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  1  MPGQELRTVN GSQMLLVLLV LSWLPHGGAL SLAEASRASF PGPSELHSED
 51  SRFRELRKRY EDLLTRLRAN QSWEDSNTDL VPAPAVRILT PEVRLGSGGH
101  LHLRISRAAL PEGLPEASRL HRALFRLSPT ASRSWDVTRP LRRQLSLARP
151  QAPALHLRLS PPPSQSDQLL AESSSARPQL ELHLRPQAAR GRRRARARNG
201  DHCPLGPGRC CRLHTVRASL EDLGWADWVL SPREVQVTMC IGACPSQFRA
251  ANMHAQIKTS LHRLKPDTVP APCCVPASYN PMVLIQKTDT GVSLQTYDDL
301  LAKDCHCI     (SEQ ID NO: 1)
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FIGURE 1

(57) Abstract: In certain aspects, the present disclosure relates to polypeptides comprising a truncated, ligand-binding portion of the extracellular domain of TβRII polypeptide useful to selectively antagonize a TβRII ligand. The disclosure further provides compositions and methods for use in treating or preventing TGFβ associated disorders.

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TGF-BETA RECEPTOR TYPE II VARIANTS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims priority from United States provisional application 61/868,713, filed on August 22, 2013; United States provisional application 61/906,270, filed on November 19, 2013; and United States provisional application 61/906,849, filed November 20, 2013. The disclosure of the priority application is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] Members of the transforming growth factor-beta (TGFβ) superfamily are pleiotropic cytokines involved in essential cellular functions such as proliferation, differentiation, apoptosis, motility, extracellular matrix production, tissue remodeling, angiogenesis, immune response, cell adhesion, and also play a key role in pathophysiology of disease states as different as chronic inflammatory conditions and cancer. Members of the TGFβ superfamily have been classified into major family groupings, which include TGFβs, bone morphogenetic proteins (BMP), osteogenic proteins (OP), growth and differentiation factors (GDF), inhibins/activins, mullerian inhibitory substances (MIS) and glial derived neurotrophic factors (GDNF).

[0003] TGFβ superfamily members transduce their signals across the plasma membrane by inducing the formation of heteromeric complexes of specific type I and type II serine/threonine kinase receptors, which in turn activate a particular subset of SMAD proteins (some inhibitory and some excitatory). The SMAD molecule compounds relay the signals into the nucleus where they direct transcriptional responses in concert with other proteins.

[0004] Dysfunctional TGFβ superfamily signaling has been linked to several clinical disorders including cancer, fibrosis, bone diseases, diabetic nephropathy, as well as chronic vascular diseases such as atherosclerosis.

[0005] Thus, it is an object of the present disclosure to provide compositions and methods for modulating TGFβ superfamily signaling.

SUMMARY OF THE INVENTION

[0006] In part, the disclosure provides TβRII polypeptides and the use of such TβRII polypeptides as selective antagonists for GDF15, TGFβ1 or TGFβ3. As described herein, polypeptides comprising part or all of the TβRII extracellular domain (ECD), with or without additional mutations, bind to and/or inhibit GDF15, TGFβ1 or TGFβ3 with varying affinities. Thus, in certain aspects, the disclosure provides TβRII polypeptides for use in selectively inhibiting TGFβ superfamily associated disorders.

[0007] In certain aspects, the disclosure provides polypeptides comprising mutations and/or truncations in the extracellular domain of TβRII. In certain aspects, the disclosure provides a TβRII fusion polypeptide comprising a first amino acid sequence from the extracellular domain of TβRII and a heterologous amino acid sequence, wherein the first amino acid sequence comprises or consists of an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical or identical to a) a sequence beginning at any of positions 23 to 35 of SEQ ID NO: 5 and ending at any of positions 153 to 159 of SEQ ID NO: 5 or b) a sequence beginning at any of positions 23 to 60 of SEQ ID NO: 6 and ending at any of positions 178 to 184 of SEQ ID NO: 6.

[0008] In certain aspects the disclosure provides polypeptides comprising a wild-type or altered and/or truncated extracellular domain of TβRII fused to at least a portion of the Fc domain of a human IgG2. Thus in certain aspects, the disclosure provides a TβRII fusion polypeptide comprising a first amino acid sequence from the extracellular domain of TβRII and a heterologous amino acid sequence, wherein the first amino acid sequence comprises or consists of an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical or identical to a) a sequence beginning at any of positions 23 to 35 of SEQ ID NO: 5 and ending at any of positions 153 to 159 of SEQ ID NO: 5 or b) a sequence beginning at any of positions 23 to 60 of SEQ ID NO: 6 and ending at any of positions 178 to 184 of SEQ ID NO: 6, and wherein the polypeptide comprises a second polypeptide sequence that comprises at least a constant domain of a human IgG2 and may optionally comprise or consist of an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical to SEQ ID NO: 19, and wherein an linker is optionally positioned between the first polypeptide and the second polypeptide. An example of the is provided as SEQ ID NO:50 and is encoded by the nucleic acid sequence of SEQ ID NO:51.

In certain embodiments, the disclosure provides polypeptides with an amino acid sequence that comprises or consists of an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%. 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:50. In certain embodiments, the disclosure provides polypeptides that are encoded by a nucleic acid sequence that comprises or consists of a nucleic acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%. 98%, 99% or 100% identical to the nucleic acid sequence of SEQ ID NO:51.

[0009] In some embodiments, the first amino acid sequence comprises or consists of the sequence beginning at position 23 of SEQ ID NO: 5 and ending at position 159 of SEQ ID NO: 5. In some embodiments, the first amino acid sequence comprises or consists of the sequence beginning at position 29 of SEQ ID NO: 5 and ending at position 159 of SEQ ID NO: 5. In some embodiments, the first amino acid sequence comprises or consists of the sequence beginning at position 35 of SEQ ID NO: 5 and ending at position 159 of SEQ ID NO: 5. In some embodiments, the first amino acid sequence comprises or consists of the sequence beginning at position 23 of SEQ ID NO: 5 and ending at position 153 of SEQ ID NO: 5. In some embodiments, the first amino acid sequence comprises or consists of the sequence beginning at position 29 of SEQ ID NO: 5 and ending at position 153 of SEQ ID NO: 5. In some embodiments, the first amino acid sequence comprises or consists of the sequence beginning at position 35 of SEQ ID NO: 5 and ending at position 153 of SEQ ID NO: 5.

[0010] In some embodiments, the first amino acid sequence comprises or consists of the sequence beginning at position 23 of SEQ ID NO: 6 and ending at positions 184 of SEQ ID NO: 6. In some embodiments, the first amino acid sequence comprises or consists of the sequence beginning at position 29 of SEQ ID NO: 6 and ending at position 184 of SEQ ID NO: 6. In some embodiments, the first amino acid sequence comprises or consists of the sequence beginning at position 23 of SEQ ID NO: 6 and ending at position 178 of SEQ ID NO: 6. In some embodiments, the first amino acid sequence comprises or consists of the sequence beginning at position 29 of SEQ ID NO: 6 and ending at position 178 of SEQ ID NO: 6.

[0011] In some embodiments, the first amino acid sequence comprises or consists of a sequence that has a D at the position corresponding to position 36 of SEQ ID NO: 47 and/or a K at the position corresponding to position 76 of SEQ ID NO: 47.

[0012] In certain aspects, the disclosure provides a TβRII fusion polypeptide comprising a first amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical or identical to the sequence of SEQ ID NO: 7 or SEQ ID NO: 13, or active fragment thereof, and a second heterologous portion, wherein the first amino acid sequence has a D at the position corresponding to position 36 of SEQ ID NO: 47 and/or a K at the position corresponding to position 76 of SEQ ID NO: 47.

[0013] In some embodiments, the first amino acid sequence comprises an N-terminal truncation of 1-12 amino acids corresponding to amino acids 1-12 of SEQ ID NO: 7 or 1-37 amino acids corresponding to amino acids 1-37 of SEQ ID NO: 13. In some embodiments, the first amino acid sequence comprises an N-terminal truncation of 6 amino acids corresponding to amino acids 1-6 of SEQ ID NO: 7 or SEQ ID NO: 13. In some embodiments, the first amino acid sequence comprises an N-terminal truncation of 12 amino acids corresponding to amino acids 1-12 of SEQ ID NO: 7 or 37 amino acids corresponding to amino acids 1-37 of SEQ ID NO: 13. In some embodiments, the first amino acid sequence comprises a C-terminal truncation of 1-6 amino acids corresponding to amino acids 137-132 of SEQ ID NO: 7 or amino acids 162-157 of SEQ ID NO: 13. In some embodiments, the first amino acid sequence comprises a C-terminal truncation of 6 amino acids corresponding to amino acids 132-137 of SEQ ID NO: 7 or amino acids 157-162 of SEQ ID NO: 13. In some embodiments, the first amino acid sequence comprises an insertion corresponding to SEQ ID NO: 18 between the residues corresponding to positions 117 and 118 of SEQ ID NO: 47.

[0014] In some embodiments, the heterologous portion comprises one or more polypeptide portions that enhance one or more of: in vivo stability, in vivo half life, uptake/administration, tissue localization or distribution, formation of protein complexes, and/or purification. In some embodiments, the heterologous portion comprises a polypeptide portion selected from: an immunoglobulin Fc domain and a serum albumin. In a further embodiment, the immunoglobulin Fc domain is joined to the TβRII polypeptide by a linker.

[0015] In some embodiments, the polypeptide includes one or more modified amino acid residues selected from: a glycosylated amino acid, a PEGylated amino acid, a farnesylated amino acid, an acetylated amino acid, a biotinylated amino acid, an amino acid

conjugated to a lipid moiety, and an amino acid conjugated to an organic derivatizing agent. In some embodiments, the polypeptide is glycosylated.

[0016] In certain aspects, the disclosure provides a TβRII fusion polypeptide comprising a first amino acid sequence consisting of a portion of the extracellular domain of TβRII that comprises an amino acid sequence that is at least 80%, at least 85%, at least 90%, or at least 95% identical to an amino acid sequence selected from SEQ ID NOs: 7-17 and 47-49, and a second heterologous portion. In certain aspects, the disclosure provides a TβRII fusion polypeptide comprising a first amino acid sequence consisting of a portion of the extracellular domain of TβRII that comprises an amino acid sequence that is at least 96% identical to an amino acid sequence selected from SEQ ID NOs: 7-17 and 47-49, and a second heterologous portion. In certain aspects, the disclosure provides a TβRII fusion polypeptide comprising a first amino acid sequence consisting of a portion of the extracellular domain of TβRII that comprises an amino acid sequence that is at least 97% identical to an amino acid sequence selected from SEQ ID NOs: 7-17 and 47-49, and a second heterologous portion. In certain aspects, the disclosure provides a TβRII fusion polypeptide comprising a first amino acid sequence consisting of a portion of the extracellular domain of TβRII that comprises an amino acid sequence that is at least 98% identical to an amino acid sequence selected from SEQ ID NOs: 7-17 and 47-49, and a second heterologous portion. In certain aspects, the disclosure provides a TβRII fusion polypeptide comprising a first amino acid sequence consisting of a portion of the extracellular domain of TβRII that comprises an amino acid sequence that is at least 99% identical to an amino acid sequence selected from SEQ ID NOs: 7-17 and 47-49, and a second heterologous portion. In certain aspects, the disclosure provides a TβRII fusion polypeptide comprising a first amino acid sequence consisting of a portion of the extracellular domain of TβRII that comprises an amino acid sequence is an amino acid sequence selected from SEQ ID NOs: 7-17 and 47-49 and a second heterologous portion.

[0017] In certain aspects, the disclosure provides a polypeptide comprising or consisting of an amino acid sequence that is at least 80%, at least 85%, at least 90%, or at least 95% identical to an amino acid sequence selected from SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37, 39, 41 and 43 or the portion thereof with the leader sequence removed, e.g., a polypeptide comprising or consisting of an amino acid sequence that is at least 80%, at least 85%, at least 90%, or at least 95% identical to an amino acid sequence selected from SEQ ID

NOs: 53, 54, 55, 56, 57, 58, 59, 60, 61, and 62. In certain aspects, the disclosure provides a polypeptide comprising or consisting of an amino acid sequence that is at least 96% identical to an amino acid sequence selected from SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37, 39, 41 and 43 or the portion thereof with the leader sequence removed, e.g., a polypeptide comprising or consisting of an amino acid sequence that is at least 96% identical to an amino acid sequence selected from SEQ ID NOs: 53, 54, 55, 56, 57, 58, 59, 60, 61, and 62. In certain aspects, the disclosure provides a polypeptide comprising or consisting of an amino acid sequence that is at least 97% identical to an amino acid sequence selected from SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37, 39, 41 and 43 or the portion thereof with the leader sequence removed, e.g., a polypeptide comprising or consisting of an amino acid sequence that is at least 97% identical to an amino acid sequence selected from SEQ ID NOs: 53, 54, 55, 56, 57, 58, 59, 60, 61, and 62. In certain aspects, the disclosure provides a polypeptide comprising or consisting of an amino acid sequence that is at least 98% identical to an amino acid sequence selected from SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37, 39, 41 and 43 or the portion thereof with the leader sequence removed, e.g., a polypeptide comprising or consisting of an amino acid sequence that is at least 98% identical to an amino acid sequence selected from SEQ ID NOs: 53, 54, 55, 56, 57, 58, 59, 60, 61, and 62. In certain aspects, the disclosure provides a polypeptide comprising or consisting of an amino acid sequence that is at least 99% identical to an amino acid sequence selected from SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37, 39, 41 and 43 or the portion thereof with the leader sequence removed, e.g., a polypeptide comprising or consisting of an amino acid sequence that is at least 99% identical to an amino acid sequence selected from SEQ ID NOs: 53, 54, 55, 56, 57, 58, 59, 60, 61, and 62. In certain aspects, the disclosure provides a polypeptide comprising or consisting of an amino acid sequence selected from SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37, 39, 41 and 43 or the portion thereof with the leader sequence removed, e.g., a polypeptide comprising or consisting of an amino acid sequence selected from SEQ ID NOs: 53, 54, 55, 56, 57, 58, 59, 60, 61, and 62.

[0018] In certain aspects, the disclosure provides a TβRII polypeptide comprising of an amino acid sequence encoded by a nucleic acid that hybridizes under stringent conditions to a complement of a nucleotide sequence selected from SEQ ID NOs: 26, 28, 30, 32, 34, 36, 38, 40, 42 and 44.

[0019] In each of the foregoing, the TβRII polypeptide may be selected that it does not include a full-length TβRII ECD. A TβRII polypeptide may be used as a monomeric

protein or in a dimerized form. A TβRII polypeptide may also be fused to a second polypeptide portion to provide improved properties, such as increased half-life or greater ease of production or purification. A fusion may be direct or a linker may be inserted between the TβRII polypeptide and any other portion. A linker may be structured or unstructured and may consist of 1, 2, 3, 4, 5, 10, 15, 20, 30, 50 or more amino acids, optionally relatively free of secondary structure.

[0020] In some embodiments, a TβRII polypeptide of the disclosure binds human GDF15 with an equilibrium dissociation constant (K_D) less than 1 x 10^{-8} M.

[0021] In some embodiments, a TβRII polypeptide of the disclosure has a glycosylation pattern characteristic of expression of the polypeptide in CHO cells.

[0022] In some embodiments, the disclosure provides a homodimer comprising two TβRII polypeptides of the disclosure.

[0023] In some embodiments, the disclosure provides an isolated polynucleotide comprising a coding sequence for the TβRII polypeptides of the disclosure. In some embodiments, the disclosure provides a recombinant polynucleotide comprising a promoter sequence operably linked to the isolated polynucleotide. In some embodiments, the disclosure provides a cell transformed with an isolated polynucleotide or a recombinant polynucleotide of the disclosure. In some embodiments, the cell is a mammalian cell. In some embodiments, the cell is a CHO cell or a human cell. In some embodiments, the cell is an HEK-293 cell.

[0024] In certain aspects, the disclosure provides a pharmaceutical preparation comprising the TβRII polypeptides or homodimers of the disclosure and a pharmaceutically acceptable excipient.

[0025] In certain aspects, the disclosure provides a method of modulating the response of a cell to a TGFβ superfamily member, the method comprising exposing the cell to a TβRII polypeptide or homodimer of the disclosure.

[0026] In certain aspects, the disclosure provides a method of treating a disease or condition associated with a TGFβ superfamily member in a patient in need thereof, the method comprising administering to the patient an effective amount of the TβRII polypeptides or homodimers of the disclosure. In some embodiments, the TGFβ superfamily member is TGFβ1, TGFβ3 or GDF15.

[0027] In some embodiments, the disease or condition is a cancer. In some embodiments, the cancer is selected from stomach cancer, intestinal cancer, skin cancer, breast cancer, melanoma, bone cancer and thyroid cancer.

[0028] In some embodiments, the disease or condition is a fibrotic or sclerotic disease or disorder. In some embodiments, the fibrotic or sclerotic disease or disorder is selected from scleroderma, atherosclerosis, liver fibrosis, diffuse systemic sclerosis, glomerulonephritis, neural scarring, dermal scarring, radiation-induced fibrosis, hepatic fibrosis, and myelofibrosis.

[0029] In some embodiments, the disease or condition is heart disease.

[0030] In some embodiments, the disease or condition is selected from hereditary hemorrhagic telangiectasia (HHT), Marfan syndrome, Loeys-Dietz syndrome, familial thoracic aortic aneurysm syndrome, arterial tortuosity syndrome, pre-eclampsia, atherosclerosis, restenosis, and hypertrophic cardiomyopathy/congestive heart failure.

[0031] In certain aspects, the disclosure provides an antibody, or antigen binding fragment thereof, that binds to GDF15 and blocks the interaction between GDF15 and TβRII.

[0032] In certain aspects, the disclosure provides a GDF15 polypeptide comprising the amino acid sequence of SEQ ID NO: 1 or a fragment thereof that binds TβRII, wherein the GDF15 polypeptide is at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% pure, with respect to protein contaminants.

[0033] In certain aspects, the disclosure provides a GDF15 polypeptide comprising the amino acid sequence of SEQ ID NO: 1 or a fragment thereof that binds to a TβRII

polypeptide of the disclosure, wherein the GDF15 polypeptide is at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% pure, with respect to protein contaminants.

[0034] In some embodiments, the GDF15 polypeptide binds TβRII with an equilibrium dissociation constant (K_D) of no greater than 10^{-8} M. In some embodiments, the GDF15 polypeptide binds to a TβRII polypeptide of the disclosure with an equilibrium dissociation constant (K_D) of no greater than 10^{-8} M.

[0035] In some embodiments, the GDF15 polypeptide is produced by expression in CHO cells.

[0036] In certain aspects, the disclosure provides a method of concentrating or purifying GDF15, comprising contacting a sample containing GDF15 with a TβRII polypeptide of the disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0037] **Figure 1** shows the amino acid sequence of native precursor for human GDF15 (NCBI reference seq: NP_004855.2). Solid underline indicates mature GDF15 (residues 197-308), with N-terminus determined by sequencing. Dotted underline denotes leader (residues 1-29).

[0038] **Figure 2** shows a nucleotide sequence encoding native precursor for human GDF15. Solid underline indicates the sequence encoding mature GDF15 (nucleotides 589-924), and dotted underline denotes the sequence encoding the leader (nucleotides 1-87). A silent mutation (G456A) used to disrupt a SfoI site in NM_004864.2 is double underlined.

[0039] **Figure 3** shows the amino acid sequence of native precursor for murine GDF15 (NP_035949.2). Solid underline indicates mature GDF15 (residues 192-303), with N-terminus determined by sequencing. Dotted underline denotes leader (residues 1-30).

[0040] **Figure 4** shows a nucleotide sequence encoding native precursor for murine GDF15 (derived from NM_011819.2). Solid underline indicates the sequence encoding mature GDF15 (nucleotides 574-909), and dotted underline denotes the sequence encoding the leader (nucleotides 1-90).

[0041] **Figure 5** shows the amino acid sequence of native precursor for the B (short) isoform of human TGFβ receptor type II (hTβRII) (NP_003233.4). Solid underline indicates the mature extracellular domain (ECD) (residues 23-159), and double underline indicates valine that is replaced in the A (long) isoform. Dotted underline denotes leader (residues 1-22).

[0042] **Figure 6** shows the amino acid sequence of native precursor for the A (long) isoform of human TβRII (NP_001020018.1). Solid underline indicates the mature ECD (residues 23-184), and double underline indicates the splice-generated isoleucine substitution. Dotted underline denotes leader (residues 1-22).

[0043] **Figure 7** shows N-terminal alignment of $hT\beta RII_{short}$ truncations and their $hT\beta RII_{long}$ counterparts. The 25-amino-acid insertion present in $hT\beta RII_{long}$ truncations is underlined. Note that the splicing process causes the valine flanking the insertion site in the short isoform to be replaced by an isoleucine in the long isoform. Boxed sequence denotes leader.

DETAILED DESCRIPTION OF THE INVENTION

1. Overview

[0044] Proteins described herein are the human forms, unless otherwise specified. NCBI references for the proteins are as follows: human TβRII isoform A ($hT\beta RII_{long}$), NP_001020018.1; human TβRII isoform B ($hT\beta RII_{short}$), NP_003233.4; human GDF15, NP_004855.2; murine GDF15, NP_035949.2. Sequences of native TβRII and GDF15 proteins from human and mouse are set forth in Figures 1-6.

[0045] The TGFβ superfamily contains a variety of growth factors that share common sequence elements and structural motifs. These proteins are known to exert biological effects on a large variety of cell types in both vertebrates and invertebrates. Members of the superfamily perform important functions during embryonic development in pattern formation and tissue specification and can influence a variety of differentiation processes, including adipogenesis, myogenesis, chondrogenesis, cardiogenesis, hematopoiesis, neurogenesis, and epithelial cell differentiation. By manipulating the activity of a member of the TGFβ family, it is often possible to cause significant physiological changes in an organism. For example, the Piedmontese and Belgian Blue cattle breeds carry

a loss-of-function mutation in the GDF8 (also called myostatin) gene that causes a marked increase in muscle mass. Grobet et al., Nat Genet. 1997, 17(1):71-4. Similarly, in humans, inactive alleles of GDF8 are associated with increased muscle mass and, reportedly, exceptional strength. Schuelke et al., N Engl J Med 2004, 350:2682-8.

[0046] TGFβ signals are mediated by heteromeric complexes of type I (e.g. TβRI) and type II (e.g. TβRII) serine/ threonine kinase receptors, which phosphorylate and activate downstream SMAD proteins upon ligand stimulation (Massagué, 2000, Nat. Rev. Mol. Cell Biol. 1:169-178). These type I and type II receptors are transmembrane proteins, composed of a ligand-binding extracellular domain with cysteine-rich region, a transmembrane domain, and a cytoplasmic domain with predicted serine/threonine specificity. Type I receptors are essential for signaling; and type II receptors are required for binding ligands and for expression of type I receptors. Type I and II receptors form a stable complex after ligand binding, resulting in phosphorylation of type I receptors by type II receptors. TGFβ has three mammalian isoforms, TGFβ1, TGFβ2 and TGFβ3, each with distinct functions in vivo. The binding of TGFβs to TβRII is a crucial step in initiating activation of the TGFβ signaling pathway, leading to phosphorylation of SMAD2, and translocation of the activated SMAD2/SMAD4 complex to the nucleus to modulate gene expression.

[0047] Growth differentiation factor 15 (GDF15) is a member of the TGFβ family. Like other ligands in the TGFβ superfamily, which contain a characteristic cysteine knot motif, mature GDF15 is synthesized with a larger prodomain (Harrison et al., Growth Factors 29:174, 2011; Shi et al., Nature 474:343, 2011) that is removed through cleavage by a furin-like protease at the canonical RXXR site to generate mature dimeric GDF15. GDF15 has been described in the literature as macrophage inhibitory cytokine-1 (MIC-1), placental bone morphogenic protein (PLAB), placental transforming growth factor beta (PTGFβ), prostate derived factor (PDF), and non-steroidal anti-inflammatory activated gene-1 (NAG-1) reflecting the different functions that have been implied for this protein. GDF15 has been linked to several physiologic and pathologic conditions. For example, GDF15 is highly expressed in the placenta, and is necessary for the maintenance of pregnancy. GDF15 concentration is also notably increased in the serum of patients with prostate, colorectal, or pancreatic cancer, as well as glioma. GDF15 has not been shown biochemically to bind or interact directly with any receptor. The present disclosure relates in part to the discovery that the TGFβ type II receptor, TβRII, binds to GDF15 with high affinity and is a functional

receptor for GDF15. TβRII fusion polypeptides, and other polypeptides containing a ligand-binding portion of TβRII are demonstrated herein to inhibit GDF15-induced gene activation. The potent inhibition of GDF15 signaling provides evidence that TβRII is a functional type II receptor for GDF15, opening a new avenue for therapeutic interventions in this signaling pathway. Therefore, in part, the disclosure identifies a physiological, high-affinity receptor for GDF15 polypeptides.

[0048] Surprisingly, soluble TβRII polypeptides are shown herein to have highly specific, high-affinity binding for GDF15. TβRII is the known type II receptor for TGFβ and binds with high affinity to TGFβ1 and TGFβ3. Human TβRII occurs naturally in at least two isoforms – A (long) and B (short) – generated by alternative splicing in the extracellular domain (ECD) (Figures 6 and 5 and SEQ ID NOS: 6 and 5). The long isoform has a 25-amino-acid insertion and the splicing process causes the valine flanking the insertion site in the short isoform to be replaced by an isoleucine in the long isoform. Soluble receptor ectodomains can function as scavengers or ligand traps to inhibit ligand-receptor interactions. Ligand traps such as soluble TβRII-Fc fusion proteins incorporating the native TβRII extracellular domain (ectodomain) will function as pan-inhibitors against TβRII ligands, including, TGFβ1, TGFβ3 and based on the findings disclosed herein, GDF15. While in some therapeutic settings this broader spectrum of ligand-binding and signal inhibition may be advantageous, in other settings a more selective molecule may be superior. It is highly desirable for ligand traps such as TβRII ectodomain polypeptides to exhibit selective ligand-binding profiles. The present disclosure relates to the surprising discovery that polypeptides comprising a truncated portion of the extracellular domain of TβRII and/or mutations within the extracellular domain have differential inhibitory effects on cell signaling by GDF15, TGFβ1 or TGFβ3. In part, the disclosure provides ligand traps, generated by a series of mutations and/or truncations in the extracellular domain of TβRII, that exhibit varying ligand-binding profiles distinct from that of the native TβRII extracellular domain. The variant TβRII polypeptides disclosed herein provide advantageous properties relative to the native full-length extracellular domain, and may be used to selectively inhibit pathways mediated by the different TβRII ligands in vivo.

