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(71) Applicant: ZYMOCENETICS, INC. [US/US]; 1201 Eastlake
   Avenue East, Seattle, WA 98102 (US).
(72) Inventor: SHEPPARD, Paul, O.; 20717 N.E. 2nd Street,
   Redmond, WA 98053 (US).
(74) Agent: LINGENFELTER, Susan, E.; ZymoGenetics, Inc., 1201
   Eastlake Avenue East, Seattle, WA 98102 (US).

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(57) Abstract

Novel ligand polypeptides, polymolecules encoding the polypeptides, and related compositions, antibodies and methods are disclosed.
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DESCRIPTION

SECRETED PROTEIN ZSIG-11

BACKGROUND OF THE INVENTION

Proteins secreted from cells can act as intercellular signaling molecules which control the ontogeny and maintenance of tissue form and function. These secreted proteins control, among other things, proliferation, differentiation, migration, and expression of cells of multicellular organisms and act in concert to form cells, tissues and organs, and to repair and regenerate damaged tissue. Examples of secreted proteins include hormones and polypeptide growth factors which include steroid hormones (e.g. estrogen, testosterone), parathyroid hormone, follicle stimulating hormone, the interleukins, platelet derived growth factor (PDGF), epidermal growth factor (EGF), granulocyte-macrophage colony stimulating factor (GM-CSF), erythropoietin (EPO) and calcitonin, among others. Hormones and growth factors influence cellular metabolism by binding to receptors. Receptors may be integral membrane proteins that are linked to signaling pathways within the cell, such as second messenger systems. Other classes of receptors are soluble molecules, such as the transcription factors.

The present invention provides novel secreted proteins, agonists, antagonists and receptors of such proteins, as well as related compositions and methods. It is to these secreted proteins and the polynucleotides encoding them that the present invention is directed.

SUMMARY OF THE INVENTION

Within one aspect of the invention is provided an isolated polynucleotide molecule that encodes a polypeptide,
wherein the polynucleotide molecule is selected from the group consisting of: a) a polynucleotide encoding a polypeptide comprising a sequence of amino acid residues that is at least 80% identical to the amino acid sequence as shown in SEQ ID NO:2, from amino acid residue 26 (Gly) to amino acid residue 313 (Glu), and specifically binds with an antibody that specifically binds with a polypeptide having the amino acid sequence of SEQ ID NO:2; b) a polynucleotide molecule having the sequence of SEQ ID NO:6; and c) a polynucleotide molecule that hybridizes under stringent conditions to a polynucleotide molecule having the nucleotide sequence of SEQ ID NO:1, or the complement of SEQ ID NO:1. Within one embodiment any difference between the amino acid sequence encoded by the polynucleotide molecule and the corresponding amino acid sequence of SEQ ID NO:2 is due to a conservative amino acid substitution. Within another embodiment the polypeptide further comprises an affinity tag or binding domain. Within yet another embodiment the polynucleotide molecule comprises nucleotides 138-1001 of SEQ ID NO:1.

Within another aspect, the invention provides a polynucleotide molecule encoding a fusion protein consisting essentially of a first portion and a second portion joined by a peptide bond, the first portion comprising a polypeptide comprising a sequence of amino acid residues that is at least 80% identical to the amino acid sequence as shown in SEQ ID NO:2, from amino acid residue 26 (Gly) to amino acid residue 313 (Glu), and specifically binds with an antibody that specifically binds with a polypeptide having the amino acid sequence of SEQ ID NO:2; and the second portion comprising another polypeptide.

Within another aspect is provided a polynucleotide encoding a fusion protein comprising a secretory signal sequence having the amino acid sequence of amino acid residues 1-25 of SEQ ID NO:2, wherein the secretory signal sequence is operably linked to an additional polypeptide.
Within yet another aspect the invention provides an expression vector comprising the following operably linked elements: a transcription promoter; a polynucleotide molecule that encodes a polypeptide, wherein the polynucleotide molecule is selected from the group consisting of: a) a polynucleotide encoding a polypeptide comprising a sequence of amino acid residues that is at least 80% identical to the amino acid sequence as shown in SEQ ID NO:2, from amino acid residue 26 (Gly) to amino acid residue 313 (Glu), and specifically binds with an antibody that specifically binds with a polypeptide having the amino acid sequence of SEQ ID NO:2; b) a polynucleotide molecule having the sequence of SEQ ID NO:6; and c) a polynucleotide molecule that hybridizes under stringent conditions to a polynucleotide molecule having the nucleotide sequence of SEQ ID NO:1, or the complement of SEQ ID NO:1; and a transcription terminator. Within one embodiment the polypeptide further comprises a secretory signal sequence operably linked to the DNA segment. Within a related embodiment the secretory signal sequence comprises amino acid residues 1-25 of SEQ ID NO:2. Within another embodiment the polynucleotide encodes a polypeptide covalently linked amino terminally or carboxy terminally to an affinity tag.

The invention also provides a cultured cell into which has been introduced an expression vector comprising the following operably linked elements: a transcription promoter; a polynucleotide molecule that encodes a polypeptide, wherein the polynucleotide molecule is selected from the group consisting of: a) a polynucleotide encoding a polypeptide comprising a sequence of amino acid residues that is at least 80% identical to the amino acid sequence as shown in SEQ ID NO:2, from amino acid residue 26 (Gly) to amino acid residue 313 (Glu), and specifically binds with an antibody that specifically binds with a polypeptide having the amino acid sequence of SEQ ID NO:2; b) a polynucleotide molecule having the sequence of SEQ ID NO:6; and c) a
polynucleotide molecule that hybridizes under stringent conditions to a polynucleotide molecule having the nucleotide sequence of SEQ ID NO:1, or the complement of SEQ ID NO:1; and a transcription terminator, wherein the cultured cell expresses the polypeptide encoded by the polynucleotide segment.

Within another aspect is provided a method of producing a polypeptide comprising: culturing a cell into which has been introduced an expression vector comprising the following operably linked elements: a transcription promoter; a polynucleotide molecule that encodes a polypeptide, wherein the polynucleotide molecule is selected from the group consisting of: a) a polynucleotide encoding a polypeptide comprising a sequence of amino acid residues that is at least 80% identical to the amino acid sequence as shown in SEQ ID NO:2 from, amino acid residue 26 (Gly) to amino acid residue 313 (Glu), and specifically binds with an antibody that specifically binds with a polypeptide having the amino acid sequence of SEQ ID NO:2; b) a polynucleotide molecule having the sequence of SEQ ID NO:6; and c) a polynucleotide molecule that hybridizes under stringent conditions to a polynucleotide molecule having the nucleotide sequence of SEQ ID NO:1, or the complement of SEQ ID NO:1; and a transcription terminator; whereby the cell expresses the polypeptide encoded by the polynucleotide segment; and recovering the expressed polypeptide. Within one embodiment the expression vector further comprises a secretory signal sequence operably linked to the polynucleotide segment, the cultured cell secretes the polypeptide into a culture medium, and the polypeptide is recovered from the culture medium.

The invention further provides an isolated polypeptide comprising a sequence of amino acid residues that is at least 80% identical to the amino acid sequence as shown in SEQ ID NO:2, from amino acid residue 26 (Gly) to amino acid residue 313 (Glu), and specifically binds with an antibody that specifically binds with a polypeptide having
the amino acid sequence of SEQ ID NO:2. Within one embodiment the polypeptide comprises a sequence of amino acid residues that is at least 90% identical to the amino acid sequence as shown in SEQ ID NO:2, from amino acid residue 26 (Gly) to amino acid residue 313 (Glu), and specifically binds with an antibody that specifically binds with a polypeptide having the amino acid sequence of SEQ ID NO:2. Within a related embodiment the polypeptide comprises a sequence of amino acid residues that is at least 95% identical to the amino acid sequence as shown in SEQ ID NO:2, from amino acid residue 26 (Gly) to amino acid residue 313 (Glu), and specifically binds with an antibody that specifically binds with a polypeptide having the amino acid sequence of SEQ ID NO:2. Within another embodiment the polypeptide further comprises an affinity tag or binding domain.

Within another aspect is provided an isolated polypeptide comprising the amino acid sequence of SEQ ID NO:2.

Within yet another aspect is provided an isolated polypeptide comprising the amino acid sequence of SEQ ID NO:2, from amino acid residue 1 (Met) to amino acid residue 25 (Ala).

The invention also provided an antibody or antibody fragment that specifically binds to a polypeptide as described above. Within one embodiment the antibody is selected from the group consisting of: a) polyclonal antibody; b) murine monoclonal antibody; c) humanized antibody derived from b); and d) human monoclonal antibody.

Within a related embodiment the antibody fragment is selected from the group consisting of F(ab'), F(ab), Fab', Fab, Fv, scFv, and minimal recognition unit. Within another embodiment is an anti-idiotype antibody that specifically binds to the antibody as described above.

Within another aspect is provided a pharmaceutical composition comprising a polypeptide, the polypeptide comprising a sequence of amino acid residues
that is at least 80% identical to the amino acid sequence as shown in SEQ ID NO:2, from amino acid residue 26 (Gly) to amino acid residue 313 (Glu), and specifically binds with an antibody that specifically binds with a polypeptide having the amino acid sequence of SEQ ID NO:2; in combination with a pharmaceutically acceptable vehicle.

Within still another aspect is provided a method of detecting the presence of ZSIG-11 RNA is a biological sample, comprising the steps of: a) contacting a ZSIG-11 nucleic acid probe under stringent hybridizing conditions with either i) test RNA molecules isolated from the biological sample, or ii) nucleic acid molecules synthesized from the isolated RNA molecules, wherein the probe has a nucleotide sequence comprising a portion of the nucleotide sequence of SEQ ID NO:1, or its complement, and b) detecting the formation of hybrids of the nucleic acid probe and either the test RNA molecules or the synthesized nucleic acid molecules, wherein the presence of the hybrids indicates the presence of ZSIG-11 RNA is the biological sample.

Within still another aspect is provided a method of detecting the presence of ZSIG-11 in a biological sample, comprising the steps of: a) contacting the biological sample with an antibody, or an antibody fragment, that specifically binds with a polypeptide having the amino acid sequence of SEQ ID NO:2, wherein the contacting is performed under conditions that allow the binding of the antibody or antibody fragment to the biological sample, and b) detecting any of the bound antibody or bound antibody fragment. In one embodiment the antibody or the antibody fragment further comprises a detectable label selected from the group consisting of radioisotope, fluorescent label, chemiluminescent label, enzyme label, bioluminescent label, and colloidal gold.
DETAILED DESCRIPTION OF THE INVENTION

Prior to setting forth the invention, it may be helpful to an understanding thereof to set forth definitions of certain terms to be used hereinafter:

Affinity tag: is used herein to denote a polypeptide segment that can be attached to a second polypeptide to provide for purification or detection of the second polypeptide or provide sites for attachment of the second polypeptide to a substrate. In principal, any peptide or protein for which an antibody or other specific binding agent is available can be used as an affinity tag. Affinity tags include a poly-histidine tract, protein A (Nilsson et al., EMBO J. 4:1075, 1985; Nilsson et al., Methods Enzymol. 198:3, 1991), glutathione S transferase (Smith and Johnson, Gene 67:31, 1988), Glu-Glu affinity tag (Grussenmeyer et al., Proc. Natl. Acad. Sci. USA 82:7952-4, 1985), substance P, Flag™ peptide (Hopp et al., Biotechnology 6:1204-10, 1988), streptavidin binding peptide, or other antigenic epitope or binding domain. See, in general, Ford et al., Protein Expression and Purification 2: 95-107, 1991. DNAs encoding affinity tags are available from commercial suppliers (e.g., Pharmacia Biotech, Piscataway, NJ).

Allelic variant: Any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation arises naturally through mutation, and may result in phenotypic polymorphism within populations. Gene mutations can be silent (i.e., no change in the encoded polypeptide), or may encode polypeptides having altered amino acid sequence. The term "allelic variant" is also used herein to denote a protein encoded by an allelic variant of a gene. Also included are the same protein from the same species which differs from a reference amino acid sequence due to allelic variation. Allelic variation refers to naturally occurring differences among individuals in genes encoding a given protein.
Amino-terminal and carboxyl-terminal: are used herein to denote positions within polypeptides. Where the context allows, these terms are used with reference to a particular sequence or portion of a polypeptide to denote proximity or relative position. For example, a certain sequence positioned carboxyl-terminal to a reference sequence within a polypeptide is located proximal to the carboxyl terminus of the reference sequence, but is not necessarily at the carboxyl terminus of the complete polypeptide.

Complement/anti-complement pair: Denotes non-identical moieties that form a non-covalently associated, stable pair under appropriate conditions. For instance, biotin and avidin (or streptavidin) are prototypical members of a complement/anti-complement pair. Other exemplary complement/anti-complement pairs include receptor/ligand pairs, antibody/antigen (or hapten or epitope) pairs, sense/antisense polynucleotide pairs, and the like. Where subsequent dissociation of the complement/anti-complement pair is desirable, the complement/anti-complement pair preferably has a binding affinity of <10^-9 M.

Complements of polynucleotide molecules: Denotes polynucleotide molecules having a complementary base sequence and reverse orientation as compared to a reference sequence. For example, the sequence 5' ATGCACGGG 3' is complementary to 5' CCCGTGCAT 3'.

Degenerate nucleotide sequence: As applied herein denotes a sequence of nucleotides that includes one or more degenerate codons (as compared to a reference polynucleotide molecule that encodes a polypeptide). Degenerate codons contain different triplets of nucleotides, but encode the same amino acid residue (i.e., GAU and GAC triplets each encode Asp).

Expression vector: A DNA molecule, linear or circular, that comprises a segment encoding a polypeptide of interest operably linked to additional segments that
provide for its transcription. Such additional segments may include promoter and terminator sequences, and optionally one or more origins of replication, one or more selectable markers, an enhancer, a polyadenylation signal, and the like. Expression vectors are generally derived from plasmid or viral DNA, or may contain elements of both.

Isolated: when applied to a polynucleotide, denotes that the polynucleotide has been removed from its natural genetic milieu and is thus free of other extraneous or unwanted coding sequences, and is in a form suitable for use within genetically engineered protein production systems. Such isolated molecules are those that are separated from their natural environment and include cDNA and genomic clones. Isolated DNA molecules of the present invention are free of other genes with which they are ordinarily associated, but may include naturally occurring 5' and 3' untranslated regions such as promoters and terminators. The identification of associated regions will be evident to one of ordinary skill in the art (see for example, Dynan and Tijan, *Nature* 316:774-78, 1985).

Isolated polypeptide or protein: is a polypeptide or protein that is found in a condition other than its native environment, such as apart from blood and animal tissue. In a preferred form, the isolated polypeptide is substantially free of other polypeptides, particularly other polypeptides of animal origin. It is preferred to provide the polypeptides in a highly purified form, i.e. greater than 95% pure, more preferably greater than 99% pure. When used in this context, the term "isolated" does not exclude the presence of the same polypeptide in alternative physical forms, such as dimers or alternatively glycosylated or derivatized forms.

Operably linked: As applied to nucleotide segments, the term "operably linked" indicates that the segments are arranged so that they function in concert for their intended purposes, e.g., transcription initiates in
the promoter and proceeds through the coding segment to the terminator.

Ortholog: denotes a polypeptide or protein obtained from one species that is the functional counterpart of a polypeptide or protein from a different species. Sequence differences among orthologs are the result of speciation.

Paralogs: are distinct but structurally related proteins made by an organism. Paralogs are believed to arise through gene duplication. For example, α-globin, β-globin, and myoglobin are paralogs of each other.

Polynucleotide: Denotes a single- or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5' to the 3' end. Polynucleotides include RNA and DNA, and may be isolated from natural sources, synthesized in vitro, or prepared from a combination of natural and synthetic molecules. Sizes of polynucleotides are expressed as base pairs (abbreviated "bp"), nucleotides ("nt"), or kilobases ("kb"). Where the context allows, the latter two terms may describe polynucleotides that are single-stranded or double-stranded. When the term is applied to double-stranded molecules it is used to denote overall length and will be understood to be equivalent to the term "base pairs". It will be recognized by those skilled in the art that the two strands of a double-stranded polynucleotide may differ slightly in length and that the ends thereof may be staggered as a result of enzymatic cleavage; thus all nucleotides within a double-stranded polynucleotide molecule may not be paired. Such unpaired ends will in general not exceed 20 nt in length.

Polypeptide: is a polymer of amino acid residues joined by peptide bonds, whether produced naturally or synthetically. Polypeptides of less than about 10 amino acid residues are commonly referred to as "peptides".

Probes and/or primers: as used herein can be RNA or DNA. DNA can be either cDNA or genomic DNA.
Polynucleotide probes and primers are single or double-stranded DNA or RNA, generally synthetic oligonucleotides, but may be generated from cloned cDNA or genomic sequences or its complements. Analytical probes will generally be at least 20 nucleotides in length, although somewhat shorter probes (14-17 nucleotides) can be used. PCR primers are at least 5 nucleotides in length, preferably 15 or more nt, more preferably 20-30 nt. Short polynucleotides can be used when a small region of the gene is targeted for analysis. For gross analysis of genes, a polynucleotide probe may comprise an entire exon or more. Probes can be labeled to provide a detectable signal, such as with an enzyme, biotin, a radionuclide, fluorophore, chemiluminescer, paramagnetic particle and the like, which are commercially available from many sources, such as Molecular Probes, Inc., Eugene, OR, and Amersham Corp., Arlington Heights, IL, using techniques that are well known in the art.

Promoter: Denotes a portion of a gene containing DNA sequences that provide for the binding of RNA polymerase and initiation of transcription. Promoter sequences are commonly, but not always, found in the 5' non-coding regions of genes.

Protein: is a macromolecule comprising one or more polypeptide chains. A protein may also comprise non-peptidic components, such as carbohydrate groups. Carbohydrates and other non-peptidic substituents may be added to a protein by the cell in which the protein is produced, and will vary with the type of cell. Proteins are defined herein in terms of their amino acid backbone structures; substituents such as carbohydrate groups are generally not specified, but may be present nonetheless.

Receptor: A cell-associated protein, or a polypeptide subunit of such protein, that binds to a bioactive molecule (the "ligand") and mediates the effect of the ligand on the cell. Binding of ligand to receptor results in a change in the receptor (and, in some cases,
receptor multimerization, i.e., association of identical or different receptor subunits) that causes interactions between the effector domain(s) of the receptor and other molecule(s) in the cell. These interactions in turn lead to alterations in the metabolism of the cell. Metabolic events that are linked to receptor-ligand interactions include gene transcription, phosphorylation, dephosphorylation, cell proliferation, increases in cyclic AMP production, mobilization of cellular calcium, mobilization of membrane lipids, cell adhesion, hydrolysis of inositol lipids and hydrolysis of phospholipids. In general, receptors can be membrane bound, cytosolic or nuclear; monomeric (e.g., thyroid stimulating hormone receptor, beta-adrenergic receptor) or multimeric (e.g., PDGF receptor, growth hormone receptor, IL-3 receptor, GM-CSF receptor, G-CSF receptor, erythropoietin receptor and IL-6 receptor).

Secretary signal sequence: A DNA sequence that encodes a polypeptide (a "secretory peptide") that, as a component of a larger polypeptide, directs the larger polypeptide through a secretory pathway of a cell in which it is synthesized. The larger polypeptide is commonly cleaved to remove the secretory peptide during transit through the secretory pathway.

Soluble receptor or ligand: A receptor or a ligand polypeptide that is not bound to a cell membrane. Soluble receptors are most commonly ligand-binding receptor polypeptides that lack transmembrane and cytoplasmic domains. Soluble ligands are most commonly receptor-binding polypeptides that lack transmembrane and cytoplasmic domains. Soluble receptors or ligands can comprise additional amino acid residues, such as affinity tags that provide for purification of the polypeptide or provide sites for attachment of the polypeptide to a substrate. Many cell-surface receptors and ligands have naturally occurring, soluble counterparts that are produced by proteolysis or translated from alternatively spliced
mRNAs. Receptor and ligand polypeptides are the to be substantially free of transmembrane and intracellular polypeptide segments when they lack sufficient portions of these segments to provide membrane anchoring or signal transduction, respectively.

