catalytically active portion thereof in the collection. In some examples, the protease trap polypeptide is modified to be cleaved by a protease having a predetermined substrate specificity and/or activity for a target substrate, for example, a target substrate involved in a disease or disorder. The method can further comprise screening the complexes for substrate specificity for the cleavage sequence of the target substrate. In such examples, the identified or selected protease has an altered activity and/or specificity towards the target substrate. In one example, the stable complex is formed by covalent linkage of a selected protease with a protease trap polypeptide. The selected proteases or catalytically active portion thereof are identified or selected from the complex in the methods provided herein. The methods provided herein can further include the step of separating the complexed proteases from the uncomplexed protease members of the collection. In one example, the protease trap polypeptide is labeled for detection or separation and separation is effected by capture of complexes containing the detectable protease trap polypeptide and the protease or catalytically active portion thereof. Capture can be effected in suspension, solution or on a solid support. In instances where capture is by a solid support, the protease trap polypeptide is attached to the solid support, which can be effected before, during or subsequent to contact of the protease trap polypeptide with the collection of proteases or catalytically active portions thereof. The solid support can include, for example, a well of a 96-well plate. In some examples, the protease trap polypeptide is labeled with biotin. In other examples, the protease trap polypeptide can be labeled with a His tag and separation can be effected by capture with a metal chelating agent such as, but not limited to, nickel sulphate ($NiSO_4$), cobalt chloride ($CoCl_2$), copper sulphate ($CuSO_4$) and zinc chloride ($ZnCl_2$). The metal chelating agent can be conjugated to a solid support, such as for example, on beads such as sepharose beads or magnetic beads.

In one aspect, there is provided a modified matrix type serine protease-1 (MT-SP1) polypeptide or catalytically active fragment thereof, comprising an amino acid replacement in an MT-SP1 polypeptide, wherein:

the amino acid replacement is one or both of T98P and F99L, based on chymotrypsin numbering;

the mutant MT-SP1 polypeptide and catalytically active fragment, which contains the amino acid replacement(s), exhibit increased substrate specificity for a complement protein target substrate compared to the MT-SP1 polypeptide not containing the amino acid replacement(s).

In one aspect, there is provided a modified matrix type serine protease-1 (MT-SP1) polypeptide or a catalytically active portion thereof, comprising an amino acid replacement at a position corresponding to 60(g), based on chymotrypsin numbering, whereby substrate specificity or activity is altered compared to the MT-SP1 polypeptide not containing the amino acid replacement.

In one aspect, there is provided a pharmaceutical composition, comprising a modified protease or catalytically active portion thereof as decribed herein in a pharmaceutically acceptable excipient.

In one aspect, there is provided a nucleic acid molecule, comprising a sequence of nucleotides that encodes a modified protease or catalytically active portion thereof as described herein.

In one aspect, there is provided a vector, comprising the nucleic acid molecule as described herein.

In one aspect, there is provided a cell, comprising the vector as described herein.

In one aspect, there is provided a pharmaceutical composition, comprising a modified MT-SP1 polypeptide or catalytically active portion thereof as described herein for use in treatment of an inflammatory disease or condition or a disease or condition with an inflammatory component.

In one aspect, there is provided the use of a pharmaceutical composition comprising a modified MT-SP1 polypeptide or catalytically active portion thereof as described herein for treatment of an inflammatory disease or condition or a disease or condition with an inflammatory component.

In the methods provided herein, the method can further include a step of amplifying the protease or catalytically active portion thereof in the separated complexes. In some examples, the protease or catalytically active portion thereof in the separated complex is displayed on a phage, and amplification is effected by infecting a host cell with the phage. The host cells can include a bacteria, for example, E.coli. The amplified

protease, either from bacterial cell medium, bacterial periplasm, phage supernatant or purified protein, can be screened for specificity

CLAIMS:

1. A modified matrix type serine protease-1 (MT-SP1) polypeptide or catalytically active fragment thereof, comprising an amino acid replacement in an MT-SP1 polypeptide, wherein:

the amino acid replacement is one or both of T98P and F99L, based on chymotrypsin numbering;

the mutant MT-SP1 polypeptide and catalytically active fragment, which contains the amino acid replacement(s), exhibit increased substrate specificity for a complement protein target substrate compared to the MT-SP1 polypeptide not containing the amino acid replacement(s).

