specifically inhibit the catalytic activity of a single MMP, and that do not react with other MMPs.

SUMMARY

[0007] 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), wherein the binding proteins comprise an immunoglobulin (Ig) heavy chain (or functional fragment thereof) and an Ig light chain (or functional fragment thereof). The disclosure further provides MMP9 binding proteins that bind specifically to MMP9 and not to other, related 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 example~~embodiments~~ of the present disclosure an anti-MMP9 antibody is a specific inhibitor of the activity of MMP9. In particular, the MMP9 binding proteins disclosed herein will be useful for inhibition of MMP9 while allowing normal function of other, related matrix metalloproteinases.

[0007a] In one aspect, there is provided an isolated MMP9 binding protein comprising an immunoglobulin heavy chain polypeptide, or functional fragment thereof, and an immunoglobulin light chain polypeptide, or functional fragment thereof, wherein the MMP9 binding protein specifically binds MMP9; and wherein the immunoglobulin heavy chain polypeptide comprises complementarity determining regions (CDRs) as set forth in SEQ ID NOs: 13-15 and immunoglobulin light chain polypeptide comprises CDRs as set forth in SEQ ID NOs: 16-18.

[0007b] In another aspect, there is provided an isolated MMP9 binding protein comprising: an immunoglobulin heavy chain polypeptide, or antigen binding fragment thereof, and an immunoglobulin light chain polypeptide, or antigen binding fragment thereof, wherein the isolated MMP9 binding protein specifically binds MMP9; and wherein the isolated MMP9 binding protein binds an epitope of human MMP9 comprising amino acid residues R162, E111, D113, and I198.

[0007c] In another aspect, there is provided an isolated nucleic acid comprising a polynucleotide sequence encoding: a heavy chain polypeptide comprising complementarity-determining regions (CDRs) of SEQ ID NOs: 13-15; and/or a light chain polypeptide comprising CDRs of SEQ ID NOs: 16-18.

[0007d] In another aspect, there is provided a vector comprising the isolated nucleic acid as defined herein.

[0007e] In another aspect, there is provided a cell comprising the vector as defined herein.

[0007f] In another aspect, there is provided a pharmaceutical composition comprising the isolated MMP9 binding protein as defined herein, the vector as defined herein or the cell as defined herein.

[0007g] In another aspect, there is provided a method of detecting MMP9 expression in tissue of a patient, the method comprising: contacting a tissue sample from the patient with an isolated MMP9 binding protein as defined herein; and detecting the presence or absence of MMP9; wherein the presence of MMP9 in the tissue sample indicates the MMP9 is expressed in tissue.

[0008] Additionally~~Accordingly~~, the present disclosure provides, *inter alia*:

[0009] **1.** A MMP9 binding protein comprising an immunoglobulin heavy chain or functional fragment thereof, and an immunoglobulin light chain or functional fragment thereof, wherein the protein does not bind to a matrix metalloproteinase other than MMP9.

[0010] **2.** The protein of embodiment 1, wherein the heavy chain comprises a complementarity-determining region (CDR) selected from one or more of SEQ ID NOs: 13-15, and the light chain comprises a CDR selected from one or more of SEQ ID NOs: 16-18.

[0011] **3.** The protein of embodiment 2, wherein the heavy chain comprises a variable region selected from the group consisting of SEQ ID NOs: 3 or 5-8, and the light chain comprises a variable region selected from the group consisting of SEQ ID NOs: 4 or 9-12.

[0012] **4.** The protein of embodiment 1, wherein the heavy chain is an IgG.

[0013] **5.** The protein of embodiment 1, wherein the light chain is a kappa chain.

[0014] 6. The protein of embodiment 1, wherein the binding of the protein to MMP9 inhibits the enzymatic activity of MMP9.

[0015] 7. The protein of embodiment 6, wherein the inhibition is non-competitive.

[0016] 8. The protein of embodiment 1, wherein 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.

CLAIMS

What is claimed is:

1. A MMP9 binding protein comprising

an immunoglobulin heavy chain polypeptide, or functional fragment thereof, and

an immunoglobulin light chain polypeptide, or functional fragment thereof,

wherein the MMP9 binding protein specifically binds MMP9.

2. The MMP9 binding protein of claim 1, wherein the MMP9 binding protein binds an epitope of human MMP9 comprising amino acid residues R162, E111, D113, and I198.