Splice variant: is used herein to denote alternative forms of RNA transcribed from a gene. Splice variation arises naturally through use of alternative splicing sites within a transcribed RNA molecule, or less commonly between separately transcribed RNA molecules, and may result in several mRNAs transcribed from the same gene. Splice variants may encode polypeptides having altered amino acid sequence. The term splice variant is also used herein to denote a protein encoded by a splice variant of an mRNA transcribed from a gene.

Molecular weights and lengths of polymers determined by imprecise analytical methods (e.g., gel electrophoresis) will be understood to be approximate values. When such a value is expressed as "about" X or "approximately" X, the stated value of X will be understood to be accurate to ±10%.

All references cited herein are incorporated by reference in their entirety.

The present invention is based in part upon the discovery of a novel DNA sequence (SEQ ID NO:1) and corresponding polypeptide sequence (SEQ ID NO:2). This novel secreted protein has been designated ZSIG-11. Novel ZSIG-11 ligand-encoding polynucleotides and polypeptides of the present invention were initially identified by querying an expressed sequence tag (EST) database for secretory signal sequences characterized by an upstream methionine start site, a hydrophobic region of approximately 13 amino acids and a cleavage site, in an effort to select for secreted proteins. Polypeptides corresponding to ESTs meeting those search criteria were compared to known sequences to identify secreted proteins having homology to known ligands. Using this information, a novel 1341 bp
human cDNA fragment (SEQ ID NO:1) was obtained. The protein encoded by this DNA fragment is predicted to be a secreted protein; however, no homology to any known protein was identified. Analysis of the deduced amino acid sequence (SEQ ID NO:2) revealed a 25 amino acid residue signal sequence (residues 1-25 of SEQ ID NO:2), a beta sheet domain containing three cysteines from residues 26 to 148 or 161 of SEQ ID NO:2, two additional cysteines are found at amino acid residues 165 and 182; and a domain containing 11 potential phosphorylation sites from residues 151 or 164 to 313 of SEQ ID NO:2). There are two potential dibasic cleavage sites, following residue 150 and residue 163, for post-translational processing which may serve to regulate the activity of the mature molecule. The resulting peptides would be from amino acid residue 26 to residue 148, from amino acid residue 151 to residue 161 and from amino acid residue 163 to residue 313 of SEQ ID NO:2. Alternatively, the domains could be partitioned into a beta sheet domain containing all five cysteine residues, comprising amino acid residues 26 to 214 of SEQ ID NO:2 and a region condensed region containing phosphorylation sites, comprising amino acid residues 215 to 313 of SEQ ID NO:2. Those skilled in the art will recognize that these boundaries are approximate, and are based on comparisons and alignments with known proteins and predictions of protein folding are approximations based on primary sequence content, and may vary slightly; however, such estimates are generally accurate to within ±4 amino acid residues.

Northern blot analysis of various human tissues was performed using a 30 bp DNA probe (SEQ ID NO:3) to a site just 3' of the signal sequence encoding an N-terminal portion of the mature protein. A predominant transcript of 1.8 kb corresponding to ZSIG-11 was found. A high level of transcription was detected in testis, prostate, thyroid and heart. A moderate level of transcription was detected in skeletal muscle, pancreas, small intestine, peripheral
blood lymphocytes, brain, placenta, liver, kidney, thymus, ovary, colon, spinal cord, trachea and adrenal gland. Three transcripts of 5 kb, 2 kb and 1.5 kb corresponding to ZSIG-11 were detected in the human osteogenic cell lines HOS, MG-63, Sa052 and U205.

ZSIG-11 maps 252.51 cR_3000 from the top of the human chromosome 20 linkage group on the WICGR radiation hybrid map. Proximal and distal framework markers were D20S908 and D20S999, respectively. This positions ZSIG-11 in the 20q13.12 region on the integrated LDB chromosome 20 map.

A degenerate polynucleotide sequence that encompasses all polynucleotides that encode the ZSIG-11 polypeptide of SEQ ID NO: 2 (amino acid residue 1-313) is disclosed in SEQ ID NO:6. Thus, ZSIG-11 polypeptide-encoding polynucleotides ranging from nucleotide 1 or 76 to nucleotide 939 of SEQ ID NO:6 are contemplated by the present invention. Also contemplated by the present invention are fragments as described above with respect to SEQ ID NO:1, which are formed from analogous regions of SEQ ID NO:6, wherein nucleotides 63 to 1001 of SEQ ID NO:1 correspond to nucleotides 1 to 939 of SEQ ID NO:6 and nucleotides 138 to 1001 of SEQ ID NO:1 correspond to nucleotides 76 to 939 of SEQ ID NO:6, nucleotides 138 to 503 of SEQ ID NO:1 correspond to nucleotides 76 to 431 of SEQ ID NO:6, nucleotides 513 to 545 of SEQ ID NO:1 correspond to nucleotides 450 to 483 of SEQ ID NO:6 and nucleotides 552 to 1001 of SEQ ID NO:1 correspond to nucleotides 490 to 939 of SEQ ID NO:6. The nucleotide base codes in SEQ ID NO:6 are summarized in Table 1 below.
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<td>C</td>
<td>G</td>
</tr>
</tbody>
</table>

The degenerate codons used in SEQ ID NO:6, encompassing all possible codons for a given amino acid, are set forth in Table 2 below.
TABLE 2

<table>
<thead>
<tr>
<th>Three Letter Code</th>
<th>One Letter Code</th>
<th>Synonymous Codons</th>
<th>Degenerate Codon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cys</td>
<td>C</td>
<td>TGC TGT</td>
<td>TGY</td>
</tr>
<tr>
<td>Ser</td>
<td>S</td>
<td>AGC AGT TCA TCC TCG TCT</td>
<td>WSN</td>
</tr>
<tr>
<td>Thr</td>
<td>T</td>
<td>ACA ACC ACG ACT</td>
<td>CAN</td>
</tr>
<tr>
<td>Pro</td>
<td>P</td>
<td>CCA CCC CGG CCT</td>
<td>CCN</td>
</tr>
<tr>
<td>Ala</td>
<td>A</td>
<td>GCA GCC GCG GCT</td>
<td>GCN</td>
</tr>
<tr>
<td>Gly</td>
<td>G</td>
<td>GGA GGC GGG GGT</td>
<td>GGN</td>
</tr>
<tr>
<td>Asn</td>
<td>N</td>
<td>AAC AAT</td>
<td>AAY</td>
</tr>
<tr>
<td>Asp</td>
<td>D</td>
<td>GAC GAT</td>
<td>GAY</td>
</tr>
<tr>
<td>Glu</td>
<td>E</td>
<td>GAA GAG</td>
<td>GAR</td>
</tr>
<tr>
<td>Gln</td>
<td>Q</td>
<td>CAA CAG</td>
<td>CAR</td>
</tr>
<tr>
<td>His</td>
<td>H</td>
<td>CAC CAT</td>
<td>CAY</td>
</tr>
<tr>
<td>Arg</td>
<td>R</td>
<td>AGA AGG CGA CGC CGG CGT</td>
<td>MGN</td>
</tr>
<tr>
<td>Lys</td>
<td>K</td>
<td>AAA AAG</td>
<td>AAR</td>
</tr>
<tr>
<td>Met</td>
<td>M</td>
<td>ATG</td>
<td>ATG</td>
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<tr>
<td>Ile</td>
<td>I</td>
<td>ATA ATC ATT</td>
<td>ATH</td>
</tr>
<tr>
<td>Leu</td>
<td>L</td>
<td>CTA CTG CGT TTA TTG</td>
<td>YTN</td>
</tr>
<tr>
<td>Val</td>
<td>V</td>
<td>GTA GTC GTG GTT</td>
<td>GTN</td>
</tr>
<tr>
<td>Phe</td>
<td>F</td>
<td>TTC TTT</td>
<td>TTY</td>
</tr>
<tr>
<td>Tyr</td>
<td>Y</td>
<td>TAC TAT</td>
<td>TAY</td>
</tr>
<tr>
<td>Trp</td>
<td>W</td>
<td>TGG</td>
<td>TGG</td>
</tr>
<tr>
<td>Ter</td>
<td>.</td>
<td>TAA TAG TGA</td>
<td>TRR</td>
</tr>
<tr>
<td>Asn</td>
<td>Asp</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>Glu</td>
<td>Gln</td>
<td>Z</td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>X</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

One of ordinary skill in the art will appreciate that some ambiguity is introduced in determining a degenerate codon, representative of all possible codons encoding each amino acid. For example, the degenerate codon for serine (WSN) can, in some circumstances, encode arginine (AGR), and the degenerate codon for arginine (MGN) can, in some circumstances, encode serine (AGY). A similar
relationship exists between codons encoding phenylalanine and leucine. Thus, some polynucleotides encompassed by the degenerate sequence may encode variant amino acid sequences, but one of ordinary skill in the art can easily identify such variant sequences by reference to the amino acid sequence of SEQ ID NO:2. Such variant sequences can be tested for functionality as disclosed herein. Within preferred embodiments of the invention the isolated polynucleotides will hybridize to similar sized regions of SEQ ID NO:1, other polynucleotide probes, primers, fragments and sequences recited herein or sequences complementary thereto. Polynucleotide hybridization is well known in the art and widely used for many applications, see for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual, Second Edition*, Cold Spring Harbor, NY, 1989; Ausubel et al., eds., *Current Protocols in Molecular Biology*, John Wiley and Sons, Inc., NY, 1987; Berger and Kimmel, eds., Guide to Molecular Cloning Techniques, *Methods in Enzymology*, volume 152, 1987 and Wetmur, *Crit. Rev. Biochem. Mol. Biol.* 26:227-59, 1990. Polynucleotide hybridization exploits the ability of single stranded complementary sequences to form a double helix hybrid. Such hybrids include DNA-DNA, RNA-RNA and DNA-RNA. Hybridization will occur between sequences which contain some degree of complementarity. Hybrids can tolerate mismatched base pairs in the double helix, but the stability of the hybrid is influenced by the degree of mismatch. The $T_m$ of the mismatched hybrid decreases by 1°C for every 1-1.5% base pair mismatch. Varying the stringency of the hybridization conditions allows control over the degree of mismatch that will be present in the hybrid. The degree of stringency increases as the hybridization temperature increases and the ionic strength of the hybridization buffer decreases. Stringent hybridization conditions encompass temperatures of about 5-25°C below the thermal melting point ($T_m$) of the hybrid and a hybridization buffer having up to 1 M Na'. Higher
degrees of stringency at lower temperatures can be achieved with the addition of formamide which reduces the \( T_m \) of the hybrid about 1°C for each 1% formamide in the buffer solution. Generally, such stringent conditions include temperatures of 20-70°C and a hybridization buffer containing up to 6X SSC and 0-50% formamide. A higher degree of stringency can be achieved at temperatures of from 40-70°C with a hybridization buffer having up to 4X SSC and from 0-50% formamide. Highly stringent conditions typically encompass temperatures of 42-70°C with a hybridization buffer having up to 1X SSC and 0-50% formamide. Different degrees of stringency can be used during hybridization and washing to achieve maximum specific binding to the target sequence. Typically, the washes following hybridization are performed at increasing degrees of stringency to remove non-hybridized polynucleotide probes from hybridized complexes.

The above conditions are meant to serve as a guide and it is well within the abilities of one skilled in the art to adapt these conditions for use with a particular polypeptide hybrid. The \( T_m \) for a specific target sequence is the temperature (under defined conditions) at which 50% of the target sequence will hybridize to a perfectly matched probe sequence. Those conditions that influence the \( T_m \) include, the size and base pair content of the polynucleotide probe, the ionic strength of the hybridization solution, and the presence of destabilizing agents in the hybridization solution. Numerous equations for calculating \( T_m \) are known in the art, see for example (Sambrook et al., ibid.; Ausubel et al., ibid.; Berger and Kimmel, ibid. and Wetmur, ibid.) and are specific for DNA, RNA and DNA-RNA hybrids and polynucleotide probe sequences of varying length. Sequence analysis software such as Oligo 4.0 and Primer Premier, as well as sites on the Internet, are available tools for analyzing a given sequence and calculating \( T_m \) based on user defined criteria. Such programs can also analyze a given sequence under
defined conditions and suggest suitable probe sequences. Typically, hybridization of longer polynucleotide sequences, >50 bp, is done at temperatures of about 20-25°C below the calculated $T_m$. For smaller probes, <50 bp, hybridization is typically carried out at the $T_m$ or 5-10°C below. This allows for the maximum rate of hybridization for DNA-DNA and DNA-RNA hybrids.

The length of the polynucleotide sequence influences the rate and stability of hybrid formation.

Smaller probe sequences, <50 bp, come to equilibrium with complementary sequences rapidly, but may form less stable hybrids. Incubation times of anywhere from minutes to hours can be used to achieve hybrid formation. Longer probe sequences come to equilibrium more slowly, but form more stable complexes even at lower temperatures. Incubations are allowed to proceed overnight or longer. Generally, incubations are carried out for a period equal to three times the calculated Cot time. Cot time, the time it takes for the polynucleotide sequences to reassociate, can be calculated for a particular sequence by methods known in the art.

The base pair composition of polynucleotide sequence will effect the thermal stability of the hybrid complex, thereby influencing the choice of hybridization temperature and the ionic strength of the hybridization buffer. A-T pairs are less stable than G-C pairs in aqueous solutions containing NaCl. Therefore, the higher the G-C content, the more stable the hybrid. Even distribution of G and C residues within the sequence also contribute positively to hybrid stability. Base pair composition can be manipulated to alter the $T_m$ of a given sequence, for example, 5-methyldeoxycytidine can be substituted for deoxycytidine and 5-bromodeoxuridine can be substituted for thymidine to increase the $T_m$. 7-deaz-2'-deoxyguanosine can be substituted for deoxyguanosine to reduce dependence on $T_m$. 

Ionic concentration of the hybridization buffer also effects the stability of the hybrid. Hybridization buffers generally contain blocking agents such as Denhardt's solution (Sigma Chemical Co., St. Louis, Mo.), denatured salmon sperm DNA, milk powders (BLOTTO), heparin or SDS, and a Na\(^+\) source, such as SSC (1X SSC: 0.15 M NaCl, 15 mM sodium citrate) or SSPE (1X SSPE: 1.8 M NaCl, 10 mM NaH\(_2\)PO\(_4\), 1 mM EDTA, pH 7.7). By decreasing the ionic concentration of the buffer, the stability of the hybrid is increased. Typically, hybridization buffers contain from between 10 mM-1 M Na\(^+\). Premixed hybridization solutions are also available from commercial sources such as Clontech Laboratories (Palo Alto, CA) and Promega Corporation (Madison, WI) for use according to manufacturer's instruction. Addition of destabilizing or denaturing agents such as formamide, tetralkylammonium salts, guanidinium cations or thiocyanate cations to the hybridization solution will alter the T\(_m\) of a hybrid. Typically, formamide is used at a concentration of up to 50% to allow incubations to be carried out at more convenient and lower temperatures. Formamide also acts to reduce non-specific background when using RNA probes.

As previously noted, the isolated polynucleotides of the present invention include DNA and RNA. Methods for isolating DNA and RNA are well known in the art. It is generally preferred to isolate RNA from testis tissue, although DNA can also be prepared using RNA from other tissues or isolated as genomic DNA. Total RNA can be prepared using guanidine HCl extraction followed by isolation by centrifugation in a CsCl gradient (Chirgwin et al., Biochemistry 18:52-94, 1979). Poly (A)\(^+\) RNA is prepared from total RNA using the method of Aviv and Leder (Proc. Natl. Acad. Sci. USA 69:1408-12, 1972). Complementary DNA (cDNA) is prepared from poly(A)\(^+\) RNA using known methods. Polynucleotides encoding ZSIG-11 polypeptides are then identified and isolated by, for example, hybridization or PCR.
Those skilled in the art will recognize that the sequence disclosed in SEQ ID NO:1 represents a single allele of the human ZSIG-11 gene, and that allelic variation and alternative splicing, "splice variants", are expected to exist. Allelic variants of the DNA sequence shown in SEQ ID NO:1, including those containing silent mutations and those in which mutations result in amino acid sequence changes, are within the scope of the present invention, as are proteins which are allelic variants of SEQ ID NO:2. Splice variants of the DNA sequence shown in SEQ ID NO:1 include DNA sequences that result from mature RNA molecules created by known eukaryotic RNA splicing processes wherein intron sequence is removed and exon sequence is joined. Such DNA sequences encoding proteins which retain properties of the ZSIG-11 protein of SEQ ID NO:2 are within the scope of the present invention. Allelic variants and splice variants of these sequences can be cloned by probing cDNA or genomic libraries from different individuals or tissues according to standard procedures known in the art.

The present invention further provides counterpart ligands and polynucleotides from other species ("species orthologs"). These species would include, but are not limited to, mammalian, avian, amphibian, reptile, fish, insect and other vertebrate and invertebrate species. Of particular interest are ZSIG-11 ligand polypeptides from other mammalian species, including murine, porcine, ovine, bovine, canine, feline, equine, and other primate ligands. Species orthologs of human ZSIG-11 can be cloned using information and compositions provided by the present invention in combination with conventional cloning techniques. For example, a cDNA can be cloned using mRNA obtained from a tissue or cell type that expresses the ligand. Suitable sources of mRNA can be identified by probing Northern blots with probes designed from the sequences disclosed herein. A library is then prepared from mRNA of a positive tissue or cell line. A ZSIG-11-
encoding cDNA can then be isolated by a variety of methods, such as by probing with a complete or partial human cDNA or with one or more sets of degenerate probes based on the disclosed sequence. A cDNA can also be cloned using the polymerase chain reaction (PCR) (Mullis, U.S. Patent No. 4,683,202), using primers designed from the sequences disclosed herein. Within an additional method, the cDNA library can be used to transform or transfet host cells, and expression of the cDNA of interest can be detected with an antibody to ZSIG-11. Similar techniques can also be applied to the isolation of genomic clones.

The present invention also provides isolated polypeptides that are substantially homologous to the ligand polypeptide of SEQ ID NO:2 and its species orthologs or paralogs. By "isolated" is meant a protein or polypeptide that is found in a condition other than its native environment, such as apart from blood and animal tissue. In a preferred form, the isolated protein or polypeptide is substantially free of other proteins or polypeptides, particularly other proteins or polypeptides of animal origin. It is preferred to provide the proteins or polypeptides in a highly purified form, i.e. greater than 95% pure, more preferably greater than 99% pure. The term "substantially homologous" is used herein to denote proteins or polypeptides having 50%, preferably 60%, more preferably at least 80%, sequence identity to the sequence shown in SEQ ID NO:2 or its species orthologs or paralogs. Such proteins or polypeptides will more preferably be at least 90% identical, and most preferably 95% or more identical to SEQ ID NO:2 or its species orthologs or paralogs. Percent sequence identity is determined by conventional methods. See, for example, Altschul et al., Bull. Math. Bio. 48: 603-16, 1986 and Henikoff and Henikoff, Proc. Natl. Acad. Sci. USA 89:10915-9, 1992. Briefly, two amino acid sequences are aligned to optimize the alignment scores using a gap opening penalty of 10, a gap extension penalty of 1, and the "blosum 62" scoring
matrix of Henikoff and Henikoff (ibid.) as shown in Table 3 (amino acids are indicated by the standard one-letter codes). The percent identity is then calculated as:

\[
\frac{\text{Total number of identical matches}}{\text{length of the longer sequence plus the number of gaps introduced into the longer sequence in order to align the two sequences}} \times 100
\]
### Table 3

| A | R | N | D | C | Q | E | G | H | I | L | K | M | F | P | S | T | W | Y | V |
| 4 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 5 | -1| 5 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 6 | -2| 0 | 6 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 10| -2| -2| 1 | 6 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 8 | 0 | -2| 0 | -1| -3| -2| -2| 6 |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 25|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 15| -1| 2 | 0 | -1| -3| 1 | 1 | -2| -1| -3| -2| 5 |   |   |   |   |   |   |   |   |   |
| 5 | 0 | -2| -1| -3| -1| 0 | -2| -3| -2| 1 | 2 | -1| 5 |   |   |   |   |   |   |   |
| 6 | -2| -3| -3| -2| -3| -3| -3| -1| 0 | 0 | -3| 0 | 6 |   |   |   |   |   |   |   |
| 7 | -1| 2 | -1| -3| -1| -1| -2| -2| -3| -3| -1| -2| -4| 7 |   |   |   |   |   |   |   |
| 4 | -1| 2 | 0 | -1| -1| 0 | 0 | 0 | -1| -2| -2| 0 | -1| -2| -1| 5 |   |   |   |   |
| 5 | 0 | -4| -3| -4| -2| -3| -2| -3| -2| -3| -1| 1 | -4| -3| -2| 11|   |   |   |   |
| 7 | -2| -2| -2| -3| -2| -1| -2| -3| 2 | -1| 1 | 2 | -1| 3 | -3| -2| -2| 2 |   |   |
| 4 | 0 | -3| -3| -1| -2| -2| -3| -3| 3 | 1 | -2| 1 | -1| -2| -2| 0 | -3| -1| 4 |   |

| 20|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |


Sequence identity of polynucleotide molecules is determined by similar methods using a ratio as disclosed above.

