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~~embodiments, 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.

[0008a] In one aspect, there is provided an isolated antibody or fragment thereof, comprising a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the heavy chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 34, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 35, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 36.

[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

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.

[0022a] In one aspect, there is provided a method of detecting human MMP9 in a sample, comprising:

a) contacting the sample with the isolated antibody or fragment thereof as described herein: and,

b) detecting binding of the isolated antibody or fragment thereof to human MMP9 in the sample, thereby detecting the presence of the human MMP9.

[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)

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.

CLAIMS

What is claimed is:

1. An isolated antibody or fragment thereof, comprising a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the heavy chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 34, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 35, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 36.

2. The isolated antibody or fragment thereof of claim 1, wherein the light chain variable (VL) region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 37, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 38, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 39.

3. The isolated antibody or fragment thereof of claim 1, wherein the heavy chain variable (VH) region comprises the amino acid sequence set forth in SEQ ID NO: 32.

4. The isolated antibody or fragment thereof of claim 2, wherein the light chain variable (VL) region comprises the amino acid sequence set forth in SEQ ID NO: 33.

5. The isolated antibody or fragment thereof of claim 2, wherein the heavy chain variable (VH) region comprises the amino acid sequence set forth in SEQ ID NO: 32 and wherein the light chain variable (VL) region comprises the amino acid sequence set forth in SEQ ID NO: 33.

6. A method of detecting human MMP9 in a sample, comprising:

a) contacting the sample with the isolated antibody or fragment thereof of any one of claims 1 to 5; and,

b) detecting binding of the isolated antibody or fragment thereof to human MMP9 in the sample, thereby detecting the presence of the human MMP9.

7. The method of claim 6, further comprising the step of comparing the amount of binding detected in the sample to an amount of binding to a control sample.

8. The method of claim 6 or 7, wherein the sample is a tissue or fluid sample from a subject.

9. The method of claim 8, wherein the fluid sample is a blood, plasma, serum, whole blood, saliva, urine, or semen sample.

10. The method of claim 8, wherein the tissue sample is a formalin-fixed sample or a frozen sample.

11. The method of any one of claims 6 to 10, wherein the isolated antibody or fragment thereof is labeled with a detectable moiety.