[0049] Thus, in certain aspects, the disclosure provides TβRII polypeptides as antagonists of GDF15, TGFβ1 or TGFβ3 for use in treating various GDF15-, TGFβ1- or TGFβ3-associated disorders. While not wishing to be bound to any particular mechanism of

action, it is expected that such polypeptides act by binding to GDF15, TGFβ1 or TGFβ3 and inhibiting the ability of these ligands to form ternary signaling complexes.

[0050] The terms used in this specification generally have their ordinary meanings in the art, within the context of this invention and in the specific context where each term is used. Certain terms are discussed below or elsewhere in the specification, to provide additional guidance to the practitioner in describing the compositions and methods of the invention and how to make and use them. The scope or meaning of any use of a term will be apparent from the specific context in which the term is used.

2. TβRII Polypeptides

[0051] Naturally occurring TβRII proteins are transmembrane proteins, with a portion of the protein positioned outside the cell (the extracellular portion) and a portion of the protein positioned inside the cell (the intracellular portion). Aspects of the present disclosure encompass variant TβRII polypeptides comprising mutations within the extracellular domain and/or truncated portions of the extracellular domain of TβRII. As described above, human TβRII occurs naturally in at least two isoforms – A (long) and B (short) – generated by alternative splicing in the extracellular domain (ECD) (Figures 6 and 5 and SEQ ID NOS: 6 and 5). SEQ ID NO: 7, which corresponds to residues 23-159 of SEQ ID NO: 5, depicts the native full-length extracellular domain of the short isoform of TβRII. SEQ ID NO: 13, which corresponds to residues 23-184 of SEQ ID NO: 6, depicts the native full-length extracellular domain of the long isoform of TβRII. Unless noted otherwise, amino acid position numbering with regard to variants based on the TβRII short and long isoforms refers to the corresponding position in the native precursors, SEQ ID NO: 5 and SEQ ID NO:6, respectively.

[0052] In certain embodiments, the disclosure provides variant TβRII polypeptides. A TβRII polypeptide of the disclosure may bind to and inhibit the function of a TGFβ superfamily member, such as but not limited to, GDF15, TGFβ1 or TGFβ3. TβRII polypeptides may include a polypeptide consisting of, or comprising, an amino acid sequence at least 80% identical, and optionally at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a truncated ECD domain of a naturally occurring TβRII polypeptide, whose C-terminus occurs at any of amino acids 153-159 of SEQ ID NO: 5. TβRII polypeptides may include a polypeptide consisting of, or comprising, an amino acid sequence at least 80%

identical, and optionally at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a truncated ECD domain of a naturally occurring TβRII polypeptide, whose C-terminus occurs at any of amino acids 178-184 of SEQ ID NO: 6. Optionally, a TβRII polypeptide does not include more than 5 consecutive amino acids, or more than 10, 20, 30, 40, 50, 52, 60, 70, 80, 90, 100, 150 or 200 or more consecutive amino acids from a sequence consisting of amino acids 160-567 of SEQ ID NO: 5 or from a sequence consisting of amino acids 185-592 of SEQ ID NO: 6. The unprocessed TβRII polypeptide may either include or exclude any signal sequence, as well as any sequence N-terminal to the signal sequence. As elaborated herein, the N-terminus of the mature (processed) TβRII polypeptide may occur at any of amino acids 23-35 of SEQ ID NO: 5 or 23-60 of SEQ ID NO: 6. Examples of mature TβRII polypeptides include, but are not limited to, amino acids 23-159 of SEQ ID NO: 5 (set forth in SEQ ID NO: 7), amino acids 29-159 of SEQ ID NO: 5 (set forth in SEQ ID NO: 9), amino acids 35-159 of SEQ ID NO: 5 (set forth in SEQ ID NO: 10), amino acids 23-153 of SEQ ID NO: 5 (set forth in SEQ ID NO: 11), amino acids 29-153 of SEQ ID NO: 5 (set forth in SEQ ID NO: 48), amino acids 35-153 of SEQ ID NO: 5 (set forth in SEQ ID NO: 47), amino acids 23-184 of SEQ ID NO: 6 (set forth in SEQ ID NO: 13), amino acids 29-184 of SEQ ID NO: 6 (set forth in SEQ ID NO: 15), amino acids 60-184 of SEQ ID NO:6 (set forth in SEQ ID NO: 10), amino acids 23-178 of SEQ ID NO: 6 (set forth in SEQ ID NO: 16), amino acids 29-178 of SEQ ID NO: 6 (set forth in SEQ ID NO: 49), and amino acids 60-178 of SEQ ID NO: 6 (set forth in SEQ ID NO: 47). Likewise, a TβRII polypeptide may comprise a polypeptide that is encoded by nucleotides 73-465 of SEQ ID NO: 30, nucleotides 73-447 of SEQ ID NO: 34, nucleotides 73-465 of SEQ ID NO: 38, nucleotides 91-465 of SEQ ID NO: 38, or nucleotides 109-465 of SEQ ID NO: 38, or silent variants thereof or nucleic acids that hybridize to the complement thereof under stringent hybridization conditions (generally, such conditions are known in the art but may, for example, involve hybridization in 50% v/v formamide, 5x SSC, 2% w/v blocking agent, 0.1% N-lauroylsarcosine, and 0.3% SDS at 65°C overnight and washing in, for example, 5x SSC at about 65°C). It will be understood by one of skill in the art that corresponding variants based on the long isoform of TβRII will include nucleotide sequences encoding the 25-amino acid insertion along with a conservative Val-Ile substitution at the flanking position C-terminal to the insertion. The TβRII polypeptides accordingly may include isolated extracellular portions of TβRII polypeptides, including both the short and the long isoforms, variants thereof (including variants that comprise, for example, no more than 2, 3, 4, 5, 10, 15, 20, 25, 30, or 35 amino acid substitutions in the sequence corresponding to amino acids 23-159 of SEQ ID

NO: 5 or amino acids 23-184 of SEQ ID NO: 6), fragments thereof, and fusion proteins comprising any of the foregoing, but in each case preferably any of the foregoing TβRII polypeptides will retain substantial affinity for at least one of GDF15, TGFβ1 or TGFβ3. Generally, a TβRII polypeptide will be designed to be soluble in aqueous solutions at biologically relevant temperatures, pH levels, and osmolarity.

[0053] In some embodiments, the variant TβRII polypeptides of the disclosure comprise one or more mutations in the extracellular domain that confer an altered ligand binding profile. A TβRII polypeptide may include one, two, five or more alterations in the amino acid sequence relative to the corresponding portion of a naturally occurring TβRII polypeptide. In some embodiments, the mutation results in a substitution, insertion, or deletion at the position corresponding to position 70 of SEQ ID NO: 5. In some embodiments, the mutation results in a substitution, insertion, or deletion at the position corresponding to position 110 of SEQ ID NO: 5. Examples include, but are not limited to, an N to D substitution or a D to K substitution in the positions corresponding to positions 70 and 110, respectively, of SEQ ID NO: 5. Examples of such variant TβRII polypeptides include, but are not limited to, the sequences set forth in SEQ ID NO: 8, SEQ ID NO:14, SEQ ID NO: 12 and SEQ ID NO: 17. A TβRII polypeptide may comprise a polypeptide or portion thereof that is encoded by nucleotides 73-483 of SEQ ID NO: 26, nucleotides 73-465 of SEQ ID NO: 42 or silent variants thereof or nucleic acids that hybridize to the complement thereof under stringent hybridization conditions.

[0054] In some embodiments, the variant TβRII polypeptides of the disclosure further comprise an insertion of 36 amino acids (SEQ ID NO: 18) between the pair of glutamate residues (positions 151 and 152 of SEQ ID NO: 5, or positions 176 and 177 of SEQ ID NO: 6) located near the C-terminus of the human TβRII ECD, as occurs naturally in the human TβRII isoform C (Konrad et al., BMC Genomics 8:318, 2007).

[0055] The disclosure further demonstrates that TβRII polypeptides can be modified to selectively antagonize TβRII ligands. Data presented here show that Fc fusion proteins comprising shorter N-terminally and C-terminally truncated variants of TβRII polypeptides display differential inhibitory effects on cellular signaling mediated by GDF15, TGFβ1 and TGFβ3. Specifically, N-terminally truncated variants beginning at amino acids 29 or 35 of SEQ ID NO: 5 and carrying, respectively, a 6- or 12-amino acid N-terminal truncation of the extracellular domain, were found to inhibit GDF15 most potently, TGFβ3 least potently and

TGFβ1 to an intermediate degree, compared to the full length extracellular domain of the short isoform of TβRII. C-terminally truncated variants, ending at amino acid 153 of SEQ ID NO: 5 and carrying a 6-amino acid C-terminal truncation of the extracellular domain had no substantial effect on ligand binding and may therefore be used interchangeably with full length versions. An N to D substitution at the position corresponding to position 70 of SEQ ID NO: 5, was found to inhibit TGFβ3 potently, have intermediate effect on GDF15 and negligible effect on TGFβ1. The N70 residue represents a potential glycosylation site. Further, an Fc fusion protein comprising a D to K substitution at the position corresponding to position 110 of SEQ ID NO: 5, was found to inhibit GDF15 most potently, TGFβ1 least potently and TGFβ3 to an intermediate degree compared to compared to the full length extracellular domain of the short isoform of TβRII. The region around position 110 has not been associated with selectivity for the known TβRII ligands TGFβ1, TGFβ2 and TGFβ3. Thus, unexpectedly, TβRII polypeptides that contain mutations in the ECD, such as but not limited to, N70D and D110K (the numbering of the residues corresponds to that of SEQ ID NO: 5) and/or begin between amino acids 29 and 35 and/or terminate between amino acid 153 and amino acid 159 are all expected to be active and exhibit widely different inhibitory potencies towards the different ligands. Any of these truncated variant forms may be desirable to use, depending on the clinical or experimental setting.

[0056] In certain embodiments, a TβRII polypeptide binds to GDF15, and the TβRII polypeptide does not show substantial binding to TGFβ1 or TGFβ3. In certain embodiments, a TβRII polypeptide binds to TGFβ1, and the TβRII polypeptide does not show substantial binding to GDF15 or TGFβ3. In certain embodiments, a TβRII polypeptide binds to TGFβ3, and the TβRII polypeptide does not show substantial binding to GDF15 or TGFβ1. Binding may be assessed using purified proteins in solution or in a surface plasmon resonance system, such as a Biacore™ system.

[0057] In certain embodiments, a TβRII polypeptide inhibits GDF15 cellular signaling, and the TβRII polypeptide has an intermediate or limited inhibitory effect on TGFβ1 or TGFβ3. In certain embodiments, a TβRII polypeptide inhibits TGFβ1 cellular signaling, and the TβRII polypeptide has an intermediate or limited inhibitory effect on GDF15 or TGFβ3. In certain embodiments, a TβRII polypeptide inhibits TGFβ3 cellular signaling, and the TβRII polypeptide has an intermediate or limited inhibitory effect on

GDF15 or TGFβ1. Inhibitory effect on cell signaling can be assayed by methods known in the art.

[0058] Taken together, an active portion of a TβRII polypeptide may comprise amino acid sequences 23-153, 23-154, 23-155, 23-156, 23-157, or 23-158 of SEQ ID NO: 5, as well as variants of these sequences starting at any of amino acids 24-35 of SEQ ID NO: 5. Similarly, an active portion of a TβRII polypeptide may comprise amino acid sequences 23-178, 23-179, 23-180, 23-181, 23-182, or 23-183 of SEQ ID NO: 6, as well as variants of these sequences starting at any of amino acids 24-60 of SEQ ID NO: 6. Exemplary TβRII polypeptides comprise amino acid sequences 29-159, 35-159, 23-153, 29-153 and 35-153 of SEQ ID NO: 5 or amino acid sequences 29-184, 60-184, 23-178, 29-178 and 60-178 of SEQ ID NO: 6. Variants within these ranges are also contemplated, particularly those having at least 80%, 85%, 90%, 95%, or 99% identity to the corresponding portion of SEQ ID NO: 5 or SEQ ID NO: 6. A TβRII polypeptide may be selected that does not include the sequence consisting of amino acids 160-567 of SEQ ID NO:5 or amino acids 185-592 of SEQ ID NO:6.

[0059] As described above, the disclosure provides TβRII polypeptides sharing a specified degree of sequence identity or similarity to a naturally occurring TβRII polypeptide. To determine the percent identity of two amino acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). The amino acid residues at corresponding amino acid positions are then compared. When a position in the first sequence is occupied by the same amino acid residue as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid "identity" is equivalent to amino acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

[0060] The comparison of sequences and determination of percent identity and similarity between two sequences can be accomplished using a mathematical algorithm (Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press,

New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991).

[0061] In one embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (J Mol. Biol. (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at http://www.gcg.com). In a specific embodiment, the following parameters are used in the GAP program: either a Blosum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (Devereux, J., *et al.*, Nucleic Acids Res. 12(1):387 (1984)) (available at http://www.gcg.com). Exemplary parameters include using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. Unless otherwise specified, percent identity between two amino acid sequences is to be determined using the GAP program using a Blosum 62 matrix, a GAP weight of 10 and a length weight of 3, and if such algorithm cannot compute the desired percent identity, a suitable alternative disclosed herein should be selected.

[0062] In another embodiment, the percent identity between two amino acid sequences is determined using the algorithm of E. Myers and W. Miller (CABIOS, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

[0063] Another embodiment for determining the best overall alignment between two amino acid sequences can be determined using the FASTDB computer program based on the algorithm of Brutlag *et al.* (*Comp. App. Biosci.,* 6:237-245 (1990)). In a sequence alignment the query and subject sequences are both amino acid sequences. The result of said global sequence alignment is presented in terms of percent identity. In one embodiment, amino acid sequence identity is performed using the FASTDB computer program based on the algorithm of Brutlag *et al.* (*Comp. App. Biosci.,* 6:237-245 (1990)). In a specific embodiment, parameters employed to calculate percent identity and similarity of an amino acid alignment comprise: Matrix=PAM 150, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5 and Gap Size

Penalty=0.05.

[0064] TβRII polypeptides may additionally include any of various leader sequences at the N-terminus. Such a sequence would allow the peptides to be expressed and targeted to the secretion pathway in a eukaryotic system. See, e.g., Ernst et al., U.S. Pat. No. 5,082,783 (1992). Alternatively, a native TβRII signal sequence may be used to effect extrusion from the cell. Possible leader sequences include native leaders, tissue plasminogen activator (TPA) and honeybee mellitin (SEQ ID NOs. 22-24, respectively). Examples of TβRII-Fc fusion proteins incorporating a TPA leader sequence include SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37, 39, 41, and 43. Processing of signal peptides may vary depending on the leader sequence chosen, the cell type used and culture conditions, among other variables, and therefore actual N-terminal start sites for mature TβRII polypeptides may shift by 1, 2, 3, 4 or 5 amino acids in either the N-terminal or C-terminal direction. Examples of TβRII-Fc fusion proteins include SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 53, 54, 55, 56, 57, 58, 59, 60, 61, and 62, as shown herein with the TβRII polypeptide portion underlined (see Examples). It will be understood by one of skill in the art that corresponding variants based on the long isoform of TβRII will include the 25-amino acid insertion along with a conservative Val-Ile substitution at the flanking position C-terminal to the insertion.

[0065] In certain embodiments, the present disclosure contemplates specific mutations of the TβRII polypeptides so as to alter the glycosylation of the polypeptide. Such mutations may be selected so as to introduce or eliminate one or more glycosylation sites, such as O-linked or N-linked glycosylation sites. Asparagine-linked glycosylation recognition sites generally comprise a tripeptide sequence, asparagine-X-threonine (or asparagine-X-serine) (where "X" is any amino acid) which is specifically recognized by appropriate cellular glycosylation enzymes. The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the wild-type TβRII polypeptide (for O-linked glycosylation sites). A variety of amino acid substitutions or deletions at one or both of the first or third amino acid positions of a glycosylation recognition site (and/or amino acid deletion at the second position) results in non-glycosylation at the modified tripeptide sequence. Another means of increasing the number of carbohydrate moieties on a TβRII polypeptide is by chemical or enzymatic coupling of glycosides to the TβRII polypeptide. Depending on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine; (b) free carboxyl groups; (c) free

sulfhydryl groups such as those of cysteine; (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline; (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan; or (f) the amide group of glutamine. These methods are described in WO 87/05330 published Sep. 11, 1987, and in Aplin and Wriston (1981) CRC Crit. Rev. Biochem., pp. 259-306, incorporated by reference herein. Removal of one or more carbohydrate moieties present on a TβRII polypeptide may be accomplished chemically and/or enzymatically. Chemical deglycosylation may involve, for example, exposure of the TβRII polypeptide to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the amino acid sequence intact. Chemical deglycosylation is further described by Hakimuddin et al. (1987) Arch. Biochem. Biophys. 259:52 and by Edge et al. (1981) Anal. Biochem. 118:131. Enzymatic cleavage of carbohydrate moieties on TβRII polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura et al. (1987) Meth. Enzymol. 138:350. The sequence of a TβRII polypeptide may be adjusted, as appropriate, depending on the type of expression system used, as mammalian, yeast, insect and plant cells may all introduce differing glycosylation patterns that can be affected by the amino acid sequence of the peptide. In general, TβRII polypeptides for use in humans will be expressed in a mammalian cell line that provides proper glycosylation, such as HEK293 or CHO cell lines, although other mammalian expression cell lines, yeast cell lines with engineered glycosylation enzymes, and insect cells are expected to be useful as well.

[0066] This disclosure further contemplates a method of generating mutants, particularly sets of combinatorial mutants of a TβRII polypeptide, as well as truncation mutants; pools of combinatorial mutants are especially useful for identifying functional variant sequences. The purpose of screening such combinatorial libraries may be to generate, for example, TβRII polypeptide variants which can act as either agonists or antagonist, or alternatively, which possess novel activities all together. A variety of screening assays are provided below, and such assays may be used to evaluate variants. For example, a TβRII polypeptide variant may be screened for ability to bind to a TβRII ligand, to prevent binding of a TβRII ligand to a TβRII polypeptide or to interfere with signaling caused by a TβRII ligand. The activity of a TβRII polypeptide or its variants may also be tested in a cell-based or in vivo assay, particularly any of the assays disclosed in the Examples.

[0067] Combinatorially-derived variants can be generated which have a selective or generally increased potency relative to a TβRII polypeptide comprising an extracellular domain of a naturally occurring TβRII polypeptide. Likewise, mutagenesis can give rise to variants which have serum half-lives dramatically different than the corresponding wild-type TβRII polypeptide. For example, the altered protein can be rendered either more stable or less stable to proteolytic degradation or other processes which result in destruction of, or otherwise elimination or inactivation of, a native TβRII polypeptide. Such variants, and the genes which encode them, can be utilized to alter TβRII polypeptide levels by modulating the half-life of the TβRII polypeptides. For instance, a short half-life can give rise to more transient biological effects and can allow tighter control of recombinant TβRII polypeptide levels within the patient. In an Fc fusion protein, mutations may be made in the linker (if any) and/or the Fc portion to alter the half-life of the protein.

[0068] A combinatorial library may be produced by way of a degenerate library of genes encoding a library of polypeptides which each include at least a portion of potential TβRII polypeptide sequences. For instance, a mixture of synthetic oligonucleotides can be enzymatically ligated into gene sequences such that the degenerate set of potential TβRII polypeptide nucleotide sequences are expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (e.g., for phage display).

[0069] There are many ways by which the library of potential TβRII polypeptide variants can be generated from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be carried out in an automatic DNA synthesizer, and the synthetic genes then be ligated into an appropriate vector for expression. The synthesis of degenerate oligonucleotides is well known in the art (see for example, Narang, SA (1983) Tetrahedron 39:3; Itakura et al., (1981) Recombinant DNA, Proc. 3rd Cleveland Sympos. Macromolecules, ed. AG Walton, Amsterdam: Elsevier pp273-289; Itakura et al., (1984) Annu. Rev. Biochem. 53:323; Itakura et al., (1984) Science 198:1056; Ike et al., (1983) Nucleic Acid Res. 11:477). Such techniques have been employed in the directed evolution of other proteins (see, for example, Scott et al., (1990) Science 249:386-390; Roberts et al., (1992) PNAS USA 89:2429-2433; Devlin et al., (1990) Science 249: 404-406; Cwirla et al., (1990) PNAS USA 87: 6378-6382; as well as U.S. Patent Nos: 5,223,409, 5,198,346, and 5,096,815).

[0070] Alternatively, other forms of mutagenesis can be utilized to generate a combinatorial library. For example, TβRII polypeptide variants can be generated and isolated from a library by screening using, for example, alanine scanning mutagenesis and the like (Ruf et al., (1994) Biochemistry 33:1565-1572; Wang et al., (1994) J. Biol. Chem. 269:3095-3099; Balint et al., (1993) Gene 137:109-118; Grodberg et al., (1993) Eur. J. Biochem. 218:597-601; Nagashima et al., (1993) J. Biol. Chem. 268:2888-2892; Lowman et al., (1991) Biochemistry 30:10832-10838; and Cunningham et al., (1989) Science 244:1081-1085), by linker scanning mutagenesis (Gustin et al., (1993) Virology 193:653-660; Brown et al., (1992) Mol. Cell Biol. 12:2644-2652; McKnight et al., (1982) Science 232:316); by saturation mutagenesis (Meyers et al., (1986) Science 232:613); by PCR mutagenesis (Leung et al., (1989) Method Cell Mol Biol 1:11-19); or by random mutagenesis, including chemical mutagenesis, etc. (Miller et al., (1992) A Short Course in Bacterial Genetics, CSHL Press, Cold Spring Harbor, NY; and Greener et al., (1994) Strategies in Mol Biol 7:32-34). Linker scanning mutagenesis, particularly in a combinatorial setting, is an attractive method for identifying truncated (bioactive) forms of TβRII polypeptides.

[0071] A wide range of techniques are known in the art for screening gene products of combinatorial libraries made by point mutations and truncations, and, for that matter, for screening cDNA libraries for gene products having a certain property. Such techniques will be generally adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of TβRII polypeptides. The most widely used techniques for screening large gene libraries typically comprises cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates relatively easy isolation of the vector encoding the gene whose product was detected. Preferred assays include TβRII ligand binding assays and ligand-mediated cell signaling assays.

[0072] In certain embodiments, the TβRII polypeptides of the disclosure may further comprise post-translational modifications in addition to any that are naturally present in the TβRII polypeptides. Such modifications include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, pegylation (polyethylene glycol) and acylation. As a result, the modified TβRII polypeptides may contain non-amino acid elements, such as polyethylene glycols, lipids, mono- or poly-saccharides, and phosphates.

Effects of such non-amino acid elements on the functionality of a TβRII polypeptide may be tested as described herein for other TβRII polypeptide variants. When a TβRII polypeptide is produced in cells by cleaving a nascent form of the TβRII polypeptide, post-translational processing may also be important for correct folding and/or function of the protein. Different cells (such as CHO, HeLa, MDCK, 293, WI38, NIH-3T3 or HEK-293) have specific cellular machinery and characteristic mechanisms for such post-translational activities and may be chosen to ensure the correct modification and processing of the TβRII polypeptides.

[0073] In certain aspects, functional variants or modified forms of the TβRII polypeptides include fusion proteins having at least a portion of the TβRII polypeptides and one or more fusion domains. Well-known examples of such fusion domains include, but are not limited to, polyhistidine, Glu-Glu, glutathione S transferase (GST), thioredoxin, protein A, protein G, an immunoglobulin heavy chain constant region (Fc), maltose binding protein (MBP), or human serum albumin. A fusion domain may be selected so as to confer a desired property. For example, some fusion domains are particularly useful for isolation of the fusion proteins by affinity chromatography. For the purpose of affinity purification, relevant matrices for affinity chromatography, such as glutathione-, amylase-, and nickel- or cobalt-conjugated resins are used. Many of such matrices are available in "kit" form, such as the Pharmacia GST purification system and the QIAexpress^{TM} system (Qiagen) useful with (HIS_6) fusion partners. As another example, a fusion domain may be selected so as to facilitate detection of the TβRII polypeptides. Examples of such detection domains include the various fluorescent proteins (e.g., GFP) as well as "epitope tags," which are usually short peptide sequences for which a specific antibody is available. Well known epitope tags for which specific monoclonal antibodies are readily available include FLAG, influenza virus haemagglutinin (HA), and c-myc tags. In some cases, the fusion domains have a protease cleavage site, such as for Factor Xa or Thrombin, which allows the relevant protease to partially digest the fusion proteins and thereby liberate the recombinant proteins therefrom. The liberated proteins can then be isolated from the fusion domain by subsequent chromatographic separation. In certain preferred embodiments, a TβRII polypeptide is fused with a domain that stabilizes the TβRII polypeptide in vivo (a "stabilizer" domain). By "stabilizing" is meant anything that increases serum half life, regardless of whether this is because of decreased destruction, decreased clearance by the kidney, or other pharmacokinetic effect. Fusions with the Fc portion of an immunoglobulin are known to confer desirable pharmacokinetic properties on a wide range of proteins. Likewise, fusions to

human serum albumin can confer desirable properties. Other types of fusion domains that may be selected include multimerizing (e.g., dimerizing, tetramerizing) domains and functional domains.

[0074] As specific examples, the present disclosure provides fusion proteins comprising variants of TβRII polypeptides fused to one of three Fc domain sequences (e.g., SEQ ID NOs: 19, 20, and 21). Optionally, the Fc domain has one or more mutations at residues such as Asp-265, Lys-322, and Asn-434 (numbered in accordance with the corresponding full-length IgG). In certain cases, the mutant Fc domain having one or more of these mutations (e.g., Asp-265 mutation) has reduced ability of binding to the Fcγ receptor relative to a wildtype Fc domain. In other cases, the mutant Fc domain having one or more of these mutations (e.g., Asn-434 mutation) has increased ability of binding to the MHC class I-related Fc-receptor (FcRN) relative to a wildtype Fc domain.

[0075] It is understood that different elements of the fusion proteins may be arranged in any manner that is consistent with the desired functionality. For example, a TβRII polypeptide may be placed C-terminal to a heterologous domain, or, alternatively, a heterologous domain may be placed C-terminal to a TβRII polypeptide. The TβRII polypeptide domain and the heterologous domain need not be adjacent in a fusion protein, and additional domains or amino acid sequences may be included C- or N-terminal to either domain or between the domains.

[0076] As used herein, the term "immunoglobulin Fc domain" or simply “Fc” is understood to mean the carboxyl-terminal portion of an immunoglobulin chain constant region, preferably an immunoglobulin heavy chain constant region, or a portion thereof. For example, an immunoglobulin Fc region may comprise 1) a CH1 domain, a CH2 domain, and a CH3 domain, 2) a CH1 domain and a CH2 domain, 3) a CH1 domain and a CH3 domain, 4) a CH2 domain and a CH3 domain, or 5) a combination of two or more domains and an immunoglobulin hinge region. In a preferred embodiment the immunoglobulin Fc region comprises at least an immunoglobulin hinge region a CH2 domain and a CH3 domain, and preferably lacks the CH1 domain.

