CLAIMS

1. A method of preparing an isolated desialylated Factor VII polypeptide, the method comprising

(1) generating a recombinant cell line that coexpresses a recombinant Factor VII polypeptide and a recombinant sialidase enzyme;

(2) culturing said recombinant cell line to allow expression of both the recombinant Factor VII polypeptide and the recombinant sialidase enzyme, wherein the recombinant sialidase enzyme removes sufficient amounts of covalently attached sialic acid residues to produce a desialylated Factor VII polypeptide having a ratio of moles of conjugated sialic acid to moles of N-linked glycan selected from the group consisting of a) less than 0.05, b) less than 0.1, c) less than 1.0, d) less than 2.0, e) less than 3.0, f) less than 4.0, g) less than 5.0; and h) less than 6.0; and

(3) isolating the desialylated Factor VII polypeptide thereby produced.

2. The method of claim 1, wherein the isolated desialylated Factor VII polypeptide comprises the wild-type Factor VII amino acid sequence of SEQ ID NO: 16.

3. The method of claim 1, wherein the isolated desialylated Factor VII polypeptide comprises a variant amino acid sequence comprising two or more sequence alterations relative to SEQ ID NO: 16.

4. The method of claim 1, wherein the isolated desialylated Factor VII polypeptide comprises the wild-type Factor VII amino acid sequence of SEQ ID NO: 16 and is glycosylated at amino acid positions 145 and 322.

5. The isolated desialylated Factor VII polypeptides prepared by the method of claim 1 for use in the treatment of a mammal having a disease or a disorder wherein blood clot formation is desirable.

6. The isolated desialylated Factor VII polypeptides of claim 5, wherein the disease or disorder is selected from the group consisting of a hemorrhage, gastrointestinal bleeding, uncontrolled bleeding, bleeding in a mammal undergoing transplantation or

resection or surgery, variceal bleeding, thrombocytopenia, hemophilia, intracranial hemorrhage, aortic aneurysm, and over administration of an anticoagulant.

7. A pharmaceutical composition comprising isolated desialylated Factor VII polypeptides prepared by the method of claim 1 and a pharmaceutically acceptable excipient.

8. A method of preparing an isolated desialylated Factor VII polypeptide, the method comprising:

(1) producing a Factor VII polypeptide in a recombinant cell line that is deficient in its ability to sialylate peptides such that it produces a desialylated Factor VII polypeptide having a ratio of moles of conjugated sialic acid to moles of N-linked glycan selected from the group consisting of a) less than 0.05, b) less than 0.1, c) less than 1.0, d) less than 2.0, e) less than 3.0, f) less than 4.0, g) less than 5.0; and h) less than 6.0; and

(2) isolating the desialylated Factor VII polypeptide thereby produced.

9. The method of claim 8, wherein the isolated desialylated Factor VII polypeptide comprises the wild-type Factor VII amino acid sequence of SEQ ID NO: 16.

10. The method of claim 8, wherein the isolated desialylated Factor VII polypeptide comprises a variant amino acid sequence comprising two or more sequence alterations relative to SEQ ID NO: 16.

11. The method of claim 8, wherein the isolated desialylated Factor VII polypeptide comprises the wild-type Factor VII amino acid sequence of SEQ ID NO: 16 and is glycosylated at amino acid positions 145 and 322.

12. The isolated desialylated Factor VII polypeptides prepared by the method of claim 8 for use in treatment of a mammal having a disease or a disorder wherein blood clot formation is desirable.

13. The isolated desialylated Factor VII polypeptides of claim 12, wherein the disease or disorder is selected from the group consisting of a hemorrhage, gastrointestinal bleeding, uncontrolled bleeding, bleeding in a mammal undergoing transplantation or

resection or surgery, variceal bleeding, thrombocytopenia, hemophilia, intracranial hemorrhage, aortic aneurysm, and over administration of an anticoagulant.

14. A pharmaceutical composition comprising isolated desialylated Factor VII polypeptides prepared by the method of claim 8 and a pharmaceutically acceptable excipient.