Substantially homologous proteins and polypeptides are characterized as having one or more amino acid substitutions, deletions or additions. These changes are preferably of a minor nature, that is conservative amino acid substitutions (see Table 4) and other substitutions that do not significantly affect the folding or activity of the protein or polypeptide; small deletions, typically of one to about 30 amino acids; and small amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue, a small linker peptide of up to about 20-25 residues, or a small extension that facilitates purification (an affinity tag), such as a poly-histidine tract, protein A (Nilsson et al., EMBO J. 4:1075, 1985; Nilsson et al., Methods Enzymol. 198:3, 1991), glutathione S transferase (Smith and Johnson, Gene 67:31, 1988), maltose binding protein (Kellerman and Ferenci, Meth. Enzymol. 90:459-63, 1982; Guan et al., Gene 67:21-30, 1987), thioredoxin, ubiquitin, cellulose binding protein, T7 polymerase, an immunoglobulin heavy chain constant region or other antigenic epitope or binding domain. See, in general Ford et al., Protein Expression and Purification 2: 95-107, 1991. DNAs encoding affinity tags are available from commercial suppliers (e.g. Pharmacia Biotech, Piscataway, NJ).
Table 4

Conservative amino acid substitutions

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
</table>
| 5 | Basic: arginine  
    | lysine  
    | histidine  
    | Acidic: glutamic acid  
    | aspartic acid  
    | Polar: glutamine  
    | asparagine  
    | Hydrophobic: leucine  
    | isoleucine  
    | 10 | valine  
    | Aromatic: phenylalanine  
    | tryptophan  
    | tyrosine  
    | Small: glycine  
    | alanine  
    | 15 | serine  
    | threonine  
    | methionine  
    | 20 |  

The proteins of the present invention can also comprise non-naturally occurring amino acid residues. Non-naturally occurring amino acids include, without limitation, trans-3-methylproline, 2,4-methanoproline, cis-4-hydroxyproline, trans-4-hydroxyproline, N-methylglycine, allo-threonine, methylthreonine, hydroxyethylcysteine, hydroxyethylhomocysteine, nitroglutamine, homoglutamine, pipecolic acid, thiazolidine carboxylic acid, dehydroproline, 3- and 4-methylproline, 3,3-dimethylproline, tert-leucine, norvaline, 2-azaphenyl-alanine, 3-azaphenylalanine, 4-azaphenylalanine, and 4-fluorophenylalanine. Several methods are known in the art for incorporating non-naturally occurring amino acid residues into proteins. For example, an in vitro system can be
employed wherein nonsense mutations are suppressed using chemically aminoacylated suppressor tRNAs. Methods for synthesizing amino acids and aminoacylating tRNA are known in the art. Transcription and translation of plasmids containing nonsense mutations is carried out in a cell-free system comprising an E. coli S30 extract and commercially available enzymes and other reagents. Proteins are purified by chromatography. See, for example, Robertson et al., J. Am. Chem. Soc. 113:2722, 1991; Ellman et al., Methods Enzymol. 202:301, 1991; Chung et al., Science 259:806-9, 1993; and Chung et al., Proc. Natl. Acad. Sci. USA 90:10145-9, 1993). In a second method, translation is carried out in Xenopus oocytes by microinjection of mutated mRNA and chemically aminoacylated suppressor tRNAs (Turcatti et al., J. Biol. Chem. 271:19991-8, 1996). Within a third method, E. coli cells are cultured in the absence of a natural amino acid that is to be replaced (e.g., phenylalanine) and in the presence of the desired non-naturally occurring amino acid(s) (e.g., 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, or 4-fluorophenylalanine). The non-naturally occurring amino acid is incorporated into the protein in place of its natural counterpart. See, Koide et al., Biochem. 33:7470-6, 1994. Naturally occurring amino acid residues can be converted to non-naturally occurring species by in vitro chemical modification. Chemical modification can be combined with site-directed mutagenesis to further expand the range of substitutions (Wynn and Richards, Protein Sci. 2:395-403, 1993).

A limited number of non-conservative amino acids, amino acids that are not encoded by the genetic code, non-naturally occurring amino acids, and unnatural amino acids may be substituted for ZSIG-11 amino acid residues.

Essential amino acids in the ZSIG-11 polypeptides of the present invention can be identified according to procedures known in the art, such as site-
directed mutagenesis or alanine-scanning mutagenesis 
(Cunningham and Wells, *Science* 244: 1081-5, 1989). In the 
latter technique, single alanine mutations are introduced 
at every residue in the molecule, and the resultant mutant 
molecules are tested for biological activity (e.g., 
adhesion-modulation, differentiation-modulation or the 
like) to identify amino acid residues that are critical to 
the activity of the molecule. See also, Hilton et al., *J. 
or other biological interaction can also be determined by 
physical analysis of structure, as determined by such 
techniques as nuclear magnetic resonance, crystallography, 
electron diffraction or photoaffinity labeling, in 
conjunction with mutation of putative contact site amino 
acids. See, for example, de Vos et al., *Science* 255:306- 
12, 1992; Smith et al., *J. Mol. Biol.* 224:899-904, 1992; 

Multiple amino acid substitutions can be made 
and tested using known methods of mutagenesis and 
screening, such as those disclosed by Reidhaar-Olson and 
Natl. Acad. Sci. USA* 86:2152-6, 1989). Briefly, these 
authors disclose methods for simultaneously randomizing 
two or more positions in a polypeptide, selecting for 
functional polypeptide, and then sequencing the 
mutagenized polypeptides to determine the spectrum of 
allowable substitutions at each position. Other methods 
that can be used include phage display (e.g., Lowman et 
No. 5,223,409; Huse, WIPO Publication WO 92/06204) and 
region-directed mutagenesis (Derbyshire et al., *Gene* 

Variants of the disclosed ZSIG-11 DNA and 
polypeptide sequences can be generated through DNA 
shuffling as disclosed by Stemmer, *Nature* 370:389-91, 
1994 and WIPO Publication WO 97/20078. Briefly, variant
DNAs are generated by in vitro homologous recombination by random fragmentation of a parent DNA followed by reassembly using PCR, resulting in randomly introduced point mutations. This technique can be modified by using a family of parent DNAs, such as allelic variants or DNAs from different species, to introduce additional variability into the process. Selection or screening for the desired activity, followed by additional iterations of mutagenesis and assay provides for rapid "evolution" of sequences by selecting for desirable mutations while simultaneously selecting against detrimental changes.

Mutagenesis methods as disclosed above can be combined with high-throughput, automated screening methods to detect activity of cloned, mutagenized polypeptides in host cells. Mutagenized DNA molecules that encode active polypeptides (e.g., receptor binding) can be recovered from the host cells and rapidly sequenced using modern equipment. These methods allow the rapid determination of the importance of individual amino acid residues in a polypeptide of interest, and can be applied to polypeptides of unknown structure.

Using the methods discussed above, one of ordinary skill in the art can identify and/or prepare a variety of polypeptides that are substantially homologous to residues 26-313 of SEQ ID NO: 2 or allelic variants thereof and retain the receptor-binding properties of the wild-type protein. Such polypeptides may include additional amino acids from the signal sequence, affinity tags and the like. Such polypeptides may also include additional polypeptide segments as generally disclosed above.

In addition to the fusion proteins disclosed above, the present invention provides fusions comprising the secretory peptide of ZSIG-11 (residues 1 through 25 of SEQ ID NO:2). This secretory peptide can be used to direct the secretion of other proteins of interest by
joining a polynucleotide sequence encoding it to the 5' end of a sequence encoding a protein of interest.

The polypeptides of the present invention, including full-length polypeptides, fragments (e.g., receptor-binding fragments), and fusion polypeptides, can be produced in genetically engineered host cells according to conventional techniques. Suitable host cells are those cell types that can be transformed or transfected with exogenous DNA and grown in culture, and include bacteria, fungal cells, and cultured higher eukaryotic cells. Eukaryotic cells, particularly cultured cells of multicellular organisms, are preferred. Techniques for manipulating cloned DNA molecules and introducing exogenous DNA into a variety of host cells are disclosed by Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, NY, 1989; andAusubel et al., eds., Current Protocols in Molecular Biology, John Wiley and Sons, Inc., NY, 1987.

In general, a DNA sequence encoding a ZSIG-11 polypeptide is operably linked to other genetic elements required for its expression, generally including a transcription promoter and terminator, within an expression vector. The vector will also commonly contain one or more selectable markers and one or more origins of replication, although those skilled in the art will recognize that within certain systems selectable markers may be provided on separate vectors, and replication of the exogenous DNA may be provided by integration into the host cell genome. Selection of promoters, terminators, selectable markers, vectors and other elements is a matter of routine design within the level of ordinary skill in the art. Many such elements are described in the literature and are available through commercial suppliers.

To direct a ZSIG-11 polypeptide into the secretory pathway of a host cell, a secretory signal sequence (also known as a leader sequence, prepro sequence or pre sequence) is provided in the expression vector.
The secretory signal sequence may be that of the ZSIG-11 polypeptide, or may be derived from another secreted protein (e.g., t-PA) or synthesized de novo. The secretory signal sequence is joined to the ZSIG-11 DNA sequence in the correct reading frame. Secretory signal sequences are commonly positioned 5' to the DNA sequence encoding the polypeptide of interest, although certain signal sequences may be positioned elsewhere in the DNA sequence of interest (see, e.g., Welch et al., U.S. Patent No. 5,037,743; Holland et al., U.S. Patent No. 5,143,830).

Alternatively, the secretory signal sequence contained in the polypeptides of the present invention is used to direct other polypeptides into the secretory pathway. The present invention provides for such fusion polypeptides. A signal fusion polypeptide can be made wherein a secretory signal sequence derived from amino acid residues 1-25 of SEQ ID NO:2 is be operably linked to another polypeptide using methods known in the art and disclosed herein. The secretory signal sequence contained in the fusion polypeptides of the present invention is preferably fused amino-terminally to an additional peptide to direct the additional peptide into the secretory pathway. Such constructs have numerous applications known in the art. For example, these novel secretory signal sequence fusion constructs can direct the secretion of an active component of a normally non-secreted protein. Such fusions may be used in vivo or in vitro to direct peptides through the secretory pathway.

Cultured mammalian cells are preferred hosts within the present invention. Methods for introducing exogenous DNA into mammalian host cells include calcium phosphate-mediated transfection (Wigler et al., Cell 14:725, 1978; Corsaro and Pearson, Somatic Cell Genetics 2:603, 1981; Graham and Van der Eb, Virology 52:456, 1973), electroporation (Neumann et al., EMBO J. 1:841-45, 1982), DEAE-dextran mediated transfection (Ausubel et al., ibid), liposome-mediated transfection (Hawley-Nelson et
al., *Focus* 15:73, 1993; Ciccarone et al., *Focus* 15:80, 1993) and viral vectors (Miller and Rosman, *BioTechniques* 7:980-90, 1989; Wang and Finer, *Nature Med.* 2:714-6, 1996). The production of recombinant polypeptides in cultured mammalian cells is disclosed, for example, by Levinson et al., U.S. Patent No. 4,713,339; Hagen et al., U.S. Patent No. 4,784,950; Palmiter et al., U.S. Patent No. 4,579,821; and Ringold, U.S. Patent No. 4,656,134. Suitable cultured mammalian cells include the COS-1 (ATCC No. CRL 1650), COS-7 (ATCC No. CRL 1651), BHK (ATCC No. CRL 1632), BHK 570 (ATCC No. CRL 10314), 293 (ATCC No. CRL 1573; Graham et al., *J. Gen. Virol.* 36:59-72, 1977) and Chinese hamster ovary (e.g., CHO-K1; ATCC No. CCL 61) cell lines. Additional suitable cell lines are known in the art and available from public depositories such as the American Type Culture Collection, Rockville, Maryland. In general, strong transcription promoters are preferred, such as promoters from SV-40 or cytomegalovirus. See, e.g., U.S. Patent No. 4,956,288. Other suitable promoters include those from metallothionein genes (U.S. Patent Nos. 4,579,821 and 4,601,978) and the adenovirus major late promoter.

Drug selection is generally used to select for cultured mammalian cells into which foreign DNA has been inserted. Such cells are commonly referred to as "transfectants". Cells that have been cultured in the presence of the selective agent and are able to pass the gene of interest to their progeny are referred to as "stable transfectants." A preferred selectable marker is a gene encoding resistance to the antibiotic neomycin. Selection is carried out in the presence of a neomycin-type drug, such as G-418 or the like. Selection systems may also be used to increase the expression level of the gene of interest, a process referred to as "amplification." Amplification is carried out by culturing transfectants in the presence of a low level of the selective agent and then increasing the amount of
selective agent to select for cells that produce high levels of the products of the introduced genes. A preferred amplifiable selectable marker is dihydrofolate reductase, which confers resistance to methotrexate. Other drug resistance genes (e.g., hygromycin resistance, multi-drug resistance, puromycin acetyltransferase) can also be used.

and Rapoport, J. Biol. Chem. 270:1543-9, 1995. In addition, transfer vectors can include an in-frame fusion with DNA encoding an epitope tag at the C- or N-terminus of the expressed ZSIG-11 polypeptide, for example, a Glu-Glu epitope tag (Grussenmeyer et al., Proc. Natl. Acad. Sci. 82:7952-4, 1985). Using a technique known in the art, a transfer vector containing ZSIG-11 is transformed into E. coli, and screened for bacmids which contain an interrupted lacZ gene indicative of recombinant baculovirus. The bacmid DNA containing the recombinant baculovirus genome is isolated, using common techniques, and used to transfect Spodoptera frugiperda cells, e.g. Sf9 cells. Recombinant virus that expresses ZSIG-11 is subsequently produced. Recombinant viral stocks are made by methods commonly used the art.

The recombinant virus is used to infect host cells, typically a cell line derived from the fall armyworm, Spodoptera frugiperda. See, in general, Glick and Pasternak, Molecular Biotechnology: Principles and Applications of Recombinant DNA, ASM Press, Washington, D.C., 1994. Another suitable cell line is the High Five™ cell line (Invitrogen) derived from Trichoplusia ni (U.S. Patent #5,300,435). Commercially available serum-free media are used to grow and maintain the cells. Suitable media are Sf900 II™ (Life Technologies) or ESF 921™ (Expression Systems) for the Sf9 cells; and Ex-cell10405™ (JRH Biosciences, Lenexa, KS) or Express FiveO™ (Life Technologies) for the T. ni cells. The cells are grown up from an inoculation density of approximately 2-5 x 10^5 cells to a density of 1-2 x 10^6 cells at which time a recombinant viral stock is added at a multiplicity of infection (MOI) of 0.1 to 10, more typically near 3. Procedures used are generally described in available laboratory manuals (King and Possee, ibid.; O'Reilly et
al., ibid.; Richardson, ibid.). Subsequent purification of the ZSIG-11 polypeptide from the supernatant can be achieved using methods described herein.

Fungal cells, including yeast cells, can also be used within the present invention. Yeast species of particular interest in this regard include *Saccharomyces cerevisiae*, *Pichia pastoris*, and *Pichia methanolica*. Methods for transforming *S. cerevisiae* cells with exogenous DNA and producing recombinant polypeptides therefrom are disclosed by, for example, Kawasaki, U.S. Patent No. 4,599,311; Kawasaki et al., U.S. Patent No. 4,931,373; Brake, U.S. Patent No. 4,870,008; Welch et al., U.S. Patent No. 5,037,743; and Murray et al., U.S. Patent No. 4,845,075. Transformed cells are selected by phenotype determined by the selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient (e.g., leucine). A preferred vector system for use in *Saccharomyces cerevisiae* is the POTI vector system disclosed by Kawasaki et al. (U.S. Patent No. 4,931,373), which allows transformed cells to be selected by growth in glucose-containing media. Suitable promoters and terminators for use in yeast include those from glycolytic enzyme genes (see, e.g., Kawasaki, U.S. Patent No. 4,599,311; Kingsman et al., U.S. Patent No. 4,615,974; and Bitter, U.S. Patent No. 4,977,092) and alcohol dehydrogenase genes. See also U.S. Patents Nos. 4,990,446; 5,063,154; 5,139,936 and 4,661,454. Transformation systems for other yeasts, including *Hansenula polymorpha*, *Schizosaccharomyces pombe*, *Kluyveromyces lactis*, *Kluyveromyces fragilis*, *Ustilago maydis*, *Pichia pastoris*, *Pichia methanolica*, *Pichia guilliermondii* and *Candida maltosa* are known in the art. See, for example, Gleeson et al., *J. Gen. Microbiol.* 132:3459-65, 1986 and Cregg, U.S. Patent No. 4,882,279. *Aspergillus* cells may be utilized according to the methods of McKnight et al., U.S. Patent No. 4,935,349. Methods
for transforming *Acremonium chrysogenum* are disclosed by Sumino et al., U.S. Patent No. 5,162,228. Methods for transforming *Neurospora* are disclosed by Lambowitz, U.S. Patent No. 4,486,533.

The use of *Pichia methanolica* as host for the production of recombinant proteins is disclosed in WIPO Publications WO 97/17450, WO 97/17451, WO 98/02536, and WO 98/02565. DNA molecules for use in transforming *P. methanolica* will commonly be prepared as double-stranded, circular plasmids, which are preferably linearized prior to transformation. For polypeptide production in *P. methanolica*, it is preferred that the promoter and terminator in the plasmid be that of a *P. methanolica* gene, such as a *P. methanolica* alcohol utilization gene (AUG1 or AUG2). Other useful promoters include those of the dihydroxyacetone synthase (DHAS), formate dehydrogenase (FMD), and catalase (CAT) genes. To facilitate integration of the DNA into the host chromosome, it is preferred to have the entire expression segment of the plasmid flanked at both ends by host DNA sequences. A preferred selectable marker for use in *Pichia methanolica* is a *P. methanolica* ADE2 gene, which encodes phosphoribosyl-5-aminoimidazole carboxylase (AIRC; EC 4.1.1.21), which allows ade2 host cells to grow in the absence of adenine. For large-scale, industrial processes where it is desirable to minimize the use of methanol, it is preferred to use host cells in which both methanol utilization genes (AUG1 and AUG2) are deleted. For production of secreted proteins, host cells deficient in vacuolar protease genes (PEP4 and PRB1) are preferred. Electroporation is used to facilitate the introduction of a plasmid containing DNA encoding a polypeptide of interest into *P. methanolica* cells. It is preferred to transform *P. methanolica* cells by electroporation using an exponentially decaying, pulsed electric field having a field strength of from 2.5 to 4.5 kV/cm, preferably about
3.75 kV/cm, and a time constant ($\tau$) of from 1 to 40 milliseconds, most preferably about 20 milliseconds.

