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(71) Applicant: **NAL PHARMACEUTICAL GROUP LIMITED** [CN/CN]; Room 2109 Windsor House, 311 Gloucester Road, Causeway Bay, Hong Kong SAR, 999077 (CN).

(72) Inventor; and
(71) Applicant : **MO, Y. Joseph** [US/US]; 1 Belleview Terrace, Princeton, NJ 08540 (US).

(72) Inventor: **CHU, Chun, Kwong**; Room 1531, Herring Gull House, Shakok Estate, Shatin, NT, Hong Kong (CN).

(74) Agent: **CHOE, Yun, H.**; Lucas & Mercanti, LLP, 30 Broad Street, 21st floor, New York, NY 10004 (US).

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(54) **Title**: COMPOSITIONS CONTAINING FUSION PROTEIN OF ALBUMIN AND ANALOGS THEREOF, METHODS FOR MAKING AND USING THE SAME

(57) **Abstract**: The invention is related to fusion proteins of human somatostatin (e.g., SST-14 or SST-28) and human serum albumin, comprising a region at least 85% homologous to human somatostatin and a region at least 85% homologous to human serum albumin or a region with a partial amino acid sequence of human serum albumin, wherein linker peptide sequences may be present between somatostatin and somatostatin moieties or somatostatin and albumin moieties. Also disclosed are constructs wherein the somatostatin moiety contains multiple tandem repeats of a somatostatin sequence. In selected embodiments, the orientation of the somatostatin and albumin moieties can be varied, and such sequences may impact the binding and efficacy of the disclosed fusion proteins. Also disclosed are methods of making and using the aforementioned constructs.

COMPOSITIONS CONTAINING FUSION PROTEIN OF ALBUMIN AND ANALOGS THEREOF, METHODS FOR MAKING AND USING THE SAME

FIELD OF INVENTION

The present invention relates to a fusion protein comprising a somatostatin, or its analogue or derivatives, a linker or spacer and an albumin, or its analogue or variant.

The present invention also relates to recombinant fusion proteins containing a human serum albumin moiety, and a somatostatin moiety, separated by a spacer sequence and analogues thereof.

BACKGROUND OF THE INVENTION

Somatostatin ("SST") is a secretory product of a variety of endocrine and non-endocrine tissues and is widely distributed throughout the body. Somatostatin inhibits pituitary, pancreatic, and gastrointestinal hormone secretion release, as well as cytokine production, intestinal motility and absorption, vascular contractility, and cell proliferation. Recent studies have found that SST has use as a treatment for cancer, inhibiting tumor growth, inhibiting the proliferation of endocrine tumors, and many other solid tumors, such as breast cancer, colorectal cancer, liver cancer and lung cancer. The somatostatin molecule has two biologically active forms: somatostatin-14 (SST-14), the cyclic tetradecapeptide, and somatostatin-28 (SST-28), an N-terminally elongated form of SST-14. SST-14 is a cyclic peptide with a length of 14 residues, containing a disulfide linkage between cysteines at positions 3 and 14. SST-28 is an N-terminal extension form (28 residues) of the same precursor that is proteolytically cleaved to generate SST-14. Although the two have similar activity, their respective potency and histological characteristics vary. For example, SST-14 displays more pronounced inhibition of glucagon and gastrin, while SST-28 displays more pronounced inhibition of growth hormone and insulin action. Both forms of somatostatin exert their respective biological functions through receptors on target cells and intracellular pathways. Five subtypes of somatostatin receptors (SSTR 1–5) have been recognized, with two spliced variants for SSTR2: SSTR2A and SSTR2B, with a different carboxyl terminus.

The beneficial effects of somatostatin in the treatment of certain hypersecretory endocrine disorders, and its anti-proliferation effect on tumors are well recognized. However, the half-life of somatostatin *in vivo* is only 2–3 min due to enzymatic degradation and

endocytosis, limiting clinical utility of somatostatin. In the past decade, numerous stable somatostatin analogs have been developed. For example, octreotide and lanreotide, are used in treatment of growth hormone (GH)-secreting adenomas and carcinoids. However, therapeutic limitations still exist due to altered binding affinity to SSTRs. As a result, there remains a need in the art for somatostatin constructs that achieve high *in vivo* half-life while maintaining a desirable binding affinity to SSTRs.

Albumin, the most abundant protein in the blood plasma, is produced in the liver as a monomeric protein of 67 kDa and responsible for 80% of the colloid osmotic pressure of plasma. Human granulocyte colony stimulating factor (G- CSF), human growth hormone (GH), human insulin, human interferon-a-2b (INF-2b), and interleukin-28B (IL-28B) fused with HSA were used effectively to construct long-acting therapeutic drug candidates. However, the comparative studies between HSA fusion proteins and the parent molecules in the biological and molecular mechanisms are less reported.

Chinese patent applications CN102391376A and CN102675467A, both hereby incorporated by reference, disclose somatostatin-albumin fusion proteins. However there remains a need for further development of somatostatin-albumin fusion proteins.

SUMMARY OF THE INVENTION

The present invention provides somatostatin-albumin fusion proteins and analogues thereof and methods of producing and using the same. Constructs prepared according to the invention 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.