15. A composition of isolated desialylated Factor VII polypeptides, wherein the Factor VII polypeptides comprise the amino acid sequence of SEQ ID NO: 16 and the ratio of moles of conjugated sialic acid to moles of N-linked glycan in the composition is within a range selected from the group consisting of (a) from 1 to 5; (b) from 1 to 4; (c) from 1 to 3; (d) from 1 to 2; and (e) from 0.5 to 1.

16. The composition of claim 15, wherein the isolated desialylated Factor VII polypeptides are glycosylated at amino acid positions 145 and 322.

17. The composition of claim 15 for use in the treatment of a mammal having a disease or a disorder wherein blood clot formation is desirable.

18. The composition of claim 17, wherein the disease or disorder is selected from the group consisting of a hemorrhage, gastrointestinal bleeding, uncontrolled bleeding, bleeding in a mammal undergoing transplantation or resection or surgery, variceal bleeding, thrombocytopenia, hemophilia, intracranial hemorrhage, aortic aneurysm, and over administration of an anticoagulant.

19. Human Factor VIIa polypeptide in which the ratio of moles of conjugated sialic acid to moles of N-linked glycan is less than 1.0; wherein the human Factor VIIa polypeptide comprises the amino acid sequence of SEQ ID NO: 16 and has glycans covalently attached at amino acid residues 145 and 322, for use in treating a patient having a disease or a disorder wherein blood clot formation is desirable.

20. The human Factor VIIa polypeptide of claim 19, wherein the ratio of moles of conjugated sialic acid to moles of N-linked glycan is less than 0.1.

21. The human Factor VIIa polypeptide of claim 19, wherein the human Factor VIIa polypeptide contains no detectable amount of conjugated sialic acid.

22. The human Factor VIIa polypeptide of claim 19, wherein the half-life of the human Factor VIIa polypeptide is less than one hour.

23. The human Factor VIIa polypeptide of claim 19, wherein the half-life of the human Factor VIIa polypeptide is less than 0.5 hour.

24. The human Factor VIIa polypeptide of claim 19, wherein the disease or disorder is hemorrhage.

25. The human Factor VIIa polypeptide of claim 19, wherein the disease or disorder is selected from the group consisting of a hemorrhage, gastrointestinal bleeding, uncontrolled bleeding, bleeding while undergoing transplantation or resection or surgery, variceal bleeding, thrombocytopenia, hemophilia, intracranial hemorrhage, aortic aneurysm, and over administration of an anticoagulant.

26. The use of a human Factor VIIa polypeptide in which the ratio of moles of conjugated sialic acid to moles of N-linked glycan is less than 1.0; wherein the human Factor VIIa polypeptide comprises the amino acid sequence of SEQ ID NO: 16 and has glycans covalently attached at amino acid residues 145 and 322, in the manufacture of a medicament for treating a patient having a disease or a disorder wherein blood clot formation is desirable.

27. The use of claim 26, wherein the ratio of moles of conjugated sialic acid to moles of N-linked glycan is less than 0.1.

28. The use of claim 27, wherein the human Factor VIIa polypeptide contains no detectable amount of conjugated sialic acid.

29. The use of claim 26, wherein the half-life of the human Factor VIIa polypeptide is less than one hour.

30. The use of claim 26, wherein the half-life of the human Factor VIIa polypeptide is less than 0.5 hour.

31. The use of claim 26, wherein the disease or disorder is hemorrhage.

32. The use of claim 26, wherein the disease or disorder is selected from the group consisting of a hemorrhage, gastrointestinal bleeding, uncontrolled bleeding, bleeding while undergoing transplantation or resection or surgery, variceal bleeding, thrombocytopenia, hemophilia, intracranial hemorrhage, aortic aneurysm, and over administration of an anticoagulant.

Claims

1. The use of an isolated desialylated Factor VIIa polypeptide in the manufacture of a medicament for the treatment of a disease or a disorder wherein blood clot formation is desirable,

wherein the disease or disorder is selected from the group consisting of a hemorrhage, gastrointestinal bleeding, uncontrolled bleeding, bleeding in a mammal undergoing transplantation or resection or surgery, variceal bleeding, thrombocytopenia, hemophilia, intracranial hemorrhage, aortic aneurysm, and over administration of an anticoagulant,

and further wherein the desialylated Factor VIIa polypeptide has a ratio of moles of conjugated sialic acid to moles of N-linked glycan of less than 1.0.