[0077] In one embodiment, the class of immunoglobulin from which the heavy chain constant region is derived is IgG (Igγ) (γ subclasses 1, 2, 3, or 4). Other classes of immunoglobulin, IgA (Igα), IgD (Igδ), IgE (Igε) and IgM (Igμ), may be used. The choice of

appropriate immunoglobulin heavy chain constant region is discussed in detail in U.S. Pat. Nos. 5,541,087 and 5,726,044. The choice of particular immunoglobulin heavy chain constant region sequences from certain immunoglobulin classes and subclasses to achieve a particular result is considered to be within the level of skill in the art. The portion of the DNA construct encoding the immunoglobulin Fc region preferably comprises at least a portion of a hinge domain, and preferably at least a portion of a CH_3 domain of Fc gamma or the homologous domains in any of IgA, IgD, IgE, or IgM.

[0078] Furthermore, it is contemplated that substitution or deletion of amino acids within the immunoglobulin heavy chain constant regions may be useful in the practice of the methods and compositions disclosed herein. One example would be to introduce amino acid substitutions in the upper CH2 region to create an Fc variant with reduced affinity for Fc receptors (Cole *et al.* (1997) J. Immunol. 159:3613).

[0079] In certain embodiments, the present disclosure makes available isolated and/or purified forms of the TβRII polypeptides, which are isolated from, or otherwise substantially free of (e.g., at least 80%, 90%, 95%, 96%, 97%, 98%, or 99% free of), other proteins and/or other TβRII polypeptide species. TβRII polypeptides will generally be produced by expression from recombinant nucleic acids.

[0080] In certain embodiments, the disclosure includes nucleic acids encoding soluble TβRII polypeptides comprising the coding sequence for an extracellular portion of a TβRII protein. In further embodiments, this disclosure also pertains to a host cell comprising such nucleic acids. The host cell may be any prokaryotic or eukaryotic cell. For example, a polypeptide of the present disclosure may be expressed in bacterial cells such as *E. coli*, insect cells (e.g., using a baculovirus expression system), yeast, or mammalian cells. Other suitable host cells are known to those skilled in the art. Accordingly, some embodiments of the present disclosure further pertain to methods of producing the TβRII polypeptides.

3. Nucleic Acids Encoding TβRII Polypeptides

[0081] In certain aspects, the disclosure provides isolated and/or recombinant nucleic acids encoding any of the TβRII polypeptides, including fragments, functional variants and fusion proteins disclosed herein. SEQ ID NO: 26, 28, 30, 32, 34, 36, 38, 40, 42, and 44 encode variants of TβRII extracellular domain fused to an IgG2 Fc or an N-terminally truncated IgG1 Fc domain. The subject nucleic acids may be single-stranded or double

stranded. Such nucleic acids may be DNA or RNA molecules. These nucleic acids may be used, for example, in methods for making TβRII polypeptides or as direct therapeutic agents (e.g., in an antisense, RNAi or gene therapy approach).

[0082] In certain aspects, the subject nucleic acids encoding TβRII polypeptides are further understood to include nucleic acids that are variants of SEQ ID NOs: 26, 28, 30, 32, 34, 36, 38, 40, 42, and 44. Variant nucleotide sequences include sequences that differ by one or more nucleotide substitutions, additions or deletions, such as allelic variants.

[0083] In certain embodiments, the disclosure provides isolated or recombinant nucleic acid sequences that are at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NOs: 26, 28, 30, 32, 34, 36, 38, 40, 42, and 44. One of ordinary skill in the art will appreciate that nucleic acid sequences complementary to SEQ ID NOs: 26, 28, 30, 32, 34, 36, 38, 40, 42, and 44, and variants of SEQ ID NOs: 26, 28, 30, 32, 34, 36, 38, 40, 42, and 44 are also within the scope of this disclosure. In further embodiments, the nucleic acid sequences of the disclosure can be isolated, recombinant, and/or fused with a heterologous nucleotide sequence, or in a DNA library.

[0084] In other embodiments, nucleic acids of the disclosure also include nucleotide sequences that hybridize under highly stringent conditions to the nucleotide sequences designated in SEQ ID NOs: 26, 28, 30, 32, 34, 36, 38, 40, 42, and 44 complement sequences of SEQ ID NOs: 26, 28, 30, 32, 34, 36, 38, 40, 42, and 44, or fragments thereof. As discussed above, one of ordinary skill in the art will understand readily that appropriate stringency conditions which promote DNA hybridization can be varied. For example, one could perform the hybridization at 6.0 x sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of 2.0 x SSC at 50°C. For example, the salt concentration in the wash step can be selected from a low stringency of about 2.0 x SSC at 50°C to a high stringency of about 0.2 x SSC at 50°C. In addition, the temperature in the wash step can be increased from low stringency conditions at room temperature, about 22°C, to high stringency conditions at about 65°C. Both temperature and salt may be varied, or temperature or salt concentration may be held constant while the other variable is changed. In some embodiments, the disclosure provides nucleic acids which hybridize under low stringency conditions of 6 x SSC at room temperature followed by a wash at 2 x SSC at room temperature.

[0085] Isolated nucleic acids which differ from the nucleic acids as set forth in SEQ ID NOs: 26, 28, 30, 32, 34, 36, 38, 40, 42, and 44 due to degeneracy in the genetic code are also within the scope of the disclosure. For example, a number of amino acids are designated by more than one triplet. Codons that specify the same amino acid, or synonyms (for example, CAU and CAC are synonyms for histidine) may result in "silent" mutations which do not affect the amino acid sequence of the protein. However, it is expected that DNA sequence polymorphisms that do lead to changes in the amino acid sequences of the subject proteins will exist among mammalian cells. One skilled in the art will appreciate that these variations in one or more nucleotides (up to about 3-5% of the nucleotides) of the nucleic acids encoding a particular protein may exist among individuals of a given species due to natural allelic variation. Any and all such nucleotide variations and resulting amino acid polymorphisms are within the scope of this disclosure.

[0086] It will be appreciated by one of skill in the art that corresponding variants based on the long isoform of TβRII will include nucleotide sequences encoding the 25-amino acid insertion along with a conservative Val-Ile substitution at the flanking position C-terminal to the insertion. It will also be appreciated that corresponding variants based on either the long (A) or short (B) isoforms of TβRII will include variant nucleotide sequences comprising an insertion of 108 nucleotides, encoding a 36-amino-acid insertion (SEQ ID NO: 18), at the same location described for naturally occurring TβRII isoform C (see Exemplification).

[0087] In certain embodiments, the recombinant nucleic acids of the disclosure may be operably linked to one or more regulatory nucleotide sequences in an expression construct. Regulatory nucleotide sequences will generally be appropriate to the host cell used for expression. Numerous types of appropriate expression vectors and suitable regulatory sequences are known in the art for a variety of host cells. Typically, said one or more regulatory nucleotide sequences may include, but are not limited to, promoter sequences, leader or signal sequences, ribosomal binding sites, transcriptional start and termination sequences, translational start and termination sequences, and enhancer or activator sequences. Constitutive or inducible promoters as known in the art are contemplated by the disclosure. The promoters may be either naturally occurring promoters, or hybrid promoters that combine elements of more than one promoter. An expression construct may be present in a cell on an episome, such as a plasmid, or the expression construct may be inserted in a

chromosome. In a preferred embodiment, the expression vector contains a selectable marker gene to allow the selection of transformed host cells. Selectable marker genes are well known in the art and will vary with the host cell used.

[0088] In certain aspects disclosed herein, the subject nucleic acid is provided in an expression vector comprising a nucleotide sequence encoding a TβRII polypeptide and operably linked to at least one regulatory sequence. Regulatory sequences are art-recognized and are selected to direct expression of the TβRII polypeptide. Accordingly, the term regulatory sequence includes promoters, enhancers, and other expression control elements. Exemplary regulatory sequences are described in Goeddel; *Gene Expression Technology*: *Methods in Enzymology*, Academic Press, San Diego, CA (1990). For instance, any of a wide variety of expression control sequences that control the expression of a DNA sequence when operatively linked to it may be used in these vectors to express DNA sequences encoding a TβRII polypeptide. Such useful expression control sequences, include, for example, the early and late promoters of SV40, tet promoter, adenovirus or cytomegalovirus immediate early promoter, RSV promoters, the lac system, the trp system, the TAC or TRC system, T7 promoter whose expression is directed by T7 RNA polymerase, the major operator and promoter regions of phage lambda , the control regions for fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase, e.g., Pho5, the promoters of the yeast α-mating factors, the polyhedron promoter of the baculovirus system and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells or their viruses, and various combinations thereof. It should be understood that the design of the expression vector may depend on such factors as the choice of the host cell to be transformed and/or the type of protein desired to be expressed. Moreover, the vector's copy number, the ability to control that copy number and the expression of any other protein encoded by the vector, such as antibiotic markers, should also be considered.

[0089] A recombinant nucleic acid included in the disclosure can be produced by ligating the cloned gene, or a portion thereof, into a vector suitable for expression in either prokaryotic cells, eukaryotic cells (yeast, avian, insect or mammalian), or both. Expression vehicles for production of a recombinant TβRII polypeptide include plasmids and other vectors. For instance, suitable vectors include plasmids of the types: pBR322-derived

plasmids, pEMBL-derived plasmids, pEX-derived plasmids, pBTac-derived plasmids and pUC-derived plasmids for expression in prokaryotic cells, such as *E. coli*.

[0090] Some mammalian expression vectors contain both prokaryotic sequences to facilitate the propagation of the vector in bacteria, and one or more eukaryotic transcription units that are expressed in eukaryotic cells. The pcDNAI/amp, pcDNAI/neo, pRc/CMV, pSV2gpt, pSV2neo, pSV2-dhfr, pTk2, pRSVneo, pMSG, pSVT7, pko-neo and pHyg derived vectors are examples of mammalian expression vectors suitable for transfection of eukaryotic cells. Some of these vectors are modified with sequences from bacterial plasmids, such as pBR322, to facilitate replication and drug resistance selection in both prokaryotic and eukaryotic cells. Alternatively, derivatives of viruses such as the bovine papilloma virus (BPV-1), or Epstein-Barr virus (pHEBo, pREP-derived and p205) can be used for transient expression of proteins in eukaryotic cells. Examples of other viral (including retroviral) expression systems can be found below in the description of gene therapy delivery systems. The various methods employed in the preparation of the plasmids and in transformation of host organisms are well known in the art. For other suitable expression systems for both prokaryotic and eukaryotic cells, as well as general recombinant procedures, see *Molecular Cloning A Laboratory Manual*, 3rd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press, 2001). In some instances, it may be desirable to express the recombinant polypeptides by the use of a baculovirus expression system. Examples of such baculovirus expression systems include pVL-derived vectors (such as pVL1392, pVL1393 and pVL941), pAcUW-derived vectors (such as pAcUW1), and pBlueBac-derived vectors (such as the ß-gal containing pBlueBac III).

[0091] In certain embodiments, a vector will be designed for production of the subject TβRII polypeptides in CHO cells, such as a Pcmv-Script vector (Stratagene, La Jolla, Calif.), pcDNA4 vectors (Invitrogen, Carlsbad, Calif.) and pCI-neo vectors (Promega, Madison, Wisc.). In a preferred embodiment, a vector will be designed for production of the subject TβRII polypeptides in HEK-293 cells. As will be apparent, the subject gene constructs can be used to cause expression of the subject TβRII polypeptides in cells propagated in culture, e.g., to produce proteins, including fusion proteins or variant proteins, for purification.

[0092] This disclosure also pertains to a host cell transfected with a recombinant gene including a coding sequence (e.g., SEQ ID NOs: 26, 28, 30, 32, 34, 36, 38, 40, 42, or

44) for one or more of the subject TβRII polypeptides. The host cell may be any prokaryotic or eukaryotic cell. For example, a TβRII polypeptide disclosed herein may be expressed in bacterial cells such as *E. coli*, insect cells (e.g., using a baculovirus expression system), yeast, or mammalian cells. Other suitable host cells are known to those skilled in the art.

[0093] Accordingly, the present disclosure further pertains to methods of producing the subject TβRII polypeptides. For example, a host cell transfected with an expression vector encoding a TβRII polypeptide can be cultured under appropriate conditions to allow expression of the TβRII polypeptide to occur. The TβRII polypeptide may be secreted and isolated from a mixture of cells and medium containing the TβRII polypeptide. Alternatively, the TβRII polypeptide may be retained cytoplasmically or in a membrane fraction and the cells harvested, lysed and the protein isolated. A cell culture includes host cells, and media. Suitable media for cell culture are well known in the art. The subject TβRII polypeptides can be isolated from cell culture medium, host cells, or both, using techniques known in the art for purifying proteins, including ion-exchange chromatography, gel filtration chromatography, ultrafiltration, electrophoresis, immunoaffinity purification with antibodies specific for particular epitopes of the TβRII polypeptides and affinity purification with an agent that binds to a domain fused to the TβRII polypeptide (e.g., a protein A column may be used to purify an TβRII-Fc fusion). In a preferred embodiment, the TβRII polypeptide is a fusion protein containing a domain which facilitates its purification. As an example, purification may be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

[0094] In another embodiment, a fusion gene coding for a purification leader sequence, such as a poly-(His)/enterokinase cleavage site sequence at the N-terminus of the desired portion of the recombinant TβRII polypeptide, can allow purification of the expressed fusion protein by affinity chromatography using a Ni^{2+} metal resin. The purification leader sequence can then be subsequently removed by treatment with enterokinase to provide the purified TβRII polypeptide (e.g., see Hochuli et al., (1987) *J. Chromatography* 411:177; and Janknecht et al., *PNAS USA* 88:8972).

[0095] Techniques for making fusion genes are well known. Essentially, the joining of various DNA fragments coding for different polypeptide sequences is performed in accordance with conventional techniques, employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed to generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel et al., John Wiley & Sons: 1992).

[0096] Examples of categories of nucleic acid compounds that are antagonists of TβRII, TGFβ1, TGFβ3 and GDF15 include antisense nucleic acids, RNAi constructs and catalytic nucleic acid constructs. A nucleic acid compound may be single or double stranded. A double stranded compound may also include regions of overhang or non-complementarity, where one or the other of the strands is single-stranded. A single-stranded compound may include regions of self-complementarity, meaning that the compound forms a so-called "hairpin" or "stem-loop" structure, with a region of double helical structure. A nucleic acid compound may comprise a nucleotide sequence that is complementary to a region consisting of no more than 1000, no more than 500, no more than 250, no more than 100 or no more than 50, 35, 30, 25, 22, 20 or 18 nucleotides of the full-length TβRII nucleic acid sequence or ligand nucleic acid sequence. The region of complementarity will preferably be at least 8 nucleotides, and optionally at least 10 or at least 15 nucleotides, such as between 15 and 25 nucleotides. A region of complementarity may fall within an intron, a coding sequence, or a noncoding sequence of the target transcript, such as the coding sequence portion. Generally, a nucleic acid compound will have a length of about 8 to about 500 nucleotides or base pairs in length, such as about 14 to about 50 nucleotides. A nucleic acid may be a DNA (particularly for use as an antisense), RNA, or RNA:DNA hybrid. Any one strand may include a mixture of DNA and RNA, as well as modified forms that cannot readily be classified as either DNA or RNA. Likewise, a double-stranded compound may be DNA:DNA, DNA:RNA or RNA:RNA, and any one strand may also include a mixture of DNA and RNA, as well as modified forms that cannot readily be classified as either DNA or RNA. A nucleic acid compound may include any of a variety of modifications, including one

or modifications to the backbone (the sugar-phosphate portion in a natural nucleic acid, including internucleotide linkages) or the base portion (the purine or pyrimidine portion of a natural nucleic acid). An antisense nucleic acid compound will preferably have a length of about 15 to about 30 nucleotides and will often contain one or more modifications to improve characteristics such as stability in the serum, in a cell or in a place where the compound is likely to be delivered, such as the stomach in the case of orally delivered compounds and the lung for inhaled compounds. In the case of an RNAi construct, the strand complementary to the target transcript will generally be RNA or modifications thereof. The other strand may be RNA, DNA, or any other variation. The duplex portion of double-stranded or single-stranded "hairpin" RNAi construct will preferably have a length of 18 to 40 nucleotides in length and optionally about 21 to 23 nucleotides in length, so long as it serves as a Dicer substrate. Catalytic or enzymatic nucleic acids may be ribozymes or DNA enzymes and may also contain modified forms. Nucleic acid compounds may inhibit expression of the target by about 50%, 75%, 90%, or more when contacted with cells under physiological conditions and at a concentration where a nonsense or sense control has little or no effect. Preferred concentrations for testing the effect of nucleic acid compounds are 1, 5 and 10 micromolar. Nucleic acid compounds may also be tested for effects on, for example, angiogenesis.

4. Alterations in Fc-fusion proteins

[0097] The application further provides TβRII-Fc fusion proteins with engineered or variant Fc regions. Such antibodies and Fc fusion proteins may be useful, for example, in modulating effector functions, such as, antigen-dependent cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). Additionally, the modifications may improve the stability of the antibodies and Fc fusion proteins. Amino acid sequence variants of the antibodies and Fc fusion proteins are prepared by introducing appropriate nucleotide changes into the DNA, or by peptide synthesis. Such variants include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antibodies and Fc fusion proteins disclosed herein. Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the antibodies and Fc fusion proteins, such as changing the number or position of glycosylation sites.

[0098] Antibodies and Fc fusion proteins with reduced effector function may be produced by introducing changes in the amino acid sequence, including, but are not limited to, the Ala-Ala mutation described by Bluestone et al. (see WO 94/28027 and WO 98/47531; also see Xu et al. 2000 Cell Immunol 200; 16-26). Thus, in certain embodiments, antibodies and Fc fusion proteins of the disclosure with mutations within the constant region including the Ala-Ala mutation may be used to reduce or abolish effector function. According to these embodiments, antibodies and Fc fusion proteins may comprise a mutation to an alanine at position 234 or a mutation to an alanine at position 235, or a combination thereof. In one embodiment, the antibody or Fc fusion protein comprises an IgG4 framework, wherein the Ala-Ala mutation would describe a mutation(s) from phenylalanine to alanine at position 234 and/or a mutation from leucine to alanine at position 235. In another embodiment, the antibody or Fc fusion protein comprises an IgG1 framework, wherein the Ala-Ala mutation would describe a mutation(s) from leucine to alanine at position 234 and/or a mutation from leucine to alanine at position 235. The antibody or Fc fusion protein may alternatively or additionally carry other mutations, including the point mutation K322A in the CH2 domain (Hezareh et al. 2001 J Virol. 75: 12161-8).

[0099] In particular embodiments, the antibody or Fc fusion protein may be modified to either enhance or inhibit complement dependent cytotoxicity (CDC). Modulated CDC activity may be achieved by introducing one or more amino acid substitutions, insertions, or deletions in an Fc region (see, e.g., U.S. Pat. No. 6,194,551). Alternatively or additionally, cysteine residue(s) may be introduced in the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved or reduced internalization capability and/or increased or decreased complement-mediated cell killing. See Caron et al., J. Exp Med. 176:1191-1195 (1992) and Shopes, B. J. Immunol. 148:2918-2922 (1992), WO99/51642, Duncan & Winter Nature 322: 738-40 (1988); U.S. Pat. No. 5,648,260; U.S. Pat. No. 5,624,821; and WO94/29351.

5. GDF15- TβRII signaling

[0100] The present disclosure relates in part to the discovery that the TGFβ type II receptor (TβRII) binds to GDF15 with high affinity. Heretofore, GDF15 has not been shown biochemically to bind or interact directly with a receptor. Inadequate or inappropriate ligand purification could be a potential reason for the inactivity of commercially available GDF15. Exemplary GDF15 polypeptides demonstrating a TβRII binding activity and methods of

making and purifying such polypeptides are disclosed herein. Sequences of native precursor GDF15 proteins and nucleotides from human and mouse are set forth in Figures 1-4. Mature human GDF15 extends from residues 197 to 308 of SEQ ID NO: 1. Similarly, mature mouse GDF15 extends from residues 192 to 303 of SEQ ID NO: 3. In certain embodiments, the present disclosure makes available isolated and/or purified forms of the GDF15 polypeptides or fragments thereof, which are isolated from, or otherwise substantially free of (e.g., at least 80%, 90%, 95%, 96%, 97%, 98%, or 99% free of), other proteins and/or other GDF15 polypeptide species. The GDF15 polypeptides of the disclosure bind to TβRII with high affinity. Binding may be assessed using purified proteins in solution or in a surface plasmon resonance system, such as a Biacore™ system. The GDF15 polypeptides will have an affinity (a dissociation constant) of about 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} M or less for TβRII polypeptides. Preferably, the GDF15 polypeptides of the disclosure are isolated and purified according to methods described herein. GDF15 polypeptides will generally be produced by expression from recombinant nucleic acids.

[0101] GDF15 polypeptides may include a polypeptide consisting of, or comprising, an amino acid sequence at least 80% identical, and optionally at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the GDF15 polypeptide of SEQ ID NO: 1 or SEQ ID NO: 3, or a functional fragment thereof. GDF15 polypeptides may include a polypeptide consisting of, or comprising, an amino acid sequence at least 80% identical, and optionally at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the GDF15 polypeptide comprising residues 197 to 308 of SEQ ID NO: 1 or residues 192 to 303 of SEQ ID NO: 3, or a functional fragment thereof. The unprocessed GDF15 polypeptide may either include or exclude any signal sequence, as well as any sequence N-terminal to the signal sequence. A GDF15 polypeptide may include variants of SEQ ID NO: 1 or SEQ ID NO: 3, or portions thereof, corresponding to 197 to 308 of SEQ ID NO: 1 or residues 192 to 303 of SEQ ID NO: 3, respectively (including variants that comprise, for example, no more than 2, 3, 4, 5, 10, 15, 20, 25, 30, or 35 amino acid substitutions in the sequence of SEQ ID NO: 1 or SEQ ID NO: 3), fragments thereof, and fusion proteins comprising any of the foregoing, but in each case preferably any of the foregoing GDF15 polypeptides will possess substantial affinity for a TβRII polypeptide.

[0102] In certain embodiments, the GDF15 polypeptides of the disclosure may further comprise post-translational modifications in addition to any that are naturally present

in the GDF15 polypeptides. Such modifications include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, pegylation (polyethylene glycol) and acylation. As a result, the modified GDF15 polypeptides may contain non-amino acid elements, such as polyethylene glycols, lipids, mono- or poly-saccharides, and phosphates. Effects of such non-amino acid elements on the functionality of a GDF15 polypeptide may be tested as described herein for other GDF15 polypeptides. When a GDF15 polypeptide is produced in cells by cleaving a nascent form of the GDF15 polypeptide, post-translational processing may also be important for correct folding and/or function of the protein. Different cells (such as CHO, HeLa, MDCK, 293, WI38, NIH-3T3 or HEK-293) have specific cellular machinery and characteristic mechanisms for such post-translational activities and may be chosen to ensure the correct modification and processing of the GDF15 polypeptides.

[0103] In certain embodiments, the disclosure includes nucleic acids encoding precursor and mature GDF15 polypeptides. In further embodiments, this disclosure also pertains to a host cell comprising such nucleic acids. The host cell may be any prokaryotic or eukaryotic cell. For example, a polypeptide of the present disclosure may be expressed in bacterial cells such as *E. coli*, insect cells (e.g., using a baculovirus expression system), yeast, or mammalian cells. Other suitable host cells are known to those skilled in the art. Accordingly, some embodiments of the present disclosure further pertain to methods of producing the GDF15 polypeptides.

[0104] In certain aspects, the disclosure provides isolated and/or recombinant nucleic acids encoding any of the GDF15 polypeptides, including fragments, functional variants and fusion proteins disclosed herein. The subject nucleic acids may be single-stranded or double-stranded. Such nucleic acids may be DNA or RNA molecules. These nucleic acids may be used, for example, in methods for making GDF15 polypeptides or as direct therapeutic agents (e.g., in an antisense, RNAi or gene therapy approach).

[0105] In certain aspects, the subject nucleic acids encoding GDF15 polypeptides are further understood to include nucleic acids that are variants of SEQ ID NO: 1 or SEQ ID NO: 3. Variant nucleotide sequences include sequences that differ by one or more nucleotide substitutions, additions or deletions, such as allelic variants.

[0106] In certain embodiments, the disclosure provides isolated or recombinant nucleic acid sequences that are at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100%

identical to SEQ ID NO: 1 or SEQ ID NO: 3. One of ordinary skill in the art will appreciate that nucleic acid sequences complementary to SEQ ID NO: 1 or SEQ ID NO: 3 and variants of SEQ ID NO: 1 or SEQ ID NO: 3 are also within the scope of this disclosure. In further embodiments, the nucleic acid sequences of the disclosure can be isolated, recombinant, and/or fused with a heterologous nucleotide sequence, or in a DNA library.

[0107] In other embodiments, nucleic acids of the disclosure also include nucleotide sequences that hybridize under highly stringent conditions to the nucleotide sequences designated in SEQ ID NO: 1 or SEQ ID NO: 3, complement sequences of SEQ ID NO: 1 or SEQ ID NO: 3, or fragments thereof. As discussed above, one of ordinary skill in the art will understand readily that appropriate stringency conditions which promote DNA hybridization can be varied. For example, one could perform the hybridization at 6.0 x sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of 2.0 x SSC at 50°C. For example, the salt concentration in the wash step can be selected from a low stringency of about 2.0 x SSC at 50°C to a high stringency of about 0.2 x SSC at 50°C. In addition, the temperature in the wash step can be increased from low stringency conditions at room temperature, about 22°C, to high stringency conditions at about 65°C. Both temperature and salt may be varied, or temperature or salt concentration may be held constant while the other variable is changed. In some embodiments, the disclosure provides nucleic acids which hybridize under low stringency conditions of 6 x SSC at room temperature followed by a wash at 2 x SSC at room temperature.

[0108] Isolated nucleic acids which differ from the nucleic acids as set forth in SEQ ID NO: 1 or SEQ ID NO: 3 due to degeneracy in the genetic code are also within the scope of the disclosure. For example, a number of amino acids are designated by more than one triplet. Codons that specify the same amino acid, or synonyms (for example, CAU and CAC are synonyms for histidine) may result in "silent" mutations which do not affect the amino acid sequence of the protein. However, it is expected that DNA sequence polymorphisms that do lead to changes in the amino acid sequences of the subject proteins will exist among mammalian cells. One skilled in the art will appreciate that these variations in one or more nucleotides (up to about 3-5% of the nucleotides) of the nucleic acids encoding a particular protein may exist among individuals of a given species due to natural allelic variation. Any and all such nucleotide variations and resulting amino acid polymorphisms are within the scope of this disclosure.

