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

[0008] Among the MMP9 binding proteins, e.g., antibodies, are those 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 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.

[0009] 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 with particular antibodies, and/or activity, such as the ability to inhibit MMP9, e.g., non-competitively.

[0010] The antibodies and fragments can be part of a pharmaceutical composition, wherein the antibodies and fragments thereof that bind to Matrix Metalloproteinase 9 comprise a heavy chain variable (VH) region having a heavy chain complementary determining region (CDR) with an amino acid sequence of SEQ ID NO: 15 and a pharmaceutically acceptable excipient. In one aspect, there is provided a pharmaceutical composition, comprising: an isolated antibody or fragment thereof that binds to Matrix Metalloproteinase 9 (MMP9), comprising: a heavy chain variable (VH) region having a complementarity determining region (CDR) with an amino acid sequence of SEQ ID NO: 15; and a pharmaceutically acceptable excipient. The VH region may further comprise CDR with the amino acid sequence of SEQ ID NOs:13 and/or 14. The VH region may also comprise the amino acid sequence as set forth in SEQ ID NOs: 3, 5, 6, 7, or 8. The VH region may also comprise the amino acid sequence that has a 95% sequence identify to the amino acid sequence as set forth in SEQ ID NOs: 3, 5, 6, 7, or 8.

[0011] In another embodiment, the disclosed pharmaceutical composition comprising an antibodies and antigen-binding fragments thereof that bind to Matrix Metalloproteinase 9 comprise a light chain variable (VL) region having a light chain complementary determining region (CDR) with an amino acid sequence of SEQ ID NO: 18 and a pharmaceutically

acceptable excipient. In one aspect, there is provided a pharmaceutical composition, comprising: an isolated antibody or fragment thereof that binds to Matrix Metalloproteinase 9, comprising: a light chain variable (VL) region having a light chain complementarity determining region (CDR) with an amino acid sequence of SEQ ID NO: 18; and a pharmaceutically acceptable excipient. The VL region may further comprises with the amino acid sequence of SEQ ID NOs: 16 and/or 17. The VL region may also comprise the amino acid sequence as set forth in SEQ ID NOs: 4, 9, 10, 11, or 12. The VL region may also comprise the amino acid sequence that have a 95% sequence identify to the amino acid sequence as set forth in SEQ ID NOs: 4, 9, 10, 11, or 12. In one embodiment, 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.

[0012] In another embodiment, the MMP9 binding protein comprises a VH region comprising a CDR with an amino acid sequence selected from the group consisting of SEQ ID NOs: 13, 14, 15, 34, 35, 36 and 47; and a VL region having a CDR with an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 17, 18, 37, 38, 39, 42, 43, 44, and 48. Also, the MMP9 binding protein may comprise a VH region has the amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 6, 7, 8, 34, 35, 36, and 46; and a VL region has the amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 9, 10, 11, 12, 37, 38, 39, 42, 43, 44, and 45. In addition, the MMP9 binding protein may comprise the VH region has a 95% sequence identify to the amino acid sequence selected from the group consisting of SEQ ID NOs: 30, 32, 46, and 47; and the VL region has a 95% sequence identify to the amino acid sequence selected from the group consisting of SEQ ID NOs: 31, 33, 41, 45, and 48. Moreover, the MMP9 binding protein may comprise a VH region has the amino acid sequence selected from the group consisting of SEQ ID NOs: 30, 32, 46, and 47; and the VL region has the amino acid sequence selected from the group consisting of SEQ ID NOs: 31, 33, 41, 45, and 48.

[0012a] In one aspect, there is provided a MMP9 binding protein comprises a VH region comprising a CDR with an amino acid sequence of SEQ ID NOs: 34, 35, 36 and 47; and a VL region having a CDR with an amino acid sequence selected from the group consisting of SEQ ID NOs: 37, 38, 39, 42, 43, 44, and 48.

[0012b] In one aspect, there is provided a method of inhibiting MMP9 activity comprises: contacting a sample with the MMP9 binding protein as defined herein.

[0013] Another ~~embodiment~~ aspect ~~of the invention~~ relates to a pharmaceutical composition, comprising 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; and a pharmaceutically acceptable excipient. In one ~~aspect~~embodiment, the epitope comprises E111, D113, R162, or I198 of SEQ ID NO: 27.

[0014] In another embodiment, the disclosed pharmaceutical compositions further comprising one or more therapeutic agents selected from the group consisting of an anti-inflammatory agent, an immunotherapeutic agent, a chemotherapeutic agent, an anti-cancer agent, an anti-fibrotic agent, or a combination thereof. Examples of a therapeutic agent include but are not limited to nab-paclitaxel, mFOLFOX6, FOLFIRI, carboplatin, paclitaxel,

pemetrexed, bevacizumab, anti-lysyl oxidase-like 2 (LOXL2) antibodies, an anti-discoidin domain receptor 1 (DDR1) antibodies, or a combination thereof.