In one embodiment, the present invention provides a nucleotide sequence encoding an albumin-somatostatin fusion protein, or polypeptide sequence, comprising:

(a) a first region comprising a nucleotide sequence encoding a human somatostatin peptide (SST);

(b) a second region comprising a nucleotide sequence encoding human serum albumin, or a fragment (ALB) thereof;

(c) a spacer region (L) comprising a nucleotide sequence encoding a polypeptide of 2-100 residues in length;

wherein the spacer region (c) is present between the region (a) and region (b), or

between region (a) and region (a).

In particular embodiments, the nucleotide sequence is optionally selected to encode an albumin-somatostatin fusion protein consisting of one of formulas I-X, as follows.

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

A fusion protein according to the invention is also described as a polypeptide herein. The polypeptide according to the invention may optionally include, in certain embodiments, 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.

In one embodiment, the present invention provides a fusion protein comprising:

(a) a first region comprising of a human somatostatin peptide (SST);

(b) a second region comprising of a human serum albumin, or a fragment (ALB) thereof;

(c) a spacer region (L) comprising of a polypeptide of 2-100 residues in length;

wherein the spacer region (c) is present between the region (a) and region (b), or between region (a) and region (a).

In a further embodiment, the invention includes a fusion protein that comprises a structure selected from formulas I-X, as follows:

$SST-(L)_{x1}-ALB$ (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 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 fusion protein.

In a further embodiment of the invention, x1, x2, x3, x4 are each independently an integer selected from 1-5, or an integer from 1-4. In a further still embodiment of the invention, y1, y2, y3 are each independently an integer selected from 1-5, or from an integer from 1-4.

In a more particular embodiment of the invention, the SST moiety 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 certain embodiments, the somatostatin-albumin fusion proteins of the invention include variants of albumin, including mammalian serum albumin, such as human serum albumin, e.g., having SEQ ID NO: 25, or a sequence having at least 85 % sequence identity thereto.

The linkers or spacers of according to a further embodiment of the invention encompass peptides covalently linked to somatostatin on one terminal and to albumin on another terminal. The spacers in another embodiment of the invention include peptide sequences having from 2-100 amino acid residues.

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 embodiments, 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.

The invention also provides for 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 and lung cancer. In certain embodiments, 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 invention is described in detail below, it is to be understood that this invention 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 embodiments only, and is not intended to limit the scope of the present invention, which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art.

DETAILED DESCRIPTION OF THE INVENTION

The present invention encompasses somatostatin-albumin fusion proteins and analogues thereof and methods of producing and using the same. Constructs prepared according to the invention include an albumin (or an analog thereof) moiety, a somatostatin moiety (e.g., SST-14, SST-28), and a spacer separating the two moieties.

The somatostatin-albumin fusion proteins of the certain embodiment of the invention include variants of albumin including human serum albumin and / or derivatives of somatostatin. The spacers of another embodiment of the invention encompasses peptides covalently linked to somatostatin on one terminal and albumin on another terminal. The spacers in other embodiments of the invention include peptide sequences having 2-100 amino acids.

In one embodiment, the present invention provides a fusion protein 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;

the ALB is an albumin or its analogues or variants.

In certain embodiments, the fusion protein of the present invention is selected from among formulas I-X, as follows.

$SST\text{-}(L)_{x1}\text{-}ALB$ (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 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 fusion protein.

In yet another embodiment, the present invention provides a nucleotide sequence encoding an albumin-somatostatin fusion protein 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;

ALB is an albumin or its analogues or variants.

In certain embodiments, the nucleotide sequence of the present invention is selected to encode an albumin-somatostatin fusion protein from among,

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

Another embodiment of the present invention provides a nucleotide sequence encoding an albumin-somatostatin fusion protein, wherein the spacer sequence consists of the sequence encoding the amino acid sequence represented by SEQ ID NO: 31 or –GGGGS-.

Another certain embodiment of the present invention provides a nucleotide sequence encoding an albumin-somatostatin fusion protein, wherein the second region (b) encodes a polypeptide having at least 85% sequence identity to SEQ ID NO: 19, albumin or a fragment thereof.

One embodiment of the present invention provides a nucleotide sequence encoding an albumin-somatostatin fusion protein, wherein the first region (a) encodes a polypeptide having at least 85% sequence identity to either SEQ ID NOS: 17 or 18, SST-14, SST-28, or a fragment thereof.

The present invention also provides a nucleotide sequence encoding an albumin-somatostatin fusion protein comprising:

(a) a first region comprising a nucleotide sequence containing one or more adjacent repeats of a sequence encoding a human somatostatin peptide;

(b) a second region comprising a nucleotide sequence encoding human serum albumin, or a fragment thereof;

(c) a spacer region comprising a nucleotide sequence encoding a polypeptide of 2-100 residues in length;

wherein the spacer region is present between the first region and the second region, or or between the first region and another first region;

wherein one or more adjacent repeats of a sequence encoding a human somatostatin peptide encodes either SST-14 or SST-28, as represented by SEQ ID NOS:17 and 18, respectively, or a sequence having at least 85% identity to either of these two sequences; or

wherein the spacer sequence consists of the sequence encoding the amino acid sequence represented by SEQ ID NO: 31 or GGGGS or by SEQ ID NO: 30 $A(EAAAK)_4A$; or

wherein the region (a) consists of one or more adjacent repeats of either SST-14 or of SST-28, as represented by SEQ ID NOS: 23 and 24, respectively, or a sequence having at least 85% identity to either of these two sequences.

