular polypeptide or an epitope refers to the selective binding of the antibody to the target antigen or epitope; these terms, and methods for determining specific binding, are well understood in the art. An antibody exhibits "specific binding" for a particular target antigen or epitope if it binds with greater affinity, avidity, more readily, and/or with greater duration to that target antigen or epitope than it does with other substances. In some embodiments, the antibody that specifically binds to the polypeptide or epitope is one that that binds to that particular polypeptide or epitope without substantially binding to any other polypeptide or polypeptide epitope.

[0071] In some embodiments, the provided antibodies specifically bind to human MMP9 with a dissociation constant (K_d) equal to or lower than 100 nM, optionally lower than 10 nM, optionally lower than 1 nM, optionally lower than 0.5 nM, optionally lower than 0.1 nM, optionally lower than 0.01 nM, or optionally lower than 0.005 nM, in certain examples, between 0.1 and 0.2 nM, or between 0.1 and 10 pM, e.g., between 0.4 and 9 pm, such as between 0.4 and 8.8 pm, in the form of monoclonal antibody, scFv, Fab, or other form of antibody measured at a temperature of about 4°C, 25°C, 37°C or 42°C.

[0072] In certain embodiments, an antibody of the present disclosure binds to one or more processing sites (*e.g.*, sites of proteolytic cleavage) in MMP9, thereby effectively blocking processing of the proenzyme or preproenzyme to the catalytically active enzyme, and thus reducing the proteolytic activity of the MMP9.

[0073] In certain embodiments, an antibody according to the present disclosure binds to MMP9 with an affinity at least 2 times, at least 5 times, at least 10 times, at least 25 times, at least 50 times, at least 100 times, at least 500 times, or at least 1000 times greater than its binding affinity for another MMP. Binding affinity can be measured by any method known in the art and can be expressed as, for example, on-rate, off-rate, dissociation constant (K_d), equilibrium constant (K_{eq}) or any term in the art.

[0074] In certain embodiments, an antibody according to the present disclosure is one that inhibits the enzymatic (i.e., catalytic) activity of MMP9, such as a non-competitive inhibitor of the catalytic activity of MMP9. In certain embodiments, an antibody according to the present disclosure binds within the catalytic domain of MMP9. In additional embodiments, an antibody according to the present disclosure binds outside the catalytic domain of MMP9.

[0075] Also provided are antibodies or antigen binding fragments thereof that compete with any one or more of the anti-MMP9 antibodies or antigen binding fragments thereof described herein for binding to MMP9. Thus, the present disclosure contemplates anti-MMP9 antibodies, and functional fragments thereof, that compete for binding with, for example, an antibody having a heavy chain polypeptide of any of SEQ ID NOS: 1 or 5-8, a light chain polypeptide of SEQ ID NOS: 2 or 9-12, or combinations thereof. In one embodiment, the anti-MMP9 antibody, or functional fragment thereof, competes for binding to human MMP9 with the antibody described herein as AB0041.

Epitope Binding

[0076] Also provided are antibodies and fragments thereof that bind to the same epitope, e.g., MMP9 epitope as any one or more of the antibodies described herein. Also provided are antibodies and fragments that specifically bind to an epitope of MMP9, where the epitope includes an amino acid residue within a particular region of MMP9 or multiple regions of MMP9. Such regions can include, for example, structural loops and/or other structural domains of MMP9, such as those shown to be important for binding to exemplary antibodies described herein. Typically, the regions are defined according to amino acid residue positions on the full-length MMP9 sequence, e.g., SEQ ID NO: 27. In some examples, the epitope is outside of cysteine-switch active pocket of SEQ ID NO: 27. In some example, the epitope contains an amino acid residue (i.e., one or more amino acid residue(s)) within a region that is residues 104-202 of SEQ ID NO: 27. In one example, the epitope contains an amino acid residue (i.e., one or more amino acid residue(s)) within a region that is residues 104-119, residues 159-166, or residues 191-202 of SEQ ID NO: 27. In some aspects, the epitope includes an amino acid residue (i.e., one or more amino acid residue(s)) within a region of MMP9 that is residues 104-

119 of SEQ ID NO: 27, an an amino acid residue within a region of MMP9 that is residues 159-166 of SEQ ID NO: 27, and an amino acid residue within a region of MMP9 that is residues 191-202 of SEQ ID NO: 27. In some cases, the epitope includes E111, D113, R162, or I198 of SEQ ID NO: 27. In some cases, it includes R162 of SEQ ID NO: 27. In some cases, it includes E111, D113, R162, and I198 of SEQ ID NO: 27.

MMP9 sequence

[0077] The amino acid sequence of human MMP9 protein is as follows:

```
MSLWQPLVLV LLVLGCCFAA PRQRQSTLVL FPGDLRTNLT DRQLAEEYLY  50
RYGYTRVAEM RGESKSLGPA LLLLQKQLSL PETGELDSAT LKAMRTPRCG  100
VPDLGRFQTF EGDLKWHHHN ITYWIQNYSE DLPRAVIDDA FARAFALWSA  150
VTPLTFTRVY SRDADIVIQF GVAEHGDGYP FDGKDGLLAH AFPPGPGIQG  200
DAHFDDDELW SLGKGVVVPT RFGNADGAAC HFPFIFEGRS YSACTTDGRS  250
DGLPWCSTTA NYDTDDRFGF CPSERLYTRD GNADGKPCQF PFIFQGQSYS  300
ACTTDGRSDG YRWCATTANY DRDKLFGFCP TRADSTVMGG NSAGELCVFP  350
FTFLGKEYST CTSEGRGDGR LWCATTSNFD SDKKWGFCPD QGYSLFLVAA  400
HEFGHALGLD HSSVPEALMY PMYRFTEGPP LHKDDVNGIR HLYGPRPEPE  450
PRPPTTTTPQ PTAPPTVCPT GPPTVHPSER PTAGPTGPPS AGPTGPPTAG  500
PSTATTVPLS PVDDACNVNI FDAIAEIGNQ LYLFKDGKYW RFSEGRGSRP  550
QGPFLIADKW PALPRKLDSV FEEPLSKKLF FFSGRQVWVY TGASVLGPRR  600
LDKLGLGADV AQVTGALRSG RGKMLLFSGR RLWRFDVKAQ MVDPRSASEV  650
DRMFPGVPLD THDVFQYREK AYFCQDRFYW RVSSRSELNQ VDQVGYVTYD  700
ILQCPED  (SEQ ID NO:27)
```

[0078] Protein domains are shown schematically in Figure 3 and are indicated below:

Amino Acid #	Feature
1-19	Signal Peptide
38-98	Peptidoglycan Binding Domain
R98/C99	Cysteine-switch active pocket
112-445	Zn dependent metalloproteinase domain
223-271	Fibronectin type II domain (gelatin binding domain)
281-329	Fibronectin type II domain (gelatin binding domain)
340-388	Fibronectin type II domain (gelatin binding domain)
400-411	Zn binding region
521-565	Hemopexin-like domain
567-608	Hemopexin-like domain
613-659	Hemopexin-like domain
661-704	Hemopexin-like domain

[0079] The amino acid sequence of mature full-length human MMP9 (which is the amino acid sequence of the propolypeptide of SEQ ID NO:27 without the signal peptide) is:

APRQRQSTLVL FPGDLRTNLT DRQLAEEYLY RYGYTRVAEM RGESKSLGPA
LLLLQKQLSL PETGELDSAT LKAMRTPRCG VPDLGRFQTF EGDLKWHHHN
ITYWIQNYSE DLPRAVIDDA FARAFALWSA VTPLTFTRVY SRDADIVIQF
GVAEHGDGYP FDGKDGLLAH AFPPGPGIQG DAHFDDDELW SLGKGVVVPT
RFGNADGAAC HFPFIFEGRS YSACTTDGRS DGLPWCSTTA NYDTDDRFGF
CPSERLYTRD GNADGKPCQF PFIFQGQSYS ACTTDGRSDG YRWCATTANY
DRDKLFGFCP TRADSTVMGG NSAGELCVFP FTFLGKEYST CTSEGRGDGR
LWCATTSNFD SDKKWGFCPD QGYSLFLVAA HEFGHALGLD HSSVPEALMY
PMYRFTEGPP LHKDDVNGIR HLYGPRPEPE PRPPTTTTPQ PTAPPTVCPT
GPPTVHPSER PTAGPTGPPS AGPTGPPTAG PSTATTVPLS PVDDACNVNI
FDAIAEIGNQ LYLFKDGKYW RFSEGRGSRP QGPFLIADKW PALPRKLDSV
FEEPLSKKLF FFSGRQVWVY TGASVLGPRR LDKLGLGADV AQVTGALRSG
RGKMLLFSGR RLWRFDVKAQ MVDPRSASEV DRMFPGVPLD THDVFQYREK
AYFCQDRFYW RVSSRSELNQ VDQVGYVTYD ILQCPED (SEQ ID NO:28)

[0080] The amino acid sequence of the signal peptide is MSLWQPLVLV LLVLGCCFA (SEQ ID NO:29).

[0081] Also provided are MMP9 polypeptides, including mutant MMP9 polypeptides. Such peptides are useful, for example, in generating and selecting antibodies and fragments as provided herein. Exemplary polypeptides include those having an amino acid sequence containing residues 104-202 of SEQ ID NO: 27, and those having an amino acid sequence of SEQ ID NO: 27 with an amino acid substitution at residue 111, 113, 162, or 198 of SEQ ID NO 27 or with an amino acid substitution at all such residues. Other exemplary polypeptides include those having an amino acid sequence containing residues 111-198 of SEQ ID NO: 27, and those having an amino acid sequence containing residues 111-198 of SEQ ID NO: 27 with an amino acid substitution at residue 111, 113, 162, or 198 of SEQ ID NO 27 or with an amino acid substitution at all such residues. Such polypeptides find use, for example, in selecting antibodies that bind to epitopes containing such residues and/or for which such residues of MMP9 are important for binding, such as those described herein.

[0082] The present disclosure contemplates MMP9 binding proteins that bind any portion of MMP9, e.g., human MMP9, with MMP9 binding proteins that preferentially bind MMP9 relative to other MMPs being of particular interest.

[0083] Anti-MMP9 antibodies, and functional fragments thereof, can be generated accordingly to methods well known in the art. Exemplary anti-MMP9 antibodies are provided below.

Mouse monoclonal anti-MMP9 antibodies

[0084] A mouse monoclonal antibody to human MMP9 was obtained as described in Example 1. This antibody contains a mouse IgG2b heavy chain and a mouse kappa light chain, and is denoted AB0041.

[0085] The amino acid sequence of the AB0041 heavy chain is as follows:

MAVLVLFLCLVAFPSCVLSQVQLKESGPGLVAPSQSLSITCTVSGFSLLSY
GVHWVRQPPGKGLEWLGVIWTGGTTNYNSALMSRLSISKDDSKSQVFLK
MNSLQTDDTAIYYCARYYYGMDYWGQGTSVTVSS*AKTTPPSVYPLAPGC*
GDTTGSSVTLGCLVKGYFPESVTVTWNSGSLSSSVHTFPALLQSGLYTMSSSVT
VPSSTWPSQTVTCSVAHPASSTTVDKKLEPSGPISTINPCPPCKECHKCPAPNL
EGGPSVFIFPPNIKDVLMISLTPKVTCVVVDVSEDDPDVRISWFVNNVEVHTA
QTQTHREDYNSTIRVVSALPIQHQDWMSGKEFKCKVNNKDLPSPIERTISKIK
GLVRAPQVYILPPPAEQLSRKDVSLTCLVVGFNPGDISVEWTSNGHTEENYKD
TAPVLDSDGSYFIYSKLDIKTSKWEKTDSFSCNVRHEGLKNYYLKKTISRSPGK
(SEQ ID NO:1)

[0086] The signal sequence is underlined, and the sequence of the IgG2b constant region is presented italics.

[0087] The amino acid sequence of theAB0041 light chain is as follows:

MESQIQVFVFVFLWLSGVDGDIVMTQSHKFMSTSVGDRVSITCKASQDV
RNTVAWYQQKTGQSPKLLIYSSSYRNTGVPDRFTGSGSGTDFTFTISSVQ
AEDLAVYFCQQHYITPYTFGGGTKLEIK*RADAAPTVSIFPPSSEQLTSGGASV*
VCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDE
YERHNSYTCEATHKTSTSPIVKSFNRNEC (SEQ ID NO:2)

[0088] The signal sequence is underlined, and the sequence of the kappa constant region is presented in italics.

[0089] The following amino acid sequence comprises the framework regions and complementarity-determining regions (CDRs) of the variable region of the IgG2b heavy chain of AB0041 (with CDRs underlined):

QVQLKESGPGLVAPSQSLSITCTVSGFSLLSYGVHWVRQPPGKGLEWLGV
IWTGGTTNYNSALMSRLSISKDDSKSQVFLKMNSLQTDDTAIYYCARYY
YGMDYWGQGTSVTVSS (SEQ ID NO:3)

[0090] The following amino acid sequence comprises the framework regions and complementarity-determining regions (CDRs) of the variable region of the kappa light chain of AB0041 (with CDRs underlined):

DIVMTQSHKFMSTSVGDRVSITCKASQDVRNTVAWYQQKTGQSPKLLIY SSSYRNTGVPDRFTGSGSGTDFTFTISSVQAEDLAVYFCQQHYITPYTFGG GTKLEIK (SEQ ID NO:4)

Other exemplary mouse anti-human MMP9 antibodies (e.g., M4 and M12) are described in Example 1B. An exemplary anti-mouse MMP9 antibody (AB0046) is described in Example 1C. Other exemplary mouse anti-human MMP9 antibodies include antibodies comprise the variable regions having the sequence of SEQ ID NO: 3, and the constant regions having 95% similarity as the sequences of the IgG2b constant regions. In addition, the exemplary mouse anti-human MMP9 antibodies include antibodies comprise the variable regions having the sequence of SEQ ID NO: 4, and the constant regions having 95% similarity as the sequences of the IgG2b constant regions. Other exemplary mouse anti-human MMP9 antibodies include antibodies comprise the variable regions having the sequences of SEQ ID NOs: 3 and 4, and the constant regions having 95% similarity as the sequences of the IgG2b constant regions. Such anti-mouse antibodies are suitable for testing and assessing the MMP9-inhibition methods.

Heavy-chain variants

[0091] The amino acid sequences of the variable regions of the AB0041 heavy and light chains were separately modified, by altering framework region sequences in the heavy and light chain variable regions. The effect of these sequence alterations was to deplete the antibody of human T-cell epitopes, thereby reducing or abolishing its immunogenicity in humans.

[0092] Four heavy-chain variants were constructed, in a human IgG4 heavy chain background containing a S241P amino acid change that stabilizes the hinge domain (Angal *et al.* (1993) *Molec. Immunol.* **30:**105-108), and are denoted VH1, VH2, VH3 and VH4. The amino acid sequences of their framework regions and CDRs are as follows:

VH1

QVQLQESGPGLVKPSETLSLTCTVSGFSLLSYGVHWVRQPPGKGLEWLG VIWTGGTTNYNSALMSRLTISKDDSKSTVYLKMNSLKTEDTAIYYCARY YYGMDYWGQGTSVTVSS (SEQ ID NO:5)

VH2

QVQLQESGPGLVKPSETLSLTCTVSGFSLLSYGVHWVRQPPGKGLEWLG
VIWTGGTTNYNSALMSRLTISKDDSKNTVYLKMNSLKTEDTAIYYCARY
YYGMDYWGQGTLVTVSS (SEQ ID NO:6)

VH3

QVQLQESGPGLVKPSETLSLTCTVSGFSLLSYGVHWVRQPPGKGLEWLG
VIWTGGTTNYNSALMSRFTISKDDSKNTVYLKMNSLKTEDTAIYYCARY
YYGMDYWGQGTLVTVSS (SEQ ID NO:7)

VH4

QVQLQESGPGLVKPSETLSLTCTVSGFSLLSYGVHWVRQPPGKGLEWLG
VIWTGGTTNYNSALMSRFTISKDDSKNTLYLKMNSLKTEDTAIYYCARY
YYGMDYWGQGTLVTVSS (SEQ ID NO:8)

[0093] Figure 1 shows an alignment of the amino acid sequences of the variable regions of the humanized heavy chains and indicates the differences in amino acid sequences in the framework regions among the four variants.

Light-chain variants

[0094] Four light-chain variants were constructed, in a human kappa chain background, and are denoted Vk1, Vk2, Vk3 and Vk4. The amino acid sequences of their framework regions and CDRs are as follows:

Vk1

DIVMTQSPSFLSASVGDRVTITCKASQDVRNTVAWYQQKTGKAPKLLIYS
SSYRNTGVPDRFTGSGSGTDFTLTISSLQAEDVAVYFCQQHYITPYTFGGG
TKVEIK (SEQ ID NO:9)

Vk2

DIVMTQSPSSLSASVGDRVTITCKASQDVRNTVAWYQQKPGKAPKLLIYS
SSYRNTGVPDRFTGSGSGTDFTLTISSLQAEDVAVYFCQQHYITPYTFGGG
TKVEIK (SEQ ID NO:10)

Vk3

DIQMTQSPSSLSASVGDRVTITCKASQDVRNTVAWYQQKPGKAPKLLIYS
SSYRNTGVPDRFSGSGSGTDFTLTISSLQAEDVAVYFCQQHYITPYTFGGG
TKVEIK (SEQ ID NO:11)

Vk4

DIQMTQSPSSLSASVGDRVTITCKASQDVRNTVAWYQQKPGKAPKLLIYS
SSYRNTGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQHYITPYTFGG
GTKVEIK (SEQ ID NO:12)

[0095] Figure 2 shows an alignment of the amino acid sequences of the variable regions of the humanized light chains and indicates the differences in amino acid sequences in the framework regions among the four variants.

[0096] The humanized heavy and light chains are combined in all possible pair-wise combinations to generate a number of functional humanized anti-MMP9 antibodies. For example, provided are antibodies with a heavy chain variable (VH) region having the amino acid sequence set forth in any of SEQ ID NOs: 3, 5, 6, 7, and 8; antibodies having a light chain variable (VL) region having the amino acid sequence set forth in any of SEQ ID NOs: 4, 9, 10, 11, and 12; and antibodies with a heavy chain variable (VH) region having the amino acid sequence set forth in any of SEQ ID NOs: 3, 5, 6, 7, and 8 and a light chain variable (VL) region having the amino acid sequence set forth in any of SEQ ID NOs: 4, 9, 10, 11, and 12, as well as antibodies that compete for binding to MMP9 with such antibodies and antibodies having at least at or about 75 %, 80 %, 85 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % or more sequence identity with such antibodies. In one example, the antibody has a VH region with an amino acid sequence having at least at or about 75 %, 80 %, 85 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % or more sequence identity with SEQ ID NO: 7 and a VL region with an amino acid sequence having at least at or about 75 %, 80 %, 85 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % or more sequence identity with SEQ ID NO: 12, or a VH region of SEQ ID NO: 7 and a VL region of SEQ ID NO: 12.

[0097] Additional heavy chain variable region amino acid sequences having 75% or more, 80% or more, 90% or more, 95% or more, or 99% or more homology to the heavy chain variable region sequences disclosed herein are also provided. Furthermore, additional light chain variable region amino acid sequences having 75% or more, 80% or more, 90% or more, 95% or more, or 99% or more homology to the light chain variable region sequences disclosed herein are also provided.

[0098] Additional heavy chain variable region amino acid sequences having 75% or more, 80% or more, 90% or more, 95% or more, or 99% or more sequence identity to the heavy chain variable region sequences disclosed herein are also provided. Furthermore, additional light chain variable region amino acid sequences having 75% or more, 80% or more, 90% or more, 95% or more, or 99% or more sequence identity to the light chain variable region sequences disclosed herein are also provided.

Complementarity-determining regions (CDRs)

[0099] In some embodiments, the CDRs of the heavy chain of exemplary provided anti-MMP9 antibodies as disclosed herein have the following amino acid sequences:

CDR1: GFSLLSYGVH (SEQ ID NO:13)
CDR2: VIWTGGTTNYNSALMS (SEQ ID NO:14)
CDR3: YYYGMDY (SEQ ID NO:15)

[0100] Thus, among the provided anti-MMP9 antibodies are antibodies having a heavy chain CDR1 region with an amino acid sequence as set forth in SEQ ID NO: 13, antibodies having a heavy chain CDR2 region with an amino acid sequence set forth in SEQ ID NO: 14, and antibodies having a heavy chain CDR3 region with an amino acid sequence as set forth in SEQ ID NO: 15, and antibodies that compete for binding with or bind to the same epitope on MMP9 as such antibodies. In some cases, the antibodies contain VH CDRs having the sequences set forth in SEQ ID NO: 13, 14, and 15.

[0101] In some embodiments, the CDRs of the light chain of exemplary anti-MMP9 antibodies as disclosed herein have the following amino acid sequences:

CDR1: KASQDVRNTVA (SEQ ID NO:16)
CDR2: SSSYRNT (SEQ ID NO:17)
CDR3: QQHYITPYT (SEQ ID NO:18)

[0102] Thus, among the provided anti-MMP9 antibodies are antibodies having a light chain CDR1 region with an amino acid sequence as set forth in SEQ ID NO: 16, antibodies having a light chain CDR2 region with an amino acid sequence set forth in SEQ ID NO: 17, and antibodies having a light chain CDR3 region with an amino acid sequence as set forth in SEQ ID NO: 18, and antibodies that compete for binding with or bind to the same epitope on MMP9 as such antibodies. In some cases, the antibodies contain VL CDRs having the sequences set forth in SEQ ID NO: 16, 17, and 18.

Nucleic acids encoding anti-MMP9 antibodies

[0103] The present disclosure provides nucleic acids encoding anti-MMP9 antibodies and functional fragments thereof. Accordingly, the present disclosure provides an isolated polynucleotide (nucleic acid) encoding an antibody or antigen-binding fragment as described herein, vectors containing such polynucleotides, and host cells and expression systems for transcribing and translating such polynucleotides into polypeptides.

[0104] The present disclosure also contemplates constructs in the form of plasmids, vectors, transcription or expression cassettes which comprise at least one polynucleotide as above.

[0105] The present disclosure also provides a recombinant host cell which comprises one or more constructs as above, as well as methods of production of the antibody or antigen-binding fragments thereof described herein which method comprises expression of nucleic acid encoding a heavy chain polypeptide and a light chain polypeptide (in the same or different host cells, and from the same or different constructs) in a recombination host cell. Expression can be achieved by culturing under appropriate conditions recombinant host cells containing the nucleic acid. Following production by expression, an antibody or antigen-binding fragment can be isolated and/or purified using any suitable technique, then used as appropriate.

[0106] Systems for cloning and expression of a polypeptide in a variety of different host cells are well known. Suitable host cells include bacteria, mammalian cells, yeast and baculovirus systems. Mammalian cell lines available in the art for expression of a heterologous polypeptide include Chinese hamster ovary cells, HeLa cells, baby hamster kidney cells, NSO mouse melanoma cells and many others. A common bacterial host is E. coli.

[0107] Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including operably linked promoter sequences, terminator sequences, polyadenylation sequences, enhancer sequences, marker genes and/or other sequences as appropriate. Vectors can be plasmids, viral e.g. 'phage, or phagemid, as appropriate. For further details see, for example, Molecular Cloning: a Laboratory Manual: 2nd edition, Sambrook et al., 1989, Cold Spring Harbor Laboratory Press. Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in Short Protocols in Molecular Biology, Second Edition, Ausubel et al. eds., John Wiley & Sons, 1992. The disclosures of Sambrook et al. and Ausubel et al. are incorporated herein by reference in their entirety.

[0108] The nucleic acid encoding a polypeptide of interest is integrated into the genome of the host cell or can be maintained as a stable or transient episomal element.

[0109] Any of a wide variety of expression control sequences – sequences that control the expression of a DNA sequence operatively linked to it – can be used in these vectors to express the DNA sequences. For example, a nucleic acid encoding a polypeptide of interest can be operably linked to a promoter, and provided in an expression construct for use in methods of production of recombinant MMP9 proteins or portions thereof.

[0110] Those of skill in the art are aware that nucleic acids encoding the antibody chains disclosed herein can be synthesized using standard knowledge and procedures in molecular biology.

[0111] Examples of nucleotide sequences encoding the heavy and light chain amino acid sequences disclosed herein, are as follows:

VH1: CAGGTGCAGC TGCAGGAATC CGGCCCTGGC CTGGTCAAGC CCTCCGAGAC ACTGTCCCTG ACCTGCACCG TGTCCGGCTT CTCCCTGCTG TCCTACGGCG TGCACTGGGT CCGACAGCCT CCAGGGAAGG GCCTGGAATG GCTGGGCGTG ATCTGGACCG GCGGCACCAC CAACTACAAC TCCGCCCTGA TGTCCCGGCT GACCATCTCC AAGGACGACT CCAAGTCCAC CGTGTACCTG AAGATGAACT CCCTGAAAAC CGAGGACACC GCCATCTACT ACTGCGCCCG GTACTACTAC GGCATGGACT ACTGGGGCCA GGGCACCTCC GTGACCGTGT CCTCA (SEQ ID NO:19)

VH2: CAGGTGCAGC TGCAGGAATC CGGCCCTGGC CTGGTCAAGC CCTCCGAGAC ACTGTCCCTG ACCTGCACCG TGTCCGGCTT CTCCCTGCTG TCCTACGGCG TGCACTGGGT CCGACAGCCT CCAGGCAAAG GCCTGGAATG GCTGGGCGTG ATCTGGACCG GCGGCACCAC CAACTACAAC TCCGCCCTGA TGTCCCGGCT GACCATCTCC AAGGACGACT CCAAGAACAC CGTGTACCTG AAGATGAACT CCCTGAAAAC CGAGGACACC GCCATCTACT ACTGCGCCCG GTACTACTAC GGCATGGACT ACTGGGGCCA GGGCACCCTG GTCACCGTGT CCTCA (SEQ ID NO:20)

VH3: CAGGTGCAGC TGCAGGAATC CGGCCCTGGC CTGGTCAAGC CCTCCGAGAC ACTGTCCCTG ACCTGCACCG TGTCCGGCTT CTCCCTGCTG TCCTACGGCG TGCACTGGGT CCGACAGCCT CCAGGCAAAG GCCTGGAATG GCTGGGCGTG ATCTGGACCG GCGGCACCAC CAACTACAAC TCCGCCCTGA TGTCCCGGTT CACCATCTCC AAGGACGACT CCAAGAACAC CGTGTACCTG AAGATGAACT CCCTGAAAAC CGAGGACACC GCCATCTACT ACTGCGCCCG GTACTACTAC GGCATGGACT ACTGGGGCCA GGGCACCCTG GTCACCGTGT CCTCA (SEQ ID NO:21)

VH4: CAGGTGCAGC TGCAGGAATC CGGCCCTGGC CTGGTCAAGC CCTCCGAGAC ACTGTCCCTG ACCTGCACCG TGTCCGGCTT CTCCCTGCTG TCCTACGGCG TGCACTGGGT CCGACAGCCT CCAGGCAAAG GCCTGGAATG GCTGGGCGTG ATCTGGACCG GCGGCACCAC CAACTACAAC TCCGCCCTGA TGTCCCGGTT CACCATCTCC AAGGACGACT CCAAGAACAC CCTGTACCTG AAGATGAACT CCCTGAAAAC CGAGGACACC GCCATCTACT ACTGCGCCCG GTACTACTAC GGCATGGACT ACTGGGGCCA GGGCACCCTG GTCACCGTGT CCTCA (SEQ ID NO:22)

Vk1: GACATCGTGA TGACCCAGTC CCCCAGCTTC CTGTCCGCCT CCGTGGGCGA CAGAGTGACC ATCACATGCA AGGCCTCTCA GGACGTGCGG AACACCGTGG CCTGGTATCA GCAGAAAACC GGCAAGGCCC CCAAGCTGCT GATCTACTCC TCCTCCTACC GGAACACCGG CGTGCCCGAC CGGTTTACCG GCTCTGGCTC CGGCACCGAC TTTACCCTGA CCATCAGCTC CCTGCAGGCC GAGGACGTGG CCGTGTACTT CTGCCAGCAG CACTACATCA CCCCCTACAC CTTCGGCGGA GGCACCAAGG TGGAAATAAA A (SEQ ID NO:23)

Vk2: GACATCGTGA TGACCCAGTC CCCCTCCAGC CTGTCCGCCT CTGTGGGCGA CAGAGTGACC ATCACATGCA AGGCCTCTCA GGACGTGCGG AACACCGTGG CCTGGTATCA GCAGAAGCCC GGCAAGGCCC CCAAGCTGCT GATCTACTCC TCCTCCTACC GGAACACCGG CGTGCCCGAC CGGTTTACCG GCTCTGGCTC CGGCACCGAC TTTACCCTGA CCATCAGCTC CCTGCAGGCC GAGGACGTGG CCGTGTACTT CTGCCAGCAG CACTACATCA CCCCCTACAC CTTCGGCGGA GGCACCAAGG TGGAAATAAA A (SEQ ID NO:24)

Vk3: GACATCCAGA TGACCCAGTC CCCCTCCAGC CTGTCCGCCT CTGTGGGCGA CAGAGTGACC ATCACATGCA AGGCCTCCCA GGACGTGCGG AACACCGTGG CCTGGTATCA GCAGAAGCCC GGCAAGGCCC CCAAGCTGCT GATCTACTCC TCCTCCTACC GGAACACCGG CGTGCCCGAC CGGTTCTCTG GCTCTGGAAG CGGCACCGAC TTTACCCTGA CCATCAGCTC CCTGCAGGCC GAGGACGTGG CCGTGTACTT CTGCCAGCAG CACTACATCA CCCCCTACAC CTTCGGCGGA GGCACCAAGG TGGAAATAAA A (SEQ ID NO:25)

Vk4: GACATCCAGA TGACCCAGTC CCCCTCCAGC CTGTCCGCCT CTGTGGGCGA CAGAGTGACC ATCACATGCA AGGCCTCTCA GGACGTGCGG AACACCGTGG CCTGGTATCA GCAGAAGCCC GGCAAGGCCC CCAAGCTGCT GATCTACTCC TCCTCCTACC GGAACACCGG CGTGCCCGAC CGGTTCTCTG

GCTCTGGAAG CGGCACCGAC TTTACCCTGA CCATCAGCTC CCTGCAGGCC
GAGGACGTGG CCGTGTACTA CTGCCAGCAG CACTACATCA CCCCCTACAC
CTTCGGCGGA GGCACCAAGG TGGAAATAAA A (SEQ ID NO:26)

[0112] Because the structure of antibodies, including the juxtaposition of CDRs and framework regions in the variable region, the structure of framework regions and the structure of heavy- and light-chain constant regions, is well-known in the art; it is well within the skill of the art to obtain related nucleic acids that encode anti-MMP-9 antibodies. Accordingly, polynucleotides comprising nucleic acid sequences having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% and at least 99% homology to any of the nucleotide sequences disclosed herein are also provided. Accordingly, polynucleotides comprising nucleic acid sequences having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% and at least 99% identity to any of the nucleotide sequences disclosed herein are also provided. In one example, the polynucleotide contains at least at or about 75 %, 80 %, 85 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % or more sequence identity with SEQ ID NO: 21 or includes or is SEQ ID NO: 21 and/or contains at least at or about 75 %, 80 %, 85 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % or more sequence identity with SEQ ID NO: 26 or includes or is SEQ ID NO: 26.

Pharmaceutical Compositions

[0113] MMP9 binding proteins, as well as nucleic acid (e.g., DNA or RNA) encoding MMP9 binding proteins, can be provided as a pharmaceutical composition, e.g., combined with a pharmaceutically acceptable carrier or excipient. Such pharmaceutical compositions are useful for, for example, administration to a subject in vivo or ex vivo, and for diagnosing and/or treating a subject with the MMP9 binding proteins, such as in any of the therapeutic or diagnostic methods provided herein.

