Claims

1. A derivative of FacⅦ or its active form FacⅦa, comprising an amino acid sequence (SEQ ID NO. 4) of blood coagulation factor Ⅶ (Factor Ⅶ, FacⅦ) and a peptide linker linked at the C-terminus thereof, wherein the C-terminal amino acid residue of the peptide linker is a cysteine.

2. The derivative according to claim 1, wherein the peptide linker is a partial sequence of SOD1 (Superoxide dismutase).

3. The derivative according to claim 2, wherein the partial sequence of SOD1 is mutated by replacing of VLKG (valine-leucine-lysine-glycine) within the sequence with a self-cleavage site sequence IPRI (isoleucine-proline-arginine-isoleucine).

4. The derivative according to claim 1, wherein the derivative is composed of an amino acid sequence selected from the group consisting of SEQ ID NOs. 9, 13, 14, 20, 24, and 27.

5. A polynucleotide encoding the derivative of FacⅦ or its active form FacⅦa of any one of claims 1 and 4.

6. The polynucleotide according to claim ~~6~~5, wherein the polynucleotide is composed of a nucleotide sequence selected from the group consisting of SEQ ID NOs. 8, 12, 15, 21, 25 and 28.

7. An expression vector comprising the polynucleotide of claim ~~7~~5.

8. The expression vector according to claim 7, wherein the vector is selected from the group consisting of pX0GC-FⅦ-ATKAVC, pX0GC-FⅦ-GGGGSC, pX0GC-FⅦ-SOD1 1-149, pX0GC-FⅦ-SOD1 IPRI, pX0GC-FⅦ-SOD1 1-25 IPRI, and pX0GC-FⅦ-SOD1 1-90 IPRI.

9. A transformant comprising the expression vector of claim 7.

10. The transformant according to claim 9, wherein the transformant is identified by accession number KCTC12022BP.

11. A method for preparing a FacVII derivative, comprising the steps of:
(i) culturing the transformant of claim 9 so as to obtain a culture solution; and
(ii) recovering the FacVII derivative from the culture solution.

12. The method according to claim 11, further comprising the step of activating the recovered FacVII derivative.

13. A conjugate of FacVII or its active form FacVIIa, wherein a non-peptidyl polymer capable of extending the blood half-life is linked to the peptide linker of the derivative of FacVII or its active form FacVIIa of any one of claims 1 to 4.

14. The conjugate according to claim 13, wherein the non-peptidyl polymer is linked to the C-terminus of the derivative of FacVII or its active form FacVIIa.

15. The conjugate according to claim 13, wherein the non-peptidyl polymer has one or more reactive group(s) selected from the group consisting of an aldehyde group, a propionaldehyde group, a butyraldehyde group, a maleimide group, ortho pyridyl disulfide, thiol and succinimide derivatives.

16. The conjugate according to claim ~~13~~15, wherein the succinimide derivative is succinimidyl propionate, succinimidyl carboxymethyl, hydroxy succinimidyl or succinimidyl carbonate.

17. The conjugate according to claim 13, wherein the non-peptidyl polymer has two or three reactive ends.

18. The conjugate according to claim 13, wherein the non-peptidyl polymer has a maleimide reactive group or an aldehyde reactive group at both ends, respectively.

19. The conjugate according to claim 13, wherein the non-peptidyl polymer is selected from the group consisting of polyethylene glycol, polypropylene glycol, ethylene glycol-propylene glycol non-peptidyl copolymers, polyoxyethylated polyols, polyvinyl alcohols, polysaccharides, dextrans, polyvinyl ethyl ethers, biodegradable polymers, lipid non-peptidyl polymers, chitins, hyaluronic acids, and a combination thereof.

20. A complex of FacVII or its active form FacVIIa that is composed of the FacVII or FacVIIa derivative of any one of claims 1 to 4 linked to an immunoglobulin Fc region via a non-peptidyl polymer.

21. The complex according to claim 20, wherein the non-peptidiyl polymer is selected from the group consisting of polyethylene glycol, polypropylene glycol, ethylene glycol-propylene glycol copolymers, polyoxyethylated polyols, polyvinyl alcohols, polysaccharides, dextrans, polyvinyl ethyl ethers, biodegradable polymers, lipid polymers, chitins, hyaluronic acids, and a combination thereof.

22. The complex according to claim 20, wherein the non-peptidyl polymer has one, two or three reactive end(s).

23. The complex according to claim 20, wherein the non-peptidyl polymer has a maleimide reactive group or an aldehyde reactive group at both ends, respectively.

24. The complex according to claim 20, wherein the immunoglobulin Fc region is composed of one to four domains selected from the group consisting of CH1, CH2, CH3 and CH4 domains.

25. The complex according to claim 20, wherein the immunoglobulin Fc region further comprises a hinge region.

26. The complex according to claim 20, wherein the immunoglobulin Fc region is derived from IgG, IgA, IgD, IgE, or IgM.

27. The complex according to claim 20, wherein the immunoglobulin Fc region is an IgG4 Fc region.

28. The complex according to claim 20, wherein the immunoglobulin Fc region is a human aglycosylated IgG4 Fc region.

29. A method for preparing a FacVIIa complex, comprising the steps of:
(i) covalently linking a non-peptidyl polymer having a succinimide derivative or aldehyde reactive group at its one end to the amine group of immunoglobulin Fc at pH 5.0 ~ pH 9.0;
(ii) recovering a conjugate that includes the immunoglobulin Fc region covalently linked with the non-peptidyl polymer at the amine group, from the reaction mixture of step (i);
(iii) covalently linking the C-terminal thiol group of the FacVII derivative of any one of claims 1 to 4 to the other end of the non-peptidyl polymer in the recovered conjugate so as to produce a FacVII complex having the immunoglobulin Fc region and the FacVII derivative at each end of the non-peptidyl polymer; and
(iv) activating the FacVII complex produced in step (iii) so as to produce a FacVIIa complex having FacVIIa and the immunoglobulin Fc region linked via the non-peptidyl polymer.

