SUMMARY

The present disclosure provides somatostatin-albumin fusion proteins and analogues thereof and methods of producing and using the same. Constructs prepared according to the disclosure include an albumin (or an analog thereof) moiety, a somatostatin moiety (SST-14, SST-28), and a spacer, such as a spacer or linker peptide, separating the two moieties.

A fusion protein according to the disclosure is also described as a polypeptide herein. The polypeptide according to the disclosure may optionally include, in certain examples, one or more non-naturally occurring amino acids or amino acid residues.

The somatostatin-albumin fusion proteins and analogues thereof broadly include a human SST peptide moiety, a linker or spacer, and a human albumen moiety. The SST peptide moiety can include analogues and derivatives thereof,, that actively inhibit the activity of human growth hormone. Optionally, the SST peptide moiety is obtained from natural or synthetic sources. The albumin moiety is, e.g., human albumin and/or active fragments or subdomains thereof. The linker or spacer is selected to enhance the stability of the somatostatin-albumin fusion protein. More particularly, the somatostatin-albumin fusion proteins and analogues thereof have a structure as follows.

There is provideda fusion protein comprising:

an SST;

an L; and

an ALB,

wherein,

SST is a somatostatin, its analogue or derivative;

L is a spacer or a linker; and

ALB is an albumin, its analogue or variant.

Preferably, the inventive fusion protein is isolated and purified.

Optionally, the ALB component of the inventive fusion protein is mammalian serum albumin. In one example, the mammalian serum albumin is SEQ ID NO: 25, or a sequence having at least 85 % sequence identity thereto.

In other particular examples, the inventive fusion protein is selected from the group consisting of:

$SST\text{-}(L)_{x1}\text{-}ALB$ (I);

$ALB\text{-}(L)_{x1}\text{-}SST$ (II);

$[SST\text{-}(L)_{x1}]_{y1}\text{-}ALB$ (III);

$ALB\text{-}[(L)_{x1}\text{-}SST]_{y1}$ (IV);

$[SST\text{-}(L)_{x1}]_{y1}\text{-}ALB\text{-}[(L)_{x2}\text{-}SST]_{y2}$ (V);

$[SST\text{-}(L)_{x1}]_{y1}\text{-}ALB\text{-}[(L)_{x2}\text{-}SST]_{y2}\text{-}(L)_{x3}\text{-}ALB$ (VI);

$[SST\text{-}(L)_{x1}]_{y1}\text{-}ALB\text{-}[(L)_{x2}\text{-}SST]_{y2}\text{-}(L)_{x3}\text{-}ALB\text{-}[(L)_{x4}\text{-}SST]_{y3}$ (VII);

$ALB\text{-}(L)_{x1}\text{-}[SST\text{-}(L)_{x2}]_{y1}\text{-}ALB$ (VIII);

$ALB\text{-}(L)_{x1}\text{-}[SST\text{-}(L)_{x2}]_{y1}\text{-}ALB\text{-}[(L)_{x3}\text{-}SST]_{y2}\text{-}(L)_{x1}\text{-}ALB$ (IX); and

$ALB\text{-}(L)_{x1}\text{-}[SST\text{-}(L)_{x2}]_{y1}\text{-}ALB\text{-}[(L)_{x3}\text{-}SST]_{y2}\text{-}(L)_{x1}\text{-}ALB\text{-}[(L)_{x4}\text{-}SST]_{y3}$ (X);

wherein,

x1, x2, x3, x4, y1, y2, or y3 is independently zero or an integer selected from 1-10, or more particularly from 1-5, or an integer from 1-4, provided that there is at least one L present in the nucleotide sequence encoding an albumin-somatostatin fusion protein.

In alternative examples, the inventive fusion protein comprises an SST that is either naturally occurring or synthetically manufactured.

In a further example, the SST of the inventive fusion protein comprises one or more tandem repeats of a sequence encoding SST-14 or SST-28, represented by SEQ ID NOS: 17 or 18, respectively, or a sequence having at least 85% identity to either of these sequences.

The SST moiety is optionally SST-14 or SST-28.

In a further example, the fusion protein comprises an L that is either flexible or alpha helically structured polypeptide linker or spacer.

In a further example, the fusion protein comprises an L that is a polypeptide having 2-100 amino acids. The linkers or spacers according to a further example of the disclosure encompass peptides covalently linked to somatostatin on one terminal and to albumin on another terminal.