2. The modified MT-SP1 polypeptide or catalytically active fragment thereof of claim 1, comprising the amino acid replacement T98P.

3. The modified MT-SP1 polypeptide or catalytically active fragment thereof of claim 1 or claim 2, comprising an amino acid replacement F99L.

4. The modified MT-SP1 polypeptide or catalytically active fragment thereof of any of claims 1-3, further comprising an amino acid replacement at Y60g.

5. A modified matrix type serine protease-1 (MT-SP1) polypeptide or a catalytically active portion thereof, comprising an amino acid replacement at a position corresponding to 60(g), based on chymotrypsin numbering, whereby substrate specificity or activity is altered compared to the MT-SP1 polypeptide not containing the amino acid replacement.

6. The MT-SP1 polypeptide or catalytically active fragment thereof of any of claims 1-5, wherein:

the unmodified MT-SP1 polypeptide comprises the sequence of amino acids set forth in SEQ ID NO:253 or 515; and the catalytically active portion comprises the sequence of amino acids set forth in SEQ ID NO: 505 or 507; or

MT-SP1 polypeptide has 95% or more sequence identity with a the MT-SP1 polypeptide set forth in SEQ ID NO:253, or a catalytically active portion thereof set forth in SEQ ID NO:505.

7. The modified MT-SP1 polypeptide or catalytically active fragment thereof of any of claims 1-6, wherein the MT-SP1 polypeptide or catalytically active portion thereof comprises up to 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid replacements compared to the MT-SP1 set forth in SEQ ID NO:253 or 505.

8. The modified MT-SP1 polypeptide or catalytically active fragment thereof of any of claims 1-7, wherein the unmodified MT-SP1 consists of the sequence of amino acids set forth in SEQ ID NO:507 or 515, whereby the modified MT-SP1 polypeptide comprises an amino acid replacement corresponding to the amino acid replacement C122S, based on chymotrypsin numbering.

9. The modified MT-SP1 polypeptide or catalytically active portion thereof of any of claims 1-8, wherein the modifications increase one or both of specificity for a C2 complement protein or activity towards C2 complement protein.

10. A pharmaceutical composition, comprising a modified protease or catalytically active portion thereof of any of claims 1-9 in a pharmaceutically acceptable excipient.

11. The pharmaceutical composition of claim 10, wherein the pharmaceutical composition is formulated for systemic, oral, nasal, pulmonary, local or topical administration.

12. A nucleic acid molecule, comprising a sequence of nucleotides that encodes a modified protease or catalytically active portion thereof of any of claims 1-9.

13. A vector, comprising the nucleic acid molecule of claim 12.

14. A cell, comprising the vector of claim 13.

15. A pharmaceutical composition, comprising a modified MT-SP1 polypeptide or catalytically active portion thereof of any of claims 1-9 for use in treatment of an inflammatory disease or condition or a disease or condition with an inflammatory component.

16. Use of a pharmaceutical composition comprising a modified MT-SP1 polypeptide or catalytically active portion thereof of any of claims 1-9 for treatment of an inflammatory disease or condition or a disease or condition with an inflammatory component.

17. The pharmaceutical composition of claim 15 or the use of claim 16, wherein complement protein C2 is cleaved, resulting in decreased complement activation to thereby reduce inflammation.

18. The pharmaceutical composition or use of any of claims 15-17, wherein the disease or condition is selected from among autoimmune diseases, sepsis, Rheumatoid arthritis (RA), membranoproliferative glomerulonephritis (MPGN), Multiple Sclerosis (MS), Myasthenia gravis (MG), asthma, inflammatory bowel disease, immune complex (IC)-mediated acute inflammatory tissue injury, Alzheimer's Disease (AD), Ischemia-reperfusion injury, rejection of a transplanted organ, macular degeneration, and Guillain-Barre syndrome.

19 The pharmaceutical composition or use of claim 18, wherein the ischemia-reperfusion injury is caused by an event or treatment selected from among myocardial infarct (MI), stroke, angioplasty, coronary artery bypass graft, cardiopulmonary bypass (CPB), and hemodialysis.

CLAIMS:

1. A modified serine protease or catalytically active fragment thereof, comprising one or more mutations in any of positions corresponding to positions 30, 73, 89, and 155, based on chymotrypsin numbering, whereby substrate specificity or activity is altered compared to the protease not containing the mutation(s),wherein the protease or catalytically active fragment thereof is of the chymotrypsin family of serine proteases.