[0114] Pharmaceutically acceptable carriers are physiologically acceptable to the administered patient and retain the therapeutic properties of the antibodies or peptides with which it is administered. Pharmaceutically-acceptable carriers and their formulations are and generally described in, for example, Remington' pharmaceutical Sciences (18th Edition, ed. A. Gennaro, Mack Publishing Co., Easton, PA 1990). One exemplary pharmaceutical carrier is physiological saline. Each carrier is "pharmaceutically acceptable" in the sense of being compatible with the other ingredients of the formulation and not substantially injurious to the patient.

[0115] Pharmaceutical compositions can be formulated to be compatible with a particular route of administration, systemic or local. Thus, pharmaceutical compositions include carriers, diluents, or excipients suitable for administration by various routes.

[0116] Pharmaceutical compositions can include pharmaceutically acceptable additives. Examples of additives include, but are not limited to, a sugar such as mannitol, sorbitol, glucose, xylitol, trehalose, sorbose, sucrose, galactose, dextran, dextrose, fructose, lactose and mixtures thereof. Pharmaceutically acceptable additives can be combined with pharmaceutically acceptable carriers and/or excipients such as dextrose. Additives also include surfactants such as polysorbate 20 or polysorbate 80.

[0117] The formulation and delivery methods will generally be adapted according to the site and the disease to be treated. Exemplary formulations include, but are not limited to, those suitable for parenteral administration, e.g., intravenous, intra-arterial, intramuscular, or subcutaneous administration, or oral administration.

[0118] Pharmaceutical compositions for parenteral delivery include, for example, water, saline, phosphate buffered saline, Hank's solution, Ringer's solution, dextrose/saline, and glucose solutions. The formulations can contain auxiliary substances to approximate physiological conditions, such as buffering agents, tonicity adjusting agents, wetting agents, detergents and the like. Additives can also include additional active ingredients such as bactericidal agents, or stabilizers. For example, the solution can contain sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate or triethanolamine oleate. Additional parenteral formulations and methods are described in Bai (1997) J. Neuroimmunol. 80:65 75; Warren (1997) J. Neurol. Sci. 152:31 38; and Tonegawa (1997) J. Exp. Med. 186:507 515. The parenteral preparation can be enclosed in ampules, disposable syringes or multiple dose vials made of glass or plastic.

[0119] Pharmaceutical compositions for intradermal or subcutaneous administration can include a sterile diluent, such as water, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid, glutathione or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose.

[0120] Pharmaceutical compositions for injection include aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor ELTM (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). The carrier can be a solvent or dispersion medium containing,

for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. Fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Antibacterial and antifungal agents include, for example, parabens, chlorobutanol, phenol, ascorbic acid and thimerosal. Isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, and sodium chloride may be included in the composition. The resulting solutions can be packaged for use as is, or lyophilized; the lyophilized preparation can later be combined with a sterile solution prior to administration.

[0121] Pharmaceutically acceptable carriers can contain a compound that stabilizes, increases, or delays absorption or clearance. Such compounds include, for example, carbohydrates, such as glucose, sucrose, or dextrans; low molecular weight proteins; compositions that reduce the clearance or hydrolysis of peptides; or excipients or other stabilizers and/or buffers. Agents that delay absorption include, for example, aluminum monostearate and gelatin. Detergents can also be used to stabilize or to increase or decrease the absorption of the pharmaceutical composition, including liposomal carriers. To protect from digestion the compound can be complexed with a composition to render it resistant to acidic and enzymatic hydrolysis, or the compound can be complexed in an appropriately resistant carrier such as a liposome. Means of protecting compounds from digestion are known in the art (see, e.g., Fix (1996) Pharm Res. 13:1760 1764; Samanen (1996) J. Pharm. Pharmacol. 48:119 135; and U.S. Pat. No. 5,391,377, describing lipid compositions for oral delivery of therapeutic agents).

[0122] Compositions of the present invention can be combined with other therapeutic moieties or imaging/diagnostic moieties as provided herein. Therapeutic moieties and/or imaging moieties can be provided as a separate composition, or as a conjugated moiety present on an MMP9 binding protein.

[0123] Formulations for in vivo administration are generally sterile. In one embodiment, the pharmaceutical compositions are formulated to be free of pyrogens such that they are acceptable for administration to human patients.

[0124] Various other pharmaceutical compositions and techniques for their preparation and use will be known to those of skill in the art in light of the present disclosure. For a detailed listing of suitable pharmacological compositions and associated administrative techniques one can refer to the detailed teachings herein, which can be further supplemented by texts such as Remington: The Science and Practice of Pharmacy 20th Ed. (Lippincott, Williams & Wilkins 2003).

[0125] Pharmaceutical compositions can be formulated based on the physical characteristics of the patient/subject needing treatment, the route of administration, and the like. Such can be packaged in a suitable pharmaceutical package with appropriate labels for the distribution to hospitals and clinics wherein the label is for the indication of treating a disorder as described herein in a subject. Medicaments can be packaged as a single or multiple units. Instructions for the dosage and administration of the pharmaceutical compositions of the present invention can be included with the pharmaceutical packages and kits described below.

Methods of Use

[0126] The MMP9 binding proteins, including anti-MMP9 antibodies and fragments thereof, of the present disclosure can be used, for example, in therapeutic and diagnostic methods, such as methods of detection of MMP9 in a sample, methods of treatment (e.g., as in methods of inhibition of angiogenesis), and methods of diagnosis and prognosis. Thus, provided are diagnostic and therapeutic methods and uses of the anti-MMP9 antibodies. Examples of methods of use are described below.

Methods of Treatment

[0127] Provided herein are methods of treatment, including methods of treating diseases and disorders associated with MMP9 expression and/or activity, as well as uses of the provided antibodies and compositions in such methods. The diseases and disorders include, but are not limited to cancer, e.g., tumors (e.g., primary or metastatic tumors), such as those that express or are disposed in a tissue which expresses MMP9, and inflammatory diseases, such as inflammatory bowel diseases, rheumatoid arthritis and inflammatory myopathies.

[0128] As used herein, "treat" or "treatment" means stasis or a postponement of development of one or more symptoms associated with a disease or disorder described herein, or ameliorating existing uncontrolled or unwanted symptoms, preventing additional symptoms, or ameliorating or preventing the underlying metabolic causes of symptoms. Thus, the terms denote that a beneficial result has been conferred on a mammalian subject with a disease or symptom, or with the potential to develop such disease or symptom. A response is achieved when the patient experiences partial or total alleviation, or reduction of signs or symptoms of illness, and can include, without limitation, prolongation of survival. The expected progression-free survival times can be measured in months to years, depending on prognostic factors including the number of relapses, stage of disease, and other factors.

[0129] Also provided are pharmaceutical compositions for use in connection with such methods, such as those containing any of the antibodies or fragments thereof described herein. Compositions can be suitable for administration locally or systemically by any suitable route.

[0130] In general, MMP9 binding proteins are administered in a therapeutically effective amount, e.g., in an amount to effect inhibition of tumor growth in a subject, to inhibit metastasis, to inhibit inflammation, to inhibit tissue destruction, to inhibit MMP9 activity, or to treat the particular disease or condition associated with MMP9.

[0131] As used herein, unless otherwise specified, the term "therapeutically effective amount" or "effective amount" refers to an amount of an agent or compound or composition that when administered (either alone or in combination with another therapeutic agent, as may be specified) to a subject is effective to prevent or ameliorate the disease condition or the progression of the disease, or result in amelioration of symptoms, e.g., treatment, healing, prevention or amelioration of the relevant medical condition, or an increase in rate of treatment, healing, prevention or amelioration of such conditions. When applied to an individual active ingredient administered alone, a therapeutically effective dose refers to that ingredient alone. When applied to a combination, a therapeutically effective dose refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously. In one example, when in vivo administration of an anti-MMP9 antibody is employed, normal dosage amounts can vary from about 10 ng/kg to up to 100 mg/kg of mammal body weight or more per day, preferably about 1 μg/kg/day to 50 mg/kg/day, optionally about 100 μg/kg/day to 20 mg/kg/day, 500 μg/kg/day to 10 mg/kg/day, or 1 mg/kg/day to 10 mg/kg/day, depending upon the route of administration. In one embodiment, intravenous dosage range from about 1 mg/kg to about 30 mg/kg. In some embodiments, intravenous dosages range from at or about 1 mg/kg to at or about 14 mg/kg, such as from at or about 2 mg/kg to at or about 14 mg/kg, q14d, once every 14 days. In other embodiments, subcutaneous dosages range from at or about 1 mg/kg to at or about 28 mg/kg, such as from at or about 2 mg/kg to at or about 28 mg/kg, q14d, once every 14 days. In some embodiments, the effective amount of dosage is administered once every 7 to 28 days. In one embodiment, the effective amount of dosage is administered once every 7 days. In another embodiment, the effective amount of dosage is administered once every 28 days.

[0132] The selected dosage regimen will depend upon a variety of factors including the activity of the MMP9 binding protein, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular composition

employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

[0133] A clinician having ordinary skill in the art can readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian can start doses of the compounds of the invention employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

[0134] In some cases, the methods of treatment include parenteral administration, e.g., intravenous, intra-arterial, intramuscular, or subcutaneous administration, or oral administration of the agent, e.g., anti-MMP9 antibody or composition containing the same.

[0135] As used herein, the term "subject" means a mammalian subject. Exemplary subjects include, but are not limited to humans, monkeys, dogs, cats, mice, rats, cows, horses, goats and sheep. In some embodiments, the subject has cancer, an inflammatory disease or condition, or an autoimmune disease or condition, and can be treated with the agent of the present invention as described below.

[0136] If needed, for treatments, methods can further include additional therapies, such as in the case of cancer, surgical removal of the cancer and/or administration of an anti-cancer agent or treatment in addition to an MMP9 binding protein. Administration of such an anti-cancer agent or treatment can be concurrent with administration of the compositions disclosed herein.

Methods of Detection of MMP9

[0137] The present disclosure also contemplates methods of detecting MMP9 in a subject, e.g., to detect tumor or tumor-associated tissue expressing MMP9, or tissue or fluid or other biological sample associated with a disease as described herein, such as autoimmune or inflammatory disease. Thus, methods of diagnosing, monitoring, staging or detecting a tumor having MMP9 activity are provided.

[0138] Samples (e.g., test biological samples) from a subject (e.g., an individual suspected of having or known to have a tumor associated with MMP9 expression, or suspected of having or known to have another disease or condition), can be analyzed for MMP9 presence, absence, expression, and/or levels. For example, such samples can be collected and analyzed by detecting the presence or absence of binding of an MMP9 binding protein, such as an antibody or fragment as described herein, to substance (e.g., protein) in the sample. In some examples, the methods further include comparing the amount of binding detected to an amount of binding to a control sample, or comparing the detected level of MMP9 to a control level of MMP9. In some

cases, the methods indicate the presence, absence, or severity of an MMP9-associated disease or condition, such as one described herein.

[0139] This analysis can be performed prior to the initiation of treatment using an MMP9 binding protein as described herein, or can be done as part of monitoring of progress of cancer treatment. In some embodiments, provided are methods of treatment, carried out by performing the detection assays and initiating, altering, or discontinuing treatment of the subject, for example, based on the results of the diagnostic assay. Such diagnostic analysis can be performed using any sample, including but not limited to tissue, cells isolated from such tissues, and the like. In some cases, the methods are performed on liquid samples, such as blood, plasma, serum, whole blood, saliva, urine, or semen. Tissue samples include, for example, formalin-fixed or frozen tissue sections.

[0140] Any suitable method for detection and analysis of MMP9 can be employed. Various diagnostic assay techniques known in the art can be adapted for such purpose, such as competitive binding assays, direct or indirect sandwich assays and immunoprecipitation assays conducted in either heterogeneous or homogeneous phases.

[0141] MMP9 binding proteins for use in detection methods can be labeled with a detectable moiety. The detectable moiety directly or indirectly produces a detectable signal. For example, the detectable moiety can be any of those described herein such as, for example, a radioisotope, such as 3H, 14C, 32P, 35S, or 125I, a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate (FITC), Texas red, cyanin, photocyan, rhodamine, or luciferin, or an enzyme, such as alkaline phosphatase, β-galactosidase or horseradish peroxidase.

[0142] Detection can be accomplished by contacting a sample under conditions suitable for MMP9 binding protein binding to MMP9, and assessing the presence (e.g., level) or absence of MMP9 binding protein-MMP9 complexes. A level of MMP9 in the sample in comparison with a level of a reference sample can indicate the presence of a tumor or tumor-associated tissues having MMP9 activity. The reference sample can be a sample taken from the subject at an earlier time point or a sample from another individual.

[0143] Various aspects of the invention are further described and illustrated by way of the several examples which follow, none of which are intended to limit the scope of the invention.

EXAMPLES

Example 1A: Preparation of antibodies to human MMP-9.

[0144] The full-length human MMP9 protein without a signal peptide (SEQ ID NO. 28) was used to immunize mice. Spleen cells from immunized mice were fused with myeloma cells to generate a hybridoma library. Monoclonal cultures were prepared and screened to identify cultures expressing an anti-MMP9 monoclonal antibody.

[0145] An antibody (AB0041) was purified from one of the cultures and characterized. This antibody contained an IgG2b heavy chain and a kappa light chain. Characterization included testing for the binding of AB0041 to other human MMPs and to MMP9 proteins from other species, including cynomolgus monkey, rat and mouse. As shown in Table 2, the AB0041 antibody had greater affinity to human and cynomolgus MMP9, that it had lower affinity to rat MMP9. In addition, the AB0041 antibody did not bind to murine MMP9 or to many human non-MMP matrix metalloproteinases.

Table 2: Cross reactivity of AB0041 and AB0045

MMP Tested	Dissociation constant (Kd)	
	AB0045	AB0041
Human MMP1	>100 nM	>100 nM
Human MMP2	>100 nM	>100 nM
Mouse MMP2	>100 nM	>100 nM
Human MMP3	>100 nM	>100 nM
Human MMP7	>100 nM	>100 nM
Human MMP8	>100 nM	>100 nM
Human MMP9	0.168 ± 0.117 nM	0.133 ± 0.030 nM
Cynomolgus monkey MMP9	0.082 ± 0.022 nM	0.145 ± 0.16 nM
Mouse MMP9	>100 nM	>100 nM
Rat MMP9	0.311 ± 0.017 nM	0.332 ± 0.022 nM
Human MMP10	>100 nM	>100 nM
Human MMP12	>100 nM	>100 nM
Human MMP13	>100 nM	>100 nM

[0146] Additional characterization included assaying the binding of AB0041 to mutant mouse and human MMP9 proteins. Non-identical residues in the catalytic domain of mouse and human MMP9 proteins were identified, and forty-six non-identical amino acid residues were selected for mutagenesis. Most mutations were generated in mouse MMP9: the mouse amino acid residues were mutated to match those of human MMP9. Other mutations were generated in human MMP9: the human amino acid residues were mutated to match those of mouse MMP9. The mutated mouse or human MMP9 proteins were used in an ELISA assay.

[0147] In the ELISA assay, the AB0041 antibody was used as the primary antibody and a goat anti-mouse IgG antibody conjugated to horseradish peroxidase was used to detect the binding. The wild-type human MMP9 was used a positive control and the wild-type mouse MMP9 was used as a negative control. The results of the ELISA assay showed an arginine

residue at position 162 of the MMP9 amino acid sequence (R162) as important for the MMP9 binding of the AB0041 antibody. The results also showed the amino acid residues E111, D113, and I198 were important for the MMP9 binding of the AB0041 antibody. Based on the crystal structure of MMP9, E111, D113, R162, and I198 are grouped near each other around a Ca2+ ion binding pocket of MMP9. In this study, the AB0041 antibody was shown to specifically bind to an epitope containing amino acid residues within regions of MMP9 containing amino acid residues 104-119, 159-166, and 191-202.

[0148] In an enzymatic assay for MMP9, the AB0041 antibody was found to act as a non-competitive inhibitor of MMP9.

Example 1B: Preparation of additional antibodies to human MMP-9.

[0149] Additional hybridomas were generated, which produced antibodies having variable regions that shared identity with AB0041. One such hybridoma, designated M4, expressed an antibody containing the heavy chain (IgG2b) sequence:

[0150] MAVLVLFLCLVAFPSCVLSQVQLKESGPGLVAPSQSLSITCTVSGFSLLSYGV HWVRQPPGKGLEWLGVIWTGGSTNYNSALMSRLSISKDDSKSQVFLKMNSLQTDDTA MYYCARYYYAMDYWGQGTSVTVSS*AKTTPPSVYPLAPGCGDTTGSSVTLGCLVKGYFPE SVTVTWNSGSLSSSVHTFPALLQSGLYTMSSSVTVPSSTWPSQTVTCSVAHPASSTTVDKKLEPS GPISTINPCPPCKECHKCPAPNLEGGPSVFIFPPNIKDVLMISLTPKVTCVVVDVSEDDPDVRI SWFVNNVEVHTAQTQTHREDYNSTIRVVSALPIQHQDWMSGKEFKCKVNNKDLPSPIERTISK IKGLVRAPQVYILPPPAEQLSRKDVSLTCLVVGFNPGDISVEWTSNGHTEENYKDTAPVLDSD GSYFIYSKLDIKTSKWEKTDSFSCNVRHEGLKNYYLKKTISRSPGK* (SEQ ID NO:30)

[0151] (signal peptide set forth in underlined text, variable region set forth in plain text, and constant region set forth in italics), and the light chain (kappa) sequence:

[0152] MESQIQVFVFVFLWLSGVDGDIVMTQSHKFMFTSVGDRVSITCKASQDVRNT VAWYQQKTGQSPKLLIYSASYRNTGVPDRFTGSISGTDFTFTISSVQAEDLALYYCQQH YSTPYTFGGGTKLEVK*RADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSE RQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC* (signal peptide set forth in underlined text, variable region set forth in plain text, and constant region set forth in italics) (SEQ ID NO: 31).

[0153] The M4 antibody had a variable heavy chain with an amino acid sequence:

[0154] QVQLKESGPGLVAPSQSLSITCTVSGFSLLSYGVHWVRQPPGKGLEWLGVIW TGGSTNYNSALMSRLSISKDDSKSQVFLKMNSLQTDDTAMYYCARYYYAMDYWGQG TSVTVSS (CDRs 1, 2, and 3 (SEQ ID NOs: 34, 35, and 36, respectively) underlined) (SEQ ID NO: 32)

[0155] and a variable light chain with the amino acid sequence

[0156] DIVMTQSHKFMFTSVGDRVSITCKASQDVRNTVAWYQQKTGQSPKLLIYSAS YRNTGVPDRFTGSISGTDFTFTISSVQAEDLALYYCQQHYSTPYTFGGGTKLEVK (CDRs 1, 2, and 3 (SEQ ID NOs: 37, 38, and 39, respectively) underlined) (SEQ ID NO: 33).

[0157] Another such hybridoma, designated M12, expressed only a kappa chain, having the sequence:

[0158] QVFVYMLLWLSGVDGDIVMTQSQKFMSTSVGDRVSVTCKASQNVGTNVA WYQQKPGQSPKALIYSASYRFSGVPDRFTGSGSGTDFTLTISNVQSEDLAEYFCQQYNS YPYTFGGGTKLEIK*RADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQ NGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC* (signal peptide set forth in underlined text, variable region set forth in plain text, and constant region set forth in italics) (SEQ ID NO: 40).

[0159] The M12 antibody had a variable light chain with the amino acid sequence

[0160] DIVMTQSQKFMSTSVGDRVSVTCKASQNVGTNVAWYQQKPGQSPKALIYSA SYRFSGVPDRFTGSGSGTDFTLTISNVQSEDLAEYFCQQYNSYPYTFGGGTKLEIK (CDRs 1, 2, and 3 (SEQ ID NOs: 42, 43, and 44, respectively) underlined) (SEQ ID NO: 41).

[0161] A sequence comparison, showing differences between the M4 and M12 heavy and light chains as compared with AB0041 antibody is shown in Figure 4.

[0162] An enzymatic assay was carried out. The results demonstrated that the antibodies produced by the M4 and M12 hybridomas acted as non-competitive inhibitors of MMP9 (data not shown).

Example 1C: Preparation of antibodies to mouse MMP-9.

[0163] Another mouse antibody, AB0046, was generated. Using a process similar to that described in Example 1A, the MMP9-knockout mice (strain B6.FVB (Cg)- *Mmp9*^{tm1Tvu}/J) was immunized using targeted domains of the pro/catalytic domain fragment of murine MMP9. The AB0046 antibody had a kappa light chain with an amino acid sequence MSSAQFLGLLLLCFQGTRCDIQMTQTTSSLSASLGDRVTISCSASQGISNYLNWYQQKPD GTFKLLIYYTSILHSGVPSRFSGSGSGTDYSLTISNLEPEDIATYYCQQYGWLPRTFGGGT KLEIK*RADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTD QDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC* (SEQ ID NO: 45) (signal peptide set forth in underlined text, variable region set forth in plain text, and constant region set forth in italics) and an IgG1 heavy chain with an amino acid sequence MGWSSIILFLVATATGVHSQVQLQQPGSVLVRPGASVKLSCTASGYTFTSYWMNWVK QRPGQGLEWIGEIYPISGRTNYNEKFKVKATLTVDTSSSTAYMDLNSLTSEDSAVYYCA

RSRANWDDYWGQGTTLTVSS*AKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVT WNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIVPRDCGC KPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVDISKDDPEVQFSWFVDDVEVHTAQTQP REEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPP KEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLNVQKS NWEAGNTFTCSVLHEGLHNHHTEKSLSHSPGK* (SEQ ID NO: 46) (signal peptide set forth in underlined text, variable region set forth in plain text, and constant region set forth in italics).

[0164] The following amino acid sequence comprises the framework regions and complementarity-determining regions (CDRs) of the variable region of the IgG1 heavy chain of AB0046 (with CDRs underlined):

[0165] QVQLQQPGSVLVRPGASVKLSCTAS<u>GYTFTSYWMN</u>WVKQRPGQGLEWIG<u>EI YPISGRTNYNEKFKV</u>KATLTVDTSSSTAYMDLNSLTSEDSAVYYCAR<u>SRANWDDY</u>WG QGTTLTVSS (SEQ ID No: 47).

[0166] The following amino acid sequence comprises the framework regions and complementarity-determining regions (CDRs) of the variable region of the kappa light chain of AB0046 (with CDRs underlined):

[0167] DIQMTQTTSSLSASLGDRVTISC<u>SASQGISNYLN</u>WYQQKPDGTFKLLIY<u>YTSIL HS</u>GVPSRFSGSGSGTDYSLTISNLEPEDIATYYC<u>QQYGWLPRT</u>FGGGTKLEIK (SEQ ID No: 48)

[0168] Additional characterizations showed that the AB0046 antibody bound to mouse MMP9 non-competitively or its binding was not dependant on the concentration of mouse MMP9. The AB0046 antibody did not bind to human MMP9 or MMP2, mouse MMP2, 3, 7, 8, or 12. Using epitope analysis as described in Example 1A, it was shown that the proline residue at position 162 of the mouse MMP9 amino acid sequence (P162) (corresponding to R162 of human MMP9) was important for the MMP9 binding of the AB0046 antibody. The results suggested that the AB0046 antibody specifically bound to an epitope containing a residue within a portion of mouse MMP9 corresponding to the portion containing amino acids 159-166 of human MMP9. Thus, the AB0046 antibody was an inhibitory antibody specific to mouse MMP9 and had similar kinetics of binding and inhibition as those of AB0041. Because AB0046 is specific to mouse MMP9 and binds to an epitope as AB0041/AB0045, AB0046 is suitable for assays which uses either AB0041 or AB0045.

[0169] Further characterization showed that the AB0046 antibody was a murine IgG1 isotype, having a limited effector function in mouse.

[0170] Three other mouse anti-MMP9 antibodies were generated using similar methods, which were non-inhibitory and for which P162 was important for binding.

Example 2: Humanization of antibodies to human MMP9

[0171] The amino acid sequences of the heavy chain and light chain of the mouse AB0041 antibody were altered at certain locations in the framework (*i.e.*, non-CDR) portion of their variable regions to generate proteins that are less immunogenic in humans. These amino acid sequence changes were shown in Figures 1 and 2. The cross-reactivity of one humanized antibody, referred to as AB0045, is shown in Table 2A above.

[0172] The humanized variant anti-MMP9 antibody, AB0045 (humanized, modified IgG4 (S241P); see Example 2, above) contained the humanized AB0041 heavy chain variant **VH3** (having the sequence set forth in SEQ ID NO: 7

[0173] (QVQLQESGPGLVKPSETLSLTCTVSGFSLLSYGVHWVRQPPGKGLEWLGVI WTGGTTNYNSALMSRFTISKDDSKNTVYLKMNSLKTEDTAIYYCARYYYGMDYWGQ GTLVTVSS)

[0174] and the humanized AB0041 light chain variant VH4 (having the light chain sequence set forth in **Vk4** (having the sequence set forth in SEQ ID NO: 12

[0175] (DIQMTQSPSSLSASVGDRVTITCKASQDVRNTVAWYQQKPGKAPKLLIYSSS YRNTGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQHYITPYTFGGGTKVEIK)).

[0176] The heavy chain of the AB0045 antibody has the sequence set forth in SEQ ID NO: 49

(MGWSLILLFLVAVATRVHSQVQLQESGPGLVKPSETLSLTCTVSGFSLLSYGVHWVRQ PPGKGLEWLGVIWTGGTTNYNSALMSRFTISKDDSKNTVYLKMNSLKTEDTAIYYCAR YYYGMDYWGQGTLVTVSS*ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCP PCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKT KPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLP PSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKS RWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK* (signal sequence underlined; sequence of the constant region presented italics)); the light chain of the AB0045 antibody has the sequence set forth in SEQ ID NO: 50

(MRVPAQLLGLLLLWLPGARCDIQMTQSPSSLSASVGDRVTITCKASQDVRNTVAWYQ QKPGKAPKLLIYSSSYRNTGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQHYITPYT FGGGTKVEIK*RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC* (signal sequence underlined; sequence of the constant region presented italics)). The antibody contains 1312 amino acids in length, is composed of two heavy chains and two light chains, and has a

theoretical pI of about 7.90, extinction coefficient of about 1.50 AU/cm at 280 nm for 1 g/L, a molecular weight of about 144 kDa, and density of about 1 g/mL in formulation buffer (50-100 mg/mL product concentration).

[0177] Further characterization of this antibody is described in Example 3, below.

Example 3: Characterization of variant MMP9 antibody AB0045 and comparison to AB0041 and AB0046

[0178] As described above, AB0045 and AB0041 antibodies are non-competitive inhibitors of MMP9. Thus, both antibodies inhibit MMP9 enzymatic activity independently of substrate concentration. The AB0045 antibody binds to the same MMP9 epitope as the AB0041 antibody with an affinity in the 1 × 10-12 molar range, as shown by direct binding and surface plasmon resonance (SPR) assays. Both antibodies are specific for MMP9, with no significant non-specific binding observed against other purified protein targets including purified domains and full length forms of MMP enzymes. Both AB0045 and AB0041 antibodies are cross-reactive with native and recombinant human and recombinant rat and cynomolgus monkey MMP9.

[0179] The *in vitro* binding affinity, inhibition characteristics, and the specificity of the antibodies of AB0045, AB0041 and AB0046 for MMP9 of human and non-human origin were determined using Enzyme-Linked Immunosorbent Assay (ELISA) and an MMP9 enzymatic assay. SPR analysis was also used to generate dissociation constants (K_d) of AB0045 and AB0041.

[0180] In the ELISA assay, the K_d value of AB0045 and AB0041 antibodies for human, cynomolgus monkey, and rat MMP9 derived from ELISA were all found to be <400 pM. The ELISA data illustrated that both AB0045 and AB0041 antibodies cross-react with MMP9 from all the relevant toxicology species tested. The AB0046 antibody was shown to be specific to mouse MMP9 and therefore could be used as a surrogate antibody in mouse efficacy models. The results showed that the K_d value of the AB0045 antibodies for human MMP9 was 0.168 ± 0.117 nM and the and K_d value of the AB0041 antibody was 0.133 ± 0.030 nM. The results on the AB0046 antibodies showed it bound to mouse MMP9 with the K_d value of 0.218 ± 0.097 nM. In the SPR analysis, the results showed that the K_d values of AB0045 and AB0041 antibodies for human MMP9 were 8.8 pM and 0.4 pM, respectively.

[0181] The enzymatic inhibitory activities of AB0045, AB0041, and AB0046 antibodies were evaluated in an assay assessing MMP9-mediated cleavage of a fluorogenic peptide substrate Mca-PLGL-Dpa-AR-NH2. All three antibodies inhibited MMP9 enzyme activity. The IC_{50} values of AB0045 (0.691 ± 0.097 nM) and AB0041 (0.569 ± 0.185 nM) for human MMP9 were not statistically different. The IC_{50} value for the AB0046 inhibition of mouse MMP9 was

0.352 ± 0.03 nM. The value was not adjusted for the concentration of active enzyme that was generated during the preparation. Additional MMP9 enzymatic assay under steady-state conditions was used to determine IC_{50} and mode of inhibition. In this assay, the IC_{50} values of AB0045 ranged from 0.148 nM to 0.161 nM in a 20-fold range of substrate concentration, and in one example is 0.158 nmThe results showed that the MMP9 inhibitory activity of AB0045 was non-competitive.

Table 2B: Binding and Inhibitory Properties of AB0045, AB0041, and surrogate mouse antibody AB0046

	AB0045	**AB0041**	**AB0046**
ELISA			
Human MMP9 Dissociation constant	0.168 ± 0.117 nM	0.133 ± 0.030 nM	>100 nM
Cynomolgus monkey MMP9 Dissociation constant	0.082 ± 0.022 nM	0.145 ± 0.16 nM	>100 nM
Mouse MMP9 Dissociation constant	>100 nM	>100 nM	0.218±0.097 nM
Rat MMP9 Dissociation constant	0.311 ± 0.017 nM	0.332 ± 0.022 nM	>100 nM
SPR			
Human MMP9 Dissociation constant	8.8pM	0.4pM	ND
Activity Assay			
Human MMP9 IC_{50}	0.691 ± 0.097 nM	0.569 ± 0.185 nM	>100 nM
Cynomolgus monkey MMP9 IC_{50}	0.194 ± 0.048 nM*	0.189 ± 0.019 nM*	>100 nM
Rat MMP9 IC_{50}	8.23 ± 1.24 nM*	2.78 ± 1.17 nM *	>100 nM
Mouse MMP9 IC_{50}	>100 nM	>100 nM	0.352±0.03 nM*

[0182] The results confirmed that AB0045 and AB0041 have equivalent binding and inhibitory properties and that AB0046 can serve as a relevant mouse surrogate antibody, for example, in mouse models of human disease.

CLAIMS

What is claimed is:

1. An isolated antibody or fragment thereof, comprising: a heavy chain variable (VH) region having a heavy chain complementary determining region (CDR) with an amino acid sequence selected from the group consisting of SEQ ID NO: 13, SEQ ID NO: 14, and SEQ ID NO: 15.

2. The antibody or fragment of claim 1, wherein the VH region has a heavy chain CDR1 with the amino acid sequence of SEQ ID NO: 13, a heavy chain CDR2 with the amino acid sequence of SEQ ID NO: 14, and a heavy chain CDR3 with the amino acid sequence of SEQ ID NO: 15.

3. An isolated antibody or fragment thereof, comprising: a light chain variable (VL) region having a light chain complementary determining region (CDR) with an amino acid sequence selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17, and SEQ ID NO: 18.

4. The antibody or fragment of claim 3, wherein the VL region has a light chain CDR1 with the amino acid sequence of SEQ ID NO: 16, a light chain CDR2 with the amino acid sequence of SEQ ID NO: 17, and a light chain CDR3 with the amino acid sequence of SEQ ID NO: 18.

5. The antibody or fragment of claim 3 or claim 4, wherein the antibody further comprises a VH region having a heavy chain CDR1 with the amino acid sequence of SEQ ID NO: 13, a heavy chain CDR2 with the amino acid sequence of SEQ ID NO: 14, and a heavy chain CDR3 with the amino acid sequence of SEQ ID NO: 15.

