Claims

[Claim 1]

A SPINK2 mutant peptide that comprises the amino acid sequence set forth in SEQ ID NO: 61 (Figure 69) and inhibits the protease activity of active human KLK5.

[Claim 2]

The peptide according to claim 1, wherein the peptide inhibits the protease activity of human KLK7 or human KLK14.

[Claim 3]

The peptide according to claim 1, wherein the peptide selectively inhibits human KLK5 and optionally human KLK7 or KLK14.

[Claim 4]

The peptide according to any one of claims 1 to 3, wherein Xaa16 (X_1) is Ala, Asp, Gly, Gln, Leu, Ser, or Thr.

[Claim 5]

The peptide according to any one of claims 1 to 4, wherein Xaa17 (X_2) is Arg, Glu, Asn, Gln, or Ser.

[Claim 6]

The peptide according to any one of claims 1 to 5, wherein Xaa18 (X_3) is Asp, Gln, Ile, Thr, Trp, or Tyr.

[Claim 7]

The peptide according to any one of claims 1 to 6, wherein Xaa19 (X_4) is Arg, Gly, Met, Gln, or Thr.

[Claim 8]

The peptide according to any one of claims 1 to 7, wherein Xaa20 (X_5) is Asp, Glu, Leu, Lys, Thr, or Tyr.

[Claim 9]

The peptide according to any one of claims 1 to 8, wherein Xaa21 (X_6) is Glu, Gly, His, Leu, Ser, Gln, or Tyr.

[Claim 10]

The peptide according to any one of claims 1 to 9, wherein Xaa22 (X_7) is Asp, Gly, Gln, Ser, or Tyr.

[Claim 11]

The peptide according to any one of claims 1 to 10, wherein Xaa24 (X_8) is Ala, Asp, Glu, Gly, Asn, Ser, or Thr.

[Claim 12]

The peptide according to any one of claims 1 to 11, wherein Xaa25 (X_9) is Arg or Lys.

[Claim 13]

The peptide according to any one of claims 1 to 12, wherein Xaa26 (X_{10}) is Asp, Glu, Gln, Ser, or Val.

[Claim 14]

The peptide according to any one of claims 1 to 13, wherein Xaa27 (X_{11}) is Phe or Tyr.

[Claim 15]

The peptide according to any one of claims 1 to 14, wherein Xaa28 (X_{12}) is Asp or Glu.

[Claim 16]

The peptide according to any one of claims 1 to 15, wherein the peptide comprises the amino acids at positions 1 to 63 in the amino acid sequence set forth in any one of SEQ ID NOs: 6, 8, 10, 12, 14, 16, 18, and 20 (Figures 14, 16, 18, 20, 22, 24, 26, and 28).

[Claim 17]

The peptide according to any one of claims 1 to 3, wherein Xaa16 (X_1) is Gly, Met, or Tyr.

[Claim 18]

The peptide according to any one of claims 1 to 3 and 17, wherein Xaa17 (X_2) is Glu, Gln, or Thr.

[Claim 19]

The peptide according to any one of claims 1 to 3, 17, and 18, wherein Xaa18 (X_3) is His, Met, or Tyr.

[Claim 20]

The peptide according to any one of claims 1 to 3 and 17 to 19, wherein Xaa19 (X_4) is Ala, Arg, Lys, or Gln.

[Claim 21]

The peptide according to any one of claims 1 to 3 and 17 to 20, wherein Xaa20 (X_5) is Gly, Arg, or Ser.

[Claim 22]

The peptide according to any one of claims 1 to 3 and 17 to 21, wherein Xaa21 (X_6) is Arg, Lys, Gln, or Ser.

[Claim 23]

The peptide according to any one of claims 1 to 3 and 17 to 22, wherein Xaa22 (X_7) is Gly.

[Claim 24]

The peptide according to any one of claims 1 to 3 and 17 to 23, wherein Xaa24 (X_8) is His, Thr, or Tyr.

[Claim 25]

The peptide according to any one of claims 1 to 3 and 17 to 24, wherein Xaa25 (X_9) is His or Tyr.

[Claim 26]

The peptide according to any one of claims 1 to 3 and 17 to 25, wherein Xaa26 (X_{10}) is Asp, Glu, or His.