Prokaryotic host cells, including strains of the bacteria *Escherichia coli*, *Bacillus* and other genera are also useful host cells within the present invention. Techniques for transforming these hosts and expressing foreign DNA sequences cloned therein are well known in the art (see, e.g., Sambrook et al., *ibid.*). When expressing a ZSIG-11 polypeptide in bacteria such as *E. coli*, the polypeptide may be retained in the cytoplasm, typically as insoluble granules, or may be directed to the periplasmic space by a bacterial secretion sequence. In the former case, the cells are lysed, and the granules are recovered and denatured using, for example, guanidine isothiocyanate or urea. The denatured polypeptide can then be refolded and dimerized by diluting the denaturant, such as by dialysis against a solution of urea and a combination of reduced and oxidized glutathione, followed by dialysis against a buffered saline solution. In the latter case, the polypeptide can be recovered from the periplasmic space in a soluble and functional form by disrupting the cells (by, for example, sonication or osmotic shock) to release the contents of the periplasmic space and recovering the protein, thereby obviating the need for denaturation and refolding.

Transformed or transfected host cells are cultured according to conventional procedures in a culture medium containing nutrients and other components required for the growth of the chosen host cells. A variety of suitable media, including defined media and complex media, are known in the art and generally include a carbon source, a nitrogen source, essential amino acids, vitamins and minerals. Media may also contain such components as growth factors or serum, as required. The growth medium will generally select for cells containing the exogenously added DNA by, for example, drug selection or deficiency in an essential nutrient which is complemented by the
Selectable marker carried on the expression vector or co-transfected into the host cell.

Expressed recombinant ZSIG-11 polypeptides (or chimeric or fused ZSIG-11 polypeptides) can be purified using fractionation and/or conventional purification methods and media. Ammonium sulfate precipitation and acid or chaotrope extraction may be used for fractionation of samples. Exemplary purification steps may include hydroxyapatite, size exclusion, FPLC and reverse-phase high performance liquid chromatography. Suitable anion exchange media include derivatized dextrans, agarose, cellulose, polyacrylamide, specialty silicas, and the like. PEI, DEAE, QAE and Q derivatives are preferred, with DEAE Fast-Flow Sepharose (Pharmacia, Piscataway, NJ) being particularly preferred. Exemplary chromatographic media include those media derivatized with phenyl, butyl, or octyl groups, such as Phenyl-Sepharose FF (Pharmacia), Toyopearl butyl 650 (Toso Haas, Montgomeryville, PA), Octyl-Sepharose (Pharmacia) and the like; or polyacrylic resins, such as Amberchrom CG 71 (Toso Haas) and the like. Suitable solid supports include glass beads, silica-based resins, cellulosic resins, agarose beads, cross-linked agarose beads, polystyrene beads, cross-linked polyacrylamide resins and the like that are insoluble under the conditions in which they are to be used. These supports may be modified with reactive groups that allow attachment of proteins by amino groups, carboxyl groups, sulfhydryl groups, hydroxyl groups and/or carbohydrate moieties. Examples of coupling chemistries include cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, hydrazide activation, and carboxyl and amino derivatives for carbodiimide coupling chemistries. These and other solid media are well known and widely used in the art, and are available from commercial suppliers. Methods for binding receptor polypeptides to support media are well known in the art. Selection of a particular method is a
matter of routine design and is determined in part by the properties of the chosen support. See, for example, *Affinity Chromatography: Principles & Methods*, Pharmacia LKB Biotechnology, Uppsala, Sweden, 1988.

The polypeptides of the present invention can be isolated by exploitation of their physical properties. For example, immobilized metal ion adsorption (IMAC) chromatography can be used to purify histidine-rich proteins. Briefly, a gel is first charged with divalent metal ions to form a chelate (E. Sulkowski, *Trends in Biochem.*, 3:1-7, 1985). Histidine-rich proteins will be adsorbed to this matrix with differing affinities, depending upon the metal ion used, and will be eluted by competitive elution, lowering the pH, or use of strong chelating agents. Other methods of purification include purification of glycosylated proteins by lectin affinity chromatography and ion exchange chromatography (*Methods in Enzymol.*, Vol. 182, "Guide to Protein Purification", M. Deutscher, (ed.), Acad. Press, San Diego, 1990, pp.529-39). Alternatively, a fusion of the polypeptide of interest and an affinity tag (e.g., polyhistidine, maltose-binding protein, an immunoglobulin domain) may be constructed to facilitate purification.

Protein refolding (and optionally reoxidation) procedures may be advantageously used. It is preferred to purify the protein to >80% purity, more preferably to >90% purity, even more preferably >95%, and particularly preferred is a pharmaceutically pure state, that is greater than 99.9% pure with respect to contaminating macromolecules, particularly other proteins and nucleic acids, and free of infectious and pyrogenic agents. Preferably, a purified protein is substantially free of other proteins, particularly other proteins of animal origin.

ZSIG-11 polypeptides or fragments thereof may also be prepared through chemical synthesis. ZSIG-11 polypeptides may be monomers or multimers; glycosylated or
non-glycosylated; pegylated or non-pegylated; and may or may not include an initial methionine amino acid residue. The presence of five cysteine residues (residues 68, 92, 94, 165 and 182 of SEQ ID NO:2) suggests that covalent disulfide bonds between ZSIG-11 monomers may be formed. The cysteine residues may also be indicative of up to two internal disulfide bonds in the ZIG-11 polypeptide structure. A combination of ZSIG-11 monomer-ZSIG-11 monomer disulfide bonds and ZSIG-11 polypeptide internal disulfide bonds may also be formed.

The invention also provides ZSIG-11 fusion or chimeric proteins with human Ig or as His-tagged proteins. To facilitate purification of the secreted ligand, a N-terminal or C-terminal extension, such as a poly-histidine tag, substance P, Flag\(^\text{TM}\) peptide (Hopp et al., *Biotechnology* 6:1204-10, 1988; available from Eastman Kodak Co., New Haven, CT) or another polypeptide or protein for which an antibody or other specific binding agent is available, can be fused to the ZSIG-11 polypeptide. In an alternative approach, ZSIG-11 polypeptides can be expressed as a fusion or chimeric proteins with immunoglobulin heavy chain constant regions, typically an F\(_c\) fragment, which contains two constant region domains and a hinge region, but lacks the variable region. Such chimera are typically secreted as multimeric molecules, wherein the F\(_c\) portions are disulfide bonded to each other and two ligand polypeptides are arrayed in close proximity to each other. Fusions of this type can be used to affinity purify the cognate receptor from solution, as an in vitro assay tool, and to block signals in vitro by specifically titrating out or blocking endogenous ligand. To purify soluble receptor, a ZSIG-11-Ig fusion protein (chimera) is added to a sample containing the soluble receptor under conditions that facilitate receptor-ligand binding (typically near-physiological temperature, pH, and ionic strength). The chimera-receptor complex is then separated from the
mixture using protein A, which is immobilized on a solid support (e.g., insoluble resin beads). The receptor is then eluted using conventional chemical techniques, such as with a salt or pH gradient. In the alternative, the chimera itself can be bound to a solid support, with binding and elution carried out as above. Collected fractions can be re-fractionated until the desired level of purity is reached. For use in assays, the chimeras are bound to a support via the Fc region and used in an ELISA format. Conversely, soluble receptor-Ig fusion proteins may be made using receptors for which a ligand has not been identified. ZSIG-11 is then mixed with a receptor fusion protein and binding is assayed as described above.

An assay system that uses a ligand-binding receptor (or an antibody, one member of a complement/anti-complement pair) or a binding fragment thereof, and a commercially available biosensor instrument (BIACore™, Pharmacia Biosensor, Piscataway, NJ) may be advantageously employed. Such receptor, antibody, member of a complement/anti-complement pair or fragment is immobilized onto the surface of a receptor chip. Use of this instrument is disclosed by Karlsson, J. Immunol. Methods 145:229-40, 1991 and Cunningham and Wells, J. Mol. Biol. 234:554-63, 1993. A receptor, antibody, member or fragment is covalently attached, using amine or sulphydryl chemistry, to dextran fibers that are attached to gold film within the flow cell. A test sample is passed through the cell. If a ligand, epitope, or opposite member of the complement/anti-complement pair is present in the sample, it will bind to the immobilized receptor, antibody or member, respectively, causing a change in the refractive index of the medium, which is detected as a change in surface plasmon resonance of the gold film. This system allows the determination of on- and off-rates, from which binding affinity can be calculated, and assessment of stoichiometry of binding.

ZSIG-11 is highly expressed in testis, with lesser expression in prostate, ovaries, placenta, thyroid, adrenal gland and brain, suggesting a role in regulation of the reproductive process. The high level of expression in testis may indicate an association with conditions related to testicular tissues. Proliferation and differentiation in vitro in response to administered ZSIG-11 polypeptides, agonists, antagonists, antibodies and binding proteins disclosed herein can be measured using cultured testicular cells or in vivo by administering molecules of the present invention to the appropriate animal model. Cultured testicular cells include dolphin DB1.Tes cells (CRL-6258); mouse GC-1 spg cells (CRL-2053); TM3 cells (CRL-1714); TM4 cells (CRL-1715); and pig ST
cells (CRL-1746), available from American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD.

ZSIG-11 may also show ability to suppress basal and LPS stimulated TNFα production from monocytes.

Administration of purified ZSIG-11 to mice resulted in significant increases in circulating levels of BUN, and decreases in phosphorus and albumin and point to changes in the renal system and/or cardiac system, as described in greater detail in the examples below. This could be a primary effect targeting kidney or heart. Histopathological analysis of tissues of treated mice indicated no significant effects on the tissues examined, including kidney, heart and liver.

In addition, increases in total white blood count were also observed. Lymphocytes, monocytes and neutrophils contributed to this increase, while eosinophils and basophils did not increase. Percentages of the various types of WBCs did not change significantly, but there was a trend towards an increase in percentage of monocytes, indicating a pro-inflammatory effect of the treatment with ZSIG-11. Increased cholesterol could be related to a pro-inflammatory effect or possibly a direct effect on lipoprotein production in the liver or uptake in peripheral tissues.

In vitro and in vivo response to ZSIG-11 can also be measured using cultured cells or by administering molecules of the claimed invention to the appropriate animal model. For instance, ZSIG-11 transfected expression host cells may be embedded in an alginate environment and injected (implanted) into recipient animals. Alginate-poly-L-lysine microencapsulation, permselective membrane encapsulation and diffusion chambers have been described as a means to entrap transfected mammalian cells or primary mammalian cells. These types of non-immunogenic "encapsulations" or microenvironments permit the transfer of nutrients into
the microenvironment, and also permit the diffusion of proteins and other macromolecules secreted or released by the captured cells across the environmental barrier to the recipient animal. Most importantly, the capsules or microenvironments mask and shield the foreign, embedded cells from the recipient animal's immune response. Such microenvironments can extend the life of the injected cells from a few hours or days (naked cells) to several weeks (embedded cells).

Alginate threads provide a simple and quick means for generating embedded cells. The materials needed to generate the alginate threads are readily available and relatively inexpensive. Once made, the alginate threads are relatively strong and durable, both in vitro and, based on data obtained using the threads, in vivo. The alginate threads are easily manipulable and the methodology is scalable for preparation of numerous threads. In an exemplary procedure, 3% alginate is prepared in sterile H₂O, and sterile filtered. Just prior to preparation of alginate threads, the alginate solution is again filtered. An approximately 50% cell suspension (containing about 5 x 10⁵ to about 5 x 10⁷ cells/ml) is mixed with the 3% alginate solution. One ml of the alginate/cell suspension is extruded into a 100 mM sterile filtered CaCl₂ solution over a time period of ~15 min, forming a "thread". The extruded thread is then transferred into a solution of 50 mM CaCl₂, and then into a solution of 25 mM CaCl₂. The thread is then rinsed with deionized water before coating the thread by incubating in a 0.01% solution of poly-L-lysine. Finally, the thread is rinsed with Lactated Ringer's Solution and drawn from solution into a syringe barrel (without needle attached). A large bore needle is then attached to the syringe, and the thread is intraperitoneally injected into a recipient in a minimal volume of the Lactated Ringer's Solution.

An alternative in vivo approach for assaying proteins of the present invention involves viral delivery
systems. Exemplary viruses for this purpose include adenovirus, herpesvirus, vaccinia virus and adeno-associated virus (AAV). Adenovirus, a double-stranded DNA virus, is currently the best studied gene transfer vector for delivery of heterologous nucleic acid (for a review, see T.C. Becker et al., Meth. Cell Biol. 43:161-89, 1994; and J.T. Douglas and D.T. Curiel, Science & Medicine 4:44-53, 1997). The adenovirus system offers several advantages: adenovirus can (i) accommodate relatively large DNA inserts; (ii) be grown to high-titer; (iii) infect a broad range of mammalian cell types; and (iv) be used with a large number of available vectors containing different promoters. Also, because adenoviruses are stable in the bloodstream, they can be administered by intravenous injection. Some disadvantages (especially for gene therapy) associated with adenovirus gene delivery include: (i) very low efficiency integration into the host genome; (ii) existence in primarily episomal form; and (iii) the host immune response to the administered virus, precluding readministration of the adenoviral vector.

By deleting portions of the adenovirus genome, larger inserts (up to 7 kb) of heterologous DNA can be accommodated. These inserts may be incorporated into the viral DNA by direct ligation or by homologous recombination with a co-transfected plasmid. In an exemplary system, the essential E1 gene has been deleted from the viral vector, and the virus will not replicate unless the E1 gene is provided by the host cell (i.e., the human 293 cell line). When intravenously administered to intact animals, adenovirus primarily targets the liver. If the adenoviral delivery system has an E1 gene deletion, the virus cannot replicate in the host cells. However, the host's tissue (i.e., liver) will express and process (and, if a signal sequence is present, secrete) the heterologous protein. Secreted proteins will enter the circulation in the highly vascularized liver, and effects on the infected animal can be determined.
The adenovirus system can also be used for protein production in vitro. By culturing adenovirus-infected non-293 cells under conditions where the cells are not rapidly dividing, the cells can produce proteins for extended periods of time. For instance, BHK cells are grown to confluence in cell factories, then exposed to the adenoviral vector encoding the secreted protein of interest. The cells are then grown under serum-free conditions, which allows infected cells to survive for several weeks without significant cell division. Alternatively, adenovirus vector infected 293S cells can be grown in suspension culture at relatively high cell density to produce significant amounts of protein (see A. Garnier et al., *Cytotechnol.*, 15:145-55, 1994). With either protocol, an expressed, secreted heterologous protein can be repeatedly isolated from the cell culture supernatant. Within the infected 293S cell production protocol, non-secreted proteins may also be effectively obtained.

ZSIG-11 polypeptides can also be used to prepare antibodies that specifically bind to ZSIG-11 epitopes, peptides or polypeptides. Methods for preparing polyclonal and monoclonal antibodies are well known in the art (see, for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual, Second Edition*, Cold Spring Harbor, NY, 1989; and Hurrell, J. G. R., Ed., *Monoclonal Hybridoma Antibodies: Techniques and Applications*, CRC Press, Inc., Boca Raton, FL, 1982). As would be evident to one of ordinary skill in the art, polyclonal antibodies can be generated from a variety of warm-blooded animals, such as horses, cows, goats, sheep, dogs, chickens, rabbits, mice, and rats.

The immunogenicity of a ZSIG-11 polypeptide may be increased through the use of an adjuvant, such as alum (aluminum hydroxide) or Freund's complete or incomplete adjuvant. Polypeptides useful for immunization also include fusion polypeptides, such as fusions of ZSIG-11 or
a portion thereof with an immunoglobulin polypeptide or with maltose binding protein. The polypeptide immunogen may be a full-length molecule or a portion thereof. If the polypeptide portion is "hapten-like", such portion may be advantageously joined or linked to a macromolecular carrier (such as keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA) or tetanus toxoid) for immunization.

As used herein, the term "antibodies" includes polyclonal antibodies, affinity-purified polyclonal antibodies, monoclonal antibodies, and antigen-binding fragments thereof, such as F(ab')2, F(ab)2, Fab', Fab, Fv and scFv proteolytic fragments. Such antibody fragments can be obtained, for example, by proteolytic hydrolysis of the antibody. Antibody fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional methods. As an illustration, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted F(ab')2. This fragment can be further cleaved using a thiol reducing agent to produce 3.5S Fab' monovalent fragments. Optionally, the cleavage reaction can be performed using a blocking group for the sulfhydryl groups that result from cleavage of disulfide linkages. As an alternative, an enzymatic cleavage using pepsin produces two monovalent Fab fragments and an Fc fragment directly. These methods are described, for example, by Goldenberg, U.S. patent No. 4,331,647, Nisonoff et al., Arch Biochem. Biophys. 89:230, 1960; Porter, Biochem. J. 73:119, 1959; Edelman et al., in Methods in Enzymology Vol. 1, page 422, Academic Press, 1967; and by Coligan Coligan et al. (eds.), Current Protocols in Immunology, pages 2.8.1-2.8.10 and 2.10.1-2.10.4, John Wiley & Sons, 1997.

Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical or genetic techniques may also be
used, so long as the fragments bind to the antigen that is recognized by the intact antibody.

Genetically engineered intact antibodies or fragments, such as chimeric antibodies, Fv fragments, single chain antibodies and the like, as well as synthetic antigen-binding peptides and polypeptides, are also included. Non-human antibodies may be humanized by grafting only non-human CDRs onto human framework and constant regions, or by incorporating the entire non-human variable domains (optionally "cloaking" them with a human-like surface by replacement of exposed residues, wherein the result is a "veneered" antibody). In some instances, humanized antibodies may retain non-human residues within the human variable region framework domains to enhance proper binding characteristics. Through humanizing antibodies, biological half-life may be increased, and the potential for adverse immune reactions upon administration to humans is reduced. Humanized monoclonal antibodies directed against ZSIG-11 polypeptides could be used as a protein therapeutic, in particular for use as an immunotherapy. Alternative techniques for generating or selecting antibodies useful herein include in vitro exposure of lymphocytes to ZSIG-11 protein or peptide, and selection of antibody display libraries in phage or similar vectors (for instance, through use of immobilized or labeled ZSIG-11 protein or peptide).

ZSIG-11 polypeptides can also be used to prepare antibodies that specifically bind to ZSIG-11 epitopes, peptides or polypeptides. Antibodies are determined to be specifically binding if: 1) they exhibit a threshold level of binding activity, and/or 2) they do not significantly cross-react with related polypeptide molecules. Antibodies herein specifically bind if they bind to a ZSIG-11 polypeptide, peptide or epitope with a binding affinity ($K_a$) of $10^6$ M$^{-1}$ or greater, preferably $10^7$ M$^{-1}$ or greater, more preferably $10^8$ M$^{-1}$ or greater, and most preferably $10^9$ M$^{-1}$ or greater. The binding affinity of an
antibody can be readily determined by one of ordinary skill in the art, for example, by Scatchard analysis (Scatchard, G., Ann. NY Acad. Sci. 51: 660-72, 1949). Antibodies specifically bind if they do not significantly cross-react with related polypeptides. Antibodies do not significantly cross-react with related polypeptide molecules, for example, if they detect ZSIG-11 but not known related polypeptides using a standard Western blot analysis (Ausubel et al., ibid.). Examples of known related polypeptides are orthologs, proteins from the same species that are members of a protein family such as other known secreted proteins, mutant ZSIG-11 polypeptides, and related non-human secreted proteins. Moreover, antibodies may be "screened against" known related polypeptides to isolate a population that specifically binds ZSIG-11 polypeptides. For example, antibodies raised to ZSIG-11 are adsorbed to related polypeptides adhered to insoluble matrix; antibodies specific to ZSIG-11 will flow through the matrix under the proper buffer conditions. Such screening allows isolation of polyclonal and monoclonal antibodies non-crossreactive to closely related polypeptides (Antibodies: A Laboratory Manual, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988; Current Protocols in Immunology, Coligan, et al. (eds.), National Institutes of Health, John Wiley and Sons, Inc., 1995). Screening and isolation of specific antibodies is well known in the art (see, Fundamental Immunology, Paul (eds.), Raven Press, 1993; Getzoff et al., Adv. in Immunol. 42: 1-98, 1988; Monoclonal Antibodies: Principles and Practice, Goding, J.W. (eds.), Academic Press Ltd., 1996; Benjamin et al., Ann. Rev. Immunol. 2: 67-101, 1984).