6. The antibody or fragment of any of claims 1, 2, and 5, wherein the VH region has the amino acid sequence set forth in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 8.

7. The antibody or fragment of claim 6, wherein the VH region has the amino acid sequence set forth in SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 8.

8. The antibody or fragment of any of claims 3-7 wherein the VL region has the amino acid sequence set forth in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, or SEQ ID NO: 12.

9. The antibody or fragment of claim 8, wherein the VL region has the amino acid sequence set forth in SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, or SEQ ID NO: 12.

10. The antibody or fragment of claim 5, wherein the VH region has the amino acid sequence set forth in SEQ ID NO: 7 and the VL region has the amino acid sequence set forth in SEQ ID NO: 12.

11. An isolated antibody or fragment thereof that competes for binding to MMP9 with an antibody having a VH region with the amino acid sequence set forth in SEQ ID NO: 7 and a VL region with the amino acid sequence set forth in SEQ ID NO: 12.

12. An isolated antibody or fragment thereof that specifically binds to an epitope of MMP9, wherein the epitope comprises an amino acid residue within a region of MMP9, the region consisting of residues 104-119, residues 159-166, or residues 191-202 of SEQ ID NO: 27.

13. The antibody or fragment of claim 12, wherein the epitope comprises an amino acid residue within a region of MMP9 consisting of residues 104-119 of SEQ ID NO: 27, an amino acid residue within a region of MMP9 consisting of residues 159-166 of SEQ ID NO: 27, and an amino acid residue within a region of MMP9 consisting of residues 191-202 of SEQ ID NO: 27.

14. The antibody or fragment of claim 12 or 13, wherein the epitope comprises E111, D113, R162, or I198 of SEQ ID NO: 27.

15. The antibody or fragment of claim 14, wherein the epitope comprises R162 of SEQ ID NO: 27.

16. The antibody or fragment of claim 14, wherein the epitope comprises E111, D113, R162, and I198 of SEQ ID NO: 27.

17. The antibody or fragment of any of claims 1-16, wherein the antibody or fragment inhibits the enzymatic activity of MMP9.

18. The antibody or fragment of claim 17, wherein the inhibition of the enzymatic activity is non-competitive.

19. The antibody or fragment of any of claims 1-18, which is human or humanized.

20. The antibody or fragment of any of claims 1-19, for use in a method of inhibiting MMP9 activity in a subject, the method comprising administering to the subject the antibody or fragment in an amount effective to inhibit MMP9 activity in the subject.

21. A method of inhibiting MMP9 activity in a subject, the method comprising administering to the subject the antibody or fragment of any of claims 1-19 in an amount effective to inhibit MMP9 activity in the subject.

22. The method of claim 21, wherein the antibody or fragment thereof is administered intravenously at a dose from about 1 mg/kg to about 14 mg/kg.

23. The method of claim 21, wherein the antibody or fragment is administered subcutaneously, at a dose of about 4 mg/kg to about 28 mg/kg.

24. The method of any of claims 21-23, wherein the antibody or fragment is administered once every 7 to 28 days.

25. An isolated nucleic acid, comprising: a nucleotide sequence encoding a heavy chain polypeptide comprising CDRs with the amino acid sequences set forth in SEQ ID NOs: 13-15 or a light chain polypeptide comprising CDRs with the amino acid sequences set forth in SEQ ID NOs: 16-18.

26. The isolated nucleic acid of claim 25, wherein the nucleotide sequence encodes the heavy chain polypeptide, which has an amino acid sequence selected from the group consisting of SEQ ID NOS: 1, 3, and 5-8.

27. The isolated nucleic acid of claim 25 or 26, wherein the nucleotide sequence encodes the light chain polypeptide, which has an amino acid sequence selected from the group consisting of SEQ ID NOS: 2, 4, and 9-12.

28. The isolated nucleic acid of any of claims 25-27, wherein the nucleotide sequence comprises a sequence selected from the group consisting of SEQ ID NOs: 19-26.

29. The isolated nucleic acid of any of claims 25-28, wherein the nucleotide sequence comprises SEQ ID NO: 21 and SEQ ID NO: 26.

30. A vector, comprising the isolated nucleic acid of any of claims 25-29.

31. A cell, comprising the vector of claim 30.

32. A pharmaceutical composition, comprising the antibody or fragment thereof of any of claims 1-19.

33. A pharmaceutical composition, comprising the vector of claim 30.

34. A pharmaceutical composition, comprising the cell of claim 31.

35. The pharmaceutical composition of any of claims 32-34, for use in a method of inhibiting MMP9 activity in a subject, the method comprising administering to the subject the pharmaceutical composition in an amount effective to inhibit MMP9 activity in the subject.

36. A method of inhibiting MMP9 activity in a subject, comprising administering to the subject the pharmaceutical composition of any of claims 32-34 in an amount effective to inhibit MMP9 activity in the subject.

37. A method of detecting MMP9 expression in a test sample from a subject, the method comprising:

(a) contacting the test sample with an antibody or fragment of any of claims 1-19; and

(b) detecting binding of the antibody or fragment to protein in the sample, thereby detecting the presence of MMP9.

38. The method of claim 37, further comprising comparing the amount of binding detected in the test sample with an amount of binding of the antibody or fragment to a control sample.

39. An isolated polypeptide, having an amino acid sequence consisting essentially of residues 111-198 of SEQ ID NO: 27.

40. An isolated mutant MMP9 polypeptide, comprising an amino acid sequence containing residues 111-198 of SEQ ID NO: 27 with an amino acid substitution at residue 111, 113, 162, or 198.

41. The isolated mutant MMP9 polypeptide of claim 40, wherein the amino acid sequence contains an amino acid substitution at residues 111, 113, 162, and 198 of SEQ ID NO 27.

FIGURE 1

Anti-MMP9 humanized heavy chains

AB0041	QVQLKESGPG	LVAPSQSLSI	TCTVSGFSLL	SYGVHWVRQP	PGKGLEWLGV	
VH1	QVQLQESGPG	LVKPSETLSL	TCTVSGFSLL	SYGVHWVRQP	PGKGLEWLGV	
VH2	QVQLQESGPG	LVKPSETLSL	TCTVSGFSLL	SYGVHWVRQP	PGKGLEWLGV	
VH3	QVQLQESGPG	LVKPSETLSL	TCTVSGFSLL	SYGVHWVRQP	PGKGLEWLGV	
VH4	QVQLQESGPG	LVKPSETLSL	TCTVSGFSLL	SYGVHWVRQP	PGKGLEWLGV	
AB0041	IWTGGTTNYN	SALMSRLSIS	KDDSKSQVFL	KMNSLQTDDT	AIYYCARYYY	
VH1	IWTGGTTNYN	SALMSRLTIS	KDDSKSTVYL	KMNSLKTEDT	AIYYCARYYY	
VH2	IWTGGTTNYN	SALMSRLTIS	KDDSKNTVYL	KMNSLKTEDT	AIYYCARYYY	
VH3	IWTGGTTNYN	SALMSRFTIS	KDDSKNTVYL	KMNSLKTEDT	AIYYCARYYY	
VH4	IWTGGTTNYN	SALMSRFTIS	KDDSKNTLYL	KMNSLKTEDT	AIYYCARYYY	
AB0041	GMDYWGQGTS	VTVSS	(SEQ ID NO:3)			
VH1	GMDYWGQGTS	VTVSS	(SEQ ID NO:5)			
VH2	GMDYWGQGTL	VTVSS	(SEQ ID NO:6)			
VH3	GMDYWGQGTL	VTVSS	(SEQ ID NO:7)			
VH4	GMDYWGQGTL	VTVSS	(SEQ ID NO:8)			

FIGURE 2

Anti-MMP9 humanized light chains

```
AB0041    DIVMTQSHKF MSTSVGDRVS ITCKASQDVR NTVAWYQQKT GQSPKLLIYS
Vk1       DIVMTQSPSF LSASVGDRVT ITCKASQDVR NTVAWYQQKT GKAPKLLIYS
Vk2       DIVMTQSPSS LSASVGDRVT ITCKASQDVR NTVAWYQQKP GKAPKLLIYS
Vk3       DIQMTQSPSS LSASVGDRVT ITCKASQDVR NTVAWYQQKP GKAPKLLIYS
Vk4       DIQMTQSPSS LSASVGDRVT ITCKASQDVR NTVAWYQQKP GKAPKLLIYS

AB0041    SSYRNTGVPD RFTGSGSGTD FTFTISSVQA EDLAVYFCQQ HYITPYTFGQ
Vk1       SSYRNTGVPD RFTGSGSGTD FTLTISSLQA EDVAVYFCQQ HYITPYTFGG
Vk2       SSYRNTGVPD RFTGSGSGTD FTLTISSLQA EDVAVYFCQQ HYITPYTFGG
Vk3       SSYRNTGVPD RFSGSGSGTD FTLTISSLQA EDVAVYFCQQ HYITPYTFGG
Vk4       SSYRNTGVPD RFSGSGSGTD FTLTISSLQA EDVAVYYCQQ HYITPYTFGG

AB0041    GTKLEIK   (SEQ ID NO:4)
Vk1       GTKVEIK   (SEQ ID NO:9)
Vk2       GTKVEIK   (SEQ ID NO:10)
Vk3       GTKVEIK   (SEQ ID NO:11)
Vk4       GTKVEIK   (SEQ ID NO:12)
```

FIGURE 3

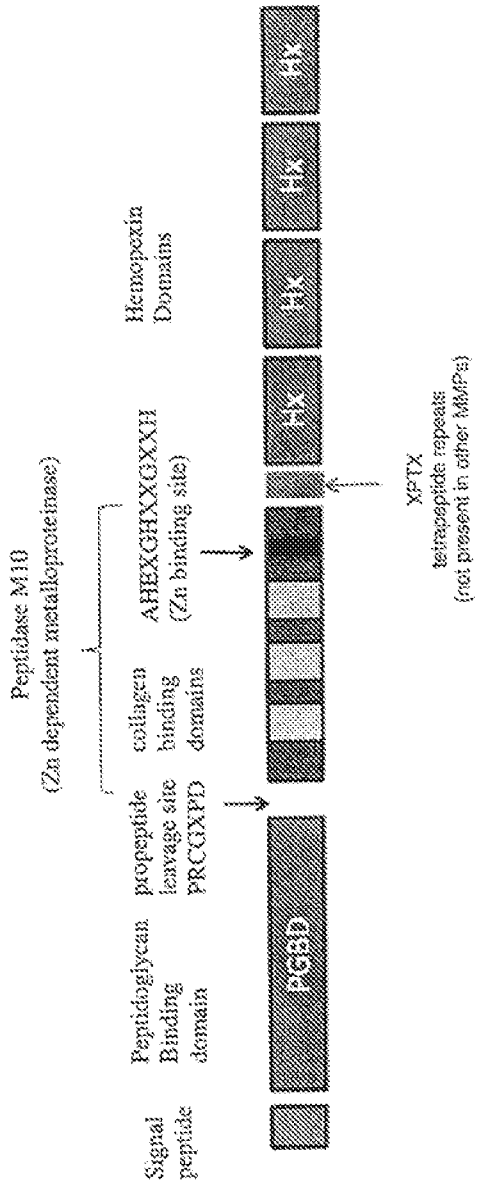
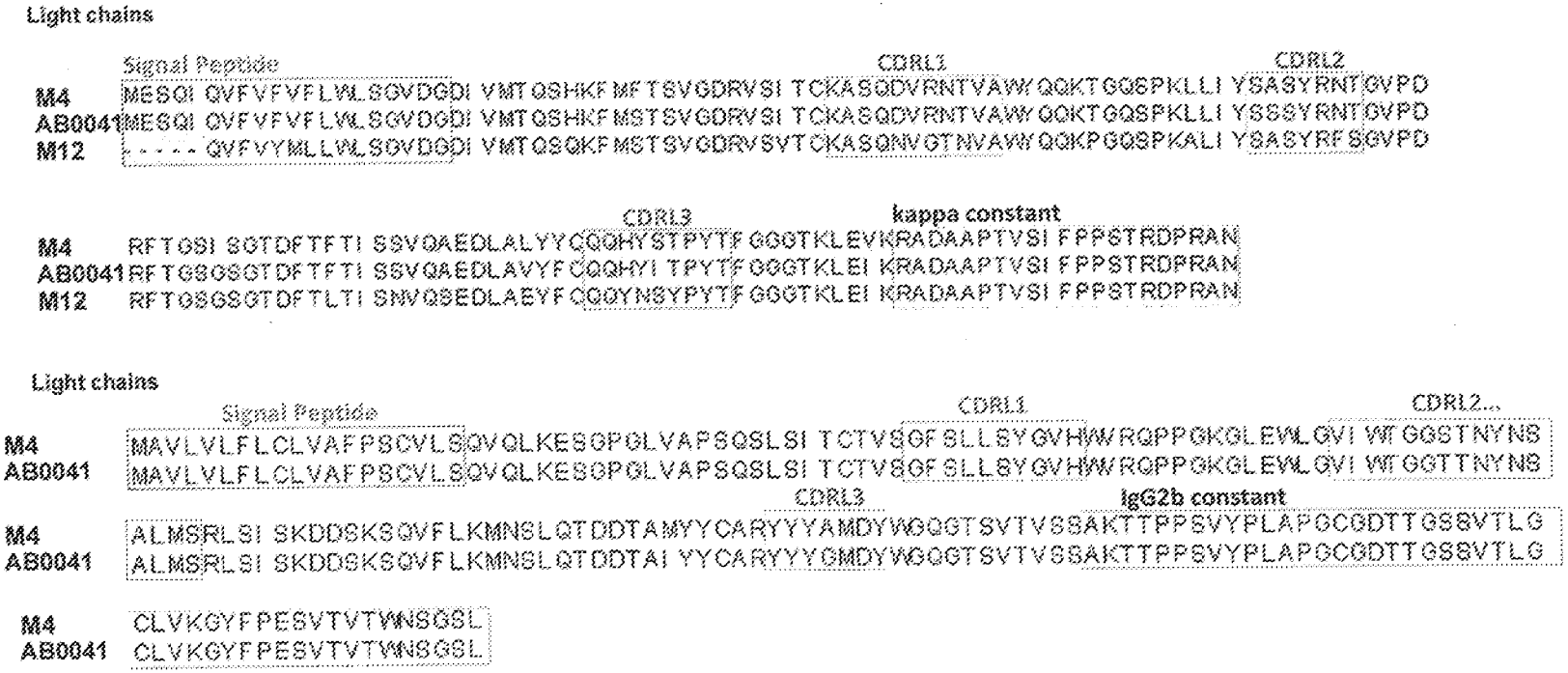


Figure 4: Comparison between AB0041, M4, and M12 heavy and light chains



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/027160

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/40 C12N9/64 A61K39/395
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009/111450 A2 (DYAX CORP [US]; DEVY LAETITIA [US]; BUCKLER DAVID [US]; COHEN EDWARD H) 11 September 2009 (2009-09-11) the whole document in particular abstract page 1, line 14 - page 12, line 21 claims 1-53; figures 1-10; examples 1-16	1-41
X A	WO 2007/094842 A2 (GENENTECH INC [US]; SIDHU SACHDEV S [US]; BIRTALAN SARA C [US]; FELLOU) 23 August 2007 (2007-08-23) the whole document in particular abstract figure 11A; sequence 413	1,19,32 2-18, 20-31, 33-41

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 October 2012	08/11/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Ferreira, Roger

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/027160

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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(19)中华人民共和国国家知识产权局

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(71)申请人 吉联亚生物科技有限公司
地址 美国加利福尼亚州

(72)发明人 维多莉亚·史密斯 斯科特·麦考利

(74)专利代理机构 北京律盟知识产权代理有限责任公司 11287
代理人 江葳

(51)Int.Cl.
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C12N 9/64(2006.01)
A61K 39/395(2006.01)

权利要求书3页 说明书29页
序列表30页 附图3页

(54)发明名称

抗基质金属蛋白酶9抗体

(57)摘要

本发明提供涉及结合到基质金属蛋白酶-9 MMP9蛋白(MMP9又称为明胶酶-B)的结合蛋白(例如抗体和其抗原结合片段)的组合物及使用方法,例如其中所述结合蛋白包含免疫球蛋白Ig重链(或其功能片段)和Ig轻链(或其功能片段)。

抗基质金属蛋白酶9人类化重链

AB0041	QVQLKESGPG LVAPSQSLSI TCTVSGFSLL SYGVHWVRQP PGKGLEWLGV	
重链可变区1	QVQLQESGPG LVKPSETLSL TCTVSGFSLL SYGVHWVRQP PGKGLEWLGV	
重链可变区2	QVQLQESGPG LVKPSETLSL TCTVSGFSLL SYGVHWVRQP PGKGLEWLGV	
重链可变区3	QVQLQESGPG LVKPSETLSL TCTVSGFSLL SYGVHWVRQP PGKGLEWLGV	
重链可变区4	QVQLQESGPG LVKPSETLSL TCTVSGFSLL SYGVHWVRQP PGKGLEWLGV	
AB0041	IWTGGTTNYN SALMSRLSIS KDDSKSQVFL KMNSLQIDDT AIYYCARYYY	
重链可变区1	IWTGGTTNYN SALMSRLTIS KDDSKSTVYL KMNSLKTEDT AIYYCARYYY	
重链可变区2	IWTGGTTNYN SALMSRLTIS KDDSKNTVYL KMNSLKTEDT AIYYCARYYY	
重链可变区3	IWTGGTTNYN SALMSRFTIS KDDSKNTVYL KMNSLKTEDT AIYYCARYYY	
重链可变区4	IWTGGTTNYN SALMSRFTIS KDDSKNTLYL KMNSLKTEDT AIYYCARYYY	
AB0041	GMDYWGQGTS VTVSS (SEQ ID NO:3)	
重链可变区1	GMDYWGQGTS VTVSS (SEQ ID NO:5)	
重链可变区2	GMDYWGQGTL VTVSS (SEQ ID NO:6)	
重链可变区3	GMDYWGQGTL VTVSS (SEQ ID NO:7)	
重链可变区4	GMDYWGQGTL VTVSS (SEQ ID NO:8)	

CN 104395345 A

1. 一种分离的抗体或其片段，其包含：具有重链互补决定区 CDR 的重链可变 VH 区，所述 CDR 具有选自由 SEQ ID NO:13、SEQ ID NO:14 及 SEQ ID NO:15 组成的群组的氨基酸序列。

2. 根据权利要求 1 所述的抗体或片段，其中所述 VH 区具有氨基酸序列为 SEQ ID NO:13 的重链 CDR1、氨基酸序列为 SEQ ID NO:14 的重链 CDR2 及氨基酸序列为 SEQ ID NO:15 的重链 CDR3。

3. 一种分离的抗体或其片段，其包含：具有轻链互补决定区 CDR 的轻链可变 VL 区，所述 CDR 具有选自由 SEQ ID NO:16、SEQ ID NO:17 及 SEQ ID NO:18 组成的群组的氨基酸序列。

4. 根据权利要求 3 所述的抗体或片段，其中所述 VL 区具有氨基酸序列为 SEQ ID NO:16 的轻链 CDR1、氨基酸序列为 SEQ ID NO:17 的轻链 CDR2 及氨基酸序列为 SEQ ID NO:18 的轻链 CDR3。

5. 根据权利要求 3 或 4 所述的抗体或片段，其中所述抗体另外包含具有氨基酸序列为 SEQ ID NO:13 的重链 CDR1、氨基酸序列为 SEQ ID NO:14 的重链 CDR2 及氨基酸序列为 SEQ ID NO:15 的重链 CDR3 的 VH 区。

6. 根据权利要求 1、2 及 5 中任一权利要求所述的抗体或片段，其中所述 VH 区具有 SEQ ID NO:1、SEQ ID NO:3、SEQ ID NO:5、SEQ ID NO:6、SEQ ID NO:7 或 SEQ ID NO:8 中所陈述的氨基酸序列。

7. 根据权利要求 6 所述的抗体或片段，其中所述 VH 区具有 SEQ ID NO:3、SEQ ID NO:5、SEQ ID NO:6、SEQ ID NO:7 或 SEQ ID NO:8 中所陈述的氨基酸序列。

8. 根据权利要求 3 到 7 中任一权利要求所述的抗体或片段，其中所述 VL 区具有 SEQ ID NO:2、SEQ ID NO:4、SEQ ID NO:9、SEQ ID NO:10、SEQ ID NO:11 或 SEQ ID NO:12 中所陈述的氨基酸序列。

9. 根据权利要求 8 所述的抗体或片段，其中所述 VL 区具有 SEQ ID NO:4、SEQ ID NO:9、SEQ ID NO:10、SEQ ID NO:11 或 SEQ ID NO:12 中所陈述的氨基酸序列。

10. 根据权利要求 5 所述的抗体或片段，其中所述 VH 区具有 SEQ ID NO:7 中所陈述的氨基酸序列并且所述 VL 区具有 SEQ ID NO:12 中所陈述的氨基酸序列。

11. 一种分离的抗体或其片段，其与具有含 SEQ ID NO:7 中所陈述的氨基酸序列的 VH 区和含 SEQ ID NO:12 中所陈述的氨基酸序列的 VL 区的抗体竞争结合到 MMP9。

12. 一种特异性结合到 MMP9 的表位的分离的抗体或其片段，其中所述表位包含在 MMP9 的区域内的氨基酸残基，所述区域由 SEQ ID NO:27 的残基 104-119、残基 159-166 或残基 191-202 组成。

13. 根据权利要求 12 所述的抗体或片段，其中所述表位包含在由 SEQ ID NO:27 的残基 104-119 组成的 MMP9 的区域内的氨基酸残基、在由 SEQ ID NO:27 的残基 159-166 组成的 MMP9 的区域内的氨基酸残基及在由 SEQ ID NO:27 的残基 191-202 组成的 MMP9 的区域内的氨基酸残基。

14. 根据权利要求 12 或 13 所述的抗体或片段，其中所述表位包含 SEQ ID NO:27 的 E111、D113、R162 或 I198。

15. 根据权利要求 14 所述的抗体或片段，其中所述表位包含 SEQ ID NO:27 的 R162。

16. 根据权利要求 14 所述的抗体或片段，其中所述表位包含 SEQ ID NO:27 的 E111、D113、R162 及 I198。

17. 根据权利要求 1 到 16 中任一权利要求所述的抗体或片段，其中所述抗体或片段抑制 MMP9 的酶活性。

18. 根据权利要求 17 所述的抗体或片段，其中所述酶活性的所述抑制是非竞争性的。

19. 根据权利要求 1 到 18 中任一权利要求所述的抗体或片段，其为人类或人类化的。

20. 根据权利要求 1 到 19 中任一权利要求所述的抗体或片段，其用于抑制个体中的 MMP9 活性的方法中，所述方法包含向所述个体投予有效抑制所述个体中的 MMP9 活性的量的所述抗体或片段。

21. 一种抑制个体中的 MMP9 活性的方法，所述方法包含向所述个体投予有效抑制所述个体中的 MMP9 活性的量的根据权利要求 1 到 19 中任一权利要求所述的抗体或片段。

22. 根据权利要求 21 所述的方法，其中所述抗体或其片段是以约 1mg/kg 到约 14mg/kg 的剂量静脉内投予。

23. 根据权利要求 21 所述的方法，其中所述抗体或片段是以约 4mg/kg 到约 28mg/kg 的剂量皮下投予。

24. 根据权利要求 21 到 23 中任一权利要求所述的方法，其中所述抗体或片段是每 7 到 28 天一次投予。

25. 一种分离的核酸，其包含 :编码包含具有 SEQ ID NO:13-15 中所陈述的氨基酸序列的 CDR 的重链多肽或包含具有 SEQ ID NO:16-18 中所陈述的氨基酸序列的 CDR 的轻链多肽的核苷酸序列。

26. 根据权利要求 25 所述的分离的核酸，其中所述核苷酸序列编码所述重链多肽，所述重链多肽具有选自由 SEQ ID NO:1、3 及 5-8 组成的群组的氨基酸序列。

27. 根据权利要求 25 或 26 所述的分离的核酸，其中所述核苷酸序列编码所述轻链多肽，所述轻链多肽具有选自由 SEQ ID NO:2、4 及 9-12 组成的群组的氨基酸序列。

28. 根据权利要求 25 到 27 中任一权利要求所述的分离的核酸，其中所述核苷酸序列包含选自由 SEQ ID NO:19-26 组成的群组的序列。

29. 根据权利要求 25 到 28 中任一权利要求所述的分离的核酸，其中所述核苷酸序列包含 SEQ ID NO:21 和 SEQ ID NO:26。

30. 一种载体，其包含根据权利要求 25 到 29 中任一权利要求所述的分离的核酸。

31. 一种细胞，其包含根据权利要求 30 所述的载体。

32. 一种医药组合物，其包含根据权利要求 1 到 19 中任一权利要求所述的抗体或其片段。

33. 一种医药组合物，其包含根据权利要求 30 所述的载体。

34. 一种医药组合物，其包含根据权利要求 31 所述的细胞。

35. 根据权利要求 32 到 34 中任一权利要求所述的医药组合物，其用于抑制个体中的 MMP9 活性的方法中，所述方法包含向所述个体投予有效抑制所述个体中的 MMP9 活性的量的所述医药组合物。

36. 一种抑制个体中的 MMP9 活性的方法，其包含向所述个体投予有效抑制所述个体中的 MMP9 活性的量的根据权利要求 32 到 34 中任一权利要求所述的医药组合物。

37. 一种检测来自个体的测试样品中的 MMP9 表达的方法，所述方法包含：

(a) 使所述测试样品与根据权利要求 1 到 19 中任一权利要求所述的抗体或片段接触；及

(b) 检测所述抗体或片段与所述样品中蛋白质的结合，由此检测 MMP9 的存在。

38. 根据权利要求 37 所述的方法，其另外包含将在所述测试样品中检测到的结合的量与所述抗体或片段结合到对照样品的量相比较。

39. 一种分离的多肽，其具有基本上由 SEQ ID NO:27 的残基 111-198 组成的氨基酸序列。

40. 一种分离的突变体 MMP9 多肽，其包含含有 SEQ ID NO:27 的残基 111-198 并且在残基 111、113、162 或 198 处具有氨基酸取代的氨基酸序列。

41. 根据权利要求 40 所述的分离的突变体 MMP9 多肽，其中所述氨基酸序列在 SEQ ID NO:27 的残基 111、113、162 及 198 处含有氨基酸取代。

抗基质金属蛋白酶 9 抗体

[0001] 有关经由 EFS-WEB 提交的序列表的参考

[0002] 如 MPEP § 1730II.B.2(a)(C) 中所批准并陈述的，以下经由 USPTO EFS-WEB 服务器提交的电子版序列表的完整内容以全文引用的方式并入本文中用于所有目的。所述序列表在电子提交的文本文档上标识如下：

[0003]

文件名	创建日期	大小（字节）
246102008540Seqlist	2012 年 2 月 29 日	65,102 字节

技术领域

[0004] 本发明属于细胞外酶、细胞外基质酶、蛋白酶及免疫学领域。

背景技术

[0005] 基质金属蛋白酶 (MMP) 属于涉及到细胞外基质形成和重塑的细胞外酶家族。这些酶含有一个保守的催化结构域，其中锌原子经三个组氨酸残基配位。已知这一家族的超过 20 个成员，组织成包括胶原酶类、明胶酶类、溶基质素类、基质溶解因子类、釉质溶解素类及膜 MMP 类在内的多个群组。

[0006] MMP2 和 MMP9 属于基质金属蛋白酶的明胶酶群组。除含有大多数 MMP 共有的信号肽、前肽、催化结构域、锌结合结构域及血红素结合蛋白样结构域外，明胶酶还含有多个纤连蛋白样结构域和一个 O- 糖基化结构域。

[0007] MMP 与多种疾病有关。然而，可用的 MMP 抑制剂部分由于毒性和缺乏功效而未取得成功。因此，需要具有特异性并且有效的 MMP 抑制剂。

发明内容

[0008] 本发明提供涉及结合到基质金属蛋白酶 -9(MMP9) 蛋白（又称为明胶酶 -B) 的结合蛋白（例如抗体和其抗原结合片段）的组合物和使用方法。所述结合蛋白通常为抗体或其片段（例如，抗原结合片段）并且通常含有免疫球蛋白 (Ig) 重链（或其功能片段）和 Ig 轻链（或其功能片段）。重链通常为 IgG，通常为人类 IgG（例如 IgG1 或 IgG4)，或其它 IgG（例如 IgG2)，或其经修饰型式。轻链通常为 κ 链。

[0009] MMP9 结合蛋白（例如抗体）为特异性结合到 MMP9 并且不结合其它基质金属蛋白酶的结合蛋白。这些 MMP9 结合蛋白可用于需要或希望获得对 MMP9 的特异性调节（例如，抑制)，例如而不直接地影响其它基质金属蛋白酶的活性的应用中。因此，在本发明的某些实施例中，抗 MMP9 抗体或其片段是 MMP9 活性的特异性抑制剂。在一些方面中，本文所揭示的 MMP9 结合蛋白将可用于抑制 MMP9，同时允许其它相关基质金属蛋白酶的正常功能。

[0010] 这些抗体和片段可参照其氨基酸序列或其部分，和 / 或各种功能（例如与 MMP9 或其特定表位的结合特异性，或与特定抗体竞争结合到 MMP9 上的表位的能力）和 / 或活性

（例如抑制 MMP9 的能力，例如非竞争性抑制）进行描述。

[0011] 所述抗体和片段包括具有重链可变 (VH) 区的那些，所述 VH 区具有含 SEQ ID NO:13、SEQ ID NO:14 或 SEQ ID NO:15 的氨基酸序列的重链互补决定区 (CDR)；具有轻链可变 (VL) 区的那些，所述 VL 区具有含 SEQ ID NO:16、SEQ ID NO:17 或 SEQ ID NO:18 的氨基酸序列的轻链互补决定区 (CDR)。示例性抗体和片段包括具有氨基酸序列为 SEQ ID NO:13 的重链 CDR1、氨基酸序列为 SEQ ID NO:14 的重链 CDR2 及氨基酸序列为 SEQ ID NO:15 的重链 CDR3 的那些；及具有 SEQ ID NO:15 的重链 CDR3 的那些。示例性抗体和片段另外包括具有氨基酸序列为 SEQ ID NO:16 的轻链 CDR1、氨基酸序列为 SEQ ID NO:17 的轻链 CDR2 及氨基酸序列为 SEQ ID NO:18 的轻链 CDR3 的那些；及具有氨基酸序列为 SEQ ID NO:18 的轻链 CDR3 的那些；以及具有 SEQ ID NO:13、14 及 15 的重链 CDR 和 SEQ ID NO:16、17 及 18 的轻链 CDR 的那些。

[0012] 示例性抗体和片段另外包括具有氨基酸序列为 SEQ ID NO:34 的重链 CDR1、氨基酸序列为 SEQ ID NO:35 的重链 CDR2 及氨基酸序列为 SEQ ID NO:36 的重链 CDR3 的那些；具有氨基酸序列为 SEQ ID NO:36 的重链 CDR3 的那些；具有氨基酸序列为 SEQ ID NO:37 的轻链 CDR1、氨基酸序列为 SEQ ID NO:38 的轻链 CDR2 及氨基酸序列为 SEQ ID NO:39 的轻链 CDR3 的那些；具有氨基酸序列为 SEQ ID NO:39 的轻链 CDR3 的那些；以及具有 SEQ ID NO:34、35 及 36 的重链 CDR 和 SEQ ID NO:37、38 及 39 的轻链 CDR 的那些。

[0013] 示例性抗体和片段另外包括具有氨基酸序列为 SEQ ID NO:42 的轻链 CDR1、氨基酸序列为 SEQ ID NO:43 的轻链 CDR2 及氨基酸序列为 SEQ ID NO:44 的轻链 CDR3 的那些；及具有氨基酸序列为 SEQ ID NO:44 的轻链 CDR3 的那些。