2. The use of claim 1, wherein the isolated desialylated Factor VIIa polypeptide comprises the wild-type Factor VIIa amino acid sequence of SEQ ID NO: 16.

3. The use of claim 1, wherein the isolated desialylated Factor VIIa polypeptide comprises a variant amino acid sequence comprising two or more sequence alterations relative to SEQ ID NO: 16.

4. The use of claim 1, wherein the isolated desialylated Factor VIIa polypeptide comprises the wild-type Factor VIIa amino acid sequence of SEQ ID NO: 16 and is glycosylated at amino acid positions 145 and 322.

5. The use of claim 1, wherein the ratio of moles of conjugated sialic acid to moles of N-linked glycan is less than 0.1.

6. The use of claim 1, wherein the isolated desialylated Factor VIIa polypeptide contains no detectable amount of conjugated sialic acid.

7. The use of claim 1, wherein the half-life of the isolated desialylated Factor VIIa polypeptide is less than one hour.

8. The use of claim 1, wherein the half-life of the isolated desialylated Factor VIIa polypeptide is less than 0.5 hour.

9. A method of preparing an isolated desialylated Factor VII polypeptide, the method comprising

(1) producing a Factor VII polypeptide in a recombinant cell line that is deficient in its ability to sialylate peptides such that it produces a desialylated Factor VII polypeptide having a ratio of moles of conjugated sialic acid to moles of N-linked glycan selected from the group consisting of a) less than 0.05, b) less than 0.1, c) less than 1.0, d) less than 2.0, e) less than 3.0, f) less than 4.0, g) less than 5.0; and h) less than 6.0; and

(2) isolating the desialylated Factor VII polypeptide thereby produced.

10. The method of claim 9, wherein the isolated desialylated Factor VII polypeptide comprises the wild-type Factor VII amino acid sequence of SEQ ID NO: 16.

11. The method of claim 9, wherein the isolated desialylated Factor VII polypeptide comprises a variant amino acid sequence comprising two or more sequence alterations relative to SEQ ID NO: 16.

12. The method of claim 9, wherein the isolated desialylated Factor VII polypeptide comprises the wild-type Factor VII amino acid sequence of SEQ ID NO: 16 and is glycosylated at amino acid positions 145 and 322.

13. An isolated variant Factor VII polypeptide comprising an amino acid sequence having at least one sequence alteration relative to the amino acid sequence of SEQ ID NO: 16, wherein the at least one sequence alteration is selected from the group consisting of P10Q, K32E, R36E, A34E, T106N, and V253N, wherein the polypeptide has a ratio of moles of conjugated sialic acid to moles of N-linked glycan between 0 and 5.0.

14. The isolated variant Factor VII polypeptide of claim 13, wherein the variant Factor VII polypeptide comprises two or more sequence alterations selected from the group consisting of P10Q, K32E, R36E, A34E, T106N, and V253N.

15. The isolated variant Factor VII polypeptide of claim 13, wherein the variant Factor VII polypeptide comprises three or more sequence alterations selected from the group consisting of P10Q, K32E, R36E, A34E, T106N, and V253N.

16. The isolated variant Factor VII polypeptide of claim 13, wherein the variant Factor VII polypeptide comprises four or more sequence alterations selected from the group consisting of P10Q, K32E, R36E, A34E, T106N, and V253N.

17. The isolated variant Factor VII polypeptide of claim 13, wherein the variant Factor VII polypeptide comprises five or more sequence alterations selected from the group consisting of P10Q, K32E, R36E, A34E, T106N, and V253N.

18. The isolated variant Factor VII polypeptide of claim 13, wherein the variant Factor VII polypeptide comprises the sequence alterations of P10Q, K32E, R36E, A34E, T106N, and V253N.

19. The isolated variant Factor VII polypeptide of claim 13, wherein the variant Factor VII polypeptide comprises the sequence alterations P10Q, K32E, T106N, and V253N.

20. The isolated variant Factor VII polypeptide of claim 13, wherein the amino acid sequence consists essentially of the amino acid sequence of SEQ ID NO: 16 having the sequence alterations P10Q, K32E, R36E, A34E, T106N, and V253N.