[0109] In certain embodiments, the recombinant nucleic acids of the disclosure may be operably linked to one or more regulatory nucleotide sequences in an expression construct. Regulatory nucleotide sequences will generally be appropriate to the host cell used for expression. Numerous types of appropriate expression vectors and suitable regulatory sequences are known in the art for a variety of host cells. Typically, said one or more regulatory nucleotide sequences may include, but are not limited to, promoter sequences, leader or signal sequences, ribosomal binding sites, transcriptional start and termination sequences, translational start and termination sequences, and enhancer or activator sequences. Constitutive or inducible promoters as known in the art are contemplated by the disclosure. The promoters may be either naturally occurring promoters, or hybrid promoters that combine elements of more than one promoter. An expression construct may be present in a cell on an episome, such as a plasmid, or the expression construct may be inserted in a chromosome. In a preferred embodiment, the expression vector contains a selectable marker gene to allow the selection of transformed host cells. Selectable marker genes are well known in the art and will vary with the host cell used.

[0110] In certain aspects disclosed herein, the subject nucleic acid is provided in an expression vector comprising a nucleotide sequence encoding a GDF15 polypeptide and operably linked to at least one regulatory sequence. Regulatory sequences are art-recognized and are selected to direct expression of the GDF15 polypeptide. Accordingly, the term regulatory sequence includes promoters, enhancers, and other expression control elements. Exemplary regulatory sequences are described in Goeddel; *Gene Expression Technology*: *Methods in Enzymology*, Academic Press, San Diego, CA (1990). For instance, any of a wide variety of expression control sequences that control the expression of a DNA sequence when operatively linked to it may be used in these vectors to express DNA sequences encoding a GDF15 polypeptide. Such useful expression control sequences, include, for example, the early and late promoters of SV40, tet promoter, adenovirus or cytomegalovirus immediate early promoter, RSV promoters, the lac system, the trp system, the TAC or TRC system, T7 promoter whose expression is directed by T7 RNA polymerase, the major operator and promoter regions of phage lambda, the control regions for fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase, e.g., Pho5, the promoters of the yeast α-mating factors, the polyhedron promoter of the baculovirus system and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells or their viruses, and various combinations thereof. It should

be understood that the design of the expression vector may depend on such factors as the choice of the host cell to be transformed and/or the type of protein desired to be expressed. Moreover, the vector's copy number, the ability to control that copy number and the expression of any other protein encoded by the vector, such as antibiotic markers, should also be considered.

[0111] A recombinant nucleic acid included in the disclosure can be produced by ligating the cloned gene, or a portion thereof, into a vector suitable for expression in either prokaryotic cells, eukaryotic cells (yeast, avian, insect or mammalian), or both. Expression vehicles for production of a recombinant GDF15 polypeptide include plasmids and other vectors. For instance, suitable vectors include plasmids of the types: pBR322-derived plasmids, pEMBL-derived plasmids, pEX-derived plasmids, pBTac-derived plasmids and pUC-derived plasmids for expression in prokaryotic cells, such as *E. coli*.

[0112] Some mammalian expression vectors contain both prokaryotic sequences to facilitate the propagation of the vector in bacteria, and one or more eukaryotic transcription units that are expressed in eukaryotic cells. The pcDNAI/amp, pcDNAI/neo, pRc/CMV, pSV2gpt, pSV2neo, pSV2-dhfr, pTk2, pRSVneo, pMSG, pSVT7, pko-neo and pHyg derived vectors are examples of mammalian expression vectors suitable for transfection of eukaryotic cells. Some of these vectors are modified with sequences from bacterial plasmids, such as pBR322, to facilitate replication and drug resistance selection in both prokaryotic and eukaryotic cells. Alternatively, derivatives of viruses such as the bovine papilloma virus (BPV-1), or Epstein-Barr virus (pHEBo, pREP-derived and p205) can be used for transient expression of proteins in eukaryotic cells. Examples of other viral (including retroviral) expression systems can be found below in the description of gene therapy delivery systems. The various methods employed in the preparation of the plasmids and in transformation of host organisms are well known in the art. For other suitable expression systems for both prokaryotic and eukaryotic cells, as well as general recombinant procedures, see *Molecular Cloning A Laboratory Manual*, 3rd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press, 2001). In some instances, it may be desirable to express the recombinant polypeptides by the use of a baculovirus expression system. Examples of such baculovirus expression systems include pVL-derived vectors (such as pVL1392, pVL1393 and pVL941), pAcUW-derived vectors (such as pAcUW1), and pBlueBac-derived vectors (such as the ß-gal containing pBlueBac III).

[0113] In a preferred embodiment, a vector will be designed for production of the subject TβRII polypeptides in CHO cells, such as a Pcmv-Script vector (Stratagene, La Jolla, Calif.), pcDNA4 vectors (Invitrogen, Carlsbad, Calif.), pCI-neo vectors (Promega, Madison, Wisc.) and UCOE™-derived vectors (Millipore). As will be apparent, the subject gene constructs can be used to cause expression of the subject GDF15 polypeptides in cells propagated in culture, e.g., to produce proteins, including fusion proteins or variant proteins, for purification.

[0114] This disclosure also pertains to a host cell transfected with a recombinant gene including a coding sequence (e.g., SEQ ID NO: 1 or SEQ ID NO:3) for one or more of the subject GDF15 polypeptides. The host cell may be any prokaryotic or eukaryotic cell. For example, a GDF15 polypeptide disclosed herein may be expressed in bacterial cells such as *E. coli*, insect cells (e.g., using a baculovirus expression system), yeast, or mammalian cells. Other suitable host cells are known to those skilled in the art. In a preferred embodiment, a GDF15 polypeptide disclosed herein is expressed in CHO cells.

[0115] Accordingly, the present disclosure further pertains to methods of producing the subject GDF15 polypeptides. For example, a host cell transfected with an expression vector encoding a GDF15 polypeptide can be cultured under appropriate conditions to allow expression of the GDF15 polypeptide to occur. The GDF15 polypeptide may be secreted and isolated from a mixture of cells and medium containing the GDF15 polypeptide. Alternatively, the TβRII polypeptide may be retained cytoplasmically or in a membrane fraction and the cells harvested, lysed and the protein isolated. A cell culture includes host cells and media. Suitable media for cell culture are well known in the art. The subject GDF15 polypeptides can be isolated from cell culture medium, host cells, or both, using techniques known in the art for purifying proteins, including ion-exchange chromatography, gel filtration chromatography, ultrafiltration, electrophoresis, immunoaffinity purification with antibodies specific for particular epitopes of the GDF15 polypeptides and affinity purification with an agent that binds to a domain fused to the GDF15 polypeptide (e.g., a protein A column may be used to purify an GDF15-Fc fusion). In a preferred embodiment, the GDF15 polypeptide is a fusion protein containing a domain which facilitates its purification. As an example, purification may be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography,

size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

[0116] In a preferred embodiment, the subject GDF15 polypeptides are purified from culture media using a series of cation-exchange column chromatography steps. Examples of the material used for the cation exchange column can be resins having substituents such as carboxymethyl (CM), sulfoethyl (SE), sulfopropyl (SP), phosphate (P) and sulfonate (S). Examples of the material used for the cation exchange column chromatography include SP Sepharose™ Fast Flow, Q Sepharose™ Fast Flow, DEAE Sepharose™ Fast Flow, Capto™ S, Capto™ DEAE (GE Healthcare), S HyperCel™ (Pall), TOYOPEARL GigaCap S-650 (TOSOH) or weak cation exchangers such as carboxymethyl. SP Sepharose™ Fast Flow and Q Sepharose™ Fast Flow are preferred.

[0117] To begin purification, parameters of the conditioned media from host cells stably expressing a GDF15 polypeptide, such as pH, ionic strength, and temperature may be adjusted if necessary. In some embodiments, a chromatography column is flushed and equilibrated with one or more solutions prior to contact with a polypeptide containing supernatant. Such solutions can include, for example, a buffer (e.g., Tris, MES, HEPES, histidine, phosphate or sodium acetate, e.g., between 1-500 mM, 25-100 mM, 15-30 mM or 20 mM), and/or salt (e.g., NaCl, $NaPO_4$ sodium acetate, or $CaCl_2$, e.g., between 0-2 M, 1-2 M or 500 mM-1M). The pH of an equilibration solution generally ranges from 3.5-10 (e.g., between pH 3.5-6, 4.0-5.5, 4.5-4.8 or 4.7). After contacting a column with a polypeptide containing fluid, the bound column can be washed. Wash solutions can include a buffer (e.g., Tris, MES, HEPES, histidine, phosphate, or sodium acetate, e.g., between 1-500 mM, 25-100 mM, 15-30 mM or 20 mM), and/or salt (e.g., NaCl, $NaPO_4$, sodium acetate, or $CaCl_2$, e.g., between 0-2 M, 1-2 M, 100 mM-1M or 100 mM-500 mM), and/or an additive (e.g. guanidine, urea, sucrose, arginine, or an arginine derivative), and/or a solvent (e.g., ethanol, acetonitrile, or polyethylene glycol). Wash solutions generally have a pH between 3.5 and 10 (e.g., a pH between 4.5-8.0). Polypeptides can be eluted from a column using a step or gradient change in pH, salt type, salt concentration, solvent type, solvent concentration, displacer type, displacer concentration, or a combination thereof. In general, to elute a polypeptide from a column, the medium is contacted with an elution buffer. In some embodiments, an elution buffer elution buffer contains a buffer (e.g., HEPES or Tris, e.g., 10-100 mM, 25-75 mM or 50 mM) and/or contains a salt (e.g., NaCl or $CaCl_2$, e.g., 0-2 M, e.g.,

10-100 mM). In some embodiments, an elution buffer may contain glycine, acetic acid, or citric acid (e.g., 20-250 mM, or 150 mM). An elution buffer may also contain acetic acid (e.g., 20 mM to about 50 mM), an additive (e.g. guanidine, urea, or sucrose, e.g., 1-10 M, 2-8 M or 6 M), and/or a solvent (e.g., ethanol, acetonitrile, polyethylene glycol, e.g., 1-10% solvent, e.g., 5% solvent). The pH of the elution buffer may range from about 5.0 to about 10.0. In some embodiments, pH can be changed (e.g., gradually) to produce a gradient elution. In some embodiments, the pH of the elution buffer is about 8.0. In some embodiments, a series of column chromatography steps are performed.

[0118] The data presented herein demonstrates that TβRII polypeptides act as antagonists of GDF15 signaling. Although soluble TβRII polypeptides, and particularly TβRII-Fc, are preferred antagonists, other types of GDF15 antagonists are expected to be useful, including anti-GDF15 antibodies, anti-TβRII antibodies, antisense, RNAi or ribozyme nucleic acids that inhibit the production of GDF15 or TβRII and other inhibitors of GDF15 or TβRII, particularly those that disrupt GDF15-TβRII binding.

[0119] An antibody that is specifically reactive with a GDF15 polypeptide and which either binds to GDF15 polypeptide so as to compete with its binding to TβRII polypeptide (binding competitively) or otherwise inhibits GDF15-mediated signaling may be used as an antagonist of GDF15 polypeptide activities. Likewise, an antibody that is specifically reactive with a TβRII polypeptide and which disrupts GDF15 binding may be used as an antagonist.

[0120] By using immunogens derived from a GDF15 polypeptide or a TβRII polypeptide, anti-protein/anti-peptide antisera or monoclonal antibodies can be made by standard protocols (see, for example, Antibodies: A Laboratory Manual ed. by Harlow and Lane (Cold Spring Harbor Press: 1988)). A mammal, such as a mouse, a hamster or rabbit can be immunized with an immunogenic form of the GDF15 polypeptide, an antigenic fragment which is capable of eliciting an antibody response, or a fusion protein. Techniques for conferring immunogenicity on a protein or peptide include conjugation to carriers or other techniques well known in the art. An immunogenic portion of a GDF15 or TβRII polypeptide can be administered in the presence of adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassays can be used with the immunogen as antigen to assess the levels of antibodies.

[0121] Following immunization of an animal with an antigenic preparation of a GDF15 polypeptide, antisera can be obtained and, if desired, polyclonal antibodies can be isolated from the serum. To produce monoclonal antibodies, antibody-producing cells (lymphocytes) can be harvested from an immunized animal and fused by standard somatic cell fusion procedures with immortalizing cells such as myeloma cells to yield hybridoma cells. Such techniques are well known in the art, and include, for example, the hybridoma technique (originally developed by Kohler and Milstein, (1975) Nature, 256: 495-497), the human B cell hybridoma technique (Kozbar et al., (1983) Immunology Today, 4: 72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., (1985) Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc. pp. 77-96). Hybridoma cells can be screened immunochemically for production of antibodies specifically reactive with a GDF15 polypeptide and monoclonal antibodies isolated from a culture comprising such hybridoma cells.

[0122] The term "antibody" as used herein is intended to include fragments thereof which are also specifically reactive with a subject polypeptide. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above for whole antibodies. For example, $F(ab)_2$ fragments can be generated by treating antibody with pepsin. The resulting $F(ab)_2$ fragment can be treated to reduce disulfide bridges to produce Fab fragments. The antibody of the present invention is further intended to include bispecific, single-chain, chimeric, humanized and fully human molecules having affinity for an TβRII or GDF15 polypeptide conferred by at least one CDR region of the antibody. An antibody may further comprise a label attached thereto and able to be detected (e.g., the label can be a radioisotope, fluorescent compound, enzyme or enzyme co-factor).

[0123] In certain embodiments, the antibody is a recombinant antibody, which term encompasses any antibody generated in part by techniques of molecular biology, including CDR-grafted or chimeric antibodies, human or other antibodies assembled from library-selected antibody domains, single chain antibodies and single domain antibodies (e.g., human V_H proteins or camelid V_{HH} proteins). In certain embodiments, an antibody of the invention is a monoclonal antibody, and in certain embodiments, the invention makes available methods for generating novel antibodies. For example, a method for generating a monoclonal antibody that binds specifically to a GDF15 polypeptide or TβRII polypeptide

may comprise administering to a mouse an amount of an immunogenic composition comprising the antigen polypeptide effective to stimulate a detectable immune response, obtaining antibody-producing cells (e.g., cells from the spleen) from the mouse and fusing the antibody-producing cells with myeloma cells to obtain antibody-producing hybridomas, and testing the antibody-producing hybridomas to identify a hybridoma that produces a monoclonal antibody that binds specifically to the antigen. Once obtained, a hybridoma can be propagated in a cell culture, optionally in culture conditions where the hybridoma-derived cells produce the monoclonal antibody that binds specifically to the antigen. The monoclonal antibody may be purified from the cell culture.

[0124] The adjective "specifically reactive with" as used in reference to an antibody is intended to mean, as is generally understood in the art, that the antibody is sufficiently selective between the antigen of interest (e.g., a GDF15 polypeptide) and other antigens that are not of interest that the antibody is useful for, at minimum, detecting the presence of the antigen of interest in a particular type of biological sample. In certain methods employing the antibody, such as therapeutic applications, a higher degree of specificity in binding may be desirable. Monoclonal antibodies generally have a greater tendency (as compared to polyclonal antibodies) to discriminate effectively between the desired antigens and cross-reacting polypeptides. One characteristic that influences the specificity of an antibody:antigen interaction is the affinity of the antibody for the antigen. Although the desired specificity may be reached with a range of different affinities, generally preferred antibodies will have an affinity (a dissociation constant) of about 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} or less. Given the high affinity between GDF15 and TβRII, it is expected that a neutralizing anti-GDF15 or anti-TβRII antibody would generally have a dissociation constant of 10^{-9} or less.

[0125] In addition, the techniques used to screen antibodies in order to identify a desirable antibody may influence the properties of the antibody obtained. For example, if an antibody is to be used for binding an antigen in solution, it may be desirable to test solution binding. A variety of different techniques are available for testing interaction between antibodies and antigens to identify particularly desirable antibodies. Such techniques include ELISAs, surface plasmon resonance binding assays (e.g., the Biacore™ binding assay, Biacore AB, Uppsala, Sweden), sandwich assays (e.g., the paramagnetic bead system of IGEN International, Inc., Gaithersburg, Maryland), western blots, immunoprecipitation assays, and immunohistochemistry.

[0126] Examples of categories of nucleic acid compounds that are GDF15 or TβRII antagonists include antisense nucleic acids, RNAi constructs and catalytic nucleic acid constructs. A nucleic acid compound may be single- or double-stranded. A double-stranded compound may also include regions of overhang or non-complementarity, where one or the other of the strands is single-stranded. A single-stranded compound may include regions of self-complementarity, meaning that the compound forms a so-called "hairpin" or "stem-loop" structure, with a region of double helical structure. A nucleic acid compound may comprise a nucleotide sequence that is complementary to a region consisting of no more than 1000, no more than 500, no more than 250, no more than 100 or no more than 50, 35, 30, 25, 22, 20 or 18 nucleotides of the full-length GDF15 nucleic acid sequence or TβRII nucleic acid sequence. The region of complementarity will preferably be at least 8 nucleotides, and optionally at least 10 or at least 15 nucleotides, such as between 15 and 25 nucleotides. A region of complementarity may fall within an intron, a coding sequence or a noncoding sequence of the target transcript, such as the coding sequence portion. Generally, a nucleic acid compound will have a length of about 8 to about 500 nucleotides or base pairs in length, such as about 14 to about 50 nucleotides. A nucleic acid may be a DNA (particularly for use as an antisense), RNA or RNA:DNA hybrid. Any one strand may include a mixture of DNA and RNA, as well as modified forms that cannot readily be classified as either DNA or RNA. Likewise, a double-stranded compound may be DNA:DNA, DNA:RNA or RNA:RNA, and any one strand may also include a mixture of DNA and RNA, as well as modified forms that cannot readily be classified as either DNA or RNA. A nucleic acid compound may include any of a variety of modifications, including one or modifications to the backbone (the sugar-phosphate portion in a natural nucleic acid, including internucleotide linkages) or the base portion (the purine or pyrimidine portion of a natural nucleic acid). An antisense nucleic acid compound will preferably have a length of about 15 to about 30 nucleotides and will often contain one or more modifications to improve characteristics such as stability in the serum, in a cell or in a place where the compound is likely to be delivered, such as the stomach in the case of orally delivered compounds and the lung for inhaled compounds. In the case of an RNAi construct, the strand complementary to the target transcript will generally be RNA or modifications thereof. The other strand may be RNA, DNA or any other variation. The duplex portion of double-stranded or single-stranded "hairpin" RNAi construct will preferably have a length of 18 to 40 nucleotides in length and optionally about 21 to 23 nucleotides in length, so long as it serves as a Dicer substrate. Catalytic or enzymatic nucleic acids may be ribozymes or DNA enzymes and may also contain modified forms. Nucleic

acid compounds may inhibit expression of the target by about 50%, 75%, 90% or more when contacted with cells under physiological conditions and at a concentration where a nonsense or sense control has little or no effect. Preferred concentrations for testing the effect of nucleic acid compounds are 1, 5 and 10 micromolar.

6. Screening Assays

[0127] In certain aspects, the present invention relates to the use of TβRII polypeptides (e.g., soluble TβRII polypeptides) and GDF15 polypeptides to identify compounds (agents) which are agonist or antagonists of the GDF15-TβRII signaling pathway. Compounds identified through this screening can be tested to assess their ability to modulate GDF15 signaling activity in vitro. Optionally, these compounds can further be tested in animal models to assess their ability to modulate tissue growth in vivo.

[0128] There are numerous approaches to screening for therapeutic agents for modulating tissue growth by targeting GDF15 and TβRII polypeptides. In certain embodiments, high-throughput screening of compounds can be carried out to identify agents that perturb GDF15 or TβRII-mediated cell signaling. In certain embodiments, the assay is carried out to screen and identify compounds that specifically inhibit or reduce binding of a TβRII polypeptide to GDF15. Alternatively, the assay can be used to identify compounds that enhance binding of a TβRII polypeptide to GDF15. In a further embodiment, the compounds can be identified by their ability to interact with a GDF15 or TβRII polypeptide.

[0129] A variety of assay formats will suffice and, in light of the present disclosure, those not expressly described herein will nevertheless be comprehended by one of ordinary skill in the art. As described herein, the test compounds (agents) of the invention may be created by any combinatorial chemical method. Alternatively, the subject compounds may be naturally occurring biomolecules synthesized in vivo or in vitro. Compounds (agents) to be tested for their ability to act as modulators of tissue growth can be produced, for example, by bacteria, yeast, plants or other organisms (e.g., natural products), produced chemically (e.g., small molecules, including peptidomimetics), or produced recombinantly. Test compounds contemplated by the present invention include non-peptidyl organic molecules, peptides, polypeptides, peptidomimetics, sugars, hormones, and nucleic acid molecules. In a specific embodiment, the test agent is a small organic molecule having a molecular weight of less than about 2,000 daltons.

[0130] The test compounds of the invention can be provided as single, discrete entities, or provided in libraries of greater complexity, such as made by combinatorial chemistry. These libraries can comprise, for example, alcohols, alkyl halides, amines, amides, esters, aldehydes, ethers and other classes of organic compounds. Presentation of test compounds to the test system can be in either an isolated form or as mixtures of compounds, especially in initial screening steps. Optionally, the compounds may be optionally derivatized with other compounds and have derivatizing groups that facilitate isolation of the compounds. Non-limiting examples of derivatizing groups include biotin, fluorescein, digoxygenin, green fluorescent protein, isotopes, polyhistidine, magnetic beads, glutathione S transferase (GST), photoactivatible crosslinkers or any combinations thereof.

[0131] In many drug screening programs which test libraries of compounds and natural extracts, high throughput assays are desirable in order to maximize the number of compounds surveyed in a given period of time. Assays which are performed in cell-free systems, such as may be derived with purified or semi-purified proteins, are often preferred as "primary" screens in that they can be generated to permit rapid development and relatively easy detection of an alteration in a molecular target which is mediated by a test compound. Moreover, the effects of cellular toxicity or bioavailability of the test compound can be generally ignored in the in vitro system, the assay instead being focused primarily on the effect of the drug on the molecular target as may be manifest in an alteration of binding affinity between a TβRII polypeptide and GDF15.

[0132] Merely to illustrate, in an exemplary screening assay of the present invention, the compound of interest is contacted with an isolated and purified TβRII polypeptide which is ordinarily capable of binding to GDF15. To the mixture of the compound and TβRII polypeptide is then added a composition containing a TβRII ligand. Detection and quantification of TβRII/GDF15 complexes provides a means for determining the compound's efficacy at inhibiting (or potentiating) complex formation between the TβRII polypeptide and GDF15. The efficacy of the compound can be assessed by generating dose response curves from data obtained using various concentrations of the test compound. Moreover, a control assay can also be performed to provide a baseline for comparison. For example, in a control assay, isolated and a purified GDF15 is added to a composition containing the TβRII polypeptide, and the formation of TβRII/GDF15 complex is quantitated in the absence of the test compound. It will be understood that, in general, the order in which

the reactants may be admixed can be varied, and can be admixed simultaneously. Moreover, in place of purified proteins, cellular extracts and lysates may be used to render a suitable cell-free assay system.

[0133] Complex formation between the TβRII polypeptide and GDF15 may be detected by a variety of techniques. For instance, modulation of the formation of complexes can be quantitated using, for example, detectably labeled proteins such as radiolabeled (e.g., ^{32}P, ^{35}S, ^{14}C or ^{3}H), fluorescently labeled (e.g., FITC), or enzymatically labeled TβRII polypeptide or GDF15, by immunoassay, or by chromatographic detection.

[0134] In certain embodiments, the present invention contemplates the use of fluorescence polarization assays and fluorescence resonance energy transfer (FRET) assays in measuring, either directly or indirectly, the degree of interaction between a TβRII polypeptide and its binding protein. Further, other modes of detection, such as those based on optical waveguides (PCT Publication WO 96/26432 and U.S. Pat. No. 5,677,196), surface plasmon resonance (SPR), surface charge sensors, and surface force sensors, are compatible with many embodiments of the invention.

[0135] Moreover, the present invention contemplates the use of an interaction trap assay, also known as the "two hybrid assay," for identifying agents that disrupt or potentiate interaction between a TβRII polypeptide and its binding protein. See for example, U.S. Pat. No. 5,283,317; Zervos et al. (1993) Cell 72:223-232; Madura et al. (1993) J Biol Chem 268:12046-12054; Bartel et al. (1993) Biotechniques 14:920-924; and Iwabuchi et al. (1993) Oncogene 8:1693-1696). In a specific embodiment, the present invention contemplates the use of reverse two hybrid systems to identify compounds (e.g., small molecules or peptides) that dissociate interactions between a TβRII polypeptide and its binding protein. See for example, Vidal and Legrain, (1999) Nucleic Acids Res 27:919-29; Vidal and Legrain, (1999) Trends Biotechnol 17:374-81; and U.S. Pat. Nos. 5,525,490; 5,955,280; and 5,965,368.

[0136] In certain embodiments, the subject compounds are identified by their ability to interact with a TβRII or GDF15 polypeptide of the invention. The interaction between the compound and the TβRII or GDF15 polypeptide may be covalent or non-covalent. For example, such interaction can be identified at the protein level using in vitro biochemical methods, including photo-crosslinking, radiolabeled ligand binding, and affinity chromatography (Jakoby WB et al., 1974, Methods in Enzymology 46: 1). In certain cases,

the compounds may be screened in a mechanism based assay, such as an assay to detect compounds which bind to a GDF15 or TβRII polypeptide. This may include a solid-phase or fluid-phase binding event. Alternatively, the gene encoding a GDF15 or TβRII polypeptide can be transfected with a reporter system (e.g., β-galactosidase, luciferase, or green fluorescent protein) into a cell and screened against the library preferably by a high-throughput screening or with individual members of the library. Other mechanism-based binding assays may be used, for example, binding assays which detect changes in free energy. Binding assays can be performed with the target fixed to a well, bead or chip or captured by an immobilized antibody or resolved by capillary electrophoresis. The bound compounds may be detected usually using colorimetric or fluorescence or surface plasmon resonance.

[0137] In certain aspects, the present invention provides methods and agents for modulating (stimulating or inhibiting) GDF15-mediated cell signaling. Therefore, any compound identified can be tested in whole cells or tissues, in vitro or in vivo, to confirm their ability to modulate GDF15 signaling. Various methods known in the art can be utilized for this purpose.