Furthermore, the present invention provides a polypeptide sequence an albumin-somatostatin fusion protein comprising:

(a) a first region comprising a polypeptide sequence of a somatostatin peptide (which may be a human somatostatin peptide);

(b) a second region comprising a polypeptide sequence of serum albumin (which may be a human serum albumin), or a fragment thereof;

(c) a spacer region comprising a polypeptide of 2-100 residues in length.

The spacer region (c) may be present between region (a) and region (b) or between region (a) and region (a). In addition, the region (a) may comprise one or more tandem repeats of a sequence encoding SST-14 or SST-28, represented by SEQ ID NOS: 17 or 18, respectively, or sequence having 85% identity to either of these sequences.

Another embodiment of the present invention provides a plasmid construct expressing an albumin-somatostatin fusion protein with any of the fusion protein or polypeptide sequences described above.

Yet another embodiment of the present invention includes a bacterial cell transformed with the plasmid construct described above.

A further embodiment of the present invention includes an isolated and purified albumin-somatostatin fusion protein having the polypeptide sequence described above (e.g., a polypeptide sequence of an albumin-somatostatin fusion protein or the plasmid construct expressing such protein).

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Table 1. A non-exclusive list of polypeptide sequences

SEQ ID NO:	Description
SEQ ID NO: 1	SST14-A(EAAAK)$_4$A-HSA-A(EAAAK)$_4$A-SST14
SEQ ID NO: 2	HSA-A(EAAAK)$_4$A-SST14
SEQ ID NO: 3	His6-GGS-HSA-GGGGS-SST14-HSA
SEQ ID NO: 4	His6-GGS-HSA-GGGGS-(SST14-GGGGS)$_2$-HSA
SEQ ID NO: 5	HSA-GGGGS-(SST14-GGGGS)$_2$- HSA
SEQ ID NO: 6	Linker GGGGGGGG
SEQ ID NO: 7	SST14-(GGGGS)$_3$-HSA
SEQ ID NO: 8	SST14-A(EAAAK)$_4$A-HSA
SEQ ID NO: 9	His6-GGS-HSA-GGGGS-SST14
SEQ ID NO: 10	SST14-GGGGS-HSA-GGS-His6
SEQ ID NO: 11	HSA-GGGGS-SST14
SEQ ID NO: 12	SST14-GGGGS-HSA
SEQ ID NO: 13	(SST14-GGGGS)$_2$- HSA
SEQ ID NO: 14	(SST14-GGGGS)$_4$- HSA
SEQ ID NO: 15	HSA-(GGGGS)$_3$-SST14
SEQ ID NO: 16	HSA-(GGGGS)$_6$-SST14
SEQ ID NO: 17	Somatostatin-14 (SST-14)

SEQ ID NO: 18	Somatostatin-28 (SST-28)
SEQ ID NO: 19	Human Serum Albumin (HSA)
SEQ ID NO: 20	MDMRVPAQLLGLLLLWLRGARC (Signal Peptide)
SEQ ID NO: 21	Linker APAPAPAPAPAPAPAPAPAP
SEQ ID NO: 22	Linker APAPAPAPAPAPAPAPAPAPAPAPAPAPAPAPAPAPAP
SEQ ID NO: 30	$A(EAAAK)_4A$ peptide
SEQ ID NO: 31	GGGGS peptide
SEQ ID NO: 32	Linker GGGGSLVPRGSGGGGS
SEQ ID NO: 33	Linker GSGSGS
SEQ ID NO: 34	Linker GGGGSLVPRGSGGGG
SEQ ID NO: 35	Linker GGGGSLVPRGSGGGGS
SEQ ID NO: 36	Linker GGSGGHMGSGG
SEQ ID NO: 37	Linker GGSGGSGGSGG
SEQ ID NO: 38	Linker GGSGGHMGSGG
SEQ ID NO: 39	Linker GGSGG
SEQ ID NO: 40	Linker GGGGSLVPRGSGGGGS
SEQ ID NO: 41	Linker GGSGGGGG
SEQ ID NO: 42	Linker GSGSGSGS
SEQ ID NO: 43	Linker GGGSEGGGSEGGGSEGGG
SEQ ID NO: 44	Linker AAGAATAA
SEQ ID NO: 45	Linker GGGGG
SEQ ID NO: 46	Linker GGSSG
SEQ ID NO: 47	Linker GSGGGTGGGSG
SEQ ID NO: 48	Linker GSGSGSGSGGSGGSGGSGGSGGSGGS

For the fusion proteins, e.g., SEQ ID NOs: 1-5, 7-10 and 13-16, it should be noted that these are encoded as pro-proteins with a 22 residue signal peptide (SEQ ID NO: 20).