Genes encoding polypeptides having potential ZSIG-11 binding domains can be obtained by screening random peptide libraries displayed on phage (phage display) or on bacteria, such as E. coli. Nucleotide sequences encoding the polypeptides can be obtained in a
number of ways, such as through random mutagenesis and random polynucleotide synthesis. These random peptide display libraries can be used to screen for peptides which interact with a known target which can be a protein or polypeptide, such as a ligand or receptor, a biological or synthetic macromolecule, or organic or inorganic substances. Techniques for creating and screening such random peptide display libraries are known in the art (Ladner et al., US Patent No:5,223,409; Ladner et al., US Patent No:4,946,778; Ladner et al., US Patent No:5,403,484 and Ladner et al., US Patent No:5,571,698) and random peptide display libraries and kits for screening such libraries are available commercially, for instance from Clontech (Palo Alto, CA), Invitrogen Inc. (San Diego, CA), New England Biolabs, Inc. (Beverly, MA) and Pharmacia LKB Biotechnology Inc. (Piscataway, NJ). Random peptide display libraries can be screened using the ZSIG-11 sequences disclosed herein to identify proteins which bind to ZSIG-11. These "binding proteins" which interact with ZSIG-11 polypeptides may be used for tagging cells; for isolating homolog polypeptides by affinity purification; they can be directly or indirectly conjugated to drugs, toxins, radionuclides and the like. These binding proteins can also be used in analytical methods such as for screening expression libraries and neutralizing ZSIG-11 activity. The binding proteins can also be used for diagnostic assays for determining circulating levels of polypeptides; for detecting or quantitating soluble polypeptides as marker of underlying pathology or disease.

A variety of assays known to those skilled in the art can be utilized to detect antibodies and binding proteins which specifically bind to ZSIG-11 proteins or peptides. Exemplary assays are described in detail in Antibodies: A Laboratory Manual, Harlow and Lane (Eds.), Cold Spring Harbor Laboratory Press, 1988. Representative examples of such assays include: concurrent immunoelectrophoresis, radioimmunoassay, radioimmuno-
precipitation, ELISA, dot blot or Western blot assay, inhibition or competition assay, and sandwich assay. In addition, antibodies can be screened for binding to wild-type versus mutant ZSIG-11 protein or peptide.

Antibodies to ZSIG-11 may be used for tagging or labeling cells. Suitable direct tags or labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent markers, chemiluminescent markers, magnetic particles and the like; indirect tags or labels may feature use of biotin-avidin or other complement/anti-complement pairs as intermediates. Uses of such tagged antibodies include immunologically tagging with a fluorescent tag for use in FACS analysis for example, and immunohistochemical tagging of cells that express human ZSIG-11 which can be used as diagnostic assays. Antibodies may also be used for isolating ZSIG-11 by affinity purification; for screening expression libraries; for generating anti-idiotypic antibodies; and as neutralizing antibodies or as antagonists to block ZSIG-11 in vitro and in vivo. Antibodies herein may also be directly or indirectly conjugated to drugs, toxins, radionuclides and the like, and these conjugates used for in vivo diagnostic or therapeutic applications.

Provided are in vitro assay methods for detecting the presence of ZSIG-11 in a biological sample, comprising the steps of: a) contacting the biological sample with an antibody, or an antibody fragment, that specifically binds with a polypeptide having the amino acid sequence of SEQ ID NO:2, wherein the contacting is performed under conditions that allow the binding of the antibody or antibody fragment to the biological sample, and b) detecting any of the bound antibody or bound antibody fragment.

In one type of in vitro assay, anti-ZSIG-11 antibodies are used in liquid phase. For example, the presence of ZSIG-11 in a biological sample can be tested by
mixing the biological sample with a trace amount of labeled ZSIG-11 and an anti-ZSIG-11 antibody under conditions that promote binding between ZSIG-11 and its antibody. Complexes of ZSIG-11 and anti-ZSIG-11 in the sample can be separated from the reaction mixture by contacting the complex with an immobilized protein which binds with the antibody, such as an Fc antibody or Staphylococcus protein A. The concentration of ZSIG-11 in the biological sample will be inversely proportional to the amount of labeled ZSIG-11 bound to the antibody and directly related to the amount of free labeled ZSIG-11.

Alternatively, in vitro assays can be performed in which anti-ZSIG-11 antibody is bound to a solid-phase carrier. For example, antibody can be attached to a polymer, such as aminodextran, in order to link the antibody to an insoluble support such as a polymer-coated bead, a plate or a tube. Other suitable in vitro assays will be readily apparent to those of skill in the art.

In another approach, anti-ZSIG-11 antibodies can be used to detect ZSIG-11 in tissue sections prepared from a biopsy specimen. Such immunochemical detection can be used to determine the relative abundance of ZSIG-11 and to determine the distribution of ZSIG-11 in the examined tissue. General immunochemistry techniques are well established (see, for example, Ponder, "Cell Marking Techniques and Their Application," in Mammalian Development: A Practical Approach, Monk (ed.), pages 115-38, IRL Press 1987; Coligan ibid.; Ausubel ibid.; and Manson (ed.), Methods In Molecular Biology, Vol.10: Immunochemical Protocols, The Humana Press, Inc., 1992).

Immunocchemical detection can be performed by contacting a biological sample with an anti-ZSIG-11 antibody, and then contacting the biological sample with a detectably labeled molecule which binds to the antibody. For example, the detectably labeled molecule can comprise an antibody moiety that binds to anti-ZSIG-11 antibody.
Alternatively, the anti-ZSIG-11 antibody can be conjugated with avidin/streptavidin (or biotin) and the detectably labeled molecule can comprise biotin (or avidin/streptavidin). Numerous variations of this basic technique are well-known to those of skill in the art.

Alternatively, an anti-ZSIG-11 antibody can be conjugated with a detectable label to form an anti-ZSIG-11 immunoconjugate. Suitable detectable labels include, for example, a radioisotope, a fluorescent label, a chemiluminescent label, an enzyme label, a bioluminescent label or colloidal gold. Methods of making and detecting such detectably-labeled immunoconjugates are well-known to those of ordinary skill in the art, and are described in more detail below.

The detectable label can be a radioisotope that is detected by autoradiography. Isotopes that are particularly useful for the purpose of the present invention are $^3$H, $^{125}$I, $^{131}$I, $^{35}$S and $^{14}$C.

Anti-ZSIG-11 immunoconjugates can also be labeled with a fluorescent compound. The presence of a fluorescently-labeled antibody is determined by exposing the immunoconjugate to light of the proper wavelength and detecting the resultant fluorescence. Fluorescent labeling compounds include fluorescein isothiocyanate, rhodamine, phycoerytherin, phycocyanin, allophycocyanin, o-phthalaldehyde and fluorescamine.

Alternatively, anti-ZSIG-11 immunoconjugates can be detectably labeled by coupling an antibody component to a chemiluminescent compound. The presence of the chemiluminescent-tagged immunoconjugate is determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of chemiluminescent labeling compounds include luminol, isoluminol, an aromatic acridinium ester, an imidazole, an acridinium salt and an oxalate ester.

Similarly, a bioluminescent compound can be used to label anti-ZSIG-11 immunoconjugates of the present
invention. Bioluminescence is a type of chemiluminescence found in biological systems in which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Bioluminescent compounds that are useful for labeling include luciferin, luciferase and aequorin.

Alternatively, anti-ZSIG-11 immunoconjugates can be detectably labeled by linking an anti-ZSIG-11 antibody component to an enzyme. When the anti-ZSIG-11-enzyme conjugate is incubated in the presence of the appropriate substrate, the enzyme moiety reacts with the substrate to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorometric or visual means. Examples of enzymes that can be used to detectably label polyspecific immunoconjugates include β-galactosidase, glucose oxidase, peroxidase and alkaline phosphatase.

Those of skill in the art will know of other suitable labels which can be employed in accordance with the present invention. The binding of marker moieties to anti-ZSIG-11 antibodies can be accomplished using standard techniques known to the art. Typical methodology in this regard is described by Kennedy et al., Clin. Chim. Acta 70:1, 1976; Schurs et al., Clin. Chim. Acta 81:1, 1977; Shih et al., Intl. J. Cancer 46:1101, 1990; Stein et al., Cancer Res. 50:1330,1990; and Coligan, supra.

Moreover, the convenience and versatility of immunochemical detection can be enhanced by using anti-ZSIG-11 antibodies that have been conjugated with avidin, streptavidin, and biotin (see, for example, Wilchek et al. (eds.), "Avidin-Biotin Technology," Methods In Enzymology, Vol. 184 (Academic Press 1990), and Bayer et al., "Immunochemical Applications of Avidin-Biotin Technology,"


The present invention also contemplates the use of immunoconjugates for in vivo detection of ZSIG-11 polypeptide. As an illustration, the method of diagnostic imaging with radiolabeled monoclonal antibodies is well-known. Examples of radioisotopes that can be bound to antibodies and are appropriate for diagnostic imaging include K-emitters and positron-emitters such as $^{99m}$Tc, $^{94}$Tc, $^{67}$Ga, $^{64}$Cu, $^{111}$In, $^{123}$I, $^{124}$I, $^{125}$I, $^{131}$I, $^{51}$Cr, $^{89}$Zr, $^{18}$F and $^{68}$Ga. Other suitable radioisotopes are known to those of skill in the art. Methods for performing immunoscintigraphy are described, for example, by Srivastava (ed.), Radiolabeled Monoclonal Antibodies For Imaging And Therapy (Plenum Press 1988), Chase, "Medical Applications of Radioisotopes," in Remington's Pharmaceutical Sciences, 18th Edition, Gennaro et al. (eds.), pp. 624-652 (Mack Publishing Co., 1990), and Brown, "Clinical Use of Monoclonal Antibodies," in Biotechnology and Pharmacy, pages 227-49, Pezzuto et al. (eds.) (Chapman & Hall 1993).

Anti-ZSIG-11 antibodies also can be labeled with paramagnetic ions for purposes of in vivo diagnosis. Elements that are particularly useful for magnetic resonance imaging include Gd, Mn, Dy and Fe ions.
ZSIG-11 ligand polypeptides may be used to identify and characterize receptors which bind ZSIG-11 polypeptides. Proteins and peptides of the present invention can be immobilized on a column and membrane preparations run over the column (Im mobilized Affinity Ligand Techniques, Hermanson et al., eds., Academic Press, San Diego, CA, 1992, 195-202). Proteins and peptides can also be radiolabeled or photoaffinity labeled as described above and specific cell-surface proteins can be identified. The soluble ligand is useful in studying the distribution of receptors on tissues or specific cell lineages, and to provide insight into receptor/ligand biology.

The invention also provides isolated and purified ZSIG-11 polynucleotide probes. Such polynucleotide probes can be RNA or DNA. DNA can be either cDNA or genomic DNA. Polynucleotide probes are single or double-stranded DNA or RNA, generally synthetic oligonucleotides, but may be generated from cloned cDNA or genomic sequences and will generally comprise at least 16 nucleotides, more often from 17 nucleotides to 25 or more nucleotides, sometimes 40 to 60 nucleotides, and in some instances a substantial portion, domain or even the entire ZSIG-11 gene or cDNA. The synthetic oligonucleotides of the present invention have at least 80% identity to a representative ZSIG-11 DNA sequence (SEQ ID NO:1) or its complement. Preferred regions from which to construct probes include the 5' and/or 3' coding sequences, receptor binding regions, signal sequences and the like.

Techniques for developing polynucleotide probes and hybridization techniques are known in the art, see for example, Ausubel et al., eds., Current Protocols in Molecular Biology, John Wiley and Sons, Inc., NY, 1991. For use as probes, the molecules can be labeled to provide a detectable signal, such as with an enzyme, biotin, a radionuclide, fluorophore, chemiluminescer, paramagnetic particle and the like, which are commercially available.
from many sources, such as Molecular Probes, Inc., Eugene, OR, and Amersham Corp., Arlington Heights, IL, using techniques that are well known in the art.

Such probes can also be used in hybridizations to detect the presence or quantify the amount of ZSIG-11 gene or mRNA transcript in a sample. ZSIG-11 polynucleotide probes could be used to hybridize to DNA or RNA targets for diagnostic purposes, using such techniques such as fluorescent in situ hybridization (FISH) or immunohistochemistry. Polynucleotide probes could be used to identify genes encoding ZSIG-11-like proteins. Such probes can also be used to screen libraries for related ZSIG-11 sequences. Such screening would be carried out under conditions of low stringency which would allow identification of sequences which are substantially homologous, but not requiring complete homology to the probe sequence. Such methods and conditions are well known in the art, see, for example, Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, NY, 1989. Such stringency conditions are described herein. Libraries may be made of genomic DNA or cDNA. Polynucleotide probes are also useful for Southern, Northern, or slot blots, colony and plaque hybridization and in situ hybridization. Mixtures of different ZSIG-11 polynucleotide probes can be prepared which would increase sensitivity or the detection of low copy number targets, in screening systems.

Nucleic acid molecules can be used to detect the expression of a ZSIG-11 gene in a biological sample. In a basic assay, a single-stranded probe molecule is incubated with RNA, isolated from a biological sample, under conditions of temperature and ionic strength that promote base pairing between the probe and target ZSIG-11 RNA species. After separating unbound probe from hybridized molecules, the amount of hybrids is detected.
A method of detecting the presence of ZSIG-11 RNA in a biological sample is provided, comprising the steps of:

a) contacting a ZSIG-11 nucleic acid probe under stringent hybridizing conditions with either
   i) test RNA molecules isolated from the biological sample, or
   ii) nucleic acid molecules synthesized from the isolated RNA molecules,

wherein the probe has a nucleotide sequence comprising a portion of the nucleotide sequence of SEQ ID NO:1, or its complement, and

b) detecting the formation of hybrids of the nucleic acid probe and either the test RNA molecules or the synthesized nucleic acid molecules,

wherein the presence of the hybrids indicates the presence of ZSIG-11 RNA is the biological sample.

Well-established hybridization methods of RNA detection include northern analysis and dot/slot blot hybridization (see, for example, Ausubel *ibid.* at pages 4-1 to 4-27, and Wu et al. (eds.), "Analysis of Gene Expression at the RNA Level," in *Methods in Gene Biotechnology*, pages 225-239, CRC Press, Inc., 1997). Nucleic acid probes can be detectably labeled with radioisotopes such as $^{32}$P or $^{35}$S. Alternatively, ZSIG-11 RNA can be detected with a nonradioactive hybridization method (see, for example, Isaac (ed.), *Protocols for Nucleic Acid Analysis by Nonradioactive Probes*, Humana Press, Inc., 1993). Typically, nonradioactive detection is achieved by enzymatic conversion of chromogenic or chemiluminescent substrates. Illustrative nonradioactive moieties include biotin, fluorescein, and digoxigenin.

ZSIG-11 oligonucleotide probes are also useful for in vivo diagnosis. As an illustration, $^{18}$F-labeled oligonucleotides can be administered to a subject and visualized by positron emission tomography (Tavitian et al., *Nature Medicine* 4:467, 1998).

One variation of PCR for diagnostic assays is reverse transcriptase-PCR (RT-PCR). In the RT-PCR technique, RNA is isolated from a biological sample, reverse transcribed to cDNA, and the cDNA is incubated with ZSIG-11 primers (see, for example, Wu et al. (eds.), "Rapid Isolation of Specific cDNAs or Genes by PCR," in Methods in Gene Biotechnology, pages 15-28, CRC Press, Inc. 1997). PCR is then performed and the products are analyzed using standard techniques.

As an illustration, RNA is isolated from a biological sample using, for example, the guanidinium-thiocyanate cell lysis procedure described above. Alternatively, a solid-phase technique can be used to isolate mRNA from a cell lysate. A reverse transcription reaction can be primed with the isolated RNA using random oligonucleotides, short homopolymers of dT, or ZSIG-11 anti-sense oligomers. Oligo-dT primers offer the advantage that various mRNA nucleotide sequences are amplified that can provide control target sequences. ZSIG-11 sequences are amplified by the polymerase chain
reaction using two flanking oligonucleotide primers that are typically 20 bases in length.

PCR amplification products can be detected using a variety of approaches. For example, PCR products can be fractionated by gel electrophoresis, and visualized by ethidium bromide staining. Alternatively, fractionated PCR products can be transferred to a membrane, hybridized with a detectably-labeled ZSIG-11 probe, and examined by autoradiography. Additional alternative approaches include the use of digoxigenin-labeled deoxyribonucleic acid triphosphates to provide chemiluminescence detection, and the C-TRAK colorimetric assay.

Another approach for detection of ZSIG-11 expression is cycling probe technology (CPT), in which a single-stranded DNA target binds with an excess of DNA-RNA-DNA chimeric probe to form a complex, the RNA portion is cleaved with RNase H, and the presence of cleaved chimeric probe is detected (see, for example, Beggs et al., J. Clin. Microbiol. 34:2985, 1996 and Bekkaoui et al., Biotechniques 20:240, 1996). Alternative methods for detection of ZSIG-11 sequences can utilize approaches such as nucleic acid sequence-based amplification (NASBA), cooperative amplification of templates by cross-hybridization (CATCH), and the ligase chain reaction (LCR) (see, for example, Marshall et al., U.S. Patent No. 5,686,272 (1997), Dyer et al., J. Virol. Methods 60:161, 1996; Ehrlich et al., Eur. J. Biochem. 243:358, 1997; and Chadwick et al., J. Virol. Methods 70:59, 1998). Other standard methods are known to those of skill in the art.

ZSIG-11 probes and primers can also be used to detect and to localize ZSIG-11 gene expression in tissue samples. Methods for such in situ hybridization are well-known to those of skill in the art (see, for example, Choo (ed.), In Situ Hybridization Protocols, Humana Press, Inc., 1994; Wu et al. (eds.), "Analysis of Cellular DNA or Abundance of mRNA by Radioactive In Situ Hybridization

Various additional diagnostic approaches are well-known to those of skill in the art (see, for example, Mathew (ed.), Protocols in Human Molecular Genetics, Humana Press, Inc., 1991; Coleman and Tsongalis, Molecular Diagnostics, Humana Press, Inc., 1996; and Elles, Molecular Diagnosis of Genetic Diseases, Humana Press, Inc., 1996).

Radiation hybrid mapping is a somatic cell genetic technique developed for constructing high-resolution, contiguous maps of mammalian chromosomes (Cox et al., Science 250:245-50, 1990). Partial or full knowledge of a gene's sequence allows the designing of PCR primers suitable for use with chromosomal radiation hybrid mapping panels. Commercially available radiation hybrid mapping panels which cover the entire human genome, such as the Stanford G3 RH Panel and the GeneBridge 4 RH Panel (Research Genetics, Inc., Huntsville, AL), are available. These panels enable rapid, PCR based, chromosomal localizations and ordering of genes, sequence-tagged sites (STTs), and other nonpolymorphic- and polymorphic markers within a region of interest. This includes establishing directly proportional physical distances between newly discovered genes of interest and previously mapped markers. The precise knowledge of a gene's position can be useful in a number of ways including: 1) determining if a sequence is part of an existing contig and obtaining additional surrounding genetic sequences in various forms such as YAC-, BAC- or cDNA clones, 2) providing a possible candidate gene for an inheritable disease which shows linkage to the same chromosomal region, and 3) for cross-referencing model organisms such as mouse which may be beneficial in helping to determine what function a
particular gene might have. ZSIG-11 gene maps 252.51 cr_3000 from the top of the human chromosome 20 linkage group on the WICGR radiation hybrid map. Proximal and distal framework markers were D20S908 and D20S99, respectively. This positions ZSIG-11 in the 20q13.12 region on the integrated LDB chromosome 20 map.