[Claim 27]

The peptide according to any one of claims 1 to 3 and 17 to 26, wherein Xaa27 (X_{11}) is Tyr.

[Claim 28]

The peptide according to any one of claims 1 to 3 and 17 to 27, wherein Xaa28 (X_{12}) is Asp or Glu.

[Claim 29]

The peptide according to any one of claims 1 to 3 and 17 to 28, wherein the peptide comprises the amino acids at positions 1 to 63 in the amino acid sequence set forth in any one of SEQ ID NOs: 22, 24, 26, and 28 (Figures 30, 32, 34, and 36).

[Claim 30]

The peptide according to any one of claims 1 to 3, wherein Xaa16 (X_1) is Gly, Ser, or Tyr.

[Claim 31]

The peptide according to any one of claims 1 to 3 and 30, wherein Xaa17 (X_2) is Asp or Gln.

[Claim 32]

The peptide according to any one of claims 1 to 3, 30, and 31, wherein Xaa18 (X_3) is Thr or Val.

[Claim 33]

The peptide according to any one of claims 1 to 3 and 30 to 32, wherein Xaa19 (X_4) is Thr or Val.

[Claim 34]

The peptide according to any one of claims 1 to 3 and 30 to 33, wherein Xaa20 (X_5) is Glu or Thr.

[Claim 35]

The peptide according to any one of claims 1 to 3 and 30 to 34, wherein Xaa21 (X_6) is His or Thr.

[Claim 36]

The peptide according to any one of claims 1 to 3 and 30 to 35, wherein Xaa22 (X_7) is Tyr.

[Claim 37]

The peptide according to any one of claims 1 to 3 and 30 to 36, wherein Xaa24 (X_8) is Asn or Ser.

[Claim 38]

The peptide according to any one of claims 1 to 3 and 30 to 37, wherein Xaa25 (X_9) is Arg.

[Claim 39]

The peptide according to any one of claims 1 to 3 and 30 to 38, wherein Xaa26 (X_{10}) is Asp or Glu.

[Claim 40]

The peptide according to any one of claims 1 to 3 and 30 to 39, wherein Xaa27 (X_{11}) is Tyr.

[Claim 41]

The peptide according to any one of claims 1 to 3 and 30 to 40, wherein Xaa28 (X_{12}) is Asp.

[Claim 42]

The peptide according to any one of claims 1 to 3 and 30 to 41, wherein the peptide comprises the amino acids at positions 1 to 63 in the amino acid sequence set forth in any one of SEQ ID NOs: 30 and 32 (Figures 38 and 40).

[Claim 43]

The peptide according to any one of claims 1 to 42, wherein the peptide has three disulfide bonds and has a three-dimensional structure characterized by including a loop structure, an α-helix, and a β-sheet.

[Claim 44]

A polynucleotide comprising a nucleotide sequence encoding the amino acid sequence comprised in the peptide according to any one of claims 1 to 43.

[Claim 45]

A vector comprising the polynucleotide according to claim 44.

[Claim 46]

A cell that comprises the polynucleotide according to claim 44 or the vector according to claim 45 or

produces the peptide according to any one of claims 1 to 43.

[Claim 47]

A method for producing a SPINK2 mutant peptide, comprising the steps (i) and (ii) below:

(i) culturing the cell according to claim 46; and

(ii) collecting the SPINK2 mutant peptide from the culture.

[Claim 48]

A method for producing the peptide according to any one of claims 1 to 43, comprising a step of preparing the peptide by chemical synthesis or *in-vitro* translation.

[Claim 49]

A SPINK2 mutant peptide obtained by the method according to claim 47 or 48.

[Claim 50]

A conjugate comprising the peptide according to any one of claims 1 to 43 and 49 wherein the peptide is bound by one and optionally more than one moiety.

[Claim 51]

The conjugate according to claim 50, wherein the one or more optional moiety comprises a second peptide that is not the SPINK2 mutant.

[Claim 52]

The conjugate according to claim 51, wherein the second peptide is located on the amino terminal side of the SPINK2 mutant.