21. The isolated variant Factor VII polypeptide of claim 13, wherein the amino acid sequence consists essentially of the amino acid sequence of SEQ ID NO: 16 having the sequence alterations P10Q, K32E, T106N, and V253N.

22. The isolated variant Factor VII polypeptide of any of claims 13-21, wherein the ratio of moles of conjugated sialic acid to moles of N-linked glycan is less than 0.1.

23. A method of preparing the isolated variant Factor VII polypeptide of claim 13, the method comprising

(1) obtaining a sialylated Factor VII polypeptide comprising an amino acid sequence having at least one sequence alteration relative to the amino acid sequence of SEQ ID NO: 16, wherein the at least one sequence alteration is selected from the group consisting of P10Q, K32E, R36E, A34E, T106N, and V253N;

(2) contacting the sialylated Factor VII polypeptide with sialidase under conditions such that sufficient amounts of covalently attached sialic acid residues are removed from the sialylated Factor VII polypeptide to produce a desialylated Factor VII polypeptide having a ratio of moles of conjugated sialic acid to moles of N-linked glycan between 0 and 5.0 and

(3) isolating the variant Factor VII polypeptide thereby produced.

24. The method of claim 23, wherein the variant Factor VII polypeptide comprises a variant sequence comprising four or more sequence alterations selected from the group consisting of P10Q, K32E, R36E, A34E, T106N, and V253N.

25. A method of preparing the isolated variant Factor VII polypeptide of claim 13, said method comprising

(1) producing a Factor VII polypeptide comprising an amino acid sequence having at least one sequence alteration relative to the amino acid sequence of SEQ ID NO: 16, wherein the at least one sequence alteration is selected from the group consisting of P10Q, K32E, R36E, A34E, T106N, and V253N, in a recombinant cell line that is deficient in its ability to sialylate peptides such that it produces a desialylated Factor VII polypeptide having a ratio of moles of conjugated sialic acid to moles of N-linked glycan between 0 and 5.0; and

(2) isolating the variant Factor VII polypeptide thereby produced.

26. The method of claim 25, wherein the variant Factor VII polypeptide comprises a variant sequence comprising four or more sequence alterations selected from the group consisting of P10Q, K32E, R36E, A34E, T106N, and V253N.

27. A method of preparing the isolated variant Factor VII polypeptide of claim 13, said method comprising

(1) obtaining a recombinant cell line that coexpresses (a) a recombinant Factor VII polypeptide comprising an amino acid sequence having at least one sequence alteration relative to the amino acid sequence of SEQ ID NO: 16, wherein the at least one sequence alteration is selected from the group consisting of P10Q, K32E, R36E, A34E, T106N, and V253N, and (b) a recombinant sialidase enzyme;

(2) culturing said recombinant cell line to allow expression of both the recombinant Factor VII polypeptide and the recombinant sialidase enzyme, wherein said recombinant sialidase enzyme removes sufficient amounts of covalently attached sialic acid residues to produce a desialylated Factor VII polypeptide having a ratio of moles of conjugated sialic acid to moles of N-linked glycan between 0 and 5.0; and

(3) isolating the variant Factor VII polypeptide thereby produced.

28. The method of claim 27, wherein the variant Factor VII polypeptide comprises a variant sequence comprising four or more sequence alterations selected from the group consisting of P10Q, K32E, R36E, A34E, T106N, and V253N.

29. An isolated variant Factor VII polypeptide selected from the group consisting of:

(1) a polypeptide comprising a Factor VII amino acid sequence having sequence alterations relative to the sequence of SEQ ID NO: 16, wherein the sequence alterations consist of (1) a glutamine residue substituted for the proline residue in position 10, (2) a glutamic acid residue substituted for the lysine residue in position 32, and (3) a sequence alteration such that N-linked glycosylation at position 145 is disrupted;

(2) a polypeptide comprising a Factor VII amino acid sequence having sequence alterations relative to the sequence of SEQ ID NO: 16, wherein the sequence alterations consist of (1) a glutamine residue substituted for the proline residue in position 10, (2) a glutamic acid residue substituted for the lysine residue in position 32, and (3) a sequence alteration such that N-linked glycosylation at position 322 is disrupted;