30. A method for preparing a FacVIIa complex, comprising the steps of:
(i) covalently linking a non-peptidyl polymer having a succinimide derivative or aldehyde reactive group at its one end to the amine group of immunoglobulin Fc at pH 5.0 ~ pH 9.0;
(ii) recovering a conjugate that includes the immunoglobulin Fc region covalently linked with the non-peptidyl polymer at the amine group, from the reaction mixture of step (i); and
(iii) covalently linking the C-terminal thiol group of the FacVIIa derivative of any one of claims 1 to 4 to the other end of the non-peptidyl polymer in the recovered conjugate so as to produce a FacVIIa complex having the immunoglobulin Fc region and the FacVIIa derivative

at each end of the non-peptidyl polymer.

31. A method for preparing a FacVIIa conjugate, comprising the steps of:

(i) covalently linking a non-peptidyl polymer having a reactive group selected from the group consisting of an aldehyde group, a propionaldehyde group, a butyraldehyde group, a maleimide group, ortho pyridyl disulfide, thiol and succinimide derivatives to the C-terminal thiol group of the FacVII derivative of any one of claims 1 to 4;

(ii) recovering the FacVII conjugate prepared by covalently linking the non-peptidyl polymer to the FacVII derivative; and

(iii) activating the recovered FacVII conjugate so as to produce a FacVIIa conjugate having the non-peptidyl polymer linked to the FacVIIa region.

32. A method for preparing a FacVIIa conjugate, comprising the steps of:

(1) covalently linking a non-peptidyl polymer having a reactive group selected from the group consisting of an aldehyde group, a propionaldehyde group, a butyraldehyde group, a maleimide group, ortho pyridyl disulfide, thiol and succinimide derivatives to the C-terminal thiol group of the FacVIIa derivative of any one of claims 1 to 4; and

(ii) recovering the FacVIIa conjugate prepared by covalently linking the non-peptidyl polymer to the FacVIIa derivative.

33. A FacVIIa complex that is composed of an immunoglobulin Fc region linked to the other end of the non-peptidyl polymer in the recovered FacVIIa conjugate of claim 31 or 32.

34. The method according to any one of claims 29 to 32, wherein activation of the FacVII derivative, FacVII conjugate, or FacVII complex is performed by on-column activation or in-solution activation.

35. The method according to any one of claims 29 to 32, wherein the non-peptidyl polymer is polyethylene glycol.

36. A FacVIIa conjugate or FacVIIa complex that is prepared by the method of any one of claims 29 to 32.

37. A complex of FacVII or its active form FacVIIa that is composed of the FacVII derivative or the FacVIIa derivative of any one of claims 1 to 4 linked with a carrier via a non-peptidyl polymer selected from the group consisting of polyethylene glycol, polypropylene glycol, ethylene glycol-propylene glycol copolymers, polyoxyethylated polyols, polyvinyl alcohols, polysaccharides, dextrans, polyvinyl ethyl ethers, biodegradable polymers, lipid polymers, chitins, hyaluronic acids, and a combination thereof.

38. The complex according to claim ~~13~~37, wherein one end of the non-peptidyl polymer constituting the FacVII conjugate or FacVIIa conjugate is linked to a carrier selected from the group consisting of antibody, albumin, and transferrin.

39. The complex according to claim 37, wherein the carrier is selected from the group consisting of antibody, albumin, and transferrin.

40. The complex according to claim 37, wherein the non-peptidyl polymer has one, two or three reactive end(s).

41. The complex according to claim 37, wherein the non-peptidyl polymer has a maleimide reactive group or an aldehyde reactive group at both ends, respectively.

42. A pharmaceutical composition for preventing or treating hemophilia, comprising the FacVII derivative or the FacVIIa derivative of any one of claims 1 to 4, the FacVII conjugate or the FacVIIa conjugate of any one of claims 13 to 19, the FacVII complex or the FacVIIa complex of any one of claims 20 to 28, or the FacVIIa conjugate or the FacVIIa complex of claim 36, as an active ingredient.

43. A pharmaceutical composition for promoting blood coagulation, comprising the FacVII derivative or the FacVIIa derivative of any one of claims 1 to 4, the FacVII conjugate

or the FacVIIa conjugate of any one of claims 13 to 19, the FacVII complex or the FacVIIa complex of any one of claims 20 to 28, or the FacVIIa conjugate or the FacVIIa complex of claim 36, as an active ingredient.

44. Use of a derivative of FacVII or its active form FacVIIa of any one of claims 1 and 4 for preparation of a medicament for preventing or treating hemophilia.

45. Use of a derivative of FacVII or its active form FacVIIa of any one of claims 1 to 4 for preparation of a medicament for promoting blood coagulation.