[Claim 53]

The conjugate according to claim 51, wherein the second peptide is located on the carboxyl terminal side of the SPINK2 mutant.

[Claim 54]

The conjugate according to any one of claims 51 to 53, wherein the second peptide is an antibody or a fragment thereof and comprises one or more Fc regions.

[Claim 55]

The conjugate according to claim 54, wherein the or each Fc region is an Fc region of human immunoglobulin, or a fragment thereof.

[Claim 56]

The conjugate according to claim 54 or 55, wherein the or each Fc region is an Fc region of IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgD, and/or IgE, or a fragment thereof.

[Claim 57]

The conjugate according to any one of claims 54 to 56, wherein the or each Fc region is an Fc region of human IgG1, or a fragment thereof.

[Claim 58]

The conjugate according to claim 57, wherein the or each Fc region of human IgG1 comprises the amino acid sequence set forth in SEQ ID NO: 87 (Figure 95).

[Claim 59]

The conjugate according to any one of claims 54 to 57, wherein the or each Fc region is a wild type or a mutant Fc region.

[Claim 60]

The conjugate according to any one of claims 51 to 59, wherein the conjugate comprises one to several aspartic acids and/or glutamic acids added to the amino terminus thereof.

[Claim 61]

The conjugate according to any one of claims 50 to 60, wherein the conjugate comprises the amino acid sequence described in (i) or (ii) below:

(i) the amino acid sequence set forth in any one of SEQ ID NOs: 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, and 96 (Figures 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, and 106); or

(ii) the amino acid sequence comprised in a conjugate that is 90% or more identical to the amino acid sequence described in (i) above and inhibits the protease activity of KLK5.

[Claim 62]

The conjugate according to any one of claims 51 to 61, wherein the SPINK2 mutant and the second peptide are linked to each other via a linker.

[Claim 63]

The conjugate according to claim 62, wherein the linker is a third peptide that is not the SPINK2 mutant or the second peptide.

[Claim 64]

A method for producing the conjugate according to any one of claims 50 to 63, comprising the steps (i) and (ii) below:

(i) culturing a cell comprising a polynucleotide comprising a nucleotide sequence encoding an amino acid sequence comprised in the conjugate or a vector in which the polynucleotide is inserted; and

(ii) collecting a SPINK2 mutant peptide conjugate or a peptide moiety comprised in the conjugate from the culture.

[Claim 65]

A method for producing the SPINK2 mutant peptide conjugate according to any one of claims 50 to 63, comprising a step of preparing the conjugate or a peptide moiety comprised in the conjugate by chemical synthesis or *in-vitro* translation.

[Claim 66]

A SPINK2 mutant peptide conjugate obtained by the method according to claim 64 or 65.

[Claim 67]

An antibody or a binding fragment thereof that binds to the peptide according to any one of claims 1 to 43 and 49.

[Claim 68]

A composition comprising the peptide according to any one of claims 1 to 43 and 49, the polynucleotide according to claim 44, the vector according to claim 45, the cell according to claim 46, the conjugate according to any one of claims 50 to 63 and 66, and/or the antibody or the binding fragment thereof according to claim 67.

[Claim 69]

A pharmaceutical composition comprising the peptide according to any one of claims 1 to 43 and 49, the polynucleotide according to claim 44, the vector according to claim 45, the cell according to claim 46, and/or the conjugate according to any one of claims 50 to 63 and 66.

[Claim 70]

The pharmaceutical composition according to claim 69, for treating or preventing a KLK5-related disease.

[Claim 71]

The pharmaceutical composition according to claim 70, wherein the KLK5-related disease is Netherton syndrome, atopic dermatitis, rosacea, UV-induced skin injury, psoriasis, asthma, spinal cord injury, cancer, or Barrett's esophagus.

[Claim 72]

The pharmaceutical composition according to any one of claims 69 to 71, for use in combination with an additional pharmaceutical product.

[Claim 73]

A composition for testing or diagnosis comprising the peptide according to any one of claims 1 to 43 and 49, the polynucleotide according to claim 44, the vector according to claim 45, the cell according to claim 46, the conjugate according to any one of claims 50 to 63 and 66, and/or the antibody or the binding fragment thereof according to claim 67.