(3) a polypeptide comprising a Factor VII amino acid sequence having sequence alterations relative to the amino acid sequence of SEQ ID NO: 16, wherein the sequence alterations consist of (1) a glutamine residue substituted for the proline residue in position 10, and (2) a glutamic acid residue substituted for the lysine residue in position 32, and (3) sequence alterations such that N-linked glycosylation at positions 145 and 322 is disrupted;

(4) a polypeptide comprising a Factor VII amino acid sequence having sequence alterations relative to the amino acid sequence of SEQ ID NO: 16, wherein the sequence alterations consist of (1) a glutamine residue substituted for the proline residue in position 10, and (2) a glutamic acid residue substituted for the lysine residue in position 32, wherein positions 145 and 322 are asparagine and have attached N-linked glycosylation;

(5) a polypeptide comprising a Factor VII amino acid sequence having sequence alterations relative to the amino acid sequence of SEQ ID NO: 16, wherein the sequence alterations consist of (1) a glutamine residue substituted for the proline residue in position 10, (2) a glutamic acid residue substituted for the lysine residue in position 32, (3) a glutamic acid residue substituted for the alanine residue in position 34, (4) a glutamic acid residue substituted for the arginine residue in position 36, and (5) a sequence alteration such that N-linked glycosylation at position 145 is disrupted;

(6) a polypeptide comprising a Factor VII amino acid sequence having sequence alterations relative to the amino acid sequence of SEQ ID NO: 16, wherein the sequence alterations consist of (1) a glutamine residue substituted for the proline residue in position 10, (2) a glutamic acid residue substituted for the lysine residue in position 32, (3) a glutamic acid residue substituted for the alanine

residue in position 34, (4) a glutamic acid residue substituted for the arginine residue in position 36, and (5) a sequence alteration such that N-linked glycosylation at position 322 is disrupted;

(7) a polypeptide comprising a Factor VII amino acid sequence having sequence alterations relative to the amino acid sequence of SEQ ID NO: 16, wherein the sequence alterations consist of (1) a glutamine residue substituted for the proline residue in position 10, (2) a glutamic acid residue substituted for the lysine residue in position 32, (3) a glutamic acid residue substituted for the alanine residue in position 34, (4) a glutamic acid residue substituted for the arginine residue in position 36, and (5) a sequence alterations such that N-linked glycosylation at positions 145 and 322 is disrupted; and

(8) a polypeptide comprising a Factor VII amino acid sequence having sequence alterations relative to the amino acid sequence of SEQ ID NO: 16, wherein the sequence alterations consist of (1) a glutamine residue substituted for the proline residue in position 10, (2) a glutamic acid residue substituted for the lysine residue in position 32, (3) a glutamic acid residue substituted for the alanine residue in position 34, and (4) a glutamic acid residue substituted for the arginine residue in position 36, wherein positions 145 and 322 are asparagine and have attached N-linked glycosylation.

30. The isolated variant Factor VII polypeptide of any of claims 13-22 or 29 for use in the treatment of a disease or a disorder wherein blood clot formation is desirable, wherein the disease or disorder is selected from the group consisting of a hemorrhage, gastrointestinal bleeding, uncontrolled bleeding, bleeding in a mammal undergoing transplantation or resection or surgery, variceal bleeding, thrombocytopenia, hemophilia, intracranial hemorrhage, aortic aneurysm, and over administration of an anticoagulant.

31. The use of the isolated variant Factor VII polypeptide of any of claims 13-22 or 29 in the manufacture of a medicament for treating a patient having a disease or a disorder wherein blood clot formation is desirable, wherein the disease or disorder is selected from the group consisting of a hemorrhage, gastrointestinal bleeding, uncontrolled bleeding, bleeding in a mammal undergoing transplantation or resection or surgery, variceal bleeding, thrombocytopenia, hemophilia, intracranial hemorrhage, aortic aneurysm, and over administration of an anticoagulant.

32. A pharmaceutical composition comprising the isolated variant Factor VII polypeptide of any of claims 13-22 or 29 and a pharmaceutically acceptable excipient.