3. The MMP9 binding protein of claim 1, wherein the MMP9 binding protein competes for binding to human MMP9 with an antibody comprising a heavy chain polypeptide of

4. The MMP9 binding protein of claim 1, wherein the MMP9 binding protein competes for binding to human MMP9 with an antibody comprising a heavy chain polypeptide comprising an amino acid sequence of SEQ ID NOS: 3 or 5-8.

5. The MMP9 binding protein of claim 1, wherein the MMP9 binding protein competes for binding to human MMP9 with an antibody comprising a light chain polypeptide comprising an amino acid sequence of SEQ ID NOS: 4 or 9-12.

6. The MMP9 binding protein of claim 1, wherein the MMP9 binding protein competes for binding to human MMP9 with an antibody comprising a heavy chain polypeptide comprising complementarity-determining regions (CDRs) of SEQ ID NOs: 13-15 and a light chain polypeptide comprising CDRs of SEQ ID NOs: 16-18.

7. The MMP9 binding protein of claim 6, wherein the heavy chain polypeptide comprises complementarity-determining regions (CDRs) of SEQ ID NOs: 13-15, and the light chain polypeptide comprises CDRs of SEQ ID NOs: 16-18.

8. The MMP9 binding protein of claim 1, wherein the MMP9 binding protein competes for binding to human MMP9 with an antibody comprising a heavy chain polypeptide comprising a variable region selected from the group consisting of SEQ ID NOs: 3 or 5-8, and a light chain polypeptide comprising a variable region selected from the group consisting of SEQ ID NOs: 4 or 9-12.

9. The MMP9 binding protein of claim 1, wherein the heavy chain polypeptide is an IgG.

10. The MMP9 binding protein of claim 1, wherein the binding of the MMP9 binding protein to MMP9 inhibits the enzymatic activity of MMP9.

11. The MMP9 binding protein of claim 10, wherein the inhibition is non-competitive.

12. The MMP9 binding protein of claim 1, wherein the MMP9 binding protein competes for binding to human MMP9 with an antibody comprising a heavy chain polypeptide encoded by a polynucleotide having a nucleotide sequence selected from the group consisting of SEQ ID NOs: 19-22 and a light chain polypeptide encoded by a polynucleotide having a nucleotide sequence selected from the group consisting of SEQ ID NOs: 23-26.

13. An isolated nucleic acid comprising a nucleotide sequence encoding:

a heavy chain polypeptide comprising complementarity-determining regions (CDRs) of SEQ ID NOs: 13-15; and/or

a light chain polypeptide comprising CDRs of SEQ ID NOs: 16-18.

14. The isolated nucleic acid of claim 13, wherein the heavy chain polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:1, 3, and 5-8.

15. The isolated nucleic acid of claim 13, wherein the light chain polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, and 9-12.

16. The isolated nucleic acid of claim 13 comprising a sequence selected from the group consisting of SEQ ID NOs: 19-26.

17. A vector comprising the isolated nucleic acid of claim 13.

18. A cell comprising the vector of claim 17.

19. A pharmaceutical composition comprising the MMP9 binding protein of claim 1.

20. A pharmaceutical composition comprising the vector of claim 17.

RECTIFIED SHEET (RULE 91) ISA/US

21. A pharmaceutical composition comprising the cell of claim 18.

22. A method of inhibiting MMP9 activity in a subject having a tumor or tumor-associated tissue having MMP9 activity, the method comprising:

administering to the subject the pharmaceutical composition of claim 19 in an amount effective to inhibit MMP9 activity;

wherein MMP9 activity is inhibited in the subject.

23. A method of detecting MMP9 expression in tissue of a patient, the method comprising:

contacting a tissue sample from the patient with an MMP9 binding protein of claim 1; and

detecting the presence or absence of MMP9;

wherein the presence of MMP9 in the tissue sample indicates the MMP9 is expressed in tissue.

RECTIFIED SHEET (RULE 91) ISA/US

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS

1. An isolated MMP9 binding protein comprising

an immunoglobulin heavy chain polypeptide, or functional fragment thereof, and

an immunoglobulin light chain polypeptide, or functional fragment thereof,

wherein the MMP9 binding protein specifically binds MMP9; and

wherein the immunoglobulin heavy chain polypeptide comprises complementarity determining regions (CDRs) as set forth in SEQ ID NOs: 13-15 and immunoglobulin light chain polypeptide comprises CDRs as set forth in SEQ ID NOs: 16-18.