[0014] 所述抗体和片段另外包括具有含 SEQ ID NO:3、SEQ ID NO:5、SEQ ID NO:6、SEQ ID NO:7 或 SEQ ID NO:8 中所陈述的氨基酸序列的 VH 区的那些；及具有含 SEQ ID NO:4、SEQ ID NO:9、SEQ ID NO:10、SEQ ID NO:11 或 SEQ ID NO:12 中所陈述的氨基酸序列的 VL 区的那些；以及具有含 SEQ ID NO:3、SEQ ID NO:5、SEQ ID NO:6、SEQ ID NO:7 或 SEQ ID NO:8 中所陈述的氨基酸序列的 VH 区和含 SEQ ID NO:4、SEQ ID NO:9、SEQ ID NO:10、SEQ ID NO:11 或 SEQ ID NO:12 中所陈述的氨基酸序列的 VL 区的抗体和片段。在一个特定实例中，所述抗体或片段具有 SEQ ID NO:7 的 VH 区和 SEQ ID NO:12 的 VL 区，或与这些序列具有至少或约 75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或更高序列一致性。其另外包括具有含 SEQ ID NO:32 或 47 中所陈述的氨基酸序列的 VH 区的那些，及具有含 SEQ ID NO:33 中或 SEQ ID NO:41 中或 SEQ ID NO:48 中所陈述的氨基酸序列的 VL 区的那些，及其组合，以及与这些序列具有至少或约 75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或更高序列一致性的序列。

[0015] 所述抗体和片段另外包括具有含 SEQ ID NO:1 中所陈述的氨基酸序列的 VH 区，和 / 或具有含 SEQ ID NO:2 中所陈述的氨基酸序列的 VL 区，和 / 或与这些序列具有至少或约 75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或更高序列一致性的 VH 或 VL 区。

[0016] 在一些情形中，所述重链是由具有选自由 SEQ ID NO:19-22 组成的群组的核苷酸序列的多核苷酸编码，并且所述轻链是由具有选自由 SEQ ID NO:23-26 组成的群组的核苷酸序列的多核苷酸编码。

[0017] 在一些实施例中，所述抗体或其片段抑制（例如通过非竞争性抑制）MMP9 的酶活性。

[0018] 所述抗体和片段另外包括特异性结合到 MMP9 的指定表位的那些。在一些情形中，所述表位是由上述抗体中的任一种特异性结合的表位。在一个实例中，所述表位含有在 SEQ ID NO:27 的半胱氨酸开关活性袋外部的氨基酸残基（即，一或多个氨基酸残基）。在某些实例中，所述表位包括在 MMP9 的指定区域内的氨基酸残基（即，一或多个氨基酸残基），例如其中所述区域是 SEQ ID NO:27 的残基 104-202。在一些实例中，所述表位包括在 MMP9 的指定区域内的氨基酸残基（即，一或多个氨基酸残基），例如其中所述区域是 SEQ ID NO:27 的残基 104-119、残基 159-166 或残基 191-202。在一个实例中，所述表位包括在作为 SEQ ID NO:27 的残基 104-119 的 MMP9 区域内的氨基酸残基（即，一或多个氨基酸残基）、在作为 SEQ ID NO:27 的残基 159-166 的 MMP9 区域内的氨基酸残基，及在作为 SEQ ID NO:27 的残基 191-202 的 MMP9 区域内的氨基酸残基。在一些情形中，所述表位包括 SEQ ID NO:27 的 E111、D113、R162 或 I198。在一些情形中，其包括 SEQ ID NO:27 的 R162。在一些情形中，其包括 SEQ ID NO:27 的 E111、D113、R162 及 I198。

[0019] 在一些情形中，所述抗体或片段是人类或人类化的。

[0020] 在一些实例中，如在约 4℃、25℃、37℃或 42℃温度下所测量，所述抗体和片段以单克隆抗体、scFv、Fab 形式或其它抗体形式以等于或小于 100nM，任选地小于 10nM，任选地小于 1nM，任选地小于 0.5nM，任选地小于 0.1nM，任选地小于 0.01nM 或任选地小于 0.005nM，在某些实例中介于 0.1 与 0.2nM 之间，或介于 0.1 与 10pM 之间，例如介于 0.4 与 9pm 之间，例如介于 0.4 与 8.8pm 之间的解离常数 (K_d) 特异性结合到人类 MMP9。

[0021] 所提供的抗体和片段还是与上述抗体中任一种具有至少或约 75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或更高序列一致性，或含有与上述抗体的对应部分具有至少或约 75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或更高序列一致性的各种部分，例如具有与 SEQ ID NO:7 具此类一致性的 VH 区和与 SEQ ID NO:12 具此类一致性的 VL 区的那些。还提供了与上述抗体中的任一种竞争结合到 MMP9 的抗体，例如与具有含 SEQ ID NO:7 中所陈述的氨基酸序列的 VH 区和含 SEQ ID NO:12 中所陈述的氨基酸序列的 VL 区的抗体竞争结合到 MMP9 的抗体。

[0022] 还提供了编码所述抗体和片段的分离的核酸，例如包括上述抗体和片段中任一种的编码序列的核酸。所提供的核酸为含有一种核苷酸序列的核酸，所述核苷酸序列编码包含具有 SEQ ID NO:13-15 中所陈述的氨基酸序列的 CDR 的重链多肽和 / 或包含具有 SEQ ID NO:16-18 中所陈述的氨基酸序列的 CDR 的轻链多肽。在一个实例中，所述核苷酸序列编码所述重链多肽，所述重链多肽具有选自由 SEQ ID NO:1、3 及 5-8 组成的群组的氨基酸序列。在另一个实例中，所述核苷酸序列编码所述轻链多肽，所述轻链多肽具有选自由 SEQ ID NO:2、4 及 9-12 组成的群组的氨基酸序列。在一个实例中，所述核苷酸序列包括选自由 SEQ ID NO:19-26，例如 SEQ ID NO:21、SEQ ID NO:26 或 SEQ ID NO:21 及 26 组成的群组的序列。还提供了含有这些核酸的载体和包括其的细胞，例如宿主细胞。

[0023] 还提供了包括所述抗体、片段、核酸、载体及细胞的医药组合物。在一些实例中，所述医药组合物另外包括载体或赋形剂，例如医药学上可接受或生物学上可接受的载剂或赋形剂。在一些情形中，所述医药组合物被用于所提供的治疗方法和应用中。

[0024] 还提供了所述抗体、片段、核酸、载体、细胞及组合物例如用于治疗学（例如抑制个体中的 MMP9）和诊断学（例如用于检测个体中的 MMP9）中的方法和用途。

[0025] 举例来说，提供了涉及 MMP9 的检测的诊断和预后方法，及用于这些方法中的药剂（例如上述抗 MMP9 抗体和其它 MMP9 结合蛋白中的任一种）。在一些情形中，所述诊断方法检测来自个体的测试样品中的 MMP9 表达。这些方法可例如通过使测试样品与如本文所述的抗体或片段（例如上述抗体或片段中的任一种）接触并检测所述抗体或片段与所述样品中蛋白质的结合，由此检测 MMP9 的存在来进行。在一些情形中，首先获得或提供样品。在一些实例中，所述方法包括将所检测的 MMP9 的量或水平与对照水平或量相比较，例如通过将在所述测试样品中检测到的结合的量与所述抗体或片段结合到对照样品的量相比较。在一些情形中，所述方法涉及简单地比较 MMP9 的测试水平与对照水平。在一些情形中，较高的测试水平（如与对照水平相比较）指示疾病或病况。

[0026] 在一些情形中，由所述方法检测到 MMP9 指示所述个体中疾病或病况的存在，例如 MMP9 相关性疾病或病况。在一些情形中，所述方法另外包括基于所述方法的结果，例如基于在所述样品中检测到的 MMP9 的水平，治疗所述个体，或调整（即，改变或中断）所述个体的治疗。生物样品为组织、从这些组织分离的细胞等。在一些情形中，所述方法是对例如血液、血浆、血清、全血、唾液、尿液或精液等液体样品进行的。组织样品包括例如福尔马林固定或冷冻的组织切片。

[0027] 还提供了例如使用非竞争性抑制 MMP9 的药剂抑制个体中的 MMP9 活性和 / 或治疗个体的疾病或病况的方法，及用于这些方法中的药剂（例如上述抗 MMP9 抗体和其它 MMP9 结合蛋白中的任一种）。所述方法一般是通过向个体投予例如有效量的 MMP9 结合蛋白，例如，如本文所提供的 MMP9 结合抗体或其片段来进行。所述抗体或片段一般特异性结合到并且非竞争性抑制 MMP9，例如，由此使个体中的 MMP9 活性得到抑制。在一些情形中，所述抗体或片段为结合在半胱氨酸开关活性袋外部的 MMP9，例如结合于上述表位之一中的抗体或片段。在一些情形中，所述抗体或片段实质上不结合到除 MMP9 外的 MMP 蛋白和 / 或实质上不结合到 MMP2。

[0028] 所述个体一般是患有疾病或病况，通常与 MMP9 表达和 / 或活性的增加或降低有关的疾病或病况者。在某些情形中，所述个体是患有与 MMP9 表达和 / 或活性增加有关的疾病或病况者。在其它情形中，所述个体是患有与 MMP9 表达和 / 或活性降低有关的疾病或病况者。

[0029] 还提供了 MMP9 多肽，包括突变体 MMP9 多肽，例如含有 SEQ ID NO:27 的残基 111-198 的多肽，及具有含 SEQ ID NO:27 的残基 111-198 并且在 SEQ ID NO:27 的残基 111、113、162 或 198 处具有氨基酸取代或在所有这些残基处具有氨基酸取代的氨基酸序列的多肽。

[0030] 还提供了上述抗体、核酸、载体、细胞及组合物中的任一种在上述治疗和诊断方法中的用途。

附图说明

[0031] 图 1 示出了小鼠单克隆抗 MMP9 抗体 (AB0041) 的重链可变区的氨基酸序列，以及重链的人类化变体 (VH1-VH4) 的氨基酸序列，其经比对以显示由人类化引起的构架氨基酸

序列的差异。CDR 是以斜体显示，并且人类化变体中与亲本小鼠单克隆抗体不同的氨基酸加下划线。

[0032] 图 2 示出了小鼠单克隆抗 MMP9 抗体 (AB0041) 的轻链可变区的氨基酸序列，以及这一轻链的人类化变体 (VH1-VH4) 的氨基酸序列，其经比对以显示由人类化引起的构架氨基酸序列的差异。CDR 是以斜体显示，并且人类化变体中与亲本小鼠单克隆抗体不同的氨基酸加下划线。

[0033] 图 3 示出了 MMP9 蛋白的示意图。

[0034] 图 4 示出了指定为 AB0041、M4 及 M12 的抗体的重链和轻链的氨基酸序列之间的比较。

具体实施方式

[0035] 除非另作指示，否则本发明的实践采用了细胞生物学、毒理学、分子生物学、生物化学、细胞培养、免疫学、肿瘤学、重组 DNA 领域及的相关领域中的标准方法和常规技术，其在所属领域的技术范围内。这些技术描述于文献中并由此是所属领域技术人员可用的。参见例如，艾伯特 B.(Alberts,B.) 等人，“细胞分子生物学 (Molecular Biology of the Cell),”第 5 版，加兰德科技出版社 (Garland Science)，纽约州纽约 (New York,NY),2008；沃特 D.(Voet,D.) 等人，“基础生物化学：在分子水平上的生命 (Fundamentals of Biochemistry:Life at the Molecular Level),” 第 3 版，约翰威立出版公司 (John Wiley&Sons)，新泽西州霍博肯 (Hoboken,NJ),2008；萨姆布鲁克 J.(Sambrook,J.) 等人，“分子克隆实验指南 (Molecular Cloning:A Laboratory Manual),”第 3 版，冷泉港实验室出版社 (Cold Spring Harbor Laboratory Press),2001；奥苏贝尔 F.(Ausubel,F.) 等人，“现代分子生物学实验技术 (Current Protocols in Molecular Biology),”约翰威立出版公司 (John Wiley&Sons)，纽约 (New York),1987 及定期更新；弗雷谢尼 R.I.(Freshney,R.I.)，“动物细胞培养：基本技术指南 (Culture of Animal Cells:A Manual of Basic Technique),”第 4 版，约翰威立出版公司，新泽西州桑莫塞县 (Somerset,NJ),2000；及丛书“酶学方法 (Methods in Enzymology),”学术出版社 (Academic Press)，加利福尼亚州圣地亚哥 (San Diego,CA)。还参见例如，“现代免疫学实验技术 (Current Protocols in Immunology),”(R. 凯克 (R.Coico)，丛书编辑)，威立公司 (Wiley),2010 年 8 月最新更新。

[0036] 某些 MMP 在肿瘤生长、转移、炎症、自体免疫性及血管疾病中起到作用。参见例如，胡 (Hu) 等人 (2007) 自然评述：药物发现 (Nature Reviews:Drug Discovery)6:480-498。因此，在某些治疗环境中需要抑制一或多种特定 MMP 的活性。尽管在序列水平上共有显著同源性，但 MMP9 和 MMP2 两种明胶酶的表达和功能作用明显不同。MMP9 表达是由多种疾病相关性细胞因子和生长因子诱导的。另外，MMP9 基因敲除小鼠在多种疾病模型中得到保护，而 MMP2 的表达更具组成性并且 MMP2 基因消除动物倾向于具有极少保护作用。一些研究已显示，MMP2 基因敲除小鼠在激发模型中展现较差的疾病。对于一些疾病或病症，超过一种 MMP 的活性受到抑制。在临床研究中，针对超过一种 MMP 的抑制剂已引起非所需的不良作用，例如毒性或缺乏功效。经显示，某些 MMP(例如 MMP2) 的活性通常是正常组织动态平衡所需的和 / 或防止疾病。某些可用的 MMP 抑制剂已引起副作用。

[0037] 所提供的实施例有特异性抑制单一 MMP 或多种所选 MMP（例如 MMP9）的催化活性并且不与某些其它 MMP 或任何其它 MMP 反应或抑制某些其它 MMP 或任何其它 MMP 的药剂，包括治疗剂，例如抗体和其抗原结合片段。所提供的实施例还有其用于各种疾病的治疗的方法和用途。

[0038] MMP9 结合蛋白

[0039] 本发明提供了结合到基质金属蛋白酶 -9(MMP9) 蛋白 (MMP9 又称为明胶酶 -B)，例如人类 MMP9，例如具有 SEQ ID NO:27 或 SEQ ID NO:28 中所陈述的氨基酸序列的人类 MMP9 的结合蛋白，例如抗体和其片段（例如抗原结合片段）。本发明的结合蛋白一般包含免疫球蛋白 (Ig) 重链（或其功能片段）和 Ig 轻链（或其功能片段）。

[0040] 本发明还提供了特异性结合到 MMP9 并且不结合例如 MMP1、MMP2、MMP3、MMP7、MMP9、MMP10、MMP12 及 MMP13 等其它基质金属蛋白酶的 MMP9 结合蛋白。因此，此类特异性 MMP9 结合蛋白一般不与非 MMP9 基质金属蛋白酶显著地或可检测地交叉反应。特异性结合 MMP9 的 MMP9 结合蛋白可用于需要或希望获得对 MMP9 的特异性调节（例如，抑制），例如而不直接地影响其它基质金属蛋白酶的活性的应用中。

[0041] 在本发明的某些实施例中，抗 MMP9 抗体为 MMP9 活性的一种抑制剂并且可为特异性 MMP9 抑制剂。在一个实施例中，本文所揭示的 MMP9 结合蛋白可用于抑制 MMP9，同时不影响其它基质金属蛋白酶。“MMP 抑制剂”或“MMP9 活性的抑制剂”可为直接或间接抑制 MMP9 活性，包括（但不限于）酶加工、抑制 MMP9 对其底物的作用（例如，通过抑制底物结合、底物裂解等）等的抗体或其抗原结合片段。

[0042] 本发明还提供了特异性结合到非小鼠 MMP9（例如人类 MMP9、食蟹猴 MMP9 及大鼠 MMP9）的 MMP9 结合蛋白。

[0043] 本发明还提供了充当非竞争性抑制剂的 MMP9 结合蛋白（例如抗 MMP9 抗体和其功能片段）。“非竞争性抑制剂”是指结合于远离酶的底物结合位点的位点，并因此可结合所述酶并且影响抑制活性，而不管所述酶是否结合到其底物的一种抑制剂。非竞争性或变构抑制作用一般与底物缔合或浓度无关。此类非竞争性抑制剂可例如提供一定水平的抑制作用，所述抑制作用可与底物浓度实质上无关。

[0044] 本发明的 MMP9 结合蛋白（例如抗体和其功能片段）包括结合 MMP9，特别是人类 MMP9，并且具有与本文所揭示的重链多肽具有至少约 80％、85％、90％、95％或更高氨基酸序列一致性的重链多肽（或其功能片段）的结合蛋白。

[0045] 本发明的 MMP9 结合蛋白（例如抗体和其功能片段）包括结合 MMP9，特别是人类 MMP9，并且具有与本文所揭示的重链多肽具有至少约 80％、85％、90％、95％或更高氨基酸序列一致性的轻链多肽（或其功能片段）的结合蛋白。

[0046] 本发明的 MMP9 结合蛋白（例如抗体和其功能片段）包括结合 MMP9，特别是人类 MMP9，并且具有如本文所揭示的重链多肽的互补决定区（“CDR”）的重链多肽（或其功能片段）以及轻链多肽（或其功能片段）的 CDR 的结合蛋白。

[0047] 如本文所使用，“同源性”或“一致性”或“相似性”在核酸和多肽的情形中是指分别基于氨基酸序列或核酸序列比对，两个多肽或两个核酸分子之间的关系。同源性和一致性可各自通过比较出于比较的目的对准的每个序列的位置来测定。当所比较的序列中的相同位置被相同碱基或氨基酸占据时，则这些分子在所述位置相同；当相同位点被相同或相

似的氨基酸残基（例如空间性和/或电子性质类似）占据时，则这些分子可称为在所述位置同源（相似）。以同源性/相似性或一致性百分比表示是指在多个位置处所比较的序列共有的一致或相似氨基酸的数量的函数。在比较两个序列时，残基（氨基酸或核酸）的不存在或额外残基的存在也会降低一致性和同源性/相似性。

[0048] 如本文所使用，“一致性”意谓当将两个或两个以上序列对准以使序列匹配达到最大（即，考虑空位和插入）时，在这些序列中相应位置处一致核苷酸或氨基酸残基的百分比。序列一般在指定区域内，例如至少约20、25、30、35、40、45、50、55、60、65或更多个氨基酸或核苷酸长的区域内对准以达到最大对应，并且可长达参考氨基酸或核苷酸的全长。为进行序列比较，通常一个序列充当参考序列，以与测试序列相比较。当使用序列比较算法时，将测试序列和参考序列输入计算机程序中，必要时，指定子序列座标，并且指定序列算法的程序参数。随后序列比较算法基于指定的程序参数计算测试序列相对于参考序列的序列一致性百分比。

[0049] 适用于测定序列一致性百分比的算法的实例为BLAST和BLAST 2.0算法，其分别描述于奥兹楚尔(Altschul)等人(1990)分子生物学杂志(J.Mol.Biol.)215:403-410及奥兹楚尔等人(1977)核酸研究(Nucleic Acids Res.)25:3389-3402中。执行BLAST分析的软件可经由国家生物技术资讯中心(National Center for Biotechnology Information；www.ncbi.nlm.nih.gov)公开获得。其它示例性算法包括ClustalW(海金思D.(Higgins D.)等人(1994)核酸研究(Nucleic Acids Res)22:4673-4680)，在www.ebi.ac.uk/Tools/clustalw/index.html上可得。

[0050] 不一致的残基位置可因保守氨基酸取代而不同。保守氨基酸取代是指具有类似侧链的残基的可互换性。举例来说，一组具有脂肪族侧链的氨基酸为甘氨酸、丙氨酸、缬氨酸、亮氨酸和异亮氨酸；一组具有脂肪族羟基侧链的氨基酸为丝氨酸和苏氨酸；一组具有含酰胺的侧链的氨基酸为天冬酰胺和谷氨酰胺；一组具有芳香族侧链的氨基酸为苯丙氨酸、酪氨酸及色氨酸；一组具有碱性侧链的氨基酸为赖氨酸、精氨酸和组氨酸；并且一组具有含硫侧链的氨基酸为半胱氨酸和甲硫氨酸。

[0051] 两个核酸之间的序列一致性也可以关于两个分子彼此在严格条件下的杂交来描述。杂交条件是遵循所属领域中的标准方法选择（参见例如，萨姆布鲁克(Sambrook)等人，分子克隆实验指南，第二版，(1989)纽约冷泉港(Cold Spring Harbor,N.Y.))。严格杂交条件的一个实例是在50℃或更高温度及0.1×SSC(15mM氯化钠/1.5mM柠檬酸钠）下杂交。严格杂交条件的另一个实例为在42℃下于以下溶液中过夜培育：50％甲酰胺、5×SSC(150mM NaCl、15mM柠檬酸三钠）、50mM磷酸钠(pH7.6)、5×丹哈特氏溶液(Denhardt's solution)、10％硫酸葡聚糖及20mg/ml变性剪切的鲑鱼精DNA；随后在约65℃下于0.1×SSC中洗涤过滤器。严格杂交条件是至少与上述代表性条件同样严格的杂交条件，其中如果这些条件与上述特定严格条件至少约80％同样严格，通常至少90％同样严格，就认为其为至少同样严格的。

[0052] 因此，本发明提供了例如这样一些抗体或其抗原结合片段，其包含与本文所述的重链可变区的氨基酸序列(例如SEQ ID NO:1或5-8)具有至少80％、85％、90％、95％或更高氨基酸序列一致性的重链可变区多肽，及与如本文所陈述的轻链多肽的氨基酸序列(例如SEQ ID NO:2或9-12)具有至少80％、85％、90％、95％或更高氨基酸序列一致性的可变

轻链多肽。

[0053] 本发明的抗 MMP9 抗体的实例将于以下更详细地描述。

[0054] 抗体

[0055] MMP9 结合蛋白包括抗体和其功能片段，例如特异性结合到 MMP9 的那些。如本文所使用，术语“抗体”意谓包含特异性结合抗原表位的肽序列（例如可变区序列）的分离的或重组多肽结合剂。所述术语是以其最广泛意义使用并且特别涵盖单克隆抗体（包括全长单克隆抗体）、多克隆抗体、人类抗体、人类化抗体、嵌合抗体、纳米抗体、双功能抗体、多特异性抗体（例如双特异性抗体），及抗体片段，包括（但不限于）Fv、scFv、Fab、Fab’、$F(ab')_2$ 及 Fab_2，只要其展现所需的生物活性即可。术语“人类抗体”是指除可能的非人类 CDR 区外，含有人类来源的序列的抗体，并且并非暗指存在免疫球蛋白分子的完整结构，只是指所述抗体在人体中具有极少免疫原性作用（即，不会诱导产生针对其自身的抗体）。

[0056] “抗体片段”包含全长抗体的一部分，例如全长抗体的抗原结合区或可变区。此类抗体片段也可以在本文中称为“功能片段”或“抗原结合片段”。抗体片段的实例包括 Fab、Fab’、$F(ab')_2$ 及 Fv 片段；双功能抗体；线性抗体（扎帕塔 (Zapata) 等人 (1995) 蛋白质工程 (Protein Eng.)8(10):1057-1062)；单链抗体分子；及由抗体片段形成的多特异性抗体。木瓜蛋白酶消化抗体产生两个相同的抗原结合片段，称为“Fab”片段，其各自具有单个抗原结合位点；及一个残留的“Fc”片段，其名称反映了易于结晶的能力。胃蛋白酶处理得到 $F(ab')_2$ 片段，其具有两个抗原组合位点并且仍能够交联抗原。

[0057] “Fv”是含有完整抗原识别和结合位点的最小抗体片段。这一区域是由紧密非共价缔合的一个重链可变结构域和一个轻链可变结构域构成的二聚物组成。在这种构型中，每个可变结构域的三个互补决定区 (CDR) 相互作用以在 V_H-V_L 二聚物的表面上界定抗原结合位点。六个 CDR 共同地赋予抗体抗原结合特异性。然而，每个单一可变结构域（或仅包含对于抗原具特异性的六个 CDR 中的三个的分离的 V_H 或 V_L 区）能够识别并结合抗原，不过亲和力一般低于完整 F_v 片段。

[0058] “F_{ab}”片段除重链和轻链可变区外，还含有轻链恒定结构域和重链的第一恒定结构域 (CH_1)。Fab 片段最初是在木瓜蛋白酶消化抗体之后观察到。Fab’ 片段与 Fab 片段的不同之处在于，F(ab’) 片段在重链 CH_1 结构域的羧基末端处含有若干其它残基，包括抗体铰链区中的一或多个半胱氨酸。$F(ab')_2$ 片段含有在铰链区附近通过二硫键接合的两个 Fab 片段，并且最初是在胃蛋白酶消化抗体之后观察到。Fab’-SH 在本文中指定为 Fab’ 片段，其中恒定结构域的半胱氨酸残基带有游离硫醇基。抗体片段的其它化学偶合也是已知的。

[0059] 来自任何脊椎动物物种的抗体（免疫球蛋白）的“轻链”基于其恒定结构域的氨基酸序列可以被分配到两种明显相异的类型之一中，称为 κ 和 λ。取决于重链恒定结构域的氨基酸序列，可将免疫球蛋白分为五个主要类别：IgA、IgD、IgE、IgG 及 IgM，并且这些类别中的若干类别可进一步细分成子类（同型），例如 IgG1、IgG2、IgG3、IgG4、IgA1 及 IgA2。

[0060] “单链 Fv”或“sFv”或“scFv”抗体片段包含抗体的 V_H 和 V_L 结构域，其中这些结构域存在于单一多肽链中。在一些实施例中，Fv 多肽进一步包含在 V_H 与 V_L 结构域之间的多肽连接子，所述连接子使 sFv 能够形成抗原结合所需的结构。关于 sFv 的评述，参见普鲁可敦 (Pluckthun)，单克隆抗体药理学 (The Pharmacology of Monoclonal Antibodies)，第 113 卷（罗森博格 (Rosenburg) 和摩尔 (Moore) 编）斯普林格-维拉格出

版社 (Springer-Verlag), 纽约 (New York), 第 269-315 页 (1994)。

[0061] 术语“双功能抗体”是指具有两个抗原结合位点的小抗体片段，这些片段包含连接到同一多肽链 (V_H-V_L) 中的轻链可变结构域 (V_L) 的重链可变结构域 (V_H)。通过使用太短而无法使同一链中的两个结构域之间配对的连接子，迫使这些结构域与另一条链的互补结构域配对，由此产生两个抗原结合位点。双功能抗体另外描述于例如 EP404,097 ;WO 93/11161 ;及霍林格 (Hollinger) 等人 (1993) 美国国家科学院院刊 (Proc.Natl.Acad. Sci.USA)90:6444-6448 中。

[0062] “分离的”抗体为从自然环境的组分鉴别并且分离和 / 或回收的抗体。其自然环境的组分可包括酶、激素及其它蛋白质或非蛋白质溶质。在一些实施例中，分离的抗体被纯化 (1) 达到如通过劳里法 (Lowry method) 所测定的超过 95 重量%，例如超过 99 重量%的抗体 ;(2) 达到例如通过使用旋杯式顺序分析仪足以获得 N 末端或内部氨基酸序列的至少 15 个残基的程度 ;或 (3) 达到通过在还原或非还原条件下进行凝胶电泳 (例如 SDS-PAGE) 并且通过考马斯蓝 (Coomassie blue) 或银染色检测的均质性。术语“分离的抗体”包括一种在重组细胞内原位的抗体，因为所述抗体的自然环境的至少一种组分将不存在。在某些实施例中，分离的抗体是通过至少一个纯化步骤制备。

[0063] 如本文所使用，“免疫反应性”是指对氨基酸残基的序列 (“结合位点”或“表位”) 具有特异性，但又与其它肽 / 蛋白质交叉反应，在将其调配用于投予人类使用的水平下无毒的抗体或其片段。“表位”是指抗原中能够与抗体或其抗原结合片段形成结合相互作用的部分。表位可为线性肽序列 (即，“连续的”) 或可由非邻接的氨基酸序列 (即，“构象”或“不连续”) 构成。术语“优先结合”意谓结合剂结合到结合位点的亲和力高于其结合不相关氨基酸序列的亲和力。

[0064] 抗 MMP9 抗体可关于重链和轻链的 CDR 进行描述。如本文所使用，术语“CDR”或“互补决定区”打算意谓在重链多肽与轻链多肽的可变区内发现的非邻接抗原组合位点。这些特定区域已描述于卡贝特 (Kabat) 等人，生物化学杂志 (J.Biol. Chem.)252:6609-6616(1977) ;卡贝特等人，美国卫生和公众服务部 (U.S.Dept.of Health and Human Services), “免疫相关蛋白质的序列 (Sequences of proteins of immunological interest)”(1991) ;查赛 (Chothia) 等人，分子生物学杂志 (J.Mol.Biol.)196:901-917(1987) ;及麦克卡伦 (MacCallum) 等人，分子生物学杂志 ,262:732-745(1996) 中，其中当彼此相比较时，这些定义包括氨基酸残基的重叠或亚组。尽管如此，用以指抗体或移植抗体或其变体的 CDR 的任一定义的应用打算在如本文所定义和使用的术语的范围内。涵盖如以上引用的每一参考文献所定义的 CDR 的氨基酸残基陈述于下表 1 中供比较。

[0065] 表 1 :CDR 定义

[0066]

	卡贝特[1]	查赛[2]	麦克卡伦[3]
V_H CDR1	31-35	26-32	30-35
V_H CDR2	50-65	53-55	47-58
V_H CDR3	95-102	96-101	93-101
V_L CDR1	24-34	26-32	30-36
V_L CDR2	50-56	50-52	46-55
V_L CDR3	89-97	91-96	89-96

[0067] [1] 残基编号遵循卡贝特等人（同上文）的命名法

[0068] [2] 残基编号遵循查赛等人（同上文）的命名法

[0069] [3] 残基编号遵循麦克卡伦等人（同上文）的命名法

[0070] 如本文所使用，当提及抗体可变区时使用的术语“构架”打算意谓在抗体可变区内的除 CDR 区外的所有氨基酸残基。可变区构架一般是长度在约 100-120 个氨基酸之间的不连续氨基酸序列，但打算仅提到除 CDR 外的氨基酸。如本文所使用，术语“构架区”打算意谓所述构架被 CDR 分开的每个结构域。

[0071] 在一些实施例中，抗体是人类化抗体或人类抗体。人类化抗体包括来自受体互补决定区 (CDR) 的残基被来自非人类物种（供体抗体；例如小鼠、大鼠或兔）的 CDR 的残基置换的人类免疫球蛋白（受体抗体），其具有所需特异性、亲和力和能力。因此，非人类（例如，鼠类）抗体的人类化形式为含有源自于非人类免疫球蛋白的最小序列的嵌合免疫球蛋白。非人类序列主要位于可变区中，特别是互补决定区 (CDR) 中。在一些实施例中，人类免疫球蛋白的 Fv 构架残基被相应非人类残基置换。人类化抗体也可包含在受体抗体和引入的 CDR 或构架序列中都未发现的残基。在某些实施例中，人类化抗体包含实质上所有的至少一个并且通常两个可变结构域，其中所有或实质上所有的 CDR 都与非人类免疫球蛋白的 CDR 对应并且所有或实质上所有的构架区都为人类免疫球蛋白共同序列的构架区。出于本发明的目的，人类化抗体也可包括免疫球蛋白片段，例如 Fv、Fab、Fab’、$F(ab')_2$ 或抗体的其它抗原结合子序列。

[0072] 人类化抗体还可包含免疫球蛋白恒定区 (Fc) 的至少一部分，通常为人类免疫球蛋白恒定区的至少一部分。例如，参见琼斯 (Jones) 等人 (1986) 自然 (Nature)321:522-525；瑞奇曼 (Riechmann) 等人 (1988) 自然 332:323-329；及普雷斯塔 (Presta)(1992) 结构生物学新观点 (Curr.Op.Struct.Biol.)2:593-596。

