

CLAIMS

1. A modified porcine pancreatic elastase (PPE) protein, comprising at least one amino acid alteration relative to a wild-type PPE protein (SEQ ID NO: 4), wherein the at least one alteration is at a residue selected from one or more of Q211, T55, D74, R75, S214, R237, and N241, the residue numbering being defined by SEQ ID NO: 1 (wild-type PPE proprotein).
2. The modified PPE protein of claim 1, wherein the at least one amino acid alteration is selected from one or more of Q211F, T55A, D74A, R75A, R75E, Q211A, S214A, R237A, N241A, and N241Y, the residue numbering being defined by SEQ ID NO: 1.
3. The modified PPE protein of claim 1 or 2, comprising, consisting, or consisting essentially of an amino acid sequence that is at least 80, 85, 90, 95, 98, or 100% identical to a sequence selected from **Table S2**, and which retains the at least one amino acid alteration.
4. The modified PPE protein of any one of claims 1-3, which is selected from:
 - a modified PPE protein that comprises, consists, or consists essentially of SEQ ID NO: 5, or an amino acid sequence that is at least 80, 85, 90, 95, 98, or 99% identical to SEQ ID NO: 5, and which retains the Q211F amino acid substitution;
 - a modified PPE protein that comprises, consists, or consists essentially of SEQ ID NO: 6, or an amino acid sequence that is at least 80, 85, 90, 95, 98, or 99% identical to SEQ ID NO: 6, and which retains the T55A amino acid substitution;
 - a modified PPE protein that comprises, consists, or consists essentially of SEQ ID NO: 7, or an amino acid sequence that is at least 80, 85, 90, 95, 98, or 99% identical to SEQ ID NO: 7, and which retains the N241A amino acid substitution;
 - a modified PPE protein that comprises, consists, or consists essentially of SEQ ID NO: 8, or an amino acid sequence that is at least 80, 85, 90, 95, 98, or 99% identical to SEQ ID NO: 8, and which retains the N241Y amino acid substitution;
 - a modified PPE protein that comprises, consists, or consists essentially of SEQ ID NO: 9, or an amino acid sequence that is at least 80, 85, 90, 95, 98, or 99% identical to SEQ ID NO: 9, and which retains the R75A amino acid substitution;
 - a modified PPE protein that comprises, consists, or consists essentially of SEQ ID NO: 10, or an amino acid sequence that is at least 80, 85, 90, 95, 98, or 99% identical to SEQ ID NO: 10, and which retains the R75E amino acid substitution;
 - modified PPE protein that comprises, consists, or consists essentially of SEQ ID NO: 11, or an amino acid sequence that is at least 80, 85, 90, 95, 98, or 99% identical to SEQ ID NO: 11, and which retains the Q211A amino acid substitution;

a modified PPE protein that comprises, consists, or consists essentially of SEQ ID NO: 12, or an amino acid sequence that is at least 80, 85, 90, 95, 98, or 99% identical to SEQ ID NO: 12, and which retains the R237A amino acid substitution;

a modified PPE protein that comprises, consists, or consists essentially of SEQ ID NO: 13, or an amino acid sequence that is at least 80, 85, 90, 95, 98, or 99% identical to SEQ ID NO: 13, and which retains the S214A amino acid substitution; and

a modified PPE protein that comprises, consists, or consists essentially of SEQ ID NO: 14, or an amino acid sequence that is at least 80, 85, 90, 95, 98, or 99% identical to SEQ ID NO: 14, and which retains the D74A amino acid substitution

5. The modified PPE protein of any one of claims 1-4, which has increased cancer cell-killing activity relative to that of the wild-type PPE protein (SEQ ID NO: 4).

6. The modified PPE protein of claim 5, which has increased cancer cell-killing activity of about or at least about 2-fold, 5-fold, 10-fold, 50-fold, 100-fold, 500-fold, or 1000-fold or more, relative to the cancer cell-killing activity of the wild-type PPE protein (SEQ ID NO: 4).

7. The modified PPE protein of claim 5 or 6, wherein the increased cancer cell-killing activity is in the absence of a human A1AT protein, in vitro or in vivo.

8. The modified PPE protein of claim 5 or 6, wherein the increased cancer cell-killing activity is in the presence of a human A1AT protein, in vitro or in vivo.

9. The modified PPE protein any one of claims 1-8, which has reduced binding to or interaction with a human alpha-1 antitrypsin (A1AT) protein relative to that of the wild-type PPE protein (SEQ ID NO: 4).