Somatostatin-Albumin Fusion Proteins

The invention encompasses polypeptide constructs wherein the somatostatin moiety is encoded by a nucleotide having at least 85% sequence identity to the nucleotide sequence of endogenous human SST-14 or SST-28 (SEQ ID Nos: 23 and 24, respectively).

The invention also encompasses polypeptide constructs wherein the human serum albumin moiety is encoded by a nucleotide having at least 85% sequence identity to the nucleotide sequence of endogenous human serum albumin (SEQ ID NO: 25). The invention further encompasses polypeptide constructs wherein the human serum albumin moiety is a fragment of the endogenous human serum albumin protein, *e.g.*, where it is encoded by a nucleotide consisting of a subsequence of SEQ ID NO: 25. For example, the human serum albumin fragment optionally includes one or more of the three human serum albumin globular domains, each of which contains two subdomains, denominated subdomain IA, IB, IIA, IIB, IIIA, and IIIB (Dockal, 1999, *The Journal Of Biological Chemistry*, 274(41): 29303–29310).

The invention also encompasses polypeptide constructs wherein the somatostatin moiety has a polypeptide sequence at least 85% sequence identity, preferably at least 90% to the polypeptide sequence of endogenous SST-14 or SST-28 (SEQ ID NOs:17 and 18, respectively).

The invention also encompasses polypeptide constructs wherein the human serum albumin moiety has a polypeptide sequence at least 85% sequence identity to the polypeptide sequence of mature human serum albumin (SEQ ID NO: 19).

The invention also encompasses a fusion protein comprising a signal peptide, a purification tag (His-6), a first linker, a human serum albumin moiety, a second linker and a somatostatin moiety. In one embodiment, the fusion protein is a polypeptide is represented by SEQ ID NO: 9 or a sequence having 85% sequence identity to the same.

The invention also encompasses a fusion protein comprising a somatostatin moiety, a first linker, a human serum albumin moiety, a second linker, a somatostatin moiety and a purification tag (His-6). In one embodiment, the fusion protein is a polypeptide is represented by SEQ ID NO: 10 or a sequence having 85% sequence identity to the same.

The invention also encompasses a nucleotide sequence (SEQ ID NO: 11) encoding a fusion protein comprising an N-terminal human serum albumin moiety and a C-terminal somatostatin moiety separated by a peptide spacer. The invention further encompasses nucleotide sequences encoding an albumin-somatostatin fusion construct which have 85% sequence identity to SEQ ID NO: 11.

The invention also encompasses a nucleotide sequence (SEQ ID NO: 12) encoding a fusion protein comprising an N-terminal somatostatin moiety and a C-terminal human serum albumin moiety separated by a peptide spacer. The invention further encompasses nucleotide sequences encoding an albumin-somatostatin fusion construct which have 85% sequence identity to SEQ ID NO: 12.

The invention also encompasses polypeptide constructs wherein the somatostatin moiety comprises two or more copies of the SST-14 or SST-28 sequence arranged in tandem, *i.e.*, "$(SST\text{-}14)_2$" or "$(SST\text{-}14)_3$"or "$(SST\text{-}28)_2$" or "$(SST\text{-}28)_3$", respectively. Optionally, a linker sequence is included between the two or more tandem somatostatin moieties, and/or a signal peptide sequence is included at the N-terminus of the fusion protein.

The invention also encompasses polypeptide constructs wherein the somatostatin moiety comprises two or more copies of the SST-14 sequence arranged in a way that at least one copy of the SST14 is linked on both sides of albumin, respectively. Optionally, a linker sequence is included between the two or more tandem somatostatin moieties and between somatostatin and albumin, and/or a signal peptide sequence is included at the N-terminus of the fusion protein. For example, the polypeptide construct may include a signal peptide, two SST-14 moieties separated by a spacer, a second spacer, and an HSA moiety as represented. Optionally, the construct omits the N-terminal signal peptide.

The invention also encompasses polypeptide constructs wherein the somatostatin moiety comprises two or three copies of the SST-28 sequence arranged in tandem, *i.e.*, "$(SST\text{-}28)_2$" or "$(SST\text{-}28)_3$", respectively. Optionally, a linker sequence is included between the two or more tandem somatostatin moieties.

The invention also encompasses polypeptide constructs comprising any of the albumin-somatostatin fusion proteins described in the preceding paragraphs, where the albumin-somatostatin fusion protein has an *in vivo* half-life longer than the endogenous SST-14 or SST-28 peptides.

The invention also encompasses polypeptide constructs comprising any of the albumin-somatostatin fusion proteins described in the preceding paragraphs, wherein the albumin-somatostatin fusion protein has an approximately equal or a greater binding affinity for a somatostatin receptor compared to endogenous SST-14 or SST-28.

The invention also encompasses albumin-somatostatin fusion proteins comprising an N-terminal albumin moiety as represented by SEQ ID NO: 15, SEQ ID NO: 16, and SEQ ID NO: 2, an internal SST moiety and a C-terminal Albumin moiety as represented by SEQ ID NO: 7 and SEQ ID NO: 8. Optionally, the N-terminus may further include a signal peptide. Optionally, one or more of the albumin and SST domains may each be separated by an independently selected linker sequence as represented by SEQ ID NO: 1.