7. Exemplary Therapeutic Uses

[0138] As used herein, a therapeutic that "prevents" a disorder or condition refers to a compound that, in a statistical sample, reduces the occurrence of the disorder or condition in the treated sample relative to an untreated control sample, or delays the onset or reduces the severity of one or more symptoms of the disorder or condition relative to the untreated control sample. The term "treating" as used herein includes amelioration or elimination of the condition once it has been established. In either case, prevention or treatment may be discerned in the diagnosis provided by a physician and the intended result of administration of the therapeutic agent.

[0139] The disclosure provides methods of treating or preventing a disease or condition associated with a TGFβ superfamily member by administering to a subject an effective amount of a TβRII polypeptide, including a TβRII-Fc fusion protein or nucleic acid antagonists (e.g., antisense or siRNA) of the foregoing, hereafter collectively referred to as "therapeutic agents". In some embodiments the disease or condition is associated with dysregulated GDF15, TGFβ1 or TGFβ3 signaling. Also provided are methods and

compositions for treating certain cardiovascular or vascular disorders. In addition, the disclosure provides methods and compositions for treating or preventing cancer. In addition, the disclosure provides methods and compositions for treating or preventing fibrotic disorders and conditions.

[0140] In particular, polypeptide therapeutic agents of the present disclosure are useful for treating or preventing chronic vascular or cardiovascular diseases. Exemplary disorders of this kind include, but are not limited to, heart disease (including myocardial disease, myocardial infarct, angina pectoris, and heart valve disease); renal disease (including chronic glomerular inflammation, diabetic renal failure, and lupus-related renal inflammation); disorders associated with atherosclerosis or other types of arteriosclerosis (including stroke, cerebral hemorrhage, subarachnoid hemorrhage, angina pectoris, and renal arteriosclerosis); thrombotic disorders (including cerebral thrombosis, thrombotic intestinal necrosis); complications of diabetes (including diabetes-related retinal disease, cataracts, diabetes-related renal disease, diabetes-related neuropathology, diabetes-related gangrene, and diabetes-related chronic infection); vascular inflammatory disorders (systemic lupus erythematosus, joint rheumatism, joint arterial inflammation, large-cell arterial inflammation, Kawasaki disease, Takayasu arteritis, Churg-Strauss syndrome, and Henoch-Schoenlein purpura); diabetic vasculopathies; and cardiac disorders such as congenital heart disease, cardiomyopathy (e.g., dilated, hypertrophic, restrictive cardiomyopathy), and congestive heart failure. Exemplary disorders further include, but are not limited to, hereditary hemorrhagic telangiectasia (HHT), Marfan syndrome, Loeys-Dietz syndrome, familial thoracic aortic aneurysm syndrome, arterial tortuosity syndrome, pre-eclampsia, and restenosis.

[0141] The TβRII polypeptide can be administered to the subject alone, or in combination with one or more agents or therapeutic modalities, e.g., therapeutic agents, which are useful for treating TGFβ associated cardiovascular disorders and/or conditions. In certain embodiments, the second agent or therapeutic modality is chosen from one or more of: angioplasty, beta blockers, anti-hypertensives, cardiotonics, anti-thrombotics, vasodilators, hormone antagonists, endothelin antagonists, calcium channel blockers, phosphodiesterase inhibitors, angiotensin type 2 antagonists and/or cytokine blockers/inhibitors

[0142] In particular, polypeptide therapeutic agents of the present disclosure are useful for treating or preventing a cancer (tumor). The terms "cancer" and "cancerous" refer to or describe, the physiological condition in mammals that is typically characterized by unregulated cell growth/proliferation. Examples of cancer, or neoplastic disorders, include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, stomach cancer, intestinal cancer, skin cancer, bone cancer, gastric cancer, melanoma, and various types of head and neck cancer, including squamous cell head and neck cancer. Other examples of neoplastic disorders and related conditions include esophageal carcinomas, thecomas, arrhenoblastomas, endometrial hyperplasia, endometriosis, fibrosarcomas, choriocarcinoma, nasopharyngeal carcinoma, laryngeal carcinomas, hepatoblastoma, Kaposi's sarcoma, skin carcinomas, hemangioma, cavernous hemangioma, hemangioblastoma, retinoblastoma, astrocytoma, glioblastoma, Schwannoma, oligodendroglioma, medulloblastoma, neuroblastomas, rhabdomyosarcoma, osteogenic sarcoma, leiomyosarcomas, urinary tract carcinomas, Wilm's tumor, renal cell carcinoma, prostate carcinoma, abnormal vascular proliferation associated with phakomatoses, and Meigs' syndrome. A cancer that is particularly amenable to treatment with the therapeutic agents described herein may be characterized by one or more of the following: the cancer has elevated TβRII levels detectable in the tumor or the serum, increased GDF15, TGFβ1 or TGFβ3 expression levels or biological activity, is metastatic or at risk of becoming metastatic, or any combination thereof.

[0143] In certain embodiments of such methods, one or more polypeptide therapeutic agents can be administered, together (simultaneously) or at different times (sequentially). In addition, polypeptide therapeutic agents can be administered with another type of compounds for treating cancer or for inhibiting angiogenesis.

[0144] In certain embodiments, the subject methods of the disclosure can be used alone. Alternatively, the subject methods may be used in combination with other conventional anti-cancer therapeutic approaches directed to treatment or prevention of proliferative disorders (e.g., tumor). For example, such methods can be used in prophylactic

cancer prevention, prevention of cancer recurrence and metastases after surgery, and as an adjuvant of other conventional cancer therapy. The present disclosure recognizes that the effectiveness of conventional cancer therapies (e.g., chemotherapy, radiation therapy, phototherapy, immunotherapy, and surgery) can be enhanced through the use of a subject polypeptide therapeutic agent.

[0145] A wide array of conventional compounds have been shown to have anti-neoplastic or anti-cancer activities. These compounds have been used as pharmaceutical agents in chemotherapy to shrink solid tumors, prevent metastases and further growth, or decrease the number of malignant cells in leukemic or bone marrow malignancies. Although chemotherapy has been effective in treating various types of malignancies, many anti-neoplastic compounds induce undesirable side effects. It has been shown that when two or more different treatments are combined, the treatments may work synergistically and allow reduction of dosage of each of the treatments, thereby reducing the detrimental side effects exerted by each compound at higher dosages. In other instances, malignancies that are refractory to a treatment may respond to a combination therapy of two or more different treatments.

[0146] When a therapeutic agent disclosed herein is administered in combination with another conventional anti-neoplastic agent, either concomitantly or sequentially, such therapeutic agent may enhance the therapeutic effect of the anti-neoplastic agent or overcome cellular resistance to such anti-neoplastic agent. This allows decrease of dosage of an anti-neoplastic agent, thereby reducing the undesirable side effects, or restores the effectiveness of an anti-neoplastic agent in resistant cells.

[0147] According to the present disclosure, the polypeptide therapeutic agents described herein may be used in combination with other compositions and procedures for the treatment of diseases. For example, a tumor may be treated conventionally with surgery, radiation or chemotherapy combined with the TβRII polypeptide, and then the TβRII polypeptide may be subsequently administered to the patient to extend the dormancy of micrometastases and to stabilize any residual primary tumor.

[0148] In certain aspects of the invention, other therapeutic agents useful for combination tumor therapy with a TβRII polypeptide include other cancer therapies: e.g., surgery, cytotoxic agents, radiological treatments involving irradiation or administration of

radioactive substances, chemotherapeutic agents, anti-hormonal agents, growth inhibitory agents, anti-neoplastic compositions, and treatment with anti-cancer agents listed herein and known in the art, or combinations thereof.

[0149] The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (e.g., At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32} and radioactive isotopes of Lu), chemotherapeutic agents e.g. methotrexate, adriamicin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents, enzymes and fragments thereof such as nucleolytic enzymes, antibiotics, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof, and the various antitumor or anticancer agents disclosed below. Other cytotoxic agents are described below. A tumoricidal agent causes destruction of tumor cells.

[0150] A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and CYTOXAN® cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethiylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan (HYCAMTIN®), CPT-11 (irinotecan, CAMPTOSAR®), acetylcamptothecin, scopolectin, and 9-aminocamptothecin); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CBl-TMl); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cyclophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and

ranimnustine; antibiotics such as the enediyne antibiotics (e.g., calicheamicin, especially calicheamicin gammall and calicheamicin omegall (see, e.g., Agnew, Chem Intl. Ed. Engl., 33: 183-186 (1994)); dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN® doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as folinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine (ELDISINE®, FILDESIN®); dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); thiotepa; taxoids, e.g., TAXOL® paclitaxel (Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAXANE™ Cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumberg, Illinois), and TAXOTERE® doxetaxel (Rhóne-Poulenc Rorer, Antony, France); chlorambucil; gemcitabine (GEMZAR®); 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine (VELBAN®); platinum; etoposide (VP- 16); ifosfamide; mitoxantrone; vincristine (ONCOVIN®); oxaliplatin; leucovovin; vinorelbine

(NAVELBINE®); novantrone; edatrexate; daunomycin; aminopterin; ibandronate; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine (XELODA®); pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone, and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATIN™) combined with 5-FU and leucovovin.

[0151] Also included in this definition are anti-hormonal agents that act to regulate, reduce, block, or inhibit the effects of hormones that can promote the growth of cancer, and are often in the form of systemic, or whole-body treatment. They may be hormones themselves. Examples include anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX® tamoxifen), EVISTA® raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY1 17018, onapristone, and FARESTON® toremifene; anti-progesterones; estrogen receptor down-regulators (ERDs); agents that function to suppress or shut down the ovaries, for example, luteinizing hormone-releasing hormone (LHRH) agonists such as LUPRON® and ELIGARD® leuprolide acetate, goserelin acetate, buserelin acetate and tripterelin; other anti-androgens such as flutamide, nilutamide and bicalutamide; and aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, MEGASE® megestrol acetate, AROMASIN® exemestane, formestane, fadrozole, RIVIS OR® vorozole, FEMARA® letrozole, and ARIMIDEX® anastrozole. In addition, such definition of chemotherapeutic agents includes bisphosphonates such as clodronate (for example, BONEFOS® or OSTAC®), DIDROC AL® etidronate, NE-58095, ZOMET A® zoledronic acid/zoledronate, FOSAMAX® alendronate, AREDIA® pamidronate, SKELID® tiludronate, or ACTONEL® risedronate; as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); antisense oligonucleotides, particularly those that inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation, such as, for example, PKC-alpha, Raf, H-Ras, and epidermal growth factor receptor (EGF-R); vaccines such as THERATOPE® vaccine and gene therapy vaccines, for example, ALLOVECTIN® vaccine, LEUVECTIN® vaccine, and VAXID® vaccine; LURTOTECAN® topoisomerase 1 inhibitor; ABARELIX® rmRH; lapatinib ditosylate (an ErbB-2 and EGFR dual tyrosine kinase small-molecule inhibitor also

known as GW572016); and pharmaceutically acceptable salts, acids or derivatives of any of the above.

[0152] A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell either in vitro or in vivo. Thus, the growth inhibitory agent may be one which significantly reduces the percentage of cells in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce Gl arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxanes, and topoisomerase II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest Gl also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in The Molecular Basis of Cancer, Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami et al. (WB Saunders: Philadelphia, 1995), especially p. 13. The taxanes (paclitaxel and docetaxel) are anticancer drugs both derived from the yew tree. Docetaxel (TAXOTERE®, Rhone -Poulenc Rorer), derived from the European yew, is a semisynthetic analogue of paclitaxel (TAXOL®, Bristol-Myers Squibb). Paclitaxel and docetaxel promote the assembly of microtubules from tubulin dimers and stabilize microtubules by preventing depolymerization, which results in the inhibition of mitosis in cells.

[0153] In still other embodiments, TβRII polypeptides may be useful in the treatment or prevention of fibrosis. As used herein, the term "fibrosis" refers to the aberrant formation or development of excess fibrous connective tissue by cells in an organ or tissue. Although processes related to fibrosis can occur as part of normal tissue formation or repair, dysregulation of these processes can lead to altered cellular composition and excess connective tissue deposition that progressively impairs to tissue or organ function. The formation of fibrous tissue can result from a reparative or reactive process. Fibrotic disorders or conditions include, but are not limited to, fibroproliferative disorders associated with vascular diseases, such as cardiac disease, cerebral disease, and peripheral vascular disease, as well as tissues and organ systems including the heart, skin, kidney, peritoneum, gut, and liver (as disclosed in, e.g., Wynn, 2004, Nat Rev 4:583-594, incorporated herein by reference). Exemplary disorders that can be treated include, but are not limited to, renal

fibrosis, including nephropathies associated with injury/fibrosis, e.g., chronic nephropathies associated with diabetes (e.g., diabetic nephropathy), lupus, scleroderma, glomerular nephritis, focal segmental glomerular sclerosis, and IgA nephropathy; gut fibrosis, e.g., scleroderma, and radiation-induced gut fibrosis; liver fibrosis, e.g., cirrhosis, alcohol-induced liver fibrosis, biliary duct injury, primary biliary cirrhosis, infection or viral-induced liver fibrosis, congenital hepatic fibrosis and autoimmune hepatitis; and other fibrotic conditions, such as cystic fibrosis, endomyocardial fibrosis, mediastinal fibrosis, sarcoidosis, scleroderma, spinal cord injury/fibrosis, myelofibrosis, vascular restenosis, atherosclerosis, , injection fibrosis (which can occur as a complication of intramuscular injections, especially in children), endomyocardial fibrosis, retroperitoneal fibrosis, and nephrogenic systemic fibrosis.

[0154] As used herein, the terms "fibrotic disorder", "fibrotic condition," and "fibrotic disease," are used interchangeably to refer to a disorder, condition or disease characterized by fibrosis. Examples of fibrotic disorders include, but are not limited to sclerotic disorders (e.g., scleroderma, atherosclerosis, diffuse systemic sclerosis), vascular fibrosis, pancreatic fibrosis, liver fibrosis (e.g., cirrhosis), renal fibrosis, musculoskeletal fibrosis, cardiac fibrosis (e.g., endomyocardial fibrosis, idiopathic myocardiopathy), skin fibrosis (e.g., scleroderma, post-traumatic, operative cutaneous scarring, keloids and cutaneous keloid formation), eye fibrosis (e.g., glaucoma, sclerosis of the eyes, conjunctival and corneal scarring, and pterygium), myelofibrosis, progressive systemic sclerosis (PSS), chronic graft-versus-host disease, Peyronie's disease, post-cystoscopic urethral stenosis, idiopathic and pharmacologically induced retroperitoneal fibrosis, mediastinal fibrosis, proliferative fibrosis, neoplastic fibrosis, Dupuytren's disease, strictures, neural scarring, dermal scarring and radiation induced fibrosis.

[0155] As used herein, inhibition of the fibrotic response of a cell, includes, but is not limited to the inhibition of the fibrotic response of one or more cells within the liver (or liver tissue); one or more cells within the kidney (or renal tissue); one or more cells within muscle tissue; one or more cells within the heart (or cardiac tissue); one or more cells within the pancreas; one or more cells within the skin; one or more cells within the bone, one or more cells within the vasculature, one or more stem cells, or one or more cells within the eye.

[0156] The present invention contemplates the use of TβRII polypeptides in combination with one or more other therapeutic modalities. Thus, in addition to the use of

TβRII polypeptides, one may also administer to the subject one or more "standard" therapies for treating fibrotic disorders. For example, the TβRII polypeptides can be administered in combination with (i.e., together with) cytotoxins, immunosuppressive agents, radiotoxic agents, and/or therapeutic antibodies. Particular co-therapeutics contemplated by the present invention include, but are not limited to, steroids (e.g., corticosteroids, such as Prednisone), immune-suppressing and/or anti-inflammatory agents (e.g., gamma-interferon, cyclophosphamide, azathioprine, methotrexate, penicillamine, cyclosporine, colchicine, antithymocyte globulin, mycophenolate mofetil, and hydroxychloroquine), cytotoxic drugs, calcium channel blockers (e.g., nifedipine), angiotensin converting enzyme inhibitors (ACE) inhibitors, para-aminobenzoic acid (PABA), dimethyl sulfoxide, transforming growth factor beta (TGFβ) inhibitors, interleukin-5 (IL-5) inhibitors, and pan caspase inhibitors.

[0157] Additional anti-fibrotic agents that may be used in combination with TβRII polypeptides include, but are not limited to, lectins (as described in, for example, U.S. Patent No.: 7,026,283, the entire contents of which is incorporated herein by reference), as well as the anti-fibrotic agents described by Wynn et al (2007, J Clin Invest 117:524-529, the entire contents of which is incorporated herein by reference). For example, additional anti-fibrotic agents and therapies include, but are not limited to, various anti-inflammatory/ immunosuppressive/ cytotoxic drugs (including colchicine, azathioprine, cyclophosphamide, prednisone, thalidomide, pentoxifylline and theophylline), TGFβ signaling modifiers (including relaxin, SMAD7, HGF, and BMP7, as well as TGFβl, TβRI, TβRII, EGR-I, and CTGF inhibitors), cytokine and cytokine receptor antagonists (inhibitors of IL-lβ, IL-5, IL-6, IL- 13, IL-21, IL-4R, IL-13Rαl, GM-CSF, TNF-α, oncostatin M, WlSP-I, and PDGFs), cytokines and chemokincs (IFN-γ, IFN-α/β, IL-12, IL-10, HGF, CXCLl0, and CXCL11), chemokine antagonists (inhibitors of CXCLl, CXCL2, CXCL12, CCL2, CCL3, CCL6, CCL17, and CCL18), chemokine receptor antagonists (inhibitors of CCR2, CCR3, CCR5, CCR7, CXCR2, and CXCR4), TLR antagonists (inhibitors of TLR3, TLR4, and TLR9), angiogenesis antagonists (VEGF-specific antibodies and adenosine deaminase replacement therapy), antihypertensive drugs (beta blockers and inhibitors of ANG 11, ACE, and aldosterone), vasoactive substances (ET-1 receptor antagonists and bosetan), inhibitors of the enzymes that synthesize and process collagen (inhibitors of prolyl hydroxylase), B cell antagonists (rituximab), integrin/adhesion molecule antagonists (molecules that block αlβl and αvβ6 integrins, as well as inhibitors of integrin-linked kinase, and antibodies specific for ICAM-I and VCAM-I), proapoptotic drugs that target myofibroblasts, MMP inhibitors

(inhibitors of MMP2, MMP9, and MMP12), and TlMP inhibitors (antibodies specific for TIMP-1).

[0158] The TβRII polypeptide and the co-therapeutic agent or co-therapy can be administered in the same formulation or separately. In the case of separate administration, the TβRII polypeptide can be administered before, after, or concurrently with the co-therapeutic or co-therapy. One agent may precede or follow administration of the other agent by intervals ranging from minutes to weeks. In embodiments where two or more different kinds of therapeutic agents are applied separately to a subject, one would generally ensure that a significant period of time did not expire between the time of each delivery, such that these different kinds of agents would still be able to exert an advantageously combined effect on the target tissues or cells.

8. Pharmaceutical Compositions

[0159] The therapeutic agents described herein (e.g., TβRII polypeptides) may be formulated into pharmaceutical compositions. Pharmaceutical compositions for use in accordance with the present disclosure may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients. Such formulations will generally be substantially pyrogen-free, in compliance with most regulatory requirements.

[0160] In certain embodiments, the therapeutic method of the disclosure includes administering the composition systemically, or locally as an implant or device. When administered, the therapeutic composition for use in this disclosure is in a pyrogen-free, physiologically acceptable form. Therapeutically useful agents other than the TβRII signaling antagonists which may also optionally be included in the composition as described above, may be administered simultaneously or sequentially with the subject compounds (e.g., TβRII polypeptides) in the methods disclosed herein.

[0161] Typically, protein therapeutic agents disclosed herein will be administered parentally, and particularly intravenously or subcutaneously. Pharmaceutical compositions suitable for parenteral administration may comprise one or more TβRII polypeptides in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with

the blood of the intended recipient or suspending or thickening agents. Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the disclosure include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

[0162] The compositions and formulations may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration

[0163] Further, the composition may be encapsulated or injected in a form for delivery to a target tissue site. In certain embodiments, compositions of the present invention may include a matrix capable of delivering one or more therapeutic compounds (e.g., TβRII polypeptides) to a target tissue site, providing a structure for the developing tissue and optimally capable of being resorbed into the body. For example, the matrix may provide slow release of the TβRII polypeptides. Such matrices may be formed of materials presently in use for other implanted medical applications.

[0164] The choice of matrix material is based on biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties. The particular application of the subject compositions will define the appropriate formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalcium phosphate, hydroxyapatite, polylactic acid and polyanhydrides. Other potential materials are biodegradable and biologically well defined, such as bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are non-biodegradable and chemically defined, such as sintered hydroxyapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalcium phosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size, particle shape, and biodegradability.

[0165] In certain embodiments, methods of the invention can be administered for orally, e.g., in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of an agent as an active ingredient. An agent may also be administered as a bolus, electuary or paste.

[0166] In solid dosage forms for oral administration (capsules, tablets, pills, dragees, powders, granules, and the like), one or more therapeutic compounds of the present invention may be mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose, and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

[0167] Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups, and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofuryl

alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming, and preservative agents.

[0168] Suspensions, in addition to the active compounds, may contain suspending agents such as ethoxylated isostearyl alcohols, polyoxyethylene sorbitol, and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

[0169] The compositions of the invention may also contain adjuvants, such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption, such as aluminum monostearate and gelatin.

[0170] It is understood that the dosage regimen will be determined by the attending physician considering various factors which modify the action of the subject compounds of the invention (e.g., TβRII polypeptides). The various factors include, but are not limited to, the patient's age, sex, and diet, the severity disease, time of administration, and other clinical factors. Optionally, the dosage may vary with the type of matrix used in the reconstitution and the types of compounds in the composition. The addition of other known growth factors to the final composition, may also affect the dosage. Progress can be monitored by periodic assessment of bone growth and/or repair, for example, X-rays (including DEXA), histomorphometric determinations, and tetracycline labeling.

[0171] In certain embodiments, the present invention also provides gene therapy for the in vivo production of TβRII polypeptides. Such therapy would achieve its therapeutic effect by introduction of the TβRII polynucleotide sequences into cells or tissues having the disorders as listed above. Delivery of TβRII polynucleotide sequences can be achieved using a recombinant expression vector such as a chimeric virus or a colloidal dispersion system.

Preferred for therapeutic delivery of TβRII polynucleotide sequences is the use of targeted liposomes.

[0172] Various viral vectors which can be utilized for gene therapy as taught herein include adenovirus, herpes virus, vaccinia, or, preferably, an RNA virus such as a retrovirus. Preferably, the retroviral vector is a derivative of a murine or avian retrovirus. Examples of retroviral vectors in which a single foreign gene can be inserted include, but are not limited to: Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV). A number of additional retroviral vectors can incorporate multiple genes. All of these vectors can transfer or incorporate a gene for a selectable marker so that transduced cells can be identified and generated. Retroviral vectors can be made target-specific by attaching, for example, a sugar, a glycolipid, or a protein. Preferred targeting is accomplished by using an antibody. Those of skill in the art will recognize that specific polynucleotide sequences can be inserted into the retroviral genome or attached to a viral envelope to allow target specific delivery of the retroviral vector containing the TβRII polynucleotide. In a preferred embodiment, the vector is targeted to bone or cartilage.

[0173] Alternatively, tissue culture cells can be directly transfected with plasmids encoding the retroviral structural genes gag, pol and env, by conventional calcium phosphate transfection. These cells are then transfected with the vector plasmid containing the genes of interest. The resulting cells release the retroviral vector into the culture medium.

[0174] Another targeted delivery system for TβRII polynucleotides is a colloidal dispersion system. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. The preferred colloidal system of this invention is a liposome. Liposomes are artificial membrane vesicles which are useful as delivery vehicles in vitro and in vivo. RNA, DNA and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (see e.g., Fraley, et al., Trends Biochem. Sci., 6:77, 1981). Methods for efficient gene transfer using a liposome vehicle, are known in the art, see e.g., Mannino, et al., Biotechniques, 6:682, 1988. The composition of the liposome is usually a combination of phospholipids, usually in combination with steroids, especially cholesterol. Other phospholipids or other lipids may also be used. The physical

characteristics of liposomes depend on pH, ionic strength, and the presence of divalent cations.

[0175] Examples of lipids useful in liposome production include phosphatidyl compounds, such as phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids, cerebrosides, and gangliosides. Illustrative phospholipids include egg phosphatidylcholine, dipalmitoylphosphatidylcholine, and distearoylphosphatidylcholine. The targeting of liposomes is also possible based on, for example, organ-specificity, cell-specificity, and organelle-specificity and is known in the art.

[0176] The disclosure provides formulations that may be varied to include acids and bases to adjust the pH; and buffering agents to keep the pH within a narrow range.

EXEMPLIFICATION

[0177] The invention now being generally described, it will be more readily understood by reference to the following examples, which are included merely for purposes of illustration of certain embodiments of the present invention, and are not intended to limit the invention.

Example 1. Generation of bioactive GDF15

[0178] GDF15 (also known as macrophage-inhibitory cytokine-1) has not been shown biochemically to bind or interact directly with any receptor. Applicants first tried without success to identify a native receptor with high-affinity binding to GDF15 using commercially available human GDF15 (R&D Systems) produced in mammalian CHO cells. Like other ligands in the TGFβ superfamily, which contain a characteristic cysteine knot motif, mature GDF15 is synthesized with a larger prodomain (Harrison et al., Growth Factors 29:174, 2011; Shi et al., Nature 474:343, 2011) that is removed through cleavage by a furin-like protease at the canonical RXXR site to generate mature dimeric GDF15. Since inadequate or inappropriate ligand purification could be a potential reason for inactivity of commercially available GDF15, Applicants tested different purification procedures for GDF15.

Stable Expression of GDF15 in CHO Cells

[0179] Applicants used CHO cells to express human GDF15 (hGDF15) and murine GDF15 (mGDF15) for further studies. The amino acid sequence of native precursor for

hGDF15 is shown in **Figure 1**, and a corresponding nucleotide sequence (with a silent, single nucleotide substitution compared to the native sequence) is shown in **Figure 2**. The native amino acid and nucleotide sequences for mGDF15 precursor are shown in **Figures 3** and **4**, respectively. For expression in CHO cells, UCOE™-based constructs encoding human or murine GDF15 precursor were stably transfected into a CHO-PACE cell line. Clones were selected in methotrexate levels of 10 nM, 20 nM, and 50 nM, and any clones that formed colonies (one or two per methotrexate concentration) were then pooled. No gene amplification was performed since it is difficult to amplify UCOE™ pools while maintaining stability of expression. Instead of dilution cloning, high-expressing pools were identified and used for generating hGDF15 and mGDF15.