10. The modified PPE protein of claim 9, which has reduced binding to the human A1AT of about or at least about 2-fold, 5-fold, 10-fold, 50-fold, 100-fold, 500-fold, or 1000-fold or more, relative to the binding of the wild-type PPE protein to the human A1AT protein.

11. The modified PPE protein of any one of claims 1-10, which has about or at least about 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000% or more of the serine protease activity of the wild-type PPE.

12. The modified PPE protein of any one of claims 1-10, which has increased serine protease activity relative to the wild-type PPE.

13. The modified PPE protein of claim 12, wherein the serine protease activity is about or at least about 2-fold, 5-fold, 10-fold, 50-fold, 100-fold, 500-fold, or 1000-fold or more higher than the serine protease activity of wild-type PPE, as measured in the absence of a human A1AT protein.

13. The modified PPE protein of claim 11, wherein the serine protease activity is about or at least about 2-fold, 5-fold, 10-fold, 50-fold, 100-fold, 500-fold, or 1000-fold or more higher than the serine protease activity of wild-type PPE, as measured in the presence of a human A1AT protein.

14. A modified PPE proprotein, comprising in an N-terminal to C-terminal orientation, a signal peptide (optionally SEQ ID NO: 2), an activation peptide (optionally SEQ ID NO: 3), and a modified PPE protein as defined in any one of claims 1-13, wherein the modified PPE proprotein is activatable via protease cleavage of the activation peptide to generate an enzymatically-active, modified PPE protein.

15. A recombinant nucleic acid molecule encoding the modified PPE protein or proprotein of any one of claims 1-14, a vector comprising the recombinant nucleic acid molecule, or a host cell comprising the recombinant nucleic acid molecule or the vector.

16. A method of producing a modified PPE protein or proprotein, comprising culturing the host cell of claim 15 under culture conditions suitable for the expression of the modified PPE protein or proprotein, and isolating the modified PPE protein or proprotein from the culture.

17. A pharmaceutical composition, comprising the modified PPE protein or proprotein of any one of claims 1-14, or an expressible polynucleotide encoding the modified PPE protein or proprotein, and a pharmaceutically acceptable carrier.

18. A method of treating, ameliorating the symptoms of, and/or reducing the progression of, a cancer in a subject in need thereof, comprising administering the pharmaceutical composition of claim 17 to the subject.

19. The method of claim 18, wherein the cancer is a primary cancer or a metastatic cancer, and is selected from one or more of melanoma (optionally metastatic melanoma), breast cancer (optionally triple-negative breast cancer, TNBC), kidney cancer (optionally renal cell carcinoma), pancreatic cancer, bone cancer, prostate cancer, small cell lung cancer, non-small cell lung cancer (NSCLC), mesothelioma, leukemia (optionally lymphocytic leukemia, chronic myelogenous leukemia, acute myeloid leukemia, or relapsed acute myeloid leukemia), multiple

myeloma, lymphoma, hepatoma (hepatocellular carcinoma), sarcoma, B-cell malignancy, ovarian cancer, colorectal cancer, glioma, glioblastoma multiforme, meningioma, pituitary adenoma, vestibular schwannoma, primary CNS lymphoma, primitive neuroectodermal tumor (medulloblastoma), bladder cancer, uterine cancer, esophageal cancer, brain cancer, head and neck cancers, cervical cancer, testicular cancer, thyroid cancer, and stomach cancer.

20. The method of claim 18 or 19, wherein the pharmaceutical compositions comprises the modified PPE proprotein, which is activated by protease cleavage of the activation peptide in a cancer tissue or tumor site of the subject in need thereof, to generate an enzymatically-active, modified PPE protein.

21. The method of any one of claims 18-20, wherein administering the pharmaceutical composition increases cancer cell-killing in the subject by about or at least about 2-fold, 5-fold, 10-fold, 50-fold, 100-fold, 500-fold, or 1000-fold or more relative to a control or reference.

22. The method of any one of claims 18-21, wherein administering the pharmaceutical composition results in tumor regression in the subject, optionally as indicated by a statistically significant decrease in the amount of viable tumor or tumor mass, optionally at least about a 10%, 20%, 30%, 40%, 50% or more decrease in tumor mass.

23. The method of any one of claims 18-22, comprising administering the pharmaceutical composition to the subject by parenteral administration or by intra-tumoral administration.

24. The method of claim 23, wherein the parenteral administration is intravenous administration.