In some embodiments, the SST moiety may comprise a pair or plurality of tandem SST sequences, *e.g.*, $(SST\text{-}14)_2$ or $(SST\text{-}28)_3$, with or without intervening spacing sequences between the two or more tandem SST repeats. Optionally, one or more purification tag sequences may be included in the sequence between two moieties or at the N or C-terminus in order to assist with purification of the fusion protein. An alternative embodiment includes a pair of SST-14 moieties separated by a spacer, as represented by SEQ ID NO: 4. A further embodiment may omit the purification tag (e.g., His6) as shown by the polypeptide sequence represented by SEQ ID NO: 5.

Somatostatin

The somatostatin for use with the present invention may be any somatostatin, its analogue or derivative. It may be a human somatostatin, any other isolated or naturally occurring somatostatin. The SST moiety can be an analogue such as octreotide, lanreotide, pasireotide, seglitide, or vapreotide.

The invention also encompasses polypeptide constructs wherein the somatostatin moiety comprises a somatostatin analog. Preferably, such an analog is suitable for expression, as part of a fusion protein, in a recombinant host cell. It is understood that a suitable somatostatin analog sequence may be used in place of the SST-14 or SST-28 sequences included in any of the examples disclosed herein.

The invention also encompasses polypeptide constructs wherein the somatostatin moiety comprises two or more tandem repeats of a somatostatin polypeptide sequence e.g.,

SST-14 or SST-28; SEQ ID NOS: 17 and 18, respectively. Each of the repeated somatostatin polypeptide sequences may be a polypeptide sequence having at least 85% sequence identity to SST-14 or SST-28. These repeated variant sequences are independently selected, *i.e.*, in some embodiments the repeats are identical, whereas in other embodiments they are unique.

Albumin

The albumin for use with the present invention may be any albumin, its analogue or variant. The albumin may be human serum albumin, or any other isolated or naturally occurring albumin.

The invention also encompasses polypeptide constructs wherein the human serum albumin moiety comprises a polypeptide sequence variant with alternative arrangement or number of disulfide bonds due to the presence of additional or fewer cysteine residues than the natural form (SEQ ID NO: 25).

Spacer or Linker

As described earlier, a spacer or linker can be used with the present invention. The spacer or linker may be independent of the somatostatin or albumin.

The invention also encompasses polypeptide constructs wherein the peptide spacer of alternatively referred to as a linker, consists of a polypeptide sequence of from about 2 to about 100 amino acid residues in length. The invention further encompasses polypeptide constructs wherein the peptide spacer is from about 2 to about 50 amino acid residues in length, preferably from about 2 to about from 30, or more preferably from about 3 to about 20 amino acid residues in length.

The invention also encompasses polypeptide constructs wherein the peptide spacer (alternatively referred to as a linker) has the polypeptide sequence "GGGGS" (SEQ ID NO: 31). Polypeptides rich in Gly, Ser or Thr offer special advantages: (i) rotational freedom of the polypeptide backbone, so that the adjacent domains are free to move relative to on another; (ii) enhanced solubility; (iii) resistance to proteolysis. In addition, many natural linkers exhibited alpha-helical structures. The alpha-helical structure is more rigid and stable than Gly rich linker. An empirical rigid linker with the sequence of $A(EAAAK)_4A$ (SEQ ID NO: 30) can be used to separate functional domains. In addition to the role of linking protein

domains together, artificial linkers may offer other advantages to the production of fusion proteins, such as improving biological activity, increasing protein expression, and achieving desirable pharmacokinetic profiles.

Table 2. A non-exhaustive list of linker sequences that may be used in the fusion protein constructs of the present invention.
GGGGSLVPRGSGGGGS (SEQ ID NO: 32)
GSGSGS (SEQ ID NO: 33)
GGGGS**LVPRG**SGGGG (thrombin proteolytic site is underlined) (SEQ ID NO: 34)
GGGGS**LVPRG**SGGGGS (thrombin proteolytic site is underlined) (SEQ ID NO: 35)
GGSGGHMGSGG (SEQ ID NO: 36)
GGSGGSGGSGG (SEQ ID NO: 37)
GGSGGHMGSGG (SEQ ID NO: 38)
GGSGG (SEQ ID NO: 39)
GGGGS**LVPRGS**GGGGS (thrombin proteolytic site is underlined) (SEQ ID NO: 40)
GGSGGGGG (SEQ ID NO: 41)
GSGSGSGS (SEQ ID NO: 42)
GGGSEGGGSEGGGSEGGG (SEQ ID NO: 43)
AAGAATAA (SEQ ID NO: 44)
GGGGG (SEQ ID NO: 45)
GGSSG (SEQ ID NO: 46)
GSGGGTGGGSG (SEQ ID NO: 47)
GT
GSGSGSGSGGSGGSGGSGGSGGSGGS (SEQ ID NO: 48)
GGS
GGGGGGGG (SEQ ID NO: 6)

$A(EAAAK)_4A$ (SEQ ID NO: 20)
APAPAPAPAPAPAPAPAPAP (SEQ ID NO: 21)
APAPAPAPAPAPAPAPAPAPAPAPAPAPAPAPAPAP (SEQ ID NO: 22)

Preparation of Somatostatin-Albumin fusion protein

An embodiment of the invention provides a method for preparation of the somatostatin-albumin fusion protein. In embodiment, the somatostatin-albumin fusion protein of the invention is prepared by expression of a recombinant fusion protein containing the gene encoding introducing the vector into a host. For example, the fusion protein is obtained by expression in a host such as yeast. For example, *Pichia pastoris* GS115 may be used as a suitable expression host, and the vector used to construct the recombinant expression is pPIC9K. In addition, mammalian lines such as CHO or HEK293 can be used as a preferred expression host.