Purification of Human GDF15

[0180] To begin purification, conditioned media from CHO cells stably expressing hGDF15 was adjusted to pH 4.7 with acetic acid. After incubation of media for 10 min at ambient temperature, precipitate was removed by centrifugation. Supernatant was filtered with a 0.8 μm disposable filter. An SP Sepharose™ Fast Flow column (GE Healthcare) was equilibrated with buffers A (20 mM sodium acetate, pH 4.7) and B (20 mM sodium acetate, 1M NaCl, pH 4.7). Loading was performed at 100 cm/hr. The column was washed with 20% B (200 mM NaCl) until no more protein eluted from the column and then washed back to 0% B to remove any residual salt. Protein was eluted with 50 mM Tris, 6M urea, pH 8.0 (Tris+urea pool) until no more protein eluted from the column, followed by elution with 50 mM Tris, 6M urea, 1M NaCl, pH 8.0 (Tris+urea+salt pool). Each pool was dialyzed in 50 mM 4-morpholineethanesulfonic acid (MES, pH 6.5) overnight at 4°C.

[0181] GDF15 found in the Tris+urea+salt pool was degraded based on Western blot analysis, so this pool was discarded. The Tris+urea pool was loaded on a Q Sepharose™ Fast Flow column (GE Healthcare) previously equilibrated with buffers A (50 mM MES, pH 6.5) and B (50 mM MES, 1M NaCl, pH 6.5). The flow-through was collected, and the column was washed with 10% B (100 mM NaCl), followed by a 10-50% B gradient (100-500 mM NaCl) over five column volumes at 120 cm/hr. After evaluation of the flow-through and wash fractions by Western blot, protein was found mainly in the flow-through. The flow-through was injected on a reverse-phase preparative C4 column (Vydac) attached to a HPLC, with buffers A (water/0.1% TFA) and B (acetonitrile/0.1% TFA). A 25-40% B gradient over 1 h at 4.5 mL/min produced the best resolution. Collected fractions were evaluated by SDS-

PAGE gel (Sypro Ruby) and Western blot to select those for concentration in a centrifugal evaporator.

Purification of Murine GDF15

[0182] The pH of the conditioned media was adjusted to pH 4.7 with acetic acid. After incubation of media for 10 min at ambient temperature, precipitate was removed by centrifugation. Supernatant was filtered with a 0.8 μm disposable filter. An SP Sepharose™ Fast Flow column (GE Healthcare) was equilibrated with buffers A (20 mM sodium acetate, pH 4.7) and B (20 mM sodium acetate, 1M NaCl, pH 4.7). Loading was performed at 100-150 cm/hr, and the column was washed with buffer A until no more protein eluted from the column. A wash was performed at 60% B (600 mM NaCl) for 3-4 column volumes, followed by elution with 100% B (1M NaCl) for 3-4 column volumes. Elution continued with 50 mM Tris, 6M urea, pH 8.0, to remove any protein still bound to the resin.

[0183] Non-reduced samples of SP-column fractions were analyzed by Western blot. Although most protein was found in the Tris-eluted fractions, previous experiments have indicated that mGDF15 found in these fractions is essentially inactive, so it was not used for further purification. Instead, purification was continued with protein found in the 100% B elution (salt-elution pool). This pool was injected on a reverse-phase preparative C4 column (Vydac) attached to a HPLC. Buffer A was water/0.1% TFA and buffer B was acetonitrile/0.1% TFA. Protein was eluted with a 25-40% B gradient over 1 h at 4.5 mL/min. After evaluation of the reverse-phase column fractions by SDS-PAGE gel (Sypro Ruby) and Western blot, the fractions containing pure mGDF15 were pooled and concentrated in a centrifugal evaporator.

[0184] The identities of hGDF15 and mGDF15 were each confirmed by N-terminal sequencing. Both types of purified GDF15 stimulated SMAD2/3 phosphorylation in two different cell lines, thereby providing confirmation of ligand activity.

Example 2. Identification of a TGFβ superfamily receptor with high-affinity binding to GDF15

[0185] Once active GDF15 protein was obtained, receptor-Fc fusion proteins comprising TGFβ superfamily receptors were screened for binding to human or murine GDF15 that was generated and purified as described in Example 1. These fusion proteins incorporated an IgG1 Fc domain and were either purchased from R&D Systems or generated in-house. Among the five type II receptors (TGFβ receptor type II, activin receptor type IIA,

activin receptor type IIB, BMP receptor type II, and MIS receptor type II), only TGFβ receptor type II (TβRII) exhibited detectable binding to GDF15 (k_a = 2.92 x 10^5 $M^{-1}s^{-1}$; k_d = 0.001 s^{-1}), as determined by surface plasmon resonance with captured receptor-Fc fusion proteins. hGDF15 bound to captured hTβRII-Fc at 37°C with an equilibrium dissociation constant (K_D) of 9.56 nM. None of the seven type I receptors (ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, and ALK7) displayed detectable binding to GDF15 (mGDF15 at 20 nM or 200 nM).

[0186] Human TβRII occurs naturally in at least two isoforms – A (long) and B (short) – generated by alternative splicing in the extracellular domain (ECD) (**Figures 5, 6**). The hTβRII-hG1Fc fusion protein (R&D Systems) used for the screening described above incorporates the wild-type $T\beta RII_{short}$ isoform. In a follow-up analysis, the affinity of mGDF15 binding to a fusion protein incorporating the wild-type $T\beta RII_{long}$ isoform (R&D Systems) was found by surface plasmon resonance to be very similar to that of the fusion protein incorporating the $T\beta RII_{short}$ isoform (K_Ds at 37°C were 2.7 nM and 4.8 nM, respectively). Having observed general equivalence of these short and long isoforms with regard to GDF15 binding, Applicants then generated a receptor-Fc fusion protein consisting of the wild-type ECD of $hT\beta RII_{short}$ (SEQ ID NO: 7) fused at its C-terminus with a human IgG2 Fc domain via a minimal linker. Unless noted otherwise, amino acid position numbering with regard to variants based on the TβRII short and long isoforms refers to the corresponding position in the native precursors, SEQ ID NO: 5 and SEQ ID NO: 6, respectively.

[0187] Given the high-affinity binding of TβRII to GDF15, we tested whether TβRII could be used as an inhibitor of GDF15. The fusion protein $hT\beta RII_{short}$(23-159)-hG2Fc was tested in A549 cells transfected with a reporter gene containing a CAGA-12 promoter construct and was found to inhibit hGDF15-induced gene activation in such cells with an IC_{50} of 0.15-0.5 nM. Potent inhibition of GDF15 signaling by the $hT\beta RII_{short}$ ECD provides additional evidence that TβRII is the high-affinity receptor for GDF15. Even though GDF15 exhibited no detectable binding to ALK5 under cell-free conditions, suppression of endogenous ALK5 mRNA by siRNA methodology markedly reduced mGDF15-mediated signaling in A549 cells (a human pulmonary epithelial cell line) compared to control treatment. In contrast, suppression of other type I receptors (ALK2, ALK3, ALK4, and ALK7) by siRNA methodology failed to alter GDF15-mediated signaling in A549 cells. This result indicates that the GDF15 ternary signaling complex includes

ALK5 (TGFβ receptor type I) as its type I receptor and thus provides corroborating evidence for TβRII as a functional type II receptor for GDF15.

Example 3. Generation of receptor fusion protein variants

TβRII ECD variants

[0188] Since TβRII also binds with high affinity to TGFβ1 and TGFβ3, native TβRII-Fc fusion protein affects signaling of these ligands as well as GDF15. While in some therapeutic settings this broader spectrum of ligand binding may be advantageous, in other settings a more selective molecule may be superior. Therefore, Applicants sought polypeptides with enhanced or reduced selectivity for GDF15 by generating fusion proteins comprising variants of human TβRII ECD. The wild-type hTβRII$_{short}$(23-159) sequence shown below (SEQ ID NO: 7) served as the basis for five receptor ECD variants listed below (SEQ ID NO: 8-12). A wild type hTβRII$_{short}$(23-159) was fused to an Fc portion of IgG2 to generate a novel, base Fc fusion construct. See SEQ ID Nos. 50, 51 and 52, below.

```
  1  TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSNCS
 51  ITSICEKPQE VCVAVWRKND ENITLETVCH DPKLPYHDFI LEDAASPKCI
101  MKEKKKPGET FFMCSCSSDE CNDNIIFSEE YNTSNPD (SEQ ID NO: 7)
```

(1) The hTβRII$_{short}$(23-159/D110K) amino acid sequence shown below (SEQ ID NO: 8), in which the substituted residue is underlined.

```
  1  TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSNCS
 51  ITSICEKPQE VCVAVWRKND ENITLETVCH DPKLPYHKFI LEDAASPKCI
101  MKEKKKPGET FFMCSCSSDE CNDNIIFSEE YNTSNPD (SEQ ID NO: 8)
```

(2) The N-terminally truncated hTβRII$_{short}$(29-159) amino acid sequence shown below (SEQ ID NO: 9).

```
  1  QKSVNNDMIV TDNNGAVKFP QLCKFCDVRF STCDNQKSCM SNCSITSICE
 51  KPQEVCVAVW RKNDENITLE TVCHDPKLPY HDFILEDAAS PKCIMKEKKK
101  PGETFFMCSC SSDECNDNII FSEEYNTSNP D   (SEQ ID NO: 9)
```

(3) The N-terminally truncated hTβRII$_{short}$(35-159) amino acid sequence shown below (SEQ ID NO: 10).

```
  1  DMIVTDNNGA VKFPQLCKFC DVRFSTCDNQ KSCMSNCSIT SICEKPQEVC
 51  VAVWRKNDEN ITLETVCHDP KLPYHDFILE DAASPKCIMK EKKKPGETFF
101  MCSCSSDECN DNIIFSEEYN TSNPD     (SEQ ID NO: 10)
```

(4) The C-terminally truncated hTβRII$_{short}$(23-153) amino acid sequence shown below (SEQ ID NO: 11).

```
  1  TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSNCS
 51  ITSICEKPQE VCVAVWRKND ENITLETVCH DPKLPYHDFI LEDAASPKCI
101  MKEKKKPGET FFMCSCSSDE CNDNIIFSEE Y   (SEQ ID NO: 11)
```

(5) The C-terminally truncated hTβRII$_{short}$(23-153/N70D) amino acid sequence shown below (SEQ ID NO: 12), in which the substituted residue is underlined.

```
  1  TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSDCS
 51  ITSICEKPQE VCVAVWRKND ENITLETVCH DPKLPYHDFI LEDAASPKCI
101  MKEKKKPGET FFMCSCSSDE CNDNIIFSEE Y   (SEQ ID NO: 12)
```

[0189] Applicants also envision five corresponding variants (SEQ ID NO: 14-17) based on the wild-type hTβRII$_{long}$(23-184) sequence shown below (SEQ ID NO: 13), in which the 25 amino-acid insertion is underlined. Note that splicing results in a conservative amino acid substitution (Val→Ile) at the flanking position C-terminal to the insertion. Sequence relationships among several hTβRII$_{short}$ variants and their hTβRII$_{long}$ counterparts are indicated in **Figure 7**.

```
  1  TIPPHVQKSD VEMEAQKDEI ICPSCNRTAH PLRHINNDMI VTDNNGAVKF
 51  PQLCKFCDVR FSTCDNQKSC MSNCSITSIC EKPQEVCVAV WRKNDENITL
101  ETVCHDPKLP YHDFILEDAA SPKCIMKEKK KPGETFFMCS CSSDECNDNI
151  IFSEEYNTSN PD   (SEQ ID NO: 13)
```

(1) The hTβRII$_{long}$(23-184/D135K) amino acid sequence shown below (SEQ ID NO: 14), in which the substituted residue is double underlined.

```
  1  TIPPHVQKSD VEMEAQKDEI ICPSCNRTAH PLRHINNDMI VTDNNGAVKF
 51  PQLCKFCDVR FSTCDNQKSC MSNCSITSIC EKPQEVCVAV WRKNDENITL
101  ETVCHDPKLP YHKFILEDAA SPKCIMKEKK KPGETFFMCS CSSDECNDNI
151  IFSEEYNTSN PD   (SEQ ID NO: 14)
```

(2) The N-terminally truncated hTβRII$_{long}$(29-184) amino acid sequence shown below (SEQ ID NO: 15).

```
  1  QKSDVEMEAQ KDEIICPSCN RTAHPLRHIN NDMIVTDNNG AVKFPQLCKF
```

```
 51  CDVRFSTCDN QKSCMSNCSI TSICEKPQEV CVAVWRKNDE NITLETVCHD
101  PKLPYHDFIL EDAASPKCIM KEKKKPGETF FMCSCSSDEC NDNIIFSEEY
151  NTSNPD     (SEQ ID NO: 15)
```

(3) The N-terminally truncated hTβRII$_{long}$(60-184) amino acid sequence shown below (same as SEQ ID NO: 10).

```
  1  DMIVTDNNGA VKFPQLCKFC DVRFSTCDNQ KSCMSNCSIT SICEKPQEVC
 51  VAVWRKNDEN ITLETVCHDP KLPYHDFILE DAASPKCIMK EKKKPGETFF
101  MCSCSSDECN DNIIFSEEYN TSNPD      (same as SEQ ID NO: 10)
```

(4) The C-terminally truncated hTβRII$_{long}$(23-178) amino acid sequence shown below (SEQ ID NO: 16).

```
  1  TIPPHVQKSD VEMEAQKDEI ICPSCNRTAH PLRHINNDMI VTDNNGAVKF
 51  PQLCKFCDVR FSTCDNQKSC MSNCSITSIC EKPQEVCVAV WRKNDENITL
101  ETVCHDPKLP YHDFILEDAA SPKCIMKEKK KPGETFFMCS CSSDECNDNI
151  IFSEEY     (SEQ ID NO: 16)
```

(5) The C-terminally truncated hTβRII$_{long}$(23-178/N95D) amino acid sequence shown below (SEQ ID NO: 17), in which the substituted residue is double underlined.

```
  1  TIPPHVQKSD VEMEAQKDEI ICPSCNRTAH PLRHINNDMI VTDNNGAVKF
 51  PQLCKFCDVR FSTCDNQKSC MSDCSITSIC EKPQEVCVAV WRKNDENITL
101  ETVCHDPKLP YHDFILEDAA SPKCIMKEKK KPGETFFMCS CSSDECNDNI
151  IFSEEY     (SEQ ID NO: 17)
```

[0190] Additional TβRII ECD variants include:

(A) The N- and C-terminally truncated hTβRII$_{short}$(35-153) or hTβRII$_{long}$(60-178) amino acid sequence shown below (SEQ ID NO: 47).

```
  1  DMIVTDNNGA VKFPQLCKFC DVRFSTCDNQ KSCMSNCSIT SICEKPQEVC
 51  VAVWRKNDEN ITLETVCHDP KLPYHDFILE DAASPKCIMK EKKKPGETFF
101  MCSCSSDECN DNIIFSEEY      (SEQ ID NO: 47)
```

(B) The N- and C-terminally truncated hTβRII$_{short}$(29-153) amino acid sequence shown below (SEQ ID NO: 48).

```
  1  QKSVNNDMIV TDNNGAVKFP QLCKFCDVRF STCDNQKSCM SNCSITSICE
 51  KPQEVCVAVW RKNDENITLE TVCHDPKLPY HDFILEDAAS PKCIMKEKKK
```

101 PGETFFMCSC SSDECNDNII FSEEY (SEQ ID NO: 48)

(C) The N- and C-terminally truncated hTβRII$_{long}$(29-178) amino acid sequence shown below (SEQ ID NO: 49).

1 QKSDVEMEAQ KDEIICPSCN RTAHPLRHIN NDMIVTDNNG AVKFPQLCKF
51 CDVRFSTCDN QKSCMSNCSI TSICEKPQEV CVAVWRKNDE NITLETVCHD
101 PKLPYHDFIL EDAASPKCIM KEKKKPGETF FMCSCSSDEC NDNIIFSEEY
(SEQ ID NO: 49)

[0191] Any of the above variants (SEQ ID NO: 8-12, 14-17, and 47-49) could incorporate an insertion of 36 amino acids (SEQ ID NO: 18) between the pair of glutamate residues (positions 151 and 152 of SEQ ID NO: 5, or positions 176 and 177 of SEQ ID NO: 6) located near the C-terminus of the hTβRII ECD, as occurs naturally in the hTβRII isoform C (Konrad et al., BMC Genomics 8:318, 2007).

GRCKIRHIGS NNRLQRSTCQ NTGWESAHVM KTPGFR (SEQ ID NO: 18)

[0192] As an example, the paired glutamate residues flanking the optional insertion site are denoted below (underlined) for the hTβRII$_{short}$(29-159) variant (SEQ ID NO: 9).

1 QKSVNNDMIV TDNNGAVKFP QLCKFCDVRF STCDNQKSCM SNCSITSICE
51 KPQEVCVAVW RKNDENITLE TVCHDPKLPY HDFILEDAAS PKCIMKEKKK
101 PGETFFMCSC SSDECNDNII FSEEYNTSNP D (SEQ ID NO: 9)

Fc domain variants

[0193] hTβRII-hFc fusion proteins were generated in which five hTβRII$_{short}$ variants described above were each fused at their C-terminus (via a minimal linker) to a human IgG2 Fc domain, which has the following amino acid sequence (SEQ ID NO: 19):

1 VECPPCPAPP VAGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVQ
51 FNWYVDGVEV HNAKTKPREE QFNSTFRVVS VLTVVHQDWL NGKEYKCKVS
101 NKGLPAPIEK TISKTKGQPR EPQVYTLPPS REEMTKNQVS LTCLVKGFYP
151 SDIAVEWESN GQPENNYKTT PPMLDSDGSF FLYSKLTVDK SRWQQGNVFS
201 CSVMHEALHN HYTQKSLSLS PGK (SEQ ID NO: 19)

[0194] Applicants envision hTβRII-hFc fusion proteins comprising alternative Fc domains, including full-length human IgG1 Fc (hG1Fc) (SEQ ID NO: 20, below) and N-terminally truncated human IgG1 Fc ($hG1Fc_{short}$) (SEQ ID NO: 21, below). Optionally, a polypeptide unrelated to an Fc domain could be attached in place of the Fc domain.

```
  1  GGPKSCDKTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV
 51  DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS VLTVLHQDWL
101  NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS REEMTKNQVS
151  LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK
201  SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK   (SEQ ID NO: 20)
```

```
  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ VSLTCLVKGF
151  YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
201  FSCSVMHEAL HNHYTQKSLS LSPGK   (SEQ ID NO: 21)
```

Leader sequence variants

[0195] The following three leader sequences were considered:

(1) Native: MGRGLLRGLWPLHIVLWTRIAS (SEQ ID NO: 22)

(2) Tissue plasminogen activator (TPA): MDAMKRGLCCVLLLCGAVFVSP (SEQ ID NO: 23)

(3) Honey bee melittin (HBML): MKFLVNVALVFMVVYISYIYA (SEQ ID NO: 24)

Expression of hTβRII-hFc fusion proteins

[0196] The selected hTβRII-hFc protein variants incorporate the TPA leader and have the unprocessed amino-acid sequences shown in SEQ ID NOs: 25, 29, 33, 37, and 41 (see Example 5). Corresponding nucleotide sequences for these variants are SEQ ID NOs: 26, 30, 34, 38, and 42. Selected hTβRII-hFc variants, each with a G2Fc domain (SEQ ID NO: 19), were expressed in HEK-293 cells and purified from conditioned media by filtration and protein A chromatography. Purity of samples for reporter gene assays was evaluated by SDS-PAGE and Western blot analysis.

[0197] Applicants envision additional hTβRII-hFc protein variants with the unprocessed amino-acid sequences shown in SEQ ID NOs: 27, 31, 35, 39, and 43, and corresponding nucleotide sequences shown in SEQ ID NOs: 28, 32, 36, 40, and 44.

[0198] The amino acid sequence of the wild-type short construct $hT\beta RII_{short}$(23-159)-hG2Fc (SEQ ID NO: 50 is shown below.

TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSNCS
ITSICEKPQE VCVAVWRKND ENITLETVCH DPKLPYHDFI LEDAASPKCI
MKEKKKPGET FFMCSCSSDE CNDNIIFSEE YNTSNPDTGG GVECPPCPAP
PVAGPSVFLF PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV QFNWYVDGVE
VHNAKTKPRE EQFNSTFRVV SVLTVVHQDW LNGKEYKCKV SNKGLPAPIE
KTISKTKGQP REPQVYTLPP SREEMTKNQV SLTCLVKGFY PSDIAVEWES
NGQPENNYKT TPPMLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH
NHYTQKSLSL SPGK (SEQ ID NO: 50)

[0199] This protein was expressed from a construct including a TPA leader sequence, as shown below (SEQ ID NO:52). Dotted underline denotes leader, and solid underline denotes linker.

1 MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSVNNDMIV TDNNGAVKFP
51 QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAVW RKNDENITLE
101 TVCHDPKLPY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
151 FSEEYNTSNP DTGGGVECPP CPAPPVAGPS VFLFPPKPKD TLMISRTPEV
201 TCVVVDVSHE DPEVQFNWYV DGVEVHNAKT KPREEQFNST FRVVSVLTVV
251 HQDWLNGKEY KCKVSNKGLP APIEKTISKT KGQPREPQVY TLPPSREEMT
301 KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPMLD SDGSFFLYSK
351 LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK
(SEQ ID NO: 52)

[0200] The nucleic acid sequence encoding SEQ ID NO:52 is shown below:

1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
51 AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG
101 TTAATAACGA CATGATAGTC ACTGACAACA ACGGTGCAGT CAAGTTTCCA
151 CAACTGTGTA AATTTTGTGA TGTGAGATTT TCCACCTGTG ACAACCAGAA
201 ATCCTGCATG AGCAACTGCA GCATCACCTC CATCTGTGAG AAGCCACAGG
251 AAGTCTGTGT GGCTGTATGG AGAAAGAATG ACGAGAACAT AACACTAGAG
301 ACAGTTTGCC ATGACCCCAA GCTCCCCTAC CATGACTTTA TTCTGGAAGA

```
 351  TGCTGCTTCT CCAAAGTGCA TTATGAAGGA AAAAAAAAAG CCTGGTGAGA
 401  CTTTCTTCAT GTGTTCCTGT AGCTCTGATG AGTGCAATGA CAACATCATC
 451  TTCTCAGAAG AATATAACAC CAGCAATCCT GACACCGGTG GTGGAGTCGA
 501  GTGCCCACCG TGCCCAGCAC CACCTGTGGC AGGACCGTCA GTCTTCCTCT
 551  TCCCCCCAAA ACCCAAGGAC ACCCTCATGA TCTCCCGGAC CCCTGAGGTC
 601  ACGTGCGTGG TGGTGGACGT GAGCCACGAA GACCCCGAGG TCCAGTTCAA
 651  CTGGTACGTG GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCACGGG
 701  AGGAGCAGTT CAACAGCACG TTCCGTGTGG TCAGCGTCCT CACCGTCGTG
 751  CACCAGGACT GGCTGAACGG CAAGGAGTAC AAGTGCAAGG TCTCCAACAA
 801  AGGCCTCCCA GCCCCCATCG AGAAAACCAT CTCCAAAACC AAAGGGCAGC
 851  CCCGAGAACC ACAGGTGTAC ACCCTGCCCC CATCCCGGGA GGAGATGACC
 901  AAGAACCAGG TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT ACCCCAGCGA
 951  CATCGCCGTG GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACAAGA
1001  CCACACCTCC CATGCTGGAC TCCGACGGCT CCTTCTTCCT CTACAGCAAG
1051  CTCACCGTGG ACAAGAGCAG GTGGCAGCAG GGGAACGTCT TCTCATGCTC
1101  CGTGATGCAT GAGGCTCTGC ACAACCACTA CACGCAGAAG AGCCTCTCCC
1151  TGTCTCCGGG TAAA                (SEQ ID NO: 51)
```

Example 4. Differential ligand inhibition by receptor fusion protein variants in cell-based assay

[0201] A reporter gene assay in A549 cells was used to determine the ability of hTβRII-hFc variants to inhibit activity of GDF15, TGFβ1, TGFβ2, and TGFβ3. This assay is based on a human lung carcinoma cell line transfected with a pGL3(CAGA)12 reporter plasmid (Dennler et al, 1998, EMBO 17: 3091-3100) as well as a Renilla reporter plasmid (pRLCMV) to control for transfection efficiency. The CAGA motif is present in the promoters of TGFβ-responsive genes (for example, PAI-1), so this vector is of general use for factors signaling through SMAD2 and SMAD3.

[0202] On the first day of the assay, A549 cells (ATCC®: CCL-185™) were distributed in 48-well plates at $6.5x10^4$ cells per well. On the second day, a solution containing 10 μg pGL3(CAGA)12, 100ng pRLCMV, 30 μl X-tremeGENE 9 (Roche Applied Science), and 970 μl OptiMEM (Invitrogen) was preincubated for 30 min, then added to Eagle's minimum essential medium (EMEM, ATCC®) supplemented with 0.1% BSA, which was applied to the plated cells (500 μl/well) for incubation overnight at room temperature. On the third day, medium was removed, and cells were incubated overnight at 37°C with a mixture of ligands and inhibitors prepared as described below.

[0203] Serial dilutions of test articles were made in a 48-well plate in a 200 µl volume of assay buffer (EMEM + 0.1 % BSA). An equal volume of assay buffer containing the test ligand was added to obtain a final ligand concentration equal to the EC50 determined previously. Human GDF15 and murine GDF15 were generated in-house (see above), while human TGFβ1, human TGFβ2, and human TGFβ3 were obtained from PeproTech. Test solutions were incubated at 37°C for 30 minutes, then 250 µl of the mixture was added to all wells. Each concentration of test article was determined in duplicate. After incubation with test solutions overnight, cells were rinsed with phosphate-buffered saline, then lysed with passive lysis buffer (Promega E1941) and stored overnight at -70°C. On the fourth and final day, plates were warmed to room temperature with gentle shaking. Cell lysates were transferred in duplicate to a chemiluminescence plate (96-well) and analyzed in a luminometer with reagents from a Dual-Luciferase Reporter Assay system (Promega E1980) to determine normalized luciferase activity.

[0204] This assay was used to screen receptor fusion protein variants for potential inhibitory effects on cell signaling by TβRII ligands. Consistent with previous reports concerning wild-type $T\beta RII_{short}$-Fc and $T\beta RII_{long}$-Fc (del Re et al., J Biol Chem 279:22765, 2004), none of the variants tested were able to inhibit TGFβ2, even at high concentrations. However, hTβRII-hFc variants unexpectedly showed differential inhibition of cellular signaling mediated by GDF15, TGFβ1, and TGFβ3. Compared with wild-type $T\beta RII_{short}$(23-159)-G2Fc, the $T\beta RII_{short}$(23-159/D110K)-G2Fc variant exhibited potent inhibition of GDF15 but loss of inhibition of TGFβ1 and greatly reduced inhibition (~50 fold) of TGFβ3 (see table below). Position 110 is located in the "hook" region of TβRII (Radaev et al., J Biol Chem 285:14806, 2010) but has not been suggested to confer selectivity among the recognized TβRII ligands TGFβ1, TGFβ2, and TGFβ3. Thus, this variant displays a profile of differential ligand inhibition in which GDF15 is inhibited most potently, TGFβ1 least potently, and TGFβ3 to an intermediate degree.