ZSIG-11 oligonucleotide probes can be used to determine if the ZSIG-11 gene is present on chromosome 20 or if a mutation has occurred. Detectable chromosomal aberrations at the ZSIG-11 gene locus include, but are not limited to, aneuploidy, gene copy number changes, insertions, deletions, restriction site changes and rearrangements. Such aberrations can be detected using polynucleotides of the present invention by employing molecular genetic techniques, such as restriction fragment length polymorphism (RFLP) analysis, short tandem repeat (STR) analysis employing PCR techniques, and other genetic linkage analysis techniques known in the art (Sambrook et al., ibid.; Ausubel et. al., ibid.; A.J. Marian, Chest 108:255-65, 1995).

ZSIG-11 polypeptides may be used within diagnostic systems. Antibodies or other agents that specifically bind to ZSIG-11 may be used to detect the presence of circulating ZSIG-11 polypeptides and/or receptors. More specifically, the present invention contemplates methods for detecting ZSIG-11 polypeptides comprising:

exposing a solution possibly containing ZSIG-11 polypeptide to an antibody attached to a solid support, wherein the antibody binds to a first epitope of a ZSIG-11 polypeptide;

washing the immobilized antibody-polypeptide to remove unbound contaminants;

exposing the immobilized antibody-polypeptide to a second antibody directed to a second epitope of a ZSIG-11 polypeptide-antibody complex, wherein the second antibody is associated with a detectable label; and
detecting the detectable label. Serum or biopsy ZSIG-11 polypeptide concentration (relative to normal serum or tissue concentration) may be indicative of dysfunction related to altered levels of ZSIG-11 polypeptides.

Other acceptable detection methods are well known in the art and include, for example, enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay. Immunohistochemically labeled antibodies can be used to detect ZSIG-11 ligand in tissue samples. ZSIG-11 levels can also be monitored by such methods as RT-PCR, where ZSIG-11 mRNA can be detected and quantified. Such methods could be used as diagnostic tools to monitor and quantify receptor or ligand polypeptide levels. The information derived from such detection methods would provide insight into the significance of ZSIG-11 polypeptides in various diseases, and as such would serve as diagnostic methods for those diseases in which altered levels of ZSIG-11 are significant.

The present invention also provides methods for studying known or identifying new prohormone convertases, or endoproteases, enzymes which process prohormones and protein precursors. Precursor proteins are cleaved or processed into active form through the action of prohormone convertases (endoproteases). The most prevalent cleavage or processing site is a dibasic amino acid prohormone convertase site. There are only a few dibasic amino acid combinations, including lys-lys, arg-arg, arg-lys and lys-arg. Non-dibasic cleavage and processing sites have also been observed, for example, Asn-Arg is a non-dibasic site found in gastrin. ZSIG-11 polypeptides may be processed by prohormone convertases into an active form. Known prohormone convertases include, but are not limited to, prohormone convertase 3 (PC3), prohormone convertase 2 (PC2), furin, or similar convertases of the furin family such as prohormone convertase 4 (PC4) and PACE4.
Prohormone convertases sometimes exhibit tissue specificity. As a result, ZSIG-11 polypeptides, which are expressed at high levels in testis, for example, are likely to be processed by prohormone convertases exhibiting testis specificity. In such methods of the present invention, ZSIG-11 polypeptides or fragments (substrate) may be incubated with known or suspected prohormone convertases (enzyme) to produce a 122 amino acid residue fragment from amino acid residue 26 to amino acid residue 148, a 10 amino acid fragment from amino acid residue 151 to amino acid residue 161, and a 150 amino acid fragment from amino acid residue 163 to amino acid residue 313 of SEQ ID NO:2. The enzyme and substrate are incubated together or co-expressed in a test cell for a time sufficient to achieve cleavage/processing of the ZSIG-11 polypeptide or fragment or fusion thereof. Detection and/or quantification of cleavage products follows, using procedures that are known in the art. For example, enzyme kinetics techniques, measuring the rate of cleavage, can be used to study or identify prohormone convertases capable of cleaving ZSIG-11 polypeptides, fragments or fusion proteins of the present invention.

Therefore the present invention also provides post-translationally modified polypeptides or polypeptide fragments having the amino acid sequence from amino acid residue 26 to amino acid residue 148 of SEQ ID NO:2; the amino acid sequence from amino acid residue 151 to amino acid residue 161 of SEQ ID NO:2 and the amino acid sequence from amino acid residue 163 to amino acid residue 313 of SEQ ID NO:2. Examples of post translational modifications include proteolytic cleavage, glycosylation, disulfide bonding and hydroxylation.

The present invention also provides antisense polynucleotide compositions that are complementary to a segment of the polynucleotides set forth in SEQ ID NO: 1. Such synthetic antisense oligonucleotides are designed to bind to mRNA encoding ZSIG-11 polypeptides and to inhibit
translation of such mRNA. Such antisense oligonucleotides are used to inhibit expression of ZSIG-11 polypeptide-encoding genes in cell culture or in a subject.

Transgenic mice, engineered to express the ZSIG-11 gene, and mice that exhibit a complete absence of ZSIG-11 gene function, referred to as "knockout mice" (Snouwaert et al., Science 257:1083, 1992), may also be generated (Lowell et al., Nature 366:740-2, 1993). These mice may be employed to study the ZSIG-11 gene and the protein encoded thereby in an in vivo system.

The invention also provides antagonists, which either bind to ZSIG-11 polypeptides or, alternatively, to a receptor to which ZSIG-11 polypeptides bind, thereby inhibiting or eliminating the function of ZSIG-11. Such ZSIG-11 antagonists would include antibodies; oligonucleotides which bind either to the ZSIG-11 polypeptide or to its receptor; natural or synthetic analogs of ZSIG-11 polypeptides which retain the ability to bind the receptor but do not result in either ligand or receptor signaling. Such analogs could be peptides or peptide-like compounds. One such peptide would comprise amino acid residues 26 to 214 of SEQ ID NO:2 which contains the five cysteine residues. Natural or synthetic small molecules which bind to receptors of ZSIG-11 polypeptides and prevent signaling are also contemplated as antagonists. As such, ZSIG-11 antagonists would be useful as therapeutics for treating certain disorders where blocking signal from either a ZSIG-11 ligand or receptor would be beneficial.

The ZSIG-11 polynucleotides and/or polypeptides disclosed herein can be useful as therapeutics, wherein ZSIG-11 agonists (including ZSIG-11 polypeptides (such as a polypeptide comprising an amino acid sequence from amino acid residue 24 to 214 of SEQ ID NO:2), substrates, cofactors, and the like) and antagonists could modulate one or more biological processes in cells, tissues and/or biological fluids. ZSIG-11 is expressed in a wide variety
of tissues, especially those of the endocrine system. The ZSIG-11 polypeptides, agonists and antagonists could be employed in therapeutic protocols for treatment of growth, metabolism and/or reproduction related disorders. For example, ZSIG-11 polypeptides or ZSIG-11 antagonists may be useful as a fertility inducing therapeutic. Such polypeptides and antagonists could be used in methods of assisted reproduction, such as artificial insemination or in vitro fertilization in humans and animals, to enhance the likelihood of successful fertilization. *In vivo*, ZSIG-11 polypeptides or antagonists would find application in the treatment of infertility. The high level of expression of ZSIG-11 in the testis suggests a role in spermatogenesis and/or sperm maturation, perhaps as a proliferation or differentiation factor. ZSIG-11 is also expressed in ovaries. Regulation of reproductive function in males and females is controlled in part by feedback inhibition of the hypothalamus and anterior pituitary by blood-bone hormones. Testis proteins, such as activins and inhibins, have been shown to regulate secretion of active molecules including follicle stimulating hormone (FSH) for the pituitary (Ying, *Endocr. Rev.* 9:267-93, 1988; Plant et al., *Hum. Reprod.* 8:41-44, 1993). Inhibins, also expressed in the ovaries, have also been shown to regulate ovarian functions (Woodruff et al., *Endocr.* 132:2332-42, 1993; Russell et al., *J. Reprod. Fertil.* 100:115-22, 1994). Relaxin has also been shown to be a systemic and local acting hormone regulating follicular and uterine growth (Bagnell et al., *J. Reprod. Fertil.* 48:127-38, 1993), as such, the polypeptides of the present invention may also have effects on female gametes and reproductive tract. These functions may also be associated with the ZSIG-11 proteins and may be used to regulate testicular or ovarian functions.

The invention also provides nucleic acid-based therapeutic treatment. If a mammal has a mutated or lacks a ZSIG-11 gene, the ZSIG-11 gene can be introduced into
the cells of the mammal. In one embodiment, a gene encoding a ZSIG-11 polypeptide is introduced in vivo in a viral vector. Such vectors include an attenuated or defective DNA virus, such as but not limited to herpes simplex virus (HSV), papillomavirus, Epstein Barr virus (EBV), adenovirus, adeno-associated virus (AAV), and the like. Defective viruses, which entirely or almost entirely lack viral genes, are preferred. A defective virus is not infective after introduction into a cell. Use of defective viral vectors allows for administration to cells in a specific, localized area, without concern that the vector can infect other cells. Examples of particular vectors include, but are not limited to, a defective herpes virus 1 (HSV1) vector (Kaplitt et al., Molec. Cell. Neurosci. 2:320-30, 1991), an attenuated adenovirus vector, such as the vector described by Stratford-Perricaudet et al. (J. Clin. Invest. 90:626-30, 1992), and a defective adeno-associated virus vector (Samulski et al., J. Virol. 61:3096-101, 1987; Samulski et al., J. Virol. 63:3822-28, 1989).

In another embodiment, the gene can be introduced in a retroviral vector, e.g., as described in Anderson et al., U.S. Patent No. 5,399,346; Mann et al., Cell 33:153, 1983; Temin et al., U.S. Patent No. 4,650,764; Temin et al., U.S. Patent No. 4,980,289; Markowitz et al., J. Virol. 62:1120, 1988; Temin et al., U.S. Patent No. 5,124,263; Dougherty et al., WIPO Publication WO 95/07358; and Kuo et al., Blood 82:845-52, 1993.

Alternatively, the vector can be introduced by lipofection in vivo using liposomes. Synthetic cationic lipids can be used to prepare liposomes for in vivo transfection of a gene encoding a marker (Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-17, 1987; and Mackey et al., Proc. Natl. Acad. Sci. USA 85:8027-31, 1988). The use of lipofection to introduce exogenous genes into specific organs in vivo has certain practical advantages. Molecular
targeting of liposomes to specific cells represents one area of benefit. It is clear that directing transfection to particular cells represents one area of benefit. It is clear that directing transfection to particular cell types would be particularly advantageous in a tissue with cellular heterogeneity, such as the pancreas, liver, kidney, and brain. Lipids may be chemically coupled to other molecules for the purpose of targeting. Targeted peptides, e.g., hormones or neurotransmitters, and proteins such as antibodies, or non-peptide molecules could be coupled to liposomes chemically.

It is possible to remove the cells from the body and introduce the vector as a naked DNA plasmid and then re-implant the transformed cells into the body. Naked DNA vector for gene therapy can be introduced into the desired host cells by methods known in the art, e.g., transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, use of a gene gun or use of a DNA vector transporter (see, for example, Wu et al., J. Biol. Chem. 267:963-7, 1992; Wu et al., J. Biol. Chem. 263:14621-24, 1988).

The ZSIG-11 polypeptides are also contemplated for pharmaceutical use. Pharmaceutically effective amounts of ZSIG-11 polypeptides, agonists or ZSIG-11 antagonists of the present invention can be formulated with pharmaceutically acceptable carriers for parenteral, oral, nasal, rectal, topical, transdermal administration or the like, according to conventional methods. Formulations may further include one or more diluents, fillers, emulsifiers, preservatives, buffers, excipients, and the like, and may be provided in such forms as liquids, powders, emulsions, suppositories, liposomes, transdermal patches and tablets, for example. Slow or extended-release delivery systems, including any of a number of biopolymers (biological-based systems), systems employing liposomes, and polymeric delivery systems, can
also be utilized with the compositions described herein to provide a continuous or long-term source of the ZSIG-11 polypeptide or antagonist. Such slow release systems are applicable to formulations, for example, for oral, topical and parenteral use. The term "pharmaceutically acceptable carrier" refers to a carrier medium which does not interfere with the effectiveness of the biological activity of the active ingredients and which is not toxic to the host or patient. One skilled in the art may formulate the compounds of the present invention in an appropriate manner, and in accordance with accepted practices, such as those disclosed in Remington's Pharmaceutical Sciences, Gennaro (ed.), Mack Publishing Co., Easton, PA 1990.

As used herein a "pharmaceutically effective amount" of a ZSIG-11 polypeptide, agonist or antagonist is an amount sufficient to induce a desired biological result. The result can be alleviation of the signs, symptoms, or causes of a disease, or any other desired alteration of a biological system. For example, an effective amount of a ZSIG-11 polypeptide is that which provides either subjective relief of symptoms or an objectively identifiable improvement as noted by the clinician or other qualified observer. Effective amounts of the ZSIG-11 polypeptides can vary widely depending on the disease or symptom to be treated. The amount of the polypeptide to be administered and its concentration in the formulations, depends upon the vehicle selected, route of administration, the potency of the particular polypeptide, the clinical condition of the patient, the side effects and the stability of the compound in the formulation. Thus, the clinician will employ the appropriate preparation containing the appropriate concentration in the formulation, as well as the amount of formulation administered, depending upon clinical experience with the patient in question or with similar patients. Such amounts will depend, in part, on the
particular condition to be treated, age, weight, and
general health of the patient, and other factors evident
to those skilled in the art. Typically a dose will be in
the range of 0.1-100 mg/kg of subject. Doses for specific
compounds may be determined from in vitro or ex vivo
studies in combination with studies on experimental
animals. Concentrations of compounds found to be
effective in vitro or ex vivo provide guidance for animal
studies, wherein doses are calculated to provide similar
concentrations at the site of action.

The dosages of the present compounds used to
practice the invention include dosages effective to result
in the desired effects. Estimation of appropriate dosages
effective for the individual patient is well within the
skill of the ordinary prescribing physician or other
appropriate health care practitioner. As a guide, the
clinician can use conventionally available advice from a
source such as the Physician's Desk Reference, 48th
Edition, Medical Economics Data Production Co., Montvale,

Preferably the compositions are presented for
administration in unit dosage forms. The term "unit
dosage form" refers to physically discrete units suitable
as unitary dosed for human subjects and animals, each unit
containing a predetermined quantity of active material
calculated to produce a desired pharmaceutical effect in
association with the required pharmaceutical diluent,
carrier or vehicle. Examples of unit dosage forms include
vials, ampules, tablets, caplets, pills, powders,
granules, eyedrops, oral or ocular solutions or
suspensions, ocular ointments, and oil-in-water emulsions.
Means of preparation, formulation and administration are
known to those of skill, see generally Remington's

The invention is further illustrated by the
following non-limiting examples.
EXAMPLES

Example 1

**Identification of ZSIG-11**

Novel ZSIG-11 ligand-encoding polynucleotides and polypeptides of the present invention were initially identified by querying an EST database for initially identified sequences. To identify the corresponding cDNA, a clone considered likely to contain the entire contig was amplified using a QIAwells 8 plasmid kit (Qiagen, Inc., Chatsworth, CA) according to manufacturer’s instructions, a 5 ml overnight culture in LB + 50 mg/ml ampicillin was prepared. The template was sequenced on an Applied Biosystems™ model 377 DNA sequencer (Perkin-Elmer Cetus, Norwalk, Ct.) using the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer Corp.) according to the manufacturer’s instructions. Oligonucleotides ZC976 (SEQ ID NO:7) and ZC447 (SEQ ID NO:8) promoters on the vector were used as sequencing primers. Oligonucleotides ZC11300 (SEQ ID NO:), ZC11796 (SEQ ID NO:9), ZC11797 (SEQ ID NO:10), ZC11873 (SEQ ID NO:11), ZC11874 (SEQ ID NO:12), ZC13966 (SEQ ID NO:13) and ZC13967 (SEQ ID NO:14) were used to complete the sequence from the clone. Sequencing reactions were carried out in a Hybaid OmniGene Temperature Cycling System (National Labnet Co., Woodbridge, NY). Sequencher™ 3.0 sequence analysis software (Gene Codes Corporation, Ann Arbor, MI) was used for data analysis. The resulting 1,341 bp sequence is disclosed in SEQ ID NO:1.
Example 2

Tissue Distribution

Human Multiple Tissue Northern Blots (MTN I, MTN II, and MTN III; Clontech) were probed to determine the tissue distribution of human ZSIG-11 expression. A 30 bp DNA probe (ZC11,300; SEQ. ID. NO.3) derived from the published sequence for LIN461779.R, which is located just 3' of the signal sequence cleavage site at the 5' end of the mature ZSIG-11 polypeptide as represented in SEQ. ID. NO. 2, was radioactively labeled with $^{32}$P using T4 polynucleotide kinase and forward reaction buffer (GIBCO BRL, Gaithersburg, MD) according to the manufacturer's specifications. The probe was purified using a NUCTRAP push column (Stratagene Cloning Systems, La Jolla, CA). ExpressHyb™ (Clontech) solution was used for prehybridization and as a hybridizing solution for the Northern blots. Hybridization took place overnight at 42°C using $2 \times 10^6$ cpm/ml of labeled probe, and the blots were then washed at 70°C in 1X SSC, 0.1% SDS. A 1.8 kb transcript was detected in numerous tissues. The highest level was in testis, with high levels in prostate, thyroid and heart, and lesser levels in skeletal muscle, pancreas, small intestine, peripheral blood lymphocytes, brain, placenta, liver, kidney, liver, thymus, ovary, colon, spinal cord, trachea and adrenal gland.

A human bone Northern was also probed to determine expression of ZSIG-11 in osteogenic cell lines. Total RNA was prepared from each of HOS cells (a human osteogenic sarcoma clone, ATCC CRL-1547), MG-63 cells (a human osteosarcoma, ATCC CRL-1427) and Saos2 (human osteogenic sarcoma, ATCC HTB-85) and U205 cells (human primary osteogenic sarcoma ATCC HTB-96) using a guanidine isothiocyanate, phenol, chloroform mixture according to Chomczynski and Sacchi (Anal. Biochem. 162:156-9, 1987). Poly(A)+ RNA was isolated using oligo d(T) cellulose
chromatography (Aviv and Leder, Proc. Natl. Acad. Sci. USA. 69: 1408-12, 1972). Northern blot analysis was then performed as follows.

About 2 µg of each of the poly A+ RNAs was denatured in 2.2 M formaldehyde/phosphate buffer (50 mM Na$_2$HPO$_4$, 50 mM NaH$_2$PO$_4$, 50 mM NaOAc, 1 mM EDTA and 2.2 M formaldehyde) and separated by 1.5% agarose mini gel electrophoresis in formaldehyde/phosphate buffer. The RNA was blotted overnight onto a nytran filter (Schleicher & Schuell, Keene, NH), and the filter was UV crosslinked (1,200 µJoules) in a STRATALINKER® UV crosslinker (Stratagene Cloning Systems). Hybridization took place overnight at 55°C using 2.2 x 10$^6$ cpm/ml of labeled probe. The blots were then washed at 50°C in 0.1X SSC, 0.1% SDS and exposed to film for two days. Three transcript signals (5kb, 2kb and 1.5kb) were detected corresponding to ZSIG-11 were detected in all four bone tissues.