The invention also encompasses plasmid constructs capable of expressing an albumin somatostatin fusion protein comprising a nucleotide sequence encoding a somatostatin albumin fusion protein as described in any of the preceding paragraphs. For example, suitable plasmid constructs include, but are not limited to, the pcDNA3.1 vector represented by SEQ ID NO: 26 with a DNA sequence encoding any of the albumin-somatostatin fusion proteins disclosed herein ligated into the multiple cloning site of this vector. Other suitable protein expression vectors known in the art may be selected based upon the expression host (*e.g.*, an expression vector with a mammalian promoter system would be suitable for expression in a human cell line whereas a yeast or bacterial expression plasmid would be selected if expression in either of these organisms was desired.

The invention also encompasses a bacterial or yeast protein expression system comprising a bacterial or yeast cell transformed with a plasmid construct comprising a nucleotide sequence that encodes a somatostatin albumin fusion protein, as described in any of the preceding paragraphs. Suitable bacterial strains include, for example, *Escherichia coli*. Suitable yeast strains include, for example, *Pichia pastoris*. An exemplary plasmid construct includes pPIC9K (Invitrogen) as represented by SEQ ID NO: 27, with a nucleotide sequence

encoding any of the albumin-somatostatin fusion proteins described herein incorporated into the multiple cloning site of the vector.

The invention also encompasses isolated and purified fusion somatostatin fusion proteins having a polypeptide sequences as described in any of the preceding paragraphs.

Table 3. A list of nucleotide sequences in certain embodiments of the invention

SEQ ID NO:	Nucleotide Sequence Encodes the following:	Description
SEQ ID NO: 23	SST14	Somatostatin-14 (SST-14)
SEQ ID NO: 24	SST28	Somatostatin-28 (SST-28)
SEQ ID NO: 25	Human Serum Albumin mature form	Human Serum Albumin (HSA)
SEQ ID NO: 26	pcDNA3.1(+) Vector	pcDNA3.1(+) Vector mammalian expression vector
SEQ ID NO: 27	pPIC9K Vector	yeast expression vector
SEQ ID NO: 28	GGGGS	GGGGS Linker
SEQ ID NO: 29	$A(EAAAK)_4A$	alpha-helical linker

When the SST is a somatostatin analogue, an alternative method known in the filed can be employed to prepare the conjugate.

Utility of Somatostatin-Albumin Fusion Protein

The fusion protein of the present invention can be used to treat conditions for which somatostatin is art-known to be employed. As such, the invention also encompasses methods of treating cancer in a human subject by administering an isolated and purified albumin-somatostatin fusion protein as described in any of the preceding paragraphs, wherein the cancer is selected from breast cancer, colorectal cancer, liver cancer and lung cancer.

The invention also encompasses methods of treating cancer in a human subject by administering a composition containing the fusion protein of the present invention, such as an

isolated and purified albumin-somatostatin fusion protein as described in any of the preceding paragraphs. The composition can also include a suitable carrier.

Eleven SST14-Albumin fusion protein constructs with various linker sequences were designed. Eight of these constructs were made into a fusion gene within a plasmid and produced by HEK 293 transient expression at 100 mL scale. The proteins were collected from the culture media, purified through albumin-based affinity purification, and dialyzed to a storage buffer. These fusion proteins were evaluated for their binding affinity to SSTR2 receptor, and also for cell-based activity in inhibiting cAMP production in a SSTR2-overexpression CHO-K1 cell line. The results of these studies indicated that the length and type of linkers significantly affected the SSTR2 receptor binding affinity, the in-vitro cell-based functional activity, and the fusion protein production yield.

EXAMPLES

Selected embodiments of the invention will be described in further detail with reference to the following experimental and comparative examples. These examples are for illustrative purposes only and are not intended to limit the scope of the invention.

EXAMPLE 1: EXPRESSION IN MAMMALIAN SYSTEMS

Example 1-1. Recombinant gene synthesis

Eight constructs were prepared (see Table 4). First, the gene was synthesized and the target sequence then inserted into the pcDNA3.1 vector.