[0073] 用于使非人类抗体人类化的方法是所属领域中已知的。一般来说，人类化抗体在其中引入了一或多个来自非人类来源的氨基酸残基。这些非人类氨基酸残基通常称为“输入”或“供体”残基，这些残基通常是从“输入”或“供体”可变结构域获得。举例来说，人类化可基本上根据维特 (Winter) 和其同事的方法，通过用啮齿动物 CDR 或 CDR 序列取代人类抗体的相应序列来进行。例如，参见琼斯等人，同上文；瑞奇曼等人，同上文；维霍恩 (Verhoeyen) 等人 (1988) 科学 (Science)239:1534-1536。因此，此类“人类化”抗体包括嵌合抗体（美国专利第 4,816,567 号），其中实质上少于完整人类可变结构域被来自非人类物种的相应序列取代。在某些实施例中，人类化抗体是一些 CDR 残基和任选地一些构架区残基被来自啮齿动物抗体（例如鼠类单克隆抗体）中类似位点的残基取代的人类抗体。

[0074] 人类抗体也可例如通过使用噬菌体展示文库来产生。霍格波姆 (Hoogenboom) 等人 (1991) 分子生物学杂志 (J.Mol.Biol),227:381；马克斯 (Marks) 等人 (1991) 分子生物

学杂志,222:581。用于制备人类单克隆抗体的其它方法描述于科勒(Cole)等人(1985)“单克隆抗体和癌症疗法(Monoclonal Antibodies and Cancer Therapy),”艾伦R.利斯(Alan R.Liss),第77页;及博纳(Boerner)等人(1991)免疫学杂志(J.Immunol.)147:86-95中。

[0075] 还可以通过将人类免疫球蛋白基因座引入转基因动物(例如小鼠)中来制备人类抗体,在这些转基因动物中,已经使内源免疫球蛋白基因部分地或完全地失活。在免疫激发之后,观察到人类抗体的产生,其在所有方面都与在人类所见非常类似,包括基因重排、组装及抗体谱系。这一方法描述于例如美国专利第5,545,807号、第5,545,806号、第5,569,825号、第5,625,126号、第5,633,425号、第5,661,016号,以及以下科技出版物中:马克斯(Marks)等人(1992)生物技术(Bio/Technology)10:779-783(1992);朗伯格(Lonberg)等人(1994)自然,368:856-859;莫里森(Morrison)(1994)自然,368:812-813;费西沃德(Fishwald)等人(1996)自然-生物技术(Nature Biotechnology)14:845-851;纽伯格(Neuberger)(1996)自然-生物技术,14:826;及朗伯格等人(1995)国际免疫学评论(Intern.Rev.Immunol.)13:65-93。

[0076] 抗体可使用如以上所描述的已知的选择和/或诱变方法进行亲和力成熟。在一些实施例中,亲和力成熟的抗体的亲和力是用于制备所述成熟抗体的起始抗体(一般为鼠类、兔、鸡、人类化或人类抗体)的亲和力的五倍或更高、十倍或更高、二十倍或更高,或三十倍或更高。

[0077] 抗体还可以为双特异性抗体。双特异性抗体是单克隆的,并且可为对至少两种不同抗原具有结合特异性的人类或人类化抗体。在本情形中,所述两种不同结合特异性可针对两种不同的MMP,或针对单一MMP(例如MMP9)上的两个不同表位。

[0078] 如本文所揭示的抗体还可以是免疫偶联物。所述免疫偶联物包含与第二分子(例如报导子)偶联的抗体(例如针对MMP9的抗体)。免疫偶联物还可包含与细胞毒性剂(例如化学治疗剂)、毒素(例如,细菌、真菌、植物或动物起源的酶活性毒素,或其片段)或放射性同位素(即,放射性偶联物)偶联的抗体。

[0079] “特异性结合到”特定多肽或表位,或对特定多肽或表位“具特异性”的抗体是指所述抗体选择性结合到目标抗原或表位;这些术语及用于确定特异性结合的方法是所属领域中众所周知的。如果抗体结合特定目标抗原或表位的亲和力、亲合力、容易性和/或持续时间高于其与其它物质的结合,那么其展现对所述目标抗原或表位的“特异性结合”。在一些实施例中,特异性结合到多肽或表位的抗体是结合到所述特定多肽或表位,而不实质上结合到任何其它多肽或多肽表位的抗体。

[0080] 在一些实施例中,如在约4℃、25℃、37℃或42℃温度下所测量,所提供的抗体以单克隆抗体、scFv、Fab形式或其它抗体形式以等于或小于100nM,任选地小于10nM,任选地小于1nM,任选地小于0.5nM,任选地小于0.1nM,任选地小于0.01nM或任选地小于0.005nM,在某些实例中介于0.1与0.2nM之间,或介于0.1与10pM之间,例如介于0.4与9pm之间,例如介于0.4与8.8pm之间的解离常数(K_d)特异性结合到人类MMP9。

[0081] 在某些实施例中,本发明的抗体结合到MMP9中的一或多个加工位点(例如,蛋白水解裂解位点),由此有效地阻断酶原或前酶原加工成催化活性酶,并且因此降低MMP9的蛋白水解活性。

[0082] 在某些实施例中,根据本发明的抗体结合到MMP9的亲和力是其对另一MMP的结合

亲和力的至少 2 倍、至少 5 倍、至少 10 倍、至少 25 倍、至少 50 倍、至少 100 倍、至少 500 倍或至少 1000 倍。结合亲和力可通过所属领域中已知的任何方法测量并且可以例如缔合速率、解离速率、解离常数 (K_d)、平衡常数 (K_{eq}) 或所属领域中的任何术语表示。

[0083] 在某些实施例中，根据本发明的抗体是抑制 MMP9 的酶活性（即，催化活性）的抗体，例如 MMP9 催化活性的非竞争性抑制剂。在某些实施例中，根据本发明的抗体在 MMP9 的催化结构域内结合。在其它实施例中，根据本发明的抗体在 MMP9 的催化结构域外部结合。

[0084] 还提供了与本文描述的一或多种抗 MMP9 抗体或其抗原结合片段竞争结合到 MMP9 的抗体或其抗原结合片段。因此，本发明涵盖与例如具有 SEQ ID NO:1 或 5-8 中任一种的重链多肽、SEQ ID NO:2 或 9-12 的轻链多肽或其组合的抗体竞争结合的抗 MMP9 抗体及其功能片段。在一个实施例中，抗 MMP9 抗体或其功能片段与本文描述为 AB0041 的抗体竞争结合到人类 MMP9。

[0085] 表位结合

[0086] 还提供了结合到与任一种或多种本文所述的抗体相同的表位（例如 MMP9 表位）的抗体和其片段。还提供了特异性结合到 MMP9 表位的抗体和片段，其中所述表位包括在 MMP9 的一个特定区域或 MMP9 的多个区域内的氨基酸残基。这些区域可包括例如 MMP9 的结构环和 / 或其它结构性结构域，例如经显示对结合到本文所述的示例性抗体很重要的那些。通常，这些区域是根据氨基酸残基在全长 MMP9 序列（例如 SEQ ID NO:27）上的位置界定。在一些实例中，所述表位是在 SEQ ID NO:27 的半胱氨酸开关活性袋的外部。在一些实例中，所述表位含有在SEQ ID NO:27的残基104-202的区域内的氨基酸残基(即，一或多个氨基酸残基)。在一个实例中，所述表位含有在SEQ ID NO:27的残基104-119、残基159-166或残基 191-202 的区域内的氨基酸残基（即，一或多个氨基酸残基）。在一些方面中，所述表位包括在作为 SEQ ID NO:27 的残基 104-119 的 MMP9 区域内的氨基酸残基（即，一或多个氨基酸残基）、在作为 SEQ ID NO:27 的残基 159-166 的 MMP9 区域内的氨基酸残基，及在作为 SEQ ID NO:27 的残基 191-202 的 MMP9 区域内的氨基酸残基。在一些情形中，所述表位包括 SEQ ID NO:27 的 E111、D113、R162 或 I198。在一些情形中，其包括 SEQ ID NO:27 的 R162。在一些情形中，其包括 SEQ ID NO:27 的 E111、D113、R162 及 I198。

[0087] MMP9 序列

[0088] 人类 MMP9 蛋白的氨基酸序列如下：

[0089]

```
MSLWQPLVLV LLVLGCCFAA PRQRQSTLVL FPGDLRTNLT DRQLAEEYLY  50
RYGYTRVAEM RGESKSLGPA LLLLQKQLSL PETGELDSAT LKAMRTPRCG  100
VPDLGRFQTF EGDLKWHHHN ITYWIQNYSE DLPRAVIDDA FARAFALWSA  150
VTPLTFTRVY SRDADIVIQF GVAEHGDGYP FDGKDGLLAH AFPPGPGIQG  200
DAHFDDDELW SLGKGVVVPT RFGNADGAAC HFPFIFEGRS YSACTTDGRS  250
DGLPWCSTTA NYDTDDRFGF CPSERLYTRD GNADGKPCQF PFIFQGQSYS  300
ACTTDGRSDG YRWCATTANY DRDKLFGFCP TRADSTVMGG NSAGELCVFP  350
FTFLGKEYST CTSEGRGDGR LWCATTSNFD SDKKWGFCPD QGYSLFLVAA  400
HEFGHALGLD HSSVPEALMY PMYRFTEGPP LHKDDVNGIR HLYGPRPEPE  450
PRPPTTTTPQ PTAPPTVCPT GPPTVHPSER PTAGPTGPPS AGPTGPPTAG  500
PSTATTVPLS PVDDACNVNI FDAIAEIGNQ LYLFKDGKYW RFSEGRGSRP  550
QGPFLIADKW PALPRKLDSV FEEPLSKKLF FFSGRQVWVY TGASVLGPRR  600
LDKLGLGADV AQVTGALRSG RGKMLLFSGR RLWRFDVKAQ MVDPRSASEV  650
DRMFPGVPLD THDVFQYREK AYFCQDRFYW RVSSRSELNQ VDQVGYVTYD  700
ILQCPED  (SEQ ID NO:27)
```

[0090] 蛋白质结构域示意性显示于图 3 中并且指示于下：

[0091]

氨基酸编号	特征
1-19	信号肽
38-98	肽聚糖结合结构域
R98/C99	半胱氨酸开关活性袋
112-445	Zn 依赖性金属蛋白酶结构域
223-271	纤连蛋白 II 型结构域(明胶结合结构域)
281-329	纤连蛋白 II 型结构域(明胶结合结构域)
340-388	纤连蛋白 II 型结构域(明胶结合结构域)
400-411	Zn 结合区
521-565	血红素结合蛋白样结构域
567-608	血红素结合蛋白样结构域
613-659	血红素结合蛋白样结构域
661-704	血红素结合蛋白样结构域

[0092] 成熟全长人类 MMP9 的氨基酸序列（其为不含信号肽的具有 SEQ ID NO:27 的前肽的氨基酸序列）为：

[0093]

APRQRQSTLVL FPGDLRTNLT DRQLAEEYLY RYGYTRVAEM RGESKSLGPA
LLLLQKQLSL PETGELDSAT LKAMRTPRCG VPDLGRFQTF EGDLKWHHHN
ITYWIQNYSE DLPRAVIDDA FARAFALWSA VTPLTFTRVY SRDADIVIQF
GVAEHGDGYP FDGKDGLLAH AFPPGPGIQG DAHFDDDELW SLGKGVVVPT
RFGNADGAAC HFPFIFEGRS YSACTTDGRS DGLPWCSTTA NYDTDDRFGF
CPSERLYTRD GNADGKPCQF PFIFQGQSYS ACTTDGRSDG YRWCATTANY
DRDKLFGFCP TRADSTVMGG NSAGELCVFP FTFLGKEYST CTSEGRGDGR
LWCATTSNFD SDKKWGFCPD QGYSLFLVAA HEFGHALGLD HSSVPEALMY
PMYRFTEGPP LHKDDVNGIR HLYGPRPEPE PRPPTTTTPQ PTAPPTVCPT
GPPTVHPSER PTAGPTGPPS AGPTGPPTAG PSTATTVPLS PVDDACNVNI
FDAIAEIGNQ LYLFKDGKYW RFSEGRGSRP QGPFLIADKW PALPRKLDSV
FEEPLSKKLF FFSGRQVWVY TGASVLGPRR LDKLGLGADV AQVTGALRSG
RGKMLLFSGR RLWRFDVKAQ MVDPRSASEV DRMFPGVPLD THDVFQYREK
AYFCQDRFYW RVSSRSELNQ VDQVGYVTYD ILQCPED (SEQ ID NO:28)

[0094] 信号肽的氨基酸序列为 MSLWQPLVLV LLVLGCCFA(SEQ ID NO:29)。

[0095] 还提供了 MMP9 多肽，包括突变体 MMP9 多肽。这些肽可用于例如产生并选择如本文所提供的抗体和片段。示例性多肽包括具有含 SEQ ID NO:27 的残基 104-202 的氨基酸序列的多肽，及具有 SEQ ID NO:27 的氨基酸序列并且在 SEQ ID NO:27 的残基 111、113、162 或 198 处具有氨基酸取代或在所有这些残基处具有氨基酸取代的多肽。其它示例性多肽包括具有含 SEQ ID NO:27 的残基 111-198 的氨基酸序列的多肽，及具有含 SEQ ID NO:27 的残基 111-198 的氨基酸序列并且在 SEQ ID NO:27 的残基 111、113、162 或 198 处具有氨基酸取代或在所有这些残基处具有氨基酸取代的多肽。这些多肽可用于例如选择结合到含这

些残基的表位和 / 或 MMP9 的这些残基对于结合很重要的抗体，例如本文所述的抗体。

[0096] 本发明涵盖结合 MMP9（例如人类 MMP9）的任何部分的 MMP9 结合蛋白，其中相对于其它 MMP，优先结合 MMP9 的 MMP9 结合蛋白是特别值得关注的。

[0097] 抗 MMP9 抗体和其功能片段可根据所属领域中众所周知的方法产生。示例性抗 MMP9 抗体提供于下。

[0098] 小鼠单克隆抗 MMP9 抗体

[0099] 针对人类 MMP9 的小鼠单克隆抗体是如实例 1 中所述获得。这一抗体含有小鼠 IgG2b 重链和小鼠 κ 轻链，并且称为 AB0041。

[0100] AB0041 重链的氨基酸序列如下：

[0101] MAVLVLFLCLVAFPSCVLSQVQLKESGPGLVAPSQSLSITCTVSGFSLLSYGVHWVRQPPGKGLEWLGVIWTGGTTNYNSALMSRLSISKDDSKSQVFLKMNSLQTDDTAIYYCARYYYGMDYWGQGTSVTVSSAKTTPPSVYPLAPGCGDTTGSSVTLGCLVKGYFPESVTVTWNSGSLSSSVHTFPALLQSGLYTMSSSVTVPSSTWPSQTVTCSVAHPASSTTVDKKLEPSGPISTINPCPPCKECHKCPAPNLEGGPSVFIFPPNIKDVLMISLTPKVTCVVVDVSEDDPDVRISWFVNNVEVHTAQTQTHREDYNSTIRVVSALPIQHQDWMSGKEFKCKVNNKDLPSPIERTISKIKGLVRAPQVYILPPPAEQLSRKDVSLTCLVVGFNPGDISVEWTSNGHTEENYKDTAPVLDSDGSYFIYSKLDIKTSKWEKTDSFSCNVRHEGLKNYYLKKTISRSPGK(SEQ ID NO:1)。

[0102] 信号序列加下划线，并且 IgG2b 恒定区的序列以斜体表示。

[0103] AB0041 轻链的氨基酸序列如下：

[0104] MESQIQVFVFVFLWLSGVDGDIVMTQSHKFMSTSVGDRVSITCKASQDVRNTVAWYQQKTGQSPKLLIYSSSYRNTGVPDRFTGSGSGTDFTFTISSVQAEDLAVYFCQQHYITPYTFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC(SEQ ID NO:2)。

[0105] 信号序列加下划线，并且 κ 恒定区的序列以斜体表示。

[0106] 以下氨基酸序列包含 AB0041 的 IgG2b 重链可变区的构架区和互补决定区 (CDR)（其中 CDR 加下划线）：

[0107] QVQLKESGPGLVAPSQSLSITCTVSGFSLLSYGVHWVRQPPGKGLEWLGVIWTGGTTNYNSALMSRLSISKDDSKSQVFLKMNSLQTDDTAIYYCARYYYGMDYWGQGTSVTVSS(SEQ ID NO:3)。

[0108] 以下氨基酸序列包含 AB0041 的 κ 轻链可变区的构架区和互补决定区 (CDR)（其中 CDR 加下划线）：

[0109] DIVMTQSHKFMSTSVGDRVSITCKASQDVRNTVAWYQQKTGQSPKLLIYSSSYRNTGVPDRFTGSGSGTDFTFTISSVQAEDLAVYFCQQHYITPYTFGGGTKLEIK(SEQ IDNO:4)。

[0110] 其它示例性小鼠抗人类 MMP9 抗体（例如 M4 和 M12）描述于实例 1B 中。示例性抗小鼠 MMP9 抗体 (AB0046) 描述于实例 1C 中。其它示例性小鼠抗人类 MMP9 抗体包括了包含具有 SEQ ID NO:3 的序列的可变区及与 IgG2b 恒定区序列具有 95％相似性的恒定区的抗体。此外，示例性小鼠抗人类 MMP9 抗体包括了包含具有 SEQ ID NO:4 的序列的可变区及与 IgG2b 恒定区序列具有 95％相似性的恒定区的抗体。其它示例性小鼠抗人类 MMP9 抗体包括了包含具有 SEQ ID NO:3 和 4 的序列的可变区及与 IgG2b 恒定区序列具有 95％相似性的恒定区的抗体。这些抗小鼠抗体适用于测试并评估 MMP9 抑制方法。

[0111] 重链变体

[0112] 通过改变 AB0041 重链和轻链可变区中的构架区序列来单独地修饰所述重链和轻链可变区的氨基酸序列。这些序列改变的影响是使人类 T 细胞表位的抗体耗尽，从而减少或消除其在人体中的免疫原性。

[0113] 在含有使铰链结构域稳定的 S241P 氨基酸改变的人类 IgG4 重链背景下构建四个重链变体（安格 (Angal) 等人 (1993) 分子免疫学 (Molec. Immunol.)30 :105-108)，并且称为 VH1、VH2、VH3 及 VH4。其构架区和 CDR 的氨基酸序列如下：

[0114] VH1

[0115] QVQLQESGPGLVKPSETLSLTCTVSGFSLLSYGVHWVRQPPGKGLEWLGVIWTGGTTNYNSALMSRLTISKDDSKSTVYLKMNSLKTEDTAIYYCARYYYGMDYWGQGTSVTVSS(SEQ ID NO :5)

[0116] VH2

[0117] QVQLQESGPGLVKPSETLSLTCTVSGFSLLSYGVHWVRQPPGKGLEWLGVIWTGGTTNYNSALMSRLTISKDDSKNTVYLKMNSLKTEDTAIYYCARYYYGMDYWGQGTLVTVSS(SEQ ID NO :6)

[0118] VH3

[0119] QVQLQESGPGLVKPSETLSLTCTVSGFSLLSYGVHWVRQPPGKGLEWLGVIWTGGTTNYNSALMSRFTISKDDSKNTVYLKMNSLKTEDTAIYYCARYYYGMDYWGQGTLVTVSS(SEQ ID NO :7)

[0120] VH4

[0121] QVQLQESGPGLVKPSETLSLTCTVSGFSLLSYGVHWVRQPPGKGLEWLGVIWTGGTTNYNSALMSRFTISKDDSKNTLYLKMNSLKTEDTAIYYCARYYYGMDYWGQGTLVTVSS(SEQ ID NO:8)

[0122] 图 1 示出了人类化重链可变区的氨基酸序列的比对并且指示这四个变体的构架区中的氨基酸序列存在差异。

[0123] 轻链变体

[0124] 在人类 κ 链背景下构建四个轻链变体，并且称为 Vk1、Vk2、Vk3 及 Vk4。其构架区和 CDR 的氨基酸序列如下：

[0125] Vk1

[0126] DIVMTQSPSFLSASVGDRVTITCKASQDVRNTVAWYQQKTGKAPKLLIYSSSYRNTGVPDRFTGSGSGTDFTLTISSLQAEDVAVYFCQQHYITPYTFGGGTKVEIK(SEQ ID NO :9)

[0127] Vk2

[0128] DIVMTQSPSSLSASVGDRVTITCKASQDVRNTVAWYQQKPGKAPKLLIYSSSYRNTGVPDRFTGSGSGTDFTLTISSLQAEDVAVYFCQQHYITPYTFGGGTKVEIK(SEQ ID NO :10)

[0129] Vk3

[0130] DIQMTQSPSSLSASVGDRVTITCKASQDVRNTVAWYQQKPGKAPKLLIYSSSYRNTGVPDRFSGSGSGTDFTLTISSLQAEDVAVYFCQQHYITPYTFGGGTKVEIK(SEQ ID NO :11)

[0131] Vk4

[0132] DIQMTQSPSSLSASVGDRVTITCKASQDVRNTVAWYQQKPGKAPKLLIYSSSYRNTGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQHYITPYTFGGGTKVEIK(SEQ ID NO :12)

[0133] 图 2 示出了人类化轻链可变区的氨基酸序列的比对并且指示这四个变体的构架区中的氨基酸序列存在差异。

[0134] 人类化重链和轻链以所有可能的逐对组合方式组合以产生多种功能性人类化抗 MMP9 抗体。举例来说，提供了含具有 SEQ ID NO :3、5、6、7 及 8 中的任一个中所陈述的氨基

酸序列的重链可变 (VH) 区的抗体；含具有 SEQ ID NO：4、9、10、11 及 12 中的任一个中所陈述的氨基酸序列的轻链可变 (VL) 区的抗体；及含具有 SEQ ID NO：3、5、6、7 及 8 中的任一个中所陈述的氨基酸序列的重链可变 (VH) 区及具有 SEQ ID NO：4、9、10、11 及 12 中的任一个中所陈述的氨基酸序列的轻链可变 (VL) 区的抗体，以及与这些抗体竞争结合到 MMP9 的抗体和与这些抗体具有至少或约 75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或更高序列一致性的抗体。在一个实例中，所述抗体具有 VH 区，所述 VH 区的氨基酸序列与 SEQ ID NO：7 具有至少或约 75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或更高的序列一致性；及 VL 区，所述 VL 区的氨基酸序列与 SEQ ID NO：12 具有至少或约 75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或更高序列一致性；或具有 SEQ ID NO：7 的 VH 区和 SEQ ID NO：12 的 VL 区。

[0135] 还提供了与本文所揭示的重链可变区序列具有 75％或更高，80％或更高，90％或更高，95％或更高，或 99％或更高同源性的其它重链可变区氨基酸序列。此外，还提供了与本文所揭示的轻链可变区序列具有 75％或更高，80％或更高，90％或更高，95％或更高，或 99％或更高同源性的其它轻链可变区氨基酸序列。

[0136] 还提供了与本文所揭示的重链可变区序列具有 75％或更高，80％或更高，90％或更高，95％或更高，或 99％或更高序列一致性的其它重链可变区氨基酸序列。此外，还提供了与本文所揭示的轻链可变区序列具有 75％或更高，80％或更高，90％或更高，95％或更高，或 99％或更高序列一致性的其它轻链可变区氨基酸序列。

[0137] 互补决定区 (CDR)

[0138] 在一些实施例中，如本文所揭示的示例性提供的抗 MMP9 抗体的重链 CDR 具有以下氨基酸序列：

[0139] CDR1：GFSLLSYGVH(SEQ ID NO：13)

[0140] CDR2：VIWTGGTTNYNSALMS(SEQ ID NO：14)

[0141] CDR3：YYYGMDY(SEQ ID NO：15)。

[0142] 因此，所提供的抗 MMP9 抗体为具有含如 SEQ ID NO：13 中所陈述的氨基酸序列的重链 CDR1 区的抗体、具有含如 SEQ ID NO：14 中所陈述的氨基酸序列的重链 CDR2 区的抗体，及具有含如 SEQ ID NO：15 中所陈述的氨基酸序列的重链 CDR3 区的抗体，以及与这些抗体竞争结合或结合到 MMP9 上与这些抗体相同的表位的抗体。在一些情形中，所述抗体含有具 SEQ ID NO：13、14 及 15 中所陈述的序列的 VH CDR。

[0143] 在一些实施例中，如本文所揭示的示例性抗 MMP9 抗体的轻链 CDR 具有以下氨基酸序列：

[0144] CDR1：KASQDVRNTVA(SEQ ID NO：16)

[0145] CDR2：SSSYRNT(SEQ ID NO：17)

[0146] CDR3：QQHYITPYT(SEQ ID NO：18)。

[0147] 因此，所提供的抗 MMP9 抗体为具有含如 SEQ ID NO：16 中所陈述的氨基酸序列的轻链 CDR1 区的抗体、具有含如 SEQ ID NO：17 中所陈述的氨基酸序列的轻链 CDR2 区的抗体，及具有含如 SEQ ID NO：18 中所陈述的氨基酸序列的轻链 CDR3 区的抗体，以及与这些抗体竞争结合或结合到 MMP9 上与这些抗体相同的表位的抗体。在一些情形中，所述抗体含有具

SEQ ID NO:16、17 及 18 中所陈述的序列的 VL CDR。

[0148] 编码抗 MMP9 抗体的核酸

[0149] 本发明提供了编码抗 MMP9 抗体和其功能片段的核酸。因此，本发明提供了一种编码如本文所述的抗体或抗原结合片段的分离的多核苷酸（核酸）、含有这些多核苷酸的载体及用于将这些多核苷酸转录并翻译成多肽的宿主细胞和表达系统。

[0150] 本发明还涵盖呈质粒、载体、转录或表达盒形式的构建体，其包含至少一种如上述的多核苷酸。

[0151] 本发明还提供一种包含一或多种如上述的构建体的重组宿主细胞，以及制造本文所述的抗体或其抗原结合片段的方法，所述方法包含在重组宿主细胞中表达编码重链多肽和轻链多肽的核酸（在相同或不同宿主细胞中并且来自相同或不同构建体）。表达可以通过在适当条件下培养含有所述核酸的重组宿主细胞来实现。在通过表达进行制造之后，可使用任何适合的技术分离和 / 或纯化抗体或抗原结合片段，随后在适当时使用。

[0152] 用于在多种不同宿主细胞中克隆并表达多肽的系统是众所周知的。适合宿主细胞包括细菌、哺乳动物细胞、酵母及杆状病毒系统。所属领域中可用于表达异源多肽的哺乳动物细胞系包括中国仓鼠卵巢细胞、海拉细胞 (HeLa cell)、幼仓鼠肾细胞、NSO 小鼠黑素瘤细胞及许多其它细胞。常用的细菌宿主是大肠杆菌。

[0153] 所选择或构建的适合载体可含有适当调控序列，包括可操作地连接的启动子序列、终止子序列、聚腺苷酸化序列、增强子序列、标记物基因和 / 或（适当时）其它序列。适当时，载体可为质粒、病毒，例如噬菌体或噬菌粒。有关进一步详情，参见例如，分子克隆实验指南：第 2 版，萨姆布鲁克等人，1989, 冷泉港实验室出版社 (Cold Spring Harbor Laboratory Press)。许多已知的用于操作核酸，例如制备核酸构建体、诱变、测序、将 DNA 引入细胞中和基因表达，以及蛋白质分析的技术和方案详细描述于精编分子生物学实验指南 (Short Protocols in Molecular Biology)，第二版，奥苏贝尔等人编，约翰威立出版公司 (John Wiley&Sons)，1992 中。萨姆布鲁克等人和奥苏贝尔等人的揭示内容以全文引用的方式并入本文中。

[0154] 编码所关注多肽的核酸被整合到宿主细胞基因组中或可维持稳定或短暂游离体元件形式。

[0155] 多种表达控制序列（即，控制可操作地连接到的 DNA 序列的表达的序列）中的任一种都可用于这些载体中以表达 DNA 序列。举例来说，编码所关注多肽的核酸可以可操作地连接到启动子，并且提供于表达构建体中以用于制造重组 MMP9 蛋白或其部分的方法中。

[0156] 所属领域技术人员应了解，编码本文所揭示的抗体链的核酸可使用分子生物学中的标准知识和程序合成。

[0157] 编码本文所揭示的重链和轻链氨基酸序列的核苷酸序列的实例如下：

[0158]

VH1: CAGGTGCAGC TGCAGGAATC CGGCCCTGGC CTGGTCAAGC CCTCCGAGAC ACTGTCCCTG ACCTGCACCG TGTCCGGCTT CTCCCTGCTG TCCTACGGCG TGCACTGGGT CCGACAGCCT CCAGGGAAGG GCCTGGAATG GCTGGGCGTG ATCTGGACCG GCGGCACCAC CAACTACAAC TCCGCCCTGA TGTCCCGGCT GACCATCTCC AAGGACGACT CCAAGTCCAC CGTGTACCTG AAGATGAACT CCCTGAAAAC CGAGGACACC GCCATCTACT ACTGCGCCCG GTACTACTAC GGCATGGACT ACTGGGGCCA GGGCACCTCC GTGACCGTGT CCTCA **(SEQ ID NO:19)**

[0159]

VH2: CAGGTGCAGC TGCAGGAATC CGGCCCTGGC CTGGTCAAGC CCTCCGAGAC ACTGTCCCTG ACCTGCACCG TGTCCGGCTT CTCCCTGCTG TCCTACGGCG TGCACTGGGT CCGACAGCCT CCAGGCAAAG GCCTGGAATG GCTGGGCGTG ATCTGGACCG GCGGCACCAC CAACTACAAC TCCGCCCTGA TGTCCCGGCT GACCATCTCC AAGGACGACT CCAAGAACAC CGTGTACCTG AAGATGAACT CCCTGAAAAC CGAGGACACC GCCATCTACT ACTGCGCCCG GTACTACTAC GGCATGGACT ACTGGGGCCA GGGCACCCTG GTCACCGTGT CCTCA (SEQ ID NO:20)

VH3: CAGGTGCAGC TGCAGGAATC CGGCCCTGGC CTGGTCAAGC CCTCCGAGAC ACTGTCCCTG ACCTGCACCG TGTCCGGCTT CTCCCTGCTG TCCTACGGCG TGCACTGGGT CCGACAGCCT CCAGGCAAAG GCCTGGAATG GCTGGGCGTG ATCTGGACCG GCGGCACCAC CAACTACAAC TCCGCCCTGA TGTCCCGGTT CACCATCTCC AAGGACGACT CCAAGAACAC CGTGTACCTG AAGATGAACT CCCTGAAAAC CGAGGACACC GCCATCTACT ACTGCGCCCG GTACTACTAC GGCATGGACT ACTGGGGCCA GGGCACCCTG GTCACCGTGT CCTCA (SEQ ID NO:21)

VH4: CAGGTGCAGC TGCAGGAATC CGGCCCTGGC CTGGTCAAGC CCTCCGAGAC ACTGTCCCTG ACCTGCACCG TGTCCGGCTT CTCCCTGCTG TCCTACGGCG TGCACTGGGT CCGACAGCCT CCAGGCAAAG GCCTGGAATG GCTGGGCGTG ATCTGGACCG GCGGCACCAC CAACTACAAC TCCGCCCTGA TGTCCCGGTT CACCATCTCC AAGGACGACT CCAAGAACAC CCTGTACCTG AAGATGAACT CCCTGAAAAC CGAGGACACC GCCATCTACT ACTGCGCCCG GTACTACTAC GGCATGGACT ACTGGGGCCA GGGCACCCTG GTCACCGTGT CCTCA (SEQ ID NO:22)