The terms "linker" or "spacer" are used interchangeably herein to refer to short amino acid sequences used to separate multiple domains in a single protein. Absence of linkers between two or more discrete domains in a protein may result in reduced or improper functionality of the protein domains *e.g.*, a reduction in catalytic activity or binding affinity for a receptor/ligand, due to the steric hindrance. Linking protein domains in the chimeric

proteins using an artificial linker can increase the space between the domains. Preferably, the linker or spacer is selected independently of the somatostatin and albumin.

The linker L is either a flexible or alpha helically structured polypeptide linker or spacer. In certain examples, L contains at least one GGGGS, $A(EAAAK)_4A$, (AP)n, wherein n is an integer selected from 10-34, (G)8, (G)5, or any combination thereof.

The albumin-somatostatin fusion constructs described herein may also include a signal peptide sequence ("SP"). Signal peptides are understood to refer to short amino acid sequences present at the N-terminus of a polypeptide that direct the cellular placement of a newly-synthesized protein. For example, signal peptides may lead to a protein being localized to a given intracellular region (*e.g.*, the nucleus), inserted into a membrane (*e.g.*, the cell membrane or the endoplasmic reticulum) or secreted from the cell. Besides directing localization, signal peptides may also be incorporated into recombinant proteins in order to improve stability, modify expression levels, and to aid in the proper folding of the recombinant proteins. The signal peptide sequence of the precursor protein is usually removed by signal peptidase in the host cell to produce a mature protein.

The albumin-somatostatin fusion constructs described herein may also include an affinity or purification tag as part of the polypeptide sequence to facilitate purification. Such tags are used as part of affinity chromatographic methods, *e.g.*, high performance liquid chromatography (HPLC) in order to purify a protein sample from a crude biological source. Suitable purification tags include, but are not limited to: poly-histidine (*e.g.*, His-6 or H6), glutathione-S-transferase (GST), maltose-binding protein (MBP), chitin binding protein (CBP), FLAG-tag (FLAG octapeptide). When it is necessary to remove the affinity tag from the fusion protein, specific enzymatic cleavage site can be introduced in the linker region. Enzymes commonly used for removal of affinity tags include, but are not limited to: factor Xa, entrokinase, thrombin, TEV protease, and rhinovirus 3C protease.

In a further example, there is provided a nucleotide sequence encoding a polypeptide comprising:

an SST;

an L; and

an ALB,

wherein,

SST is a somatostatin or its analogues or derivatives;

L is a spacer or a linker; and

ALB is an albumin or its analogues or variants.

In particular examples the inventive nucleotide encodes a polypeptide that is selected from the group consisting of,

$SST\text{-}(L)_{x1}\text{-}ALB$ (I);

$ALB\text{-}(L)_{x1}\text{-}SST$ (II);

$[SST\text{-}(L)_{x1}]_{y1}\text{-}ALB$ (III);

$ALB\text{-}[(L)_{x1}\text{-}SST]_{y1}$ (IV);

$[SST\text{-}(L)_{x1}]_{y1}\text{-}ALB\text{-}[(L)_{x2}\text{-}SST]_{y2}$ (V);

$[SST\text{-}(L)_{x1}]_{y1}\text{-}ALB\text{-}[(L)_{x2}\text{-}SST]_{y2}\text{-}(L)_{x3}\text{-}ALB$ (VI);

$[SST\text{-}(L)_{x1}]_{y1}\text{-}ALB\text{-}[(L)_{x2}\text{-}SST]_{y2}\text{-}(L)_{x3}\text{-}ALB\text{-}[(L)_{x4}\text{-}SST]_{y3}$ (VII);

$ALB\text{-}(L)_{x1}\text{-}[SST\text{-}(L)_{x2}]_{y1}\text{-}ALB$ (VIII);

$ALB\text{-}(L)_{x1}\text{-}[SST\text{-}(L)_{x2}]_{y1}\text{-}ALB\text{-}[(L)_{x3}\text{-}SST]_{y2}\text{-}(L)_{x1}\text{-}ALB$ (IX); and

$ALB\text{-}(L)_{x1}\text{-}[SST\text{-}(L)_{x2}]_{y1}\text{-}ALB\text{-}[(L)_{x3}\text{-}SST]_{y2}\text{-}(L)_{x1}\text{-}ALB\text{-}[(L)_{x4}\text{-}SST]_{y3}$ (X);

wherein,

each of x1, x2, x3, x4, y1, y2, or y3 is independently zero or an integer selected from 1-10, or more particularly from 1-5 or from 1-4, provided that there is at least one L present in the polypeptide.

In a further example, the nucleotide sequence encodes a fusion protein wherein the SST comprises one or more tandem repeats of a sequence encoding SST-14 or SST-28, represented by SEQ ID NOS: 17 or 18, respectively, or a sequence having at least 85% identity to either of these sequences.