Example 1-2. Plasmid generation

Maxi-prep or Mega-prep was used to generate ~20 mg of each DNA

Example 1-3. Transfection and protein production

(A) Suspension cell method

FreeStyle™ 293-F Cells were seeded at 0.55-0.6×10^6 cells/mL in a flask. After about 24 hours, the cells were seeded in a shake flask at 1.1-1.2×10^6 cells/mL. DNA was prepared at 500 ug DNA / 80 mL in a FreeStyle medium. Polyethylenimine (PEI) was prepared at 1.8

mL PEI per 80 mL in a FreeStyle medium. DNA was mixed in the FreeStyle medium, and the effective amount of PEI was added to the DNA solution, and the mixture is vortexed incubated for about 15 minutes at room temperature to form DNA-PEI complex. An 80 mL of the incubated DNA-PEI complex is added to a cell culture. About 3 hours later, TC Yeastolate feed (BD) is added to have the final concentration of 4 gram / liter of culture. After about 7-8 days, the medium is harvested by centrifugation.

(B) Adherent cell method

About 24 hours before transfection, HEK293 cells were seeded to 50-90 % confluency in a flask, and complete medium is added. After about 24 hours, cells were washed followed by adding basal medium.

DNA and PEI solutions are prepared by adding DNA to a serum free medium. The PEI solution was added to the DNA solution and incubated for 15 minutes to form DNA-PEI complex at room temperature.

The PEI/DNA mixture was added to cells, and the mixture incubated for about 4-6 hours at 37 °C. The medium was removed and fresh medium with Glutamine and serum was added, followed by incubating at 37 °C for 4 days.

The medium was harvested after about 4 days, by centrifuging to collect the supernatant. The precipitate was replenished with fresh medium with L-Glutamine for another 3-day incubation to repeat the harvesting process.

Example 1-4: Protein Concentration, Ni-NTA Purification and Buffer Exchange

The collected medium was concentrated by TFF system (Millipore) to a certain volume depending on purification methods (either continuous chromatography or manual batch purification).

The concentrated proteins was incubated with fresh Ni-NTA resin at about 4 °C in binding buffer and washed with wash buffer using either chromatography or batch system. The protein was eluted with elute buffer and fractions were collected and concentrated to recover the purified protein. The protein can be further purified using size exclusion chromatography purification.

The buffer of the final eluate can be exchanged by dialysis to a desired buffer.

EXAMPLE 2: YIELDS OF SEVERAL SST-ALBUMIN FUSION PROTEINS

The SST-HSA fusion proteins were all expressed in soluble form with high yield. The length or the nature of the linkers can affect the protein yield and solubility of the fusion proteins. The results indicated that the production yield slightly decreased as the fusion protein constructs became longer and more complex. However, all the constructs exhibited yield for scale up production.

Table 4. SST14-HSA fusion protein expression yield

Sequence ID	Design	Total amino acids	MW (kDa)	Production Yield (g/L)
SEQ ID NO: 1	SST14-A(EAAAK)$_4$A-HSA-A(EAAAK)$_4$A-SST14	657	73.8364	0.26
SEQ ID NO: 2	HSA A(EAAAK)$_4$A-SST14	621	70.1543	0.27
SEQ ID NO: 7	SST14-(GGGGS)$_3$-HSA	614	69.112	0.33
SEQ ID NO: 8	SST14-A(EAAAK)$_4$A-HSA	621	70.1543	0.25
SEQ ID NO: 9	H6-GGGGS-HSA-GGGGS-SST14	613	69.4874	0.30
SEQ ID NO: 10	SST14-GGGGS-HSA-GGGGS-H6	613	69.4874	0.41
SEQ ID NO: 15	HSA-(GGGGS)3-SST14	614	69.112	0.28
SEQ ID NO: 16	HSA-(GGGGS)6-SST14	629	70.1119	0.29

EXAMPLE 3: BINDING AFFINITY OF SEVERAL SST-ALBUMIN FUSION PROTEINS

This assay measures binding of [^{125}I]Somatostatin to human somatostatin sst2 receptors. CHO-K$_1$ cells stably transfected with a plasmid encoding the human somatostatin sst2 receptor are used to prepare membranes in modified HEPES pH 7.4 buffer using standard techniques. A 0.1 mg aliquot of membrane is incubated with 0.03 nM

[^{125}I]Somatostatin and tested fusion proteins for 240 minutes at 25 °C. Non-specific binding is estimated in the presence of 1 μM Somatostatin. Membranes are filtered and washed 3 times and the filters are counted to determine [^{125}I]Somatostatin specifically bound.

The competitive binding study ^{125}I-Tyr-somatostatin versus the fusion proteins demonstrated the following results. The efficiency of the inhibition varies depending on the construct of the fusion proteins. The fusion protein construct (SEQ ID NO: 1) with two alpha-helical linker, A(EAAAK)$_4$A, showed 100 % inhibition of the somatostatin and its receptor interaction. The SEQ ID NO: 1 construct has two somatostatin moiety on both N and C terminal sides of human serum albumin. The smaller construct with one somatostatin on the C terminal side of human serum albumin linked by the same alpha-helical linker (SEQ ID NO: 2) showed 96 % inhibition. The same construct with the more flexible GGGGS linker showed lower inhibition of 82 – 85 % depending on the length. The length of GGGGS linkers also affected the inhibition. The construct with five amino acid GGGGS linker (SEQ ID NO: 9 and SEQ ID NO: 8) showed 57-59 % inhibition whereas the constructs with 15 amino acid (SEQ ID NO: 15) or 30 amino acid GGGGS linkers (SEQ ID NO: 16) showed over 80 %, suggesting that longer than five amino acid GGGGS would be more advantageous to SST function. A more rigid A(EAAAK)$_4$A (a-helical) linker would be more efficient in binding than flexible GGGGS linker. A multiple SST can increase the effective concentration of the ligand for SST receptor binding. The position of Histidine purification tag may not affect the binding. Changing the orientation or position of albumin in the fusion protein may further increase the efficiency of the protein binding.