2. The isolated MMP9 binding protein according to claim 1, wherein:

a) the MMP9 binding protein binds an epitope of human MMP9 comprising amino acid residues R162, E111, D113, and I198; or

b) the MMP9 binding protein comprises a heavy chain polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 3, 5, 6, 7, and 8; or

c) the isolated MMP9 binding protein comprises a light chain polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 9, 10, 11, and 12; or

d) the isolated MMP9 binding protein comprises a heavy chain polypeptide comprising a variable region selected from the group consisting of SEQ ID NOs: 3, 5, 6, 7, and 8, and a light chain polypeptide comprising a variable region selected from the group consisting of SEQ ID NOs: 4, 9, 10, 11, and 12; or

e) the isolated MMP9 binding protein comprises a heavy chain polypeptide of SEQ ID NO: 7 and a light chain polypeptide of SEQ ID NO: 12.

3. The isolated MMP9 binding protein according to any one of claims 1-2, wherein the heavy chain polypeptide is an IgG.

4. The isolated MMP9 binding protein according to any one of claims 1-3, wherein the binding of the isolated MMP9 binding protein to MMP9 inhibits the enzymatic activity of MMP9.

5. The isolated MMP9 binding protein of claim 4, wherein the inhibition is non-competitive.

6. The isolated MMP9 binding protein according to any one of claims 1-5, wherein the isolated MMP9 binding protein comprises a heavy chain polypeptide encoded by a polynucleotide having a nucleotide sequence selected from the group consisting of SEQ ID NOs: 19, 20, 21, and 22 and a light chain polypeptide encoded by a polynucleotide having a nucleotide sequence selected from the group consisting of SEQ ID NOs: 23, 24, 25, and 26.

7. The isolated MMP9 binding protein of any one of claims 1-6, wherein the MMP9 binding protein is a humanized or chimeric antibody.

8. An isolated MMP9 binding protein comprising:

an immunoglobulin heavy chain polypeptide, or antigen binding fragment thereof, and an immunoglobulin light chain polypeptide, or antigen binding fragment thereof,

wherein the isolated MMP9 binding protein specifically binds MMP9; and

wherein the isolated MMP9 binding protein binds an epitope of human MMP9 comprising amino acid residues R162, E111, D113, and I198.

9. The isolated MMP9 binding protein of claim 8, wherein:

a) the immunoglobulin heavy chain polypeptide comprises complementarity determining regions (CDRs) as set forth in SEQ ID NOs: 13-15; or

b) the immunoglobulin light chain polypeptide comprises CDRs as set forth in SEQ ID NOs: 16-18; or

c) the heavy chain polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 3, 5, 6, 7 and 8; or

d) the light chain polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 9, 10, 11, and 12.

10. The isolated MMP9 binding protein of any one of claims 8-9, wherein the binding of the MMP9 binding protein to MMP9 inhibits the enzymatic activity of MMP9.

11. The isolated MMP9 binding protein of claim 10, wherein the inhibition is non-competitive.

12. The isolated MMP9 binding protein of any one of claims 1-11, wherein the MMP9 binding protein is a humanized or chimeric antibody.

13. An isolated nucleic acid comprising a polynucleotide sequence encoding:

a heavy chain polypeptide comprising complementarity-determining regions (CDRs) of SEQ ID NOs: 13-15; and/or

a light chain polypeptide comprising CDRs of SEQ ID NOs: 16-18.

14. The isolated nucleic acid according to claim 13, wherein:

a) the polynucleotide sequence encodes the heavy chain polypeptide comprising an amino acid sequence selected from the group consisting of any one of SEQ ID NOs: 1, 3, 5, 6, 7, and 8; or

b) the polynucleotide sequence encodes light chain polypeptide comprising an amino acid sequence selected from the group consisting of any one of SEQ ID NOs: 2, 4, 9, 10, 11, and 12.

15. The isolated nucleic acid according to any one of claims 13-14 comprising a polynucleotide sequence selected from the group consisting of SEQ ID NOs: 19, 20, 21, 22, 23, 24, 25, and 26.

16. A vector comprising the isolated nucleic acid of any one of claims 13 - 15.

17. A cell comprising the vector of claim 16.

18. A pharmaceutical composition comprising the isolated MMP9 binding protein of any one of claims 1 – 12, the vector of claim 16 or the cell of claim 17.

19. A method of detecting MMP9 expression in tissue of a patient, the method comprising:

contacting a tissue sample from the patient with an isolated MMP9 binding protein of any one of claims 1 - 12; and

detecting the presence or absence of MMP9;

wherein the presence of MMP9 in the tissue sample indicates the MMP9 is expressed in tissue.