[Claim 74]

The method according to any one of claims 47, 48, 64, and 65, comprising a step of performing affinity purification using the antibody or the binding fragment thereof according to claim 67.

[Claim 75]

A method for identifying a KLK5 inhibitory SPINK2 mutant peptide, comprising the steps (i) to (iii) below:

(i) incubating a KLK5 protease and a substrate in the presence and absence of a test SPINK2 mutant peptide;

(ii) measuring the protease activity of KLK5 in the presence and absence of the test SPINK2 mutant peptide; and

(iii) determining the peptide to be positive when the protease activity of KLK5 in the presence of the peptide is lower than the protease activity of KLK5 in the absence of the peptide.

[Claim 76]

A method for identifying a KLK5 inhibitory compound, comprising the steps (i) to (iii) below, using a peptide comprising the amino acid sequence set forth in any one of SEQ ID NOs: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, and 32 (Figures 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, and 40) or a conjugate comprising the peptide as a reference compound:

(i) incubating a KLK5 protease and a substrate in the presence and absence of a test compound;

(ii) measuring the protease activity of KLK5 in the presence and absence of the test compound; and

(iii) determining the test compound to be positive when the protease activity of KLK5 in the presence of the test compound is lower than the protease activity of KLK5 in the absence of the test compound.

[Claim 77]

A method for identifying a KLK5 inhibitory compound, comprising the steps (i) to (iii) below:

(i) measuring a protease inhibitory activity against KLK5 of a test compound;

(ii) measuring a protease inhibitory activity against KLK5 of a reference compound that is a peptide comprising the amino acid sequence set forth in any one of SEQ ID NOs: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, and 32 (Figures 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, and 40) or a conjugate comprising the peptide; and

(iii) determining the test compound to be positive when the protease inhibitory activity against KLK5 of the test compound is equivalent to or higher than the protease inhibitory activity against KLK5 of the reference compound.

[Claim 78]

A method of measuring a KLK5 protease activity, comprising the steps (i) and (ii) below, using a peptide comprising the amino acid sequence set forth in any one of SEQ ID NOs: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, and 32 (Figures 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, and 40) or a conjugate comprising the peptide as a reference compound:

(i) incubating a KLK5 protease with a substrate, and optionally another component; and

(ii) measuring an amount of the substrate and/or an amount of a product after step (i).

[Claim 79]

A KLK5 inhibitory SPINK2 mutant peptide or a SPINK2 mutant peptide conjugate, having a dissociation constant (K_D) for KLK5, as measured by surface plasmon resonance analysis by immobilizing the peptide or the conjugate and adding KLK5 thereto, of 1×10^{-9} M or less.

[Claim 80]

A conjugate comprising the amino acid sequence set forth in SEQ ID NO: 34 (Figure 42).

[Claim 81]

A conjugate comprising the amino acid sequence set forth in SEQ ID NO: 36 (Figure 44).

[Claim 82]

A conjugate comprising the amino acid sequence set forth in SEQ ID NO: 38 (Figure 46).

[Claim 83]

A conjugate comprising the amino acid sequence set forth in SEQ ID NO: 40 (Figure 48).

[Claim 84]

A conjugate comprising the amino acid sequence set forth in SEQ ID NO: 42 (Figure 50).

[Claim 85]

A conjugate comprising the amino acid sequence set forth in SEQ ID NO: 44 (Figure 52).

[Claim 86]

A conjugate comprising the amino acid sequence set forth in SEQ ID NO: 46 (Figure 54).

[Claim 87]

A conjugate comprising the amino acid sequence set forth in SEQ ID NO: 48 (Figure 56).

[Claim 88]

A conjugate comprising the amino acid sequence set forth in SEQ ID NO: 50 (Figure 58).

[Claim 89]

A conjugate comprising the amino acid sequence set forth in SEQ ID NO: 52 (Figure 60).

[Claim 90]

A conjugate comprising the amino acid sequence set forth in SEQ ID NO: 54 (Figure 62).

[Claim 91]

A conjugate comprising the amino acid sequence set forth in SEQ ID NO: 56 (Figure 64).

[Claim 92]

A conjugate comprising the amino acid sequence set forth in SEQ ID NO: 58 (Figure 66).