Table 5. Inhibition of ^{125}I-Tyr^1-somatostatin binding on SSTR2 by the fusion proteins

Sequence ID	**construct**	**Inhibition % at 0.1 μM**	**IC_{50} (nM)**
SEQ ID NO: 1	SST14-A(EAAAK)$_4$A-Albumin-A(EAAAK)$_4$A-SST14	100	2.38
SEQ ID NO: 2	Albumin-A(EAAAK)$_4$A-SST14	96	9.41
SEQ ID NO: 7	SST14-(GGGGS)$_3$-Albumin	70	
SEQ ID NO: 8	SST14-A(EAAAK)$_4$A-Albumin	79	

SEQ ID NO: 9	His6-GGS-Albumin-GGGGS-SST14	59	
SEQ ID NO: 10	SST14-GGGGS-Albumin-His6	57	
SEQ ID NO: 15	Albumin-$(GGGGS)_3$-SST14	85	33
SEQ ID NO: 16	Albumin-$(GGGGS)_6$-SST14	82	
SEQ ID NO: 17	SST-14		0.0069

EXAMPLE 4. INHIBITION OF SEVERAL SST-ALBUMIN FUSION PROTEINS TO CAMP ACCUMULATION IN SSTR2-EXPRESSING CELLS

Human recombinant somatostatin sst2a receptors expressed in CHO-K1 cells are used. Test compound and/or vehicle is incubated with the cells (2 × 105 /ml) in incubation buffer for 20 minutes at 37 °C. Test compound-induced decrease of cAMP by 50 percent or more (50%) relative to the 10 nM Octreotide response indicates possible sst2a receptor agonist activity.

The inhibition of the accumulation of cAMP has been observed in SST receptor type 2 expressing CHO-K1 cells. The value of EC_{50} is 260 nM. The constructs that have longer linkers (SEQ ID NOS: 1, 15, and 2) have lower EC_{50} values, which coincided with the binding assay data. The alpha-helical linker appeared to be more efficient in the inhibition of cAMP production, when Albumin-$(GGGGS)_3$-SST14 and Albumin-$(GGGGS)_3$-SST14 EC_{50} values are compared.

Table 6. EC50 value for the inhibition of cAMP production

Sequence ID	**construct**	**EC_{50} values of the inhibition of cAMP production (nM)**
SEQ ID NO: 1	SST14-A$(EAAAK)_4$A-Albumin-A$(EAAAK)_4$A-SST14	5.14
SEQ ID NO: 2	Albumin-A$(EAAAK)_4$A-SST14	17.6
SEQ ID NO: 9	H6-GGGGS-Albumin-GGGGS-SST14	260
SEQ ID NO: 15	Albumin-$(GGGGS)_3$-SST14	23
	Octreotide	0.041

CLAIMS

We Claim:

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,

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 peptide having 2-100 amino acids.

8. The fusion protein of claim 6, wherein the peptide 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,

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

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. A method of treating a disease or disorder of endocrine release in a human subject by administering an effective amount of a pharmaceutical composition comprising the fusion protein of claim 1, wherein the disease or disorder of endocrine release is a condition that responds to the administration of somatostatin.

20. The method 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 and lung cancer.

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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/19950

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 38/31, A61P 3/10 (2016.01)
CPC - A61K 38/38, A61K 38/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 38/31, A61P 3/10 (2016.01)
CPC - A61K 38/38, A61K 38/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 514/7.1, 514/6.9
Keyword search; search terms below

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Scholar, Google Patents. somatostain, fusion protein, SST-14, SST-28, albumin, linker, glycine linker, polyglycine linker, serum albumin, tandem repeat, repeat, cancer, somatostain receptor, GGGGS, A(EAAAK)4A, (AP)n, endocrine disease/disease

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2013/0157933 A1 (ROSEN et al.) 20 June 2013 (20.06.2013) Table 1, Table 2, para [0040], [0044], [0072], [0212], [0284], [0287], [0302], [0304], [0382], [0382], [0385], [0549], [0714], [0715], [0716], [0775], [0816], [1481], [1574]	1-20 ---------- 21
Y	EVANS et al. Analysis of somatostatin receptor subtype mRNA expression in human breast cancer. Br. J Cancer. 31 December 1997 (31.12.1997) Volume 75 Issue 6 pp 798-803. Abstract.	21
A	DQ986150.1, GenBank Accession No: DQ986150.1, Serum albumin mRNA, complete cds, [Homo sapiens], 11 October 2006 [online]. [Retrieved on 19 May 2016]. Retrieved from Internet <URL: http://www.ncbi.nlm.nih.gov/nuccore/DQ986150.1> Entire document	10

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 May 2016 (17.05.2016)	21 JUN 2016
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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