2. The modified protease or catalytically active fragment thereof of claim 1, wherein the protease is a modified urinary plasminogen activator (u-PA).

3. A modified urinary plasminogen activator (u-PA) polypeptide or catalytically active fragment thereof, comprising an amino acid replacement in a position corresponding to a position selected from among positions 21, 24, 30, 38, 39, 61(A), 72, 73, 75, 80, 82, 84, 89, 92, 132, 133, 137, 138, 155, 156, 158, 159, 160, 187 and 217, based on chymotrypsin numbering, whereby substrate specificity or activity is altered compared to the u-PA polypeptide not containing the amino acid replacement(s).

4. The modified u-PA polypeptide or catalytically active fragment thereof of claim 3, wherein the amino acid replacement is at an amino acid residue selected from among F21, I24, F30, V38, T39, Y61(A), R72, L73, S75, E80, K82, E84, I89, K92, F132, G133, E137, I138, L155, K156, T158, V159, V160, K187, and R217 of a u-PA polypeptide, based on chymotrypsin numbering.

5. The modified u-PA polypeptide or catalytically active fragment thereof of any of claims 3 or claim 4, comprising an amino acid replacement selected from among F21V, I24L, F30I, F30V, F30L, F30T, F30G, F30M, V38D, T39A, Y61(A)H, R72G, L73A, L73P, S75P, E80G, K82E, E84K, I89V, K92E, F132L, G133D, E137G, I138T, L155P, L155V, L155M, K156Y, T158A, V159A, V160A, K187E, and R217C.

6. The modified u-PA polypeptide or catalytically active fragment thereof of any of claims 2-5, wherein:

the u-PA polypeptide has 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity with a wild-type u-PA polypeptide set forth in SEQ ID NO:433; and

the polypeptide includes the one or more modifications.

7. The modified u-PA polypeptide or catalytically active fragment thereof of any of claims 1-5, wherein the amino acid modifications are in a u-PA polypeptide set forth in SEQ ID NO:433.

8. The modified u-PA polypeptide or catalytically active fragment thereof of any of claims 3-7, whereby substrate specificity to VEFGR-2, or a sequence thereof, is altered.

9. The modified u-PA polypeptide or catalytically active fragment thereof of claim 8, comprising an amino acid replacement selected from among V38D, F30I, F30T, F30L, F30V, F30G, F30M, R72G, L73A, L73P, S75P, I89V, F132L, G133D, E137G, I138T, L155P, L155V, L155M, V160A, and R217C, based on chymotrypsin numbering.

10. The modified u-PA polypeptide or catalytically active fragment thereof of any of claims 3-9, wherein the u-PA polypeptide or catalytically active portion thereof contains amino acid replacements selected from among L73A/I89V; S75P/I89V/I138T; R72G/L155P; F132L/V160A; L73A/I89V/F30T; L73A/I89V/F30L; L73A/I89V/F30V; L73A/I89V/F30G; L73A/I89V/L155V; L73A/I89V/F30M; L73A/I89V/L155M; L73A/I89V/F30L/L155M; and L73A/I89V/F30G/L155M.

11. The modified u-PA polypeptide or catalytically active fragment thereof of any of claims 3-9 that has a sequence of amino acids set forth in any of SEQ ID NOS: 434-459, or fragments thereof of contiguous amino acids containing the mutation and having catalytic activity.

12. The modified u-PA polypeptide or catalytically active fragment thereof of any of claims 3-7, whereby substrate specificity to a t-PA substrate, or sequence thereof, is altered.

13. The modified u-PA polypeptide or catalytically active fragment thereof of claim 12, comprising an amino acid replacement selected from among F21V, I24L, F30V, F30L, T39A, Y61(A)H, E80G, K82E, E84K, I89V, K92E, K156T, T158A, V159A, and K187E, based on chymotrypsin numbering.

14. The modified u-PA polypeptide or catalytically active fragment thereof of any of claims 3-13, wherein the u-PA polypeptide or catalytically active portion thereof contains amino acid replacements selected from among F30V/Y61(A)H; F30V/K82E; F30V/K156T; F30V/K82E/V159A; F30V/K82E/T39A/V159A; F30V/K82E/T158A/V159A; F30V/Y61(A)H/K92E; F30V/K82E/V159A/E80G/I89V/K187E; and F30V/K82E/V159A/E80G/E84K/I89V/K187E.