The disclosure is further contemplated to include an expression vector, e.g., a plasmid construct, a host cell comprising the expression vector, that is capable of expressing the inventive albumin-somatostatin fusion protein. The host cell can be a suitable bacterial host cell, a suitable mammalian host cell, a suitable plant host cell, or a suitable insect host cell.

In one aspect, the invention refers to use of the fusion protein as described herein in the manufacture of a medicament for treating a disease or disorder of endocrine release, wherein an effective amount of a pharmaceutical composition comprising said fusion protein is to be administered in a human subject, and wherein the disease or disorder of endocrine release is a condition that responds to the administration of somatostatin.

In a second aspect, the invention refers to the fusion protein as described herein for use in therapy.

There are also provided methods of treating a disease or disorder of endocrine release in a mammal, such as in a human subject, by administering an effective amount of a pharmaceutical composition comprising the inventive fusion protein, wherein the disease or disorder of endocrine release is a condition that responds to the administration of somatostatin.

For example, the disease or disorder is a cancer selected from the group consisting of breast cancer, colorectal cancer, liver cancer, endocrine cancer, neuroendocrine cancers, pancreatic cancer, prostate cancer, brain cancer, and lung cancer. In certain examples, the cancer expresses somatostatin receptor type 1, 2, 3, 4 or 5.

It should also be understood that singular forms such as "a," "an," and "the" are used throughout this application for convenience, however, except where context or an explicit statement indicates otherwise, the singular forms are intended to include the plural. Further, it should be understood that every journal article, patent, patent application, publication, and the like that is mentioned herein is hereby incorporated by reference in its entirety and for all purposes.

All numerical ranges should be understood to include each and every numerical point within the numerical range, and should be interpreted as reciting each and every numerical point individually. The endpoints of all ranges directed to the same component or property are inclusive, and intended to be independently combinable.

As used herein, the term "about" means within 10% of the reported numerical value, preferably within 5% of the reported numerical value.

The phrase "consisting essentially of" means that the composition or method may include additional ingredients and/or steps, but only if the additional ingredients and/or steps do not materially alter the basic and novel characteristics of the claimed composition or

method.

The SST and albumin fusion proteins of present application provide advantages over natural SST of (a) higher *in vivo* stability, (b) higher binding affinity to SST receptors, (c) higher protein expression yield, and (d) better pharmacokinetic/pharmacodynamics behavior.

Before the present disclosure is described in detail below, it is to be understood that this disclosure is not limited to the particular methodology, protocols and reagents described herein, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular examples only, and is not intended to limit the scope of the present disclosure, which will be limited only by the appended claims. Unless defined

CLAIMS

1. A fusion protein comprising:

an SST;

an L; and

an ALB,

wherein,

SST is a somatostatin, its analogue or derivative;

L is a spacer or a linker; and

ALB is an albumin, its analogue or variant.

2. The fusion protein of claim 1, selected from the group consisting of:

$SST\text{-}(L)_{x1}\text{-}ALB$ (I);

$ALB\text{-}(L)_{x1}\text{-}SST$ (II);

$[SST\text{-}(L)_{x1}]_{y1}\text{-}ALB$ (III);

$ALB\text{-}[(L)_{x1}\text{-}SST]_{y1}$ (IV);

$[SST\text{-}(L)_{x1}]_{y1}\text{-}ALB\text{-}[(L)_{x2}\text{-}SST]_{y2}$ (V);

$[SST\text{-}(L)_{x1}]_{y1}\text{-}ALB\text{-}[(L)_{x2}\text{-}SST]_{y2}\text{-}(L)_{x3}\text{-}ALB$ (VI);

$[SST\text{-}(L)_{x1}]_{y1}\text{-}ALB\text{-}[(L)_{x2}\text{-}SST]_{y2}\text{-}(L)_{x3}\text{-}ALB\text{-}[(L)_{x4}\text{-}SST]_{y3}$ (VII);

$ALB\text{-}(L)_{x1}\text{-}[SST\text{-}(L)_{x2}]_{y1}\text{-}ALB$ (VIII);

$ALB\text{-}(L)_{x1}\text{-}[SST\text{-}(L)_{x2}]_{y1}\text{-}ALB\text{-}[(L)_{x3}\text{-}SST]_{y2}\text{-}(L)_{x1}\text{-}ALB$ (IX); and

$ALB\text{-}(L)_{x1}\text{-}[SST\text{-}(L)_{x2}]_{y1}\text{-}ALB\text{-}[(L)_{x3}\text{-}SST]_{y2}\text{-}(L)_{x1}\text{-}ALB\text{-}[(L)_{x4}\text{-}SST]_{y3}$ (X);

wherein,

x1, x2, x3, x4, y1, y2, or y3 is independently zero or an integer selected from 1-10, provided that there is at least one L present in the nucleotide sequence encoding an albumin-somatostatin fusion protein.