Vk1: GACATCGTGA TGACCCAGTC CCCCAGCTTC CTGTCCGCCT CCGTGGGCGA CAGAGTGACC ATCACATGCA AGGCCTCTCA GGACGTGCGG AACACCGTGG CCTGGTATCA GCAGAAAACC GGCAAGGCCC CCAAGCTGCT GATCTACTCC TCCTCCTACC GGAACACCGG CGTGCCCGAC CGGTTTACCG GCTCTGGCTC CGGCACCGAC TTTACCCTGA CCATCAGCTC CCTGCAGGCC GAGGACGTGG CCGTGTACTT CTGCCAGCAG CACTACATCA CCCCCTACAC CTTCGGCGGA GGCACCAAGG TGGAAATAAA A (SEQ ID NO:23)

[0160]

Vk2: GACATCGTGA TGACCCAGTC CCCCTCCAGC CTGTCCGCCT
CTGTGGGCGA CAGAGTGACC ATCACATGCA AGGCCTCTCA GGACGTGCGG
AACACCGTGG CCTGGTATCA GCAGAAGCCC GGCAAGGCCC CCAAGCTGCT
GATCTACTCC TCCTCCTACC GGAACACCGG CGTGCCCGAC CGGTTTACCG
GCTCTGGCTC CGGCACCGAC TTTACCCTGA CCATCAGCTC CCTGCAGGCC
GAGGACGTGG CCGTGTACTT CTGCCAGCAG CACTACATCA CCCCCTACAC
CTTCGGCGGA GGCACCAAGG TGGAAATAAA A **(SEQ ID NO:24)**

Vk3: GACATCCAGA TGACCCAGTC CCCCTCCAGC CTGTCCGCCT
CTGTGGGCGA CAGAGTGACC ATCACATGCA AGGCCTCCCA GGACGTGCGG
AACACCGTGG CCTGGTATCA GCAGAAGCCC GGCAAGGCCC CCAAGCTGCT
GATCTACTCC TCCTCCTACC GGAACACCGG CGTGCCCGAC CGGTTCTCTG
GCTCTGGAAG CGGCACCGAC TTTACCCTGA CCATCAGCTC CCTGCAGGCC
GAGGACGTGG CCGTGTACTT CTGCCAGCAG CACTACATCA CCCCCTACAC
CTTCGGCGGA GGCACCAAGG TGGAAATAAA A **(SEQ ID NO:25)**

Vk4: GACATCCAGA TGACCCAGTC CCCCTCCAGC CTGTCCGCCT
CTGTGGGCGA CAGAGTGACC ATCACATGCA AGGCCTCTCA GGACGTGCGG
AACACCGTGG CCTGGTATCA GCAGAAGCCC GGCAAGGCCC CCAAGCTGCT
GATCTACTCC TCCTCCTACC GGAACACCGG CGTGCCCGAC CGGTTCTCTG
GCTCTGGAAG CGGCACCGAC TTTACCCTGA CCATCAGCTC CCTGCAGGCC
GAGGACGTGG CCGTGTACTA CTGCCAGCAG CACTACATCA CCCCCTACAC
CTTCGGCGGA GGCACCAAGG TGGAAATAAA A **(SEQ ID NO:26)**

[0161] 由于抗体结构，包括可变区中的 CDR 和构架区的并置、构架区的结构以及重链和轻链恒定区的结构是所属领域中众所周知的；故获得编码抗 MMP-9 抗体的相关核酸正在所属领域的技能范围内。因此，还提供了包含与本文所揭示的核苷酸序列中的任一种具有至少 75%、至少 80%、至少 85%、至少 90%、至少 95%、至少 98%及至少 99%同源性的核酸序列的多核苷酸。因此，还提供了包含与本文所揭示的核苷酸序列中的任一种具有至少 75%、至少 80%、至少 85%、至少 90%、至少 95%、至少 98%及至少 99%一致性的核酸序列的多核苷酸。在一个实例中，所述多核苷酸与 SEQ ID NO:21 含有至少或约 75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或更高序列一致性，或包括或为 SEQ ID NO:21，和 / 或与 SEQ ID NO:26 含有至少或约 75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或更高序列一致性，或包括或为 SEQ ID NO:26。

[0162] 医药组合物

[0163] 提供的 MMP9 结合蛋白，以及编码 MMP9 结合蛋白的核酸（例如 DNA 或 RNA）可为例如与医药学上可接受的载剂或赋形剂组合的医药组合物形式。所述医药组合物可用于例如在体内或离体投予个体，并且用于用 MMP9 结合蛋白诊断和 / 或治疗个体，例如用于本文所提供的治疗或诊断方法中的任一种中。

[0164] 医药学上可接受的载剂为经投药的患者生理学上可接受的并且保持与其一起投予的抗体或肽的治疗特性。医药学上可接受的载剂及其调配物大体上描述于例如雷氏药学大全(Remington'pharmaceutical Sciences)(第18版,A. 杰纳罗(A.Gennaro)编,默克出版公司(Mack Publishing Co.),宾夕法尼亚州伊斯顿(Easton,PA)1990)中。一种示例性医药载剂是生理盐水。在与所述调配物的其它成分可相容并且对患者实质上无害的意义上看,每种载剂为"医药学上可接受的"。

[0165] 医药组合物可经调配以与特定投药途径(全身或局部)相容。因此,医药组合物包括适于通过各种途径投药的载剂、稀释剂或赋形剂。

[0166] 医药组合物可包括医药学上可接受的添加剂。添加剂的实例包括(但不限于)糖,例如甘露糖醇、山梨糖醇、葡萄糖、木糖醇、海藻糖、山梨糖、蔗糖、半乳糖、葡聚糖、右旋糖、果糖、乳糖及其混合物。医药学上可接受的添加剂可与医药学上可接受的载剂和/或赋形剂(例如右旋糖)组合。添加剂还包括表面活性剂,例如聚山梨醇酯20或聚山梨醇酯80。

[0167] 所述调配物和递送方法一般将根据有待治疗的部位和疾病来调整。示例性调配物包括(但不限于)适于肠胃外投药(例如静脉内、动脉内、肌肉内或皮下投药)或经口投药的调配物。

[0168] 供肠胃外递送的医药组合物包括例如水、生理盐水、磷酸盐缓冲的生理盐水、汉克氏溶液(Hank's solution)、林格氏溶液(Ringer's solution)、右旋糖/生理盐水及葡萄糖溶液。所述调配物可含有用于模拟生理条件的辅助物质,例如缓冲剂、张力调节剂、润湿剂、清洁剂等。添加剂还可包括其它活性成分,例如杀菌剂或稳定剂。举例来说,所述溶液可含有乙酸钠、乳酸钠、氯化钠、氯化钾、氯化钙、脱水山梨糖醇单月桂酸酯或三乙醇胺油酸酯。其它肠胃外调配物和方法描述于白(Bai)(1997)神经免疫学杂志(J.Neuroimmunol.)80:6575;瓦伦(Warren)(1997)神经科学杂志(J.Neurol.Sci.)152:3138;及托尼盖瓦(Tonegawa)(1997)实验医学杂志(J.Exp.Med.)186:507515中。肠胃外制剂可封装于由玻璃或塑料制成的安瓿、一次性注射器或多剂量小瓶中。

[0169] 供真皮内或皮下投药的医药组合物可包括无菌稀释剂,例如水、生理盐水溶液、非挥发性油、聚乙二醇、甘油、丙二醇或其它合成溶剂;抗细菌剂,例如苯甲醇或对羟基苯甲酸甲酯;抗氧化剂,例如抗坏血酸、谷胱甘肽或亚硫酸氢钠;螯合剂,例如乙二胺四乙酸;缓冲剂,例如乙酸盐、柠檬酸盐或磷酸盐;及用于调整张力的试剂,例如氯化钠或右旋糖。

[0170] 供注射的医药组合物包括水溶液(在水溶性情况下)或分散液及用于临时制备无菌可注射溶液或分散液的无菌粉末。对于静脉内投药,适合载剂包括生理盐水、抑菌性水、Cremophor ELTM(巴斯夫公司(BASF),新泽西州帕西帕尼(Parsippany,N.J.))或磷酸盐缓冲的生理盐水(PBS)。载剂可为含有例如水、乙醇、多元醇(例如,甘油、丙二醇及液体聚乙二醇等)及其适合混合物的溶剂或分散介质。可通过例如利用包衣(例如卵磷脂),在分散液的情况通过维持所需粒度及通过利用表面活性剂来维持流动性。抗细菌剂和抗真菌剂包括例如对羟基苯甲酸酯、氯丁醇、苯酚、抗坏血酸及硫柳汞。在所述组合物中可包括等渗剂,例如糖、多元醇(例如甘露糖醇、山梨糖醇)及氯化钠。所得溶液可经包装以按原样使用,或经冻干;冻干的制剂可随后在投药之前与无菌溶液组合。

[0171] 医药学上可接受的载剂可含有使吸收或清除稳定、增加或延迟的化合物。所述化合物包括例如碳水化合物,例如葡萄糖、蔗糖或葡聚糖;低分子量蛋白质;降低肽清除率

或水解的组合物；或赋形剂或其它稳定剂和/或缓冲剂。延迟吸收的试剂包括例如单硬脂酸铝和明胶。也可以使用清洁剂来使医药组合物的吸收稳定或者增加或减少，包括脂质体载剂。为了防止消化，可使所述化合物与组合物形成复合物，以使其对酸性和酶促水解具有抗性，或可使所述化合物与具有适当抗性的载剂（例如脂质体）形成复合物。保护化合物免于消化的手段是所属领域中已知的（参见例如，费克斯 (Fix)(1996) 药学研究 (Pharm Res.)13:17601764；萨曼伦 (Samanen)(1996) 药学与药理学杂志 (J.Pharm. Pharmacol.)48:119135；及美国专利第 5,391,377 号，其描述了供经口递送治疗剂的脂质组合物）。

[0172] 本发明的组合物可与如本文所提供的其它治疗部分或成像/诊断部分组合。治疗部分和/或成像部分可作为单独组合物，或作为 MMP9 结合蛋白上存在的偶联的部分提供。

[0173] 供体内投药的调配物一般为无菌的。在一个实施例中，所述医药组合物被调配成不含热原质，由此使其可接受用于投予人类患者。

[0174] 依据本发明，各种其它医药组合物及其制备和使用技术将为所属领域技术人员所知。有关适合药理学组合物和相关投药技术的详细清单，可参考本文的详细教示，其可通过例如雷氏药物学与实践 (Remington:The Science and Practice of Pharmacy) 第 20 版（利平科特威廉姆斯威尔金斯出版公司 (Lippincott,Williams&Wilkins)2003) 等课本进一步补充。

[0175] 医药组合物可基于需要治疗的患者/个体的身体特征、投药途径等进行调配。这些可包装于适合医药包装中，并附上适当标记供分配到医院和诊所，其中所述标记是用于指示治疗个体的如本文所述的病症。药物可按单一或多个单位形式包装。有关本发明的医药组合物的剂量和投药的说明书可包括在以下所述的医药包装和试剂盒中。

[0176] 使用方法

[0177] 本发明的 MMP9 结合蛋白，包括抗 MMP9 抗体及其片段可用于例如治疗和诊断方法中，例如检测样品中的 MMP9 的方法、治疗方法（例如，如在抑制血管生成的方法中）及诊断和预后方法。因此，提供了抗 MMP9 抗体的诊断和治疗方法及用途。使用方法的实例描述于下。

[0178] 治疗方法

[0179] 本文提供了治疗方法，包括治疗与 MMP9 表达和/或活性有关的疾病和病症的方法，以及所提供的抗体和组合物在这些方法中的用途。所述疾病和病症包括（但不限于）癌症，例如肿瘤（例如原发性或转移性肿瘤），例如表达或安置于表达 MMP9 的组织中的那些，以及炎症性疾病，例如炎症性肠病、类风湿性关节炎及炎症性肌病。

[0180] 如本文所使用，“治疗 (treat/treatment)”意谓与本文所述疾病或病症有关的一或多种症状的停滞或发生延缓，或改善现有的不受控制或不想要的症状、预防其它症状，或改善或预防症状的潜在代谢原因。因此，所述术语表示已赋予患有疾病或症状，或有可能发生所述疾病或症状的哺乳动物个体有益结果。当患者经历疾病的病征或症状的部分或完全减轻，或减少时，实现反应，并且可包括（不限于）存活期延长。取决于预后因子，包括复发次数、疾病的阶段及其它因素，所测量的预期无进展存活时间可为数月到数年。

[0181] 还提供了结合这些方法使用的医药组合物，例如含有本文所述抗体或其片段中的任一种的医药组合物。组合物可适于通过任何适合途径局部或全身投予。

[0182] 一般来说，MMP9 结合蛋白是以治疗有效量投予，例如以实现个体中肿瘤生长的抑制、抑制转移、抑制炎症、抑制组织破坏，或抑制 MMP9 活性，或治疗与 MMP9 相关的特定疾病或病况的量投予

[0183] 如本文所使用，除非另作具体说明，否则术语“治疗有效量”或“有效量”是指当投予（单独或与另一治疗剂组合，如可能具体说明的）个体时有效预防或改善疾病病况或疾病的进展，或使症状改善，例如使相关医学病况得到治疗、治愈、预防或改善，或使这些病况的治疗、治愈、预防或改善的几率增加的药剂或化合物或组合物的量。当应用于单独投予的个别活性成分时，治疗有效剂量仅指所述成分。当应用于组合时，治疗有效剂量是指引起治疗作用的多种活性成分的组合量，无论依序还是同时组合投予。在一个实例中，当采用抗 MMP9 抗体的体内投予时，取决于投药途径，标准剂量可在每天每公斤哺乳动物体重约 10ng 到 100mg 或更高，优选地每天约 1μg/kg 到 50mg/kg，任选地每天约 100μg/kg 到 20mg/kg、每天 500μg/kg 到 10mg/kg，或每天 1mg/kg 到 10mg/kg 间变化。在一个实施例中，静脉内剂量在约 1mg/kg 到约 30mg/kg 范围内。在一些实施例中，静脉内剂量在每 14 天一次 (q14d) 1mg/kg 或约 1mg/kg 到 14mg/kg 或约 14mg/kg 范围内，例如在 2mg/kg 或约 2mg/kg 到 14mg/kg 或约 14mg/kg 范围内。在其它实施例中，皮下剂量在每 14 天一次 (q14d) 1mg/kg 或约 1mg/kg 到 28mg/kg 或约 28mg/kg 范围内，例如在 2mg/kg 或约 2mg/kg 到 28mg/kg 或约 28mg/kg 范围内。在一些实施例中，有效剂量是每 7 到 28 天一次投予。在一个实施例中，有效剂量是每 7 天一次投予。在另一个实施例中，有效剂量是每 28 天一次投予。

[0184] 所选剂量方案将取决于多种因素，包括 MMP9 结合蛋白的活性、投药途径、投药时间、所用特定化合物的排泄速率、治疗持续时间、与所用特定组合物组合使用的其它药物、化合物和 / 或材料、所治疗的患者的年龄、性别、体重、病况、一般健康状况和前病史，及医疗技术中众所周知的类似因素。

[0185] 具有所属领域普通技术的临床医生能容易地确定所需医药组合物的有效量并开具处方。举例来说，医师或兽医可以比获得所需治疗作用所需水平低的水平的以医药组合物形式使用的本发明化合物的剂量开始并逐步增加剂量直到达成所需作用。

[0186] 在一些情形中，治疗方法包括肠胃外投药，例如静脉内、动脉内、肌肉内或皮下投药；或经口投予药剂，例如抗 MMP9 抗体或含有其的组合物。

[0187] 如本文所使用，术语“个体”意谓哺乳动物个体。示例性个体包括（但不限于）人类、猴、狗、猫、小鼠、大鼠、母牛、马、山羊及绵羊。在一些实施例中，所述个体患有癌症、炎症性疾病或病况，或自体免疫性疾病或病况，并且可用如以下所述的本发明的药剂治疗。

[0188] 必要时，为进行治疗，方法可进一步包括其它疗法，例如在癌症的情况下，除 MMP9 结合蛋白外，手术去除癌症和 / 或投予抗癌剂或治疗。此类抗癌剂或治疗的投予可与本文所揭示的组合物的投予同时进行。

[0189] MMP9 的检测方法

[0190] 本发明还涵盖检测个体中的 MMP9，例如检测表达 MMP9 的肿瘤或肿瘤相关性组织，或者与如本文所述的疾病（例如自体免疫性疾病或炎症性疾病）相关的组织或流体或其它生物样品的方法。因此，提供了诊断、监测、分期或检测具有 MMP9 活性的肿瘤的方法。

[0191] 可分析来自个体（例如，怀疑患有或已知患有与 MMP9 表达相关的肿瘤，或怀疑患有或已知患有另一疾病或病况的个体）的样品（例如，测试生物样品）中 MMP9 的存在、不

存在、表达和 / 或水平。举例来说，可收集这些样品，并通过检测 MMP9 结合蛋白（例如，如本文所述的抗体或片段）与样品中的物质（例如蛋白质）的结合的存在或不存在来进行分析。在一些实例中，所述方法另外包括将检测到的结合的量与结合到对照样品的量相比较，或将所检测的 MMP9 水平与对照 MMP9 水平相比较。在一些情形中，所述方法指示 MMP9 相关性疾病或病况（如本文所述的疾病或病况）的存在、不存在或严重程度。

[0192] 这一分析可在使用如本文所述的 MMP9 结合蛋白的治疗起始之前进行，或可作为监测癌症治疗的进展的一部分进行。在一些实施例中，提供了治疗方法，其是通过进行检测分析，并例如基于诊断分析的结果起始、改变或中断个体的治疗来进行。所述诊断分析可使用任何样品进行，包括（但不限于）组织、从所述组织分离的细胞等。在一些情形中，所述方法是对例如血液、血浆、血清、全血、唾液、尿液或精液等液体样品进行的。组织样品包括例如福尔马林固定或冷冻的组织切片。

[0193] 用于检测和分析 MMP9 的任何适合方法均可使用。所属领域中已知的各种诊断分析技术可用于所述目的，例如竞争性结合分析、直接或间接夹心分析和在非均质或均质相中进行的免疫沉淀分析。

[0194] 用于检测方法中的 MMP9 结合蛋白可用可检测部分标记。可检测部分直接或间接地产生可检测信号。举例来说，可检测部分可为本文所述部分中的任一种，例如放射性同位素，例如 3H、14C、32P、35S 或 125I ；荧光或化学发光化合物，例如荧光素异硫氰酸酯 (FITC)、德克萨斯红 (Texas red)、花青苷、光青素 (photocyan)、罗丹明 (rhodamine) 或虫荧光素 ；或酶，例如碱性磷酸酶、β - 半乳糖苷酶或辣根过氧化物酶。

[0195] 检测可通过使样品在适于 MMP9 结合蛋白结合到 MMP9 的条件下接触，并评估 MMP9 结合蛋白 -MMP9 复合物的存在（例如水平）或不存在来实现。将样品中 MMP9 的水平与参考样品中的水平相比较可指示具有 MMP9 活性的肿瘤或肿瘤相关性组织的存在。参考样品可为在较早时间点从个体取得的样品或来自另一个体的样品。

[0196] 本发明的各种方面将借助于以下若干实例进一步描述并说明，这些实例不打算限制本发明的范围。

[0197] 实例

[0198] 实例 1A ：针对人类 MMP-9 的抗体的制备 .

[0199] 使用不含信号肽的全长人类 MMP9 蛋白 (SEQ ID NO.28) 对小鼠进行免疫。将来自经免疫小鼠的脾细胞与骨髓瘤细胞融合以产生杂交瘤文库。制备单克隆培养物并进行筛选以鉴别出表达抗 MMP9 单克隆抗体的培养物。

[0200] 从一种培养物中纯化出抗体 (AB0041) 并进行表征。这一抗体含有 IgG2b 重链和 κ 轻链。表征包括测试 AB0041 与其它人类 MMP 和来自其它物种（包括食蟹猴、大鼠和小鼠）的 MMP9 蛋白的结合。如表 2 中所示，AB0041 抗体对人类和食蟹猴 MMP9 具有较高亲和力，其对大鼠 MMP9 具有较低亲和力。此外，AB0041 抗体不结合到鼠类 MMP9 或许多人类非 MMP 基质金属蛋白酶。

[0201] 表 2 ：AB0041 和 AB0045 的交叉反应性

[0202]

所测试的 MMP	解离常数(Kd)	
	AB0045	AB0041
人类 MMP1	>100 nM	>100 nM
人类 MMP2	>100 nM	>100 nM
小鼠 MMP2	>100 nM	>100 nM
人类 MMP3	>100 nM	>100 nM
人类 MMP7	>100 nM	>100 nM
人类 MMP8	>100 nM	>100 nM
人类 MMP9	0.168 ± 0.117 nM	0.133 ± 0.030 nM
食蟹猴 MMP9	0.082 ± 0.022 nM	0.145 ± 0.16 nM
小鼠 MMP9	>100 nM	>100 nM
大鼠 MMP9	0.311 ± 0.017 nM	0.332 ± 0.022 nM
人类 MMP10	>100 nM	>100 nM
人类 MMP12	>100 nM	>100 nM
人类 MMP13	>100 nM	>100 nM

[0203] 其它表征包括分析 AB0041 与突变体小鼠和人类 MMP9 蛋白的结合。鉴别出小鼠和人类 MMP9 蛋白催化结构域中的不一致残基，并选择四十六个不一致氨基酸残基进行诱变。在小鼠 MMP9 中产生大多数突变：使小鼠氨基酸残基突变以匹配人类 MMP9 的残基。在人类 MMP9 中产生其它突变：使人类氨基酸残基突变以匹配小鼠 MMP9 的残基。将突变的小鼠或人类 MMP9 蛋白用于 ELISA 分析中。

[0204] 在 ELISA 分析中，使用 AB0041 抗体作为一次抗体，并且使用偶联到辣根过氧化物酶的山羊抗小鼠 IgG 抗体来检测结合。使用野生型人类 MMP9 作为阳性对照并且使用野生型小鼠 MMP9 作为阴性对照。ELISA 分析的结果显示，在 MMP9 氨基酸序列 162 位的精氨酸残基 (R162) 对于 AB0041 抗体结合 MMP9 很重要。这些结果还显示，氨基酸残基 E111、D113 及 I198 对于 AB0041 抗体结合 MMP9 很重要。基于 MMP9 的晶体结构，E111、D113、R162 及 I198 在 MMP9 的 Ca2+ 离子结合袋周围彼此接近地集合成群。在本研究中，经显示，AB0041 抗体特异性结合到这样一种表位，其含有的氨基酸残基在含氨基酸残基 104-119、159-166 及 191-202 的 MMP9 区域内。

[0205] 在针对 MMP9 的酶分析中，发现 AB0041 抗体充当 MMP9 的非竞争性抑制剂。

[0206] 实例 1B：针对人类 MMP-9 的其它抗体的制备 .

[0207] 产生其它杂交瘤，这些杂交瘤产生具有与 AB0041 共有一致性的可变区的抗体。一种此类杂交瘤，称为 M4，表达一种抗体，其含有以下重链 (IgG2b) 序列：

[0208] MAVLVLFLCLVAFPSCVLSQVQLKESGPGLVAPSQSLSITCTVSGFSLLSYGVHWVRQPPGKGLEWLGVIWTGGSTNYNSALMSRLSISKDDSKSQVFLKMNSLQTDDTAMYYCARYYYAMDYWGQGTSVTVSSAKTTPPSVYPLAPGCGDTTGSSVTLGCLVKGYFPESVTVTWNSGSLSSSVHTFPALLQSGLYTMSSSVTVPSSTWPSQTVTCSVAHPASSTTVDKKLEPSGPISTINPCPPCKECHKCPAPNLEGGPSVFIFPPNIKDVLMISLTPKVTCVVVDVSEDDPDVRISWFVNNVEVHTAQTQTHREDYNSTIRVVSALPIQHQDWMSGKEFKCKVNNKDLPSPIERTISKIKGLVRAPQVYILPPPAEQLSRKDVSLTCLVVGFNPGDISVEWTSNGHTEENYKDTAPVLDSDGSYFIYSKLDIKTSKWEKTDSFSCNVRHEGLKNYYLKKTISRSPGK(SEQ ID NO:30)

[0209] (信号肽以加下划线文字陈述，可变区以无格式文字陈述，并且恒定区以斜体陈

述），及以下轻链（κ）序列：

[0210] MESQIQVFVFVFLWLSGVDGDIVMTQSHKFMFTSVGDRVSITCKASQDVRNTVAWYQQKTGQSPKLLIYSASYRNTGVPDRFTGSISGTDFTFTISSVQAEDLALYYCQQHYSTPYTFGGGTKLEVKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC（信号肽以加下划线文字陈述，可变区以无格式文字陈述，并且恒定区以斜体陈述）(SEQ ID NO:31)。

[0211] M4 抗体具有含以下氨基酸序列的可变重链：

[0212] QVQLKESGPGLVAPSQSLSITCTVSGFSLLSYGVHWVRQPPGKGLEWLGVIWTGGSTNYNSALMSRLSISKDDSKSQVFLKMNSLQTDDTAMYYCARYYYAMDYWGQGTSVTVSS(CDR 1、2 及 3（分别为 SEQ ID NO:34、35 及 36）加下划线）(SEQ ID NO:32)

[0213] 及含以下氨基酸序列的可变轻链：

[0214] DIVMTQSHKFMFTSVGDRVSITCKASQDVRNTVAWYQQKTGQSPKLLIYSASYRNTGVPDRFTGSISGTDFTFTISSVQAEDLALYYCQQHYSTPYTFGGGTKLEVK(CDR 1、2 及 3（分别为 SEQ ID NO:37、38 及 39）加下划线）(SEQ ID NO:33)。

[0215] 另一种此类杂交瘤，称为 M12，仅表达 κ 链，其具有以下序列：

[0216] QVFVYMLLWLSGVDGDIVMTQSQKFMSTSVGDRVSVTCKASQNVGTNVAWYQQKPGQSPKALIYSASYRFSGVPDRFTGSGSGTDFTLTISNVQSEDLAEYFCQQYNSYPYTFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC（信号肽以加下划线文字陈述，可变区以无格式文字陈述，并且恒定区以斜体陈述）(SEQ ID NO:40)。

[0217] M12 抗体具有含以下氨基酸序列的可变轻链：

[0218] DIVMTQSQKFMSTSVGDRVSVTCKASQNVGTNVAWYQQKPGQSPKALIYSASYRFSGVPDRFTGSGSGTDFTLTISNVQSEDLAEYFCQQYNSYPYTFGGGTKLEIK(CDR 1、2 及 3（分别为 SEQ ID NO:42、43 及 44）加下划线）(SEQ ID NO:41)。

[0219] 图 4 中示出了序列比较，显示了如与 AB0041 抗体相比较，M4 与 M12 重链和轻链之间的差异。

[0220] 进行酶分析。结果证实，由 M4 和 M12 杂交瘤产生的抗体充当 MMP9 的非竞争性抑制剂（数据未示出）。

[0221] 实例 1C：针对小鼠 MMP-9 的抗体的制备。

[0222] 产生另一小鼠抗体 AB0046。使用与实例 1A 中所述类似的方法，使用鼠类 MMP9 的前体 / 催化结构域片段的靶向结构域对 MMP9 基因敲除小鼠（品系 B6.FVB(Cg)-Mmp9^{tm1Tvu}/J）进行免疫。AB0046 抗体具有含以下氨基酸序列的 κ 轻链：MSSAQFLGLLLLCFQGTRCDIQMTQTTSSLSASLGDRVTISCSASQGISNYLNWYQQKPDGTFKLLIYYTSILHSGVPSRFSGSGSGTDYSLTISNLEPEDIATYYCQQYGWLPRTFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC(SEQ ID NO:45)（信号肽以加下划线文字陈述，可变区以无格式文字陈述，并且恒定区以斜体陈述）；及含以下氨基酸序列的 IgG1 重链：MGWSSIILFLVATATGVHSQVQLQQPGSVLVRPGASVKLSCTASGYTFTSYWMNWVKQRPGQGLEWIGEIYPISGRTNYNEKFKVKATLTVDTSSSTAYMDLNSLTSEDSAVYYCARSRANWDDYWGQGTTLTVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPS

ETVTCNVAHPASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVDISKDDPEVQFSW FVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPP KEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLNVQKSNWEAGNTFTCSVLHEG LHNHHTEKSLSHSPGK(SEQ ID NO:46)(信号肽以加下划线文字陈述,可变区以无格式文字陈述,并且恒定区以斜体陈述)。

[0223] 以下氨基酸序列包含 AB0046 的 IgG1 重链可变区的构架区和互补决定区 (CDR)(其中 CDR 加下划线):

[0224] QVQLQQPGSVLVRPGASVKLSCTASGYTFTSYWMNWVKQRPGQGLEWIGEIYPISGRTNYNEKFKVKAT LTVDTSSSTAYMDLNSLTSEDSAVYYCARSRANWDDYWGQGTTLTVSS(SEQ ID No:47)。

[0225] 以下氨基酸序列包含 AB0046 的 κ 轻链可变区的构架区和互补决定区 (CDR)(其中 CDR 加下划线):

[0226] DIQMTQTTSSLSASLGDRVTISCSASQGISNYLNWYQQKPDGTFKLLIYYTSILHSGVPSRFSGSGSGT DYSLTISNLEPEDIATYYCQQYGWLPRTFGGGTKLEIK(SEQ ID No:48)。

[0227] 其它表征显示,AB0046 抗体以非竞争性方式结合到小鼠 MMP9 或其结合不取决于小鼠 MMP9 的浓度。AB0046 抗体不结合到人类 MMP9 或 MMP2、小鼠 MMP2、3、7、8 或 12。使用如实例 1A 中所述的表位分析显示,在小鼠 MMP9 氨基酸序列 162 位的脯氨酸残基 (P162)(对应于人类 MMP9 的 R162) 对于 AB0046 抗体结合 MMP9 很重要。结果表明,AB0046 特异性结合到这样一种表位,其含有的残基在小鼠 MMP9 对应于人类 MMP9 的含氨基酸 159-166 的部分的一部分内。因此,AB0046 抗体是对小鼠 MMP9 具有特异性的一种抑制性抗体并且具有与 AB0041 类似的结合动力学和抑制作用。由于 AB0046 对小鼠 MMP9 具有特异性并且结合到与 AB0041/AB0045 相同的表位,故 AB0046 适合于使用 AB0041 或 AB0045 的分析。

[0228] 进一步表征显示,AB0046 抗体是一种鼠类 IgG1 同型,在小鼠中具有一种有限的效应功能。

[0229] 使用类似方法产生三种其它的小鼠抗 MMP9 抗体,其为非抑制性的并且 P162 对于结合很重要。

[0230] 实例 2:针对人类 MMP9 的抗体的人类化

[0231] 使小鼠 AB0041 抗体重链和轻链的氨基酸序列在其可变区的构架 (即,非 CDR) 部分中的某些位置处发生改变以产生在人体中具有较低免疫原性的蛋白质。这些氨基酸序列变化显示于图 1 和 2 中。上表 2A 中显示了一种称为 AB0045 的人类化抗体的交叉反应性。

[0232] 人类化变体抗 MMP9 抗体 AB0045(人类化,经修饰 IgG4(S241P);参见以上实例 2)含有人类化 AB0041 重链变体 VH3(具有 SEQ ID NO:7

[0233] (QVQLQESGPGLVKPSETLSLTCTVSGFSLLSYGVHWVRQPPGKGLEWLGVIWTGGTTNYNSALMSRFT ISKDDSKNTVYLKMNSLKTEDTAIYYCARYYYGMDYWGQGTLVTVSS) 中所陈述的序列)

[0234] 及人类化 AB0041 轻链变体 VH4(具有 Vk4(具有 SEQ ID NO:12

[0235] (DIQMTQSPSSLSASVGDRVTITCKASQDVRNTVAWYQQKPGKAPKLLIYSSSYRNTGVPDRFSGSGSG TDFTLTISSLQAEDVAVYYCQQHYITPYTFGGGTKVEIK) 中所陈述的序列) 中所陈述的轻链序列)。