Example 3
Chromosomal Localization of ZSIG-11

ZSIG-11 was mapped to chromosome 20 using the commercially available GeneBridge 4 Radiation Hybrid Panel (Research Genetics, Inc., Huntsville, AL). The GeneBridge 4 Radiation Hybrid Panel contains PCRable DNAs from each of 93 radiation hybrid clones, plus two control DNAs (the HFL donor and the A23 recipient). A publicly available WWW server (http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl) allows mapping relative to the Whitehead Institute/MIT Center for Genome Research's radiation hybrid map of the human genome (the "WICGR" radiation hybrid map) which was constructed with the GeneBridge 4 Radiation Hybrid Panel.

For the mapping of ZSIG-11 with the GeneBridge 4 RH Panel, 20 µl reactions were set up in a PCRable 96-well microtiter plate (Stratagene, La Jolla, CA) and used in a RoboCycler Gradient 96 thermal cycler (Stratagene). Each of the 95 PCR reactions consisted of 2 µl 10X KlenTaq PCR
reaction buffer (CLONTECH Laboratories, Inc., Palo Alto, CA), 1.6 μl dNTPs mix (2.5 mM each, PERKIN-ELMER, Foster City, CA), 1 μl sense primer, ZC 13,538, (SEQ ID NO:4), 1 μl antisense primer, ZC 13,537 (SEQ ID NO:5), 2 μl RediLoad (Research Genetics, Inc.), 0.4 μl 50X Advantage KlenTag Polymerase Mix (Clontech Laboratories, Inc.), 25 ng of DNA from an individual hybrid clone or control and ddH2O for a total volume of 20 μl. The reactions were overlaid with an equal amount of mineral oil and sealed. The PCR cycler conditions were as follows: an initial 1 cycle 5 minute denaturation at 95°C, 35 cycles of a 1 minute denaturation at 95°C, 1 minute annealing at 54°C and 1.5 minute extension at 72°C, followed by a final 1 cycle extension of 7 minutes at 72°C. The reactions were separated by electrophoresis on a 3% NuSieve GTG agarose gel (FMC Bioproducts, Rockland, ME).

The results showed that ZSIG-11 maps 252.51 cR 3000 from the top of the human chromosome 20 linkage group on the WICGR radiation hybrid map. Proximal and distal framework markers were D20S908 and D20S99, respectively. This positions ZSIG-11 in the 20q13.12 region on the integrated LDB chromosome 20 map (The Genetic Location Database, University of Southampton, WWW server:http://cedar.genetics.soton.ac.uk/public_html/).

Example 4

Construction of ZSIG-11 Mammalian Expression Vectors ZSIG-11CF/pZP9 and ZSIG-11NF/pZP9

Two expression vectors were prepared for the ZSIG-11 polypeptide, ZSIG-11CF/pZP9 and ZSIG-11NF/pZP9, wherein the constructs are designed to express a ZSIG-11 polypeptide with a C- or N-terminal FLAG tag (SEQ ID NO: 15).

ZSIG-11CF/pZP9

A 947 bp PCR generated ZSIG-11 DNA fragment was created using ZC13450 (SEQ ID NO:28) and ZC13445 (SEQ ID
NO:29) as PCR primers and the template described in Example 1 above. The PCR reaction was incubated at 94°C for 5 minutes, and then run for 10 cycles of 30 seconds at 94°C and 2 minutes at 75°C, followed by 15 cycles at 94°C for 30 seconds and 60°C for 2 minutes. The resultant PCR product was then run on a 0.9% GTG/TBE agarose gel with 1X TBE buffer. A band of the predicted size, 947 bp, was excised and the DNA was purified from the gel with a QUIAQUICK® column (Qiagen) according the manufacturer's instructions. The DNA was digested with the restriction enzymes Xho I and Bam HI, followed by extraction and precipitated.

The excised DNA was subcloned into plasmid CF/pZP9 which had been cut with Xho I and Bam HI. The ZSIG-11/CFpZP9 expression vector uses the native ZSIG-11 signal peptide, and the FLAG epitope (SEQ ID NO:15) is attached at the C-terminus as a purification aid. Plasmid CF/pZP9 (deposited at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD) is a mammalian expression vector containing an expression cassette having the mouse metallothionein-1 promoter, multiple restriction sites for insertion of coding sequences, a sequence encoding the flag peptide (SEQ ID NO:15), a stop codon and a human growth hormone terminator. The plasmid also has an E. coli origin of replication, a mammalian selectable marker expression unit having an SV40 promoter, enhancer and origin of replication, a DHFR gene and the SV40 terminator.

ZSIG-11NF/pZP9

A 873 bp PCR generated ZSIG-11 DNA fragment was created in accordance with the procedure set forth above using ZC13440 (SEQ ID NO:16) and ZC13439 (SEQ ID NO:17) as PCR primers. The purified PCR fragment was digested with the restriction enzymes BAM HI and Xho I, followed by extraction and precipitation.
The excised ZSIG-11 DNA was subcloned into plasmid NF/pZP9 which had been cut with Bam HI and Xho I. The ZSIG-11/NFpZP9 expression vector incorporates the TPA leader and attaches the FLAG epitope (SEQ ID NO:15) to the N-terminal of the ZSIG-11 polypeptide-encoding polynucleotide sequence. Plasmid NF/pZP9 (deposited at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD) is a mammalian expression vector containing an expression cassette having the mouse metallothionein-1 promoter, a TPA leader peptide followed by the sequence encoding the FLAG peptide (SEQ ID NO:15), multiple restriction sites for insertion of coding sequences, and a human growth hormone terminator. The plasmid also contains an E. coli origin of replication, a mammalian selectable marker expression unit having an SV40 promoter, enhancer and origin of replication, a DHFR gene and the SV40 terminator.

Ten nanograms of the restriction digested C- and N-terminal FLAG/ZSIG-11 inserts and 10 ng of the corresponding vectors were ligated at room temperature for 3 hours. One microliter of each ligation reaction was independently electroporated into DH10B competent cells (GIBCO BRL, Gaithersburg, MD) according to manufacturer's direction and plated onto LB plates containing 50 mg/ml ampicillin, and incubated overnight. Colonies were screened by PCR as described above. For ZSIG-11CF/pZP9 screens the primers were, ZC13450 (SEQ ID NO:28) and ZC13445 (SEQ ID NO:29) and for ZSIG-11NF/pZP9 screens the primers were ZC13440 (SEQ ID NO:16) and ZC13439 (SEQ ID NO:17). The insert sequence of positive clones, 873 bp fragment for ZSIG-11NF and a 947 bp fragment for ZSIG-11/CF were verified by sequence analysis. A large scale plasmid preparation was done using a QIAGEN® Maxi prep kit (Qiagen) according to manufacturer's instructions.
two 150 mm culture dishes of methotrexate resistant colonies, from each construct, were trypsinized and the cells were pooled and grown to confluence in a T162 flask. The pools were subjected to Western blot analysis and transferred to large scale culture.

Example 6

Large Scale Culture of ZSIG-11 FLAG-tagged polypeptides

One T-162 flask, containing confluent cells expressing ZSIG-11/NF, obtained from the expression procedure described above, was expanded into six T-162 flasks. One of the six resulting flasks was used to freeze down four cryovials, and the other five flasks were used to generate a Nunc cell factory.

The cells from the five T-165 flasks were used to seed a Nunc cell factory (10 layers, commercially available from VWR). Briefly, the cells from the T-162 flasks described above containing cells expressing ZSIG-11-NF were detached using trypsin, pooled, and added to 1.5 liters ESTEP1 media (668.7g/50L DMEM (Gibco), 5.5 g/50L pyruvic acid, sodium salt 96% (Mallinckrodt), 185.0 g/50L NaHCO3 (Mallinkrodt), 5.0 mg/ml and 25 ml/50L insulin (JRH Biosciences), 10.0 mg/ml and 25 ml/50L transferrin (JRH Biosciences), 2.5L/50L fetal bovine serum (characterized) (Hyclone), 1 μM MTX, with pH adjusted to 7.05 +/- 0.05 ) prewarmed to 37°C. The media containing cells was then poured into Nunc cell factories via a funnel. The cell factories were placed in a 37°C/5.0% CO2 incubator.

At 80-100% confluence, a visual contamination test (phenol red color change) was performed on the Nunc cell factories. Since no contamination was observed, supernatant from the confluent factories was poured into a small harvest container, sampled and discarded. The adherent cells were then washed once with 400 ml PBS. To detach the cells from the factories, 100 mls of trypsin
Example 5

Mammalian Expression of ZSIG-11

BHK 570 cells (ATCC No. CRL-10314) were plated in 10 cm tissue culture dishes and allowed to grow to approximately 50 to 80% confluency overnight at 37°C, 5% CO₂, in DMEM/FBS media (DMEM, Gibco/BRL High Glucose, 5% fetal bovine serum (Hyclone, Logan, UT), 1 μM L-glutamine (JRH Biosciences, Lenexa, KS), 1 μM sodium pyruvate (Gibco BRL)). The cells were then transfected with the plasmid ZSIG-11NF/pZP9 (N-terminal FLAG tag) or ZSIG-11CF/pZP9 (C-terminal FLAG tag), using Lipofectamine™ (Gibco BRL), in serum free (SF) media formulation (DMEM, 10 μg/ml transferrin, 5 μg/ml insulin, 10 μg/ml fetuin, 2 ng/ml selenium, 1% L-glutamine and 1% sodium pyruvate). Sixteen micrograms of ZSIG-11NF/pZP9 and 16 μg of ZSIG-11CF/pZP9 were separately diluted into 15 ml tubes to a total final volume of 640 μl SF media. In separate tubes, 35 μl of Lipofectamine™ (Gibco BRL) was mixed with 605 μl of SF medium. The Lipofectamine™ mix was added to the DNA mix and allowed to incubate approximately 30 minutes at room temperature. Five milliliters of SF media was added to the DNA:Lipofectamine™ mixture. The cells were rinsed once with 5 ml of SF media, aspirated, and the DNA:Lipofectamine™ mixture was added. The cells were incubated at 37°C for five hours, then 6.4 ml of DMEM/10% FBS, 1% PSN media was added to the plate. The plate was incubated at 37°C overnight and the DNA:Lipofectamine™ mixture was replaced with fresh FBS/DMEM media the next day. On day 2 post-transfection, the cells were split into the selection media (DMEM/FBS media from above with the addition of 1 μM methotrexate (Sigma Chemical Co., St. Louis, Mo.)) in 150 mm plates at 1:10, 1:20 and 1:50. The plates were refed at day 5 post-transfection with fresh selection media. Approximately 10 days post-transfection,
was added to each and removed and the cells were then incubated for 5 to 10 minutes in the residual trypsin. The ZSIG-11NF cells were collected following two, 200 ml washes of ESTEP1 media. To each of ten ESTEP1 media-containing bottles (1.5 liters each, at 37°C) was added 40 mls of collected cells. One 1.5 liter bottle was then used to fill one Nunc factory. Each cell factory was placed in a 37°C/5.0% CO₂ incubator.

At 80-90% confluence, a visual contamination test (phenol red color change) was performed on the Nunc cell factories. Since no contamination was observed, supernatant from the confluent factories were poured into a small harvest container, sampled and discarded. Cells were then washed once with 400 ml PBS. To the factories was added 1.5 liters of ESTEP2 media (668.7g/50L DMEM (Gibco), 5.5 g/50L pyruvic acid, sodium salt 96% (Mallinckrodt), 185.0 g/50L NaHCO₃ (Mallinckrodt), 5.0 mg/ml, 25 ml/50L insulin, 10.0 mg/ml and 25 ml/50L transferrin).

At approximately 48 hours a visual contamination test (phenol red color change) was performed on the Nunc cell factories. Supernatant from each factory was poured into small harvest containers, 13 liters was collected. Fresh serum-free media (1.5 liters) was poured into each Nunc cell factory, and the factories were incubated at 37°C/5.0% CO₂. One ml of supernatant harvest was transferred to a microscope slide, and subjected to microscopic analysis for contamination. The contents of the small harvest containers for each factory were pooled and immediately filtered. A second harvest was then performed, substantially as described above at 50 hours and the cell factories were discarded thereafter. An aseptically assembled filter train apparatus was used for aseptic filtration of the harvest supernatant (conditioned media). Assembly was a follows: tubing was wire-tied to an Opti-Cap filter (Millipore Corp., Bedford, MA) and a Gelman Supercap 50 filter (Gelman Sciences, Ann Arbor,
MI). The Supercap 50 filter was also attached to a sterile capped container located in a hood; tubing located upstream of the Millipore Opti-cap filter was inserted into a peristaltic pump; and the free end of the tubing was placed in the large harvest container. The peristaltic pump was run between 200 and 300 rpm, until all of the conditioned media passed through the 0.22 μm final filter into a sterile collection container. The filtrate was placed in a 4°C cold room pending purification.

Conditioned media containing ZSIG-11/NF was collected for concentration at various time points (at the 5 T-162 flask stage; 1 factory, fetal bovine serum media; 10 factories, fetal bovine serum media; 10 factories, serum free media and a second 10 factory, serum free media time point). Since the expected mass of the protein was in excess of 8 kDa, Millipore 5 kDa cut off concentrators were used. The starting volume for each sample was 15 ml, which was concentrated to a final volume of 1.5 ml. The concentrators were spun at 4°C in Beckman tabletop centrifuge at 2000 x g (3000 rpm) for 40 minutes. The concentrate was transferred to a 1.5 ml non-stick microfuge tube, and the volume was adjusted to 1 ml using flow through media to achieve a 10x concentration. To sterilize the media, the 10x concentrate was split into two Costar Spin-X tubes, and the tubes were spun at 8000 x g for two minutes in a Eppendorf 5415 microfuge (VWR, Seattle, WA).

Example 7
Baculovirus Expression of ZSIG-11

Two expression vectors were prepared to express ZSIG-11 polypeptides in insect cells: pSGCF11, designed to express a C-terminally FLAG-tagged ZSIG-11 polypeptide and pSGNF11 designed to express a N-terminally FLAG-tagged polypeptide.
pFSGCF11

A 977 bp PCR generated ZSIG-11 DNA fragment excised from the mammalian expression vector ZSIG-11CF/pZP9, described above, using restriction enzymes Eco RI and Xba I. The restriction digest fragment was run on a 1% Seaplaque/1% NuSieve agarose gel. The 977 bp band was excised, diluted to 0.5% agarose with 2 mM MgCl₂, melted at 65°C and ligated into a Eco RI/Xba I digested baculovirus expression vector, pZBV4L (a modification of the pFastBac expression vector, the polyhedron promoter has been removed and replaced with the late activating Basic Protein Promoter). 98.7 ng of the of the restriction digested ZSIG-11 insert and 167.4 ng of the corresponding vector were ligated overnight. The ligation mix was diluted 3 fold in TE (10 mM Tris-HCl, pH 7.5 and 1 mM EDTA) and 4 fmol of the diluted ligation mix was transformed into DH5α Library Efficiency competent cells (Life Technologies) according to manufacturer's direction by heat shock for 45 seconds in a 42°C waterbath. The ligated DNA was diluted in 450 µl of SOC media (2% Bacto Tryptone, 0.5% Bacto Yeast Extract, 10 ml 1M NaCl, 1.5 mM KCl, 10 mM MgCl₂, 10 mM MgSO₄, and 20 mM glucose) and plated onto LB plates containing 100 µg/ml ampicillin. Clones were analyzed by restriction digests and 1 µl of the positive clone was transformed into 20 µl DH10Bac Max Efficiency competent cells (GIBCO-BRL, Gaithersburg, MD) according to manufacturer's instruction, by heat shock for 45 seconds in a 42°C waterbath. The ligated DNA was diluted in 980 ml SOC media (2% Bacto Tryptone, 0.5% Bacto Yeast Extract, 10 ml 1M NaCl, 1.5 mM KCl, 10 mM MgCl₂, 10 mM MgSO₄, and 20 mM glucose) and plated onto Luria Agar plates containing 50 mg/ml kanamycin, 7 mg/ml gentamicin, 10 mg/ml tetracycline, IPTG and Blue Gal. The cells were incubated for 48 hours at 37°C. A color selection was used to identify those cells having virus that had incorporated into the plasmid (referred to as a "bacmid"). Those
colonies, which were white in color, were picked for analysis. Bacmid DNA was isolated from positive colonies using the QiaVac Miniprep8 system (Qiagen) according to the manufacturer's directions. Clones were screened for the correct insert by amplifying DNA using primers to the Basic Protein Promoter and to the SV40 terminus via PCR. Those having the correct insert were used to transfect Spodoptera frugiperda (Sf9) cells.

pSGNF11

A 1028 bp PCR generated ZSIG-11 DNA fragment excised from the mammalian expression vector ZSIG-11NF/pZP9, described above, using restriction enzymes Nco I and Xba I. The restriction digest fragment was run on a 1% SeaPlaque/1% NuSieve agarose gel. The 1028 bp band was excised, diluted to 0.5% agarose with 2 mM MgCl₂, melted at 65°C and ligated into a Nco I/Xba I digested baculovirus expression vector, pZBV3L (a modification of the pFastBac expression vector, the polyhedron promoter has been removed and replaced with the late activating Basic Protein Promoter) and transformed into DH10α cells as described above.

Transfection

Sf9 cells were seeded at 5 x 10⁶ cells per 35 mm plate and allowed to attach for 1 hour at 27°C. Five microliters of bacmid DNA was diluted with 100 μl Sf-900 II SFM. Six microliters of CellFECTIN Reagent (Life Technologies) was diluted with 100 μl Sf-900 II SMF. The bacmid DNA and lipid solutions were gently mixed and incubated 30-45 minutes at room temperature. Following the incubation, 0.8 ml Sf-900 II SFM was added to the lipid-DNA mixture. Media was aspirated from one plate of cells and the lipid-DNA mixture was added. The cells were incubated at 27°C for 4.5 hours, then 2 ml of Sf-900 II media containing penicillin/streptomycin was added to each plate. The plates were incubated at 27°C, 90% humidity, for 96 hours after which the virus was harvested.
Primary Amplification

Sf9 cells were grown in 50 ml Sf-900 II SFM in a 50 ml shake flask to an approximate density of approximately 0.04 x 10^6 cells/ml. They were then transfected with 100 µl of the virus stock from above and incubated at 27°C for 3 days after which time the virus was harvested, with titers of about 2 x 10^7 pfu/ml. To scale up, Sf9 cells were grown to a density of 1.3 x 10^6 SF9 cells/ml in five liters of SF 900 II SFM, approximately 91 hours. The cells were then transfected with the harvested virus (MOI 0.2) and incubated as above for 47.4 hours post infection.

Example 8

Purification of ZSIG-11NF from Baculovirus Infected Sf9 Cells

Unless otherwise noted, all operations were carried out at 4°C. A mixture of protease inhibitors was added to a 2000 ml sample of conditioned media from baculovirus-infected Sf9 cells, as described above, to final concentrations of 2.5 mM ethylenediaminetetraacetic acid (EDTA, Sigma Chemical Co. St. Louis, MO), 0.001 mM leupeptin (Boehringer-Mannheim, Indianapolis, IN), 0.001 mM pepstatin (Boehringer-Mannheim) and 0.4 mM Pefabloc (Boehringer-Mannheim). The sample was centrifuged at 10,000 rpm for 30 min at 4°C in a Beckman JLA-10.5 rotor (Beckman Instruments, Palo Alto, CA) in a Beckman Avanti J25I centrifuge (Beckman Instruments) to remove cell debris. To the supernatant fraction was added a 50.0 ml sample of anti-Flag Sepharose (Eastman Kodak, Rochester, NY) and the mixture was gently agitated on a Wheaton (Millville, NJ) roller culture apparatus for 18.0 h at 4°C.