15. The modified u-PA polypeptide or catalytically active fragment thereof of any of claims 3-14 that has a sequence of amino acids set forth in any of SEQ ID NOS: 460-472, or fragments thereof of contiguous amino acids containing the mutation and having catalytic activity.

16. A modified matrix type serine protease I (MT-SP1) polypeptide or a catalytically active portion thereof, comprising an amino acid replacement in a position corresponding to a position selected from among positions 23, 52, 60(g), 65, 71, 93, 95, 126, 129, 131, 136, 143, 144, 154, 164, 166, 184(a), 201, 209, 230, 234, and 244 , based on chymotrypsin numbering, whereby substrate specificity or activity is altered compared to the MT-SP1 polypeptide not containing the amino acid replacement(s).

17. The modified MT-SP1 polypeptide or catalytically active portion thereof of claim 16, wherein the amino acid replacement is at an amino acid residue selected from among D23, L52, Y60(g), T65, H71, F93, N95, A126, V129, P131, I136, H143, T144, I154, N164, T166, F184(a), S201, Q209, R230, F234, and V244 of a MT-SP1 polypeptide, based on chymotrypsin numbering.

18. The modified MT-SP1 polypeptide or catalytically active fragment thereof of claim 16 or claim 17, comprising an amino acid replacement selected from among D23E, L52M, Y60(g)s, H71R, F93L, N95K, A126T, V129D, P131S, I136T, I136V, H143R, T144I, I154V, N164D, T166A, F184(a)L, S201I, Q209L, R230W, F234L, and V244G.

19. The modified MT-SP1 polypeptide or catalytically active fragment thereof of claim 18, further comprising an amino acid replacement selected from among D23E, I41F, I41T, L52M, Y60(g)s, T65K, H71R, F93L, N95K, F97Y, F97L, T98P, F99L, A126T, V129D, P131S, I136T, I136V, H143R, T144I, I154V, N164D, T166A, L171F, P173S, Q175R, F184(a)L, Q192H, S201I, Q209L, D217V, Q221(a)L, R230W, F234L, and V244G.

20. The modified MT-SP1 polypeptide or catalytically active fragment thereof of any of claims 16-19, wherein the MT-SP1 polypeptide or catalytically active portion thereof contains amino acid replacements selected from among I136T/N164D/T166A/F184(A)L/D217V; I41F/A126T/V244G; D23E/I41F/T98P/T144I; I41F/ L171F/V244G; H143R/Q175R; I41F /I154V/V244G; I141F/L52M/ V129D/Q221(A)L; F97Y/ I136V/Q192H/S201I; H71R/ P131S/D217V; T65K/F93L/F97Y/ D217V; I41T/ P173S/Q209L and F97L/ F234L.

21. A modified matrix type serine protease I (MT-SP1) polypeptide or a catalytically active portion thereof, comprising one or more amino acid modifications selected from among T65K, F97Y, F97L, T98P, F99L, L171F, P173S, Q192H, and Q221(a)L, based on chymotrypsin numbering, whereby substrate specificity or activity is altered compared to the MT-SP1 polypeptide not containing the mutation(s).

22. The modified MT-SP1 polypeptide or catalytically active fragment thereof of claim 21, further comprising an amino acid replacement I41F, I41T, Q175R or D217V.

23. The modified MT-SP1 polypeptide or catalytically active fragment thereof of claim 22, wherein the amino acid replacement is I41F/ L171F.

24. The modified MT-SP1 polypeptide or catalytically active fragment thereof of any of claims 16-23, wherein the modifications are in an MT-SP1 polypeptide having a sequence of amino acids set forth in SEQ ID NO:253, or are in a catalytically active portion of an MT-SP1 having a sequence of amino acids set forth in SEQ ID NO: 505.

25. The modified MT-SP1 polypeptide or catalytically active fragment thereof of any of claims 1-24, wherein the modifications are in an MT-SP1 polypeptide further comprising a modification corresponding to modification of C122S in an MT-SP1 polypeptide set forth in SEQ ID NO:253, based on chymotrypsin numbering.