[0015] 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, such as any of those described above, e.g., in an effective amount. The antibody or fragment generally specifically binds to and non-competitively inhibits MMP9, typically such that MMP9 activity is inhibited in the subject. In some cases, the antibody or fragment is one that binds MMP9 outside the catalytic domain, 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. Further provided are methods of detecting or monitoring MMP9 activity comprising contacting a sample with MMP9 binding protein, and assessing the presence or absence of MMP9 binding protein – MMP9 complex; wherein the absence of the MMP9 binding protein-MMP9 complex indicates the sample does not have the MMP9 activity, and the presence of the MMP9 binding protein-MMP9 complex indicates the sample has the MMP9 activity. The MMP9 activity may be detected by using any of the MMP9 binding protein disclosed herein.

[0015a] In one aspect, there is provided a method of detecting or monitoring MMP9 activity, comprising:

contacting a sample with MMP9 binding protein,

assessing the presence or absence of MMP9 binding protein – MMP9 complex;

wherein the MMP9 binding protein comprises a VH region comprising a CDR with an amino acid sequence selected from the group consisting of SEQ ID NOs: 13, 14, 15, 34, 35, 36 and 47; and a VL region having a CDR with an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 17, 18, 37, 38, 39, 42, 43, 44, and 48;

whereby the absence of the MMP9 binding protein-MMP9 complex indicates the sample does not have the MMP9 activity, whereby the presence of the MMP9 binding protein-MMP9 complex indicates the sample has the MMP9 activity.

[0016] Also provided are methods and uses of the pharmaceutical compositions disclosed here, where a subject in need thereof that has an MMP-9-associated disease or condition is administered an effective amount of the disclosed antibodies or fragments thereof or the pharmaceutical composition comprising the disclosed antibodies or fragments thereof that is effective to inhibit MMP9 activity in the subject. Examples of MMP9-associated diseases or conditions include but are not limited to cancers, autoimmune, inflammatory, or fibrotic diseases or conditions. Examples of MMP9-associated cancers include but are not limited to pancreatic cancer, esophagogastric adenocarcinoma, non-small cell lung cancer, lung squamous cell carcinoma, lung adenocarcinoma, gastric adenocarcinoma, colorectal carcinoma, pancreatic adenocarcinoma, head and neck squamous cell carcinoma, hepatocellular carcinomacolorectal cancer, colorectal adenocarcinoma, or hepatocellular carcinoma. Examples of MMP9-associated autoimmune or inflammatory disease or condition is rheumatoid arthritis, an inflammatory bowel disease (IBD), septicemia, multiple

CLAIMS

What is claimed is:

1. A pharmaceutical composition, comprising:

an isolated antibody or fragment thereof that binds to Matrix Metalloproteinase 9 (MMP9), comprising: a heavy chain variable (VH) region having a complementary determining region (CDR) with an amino acid sequence of SEQ ID NO: 15; and

a pharmaceutically acceptable excipient.

2. The pharmaceutical composition of claim 1, wherein the VH region further comprises CDR with the amino acid sequence of SEQ ID NOs: 13 and/or 14.

3. A pharmaceutical composition, comprising:

an isolated antibody or fragment thereof that binds to Matrix Metalloproteinase 9, comprising: a light chain variable (VL) region having a light chain complementary determining region (CDR) with an amino acid sequence of SEQ ID NO: 18; and

a pharmaceutically acceptable excipient.

4. The pharmaceutical composition of any of claims 1-3, wherein the VL region further comprises with the amino acid sequence of SEQ ID NOs: 16 and/or 17.

5. The pharmaceutical composition of any of claims 1, 2, and 4, wherein the VH region has the amino acid sequence set forth in SEQ ID NOs: 3, 5, 6, 7, or 8.

6. The pharmaceutical composition of any of claims 1-5 wherein the VL region has the amino acid sequence set forth in SEQ ID NOs: 4, 9, 10, 11, or 12.

7. The pharmaceutical composition of claim 6, 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.

8. A pharmaceutical composition, comprising:

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; and a pharmaceutically acceptable excipient.

9. The pharmaceutical composition of claim 8, wherein the epitope comprises E111, D113, R162, or I198 of SEQ ID NO: 27.

10. The pharmaceutical composition of any of claims 1-9, wherein the antibody or fragment inhibits the enzymatic activity of MMP9.