[Claim 93]

A conjugate comprising the amino acid sequence set forth in SEQ ID NO: 60 (Figure 68).

[Claim 94]

A conjugate comprising the amino acid sequence set forth in SEQ ID NO: 96 (Figure 106).

Claims

1. A SPINK2 mutant peptide that inhibits the protease activity of active human KLK5, wherein the peptide comprises: (i) the amino acid sequence set forth in any one of SEQ ID Nos: 6, 8, 10, 12, 14, 16, 18, and 20; or (ii) the amino acid sequence consisting of amino acids 4 to 66 of the amino acid sequence set forth in any one of SEQ ID Nos: 34, 40 and 48.

2. A conjugate comprising the peptide according to claim 1, wherein the peptide has at least one moiety attached to the peptide.

3. The conjugate according to claim 2, wherein the at least one moiety comprises a second peptide that is not the SPINK2 mutant.

4. The conjugate according to claim 3, wherein the second peptide is located on the amino terminal side of the SPINK2 mutant.

5. The conjugate according to claim 3, wherein the second peptide is located on the carboxyl terminal side of the SPINK2 mutant.

6. The conjugate according to claim 5, wherein the second peptide is an antibody or a fragment thereof and comprises one or more Fc regions.

7. The conjugate according to claim 6, wherein each Fc region is an Fc region of human immunoglobulin, or a fragment thereof.

8. The conjugate according to claim 6, wherein each Fc region is an Fc region of IgG1, IgG2, IgG3, IgG4, IgM, IgAl, IgA2, IgD, or IgE, or a fragment thereof.

9. The conjugate according to claim 6, wherein each Fc region is an Fc region of human IgG1, or a fragment thereof.

10. The conjugate according to claim 9, wherein each Fc region of human IgG1 comprises the amino acid sequence set forth in SEQ ID NO: 87.

11. The conjugate according to claim 6, wherein each Fc region is a wild type or a mutant Fc region.

12. The conjugate according to claim 2, wherein the conjugate comprises one or more aspartic acids and/or glutamic acids added to the amino terminus thereof.

13. The conjugate according to claim 3, wherein the SPINK2 mutant and the second peptide are linked to each other via a linker.

14. The conjugate according to claim 13, wherein the linker is a third peptide that is not the SPINK2 mutant or the second peptide.

15. A pharmaceutical composition comprising the peptide according to claim 1.

16. The pharmaceutical composition according to claim 15, further comprising an additional pharmaceutical product.

17. A pharmaceutical composition comprising the conjugate according to claim 2.

18. The pharmaceutical composition according to claim 17, further comprising an additional pharmaceutical product.

19. The conjugate according to claim 2, wherein the conjugate comprises:

(a) a peptide having an amino acid sequence set forth in any one of SEQ ID Nos: 34,36,38,40,42,44,46,48,50,52,54,56,58,60, and 96; or

(b) a peptide having an amino acid sequence formed by the deletion of one amino acid from the amino acid sequence according to (a);

wherein the conjugate inhibits the protease activity of KLK5.

20. A conjugate comprising a peptide having the amino acid sequence set forth in SEQ ID No: 34or the amino acid sequence formed by the deletion of one amino acid from the amino acid sequence set forth in SEQ ID No: 34.

21. A composition comprising the conjugate according to claim 20.

22. The composition according to claim 21, wherein the composition is a pharmaceutical composition.

23. A conjugate comprising a peptide having the amino acid sequence set forth in SEQ ID No: 36 or the amino acid sequence formed by the deletion of one amino acid from the amino acid sequence set forth in SEQ ID No: 36.

24. A composition comprising the conjugate according to claim 23.

25. The composition according to claim 24, wherein the composition is a pharmaceutical composition.

26. A conjugate comprising a peptide having the amino acid sequence set forth in SEQ ID No: 38or the amino acid sequence formed by the deletion of one amino acid from the amino acid sequence set forth in SEQ ID No: 38.

27. A composition comprising the conjugate according to claim 26.

28. The composition according to claim 27, wherein the composition is a pharmaceutical composition.

29. The conjugate according to claim 6, wherein each Fc region is a mutant Fc region.