26. The modified MT-SP1 polypeptide or catalytically active fragment thereof of claim 25, wherein the modifications are in an MT-SP1 polypeptide having a sequence of amino acids set forth in SEQ ID NO: 507 or 517.

27. The modified MT-SP1 polypeptide or catalytically active fragment thereof of any of claims 16-26, wherein:

the MT-SP1 polypeptide has 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity with a wild-type MT-SP1 polypeptide set forth in SEQ ID NO:253, or a catalytically active portion thereof set forth in SEQ ID NO:505; and

the polypeptide includes the one or more modifications.

28. The modified MT-SP1 polypeptide or catalytically active fragment thereof of any of claims 16-27, wherein the modifications increase one or both of specificity for a C2 complement protein or activity towards C2 complement protein.

29. The modified MT-SP1 polypeptide or catalytically active portion thereof of any of claims 16-28 that has a sequence of amino acids set forth in any of SEQ ID NOS: 568-609.

30. A pharmaceutical composition, comprising a modified protease or catalytically active fragment thereof of any of claims 1-29.

31. The pharmaceutical composition of claim 30, further comprising a pharmaceutically acceptable excipient.

32. The pharmaceutical composition of claim 30 or claim 31, wherein the pharmaceutical composition is formulated for systemic, oral, nasal, pulmonary, local or topical administration.

33. A kit, comprising the pharmaceutical composition of any of claims 30-32, a device for administration of the composition and, optionally, instructions for administration.

34. A nucleic acid molecule, comprising a sequence of nucleotides that encodes a modified protease or catalytically active fragment thereof of any of claims 1-29.

35. A vector, comprising the nucleic acid molecule of claim 34.

36. A cell, comprising the vector of claim 35.

37. A pharmaceutical composition, comprising a modified u-PA polypeptide or catalytically active fragment thereof of any of claims 8-11 for use in treatment of a disease or condition that is mediated by VEGFR-2.

38. Use of a pharmaceutical composition comprising a modified u-PA polypeptide or catalytically active fragment thereof of any of claims 8-11 for treatment of a disease or condition that is mediated by VEGFR-2.

39. The pharmaceutical composition or use of claim 37 or claim 38, wherein the disease or condition to be treated is an angiogenic disease.

40. The pharmaceutical composition or use of claim 39, wherein the angiogenic disease is selected from among cancer, macular degeneration, inflammation, and diabetes.

41. The pharmaceutical composition or use of any of claims 37-40, further comprising another anti-tumor agent.

42. A pharmaceutical composition, comprising a modified u-PA polypeptide or catalytically active fragment thereof of any of claims 12-15 for use in treatment of a disease or condition that is treated by administration of t-PA.

43. Use of a pharmaceutical composition comprising a modified u-PA polypeptide or catalytically active fragment thereof of any of claims 12-15 for treatment of a disease or condition that is treated by t-PA.

44. The pharmaceutical composition or use of claim 42 or claim 43, wherein the disease or condition is selected from among arterial thrombosis, venous thrombosis and thromboembolism, ischemic stroke, acquired coagulation disorders, disseminated intravascular coagulation, bacterial infection and periodontitis, and neurological conditions.

45. A pharmaceutical composition, comprising a modified MT-SP1 polypeptide or catalytically active portion thereof of any of claims 16-29 for use in treatment of a disease or condition that is mediated by complement protein C2.

46. Use of a pharmaceutical composition comprising a modified MT-SP1 polypeptide or catalytically active portion thereof of any of claims 16-29 for treatment of a disease or condition that is mediated by complement protein C2.

47. The pharmaceutical composition or use of claim 45 or 46, wherein the disease or condition is selected from among autoimmune diseases, sepsis, Rheumatoid arthritis (RA), membranoproliferative glomerulonephritis (MPGN), Multiple Sclerosis (MS), Myasthenia gravis (MG), asthma, inflammatory bowel disease, immune complex (IC)-mediated acute inflammatory tissue injury, Alzheimer's Disease (AD), Ischemia-reperfusion injury, rejection of a transplanted organ, macular degeneration, and Guillan-Barre syndrome.

48. The pharmaceutical composition or use of claim 47, wherein the ischemia-reperfusion injury is caused by an event or treatment selected from among myocardial infarct (MI), stroke, angioplasty, coronary artery bypass graft, cardiopulmonary bypass (CPB), and hemodialysis.