Construct		IC_{50} (nM)		
		mGDF15 (35 ng/ml)	hTGFβ1 (640 pg/ml)	hTGFβ3 (270 pg/ml)
Full-length wild-type ECD	$T\beta RII_{short}$(23-159)-G2Fc	~ 0.12	1.73	0.14
Full-length ECD with D110K substitution	$T\beta RII_{short}$(23-159/D110K)-G2Fc	~ 0.7	ND (> 73.6)	~ 6.9

ND, not determined

[0205] In a second experiment, potencies of variants with N-terminally truncated TβRII ECD were compared with that of full-length wild-type TβRII ECD. As shown in the table below, $T\beta RII_{short}$(29-159)-G2Fc and $T\beta RII_{short}$(35-159)-G2Fc displayed a greatly diminished ability to inhibit TGFβ3 but an undiminished (N'Δ6) or only slightly diminished (N'Δ12) ability to inhibit GDF15 compared to $T\beta RII_{short}$(23-159)-G2Fc (wild-type). Effects of N-terminal truncation on inhibition of TGFβ1 compared to wild-type were intermediate in magnitude. Thus, these two variants exhibit a profile of differential ligand inhibition in which GDF15 is inhibited most potently, TGFβ3 least potently, and TGFβ1 to an intermediate degree.

Construct		IC_{50} (nM)		
		hGDF15 (70 or 112 ng/ml)	hTGFβ1 (640 pg/ml)	hTGFβ3 (270 pg/ml)
Full-length wild-type ECD	$T\beta RII_{short}$(23-159)-G2Fc	0.14 – 0.53	0.52	0.37
N'Δ6 ECD	$T\beta RII_{short}$(29-159)-G2Fc	0.40	2.05	ND (> 7.5)
N'Δ12 ECD	$T\beta RII_{short}$(35-159)-G2Fc	0.92	2.51	ND (> 7.5)

ND, not determined

[0206] In a third experiment, we determined the effect on potency of a N70D substitution in a C-terminally truncated TβRII ECD. This aspartate residue represents a potential glycosylation site. As shown in the table below, $T\beta RII_{short}$(23-153/N70D)-G2Fc displayed greatly diminished ability to inhibit TGFβ1 and virtually undiminished ability to inhibit TGFβ3 compared to $T\beta RII_{short}$(23-153)-G2Fc. Effects of N70D substitution on inhibition of GDF15 compared to both $T\beta RII_{short}$(23-153)-G2Fc and wild-type were intermediate in magnitude. Thus, the C-terminally truncated variant with N70D substitution exhibits a profile of differential ligand inhibition in which TGFβ3 is inhibited most potently, TGFβ1 least potently, and GDF15 to an intermediate degree.

Construct		IC_{50} (nM)		
		hGDF15 (70 ng/ml)	hTGFβ1 (640 pg/ml)	hTGFβ3 (270 pg/ml)
Full-length wild-type ECD	$T\beta RII_{short}$(23-159)-G2Fc	0.14	ND	ND
C'Δ6 ECD	$T\beta RII_{short}$(23-153)-G2Fc	0.18	2.62	0.14
C'Δ6 ECD with N70D substitution	$T\beta RII_{short}$(23-153/N70D)-G2Fc	2.42	17.7	0.28

[0207] Together, these results demonstrate that Applicants have generated truncations and mutations of the TβRII ECD that exhibit widely different ligand binding profiles. Notably, this demonstration reveals that properly expressed and purified GDF15 interacts directly with TβRII and can be differentially inhibited by fusion proteins comprising variants of the TβRII ECD. Activity profiles of these variants can be summarized in the following table.

Summary of Ligand Selectivity				
Construct		Degree of Ligand Inhibition		
		Potent	Moderate	Negligible
Full-length wild-type ECD	$T\beta RII_{short}$(23-159)-G2Fc	GDF15 TGFβ1 TGFβ3	---	TGFβ2
Full-length ECD with D110K substitution	$T\beta RII_{short}$(23-159/D110K)-G2Fc	GDF15	TGFβ3	TGFβ1 TGFβ2
N'Δ6 ECD	$T\beta RII_{short}$(29-159)-G2Fc	GDF15	TGFβ1	TGFβ2 TGFβ3
N'Δ12 ECD	$T\beta RII_{short}$(35-159)-G2Fc	GDF15	TGFβ1	TGFβ2 TGFβ3
C'Δ6 ECD with N70D substitution	$T\beta RII_{short}$(23-153/N70D)-G2Fc	TGFβ3	GDF15	TGFβ1 TGFβ2

[0208] We predict that the $T\beta RII_{long}$ ECD counterparts of these $T\beta RII_{short}$ ECD variants will exhibit similar ligand selectivity. In addition, a C'Δ6 truncated ECD (such as SEQ ID NOs: 11 and 16 for the $T\beta RII_{short}$ and $T\beta RII_{long}$ isoforms, respectively) can be used as a base sequence for $T\beta RII_{short}$ or $T\beta RII_{long}$ in which to introduce mutations and N-terminal truncations.

Example 5. Exemplary hTβRII-hFc nucleic acids and proteins

[0209] This example summarizes nucleic acid constructs that can be used to express TβRII constructs in HEK-293 or CHO cells, according to the methods provided herein in order to provide the proteins isolated from cell culture. In each case the mature protein isolated from cell culture will have the leader sequence (dotted underline in each sequence below) removed.

[0210] **Item 1** shows the amino acid sequence of $hT\beta RII_{short}$(23-159/D110K)-hG2Fc (SEQ ID NO: 25). Double underline indicates D110K substitution. Dotted underline denotes leader, and solid underline denotes linker.

1 MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSVNNDMIV TDNNGAVKFP

```
 51  QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAVW RKNDENITLE
101  TVCHDPKLPY HKFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
151  FSEEYNTSNP DTGGGVECPP CPAPPVAGPS VFLFPPKPKD TLMISRTPEV
201  TCVVVDVSHE DPEVQFNWYV DGVEVHNAKT KPREEQFNST FRVVSVLTVV
251  HQDWLNGKEY KCKVSNKGLP APIEKTISKT KGQPREPQVY TLPPSREEMT
301  KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPMLD SDGSFFLYSK
351  LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK
     (SEQ ID NO: 25)
```

[0211] **Item 2** shows a nucleotide sequence encoding hTβRII$_{short}$(23-159/D110K)-hG2Fc (SEQ ID NO: 26). Double underline indicates D110K substitution. Dotted underline denotes leader, and solid underline denotes linker.

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG
 101  TTAATAACGA CATGATAGTC ACTGACAACA ACGGTGCAGT CAAGTTTCCA
 151  CAACTGTGTA AATTTTGTGA TGTGAGATTT TCCACCTGTG ACAACCAGAA
 201  ATCCTGCATG AGCAACTGCA GCATCACCTC CATCTGTGAG AAGCCACAGG
 251  AAGTCTGTGT GGCTGTATGG AGAAAGAATG ACGAGAACAT AACACTAGAG
 301  ACAGTTTGCC ATGACCCCAA GCTCCCCTAC CATAAGTTTA TTCTGGAAGA
 351  TGCTGCTTCT CCAAAGTGCA TTATGAAGGA AAAAAAAAAG CCTGGTGAGA
 401  CTTTCTTCAT GTGTTCCTGT AGCTCTGATG AGTGCAATGA CAACATCATC
 451  TTCTCAGAAG AATATAACAC CAGCAATCCT GACACCGGTG GTGGAGTCGA
 501  GTGCCCACCG TGCCCAGCAC CACCTGTGGC AGGACCGTCA GTCTTCCTCT
 551  TCCCCCCAAA ACCCAAGGAC ACCCTCATGA TCTCCCGGAC CCCTGAGGTC
 601  ACGTGCGTGG TGGTGGACGT GAGCCACGAA GACCCCGAGG TCCAGTTCAA
 651  CTGGTACGTG GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCACGGG
 701  AGGAGCAGTT CAACAGCACG TTCCGTGTGG TCAGCGTCCT CACCGTCGTG
 751  CACCAGGACT GGCTGAACGG CAAGGAGTAC AAGTGCAAGG TCTCCAACAA
 801  AGGCCTCCCA GCCCCCATCG AGAAAACCAT CTCCAAAACC AAAGGGCAGC
 851  CCCGAGAACC ACAGGTGTAC ACCCTGCCCC CATCCCGGGA GGAGATGACC
 901  AAGAACCAGG TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT ACCCCAGCGA
 951  CATCGCCGTG GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACAAGA
1001  CCACACCTCC CATGCTGGAC TCCGACGGCT CCTTCTTCCT CTACAGCAAG
1051  CTCACCGTGG ACAAGAGCAG GTGGCAGCAG GGGAACGTCT TCTCATGCTC
1101  CGTGATGCAT GAGGCTCTGC ACAACCACTA CACGCAGAAG AGCCTCTCCC
1151  TGTCTCCGGG TAAA                (SEQ ID NO: 26)
```

[0212] **Item 3** shows the amino acid sequence of $hT\beta RII_{short}$(23-159/D110K)-$hG1Fc_{short}$ (SEQ ID NO: 27). Double underline indicates D110K substitution. Dotted underline denotes leader, and solid underline denotes linker.

```
  1 MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSVNNDMIV TDNNGAVKFP
 51 QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAVW RKNDENITLE
101 TVCHDPKLPY HKFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
151 FSEEYNTSNP DTGGGTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP
201 EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT
251 VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE
301 MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY
351 SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK
    (SEQ ID NO: 27)
```

[0213] **Item 4** shows a nucleotide sequence encoding $hT\beta RII_{short}$(23-159/D110K)-$hG1Fc_{short}$ (SEQ ID NO: 28). Double underline indicates D110K substitution. Dotted underline denotes leader, and solid underline denotes linker.

```
   1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51 AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG
 101 TTAATAACGA CATGATAGTC ACTGACAACA ACGGTGCAGT CAAGTTTCCA
 151 CAACTGTGTA AATTTTGTGA TGTGAGATTT TCCACCTGTG ACAACCAGAA
 201 ATCCTGCATG AGCAACTGCA GCATCACCTC CATCTGTGAG AAGCCACAGG
 251 AAGTCTGTGT GGCTGTATGG AGAAAGAATG ACGAGAACAT AACACTAGAG
 301 ACAGTTTGCC ATGACCCCAA GCTCCCCTAC CATAAGTTTA TTCTGGAAGA
 351 TGCTGCTTCT CCAAAGTGCA TTATGAAGGA AAAAAAAAAG CCTGGTGAGA
 401 CTTTCTTCAT GTGTTCCTGT AGCTCTGATG AGTGCAATGA CAACATCATC
 451 TTCTCAGAAG AATATAACAC CAGCAATCCT GACACCGGTG GTGGAACTCA
 501 CACATGCCCA CCGTGCCCAG CACCTGAACT CCTGGGGGGA CCGTCAGTCT
 551 TCCTCTTCCC CCCAAAACCC AAGGACACCC TCATGATCTC CCGGACCCCT
 601 GAGGTCACAT GCGTGGTGGT GGACGTGAGC CACGAAGACC CTGAGGTCAA
 651 GTTCAACTGG TACGTGGACG GCGTGGAGGT GCATAATGCC AAGACAAAGC
 701 CGCGGGAGGA GCAGTACAAC AGCACGTACC GTGTGGTCAG CGTCCTCACC
 751 GTCCTGCACC AGGACTGGCT GAATGGCAAG GAGTACAAGT GCAAGGTCTC
 801 CAACAAAGCC CTCCCAGCCC CCATCGAGAA AACCATCTCC AAAGCCAAAG
 851 GGCAGCCCCG AGAACCACAG GTGTACACCC TGCCCCCATC CCGGGAGGAG
 901 ATGACCAAGA ACCAGGTCAG CCTGACCTGC CTGGTCAAAG GCTTCTATCC
 951 CAGCGACATC GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG GAGAACAACT
```

```
1001   ACAAGACCAC GCCTCCCGTG CTGGACTCCG ACGGCTCCTT CTTCCTCTAT
1051   AGCAAGCTCA CCGTGGACAA GAGCAGGTGG CAGCAGGGGA ACGTCTTCTC
1101   ATGCTCCGTG ATGCATGAGG CTCTGCACAA CCACTACACG CAGAAGAGCC
1151   TCTCCCTGTC CCCGGGTAAA          (SEQ ID NO: 28)
```

[0214] **Item 5** shows the amino acid sequence of hTβRII$_{short}$(29-159)-hG2Fc (SEQ ID NO: 29). Dotted underline denotes leader, and solid underline denotes linker.

```
1   MDAMKRGLCC VLLLCGAVFV SPGAQKSVNN DMIVTDNNGA VKFPQLCKFC
51  DVRFSTCDNQ KSCMSNCSIT SICEKPQEVC VAVWRKNDEN ITLETVCHDP
101 KLPYHDFILE DAASPKCIMK EKKKPGETFF MCSCSSDECN DNIIFSEEYN
151 TSNPDTGGGV ECPPCPAPPV AGPSVFLFPP KPKDTLMISR TPEVTCVVVD
201 VSHEDPEVQF NWYVDGVEVH NAKTKPREEQ FNSTFRVVSV LTVVHQDWLN
251 GKEYKCKVSN KGLPAPIEKT ISKTKGQPRE PQVYTLPPSR EEMTKNQVSL
301 TCLVKGFYPS DIAVEWESNG QPENNYKTTP PMLDSDGSFF LYSKLTVDKS
351 RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK    (SEQ ID NO: 29)
```

[0215] **Item 6** shows a nucleotide sequence encoding hTβRII$_{short}$(29-159)-hG2Fc (SEQ ID NO: 30). Dotted underline denotes leader, and solid underline denotes linker.

```
1   ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
51  AGTCTTCGTT TCGCCCGGCG CCCAGAAGTC GGTTAATAAC GACATGATAG
101 TCACTGACAA CAACGGTGCA GTCAAGTTTC CACAACTGTG TAAATTTTGT
151 GATGTGAGAT TTTCCACCTG TGACAACCAG AAATCCTGCA TGAGCAACTG
201 CAGCATCACC TCCATCTGTG AGAAGCCACA GGAAGTCTGT GTGGCTGTAT
251 GGAGAAAGAA TGACGAGAAC ATAACACTAG AGACAGTTTG CCATGACCCC
301 AAGCTCCCCT ACCATGACTT TATTCTGGAA GATGCTGCTT CTCCAAAGTG
351 CATTATGAAG GAAAAAAAAA AGCCTGGTGA GACTTTCTTC ATGTGTTCCT
401 GTAGCTCTGA TGAGTGCAAT GACAACATCA TCTTCTCAGA AGAATATAAC
451 ACCAGCAATC CTGACACCGG TGGTGGAGTC GAGTGCCCAC CGTGCCCAGC
501 ACCACCTGTG GCAGGACCGT CAGTCTTCCT CTTCCCCCCA AAACCCAAGG
551 ACACCCTCAT GATCTCCCGG ACCCCTGAGG TCACGTGCGT GGTGGTGGAC
601 GTGAGCCACG AAGACCCCGA GGTCCAGTTC AACTGGTACG TGGACGGCGT
651 GGAGGTGCAT AATGCCAAGA CAAAGCCACG GGAGGAGCAG TTCAACAGCA
701 CGTTCCGTGT GGTCAGCGTC CTCACCGTCG TGCACCAGGA CTGGCTGAAC
751 GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC AAAGGCCTCC CAGCCCCCAT
801 CGAGAAAACC ATCTCCAAAA CCAAAGGGCA GCCCCGAGAA CCACAGGTGT
851 ACACCCTGCC CCCATCCCGG GAGGAGATGA CCAAGAACCA GGTCAGCCTG
901 ACCTGCCTGG TCAAAGGCTT CTACCCCAGC GACATCGCCG TGGAGTGGGA
```

951 GAGCAATGGG CAGCCGGAGA ACAACTACAA GACCACACCT CCCATGCTGG
1001 ACTCCGACGG CTCCTTCTTC CTCTACAGCA AGCTCACCGT GGACAAGAGC
1051 AGGTGGCAGC AGGGGAACGT CTTCTCATGC TCCGTGATGC ATGAGGCTCT
1101 GCACAACCAC TACACGCAGA AGAGCCTCTC CCTGTCTCCG GGTAAA
(SEQ ID NO: 30)

[0216] **Item 7** shows the amino acid sequence of hTβRII$_{short}$(29-159)-hG1Fc$_{short}$ (SEQ ID NO: 31). Dotted underline denotes leader, and solid underline denotes linker.

1 MDAMKRGLCC VLLLCGAVFV SPGAQKSVNN DMIVTDNNGA VKFPQLCKFC
51 DVRFSTCDNQ KSCMSNCSIT SICEKPQEVC VAVWRKNDEN ITLETVCHDP
101 KLPYHDFILE DAASPKCIMK EKKKPGETFF MCSCSSDECN DNIIFSEEYN
151 TSNPDTGGGT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV
201 VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW
251 LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SREEMTKNQV
301 SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD
351 KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK (SEQ ID NO: 31)

[0217] **Item 8** shows a nucleotide sequence encoding hTβRII$_{short}$(29-159)-hG1Fc$_{short}$ (SEQ ID NO: 32). Dotted underline denotes leader, and solid underline denotes linker.

1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
51 AGTCTTCGTT TCGCCCGGCG CCCAGAAGTC GGTTAATAAC GACATGATAG
101 TCACTGACAA CAACGGTGCA GTCAAGTTTC CACAACTGTG TAAATTTTGT
151 GATGTGAGAT TTTCCACCTG TGACAACCAG AAATCCTGCA TGAGCAACTG
201 CAGCATCACC TCCATCTGTG AGAAGCCACA GGAAGTCTGT GTGGCTGTAT
251 GGAGAAAGAA TGACGAGAAC ATAACACTAG AGACAGTTTG CCATGACCCC
301 AAGCTCCCCT ACCATGACTT TATTCTGGAA GATGCTGCTT CTCCAAAGTG
351 CATTATGAAG GAAAAAAAAA AGCCTGGTGA GACTTTCTTC ATGTGTTCCT
401 GTAGCTCTGA TGAGTGCAAT GACAACATCA TCTTCTCAGA AGAATATAAC
451 ACCAGCAATC CTGACACCGG TGGTGGAACT CACACATGCC CACCGTGCCC
501 AGCACCTGAA CTCCTGGGGG GACCGTCAGT CTTCCTCTTC CCCCCAAAAC
551 CCAAGGACAC CCTCATGATC TCCCGGACCC CTGAGGTCAC ATGCGTGGTG
601 GTGGACGTGA GCCACGAAGA CCCTGAGGTC AAGTTCAACT GGTACGTGGA
651 CGGCGTGGAG GTGCATAATG CCAAGACAAA GCCGCGGGAG GAGCAGTACA
701 ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG
751 CTGAATGGCA AGGAGTACAA GTGCAAGGTC TCCAACAAAG CCCTCCCAGC
801 CCCCATCGAG AAAACCATCT CCAAAGCCAA AGGGCAGCCC CGAGAACCAC

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 851  AGGTGTACAC CCTGCCCCCA TCCCGGGAGG AGATGACCAA GAACCAGGTC
 901  AGCCTGACCT GCCTGGTCAA AGGCTTCTAT CCCAGCGACA TCGCCGTGGA
 951  GTGGGAGAGC AATGGGCAGC CGGAGAACAA CTACAAGACC ACGCCTCCCG
1001  TGCTGGACTC CGACGGCTCC TTCTTCCTCT ATAGCAAGCT CACCGTGGAC
1051  AAGAGCAGGT GGCAGCAGGG GAACGTCTTC TCATGCTCCG TGATGCATGA
1101  GGCTCTGCAC AACCACTACA CGCAGAAGAG CCTCTCCCTG TCCCCGGGTA
1151  AA      (SEQ ID NO: 32)
```

[0218] **Item 9** shows the amino acid sequence of hTβRII$_{short}$(35-159)-hG2Fc (SEQ ID NO: 33). Dotted underline denotes leader, and solid underline denotes linker.

```
   1  MDAMKRGLCC VLLLCGAVFV SPGADMIVTD NNGAVKFPQL CKFCDVRFST
  51  CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD
 101  FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPDT
 151  GGGVECPPCP APPVAGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP
 201  EVQFNWYVDG VEVHNAKTKP REEQFNSTFR VVSVLTVVHQ DWLNGKEYKC
 251  KVSNKGLPAP IEKTISKTKG QPREPQVYTL PPSREEMTKN QVSLTCLVKG
 301  FYPSDIAVEW ESNGQPENNY KTTPPMLDSD GSFFLYSKLT VDKSRWQQGN
 351  VFSCSVMHEA LHNHYTQKSL SLSPGK      (SEQ ID NO: 33)
```

[0219] **Item 10** shows a nucleotide sequence encoding hTβRII$_{short}$(35-159)-hG2Fc (SEQ ID NO: 34). Dotted underline denotes leader, and solid underline denotes linker.

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCGACATGAT AGTCACTGAC AACAACGGTG
 101  CAGTCAAGTT TCCACAACTG TGTAAATTTT GTGATGTGAG ATTTTCCACC
 151  TGTGACAACC AGAAATCCTG CATGAGCAAC TGCAGCATCA CCTCCATCTG
 201  TGAGAAGCCA CAGGAAGTCT GTGTGGCTGT ATGGAGAAAG AATGACGAGA
 251  ACATAACACT AGAGACAGTT TGCCATGACC CCAAGCTCCC CTACCATGAC
 301  TTTATTCTGG AAGATGCTGC TTCTCCAAAG TGCATTATGA AGGAAAAAAA
 351  AAAGCCTGGT GAGACTTTCT TCATGTGTTC CTGTAGCTCT GATGAGTGCA
 401  ATGACAACAT CATCTTCTCA GAAGAATATA ACACCAGCAA TCCTGACACC
 451  GGTGGTGGAG TCGAGTGCCC ACCGTGCCCA GCACCACCTG TGGCAGGACC
 501  GTCAGTCTTC CTCTTCCCCC CAAAACCCAA GGACACCCTC ATGATCTCCC
 551  GGACCCCTGA GGTCACGTGC GTGGTGGTGG ACGTGAGCCA CGAAGACCCC
 601  GAGGTCCAGT TCAACTGGTA CGTGGACGGC GTGGAGGTGC ATAATGCCAA
 651  GACAAAGCCA CGGGAGGAGC AGTTCAACAG CACGTTCCGT GTGGTCAGCG
 701  TCCTCACCGT CGTGCACCAG GACTGGCTGA ACGGCAAGGA GTACAAGTGC
 751  AAGGTCTCCA ACAAAGGCCT CCCAGCCCCC ATCGAGAAAA CCATCTCCAA
```

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 801  AACCAAAGGG CAGCCCCGAG AACCACAGGT GTACACCCTG CCCCCATCCC
 851  GGGAGGAGAT GACCAAGAAC CAGGTCAGCC TGACCTGCCT GGTCAAAGGC
 901  TTCTACCCCA GCGACATCGC CGTGGAGTGG GAGAGCAATG GGCAGCCGGA
 951  GAACAACTAC AAGACCACAC CTCCCATGCT GGACTCCGAC GGCTCCTTCT
1001  TCCTCTACAG CAAGCTCACC GTGGACAAGA GCAGGTGGCA GCAGGGGAAC
1051  GTCTTCTCAT GCTCCGTGAT GCATGAGGCT CTGCACAACC ACTACACGCA
1101  GAAGAGCCTC TCCCTGTCTC CGGGTAAA      (SEQ ID NO: 34)
```

[0220] **Item 11** shows the amino acid sequence of hTβRII$_{short}$(35-159)-hG1Fc$_{short}$ (SEQ ID NO: 35). Dotted underline denotes leader, and solid underline denotes linker.

```
   1  MDAMKRGLCC VLLLCGAVFV SPGADMIVTD NNGAVKFPQL CKFCDVRFST
  51  CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD
 101  FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPDT
 151  GGGTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE
 201  DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY
 251  KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSREEMT KNQVSLTCLV
 301  KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ
 351  GNVFSCSVMH EALHNHYTQK SLSLSPGK        (SEQ ID NO: 35)
```

[0221] **Item 12** shows a nucleotide sequence encoding hTβRII$_{short}$(35-159)-hG1Fc$_{short}$ (SEQ ID NO: 36). Dotted underline denotes leader, and solid underline denotes linker.

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCGACATGAT AGTCACTGAC AACAACGGTG
 101  CAGTCAAGTT TCCACAACTG TGTAAATTTT GTGATGTGAG ATTTTCCACC
 151  TGTGACAACC AGAAATCCTG CATGAGCAAC TGCAGCATCA CCTCCATCTG
 201  TGAGAAGCCA CAGGAAGTCT GTGTGGCTGT ATGGAGAAAG AATGACGAGA
 251  ACATAACACT AGAGACAGTT TGCCATGACC CCAAGCTCCC CTACCATGAC
 301  TTTATTCTGG AAGATGCTGC TTCTCCAAAG TGCATTATGA AGGAAAAAAA
 351  AAAGCCTGGT GAGACTTTCT TCATGTGTTC CTGTAGCTCT GATGAGTGCA
 401  ATGACAACAT CATCTTCTCA GAAGAATATA ACACCAGCAA TCCTGACACC
 451  GGTGGTGGAA CTCACACATG CCCACCGTGC CCAGCACCTG AACTCCTGGG
 501  GGGACCGTCA GTCTTCCTCT TCCCCCCAAA ACCCAAGGAC ACCCTCATGA
 551  TCTCCCGGAC CCCTGAGGTC ACATGCGTGG TGGTGGACGT GAGCCACGAA
 601  GACCCTGAGG TCAAGTTCAA CTGGTACGTG GACGGCGTGG AGGTGCATAA
 651  TGCCAAGACA AAGCCGCGGG AGGAGCAGTA CAACAGCACG TACCGTGTGG
 701  TCAGCGTCCT CACCGTCCTG CACCAGGACT GGCTGAATGG CAAGGAGTAC
 751  AAGTGCAAGG TCTCCAACAA AGCCCTCCCA GCCCCCATCG AGAAAACCAT
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 801  CTCCAAAGCC AAAGGGCAGC CCCGAGAACC ACAGGTGTAC ACCCTGCCCC
 851  CATCCCGGGA GGAGATGACC AAGAACCAGG TCAGCCTGAC CTGCCTGGTC
 901  AAAGGCTTCT ATCCCAGCGA CATCGCCGTG GAGTGGGAGA GCAATGGGCA
 951  GCCGGAGAAC AACTACAAGA CCACGCCTCC CGTGCTGGAC TCCGACGGCT
1001  CCTTCTTCCT CTATAGCAAG CTCACCGTGG ACAAGAGCAG GTGGCAGCAG
1051  GGGAACGTCT TCTCATGCTC CGTGATGCAT GAGGCTCTGC ACAACCACTA
1101  CACGCAGAAG AGCCTCTCCC TGTCCCCGGG TAAA
      (SEQ ID NO: 36)
```

[0222] **Item 13** shows the amino acid sequence of hTβRII$_{short}$(23-153)-hG2Fc (SEQ ID NO: 37). Dotted underline denotes leader, and solid underline denotes linker.