11. The pharmaceutical composition of claim 10, wherein the inhibition of the enzymatic activity is non-competitive.

12. The pharmaceutical composition of any of claims 1-11, further comprising one or more therapeutic agents selected from the group consisting of an anti-inflammatory agent, an immunotherapeutic agent, a chemotherapeutic agent, an anti-cancer agent, an anti-fibrotic agent, or a combination thereof.

13. The pharmaceutical composition of claim 12, wherein the one or more therapeutic agent is selected from the group consisting of nab-paclitaxel, mFOLFOX6, FOLFIRI, carboplatin, paclitaxel, pemetrexed, bevacizumab, anti-lysyl oxidase-like 2 (LOXL2) antibody, an anti-discoidin domain receptor 1 (DDR1) antibody, or a combination thereof.

14. A method of inhibiting MMP9 activity in a subject, comprising administering to the subject the pharmaceutical composition of any of claims 1-13 in an amount effective to inhibit MMP9 activity in the subject suffering from an MMP9-associate disease or condition.

15. The method of claim 14, wherein the MMP9-associated disease or condition is a cancer, autoimmune, inflammatory or fibrotic disease or condition.

16. The method of claim 15, wherein the MMP9-associated cancer is pancreatic cancer, esophagogastric adenocarcinoma, non-small cell lung cancer, lung squamous cell carcinoma, lung adenocarcinoma, gastric adenocarcinoma, colorectal carcinoma, pancreatic adenocarcinoma, head and neck squamous cell carcinoma, hepatocellular carcinomacolorectal cancer, colorectal adenocarcinoma, or hepatocellular carcinoma.

17. The method of claim 16, wherein the MMP9-associated autoimmune or inflammatory disease or condition rheumatoid arthritis, an inflammatory bowel disease (IBD), septicemia, multiple sclerosis, muscular dystrophy, lupus, allergy, or asthma.

18. The method of claim 17, wherein the IBD is ulcerative colitis (UC), Crohn's disease (CD), or indeterminate colitis.

19. The method of any of claims 15-18, wherein the antibody or fragment is administered at a dose of about 1 mg/kg to about 28 mg/kg.

20. The method of any of claims 15-18, wherein the antibody or fragment is administered at a dosage of between at or about 100 and at or about 1800 mg/Kg body weight.

21. The method of claim 20, wherein the dosage is at or about 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, or 1800 mg/Kg body weight.

22. The method of any of claims 15-21, wherein the antibody or fragment is administered once every week, once every two weeks, or once every three weeks.

23. The method of any of claims 15-22, wherein the antibody or fragment is administered intravenously, intradermally, or subcutaneously.

24. The method of claims 12-23, wherein the antibody or fragment is administered concurrently or sequentially with the one or more therapeutic agents.

25. A method of detecting or monitoring MMP9 activity, comprises:

contacting a sample with MMP9 binding protein,

assessing the presence or absence of MMP9 binding protein – MMP9 complex;

wherein the MMP9 binding protein comprises a VH region comprising a CDR with an amino acid sequence selected from the group consisting of SEQ ID NOs: 13, 14, 15, 34, 35, 36 and 47; and a VL region having a CDR with an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 17, 18, 37, 38, 39, 42, 43, 44, and 48;

whereby the absence of the MMP9 binding protein-MMP9 complex indicates the sample does not have the MMP9 activity, whereby the presence of the MMP9 binding protein-MMP9 complex indicates the sample has the MMP9 activity.

26. The method of claim 25, wherein the MMP9 binding protein comprises a VH region has the amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 6, 7, 8, 34, 35, 36, and 46; and a VL region has the amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 9, 10, 11, 12, 37, 38, 39, 42, 43, 44, and 45.

27. A MMP9 binding protein comprises a VH region comprising a CDR with an amino acid sequence of SEQ ID NOs: 34, 35, 36 and 47; and a VL region having a CDR with an amino acid sequence selected from the group consisting of SEQ ID NOs: 37, 38, 39, 42, 43, 44, and 48.

28. The MMP9 binding protein of claim 27, wherein the VH region has a 95% sequence identify to the amino acid sequence selected from the group consisting of SEQ ID NOs: 30, 32, 46, and 47; and the VL region has a 95% sequence identify to the amino acid sequence selected from the group consisting of SEQ ID NOs: 31, 33, 41, 45, and 48.

29. The MMP9 binding protein of any of claims 27-28, wherein the VH region has the amino acid sequence selected from the group consisting of SEQ ID NOs: 30, 32, 46, and 47; and the VL region has the amino acid sequence selected from the group consisting of SEQ ID NOs: 31, 33, 41, 45, and 48.