3. The fusion protein of claim 1, wherein the SST is either naturally occurring or synthetically manufactured.

4. The fusion protein of claim 1 wherein the SST comprises one or more tandem repeats of a sequence encoding SST-14 or SST-28, represented by SEQ ID NOS: 17 or 18,

respectively, or a sequence having at least 85% identity to either of these sequences.

5. The fusion protein of claim 1, wherein the SST is SST-14 or SST-28.

6. The fusion protein of claim 1, wherein L is either flexible or alpha helically structured polypeptide linker or spacer.

7. The fusion protein of claim 1, wherein L is a polypeptide having 2-100 amino acids.

8. The fusion protein of claim 6, wherein the polypeptide contains at least one GGGGS, $A(EAAAK)_4A$, $(AP)_n$, wherein n is an integer selected from 10-34, $(G)_8$, $(G)_5$, or any combination thereof.

9. The fusion protein of claim 1, wherein ALB is mammalian serum albumin.

10. The fusion protein of claim 1, wherein the mammalian serum albumin is SEQ ID NO: 25, or a sequence having at least 85 % sequence identity thereto.

11. The fusion protein of claim 2, wherein x1, x2, x3, x4 are each independently an integer selected from 1-5.

12. The fusion protein of claim 2, wherein y1, y2, y3 are each independently an integer selected from 1-5.

13. A nucleotide sequence encoding a polypeptide comprising:

an SST;

an L; and

an ALB,

wherein,

SST is a somatostatin or its analogues or derivatives;

L is a spacer or a linker; and

ALB is an albumin or its analogues or variants.

14. The nucleotide sequence of claim 13, encoding a polypeptide that is selected from the group consisting of,

$SST-(L)_{x1}-ALB$ (I);

$ALB-(L)_{x1}-SST$ (II);

$[SST-(L)_{x1}]_{y1}-ALB$ (III);

$ALB-[(L)_{x1}-SST]_{y1}$ (IV);

$[SST-(L)_{x1}]_{y1}-ALB-[(L)_{x2}-SST]_{y2}$ (V);

$[SST-(L)_{x1}]_{y1}-ALB-[(L)_{x2}-SST]_{y2}-(L)_{x3}-ALB$ (VI);

$[SST-(L)_{x1}]_{y1}-ALB-[(L)_{x2}-SST]_{y2}-(L)_{x3}-ALB-[(L)_{x4}-SST]_{y3}$ (VII);

$ALB-(L)_{x1}-[SST-(L)_{x2}]_{y1}-ALB$ (VIII);

$ALB-(L)_{x1}-[SST-(L)_{x2}]_{y1}-ALB-[(L)_{x3}-SST]_{y2}-(L)_{x1}-ALB$ (IX); and

$ALB-(L)_{x1}-[SST-(L)_{x2}]_{y1}-ALB-[(L)_{x3}-SST]_{y2}-(L)_{x1}-ALB-[(L)_{x4}-SST]_{y3}$ (X);

wherein,

each of x1, x2, x3, x4, y1, y2, or y3 is independently zero or an integer selected from 1-10,

provided that there is at least one L present in the polypeptide.

15. The nucleotide sequence of claim 13, encoding the polypeptide sequence, wherein the SST comprises one or more tandem repeats of a sequence encoding SST-14 or SST-28, represented by SEQ ID NOS: 17 or 18, respectively, or a sequence having at least 85% identity to either SEQ ID NO: 17 or SEQ ID NO: 18.

16. A plasmid construct expressing an albumin-somatostatin fusion protein comprising the fusion protein of claim 1.

17. A bacterial host cell transformed with the plasmid construct of claim 16.

18. The fusion protein of claim 1 that is isolated and purified.

19. Use of the fusion protein of claim 1 in the manufacture of a medicament for treating a disease or disorder of endocrine release, wherein an effective amount of a pharmaceutical composition comprising said fusion protein is to be administered in a human subject, and wherein the disease or disorder of endocrine release is a condition that responds to the administration of somatostatin.

20. The use of claim 19, wherein the condition is a cancer selected from the group consisting of breast cancer, colorectal cancer, liver cancer, endocrine cancer, neuroendocrine cancers, pancreatic cancer, prostate cancer, brain cancer and lung cancer.

21. The use of claim 20, wherein the cancer expresses somatostatin receptor type 1, 2, 3, 4 or 5.

22. The fusion protein of claim 1 for use in therapy.