The mixture was poured into a 5.0 x 20.0 cm Econo-Column (Bio-Rad, Laboratories, Hercules, CA) and the gel was washed with 30 column volumes of phosphate buffered saline (PBS). The unretained flow-through
fraction was discarded. Once the absorbance of the effluent at 280 nM was less than 0.05, flow through the column was reduced to zero and the anti-Flag Sepharose gel was washed with 2.0 column volumes of PBS containing 0.2 mg/ml of Flag peptide (Eastman Kodak) (SEQ ID NO:15. After 1.0 h at 4°C, flow was resumed and the eluted protein was collected. This fraction is referred to as the peptide elution. The anti-Flag Sepharose gel was washed with 2.0 column volumes of 0.1M glycine, pH 2.5, and the glycine wash was collected separately. The pH of the glycine-elapsed fraction was adjusted to 7.0 by the addition of a small volume of 10X PBS and stored at 4°C for future analysis if needed.

The peptide elution was concentrated to 5.0 ml using a 5,000 molecular weight cutoff membrane concentrator (Millipore, Bedford, MA) according to the manufacturer's instructions. The concentrated peptide elution was separated from free peptide by chromatography on a 1.5 x 50 cm Sephadex G-50 (Pharmacia, Piscataway, NJ) column equilibrated in PBS at a flow rate of 1.0 ml/min using a BioCad Sprint HPLC (PerSeptive BioSystems, Framingham, MA). Two-ml fractions were collected and the absorbance at 280 nM was monitored. The first peak of material absorbing at 280 nM and eluting near the void volume of the column was collected. This material represented purified ZSIG-11-NF.

By SDS-PAGE analysis under reducing conditions, the purified ZSIG-11-NF was composed of two major Coomassie blue-stained bands of apparent molecular weights 50,000 and 45,000. Both bands showed cross-reactivity with the anti-Flag antibody on Western blots. The relative abundance of these two bands was about 3 to 1 in favor of the 50 kDa protein. Under non-reducing conditions, the SDS-PAGE and Western blotting analysis showed that the material was highly aggregated and migrated as a smear from about 70,000 Da to the top of the gel. The protein concentration of the purified proteins was performed by
BCA analysis (Pierce, Rockford, IL) and the material was aliquoted, and stored at -80°C according to our standard procedures. The concentration of ZSIG-11-NF was 0.24 mg/ml.

Example 9
Construction of ZSIG-11 Amino Terminal FLAG-Tagged
Pichia Expression Vector

Expression of ZSIG-11 in Pichia methanolica utilizes the expression system described in co-assigned WIPO publication WO 97/17450. An expression plasmid containing all or part of a polynucleotide encoding ZSIG-11 was constructed via homologous recombination. The expression vector was built from pCZR190, which contains the AUG1 promoter, followed by the alpha factor prepro (αFpp) leader sequence, followed by an amino-terminal FLAG tag (NF), a blunt-ended Sma I restriction site for insertion of the gene sequence of interest, a translational stop codon, followed by the AUG1 terminator, the ADE2 selectable marker, and finally the AUG1 3' untranslated region. Also included in this vector are the URA3 and CEN-ARS sequences required for selection and replication in S. cerevisiae, and the AmpR and colE1 ori sequences required for selection and replication in E. coli.

Two construct specific linkers were prepared and along with ZSIG-11, were homologously recombined into the yeast expression vector pCZR190. The N-terminal linker comprises 70 base pairs of the αFpp coding sequence joined to a nucleotide sequence encoding a FLAG tag (SEQ ID NO:15) followed by 70 base pairs of nucleotide sequence encoding a portion of the amino-terminus from the mature ZSIG-11 sequence. The C-terminal linker comprises about 70 base pairs of carboxy terminus coding sequence of the ZSIG-11 joined with 70 base pairs of AUG1 terminator sequence.
The N-terminal linker was synthesized by a PCR reaction. Briefly, to a final reaction volume of 100 µl was added 1 pmol each of each linker ZC14293 (SEQ ID NO:18) and ZC14280 (SEQ ID NO:19), 100 pmol of each primer ZC13497 (SEQ ID NO:20) and ZC13735 (SEQ ID NO:21), 10 µl of 10X PCR buffer (Boehringer Mannheim), 1 µl Pwo polymerase (Boehringer Mannheim), 10 µl of 0.25 mM nucleotide triphosphate mix (Perkin Elmer) and dH₂O. The PCR reaction was incubated at 94°C for 1.5 minutes, followed by 10 cycles of 30 seconds at 94°C, 1 minute at 50°C and 1 minute at 72°C, concluded with a 10 minute extension at 72°C. The resulting 140 bp double stranded, NF-tagged linker is disclosed in SEQ ID NO:22.

The C-terminal untagged ZSIG-11 linker was made via a PCR reaction as described using oligonucleotides linkers ZC14218 (SEQ ID NO:23) and ZC14734 (SEQ ID NO:24), and primers ZC14273 (SEQ ID NO:25) and ZC14355 (SEQ ID NO:26). The resulting 140 bp double stranded, C-terminal untagged linker is disclosed in SEQ ID NO:27.

The NF-ZSIG-11 plasmid was made by homologously recombinating 100 ng of Sma I digested pCZR190 acceptor vector, the 1 µg of Eco RI-Xho I ZSIG-11 cDNA donor fragment, 1 µg of N-terminal FLAG-tagged ZSIG-11 linker (SEQ ID NO:22) and 1 µg of C-terminal linker (SEQ ID NO:27) into S. cerevisiae. One hundred microliters of competent yeast cells (S. cerevisiae) was combined with 10 µl of each of the fragments and linkers and transferred to a 0.2 cm electroporation cuvette. The yeast/DNA mixture was electropulsed at 0.75 kV (5 kV/cm), 80 ohms, 25 µF. To the cuvette was added 600 µl of 1.2 M sorbitol and 300 µl aliquots of the yeast/sorbitol mixture was plated onto two URA D plates and incubated at 30°C.

After about 48 hours the Ura⁺ yeast transformants from a single plate were resuspended in 2.5 ml H₂O and spun briefly to pellet the yeast cells. The cell pellet was resuspended in 1 ml of lysis buffer (2% Triton X-100, 1% SDS, 100 mM NaCl, 10 mM Tris, pH 8.0, 1 mM EDTA). Five
hundred microliters of the lysis mixture was added to an Eppendorf tube containing 300 µl acid washed glass beads and 200 µl phenol-chloroform, vortexed for 1 minute intervals two or three times, followed by a 5 minute spin in an Eppendorf centrifuge at maximum speed. Three hundred microliters of the aqueous phase was transferred to a fresh tube and the DNA precipitated with 600 µl ethanol (EtOH), followed by a 10 minutes at 4ºC. The DNA pellet was resuspended in 100 µl H2O.

Five microliters of the resuspended DNA prep was used to transform 40 µl of electrocompetent E. coli cells (DH10B, Gibco BRL). The cells were electropulsed at 2.0 kV and 400 ohms. Following electroporation, 1 ml SOC (2% Bacto™ Tryptone (Difco, Detroit, MI), 0.5% yeast extract (Difco), 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl₂, 10 mM MgSO₄, 20 mM glucose) was added and the cells were allowed to recover for 1 hour at 37ºC prior to plating 250 µl aliquots on four LB AMP plates (LB broth (Lennox), 1.8% Bacto™ Agar (Difco), 100 mg/L Ampicillin).

Individual clones harboring the correct expression construct were identified by PCR screening. The primers used to amplify the N-tagged ZSIG-11 clone were ZC13497 (SEQ ID NO:20) and ZC13734 (SEQ ID NO:24). The insert sequence of positive clones, identified by a ~1000 bp fragment, were verified by sequence analysis. One such clone was designated pGMN18. Larger scale plasmid DNA was isolated using Qiagen maxi kits (Qiagen) and the DNA was digested with Not I to liberate the Pichia-ZSIG-11 expression cassette from the vector backbone. The Not I DNA fragment was then transformed into the Pichia methanolica expression host, PMAD16. This was done by mixing 100 µl of prepared competent PMAD16 cells with 10 µg of Not I digested NF-tagged ZSIG-11 fragment and transferred to a 0.2 cm electroporation cuvette. The yeast/DNA mixture was electropulsed at 0.75 kV, 25 µF, infinite ohms. To the cuvette was added 1 ml of 1X Yeast Nitrogen Base and 500 µl aliquots were plated
onto two ADE DS (0.056% -Ade -Trp -Thr powder, 0.67% yeast nitrogen base without amino acids, 2% D-glucose, 0.5% 200X tryptophan, threonine solution, and 18.22% D-sorbitol) plates for selection and incubated at 30°C. Transformants were then picked and screened via Western blot for high-level NF-tagged ZSIG-11 expression. The resulting NF-tagged-ZSIG-11 strains were designated PMAD16::pGMN18-16 and 21 and subjected to large scale fermentation.

Example 10

In vivo Administration of ZSIG-11

Thirty three male C57Bl mice, approximately 4 weeks old were weighed and glucose measurement was taken one day prior to injection. On day 0, the mice were divided into 4 groups and received 2 or 20 μg purified baculovirus produced N-FLAG-tagged ZSIG-11 resuspended in 0.1% BSA in PBS or vehicle by intravenous tail vein injection. Group 1, 20 μg ZSIG-11, n=9. Group 2, 2 μg ZSIG-11, n=9. Group 3, vehicle only, n=9. Group 4, untreated, n=6. The animals' weights glucose was monitored during the 2 week study. Injections were daily thereafter for a total of 14 injections including the day of sacrifice. Glucose readings were taken using the Glucometer system.

On day 13 the mice received the final protein injection were bled under ether anesthesia for complete hematology counts and clinical chemistry. The mice were sacrificed by cervical dislocation. Selected organ weights were taken and tissues were harvested for necropsy.

Total white blood count was significantly higher in the 20 ug treated group than in the vehicle group (InStat Mac Instant Statistics 2.03, GraphPad Software, San Diego, CA), P=0.0035). Significant increases in counts of lymphocytes (P<0.05), monocytes (P<0.01) and
neutrophils (P<0.05) contributed to this increase, while eosinophils and basophils did not increase. Percentages of the various types of WBCs did not change significantly, but there was a trend towards an increase in percentage of monocytes.

Statistically significant differences in clinical chemistry parameters between ZSIG-11 treated groups and the vehicle treated group were:

- A highly significant, dose responsive, increase in blood urea nitrogen (p=0.0002). There were significant decreases in phosphorus (p=0.0091) and albumin levels (p=0.0004). There was also a significant, dose responsive increase in cholesterol levels (p=0.0019).

Histopathology indicated no significant effects on the tissues examined, including kidney, heart and liver.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.
CLAIMS

What is claimed is:

1. An isolated polynucleotide molecule that encodes a polypeptide, wherein the polynucleotide molecule is selected from the group consisting of:
   
a) a polynucleotide encoding a polypeptide comprising a sequence of amino acid residues that is at least 80% identical to the amino acid sequence as shown in SEQ ID NO:2, from amino acid residue 26 (Gly) to amino acid residue 313 (Glu), and specifically binds with an antibody that specifically binds with a polypeptide having the amino acid sequence of SEQ ID NO:2;
   
b) a polynucleotide molecule having the sequence of SEQ ID NO:6; and
   
c) a polynucleotide molecule that hybridizes under stringent conditions to a polynucleotide molecule having the nucleotide sequence of SEQ ID NO:1, or the complement of SEQ ID NO:1.

2. An isolated polynucleotide molecule according to claim 1, wherein any difference between said amino acid sequence encoded by the polynucleotide molecule and said corresponding amino acid sequence of SEQ ID NO:2 is due to a conservative amino acid substitution.

3. An isolated polynucleotide molecule of claim 1, wherein said polypeptide further comprises an affinity tag or binding domain.

4. An isolated polynucleotide molecule of claim 1, comprising nucleotides 138-1001 of SEQ ID NO:1.

5. A polynucleotide molecule encoding a fusion protein consisting essentially of a first portion and a second
portion joined by a peptide bond, said first portion comprising a polypeptide comprising a sequence of amino acid residues that is at least 80% identical to the amino acid sequence as shown in SEQ ID NO:2, from amino acid residue 26 (Gly) to amino acid residue 313 (Glu), and specifically binds with an antibody that specifically binds with a polypeptide having the amino acid sequence of SEQ ID NO:2; and 
said second portion comprising another polypeptide.

6. A polynucleotide encoding a fusion protein comprising a secretory signal sequence having the amino acid sequence of amino acid residues 1-25 of SEQ ID NO:2, wherein said secretory signal sequence is operably linked to an additional polypeptide.

7. An expression vector comprising the following operably linked elements:
a transcription promoter;
a polynucleotide molecule that encodes a polypeptide, wherein said polynucleotide molecule is selected from the group consisting of:

a) a polynucleotide encoding a polypeptide comprising a sequence of amino acid residues that is at least 80% identical to the amino acid sequence as shown in SEQ ID NO:2, from amino acid residue 26 (Gly) to amino acid residue 313 (Glu), and specifically binds with an antibody that specifically binds with a polypeptide having the amino acid sequence of SEQ ID NO:2;
b) a polynucleotide molecule having the sequence of SEQ ID NO:6; and
c) a polynucleotide molecule that hybridizes under stringent conditions to a polynucleotide molecule having the nucleotide sequence of SEQ ID NO:1, or the complement of SEQ ID NO:1; and

a transcription terminator.
8. An expression vector according to claim 7 further comprising a secretory signal sequence operably linked to said DNA segment.

9. An expression vector according to claim 8, wherein said secretory signal sequence comprises amino acid residues 1-25 of SEQ ID NO:2.

10. An expression vector according to claim 7, wherein said polynucleotide encodes a polypeptide covalently linked amino terminally or carboxy terminally to an affinity tag.

11. A cultured cell into which has been introduced an expression vector comprising the following operably linked elements:
   a transcription promoter;
   a polynucleotide molecule that encodes a polypeptide, wherein said polynucleotide molecule is selected from the group consisting of:
      a) a polynucleotide encoding a polypeptide comprising a sequence of amino acid residues that is at least 80% identical to the amino acid sequence as shown in SEQ ID NO:2, from amino acid residue 26 (Gly) to amino acid residue 313 (Glu), and specifically binds with an antibody that specifically binds with a polypeptide having the amino acid sequence of SEQ ID NO:2;
      b) a polynucleotide molecule having the sequence of SEQ ID NO:6; and
      c) a polynucleotide molecule that hybridizes under stringent conditions to a polynucleotide molecule having the nucleotide sequence of SEQ ID NO:1, or the complement of SEQ ID NO:1; and
   a transcription terminator, wherein said cultured cell expresses said polypeptide encoded by said polynucleotide segment.
12. A method of producing a polypeptide comprising:
culturing a cell into which has been introduced an
expression vector comprising the following operably linked elements:
   a transcription promoter;
a polynucleotide molecule that encodes a polypeptide,
wherein said polynucleotide molecule is selected from the group
consisting of:
   a) a polynucleotide encoding a polypeptide comprising
   a sequence of amino acid residues that is at least 80%
   identical to the amino acid sequence as shown in SEQ ID NO:2
   from, amino acid residue 26 (Gly) to amino acid residue 313
   (Glu), and specifically binds with an antibody that
   specifically binds with a polypeptide having the amino acid
   sequence of SEQ ID NO:2;
   b) a polynucleotide molecule having the sequence of
   SEQ ID NO:6; and
   c) a polynucleotide molecule that hybridizes under
   stringent conditions to a polynucleotide molecule having the
   nucleotide sequence of SEQ ID NO:1, or the complement of SEQ ID
   NO:1; and
   a transcription terminator;
   whereby said cell expresses said polypeptide encoded
   by said polynucleotide segment; and
   recovering said expressed polypeptide.

13. A method according to claim 12, wherein said
expression vector further comprises a secretory signal sequence
operably linked to said polynucleotide segment, said cultured
cell secretes said polypeptide into a culture medium, and said
polypeptide is recovered from said culture medium.

14. An isolated polypeptide comprising a sequence
of amino acid residues that is at least 80% identical to the
amino acid sequence as shown in SEQ ID NO:2, from amino acid
residue 26 (Gly) to amino acid residue 313 (Glu), and
specifically binds with an antibody that specifically binds with a polypeptide having the amino acid sequence of SEQ ID NO:2.

15. An isolated polypeptide according to claim 14 wherein said polypeptide comprises a sequence of amino acid residues that is at least 90% identical to the amino acid sequence as shown in SEQ ID NO:2, from amino acid residue 26 (Gly) to amino acid residue 313 (Glu), and specifically binds with an antibody that specifically binds with a polypeptide having the amino acid sequence of SEQ ID NO:2.

16. An isolated polypeptide according to claim 14, wherein said polypeptide comprises a sequence of amino acid residues that is at least 95% identical to the amino acid sequence as shown in SEQ ID NO:2, from amino acid residue 26 (Gly) to amino acid residue 313 (Glu), and specifically binds with an antibody that specifically binds with a polypeptide having the amino acid sequence of SEQ ID NO:2.

17. An isolated polypeptide according to claim 14, further comprising an affinity tag or binding domain.

18. An isolated polypeptide comprising the amino acid sequence of SEQ ID NO:2.

19. An isolated polypeptide comprising the amino acid sequence of SEQ ID NO:2, from amino acid residue 1 (Met) to amino acid residue 25 (Ala).

20. An antibody or antibody fragment that specifically binds to a polypeptide according to claim 18.
21. An antibody according to claim 20, wherein said antibody is selected from the group consisting of:
   a) polyclonal antibody;
   b) murine monoclonal antibody;
   c) humanized antibody derived from b); and
   d) human monoclonal antibody.

22. An antibody fragment according to claim 20, wherein said antibody fragment is selected from the group consisting of F(ab'), F(ab), Fab', Fab, Fv, scFv, and minimal recognition unit.

23. An anti-idiotypic antibody that specifically binds to said antibody of claim 20.

24. A pharmaceutical composition comprising a polypeptide, said polypeptide comprising a sequence of amino acid residues that is at least 80% identical to the amino acid sequence as shown in SEQ ID NO:2, from amino acid residue 26 (Gly) to amino acid residue 313 (Glu), and specifically binds with an antibody that specifically binds with a polypeptide having the amino acid sequence of SEQ ID NO:2; in combination with a pharmaceutically acceptable vehicle.

25. A method of detecting the presence of ZSIG-11 RNA is a biological sample, comprising the steps of:
   a) contacting a ZSIG-11 nucleic acid probe under stringent hybridizing conditions with either
      i) test RNA molecules isolated from the biological sample, or
      ii) nucleic acid molecules synthesized from the isolated RNA molecules,
   wherein said probe has a nucleotide sequence comprising a portion of the nucleotide sequence of SEQ ID NO:1, or its complement, and
b) detecting the formation of hybrids of the nucleic acid probe and either the test RNA molecules or the synthesized nucleic acid molecules, wherein the presence of the hybrids indicates the presence of ZSIG-11 RNA is the biological sample.

26. A method of detecting the presence of ZSIG-11 in a biological sample, comprising the steps of:
   a) contacting the biological sample with an antibody, or an antibody fragment, that specifically binds with a polypeptide having the amino acid sequence of SEQ ID NO:2, wherein said contacting is performed under conditions that allow the binding of the antibody or antibody fragment to the biological sample, and
   b) detecting any of the bound antibody or bound antibody fragment.

27. The method according to claim 26, wherein said antibody or the antibody fragment further comprises a detectable label selected from the group consisting of radioisotope, fluorescent label, chemiluminescent label, enzyme label, bioluminescent label, and colloidal gold.
SEQUENCE LISTING

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1201 Eastlake Avenue East
Seattle, Washington 98102
United States of America

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INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/10 C12N15/62 C12N5/10 C12N1/19 C07K14/47
C07K16/18 C12O1/68 G01N33/566 A61K38/17

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 6 C12N C07K C12Q G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database consulted during the international search (name of database and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Further documents are listed in the continuation of box C. Patent family members are listed in annex.

Date of the actual completion of the international search

26 January 1999

Date of mailing of the international search report

05/02/1999

Name and mailing address of the ISA

European Patent Office, P. B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk, Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016

Authorized officer

Galli, I
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