30. A method of inhibiting MMP9 activity comprises: contacting a sample with the MMP9 binding protein of any of claims 27-29.

CLAIMS

What is claimed is:

1. A pharmaceutical composition, comprising:

an isolated antibody or fragment thereof that binds to Matrix Metalloproteinase 9 (MMP9), comprising: a heavy chain variable (VH) region having a complementarity determining region (CDR) with an amino acid sequence of SEQ ID NO: 15; and

a pharmaceutically acceptable excipient.

2. The pharmaceutical composition of claim 1, wherein the VH region further comprises CDR with the amino acid sequence of SEQ ID NOs: 13 and/or 14.

3. A pharmaceutical composition, comprising:

an isolated antibody or fragment thereof that binds to Matrix Metalloproteinase 9, comprising: a light chain variable (VL) region having a light chain complementarity determining region (CDR) with an amino acid sequence of SEQ ID NO: 18; and

a pharmaceutically acceptable excipient.

4. The pharmaceutical composition of any of claims 1-3, wherein the VL region further comprises with the amino acid sequence of SEQ ID NOs: 16 and/or 17.

5. The pharmaceutical composition of any of claims 1, 2, and 4, wherein the VH region has the amino acid sequence set forth in SEQ ID NOs: 3, 5, 6, 7, or 8.

6. The pharmaceutical composition of any of claims 1-5 wherein the VL region has the amino acid sequence set forth in SEQ ID NOs: 4, 9, 10, 11, or 12.

7. The pharmaceutical composition of claim 6, 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.

8. A pharmaceutical composition, comprising:

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; and a pharmaceutically acceptable excipient.

9. The pharmaceutical composition of claim 8, wherein the epitope comprises E111, D113, R162, or I198 of SEQ ID NO: 27.

10. The pharmaceutical composition of any of claims 1-9, wherein the antibody or fragment inhibits the enzymatic activity of MMP9.

11. The pharmaceutical composition of claim 10, wherein the inhibition of the enzymatic activity is non-competitive.

12. The pharmaceutical composition of any of claims 1-11, further comprising one or more therapeutic agents selected from the group consisting of an anti-inflammatory agent, an immunotherapeutic agent, a chemotherapeutic agent, an anti-cancer agent, an anti-fibrotic agent, or a combination thereof.

13. The pharmaceutical composition of claim 12, wherein the one or more therapeutic agent is selected from the group consisting of nab-paclitaxel, mFOLFOX6, FOLFIRI, carboplatin, paclitaxel, pemetrexed, bevacizumab, anti-lysyl oxidase-like 2 (LOXL2) antibody, an anti-discoidin domain receptor 1 (DDR1) antibody, or a combination thereof.

14. A method of detecting or monitoring MMP9 activity, comprising:

contacting a sample with MMP9 binding protein,

assessing the presence or absence of MMP9 binding protein – MMP9 complex;

wherein the MMP9 binding protein comprises a VH region comprising a CDR with an amino acid sequence selected from the group consisting of SEQ ID NOs: 13, 14, 15, 34, 35, 36 and 47; and a VL region having a CDR with an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 17, 18, 37, 38, 39, 42, 43, 44, and 48;

whereby the absence of the MMP9 binding protein-MMP9 complex indicates the sample does not have the MMP9 activity, whereby the presence of the MMP9 binding protein-MMP9 complex indicates the sample has the MMP9 activity.

15. The method of claim 14, wherein the MMP9 binding protein comprises a VH region has the amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 6, 7, 8, 34, 35, 36, and 46; and a VL region has the amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 9, 10, 11, 12, 37, 38, 39, 42, 43, 44, and 45.

16. A MMP9 binding protein comprises a VH region comprising a CDR with an amino acid sequence of SEQ ID NOs: 34, 35, 36 and 47; and a VL region having a CDR with an amino acid sequence selected from the group consisting of SEQ ID NOs: 37, 38, 39, 42, 43, 44, and 48.

17. The MMP9 binding protein of claim 16, wherein the VH region has a 95% sequence identify to the amino acid sequence selected from the group consisting of SEQ ID NOs: 30, 32, 46, and 47; and the VL region has a 95% sequence identify to the amino acid sequence selected from the group consisting of SEQ ID NOs: 31, 33, 41, 45, and 48.

18. The MMP9 binding protein of any of claims 16-17, wherein the VH region has the amino acid sequence selected from the group consisting of SEQ ID NOs: 30, 32, 46, and 47; and the VL region has the amino acid sequence selected from the group consisting of SEQ ID NOs: 31, 33, 41, 45, and 48.

19. A method of inhibiting MMP9 activity comprises: contacting a sample with the MMP9 binding protein of any of claims 16-18.