[0236] AB0045 抗体的重链具有 SEQ ID NO:49(MGWSLILLFLVAVATRVHSQVQLQESGPGLVKPSET LSLTCTVSGFSLLSYGVHWVRQPPGKGLEWLGVIWTGGTTNYNSALMSRFTISKDDSKNTVYLKMNSLKTEDTAIYY CARYYYGMDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV

LQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPS SIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK(信号序列加下划线;恒定区序列以斜体呈现))中所陈述的序列;AB0045抗体的轻链具有SEQ ID NO:50(MRVPAQLLGLLLLWLPGARCDIQ MTQSPSSLSASVGDRVTITCKASQDVRNTVAWYQQKPGKAPKLLIYSSSYRNTGVPDRFSGSGSGTDFTLTISSLQA EDVAVYYCQQHYITPYTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC(信号序列加下划线;恒定区序列以斜体呈现))中所陈述的序列。所述抗体含有1312个氨基酸的长度,由两个重链和两个轻链构成并且具有约7.90的理论pI值、对于1g/L在280nm下约1.50AU/cm的消光系数、约144kDa的分子量及在调配缓冲液中约1g/mL的密度(50-100mg/mL产物浓度)。

[0237] 这一抗体的进一步表征描述于以下实例3中。

[0238] 实例3:变体MMP9抗体AB0045的表征及与AB0041和AB0046的比较

[0239] 如以上所述,AB0045和AB0041抗体是MMP9的非竞争性抑制剂。因此,两种抗体都抑制MMP9酶活性,而与底物浓度无关。AB0045抗体结合到与AB0041抗体相同的MMP9表位,并且亲和力在1×10^{-12}摩尔浓度范围内,如通过直接结合和表面等离子共振(SPR)分析所显示。两种抗体都对MMP9具有特异性,并且未观察到针对其它纯化蛋白质目标(包括MMP酶的纯化结构域和全长形式)的显著非特异性结合。AB0045和AB0041抗体都与天然和重组人类MMP9以及重组大鼠和食蟹猴MMP9交叉反应。

[0240] 使用酶连免疫吸附剂分析(ELISA)和MMP9酶分析来测定AB0045、AB0041及AB0046抗体对于人类和非人类来源的MMP9的体外结合亲和力、抑制特征和特异性。同时使用SPR分析来产生AB0045和AB0041的解离常数(K_d)。

[0241] 在ELISA分析中,发现由ELISA得到的AB0045和AB0041抗体对于人类、食蟹猴及大鼠MMP9的K_d值都<400pM。ELISA数据说明,AB0045和AB0041抗体都与来自所测试的所有相关毒理学物种的MMP9交叉反应。经显示,AB0046抗体对小鼠MMP9具有特异性,并因此可在小鼠功效模型中用作代用抗体。结果显示,AB0045抗体对于人类MMP9的K_d值为0.168±0.117nM并且AB0041抗体的K_d值为0.133±0.030nM。有关AB0046抗体的结果显示,其结合到小鼠MMP9,并且K_d值为0.218±0.097nM。在SPR分析中,结果显示AB0045和AB0041抗体对于人类MMP9的K_d值分别为8.8pM和0.4pM。

[0242] 在评估MMP9介导的荧光肽底物Mca-PLGL-Dpa-AR-NH2裂解的分析中评价AB0045、AB0041及AB0046抗体的酶抑制活性。全部三种抗体都抑制MMP9酶活性。AB0045(0.691±0.097nM)和AB0041(0.569±0.185nM)对于人类MMP9的IC_{50}值无统计学差异。有关AB0046对小鼠MMP9的抑制作用的IC_{50}值为0.352±0.03nM。

[0243] 所述值未针对在制备期间产生的活性酶的浓度进行调整。使用在稳态条件下进行的其它MMP9酶分析来测定IC_{50}和抑制模式。在这一分析中,AB0045的IC_{50}值在0.148nM到0.161nM范围内(在20倍底物浓度范围内),并且在一个实例中为0.158nm。这些结果显示,AB0045的MMP9抑制活性是非竞争性的。

[0244] 表2B:AB0045、AB0041及代用小鼠抗体AB0046的结合和抑制特性

[0245]

	AB0045	AB0041	AB0046
ELISA			
人类 MMP9 解离常数	0.168 ± 0.117 nM	0.133 ± 0.030 nM	>100 nM
食蟹猴 MMP9 解离常数	0.082 ± 0.022 nM	0.145 ± 0.16 nM	>100 nM
小鼠 MMP9 解离常数	>100 nM	>100 nM	0.218±0.097 nM
大鼠 MMP9 解离常数	0.311 ± 0.017 nM	0.332 ± 0.022 nM	>100 nM
SPR			
人类 MMP9 解离常数	8.8pM	0.4pM	ND
活性分析			
人类 MMP9 IC_{50}	0.691 ± 0.097 nM	0.569 ± 0.185 nM	>100 nM
食蟹猴 MMP9 IC_{50}	0.194 ± 0.048 nM*	0.189 ± 0.019 nM*	>100 nM

[0246]

大鼠 MMP9 IC_{50}	8.23 ± 1.24 nM*	2.78 ± 1.17 nM*	>100 nM
小鼠 MMP9 IC_{50}	>100 nM	>100 nM	0.352±0.03 nM*

[0247] 结果证实，AB0045 和 AB0041 具有相当的结合和抑制特性，并且 AB0046 可例如在小鼠人类疾病模型中用作相关小鼠代用抗体。

[0001]

序列表

<110> 吉联亚生物科技有限公司

<120> 抗基质金属蛋白酶9抗体

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<141> Concurrently Herewith

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Val Leu Ser Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala
            20                  25                  30
Pro Ser Gln Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu
        35                  40                  45
Leu Ser Tyr Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu
    50                  55                  60
Glu Trp Leu Gly Val Ile Trp Thr Gly Gly Thr Thr Asn Tyr Asn Ser
65                  70                  75                  80
Ala Leu Met Ser Arg Leu Ser Ile Ser Lys Asp Asp Ser Lys Ser Gln
                85                  90                  95
Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Ile Tyr
            100                 105                 110
Tyr Cys Ala Arg Tyr Tyr Tyr Gly Met Asp Tyr Trp Gly Gln Gly Thr
        115                 120                 125
Ser Val Thr Val Ser Ser Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro
    130                 135                 140
Leu Ala Pro Gly Cys Gly Asp Thr Thr Gly Ser Ser Val Thr Leu Gly
145                 150                 155                 160
```

[0002]

```
Cys Leu Val Lys Gly Tyr Phe Pro Glu Ser Val Thr Val Thr Trp Asn
                165                 170                 175
Ser Gly Ser Leu Ser Ser Ser Val His Thr Phe Pro Ala Leu Leu Gln
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Ser Gly Leu Tyr Thr Met Ser Ser Ser Val Thr Val Pro Ser Ser Thr
        195                 200                 205
Trp Pro Ser Gln Thr Val Thr Cys Ser Val Ala His Pro Ala Ser Ser
    210                 215                 220
Thr Thr Val Asp Lys Lys Leu Glu Pro Ser Gly Pro Ile Ser Thr Ile
225                 230                 235                 240
Asn Pro Cys Pro Pro Cys Lys Glu Cys His Lys Cys Pro Ala Pro Asn
                245                 250                 255
Leu Glu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Asn Ile Lys Asp
            260                 265                 270
Val Leu Met Ile Ser Leu Thr Pro Lys Val Thr Cys Val Val Val Asp
        275                 280                 285
Val Ser Glu Asp Asp Pro Asp Val Arg Ile Ser Trp Phe Val Asn Asn
    290                 295                 300
Val Glu Val His Thr Ala Gln Thr Gln Thr His Arg Glu Asp Tyr Asn
305                 310                 315                 320
Ser Thr Ile Arg Val Val Ser Ala Leu Pro Ile Gln His Gln Asp Trp
                325                 330                 335
Met Ser Gly Lys Glu Phe Lys Cys Lys Val Asn Asn Lys Asp Leu Pro
            340                 345                 350
Ser Pro Ile Glu Arg Thr Ile Ser Lys Ile Lys Gly Leu Val Arg Ala
        355                 360                 365
Pro Gln Val Tyr Ile Leu Pro Pro Pro Ala Glu Gln Leu Ser Arg Lys
    370                 375                 380
Asp Val Ser Leu Thr Cys Leu Val Val Gly Phe Asn Pro Gly Asp Ile
385                 390                 395                 400
Ser Val Glu Trp Thr Ser Asn Gly His Thr Glu Glu Asn Tyr Lys Asp
                405                 410                 415
Thr Ala Pro Val Leu Asp Ser Asp Gly Ser Tyr Phe Ile Tyr Ser Lys
            420                 425                 430
Leu Asp Ile Lys Thr Ser Lys Trp Glu Lys Thr Asp Ser Phe Ser Cys
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[0003]

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Thr Ser Val Gly Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp
35 40 45
Val Arg Asn Thr Val Ala Trp Tyr Gln Gln Lys Thr Gly Gln Ser Pro
50 55 60
Lys Leu Leu Ile Tyr Ser Ser Ser Tyr Arg Asn Thr Gly Val Pro Asp
65 70 75 80
Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser
85 90 95
Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Phe Cys Gln Gln His Tyr
100 105 110
Ile Thr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
115 120 125
Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln
130 135 140
Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr
145 150 155 160
Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln
165 170 175
Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr
180 185 190
Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg
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His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro
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Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
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<220>
<221> CHAIN
<222> (1)...(115)
<223> AB0041的IgG2b重链的可变区

<220>
<221> misc_feature
<222> (26)...(35)
<223> 互补决定区(CDR)

<220>
<221> misc_feature

[0004]

<222> (50)...(65)
<223> 互补决定区(CDR)

<220>
<221> misc_feature
<222> (98)...(104)
<223> 互补决定区(CDR)

<400> 3
Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln
1 5 10 15
Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Leu Ser Tyr
20 25 30
Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45
Gly Val Ile Trp Thr Gly Gly Thr Thr Asn Tyr Asn Ser Ala Leu Met
50 55 60
Ser Arg Leu Ser Ile Ser Lys Asp Asp Ser Lys Ser Gln Val Phe Leu
65 70 75 80
Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Ile Tyr Tyr Cys Ala
85 90 95
Arg Tyr Tyr Tyr Gly Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr
100 105 110
Val Ser Ser
115

<210> 4
<211> 107
<212> PRT
<213> 小家鼠

<220>
<221> CHAIN
<222> (1)...(107)
<223> AB0041的κ轻链的可变区

<220>
<221> misc_feature
<222> (24)...(34)
<223> 互补决定区(CDR)

<220>
<221> misc_feature
<222> (50)...(56)
<223> 互补决定区(CDR)

<220>
<221> misc_feature
<222> (89)...(97)
<223> 互补决定区(CDR)

<400> 4
Asp Ile Val Met Thr Gln Ser His Lys Phe Met Ser Thr Ser Val Gly
1 5 10 15
Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp Val Arg Asn Thr

[0005]

```
                20                  25                  30
Val Ala Trp Tyr Gln Gln Lys Thr Gly Gln Ser Pro Lys Leu Leu Ile
        35                  40                  45
Tyr Ser Ser Ser Tyr Arg Asn Thr Gly Val Pro Asp Arg Phe Thr Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Val Gln Ala
65                  70                  75                  80
Glu Asp Leu Ala Val Tyr Phe Cys Gln Gln His Tyr Ile Thr Pro Tyr
                85                  90                  95
Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105
```

<210> 5
<211> 115
<212> PRT
<223> 人工序列

<220>
<223> 合成构建体

<220>
<221> VARIANT
<222> (1)...(115)
<223> VH1 重链变体

```
<400> 5
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
 1               5                  10                  15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Leu Ser Tyr
            20                  25                  30
Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
        35                  40                  45
Gly Val Ile Trp Thr Gly Gly Thr Thr Asn Tyr Asn Ser Ala Leu Met
    50                  55                  60
Ser Arg Leu Thr Ile Ser Lys Asp Asp Ser Lys Ser Thr Val Tyr Leu
65                  70                  75                  80
Lys Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Ile Tyr Tyr Cys Ala
                85                  90                  95
Arg Tyr Tyr Tyr Gly Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr
            100                 105                 110
Val Ser Ser
        115
```

<210> 6
<211> 115
<212> PRT
<223> 人工序列

<220>
<223> 合成构建体

<220>
<221> VARIANT
<222> (1)...(115)

[0006]

<223> VH2 重链变体

<400> 6
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Leu Ser Tyr
20 25 30
Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45
Gly Val Ile Trp Thr Gly Gly Thr Thr Asn Tyr Asn Ser Ala Leu Met
50 55 60
Ser Arg Leu Thr Ile Ser Lys Asp Asp Ser Lys Asn Thr Val Tyr Leu
65 70 75 80
Lys Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Ile Tyr Tyr Cys Ala
85 90 95
Arg Tyr Tyr Tyr Gly Met Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr
100 105 110
Val Ser Ser
115

<210> 7
<211> 115
<212> PRT
<223> 人工序列

<220>
<223> 合成构建体

<220>
<221> VARIANT
<222> (1)...(115)
<223> VH3 重链变体

<400> 7
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Leu Ser Tyr
20 25 30
Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45
Gly Val Ile Trp Thr Gly Gly Thr Thr Asn Tyr Asn Ser Ala Leu Met
50 55 60
Ser Arg Phe Thr Ile Ser Lys Asp Asp Ser Lys Asn Thr Val Tyr Leu
65 70 75 80
Lys Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Ile Tyr Tyr Cys Ala
85 90 95
Arg Tyr Tyr Tyr Gly Met Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr
100 105 110
Val Ser Ser
115

<210> 8
<211> 115
<212> PRT

[0007]

<223> 人工序列

<220>
<223> 合成构建体

<220>
<221> VARIANT
<222> (1)...(115)
<223> VH4 重链变体

<400> 8
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Leu Ser Tyr
20 25 30
Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45
Gly Val Ile Trp Thr Gly Gly Thr Thr Asn Tyr Asn Ser Ala Leu Met
50 55 60
Ser Arg Phe Thr Ile Ser Lys Asp Asp Ser Lys Asn Thr Leu Tyr Leu
65 70 75 80
Lys Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Ile Tyr Tyr Cys Ala
85 90 95
Arg Tyr Tyr Tyr Gly Met Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr
100 105 110
Val Ser Ser
115

<210> 9
<211> 107
<212> PRT
<223> 人工序列

<220>
<223> 合成构建体

<220>
<221> VARIANT
<222> (1)...(107)
<223> Vk1 轻链变体

<400> 9
Asp Ile Val Met Thr Gln Ser Pro Ser Phe Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Val Arg Asn Thr
20 25 30
Val Ala Trp Tyr Gln Gln Lys Thr Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Ser Ser Ser Tyr Arg Asn Thr Gly Val Pro Asp Arg Phe Thr Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Ala
65 70 75 80
Glu Asp Val Ala Val Tyr Phe Cys Gln Gln His Tyr Ile Thr Pro Tyr
85 90 95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys

[0008]

100 105

<210> 10
<211> 107
<212> PRT
<223> 人工序列

<220>
<223> 合成构建体

<220>
<221> VARIANT
<222> (1)...(107)
<223> Vk2 轻链变体

<400> 10
Asp Ile Val Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Val Arg Asn Thr
20 25 30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Ser Ser Ser Tyr Arg Asn Thr Gly Val Pro Asp Arg Phe Thr Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Ala
65 70 75 80
Glu Asp Val Ala Val Tyr Phe Cys Gln Gln His Tyr Ile Thr Pro Tyr
85 90 95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 11
<211> 107
<212> PRT
<223> 人工序列

<220>
<223> 合成构建体

<220>
<221> VARIANT
<222> (1)...(107)
<223> Vk3 轻链变体

<400> 11
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Val Arg Asn Thr
20 25 30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Ser Ser Ser Tyr Arg Asn Thr Gly Val Pro Asp Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Ala

[0009]

65 70 75 80
Glu Asp Val Ala Val Tyr Phe Cys Gln Gln His Tyr Ile Thr Pro Tyr
85 90 95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 12
<211> 107
<212> PRT
<223> 人工序列

<220>
<223> 合成构建体

<220>
<221> VARIANT
<222> (1)...(107)
<223> Vk4 轻链变体

<400> 12
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Val Arg Asn Thr
20 25 30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Ser Ser Ser Tyr Arg Asn Thr Gly Val Pro Asp Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Ala
65 70 75 80
Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln His Tyr Ile Thr Pro Tyr
85 90 95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 13
<211> 10
<212> PRT
<223> 人工序列

<220>
<223> 合成构建体

<220>
<221> misc_feature
<222> (1)...(10)
<223> 抗MMP9抗体重链的互补决定区(CDR1)

<400> 13
Gly Phe Ser Leu Leu Ser Tyr Gly Val His
1 5 10

<210> 14

[0010]

<211> 16
<212> PRT
<223> 人工序列

<220>
<223> 合成构建体

<220>
<221> misc_feature
<222> (1)...(16)
<223> 抗MMP9抗体重链的互补决定区(CDR2)

<400> 14
Val Ile Trp Thr Gly Gly Thr Thr Asn Tyr Asn Ser Ala Leu Met Ser
1 5 10 15

<210> 15
<211> 7
<212> PRT
<223> 人工序列

<220>
<223> 合成构建体

<220>
<221> misc_feature
<222> (1)...(7)
<223> 抗MMP9抗体重链的互补决定区(CDR3)

<400> 15
Tyr Tyr Tyr Gly Met Asp Tyr
1 5

<210> 16
<211> 11
<212> PRT
<223> 人工序列

<220>
<223> 合成构建体

<220>
<221> misc_feature
<222> (1)...(11)
<223> 抗MMP9抗体轻链的互补决定区(CDR1)

<400> 16
Lys Ala Ser Gln Asp Val Arg Asn Thr Val Ala
1 5 10

<210> 17
<211> 7
<212> PRT

[0011]

<223> 人工序列

<220>
<223> 合成构建体

<220>
<221> misc_feature
<222> (1)...(7)
<223> 抗MMP9抗体轻链的互补决定区(CDR2)

<400> 17
Ser Ser Ser Tyr Arg Asn Thr
1 5

<210> 18
<211> 9
<212> PRT
<223> 人工序列

<220>
<223> 合成构建体

<220>
<221> misc_feature
<222> (1)...(9)
<223> 抗MMP9抗体轻链的互补决定区(CDR3)

<400> 18
Gln Gln His Tyr Ile Thr Pro Tyr Thr
1 5

<210> 19
<211> 345
<212> DNA
<223> 人工序列

<220>
<223> 合成构建体

<220>
<221> misc_feature
<222> (1)...(345)
<223> 编码VH1重链氨基酸序列的核苷酸序列

<400> 19
caggtgcagc tgcaggaatc cggccctggc ctggtcaagc cctccgagac actgtccctg 60
acctgcaccg tgtccggctt ctccctgctg tcctacggcg tgcactgggt ccgacagcct 120
ccagggaagg gcctggaatg gctgggcgtg atctggaccg gcggcaccac caactacaac 180
tccgccctga tgtcccggct gaccatctcc aaggacgact ccaagtccac cgtgtacctg 240
aagatgaact ccctgaaaac cgaggacacc gccatctact actgcgcccg gtactactac 300
ggcatggact actggggcca gggcacctcc gtgaccgtgt cctca 345

<210> 20
<211> 345

[0012]

<212> DNA
<223> 人工序列

<220>
<223> 合成构建体

<220>
<221> misc_feature
<222> (1)...(345)
<223> 编码VH2重链氨基酸序列的核苷酸序列

<400> 20
caggtgcagc tgcaggaatc cggccctggc ctggtcaagc cctccgagac actgtccctg 60
acctgcaccg tgtccggctt ctccctgctg tcctacggcg tgcactgggt ccgacagcct 120
ccaggcaaag gcctggaatg gctgggcgtg atctggaccg gcggcaccac caactacaac 180
tccgccctga tgtcccggct gaccatctcc aaggacgact ccaagaacac cgtgtacctg 240
aagatgaact ccctgaaaac cgaggacacc gccatctact actgcgcccg gtactactac 300
ggcatggact actggggcca gggcaccctg gtcaccgtgt cctca 345

<210> 21
<211> 345
<212> DNA
<223> 人工序列

<220>
<223> 合成构建体

<220>
<221> misc_feature
<222> (1)...(345)
<223> 编码VH3重链氨基酸序列的核苷酸序列

<400> 21
caggtgcagc tgcaggaatc cggccctggc ctggtcaagc cctccgagac actgtccctg 60
acctgcaccg tgtccggctt ctccctgctg tcctacggcg tgcactgggt ccgacagcct 120
ccaggcaaag gcctggaatg gctgggcgtg atctggaccg gcggcaccac caactacaac 180
tccgccctga tgtcccggtt caccatctcc aaggacgact ccaagaacac cgtgtacctg 240
aagatgaact ccctgaaaac cgaggacacc gccatctact actgcgcccg gtactactac 300
ggcatggact actggggcca gggcaccctg gtcaccgtgt cctca 345

<210> 22
<211> 345
<212> DNA
<223> 人工序列

<220>
<223> 合成构建体

<220>
<221> misc_feature
<222> (1)...(345)
<223> 编码VH4重链氨基酸序列的核苷酸序列

<400> 22
caggtgcagc tgcaggaatc cggccctggc ctggtcaagc cctccgagac actgtccctg 60
acctgcaccg tgtccggctt ctccctgctg tcctacggcg tgcactgggt ccgacagcct 120

[0013]

ccaggcaaag gcctggaatg gctgggcgtg atctggaccg gcggcaccac caactacaac 180
tccgccctga tgtcccggtt caccatctcc aaggacgact ccaagaacac cctgtacctg 240
aagatgaact ccctgaaaac cgaggacacc gccatctact actgcgcccg gtactactac 300
ggcatggact actggggcca gggcaccctg gtcaccgtgt cctca 345

<210> 23
<211> 321
<212> DNA
<223> 人工序列

<220>
<223> 合成构建体

<220>
<221> misc_feature
<222> (1)...(321)
<223> 编码Vk1轻链氨基酸序列的核苷酸序列

<400> 23
gacatcgtga tgacccagtc ccccagcttc ctgtccgcct ccgtgggcga cagagtgacc 60
atcacatgca aggcctctca ggacgtgcgg aacaccgtgg cctggtatca gcagaaaacc 120
ggcaaggccc ccaagctgct gatctactcc tcctcctacc ggaacaccgg cgtgcccgac 180
cggtttaccg gctctggctc cggcaccgac tttaccctga ccatcagctc cctgcaggcc 240
gaggacgtgg ccgtgtactt ctgccagcag cactacatca ccccctacac cttcggcgga 300
ggcaccaagg tggaaataaa a 321

<210> 24
<211> 321
<212> DNA
<223> 人工序列

<220>
<223> 合成构建体

<220>
<221> misc_feature
<222> (1)...(321)
<223> 编码Vk2轻链氨基酸序列的核苷酸序列

<400> 24
gacatcgtga tgacccagtc cccctccagc ctgtccgcct ctgtgggcga cagagtgacc 60
atcacatgca aggcctctca ggacgtgcgg aacaccgtgg cctggtatca gcagaagccc 120
ggcaaggccc ccaagctgct gatctactcc tcctcctacc ggaacaccgg cgtgcccgac 180
cggtttaccg gctctggctc cggcaccgac tttaccctga ccatcagctc cctgcaggcc 240
gaggacgtgg ccgtgtactt ctgccagcag cactacatca ccccctacac cttcggcgga 300
ggcaccaagg tggaaataaa a 321

<210> 25
<211> 321
<212> DNA
<223> 人工序列

<220>
<223> 合成构建体

<220>

[0014]

<221> misc_feature
<222> (1)...(321)
<223> 编码Vk3轻链氨基酸序列的核苷酸序列

<400> 25
gacatccaga tgacccagtc cccctccagc ctgtccgcct ctgtgggcga cagagtgacc 60
atcacatgca aggcctccca ggacgtgcgg aacaccgtgg cctggtatca gcagaagccc 120
ggcaaggccc ccaagctgct gatctactcc tcctcctacc ggaacaccgg cgtgccccgac 180
cggttctctg gctctggaag cggcaccgac tttaccctga ccatcagctc cctgcaggcc 240
gaggacgtgg ccgtgtactt ctgccagcag cactacatca cccccctacac cttcggcgga 300
ggcaccaagg tggaaataaa a 321

<210> 26
<211> 321
<212> DNA
<223> 人工序列

<220>
<223> 合成构建体

<220>
<221> misc_feature
<222> (1)...(321)
<223> 编码Vk4轻链氨基酸序列的核苷酸序列

<400> 26
gacatccaga tgacccagtc cccctccagc ctgtccgcct ctgtgggcga cagagtgacc 60
atcacatgca aggcctctca ggacgtgcgg aacaccgtgg cctggtatca gcagaagccc 120
ggcaaggccc ccaagctgct gatctactcc tcctcctacc ggaacaccgg cgtgccccgac 180
cggttctctg gctctggaag cggcaccgac tttaccctga ccatcagctc cctgcaggcc 240
gaggacgtgg ccgtgtacta ctgccagcag cactacatca cccccctacac cttcggcgga 300
ggcaccaagg tggaaataaa a 321

<210> 27
<211> 707
<212> PRT
<213> 智人

<220>
<221> misc_feature
<222> (1)...(707)
<223> 基质金属蛋白酶9 (MMP9)

<220>
<221> PEPTIDE
<222> (1)...(19)
<223> 信号肽

<220>
<221> DOMAIN
<222> (38)...(98)
<223> 肽聚糖结合结构域

<220>
<221> SITE
<222> (98)...(99)

[0015]

<223> 前肽裂解位点

<220>
<221> DOMAIN
<222> (112)...(445)
<223> Zn依赖性金属蛋白酶结构域

<220>
<221> DOMAIN
<222> (223)...(271)
<223> 纤连蛋白II型结构域(明胶结合结构域)

<220>
<221> DOMAIN
<222> (281)...(329)
<223> 纤连蛋白II型结构域(明胶结合结构域)

<220>
<221> DOMAIN
<222> (340)...(388)
<223> 纤连蛋白II型结构域(明胶结合结构域)

<220>
<221> misc_feature
<222> (400)...(411)
<223> Zn结合区

<220>
<221> DOMAIN
<222> (521)...(565)
<223> 血红素结合蛋白样结构域

<220>
<221> DOMAIN
<222> (567)...(608)
<223> 血红素结合蛋白样结构域

<220>
<221> DOMAIN
<222> (613)...(659)
<223> 血红素结合蛋白样结构域

<220>
<221> DOMAIN
<222> (661)...(704)
<223> 血红素结合蛋白样结构域

<400> 27

Met	Ser	Leu	Trp	Gln	Pro	Leu	Val	Leu	Val	Leu	Leu	Val	Leu	Gly	Cys
1				5				10						15	
Cys	Phe	Ala	Ala	Pro	Arg	Gln	Arg	Gln	Ser	Thr	Leu	Val	Leu	Phe	Pro
			20					25					30		
Gly	Asp	Leu	Arg	Thr	Asn	Leu	Thr	Asp	Arg	Gln	Leu	Ala	Glu	Glu	Tyr
		35					40					45			
Leu	Tyr	Arg	Tyr	Gly	Tyr	Thr	Arg	Val	Ala	Glu	Met	Arg	Gly	Glu	Ser

[0016]

50 55 60
Lys Ser Leu Gly Pro Ala Leu Leu Leu Leu Gln Lys Gln Leu Ser Leu
65 70 75 80
Pro Glu Thr Gly Glu Leu Asp Ser Ala Thr Leu Lys Ala Met Arg Thr
85 90 95
Pro Arg Cys Gly Val Pro Asp Leu Gly Arg Phe Gln Thr Phe Glu Gly
100 105 110
Asp Leu Lys Trp His His His Asn Ile Thr Tyr Trp Ile Gln Asn Tyr
115 120 125
Ser Glu Asp Leu Pro Arg Ala Val Ile Asp Asp Ala Phe Ala Arg Ala
130 135 140
Phe Ala Leu Trp Ser Ala Val Thr Pro Leu Thr Phe Thr Arg Val Tyr
145 150 155 160
Ser Arg Asp Ala Asp Ile Val Ile Gln Phe Gly Val Ala Glu His Gly
165 170 175
Asp Gly Tyr Pro Phe Asp Gly Lys Asp Gly Leu Leu Ala His Ala Phe
180 185 190
Pro Pro Gly Pro Gly Ile Gln Gly Asp Ala His Phe Asp Asp Asp Glu
195 200 205
Leu Trp Ser Leu Gly Lys Gly Val Val Val Pro Thr Arg Phe Gly Asn
210 215 220
Ala Asp Gly Ala Ala Cys His Phe Pro Phe Ile Phe Glu Gly Arg Ser
225 230 235 240
Tyr Ser Ala Cys Thr Thr Asp Gly Arg Ser Asp Gly Leu Pro Trp Cys
245 250 255
Ser Thr Thr Ala Asn Tyr Asp Thr Asp Asp Arg Phe Gly Phe Cys Pro
260 265 270
Ser Glu Arg Leu Tyr Thr Arg Asp Gly Asn Ala Asp Gly Lys Pro Cys
275 280 285
Gln Phe Pro Phe Ile Phe Gln Gly Gln Ser Tyr Ser Ala Cys Thr Thr
290 295 300
Asp Gly Arg Ser Asp Gly Tyr Arg Trp Cys Ala Thr Thr Ala Asn Tyr
305 310 315 320
Asp Arg Asp Lys Leu Phe Gly Phe Cys Pro Thr Arg Ala Asp Ser Thr
325 330 335
Val Met Gly Gly Asn Ser Ala Gly Glu Leu Cys Val Phe Pro Phe Thr
340 345 350
Phe Leu Gly Lys Glu Tyr Ser Thr Cys Thr Ser Glu Gly Arg Gly Asp
355 360 365
Gly Arg Leu Trp Cys Ala Thr Thr Ser Asn Phe Asp Ser Asp Lys Lys
370 375 380
Trp Gly Phe Cys Pro Asp Gln Gly Tyr Ser Leu Phe Leu Val Ala Ala
385 390 395 400
His Glu Phe Gly His Ala Leu Gly Leu Asp His Ser Ser Val Pro Glu
405 410 415
Ala Leu Met Tyr Pro Met Tyr Arg Phe Thr Glu Gly Pro Pro Leu His
420 425 430
Lys Asp Asp Val Asn Gly Ile Arg His Leu Tyr Gly Pro Arg Pro Glu
435 440 445
Pro Glu Pro Arg Pro Pro Thr Thr Thr Thr Pro Gln Pro Thr Ala Pro
450 455 460
Pro Thr Val Cys Pro Thr Gly Pro Pro Thr Val His Pro Ser Glu Arg
465 470 475 480
Pro Thr Ala Gly Pro Thr Gly Pro Pro Ser Ala Gly Pro Thr Gly Pro
485 490 495
Pro Thr Ala Gly Pro Ser Thr Ala Thr Thr Val Pro Leu Ser Pro Val

[0017]

500 505 510
Asp Asp Ala Cys Asn Val Asn Ile Phe Asp Ala Ile Ala Glu Ile Gly
515 520 525
Asn Gln Leu Tyr Leu Phe Lys Asp Gly Lys Tyr Trp Arg Phe Ser Glu
530 535 540
Gly Arg Gly Ser Arg Pro Gln Gly Pro Phe Leu Ile Ala Asp Lys Trp
545 550 555 560
Pro Ala Leu Pro Arg Lys Leu Asp Ser Val Phe Glu Glu Pro Leu Ser
565 570 575
Lys Lys Leu Phe Phe Phe Ser Gly Arg Gln Val Trp Val Tyr Thr Gly
580 585 590
Ala Ser Val Leu Gly Pro Arg Arg Leu Asp Lys Leu Gly Leu Gly Ala
595 600 605
Asp Val Ala Gln Val Thr Gly Ala Leu Arg Ser Gly Arg Gly Lys Met
610 615 620
Leu Leu Phe Ser Gly Arg Arg Leu Trp Arg Phe Asp Val Lys Ala Gln
625 630 635 640
Met Val Asp Pro Arg Ser Ala Ser Glu Val Asp Arg Met Phe Pro Gly
645 650 655
Val Pro Leu Asp Thr His Asp Val Phe Gln Tyr Arg Glu Lys Ala Tyr
660 665 670
Phe Cys Gln Asp Arg Phe Tyr Trp Arg Val Ser Ser Arg Ser Glu Leu
675 680 685
Asn Gln Val Asp Gln Val Gly Tyr Val Thr Tyr Asp Ile Leu Gln Cys
690 695 700
Pro Glu Asp
705

<210> 28
<211> 688
<212> PRT
<213> 智人

<220>
<221> misc_feature
<222> (1)...(688)
<223> 成熟全长基质金属蛋白酶9 (MMP9)

<400> 28
Ala Pro Arg Gln Arg Gln Ser Thr Leu Val Leu Phe Pro Gly Asp Leu
1 5 10 15
Arg Thr Asn Leu Thr Asp Arg Gln Leu Ala Glu Glu Tyr Leu Tyr Arg
20 25 30
Tyr Gly Tyr Thr Arg Val Ala Glu Met Arg Gly Glu Ser Lys Ser Leu
35 40 45
Gly Pro Ala Leu Leu Leu Leu Gln Lys Gln Leu Ser Leu Pro Glu Thr
50 55 60
Gly Glu Leu Asp Ser Ala Thr Leu Lys Ala Met Arg Thr Pro Arg Cys
65 70 75 80
Gly Val Pro Asp Leu Gly Arg Phe Gln Thr Phe Glu Gly Asp Leu Lys
85 90 95
Trp His His His Asn Ile Thr Tyr Trp Ile Gln Asn Tyr Ser Glu Asp
100 105 110
Leu Pro Arg Ala Val Ile Asp Asp Ala Phe Ala Arg Ala Phe Ala Leu
115 120 125

[0018]

```
Trp Ser Ala Val Thr Pro Leu Thr Phe Thr Arg Val Tyr Ser Arg Asp
    130                 135                 140
Ala Asp Ile Val Ile Gln Phe Gly Val Ala Glu His Gly Asp Gly Tyr
145                 150                 155                 160
Pro Phe Asp Gly Lys Asp Gly Leu Leu Ala His Ala Phe Pro Pro Gly
                165                 170                 175
Pro Gly Ile Gln Gly Asp Ala His Phe Asp Asp Asp Glu Leu Trp Ser
            180                 185                 190
Leu Gly Lys Gly Val Val Val Pro Thr Arg Phe Gly Asn Ala Asp Gly
        195                 200                 205
Ala Ala Cys His Phe Pro Phe Ile Phe Glu Gly Arg Ser Tyr Ser Ala
    210                 215                 220
Cys Thr Thr Asp Gly Arg Ser Asp Gly Leu Pro Trp Cys Ser Thr Thr
225                 230                 235                 240
Ala Asn Tyr Asp Thr Asp Asp Arg Phe Gly Phe Cys Pro Ser Glu Arg
                245                 250                 255
Leu Tyr Thr Arg Asp Gly Asn Ala Asp Gly Lys Pro Cys Gln Phe Pro
            260                 265                 270
Phe Ile Phe Gln Gly Gln Ser Tyr Ser Ala Cys Thr Thr Asp Gly Arg
        275                 280                 285
Ser Asp Gly Tyr Arg Trp Cys Ala Thr Thr Ala Asn Tyr Asp Arg Asp
    290                 295                 300
Lys Leu Phe Gly Phe Cys Pro Thr Arg Ala Asp Ser Thr Val Met Gly
305                 310                 315                 320
Gly Asn Ser Ala Gly Glu Leu Cys Val Phe Pro Phe Thr Phe Leu Gly
                325                 330                 335
Lys Glu Tyr Ser Thr Cys Thr Ser Glu Gly Arg Gly Asp Gly Arg Leu
            340                 345                 350
Trp Cys Ala Thr Thr Ser Asn Phe Asp Ser Asp Lys Lys Trp Gly Phe
        355                 360                 365
Cys Pro Asp Gln Gly Tyr Ser Leu Phe Leu Val Ala Ala His Glu Phe
    370                 375                 380
Gly His Ala Leu Gly Leu Asp His Ser Ser Val Pro Glu Ala Leu Met
385                 390                 395                 400
Tyr Pro Met Tyr Arg Phe Thr Glu Gly Pro Pro Leu His Lys Asp Asp
                405                 410                 415
Val Asn Gly Ile Arg His Leu Tyr Gly Pro Arg Pro Glu Pro Glu Pro
            420                 425                 430
Arg Pro Pro Thr Thr Thr Thr Pro Gln Pro Thr Ala Pro Pro Thr Val
        435                 440                 445
Cys Pro Thr Gly Pro Pro Thr Val His Pro Ser Glu Arg Pro Thr Ala
    450                 455                 460
Gly Pro Thr Gly Pro Pro Ser Ala Gly Pro Thr Gly Pro Pro Thr Ala
465                 470                 475                 480
Gly Pro Ser Thr Ala Thr Thr Val Pro Leu Ser Pro Val Asp Asp Ala
                485                 490                 495
Cys Asn Val Asn Ile Phe Asp Ala Ile Ala Glu Ile Gly Asn Gln Leu
            500                 505                 510
Tyr Leu Phe Lys Asp Gly Lys Tyr Trp Arg Phe Ser Glu Gly Arg Gly
        515                 520                 525
Ser Arg Pro Gln Gly Pro Phe Leu Ile Ala Asp Lys Trp Pro Ala Leu
    530                 535                 540
Pro Arg Lys Leu Asp Ser Val Phe Glu Glu Pro Leu Ser Lys Lys Leu
545                 550                 555                 560
Phe Phe Phe Ser Gly Arg Gln Val Trp Val Tyr Thr Gly Ala Ser Val
                565                 570                 575
```

[0019]

Leu Gly Pro Arg Arg Leu Asp Lys Leu Gly Leu Gly Ala Asp Val Ala
580 585 590
Gln Val Thr Gly Ala Leu Arg Ser Gly Arg Gly Lys Met Leu Leu Phe
595 600 605
Ser Gly Arg Arg Leu Trp Arg Phe Asp Val Lys Ala Gln Met Val Asp
610 615 620
Pro Arg Ser Ala Ser Glu Val Asp Arg Met Phe Pro Gly Val Pro Leu
625 630 635 640
Asp Thr His Asp Val Phe Gln Tyr Arg Glu Lys Ala Tyr Phe Cys Gln
645 650 655
Asp Arg Phe Tyr Trp Arg Val Ser Ser Arg Ser Glu Leu Asn Gln Val
660 665 670
Asp Gln Val Gly Tyr Val Thr Tyr Asp Ile Leu Gln Cys Pro Glu Asp
675 680 685

<210> 29
<211> 19
<212> PRT
<213> 智人

<400> 29
Met Ser Leu Trp Gln Pro Leu Val Leu Val Leu Leu Val Leu Gly Cys
1 5 10 15
Cys Phe Ala

<210> 30
<211> 470
<212> PRT
<213> 小家鼠

<220>
<221> CHAIN
<222> (1)...(470)
<223> M4重链(IgG2b)

<400> 30
Met Ala Val Leu Val Leu Phe Leu Cys Leu Val Ala Phe Pro Ser Cys
1 5 10 15
Val Leu Ser Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala
20 25 30
Pro Ser Gln Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu
35 40 45
Leu Ser Tyr Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu
50 55 60
Glu Trp Leu Gly Val Ile Trp Thr Gly Gly Ser Thr Asn Tyr Asn Ser
65 70 75 80
Ala Leu Met Ser Arg Leu Ser Ile Ser Lys Asp Asp Ser Lys Ser Gln
85 90 95
Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Met Tyr
100 105 110
Tyr Cys Ala Arg Tyr Tyr Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr
115 120 125
Ser Val Thr Val Ser Ser Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro

[0020]

130 135 140
Leu Ala Pro Gly Cys Gly Asp Thr Thr Gly Ser Ser Val Thr Leu Gly
145 150 155 160
Cys Leu Val Lys Gly Tyr Phe Pro Glu Ser Val Thr Val Thr Trp Asn
165 170 175
Ser Gly Ser Leu Ser Ser Ser Val His Thr Phe Pro Ala Leu Leu Gln
180 185 190
Ser Gly Leu Tyr Thr Met Ser Ser Ser Val Thr Val Pro Ser Ser Thr
195 200 205
Trp Pro Ser Gln Thr Val Thr Cys Ser Val Ala His Pro Ala Ser Ser
210 215 220
Thr Thr Val Asp Lys Lys Leu Glu Pro Ser Gly Pro Ile Ser Thr Ile
225 230 235 240
Asn Pro Cys Pro Pro Cys Lys Glu Cys His Lys Cys Pro Ala Pro Asn
245 250 255
Leu Glu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Asn Ile Lys Asp
260 265 270
Val Leu Met Ile Ser Leu Thr Pro Lys Val Thr Cys Val Val Val Asp
275 280 285
Val Ser Glu Asp Asp Pro Asp Val Arg Ile Ser Trp Phe Val Asn Asn
290 295 300
Val Glu Val His Thr Ala Gln Thr Gln Thr His Arg Glu Asp Tyr Asn
305 310 315 320
Ser Thr Ile Arg Val Val Ser Ala Leu Pro Ile Gln His Gln Asp Trp
325 330 335
Met Ser Gly Lys Glu Phe Lys Cys Lys Val Asn Asn Lys Asp Leu Pro
340 345 350
Ser Pro Ile Glu Arg Thr Ile Ser Lys Ile Lys Gly Leu Val Arg Ala
355 360 365
Pro Gln Val Tyr Ile Leu Pro Pro Pro Ala Glu Gln Leu Ser Arg Lys
370 375 380
Asp Val Ser Leu Thr Cys Leu Val Val Gly Phe Asn Pro Gly Asp Ile
385 390 395 400
Ser Val Glu Trp Thr Ser Asn Gly His Thr Glu Glu Asn Tyr Lys Asp
405 410 415
Thr Ala Pro Val Leu Asp Ser Asp Gly Ser Tyr Phe Ile Tyr Ser Lys
420 425 430
Leu Asp Ile Lys Thr Ser Lys Trp Glu Lys Thr Asp Ser Phe Ser Cys
435 440 445
Asn Val Arg His Glu Gly Leu Lys Asn Tyr Tyr Leu Lys Lys Thr Ile
450 455 460
Ser Arg Ser Pro Gly Lys
465 470

<210> 31
<211> 234
<212> PRT
<213> 小家鼠

<220>
<221> CHAIN
<222> (1)...(234)
<223> M4轻链(κ)

<400> 31

[0021]

Met Glu Ser Gln Ile Gln Val Phe Val Phe Val Phe Leu Trp Leu Ser
1 5 10 15
Gly Val Asp Gly Asp Ile Val Met Thr Gln Ser His Lys Phe Met Phe
20 25 30
Thr Ser Val Gly Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp
35 40 45
Val Arg Asn Thr Val Ala Trp Tyr Gln Gln Lys Thr Gly Gln Ser Pro
50 55 60
Lys Leu Leu Ile Tyr Ser Ala Ser Tyr Arg Asn Thr Gly Val Pro Asp
65 70 75 80
Arg Phe Thr Gly Ser Ile Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser
85 90 95
Ser Val Gln Ala Glu Asp Leu Ala Leu Tyr Tyr Cys Gln Gln His Tyr
100 105 110
Ser Thr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Val Lys Arg
115 120 125
Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln
130 135 140
Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr
145 150 155 160
Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln
165 170 175
Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr
180 185 190
Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg
195 200 205
His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro
210 215 220
Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
225 230

<210> 32
<211> 115
<212> PRT
<213> 小家鼠

<400> 32
Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln
1 5 10 15
Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Leu Ser Tyr
20 25 30
Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45
Gly Val Ile Trp Thr Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Met
50 55 60
Ser Arg Leu Ser Ile Ser Lys Asp Asp Ser Lys Ser Gln Val Phe Leu
65 70 75 80
Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Met Tyr Tyr Cys Ala
85 90 95
Arg Tyr Tyr Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr
100 105 110
Val Ser Ser
115

[0022]

<210> 33
<211> 107
<212> PRT
<213> 小家鼠

<400> 33
Asp Ile Val Met Thr Gln Ser His Lys Phe Met Phe Thr Ser Val Gly
1 5 10 15
Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp Val Arg Asn Thr
20 25 30
Val Ala Trp Tyr Gln Gln Lys Thr Gly Gln Ser Pro Lys Leu Leu Ile
35 40 45
Tyr Ser Ala Ser Tyr Arg Asn Thr Gly Val Pro Asp Arg Phe Thr Gly
50 55 60
Ser Ile Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Val Gln Ala
65 70 75 80
Glu Asp Leu Ala Leu Tyr Tyr Cys Gln Gln His Tyr Ser Thr Pro Tyr
85 90 95
Thr Phe Gly Gly Gly Thr Lys Leu Glu Val Lys
100 105

<210> 34
<211> 10
<212> PRT
<213> 小家鼠

<400> 34
Gly Phe Ser Leu Leu Ser Tyr Gly Val His
1 5 10

<210> 35
<211> 10
<212> PRT
<213> 小家鼠

<400> 35
Val Ile Trp Thr Gly Gly Ser Thr Asn Tyr
1 5 10

<210> 36
<211> 7
<212> PRT
<213> 小家鼠

<400> 36
Tyr Tyr Tyr Ala Met Asp Tyr
1 5

<210> 37
<211> 11
<212> PRT

[0023]

<213> 小家鼠

<400> 37
Lys Ala Ser Gln Asp Val Arg Asn Thr Val Ala
1 5 10

<210> 38
<211> 7
<212> PRT
<213> 小家鼠

<400> 38
Ser Ala Ser Tyr Arg Asn Thr
1 5

<210> 39
<211> 9
<212> PRT
<213> 小家鼠

<400> 39
Gln Gln His Tyr Ser Thr Pro Tyr Thr
1 5

<210> 40
<211> 229
<212> PRT
<213> 小家鼠

<220>
<221> CHAIN
<222> (1)...(229)
<223> M12 κ链

<400> 40
Gln Val Phe Val Tyr Met Leu Leu Trp Leu Ser Gly Val Asp Gly Asp
1 5 10 15
Ile Val Met Thr Gln Ser Gln Lys Phe Met Ser Thr Ser Val Gly Asp
20 25 30
Arg Val Ser Val Thr Cys Lys Ala Ser Gln Asn Val Gly Thr Asn Val
35 40 45
Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Ala Leu Ile Tyr
50 55 60
Ser Ala Ser Tyr Arg Phe Ser Gly Val Pro Asp Arg Phe Thr Gly Ser
65 70 75 80
Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Asn Val Gln Ser Glu
85 90 95
Asp Leu Ala Glu Tyr Phe Cys Gln Gln Tyr Asn Ser Tyr Pro Tyr Thr
100 105 110
Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala Asp Ala Ala Pro
115 120 125
Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu Thr Ser Gly Gly
130 135 140

[0024]

Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr Pro Lys Asp Ile Asn
145 150 155 160
Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln Asn Gly Val Leu Asn
165 170 175
Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr Tyr Ser Met Ser Ser
180 185 190
Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg His Asn Ser Tyr Thr
195 200 205
Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro Ile Val Lys Ser Phe
210 215 220
Asn Arg Asn Glu Cys
225

<210> 41
<211> 107
<212> PRT
<213> 小家鼠

<400> 41
Asp Ile Val Met Thr Gln Ser Gln Lys Phe Met Ser Thr Ser Val Gly
1 5 10 15
Asp Arg Val Ser Val Thr Cys Lys Ala Ser Gln Asn Val Gly Thr Asn
20 25 30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Ala Leu Ile
35 40 45
Tyr Ser Ala Ser Tyr Arg Phe Ser Gly Val Pro Asp Arg Phe Thr Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Asn Val Gln Ser
65 70 75 80
Glu Asp Leu Ala Glu Tyr Phe Cys Gln Gln Tyr Asn Ser Tyr Pro Tyr
85 90 95
Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 42
<211> 11
<212> PRT
<213> 小家鼠

<400> 42
Lys Ala Ser Gln Asn Val Gly Thr Asn Val Ala
1 5 10

<210> 43
<211> 7
<212> PRT
<213> 小家鼠

<400> 43
Ser Ala Ser Tyr Arg Phe Ser
1 5

[0025]

<210> 44
<211> 9
<212> PRT
<213> 小家鼠

<400> 44
Gln Gln Tyr Asn Ser Tyr Pro Tyr Thr
1 5

<210> 45
<211> 233
<212> PRT
<213> 小家鼠

<220>
<221> CHAIN
<222> (1)...(233)
<223> AB0046 κ轻链

<400> 45
Met Ser Ser Ala Gln Phe Leu Gly Leu Leu Leu Leu Cys Phe Gln Gly
1 5 10 15
Thr Arg Cys Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala
20 25 30
Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Ser Ala Ser Gln Gly Ile
35 40 45
Ser Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Phe Lys
50 55 60
Leu Leu Ile Tyr Tyr Thr Ser Ile Leu His Ser Gly Val Pro Ser Arg
65 70 75 80
Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn
85 90 95
Leu Glu Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Tyr Gly Trp
100 105 110
Leu Pro Arg Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala
115 120 125
Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu
130 135 140
Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr Pro
145 150 155 160
Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln Asn
165 170 175
Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr Tyr
180 185 190
Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg His
195 200 205
Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro Ile
210 215 220
Val Lys Ser Phe Asn Arg Asn Glu Cys
225 230

<210> 46
<211> 460
<212> PRT

[0026]

<213> 小家鼠

<220>
<221> CHAIN
<222> (1)...(460)
<223> AB0046 IgG1重链

<400> 46

```
Met Gly Trp Ser Ser Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
 1               5                  10                  15
Val His Ser Gln Val Gln Leu Gln Gln Pro Gly Ser Val Leu Val Arg
            20                  25                  30
Pro Gly Ala Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Tyr Thr Phe
        35                  40                  45
Thr Ser Tyr Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu
    50                  55                  60
Glu Trp Ile Gly Glu Ile Tyr Pro Ile Ser Gly Arg Thr Asn Tyr Asn
65                  70                  75                  80
Glu Lys Phe Lys Val Lys Ala Thr Leu Thr Val Asp Thr Ser Ser Ser
                85                  90                  95
Thr Ala Tyr Met Asp Leu Asn Ser Leu Thr Ser Glu Asp Ser Ala Val
            100                 105                 110
Tyr Tyr Cys Ala Arg Ser Arg Ala Asn Trp Asp Asp Tyr Trp Gly Gln
        115                 120                 125
Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Pro Pro Ser Val
    130                 135                 140
Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser Met Val Thr
145                 150                 155                 160
Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Val Thr
                165                 170                 175
Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala Val
            180                 185                 190
Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Pro Ser
        195                 200                 205
Ser Thr Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala His Pro Ala
    210                 215                 220
Ser Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp Cys Gly Cys
225                 230                 235                 240
Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val Phe Ile Phe
                245                 250                 255
Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr Pro Lys Val
            260                 265                 270
Thr Cys Val Val Val Asp Ile Ser Lys Asp Asp Pro Glu Val Gln Phe
        275                 280                 285
Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln Thr Gln Pro
    290                 295                 300
Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser Glu Leu Pro
305                 310                 315                 320
Ile Met His Gln Asp Trp Leu Asn Gly Lys Glu Phe Lys Cys Arg Val
                325                 330                 335
Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr
            340                 345                 350
Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile Pro Pro Pro Lys
        355                 360                 365
Glu Gln Met Ala Lys Asp Lys Val Ser Leu Thr Cys Met Ile Thr Asp
    370                 375                 380
```

[0027]

Phe Phe Pro Glu Asp Ile Thr Val Glu Trp Gln Trp Asn Gly Gln Pro
385 390 395 400
Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Asp Thr Asp Gly Ser
405 410 415
Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn Trp Glu Ala
420 425 430
Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu His Asn His
435 440 445
His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys
450 455 460

<210> 47
<211> 117
<212> PRT
<213> 小家鼠

<400> 47
Gln Val Gln Leu Gln Gln Pro Gly Ser Val Leu Val Arg Pro Gly Ala
1 5 10 15
Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30
Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45
Gly Glu Ile Tyr Pro Ile Ser Gly Arg Thr Asn Tyr Asn Glu Lys Phe
50 55 60
Lys Val Lys Ala Thr Leu Thr Val Asp Thr Ser Ser Ser Thr Ala Tyr
65 70 75 80
Met Asp Leu Asn Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Ser Arg Ala Asn Trp Asp Asp Tyr Trp Gly Gln Gly Thr Thr
100 105 110
Leu Thr Val Ser Ser
115

<210> 48
<211> 107
<212> PRT
<213> 小家鼠

<400> 48
Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly
1 5 10 15
Asp Arg Val Thr Ile Ser Cys Ser Ala Ser Gln Gly Ile Ser Asn Tyr
20 25 30
Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Phe Lys Leu Leu Ile
35 40 45
Tyr Tyr Thr Ser Ile Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Pro
65 70 75 80
Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Tyr Gly Trp Leu Pro Arg
85 90 95
Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

[0028]

<210> 49
<211> 461
<212> PRT
<223> 人工序列

<220>
<223> 合成构建体

<220>
<221> CHAIN
<222> (1)...(461)
<223> AB0045重链

<400> 49

Met	Gly	Trp	Ser	Leu	Ile	Leu	Leu	Phe	Leu	Val	Ala	Val	Ala	Thr	Arg
1				5				10						15	
Val	His	Ser	Gln	Val	Gln	Leu	Gln	Glu	Ser	Gly	Pro	Gly	Leu	Val	Lys
			20					25					30		
Pro	Ser	Glu	Thr	Leu	Ser	Leu	Thr	Cys	Thr	Val	Ser	Gly	Phe	Ser	Leu
		35					40					45			
Leu	Ser	Tyr	Gly	Val	His	Trp	Val	Arg	Gln	Pro	Pro	Gly	Lys	Gly	Leu
	50					55					60				
Glu	Trp	Leu	Gly	Val	Ile	Trp	Thr	Gly	Gly	Thr	Thr	Asn	Tyr	Asn	Ser
65					70					75					80
Ala	Leu	Met	Ser	Arg	Phe	Thr	Ile	Ser	Lys	Asp	Asp	Ser	Lys	Asn	Thr
				85					90					95	
Val	Tyr	Leu	Lys	Met	Asn	Ser	Leu	Lys	Thr	Glu	Asp	Thr	Ala	Ile	Tyr
			100					105					110		
Tyr	Cys	Ala	Arg	Tyr	Tyr	Tyr	Gly	Met	Asp	Tyr	Trp	Gly	Gln	Gly	Thr
		115					120					125			
Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro
	130					135					140				
Leu	Ala	Pro	Cys	Ser	Arg	Ser	Thr	Ser	Glu	Ser	Thr	Ala	Ala	Leu	Gly
145					150					155					160
Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn
				165					170					175	
Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln
			180					185					190		
Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser
		195					200					205			
Ser	Leu	Gly	Thr	Lys	Thr	Tyr	Thr	Cys	Asn	Val	Asp	His	Lys	Pro	Ser
	210					215					220				
Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Ser	Lys	Tyr	Gly	Pro	Pro	Cys
225					230					235					240
Pro	Pro	Cys	Pro	Ala	Pro	Glu	Phe	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu
				245					250					255	
Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu
			260					265					270		
Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	Gln	Glu	Asp	Pro	Glu	Val	Gln
		275					280					285			
Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys
	290					295					300				
Pro	Arg	Glu	Glu	Gln	Phe	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu
305					310					315					320

[0029]

```
Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
                325                 330                 335
Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys
            340                 345                 350
Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
        355                 360                 365
Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
    370                 375                 380
Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
385                 390                 395                 400
Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
                405                 410                 415
Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln
            420                 425                 430
Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
        435                 440                 445
His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
    450                 455                 460
```

<210> 50
<211> 234
<212> PRT
<223> 人工序列

<220>
<223> 合成构建体

<220>
<221> CHAIN
<222> (1)...(234)
<223> AB0045轻链

<400> 50

```
Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp Leu Pro
 1                  5                  10                  15
Gly Ala Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser
            20                  25                  30
Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp
        35                  40                  45
Val Arg Asn Thr Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro
    50                  55                  60
Lys Leu Leu Ile Tyr Ser Ser Ser Tyr Arg Asn Thr Gly Val Pro Asp
65                  70                  75                  80
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
                85                  90                  95
Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln His Tyr
            100                 105                 110
Ile Thr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
        115                 120                 125
Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
    130                 135                 140
Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
145                 150                 155                 160
Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
                165                 170                 175
```

[0030]

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
180 185 190
Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
195 200 205
His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
210 215 220
Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230

抗基质金属蛋白酶9人类化重链

AB0041	QVQLKESGPG	LVAPSQSLSI	TCTVSGFSLL	SYGVHWVRQP	PGKGLEWLGV
重链可变区1	QVQLQESGPG	LVKPSETLSL	TCTVSGFSLL	SYGVHWVRQP	PGKGLEWLGV
重链可变区2	QVQLQESGPG	LVKPSETLSL	TCTVSGFSLL	SYGVHWVRQP	PGKGLEWLGV
重链可变区3	QVQLQESGPG	LVKPSETLSL	TCTVSGFSLL	SYGVHWVRQP	PGKGLEWLGV
重链可变区4	QVQLQESGPG	LVKPSETLSL	TCTVSGFSLL	SYGVHWVRQP	PGKGLEWLGV
AB0041	IWTGGTTNYN	SALMSRLSIS	KDDSKSQVFL	KMNSLQTDDT	AIYYCARYYY
重链可变区1	IWTGGTTNYN	SALMSRLTIS	KDDSKSTVYL	KMNSLKTEDT	AIYYCARYYY
重链可变区2	IWTGGTTNYN	SALMSRLTIS	KDDSKNTVYL	KMNSLKTEDT	AIYYCARYYY
重链可变区3	IWTGGTTNYN	SALMSRFTIS	KDDSKNTVYL	KMNSLKTEDT	AIYYCARYYY
重链可变区4	IWTGGTTNYN	SALMSRFTIS	KDDSKNTLYL	KMNSLKTEDT	AIYYCARYYY
AB0041	GMDYWGQGTS	VTVSS	(SEQ ID NO:3)		
重链可变区1	GMDYWGQGTS	VTVSS	(SEQ ID NO:5)		
重链可变区2	GMDYWGQGTL	VTVSS	(SEQ ID NO:6)		
重链可变区3	GMDYWGQGTL	VTVSS	(SEQ ID NO:7)		
重链可变区4	GMDYWGQGTL	VTVSS	(SEQ ID NO:8)		

图 1

抗基质金属蛋白酶9人类化轻链

AB0041	DIVMTQSHKF	MSTSVGDRVS	ITCKASQDVR	NTVAWYQQKT GQSPKLLIYS
Vk1	DIVMTQSPSF	LSASVGDRVT	ITCKASQDVR	ATVAWYQQKT GKAPKLLIYS
Vk2	DIVMTQSPSS	LSASVGDRVT	ITCKASQDVR	NTVAWYQQKP GKAPKLLIYS
Vk3	DIQMTQSPSS	LSASVGDRVT	ITCKASQDVR	NTVAWYQQKP GKAPKLLIYS
Vk4	DIQMTQSPSS	LSASVGDRVT	ITCKASQDVR	NTVAWYQQKP GKAPKLLIYS
AB0041	SSYRNTGVPD	RFTGSGSGTD	FTFTISSVQA	EDLAVYFCQQ HYITPYTFGG
Vk1	SSYRNTGVPD	RFTGSGSGTD	FTLTISSLQA	EDVAVYFCQQ HYITPYTFGG
Vk2	SSYRNTGVPD	RFTGSGSGTD	FTLTISSLQA	EDVAVYFCQQ HYITPYTFGG
Vk3	SSYRNTGVPD	RFSGSGSGTD	FTLTISSLQA	EDVAVYFCQQ HYITPYTFGG
Vk4	SSYRNTGVPD	RFSGSGSGTD	FTLTISSLQA	EDVAVYYCQQ HYITPYTFGG
AB0041	GTKLEIK	(SEQ ID NO:4)		
Vk1	GTKVEIK	(SEQ ID NO:9)		
Vk2	GTKVEIK	(SEQ ID NO:10)		
Vk3	GTKVEIK	(SEQ ID NO:11)		
Vk4	GTKVEIK	(SEQ ID NO:12)		

图 2

图 3

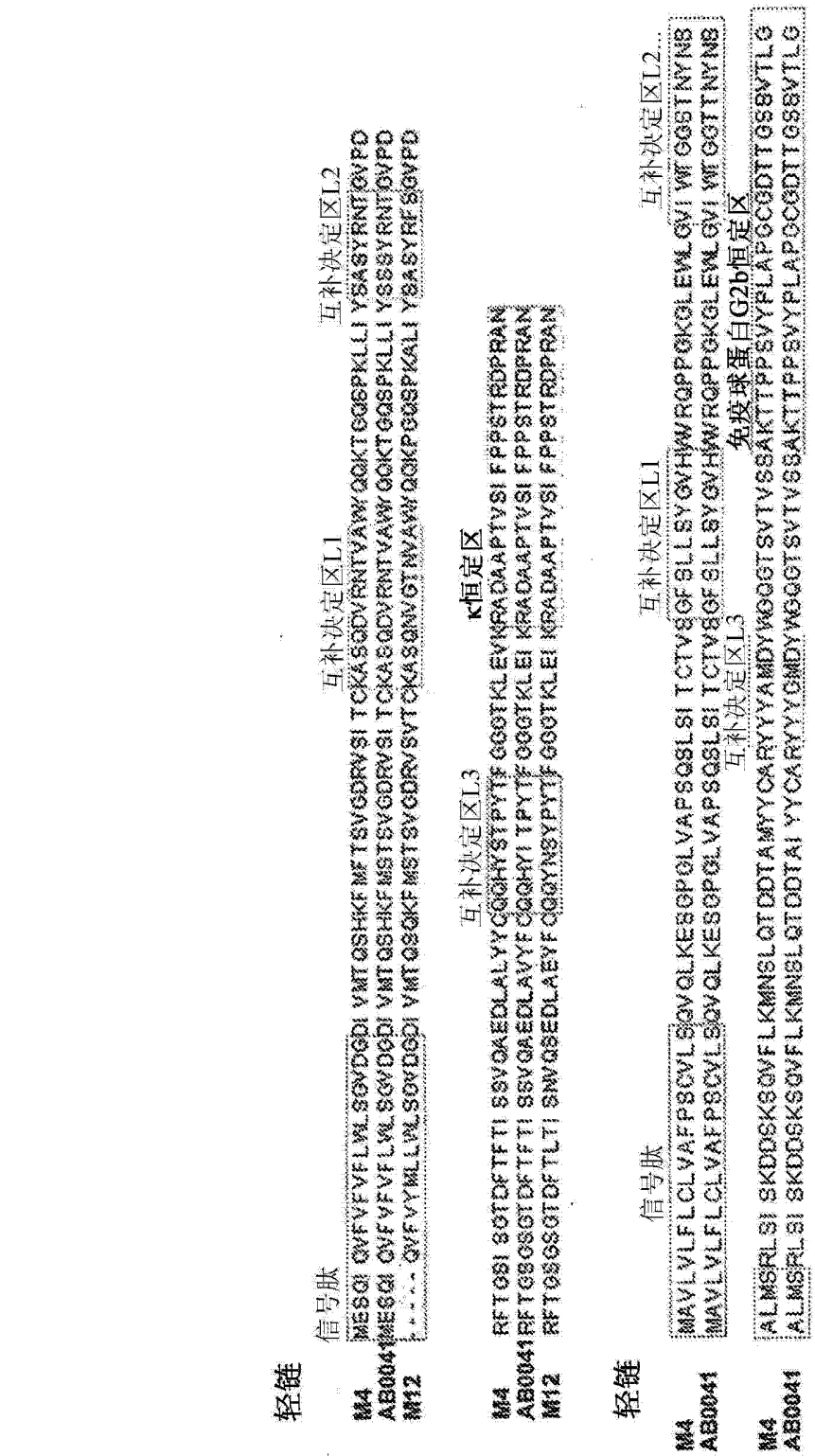

图 4 :AB0041、M4 及 M12 重链和轻链之间的比较