```
   1  MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSVNNDMIV TDNNGAVKFP
  51  QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAVW RKNDENITLE
 101  TVCHDPKLPY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
 151  FSEEYTGGGV ECPPCPAPPV AGPSVFLFPP KPKDTLMISR TPEVTCVVVD
 201  VSHEDPEVQF NWYVDGVEVH NAKTKPREEQ FNSTFRVVSV LTVVHQDWLN
 251  GKEYKCKVSN KGLPAPIEKT ISKTKGQPRE PQVYTLPPSR EEMTKNQVSL
 301  TCLVKGFYPS DIAVEWESNG QPENNYKTTP PMLDSDGSFF LYSKLTVDKS
 351  RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK    (SEQ ID NO: 37)
```

[0223] **Item 14** shows a nucleotide sequence encoding hTβRII$_{short}$(23-153)-hG2Fc (SEQ ID NO: 38). Dotted underline denotes leader, and solid underline denotes linker.

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG
 101  TTAATAACGA CATGATAGTC ACTGACAACA ACGGTGCAGT CAAGTTTCCA
 151  CAACTGTGTA AATTTTGTGA TGTGAGATTT TCCACCTGTG ACAACCAGAA
 201  ATCCTGCATG AGCAACTGCA GCATCACCTC CATCTGTGAG AAGCCACAGG
 251  AAGTCTGTGT GGCTGTATGG AGAAAGAATG ACGAGAACAT AACACTAGAG
 301  ACAGTTTGCC ATGACCCCAA GCTCCCCTAC CATGACTTTA TTCTGGAAGA
 351  TGCTGCTTCT CCAAAGTGCA TTATGAAGGA AAAAAAAAAG CCTGGTGAGA
 401  CTTTCTTCAT GTGTTCCTGT AGCTCTGATG AGTGCAATGA CAACATCATC
 451  TTCTCAGAAG AATATACCGG TGGTGGAGTC GAGTGCCCAC CGTGCCCAGC
 501  ACCACCTGTG GCAGGACCGT CAGTCTTCCT CTTCCCCCCA AAACCCAAGG
 551  ACACCCTCAT GATCTCCCGG ACCCCTGAGG TCACGTGCGT GGTGGTGGAC
 601  GTGAGCCACG AAGACCCCGA GGTCCAGTTC AACTGGTACG TGGACGGCGT
 651  GGAGGTGCAT AATGCCAAGA CAAAGCCACG GGAGGAGCAG TTCAACAGCA
 701  CGTTCCGTGT GGTCAGCGTC CTCACCGTCG TGCACCAGGA CTGGCTGAAC
```

```
 751  GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC AAAGGCCTCC CAGCCCCCAT
 801  CGAGAAAACC ATCTCCAAAA CCAAAGGGCA GCCCCGAGAA CCACAGGTGT
 851  ACACCCTGCC CCCATCCCGG GAGGAGATGA CCAAGAACCA GGTCAGCCTG
 901  ACCTGCCTGG TCAAAGGCTT CTACCCCAGC GACATCGCCG TGGAGTGGGA
 951  GAGCAATGGG CAGCCGGAGA ACAACTACAA GACCACACCT CCCATGCTGG
1001  ACTCCGACGG CTCCTTCTTC CTCTACAGCA AGCTCACCGT GGACAAGAGC
1051  AGGTGGCAGC AGGGGAACGT CTTCTCATGC TCCGTGATGC ATGAGGCTCT
1101  GCACAACCAC TACACGCAGA AGAGCCTCTC CCTGTCTCCG GGTAAA
      (SEQ ID NO: 38)
```

[0224] **Item 15** shows the amino acid sequence of $hT\beta RII_{short}$(23-153)-$hG1Fc_{short}$ (SEQ ID NO: 39). Dotted underline denotes leader, and solid underline denotes linker.

```
   1  MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSVNNDMIV TDNNGAVKFP
  51  QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAVW RKNDENITLE
 101  TVCHDPKLPY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
 151  FSEEYTGGGT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV
 201  VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW
 251  LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SREEMTKNQV
 301  SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD
 351  KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK    (SEQ ID NO: 39)
```

[0225] **Item 16** shows a nucleotide sequence encoding $hT\beta RII_{short}$(23-153)-$hG1Fc_{short}$ (SEQ ID NO: 40). Dotted underline denotes leader, and solid underline denotes linker.

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG
 101  TTAATAACGA CATGATAGTC ACTGACAACA ACGGTGCAGT CAAGTTTCCA
 151  CAACTGTGTA AATTTTGTGA TGTGAGATTT TCCACCTGTG ACAACCAGAA
 201  ATCCTGCATG AGCAACTGCA GCATCACCTC CATCTGTGAG AAGCCACAGG
 251  AAGTCTGTGT GGCTGTATGG AGAAAGAATG ACGAGAACAT AACACTAGAG
 301  ACAGTTTGCC ATGACCCCAA GCTCCCCTAC CATGACTTTA TTCTGGAAGA
 351  TGCTGCTTCT CCAAAGTGCA TTATGAAGGA AAAAAAAAAG CCTGGTGAGA
 401  CTTTCTTCAT GTGTTCCTGT AGCTCTGATG AGTGCAATGA CAACATCATC
 451  TTCTCAGAAG AATATACCGG TGGTGGAACT CACACATGCC CACCGTGCCC
 501  AGCACCTGAA CTCCTGGGGG GACCGTCAGT CTTCCTCTTC CCCCCAAAAC
 551  CCAAGGACAC CCTCATGATC TCCCGGACCC CTGAGGTCAC ATGCGTGGTG
 601  GTGGACGTGA GCCACGAAGA CCCTGAGGTC AAGTTCAACT GGTACGTGGA
 651  CGGCGTGGAG GTGCATAATG CCAAGACAAA GCCGCGGGAG GAGCAGTACA
```

```
 701  ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG
 751  CTGAATGGCA AGGAGTACAA GTGCAAGGTC TCCAACAAAG CCCTCCCAGC
 801  CCCCATCGAG AAAACCATCT CCAAAGCCAA AGGGCAGCCC CGAGAACCAC
 851  AGGTGTACAC CCTGCCCCCA TCCCGGGAGG AGATGACCAA GAACCAGGTC
 901  AGCCTGACCT GCCTGGTCAA AGGCTTCTAT CCCAGCGACA TCGCCGTGGA
 951  GTGGGAGAGC AATGGGCAGC CGGAGAACAA CTACAAGACC ACGCCTCCCG
1001  TGCTGGACTC CGACGGCTCC TTCTTCCTCT ATAGCAAGCT CACCGTGGAC
1051  AAGAGCAGGT GGCAGCAGGG GAACGTCTTC TCATGCTCCG TGATGCATGA
1101  GGCTCTGCAC AACCACTACA CGCAGAAGAG CCTCTCCCTG TCCCCGGGTA
1151  AA       (SEQ ID NO: 40)
```

[0226] **Item 17** shows the amino acid sequence of hTβRII$_{short}$(23-153/N70D)-hG2Fc (SEQ ID NO: 41). Double underline indicates N70D substitution. Dotted underline denotes leader, and solid underline denotes linker.

```
   1  MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSVNNDMIV TDNNGAVKFP
  51  QLCKFCDVRF STCDNQKSCM SDCSITSICE KPQEVCVAVW RKNDENITLE
 101  TVCHDPKLPY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
 151  FSEEYTGGGV ECPPCPAPPV AGPSVFLFPP KPKDTLMISR TPEVTCVVVD
 201  VSHEDPEVQF NWYVDGVEVH NAKTKPREEQ FNSTFRVVSV LTVVHQDWLN
 251  GKEYKCKVSN KGLPAPIEKT ISKTKGQPRE PQVYTLPPSR EEMTKNQVSL
 301  TCLVKGFYPS DIAVEWESNG QPENNYKTTP PMLDSDGSFF LYSKLTVDKS
 351  RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK    (SEQ ID NO: 41)
```

[0227] **Item 18** shows a nucleotide sequence encoding hTβRII$_{short}$(23-153/N70D)-hG2Fc (SEQ ID NO: 42). Double underline indicates N70D substitution. Dotted underline denotes leader, and solid underline denotes linker.

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG
 101  TTAATAACGA CATGATAGTC ACTGACAACA ACGGTGCAGT CAAGTTTCCA
 151  CAACTGTGTA AATTTTGTGA TGTGAGATTT TCCACCTGTG ACAACCAGAA
 201  ATCCTGCATG AGCGACTGCA GCATCACCTC CATCTGTGAG AAGCCACAGG
 251  AAGTCTGTGT GGCTGTATGG AGAAAGAATG ACGAGAACAT AACACTAGAG
 301  ACAGTTTGCC ATGACCCCAA GCTCCCCTAC CATGACTTTA TTCTGGAAGA
 351  TGCTGCTTCT CCAAAGTGCA TTATGAAGGA AAAAAAAAAG CCTGGTGAGA
 401  CTTTCTTCAT GTGTTCCTGT AGCTCTGATG AGTGCAATGA CAACATCATC
 451  TTCTCAGAAG AATATACCGG TGGTGGAGTC GAGTGCCCAC CGTGCCCAGC
 501  ACCACCTGTG GCAGGACCGT CAGTCTTCCT CTTCCCCCCA AAACCCAAGG
```

```
 551  ACACCCTCAT GATCTCCCGG ACCCCTGAGG TCACGTGCGT GGTGGTGGAC
 601  GTGAGCCACG AAGACCCCGA GGTCCAGTTC AACTGGTACG TGGACGGCGT
 651  GGAGGTGCAT AATGCCAAGA CAAAGCCACG GGAGGAGCAG TTCAACAGCA
 701  CGTTCCGTGT GGTCAGCGTC CTCACCGTCG TGCACCAGGA CTGGCTGAAC
 751  GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC AAAGGCCTCC CAGCCCCCAT
 801  CGAGAAAACC ATCTCCAAAA CCAAAGGGCA GCCCCGAGAA CCACAGGTGT
 851  ACACCCTGCC CCCATCCCGG GAGGAGATGA CCAAGAACCA GGTCAGCCTG
 901  ACCTGCCTGG TCAAAGGCTT CTACCCCAGC GACATCGCCG TGGAGTGGGA
 951  GAGCAATGGG CAGCCGGAGA ACAACTACAA GACCACACCT CCCATGCTGG
1001  ACTCCGACGG CTCCTTCTTC CTCTACAGCA AGCTCACCGT GGACAAGAGC
1051  AGGTGGCAGC AGGGGAACGT CTTCTCATGC TCCGTGATGC ATGAGGCTCT
1101  GCACAACCAC TACACGCAGA AGAGCCTCTC CCTGTCTCCG GGTAAA
      (SEQ ID NO: 42)
```

[0228] **Item 19** shows the amino acid sequence of $hT\beta RII_{short}$(23-153/N70D)-$hG1Fc_{short}$ (SEQ ID NO: 43). Double underline indicates N70D substitution. Dotted underline denotes leader, and solid underline denotes linker.

```
   1  MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSVNNDMIV TDNNGAVKFP
  51  QLCKFCDVRF STCDNQKSCM SDCSITSICE KPQEVCVAVW RKNDENITLE
 101  TVCHDPKLPY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
 151  FSEEYTGGGT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV
 201  VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW
 251  LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SREEMTKNQV
 301  SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD
 351  KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK    (SEQ ID NO: 43)
```

[0229] **Item 20** shows a nucleotide sequence encoding $hT\beta RII_{short}$(23-153/N70D)-$hG1Fc_{short}$ (SEQ ID NO: 44). Double underline indicates N70D substitution. Dotted underline denotes leader, and solid underline denotes linker.

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG
 101  TTAATAACGA CATGATAGTC ACTGACAACA ACGGTGCAGT CAAGTTTCCA
 151  CAACTGTGTA AATTTTGTGA TGTGAGATTT TCCACCTGTG ACAACCAGAA
 201  ATCCTGCATG AGCGACTGCA GCATCACCTC CATCTGTGAG AAGCCACAGG
 251  AAGTCTGTGT GGCTGTATGG AGAAAGAATG ACGAGAACAT AACACTAGAG
 301  ACAGTTTGCC ATGACCCCAA GCTCCCCTAC CATGACTTTA TTCTGGAAGA
 351  TGCTGCTTCT CCAAAGTGCA TTATGAAGGA AAAAAAAAAG CCTGGTGAGA
```

401 CTTTCTTCAT GTGTTCCTGT AGCTCTGATG AGTGCAATGA CAACATCATC
451 TTCTCAGAAG AATATACCGG TGGTGGAACT CACACATGCC CACCGTGCCC
501 AGCACCTGAA CTCCTGGGGG GACCGTCAGT CTTCCTCTTC CCCCCAAAAC
551 CCAAGGACAC CCTCATGATC TCCCGGACCC CTGAGGTCAC ATGCGTGGTG
601 GTGGACGTGA GCCACGAAGA CCCTGAGGTC AAGTTCAACT GGTACGTGGA
651 CGGCGTGGAG GTGCATAATG CCAAGACAAA GCCGCGGGAG GAGCAGTACA
701 ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG
751 CTGAATGGCA AGGAGTACAA GTGCAAGGTC TCCAACAAAG CCCTCCCAGC
801 CCCCATCGAG AAAACCATCT CCAAAGCCAA AGGGCAGCCC CGAGAACCAC
851 AGGTGTACAC CCTGCCCCCA TCCCGGGAGG AGATGACCAA GAACCAGGTC
901 AGCCTGACCT GCCTGGTCAA AGGCTTCTAT CCCAGCGACA TCGCCGTGGA
951 GTGGGAGAGC AATGGGCAGC CGGAGAACAA CTACAAGACC ACGCCTCCCG
1001 TGCTGGACTC CGACGGCTCC TTCTTCCTCT ATAGCAAGCT CACCGTGGAC
1051 AAGAGCAGGT GGCAGCAGGG GAACGTCTTC TCATGCTCCG TGATGCATGA
1101 GGCTCTGCAC AACCACTACA CGCAGAAGAG CCTCTCCCTG TCCCCGGGTA
1151 AA (SEQ ID NO: 44)

[0230] **Item 21** shows the mature amino acid sequence (i.e., without the leader sequence) of hTβRII$_{short}$(23-159/D110K)-hG2Fc (SEQ ID NO: 53). Double underline indicates D110K substitution. Single underline denotes linker.

TIPPHV QKSVNNDMIV TDNNGAVKFP
QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAVW RKNDENITLE
TVCHDPKLPY HKFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
FSEEYNTSNP DTGGGVECPP CPAPPVAGPS VFLFPPKPKD TLMISRTPEV
TCVVVDVSHE DPEVQFNWYV DGVEVHNAKT KPREEQFNST FRVVSVLTVV
HQDWLNGKEY KCKVSNKGLP APIEKTISKT KGQPREPQVY TLPPSREEMT
KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPMLD SDGSFFLYSK
LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK
(SEQ ID NO:53)

[0231] **Item 22** shows the mature amino acid sequence (i.e., without the leader sequence) of hTβRII$_{short}$(23-159/D110K)-hG1Fc$_{short}$ (SEQ ID NO: 54). Double underline indicates D110K substitution. Single underline denotes linker.

TIPPHV QKSVNNDMIV TDNNGAVKFP
QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAVW RKNDENITLE
TVCHDPKLPY HKFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII

FSEEYNTSNP DTGGGTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP
EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT
VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE
MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY
SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK (SEQ ID
NO: 54)

[0232] **Item 23** shows the mature amino acid sequence (i.e., without the leader sequence) of hTβRII$_{short}$(29-159)-hG2Fc (SEQ ID NO: 55). Single underline denotes linker.

QKSVNN DMIVTDNNGA VKFPQLCKFC
DVRFSTCDNQ KSCMSNCSIT SICEKPQEVC VAVWRKNDEN ITLETVCHDP
KLPYHDFILE DAASPKCIMK EKKKPGETFF MCSCSSDECN DNIIFSEEYN
TSNPDTGGGV ECPPCPAPPV AGPSVFLFPP KPKDTLMISR TPEVTCVVVD
VSHEDPEVQF NWYVDGVEVH NAKTKPREEQ FNSTFRVVSV LTVVHQDWLN
GKEYKCKVSN KGLPAPIEKT ISKTKGQPRE PQVYTLPPSR EEMTKNQVSL
TCLVKGFYPS DIAVEWESNG QPENNYKTTP PMLDSDGSFF LYSKLTVDKS
RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK (SEQ ID NO: 55)

[0233] **Item 24** shows the mature amino acid sequence (i.e., without the leader sequence) of hTβRII$_{short}$(29-159)-hG1Fc$_{short}$ (SEQ ID NO: 56). Single underline denotes linker.

QKSVNN DMIVTDNNGA VKFPQLCKFC
DVRFSTCDNQ KSCMSNCSIT SICEKPQEVC VAVWRKNDEN ITLETVCHDP
KLPYHDFILE DAASPKCIMK EKKKPGETFF MCSCSSDECN DNIIFSEEYN
TSNPDTGGGT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV
VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW
LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SREEMTKNQV
SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD
KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK (SEQ ID NO: 56)

[0234] **Item 25** shows the mature amino acid sequence (i.e., without the leader sequence) of hTβRII$_{short}$(35-159)-hG2Fc (SEQ ID NO: 57). Single underline denotes linker.

DMIVTD NNGAVKFPQL CKFCDVRFST
CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD
FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPDT

GGGVECPPCP APPVAGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP
EVQFNWYVDG VEVHNAKTKP REEQFNSTFR VVSVLTVVHQ DWLNGKEYKC
KVSNKGLPAP IEKTISKTKG QPREPQVYTL PPSREEMTKN QVSLTCLVKG
FYPSDIAVEW ESNGQPENNY KTTPPMLDSD GSFFLYSKLT VDKSRWQQGN
VFSCSVMHEA LHNHYTQKSL SLSPGK (SEQ ID NO: 57)

[0235] **Item 26** shows the mature amino acid sequence (i.e., without the leader sequence) of hTβRII$_{short}$(35-159)-hG1Fc$_{short}$ (SEQ ID NO: 58). Single underline denotes linker.

DMIVTD NNGAVKFPQL CKFCDVRFST
CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD
FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPDT
GGGTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE
DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY
KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSREEMT KNQVSLTCLV
KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ
GNVFSCSVMH EALHNHYTQK SLSLSPGK (SEQ ID NO: 58)

[0236] **Item 27** shows the mature amino acid sequence (i.e., without the leader sequence) of hTβRII$_{short}$(23-153)-hG2Fc (SEQ ID NO: 59). Single underline denotes linker.

TIPPHV QKSVNNDMIV TDNNGAVKFP
QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAVW RKNDENITLE
TVCHDPKLPY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
FSEEYTGGGV ECPPCPAPPV AGPSVFLFPP KPKDTLMISR TPEVTCVVVD
VSHEDPEVQF NWYVDGVEVH NAKTKPREEQ FNSTFRVVSV LTVVHQDWLN
GKEYKCKVSN KGLPAPIEKT ISKTKGQPRE PQVYTLPPSR EEMTKNQVSL
TCLVKGFYPS DIAVEWESNG QPENNYKTTP PMLDSDGSFF LYSKLTVDKS
RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK (SEQ ID NO: 59)

[0237] **Item 28** shows the mature amino acid sequence (i.e., without the leader sequence) of hTβRII$_{short}$(23-153)-hG1Fc$_{short}$ (SEQ ID NO: 60). Single underline denotes linker.

TIPPHV QKSVNNDMIV TDNNGAVKFP
QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAVW RKNDENITLE
TVCHDPKLPY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
FSEEYTGGGT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV
VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW

LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SREEMTKNQV
SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD
KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK (SEQ ID NO: 60)

[0238] **Item 29** shows the mature amino acid sequence (i.e., without the leader sequence) of $hT\beta RII_{short}$(23-153/N70D)-hG2Fc (SEQ ID NO: 61). Double underline indicates N70D substitution. Single underline denotes linker.

TIPPHV QKSVNNDMIV TDNNGAVKFP
QLCKFCDVRF STCDNQKSCM SDCSITSICE KPQEVCVAVW RKNDENITLE
TVCHDPKLPY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
FSEEYTGGGV ECPPCPAPPV AGPSVFLFPP KPKDTLMISR TPEVTCVVVD
VSHEDPEVQF NWYVDGVEVH NAKTKPREEQ FNSTFRVVSV LTVVHQDWLN
GKEYKCKVSN KGLPAPIEKT ISKTKGQPRE PQVYTLPPSR EEMTKNQVSL
TCLVKGFYPS DIAVEWESNG QPENNYKTTP PMLDSDGSFF LYSKLTVDKS
RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK (SEQ ID NO: 61)

[0239] **Item 30** shows the mature amino acid sequence (i.e., without the leader sequence) of $hT\beta RII_{short}$(23-153/N70D)-$hG1Fc_{short}$ (SEQ ID NO: 62). Double underline indicates N70D substitution. Single underline denotes linker.

TIPPHV QKSVNNDMIV TDNNGAVKFP
QLCKFCDVRF STCDNQKSCM SDCSITSICE KPQEVCVAVW RKNDENITLE
TVCHDPKLPY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
FSEEYTGGGT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV
VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW
LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SREEMTKNQV
SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD
KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK (SEQ ID NO: 62)

INCORPORATION BY REFERENCE

[0240] All publications and patents mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference.

[0241] While specific embodiments of the subject matter have been discussed, the above specification is illustrative and not restrictive. Many variations will become apparent to those skilled in the art upon review of this specification and the claims below. The full

scope of the invention should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations.

We Claim:

1. A TβRII fusion polypeptide comprising a first amino acid sequence from the extracellular domain of TβRII and a heterologous amino acid sequence, wherein the first amino acid sequence consists of an amino acid sequence at least 80% identical to:

a) a sequence beginning at any of positions 23 to 35 of SEQ ID NO: 5 and ending at any of positions 153 to 159 of SEQ ID NO: 5 or

b) a sequence beginning at any of positions 23 to 60 of SEQ ID NO: 6 and ending at any of positions 178 to 184 of SEQ ID NO: 6.

2. The TβRII fusion polypeptide of claim 1, wherein the first amino acid sequence consists of the sequence beginning at position 23 of SEQ ID NO: 5 and ending at position 159 of SEQ ID NO: 5.

3. The TβRII fusion polypeptide of claim 1, wherein the first amino acid sequence consists of the sequence beginning at position 29 of SEQ ID NO: 5 and ending at position 159 of SEQ ID NO: 5.

4. The TβRII fusion polypeptide of claim 1, wherein the first amino acid sequence consists of the sequence beginning at position 35 of SEQ ID NO: 5 and ending at position 159 of SEQ ID NO: 5.

5. The TβRII fusion polypeptide of claim 1, wherein the first amino acid sequence consists of the sequence beginning at position 23 of SEQ ID NO: 5 and ending at position 153 of SEQ ID NO: 5.

6. The TβRII fusion polypeptide of claim 1, wherein the first amino acid sequence consists of the sequence beginning at position 29 of SEQ ID NO: 5 and ending at position 153 of SEQ ID NO: 5.

7. The TβRII fusion polypeptide of claim 1, wherein the first amino acid sequence consists of the sequence beginning at position 35 of SEQ ID NO: 5 and ending at position 153 of SEQ ID NO: 5.

8. The TβRII fusion polypeptide of claim 1, wherein the first amino acid sequence consists of the sequence beginning at position 23 of SEQ ID NO: 6 and ending at positions 184 of SEQ ID NO: 6.

9. The TβRII fusion polypeptide of claim 1, wherein the first amino acid sequence consists of the sequence beginning at position 29 of SEQ ID NO: 6 and ending at position 184 of SEQ ID NO: 6.

10. The TβRII fusion polypeptide of claim 1, wherein the first amino acid sequence consists of the sequence beginning at position 23 of SEQ ID NO: 6 and ending at position 178 of SEQ ID NO: 6.

11. The TβRII fusion polypeptide of claim 1, wherein the first amino acid sequence consists of the sequence beginning at position 29 of SEQ ID NO: 6 and ending at position 178 of SEQ ID NO: 6.

12. The TβRII fusion polypeptide of any one of claims 1 to 11, wherein the first amino acid sequence consists of a sequence that has a D at the position corresponding to position 36 of SEQ ID NO: 47 and/or a K at the position corresponding to position 76 of SEQ ID NO: 47.

13. A TβRII fusion polypeptide comprising a first amino acid sequence at least 80% identical to the sequence of SEQ ID NO: 7 or SEQ ID NO: 13, or active fragment thereof, and a second heterologous portion, wherein the first amino acid sequence has a D at the position corresponding to position 36 of SEQ ID NO: 47 and/or a K at the position corresponding to position 76 of SEQ ID NO: 47.

14. The TβRII fusion polypeptide of claim 13 wherein the first amino acid sequence comprises an N-terminal truncation of 1-12 amino acids corresponding to amino acids 1-12 of SEQ ID NO: 7 or 1-37 amino acids corresponding to amino acids 1-37 of SEQ ID NO: 13.

15. The TβRII fusion polypeptide of claim 13 or 14, wherein the first amino acid sequence comprises an N-terminal truncation of 6 amino acids corresponding to amino acids 1-6 of SEQ ID NO: 7 or SEQ ID NO: 13.

16. The TβRII fusion polypeptide of claim 13 or 14, wherein the first amino acid sequence comprises an N-terminal truncation of 12 amino acids corresponding to amino acids 1-12 of SEQ ID NO: 7 or 37 amino acids corresponding to amino acids 1-37 of SEQ ID NO: 13.

17. The TβRII fusion polypeptide of any one of claims 13 to 16, wherein the first amino acid sequence comprises a C-terminal truncation of 1-6 amino acids corresponding to amino acids 137-132 of SEQ ID NO: 7 or amino acids 162-157 of SEQ ID NO: 13.

18. The TβRII fusion polypeptide of any one of claims 13 to 17, wherein the first amino acid sequence comprises a C-terminal truncation of 6 amino acids corresponding to amino acids 132-137 of SEQ ID NO: 7 or amino acids 157-162 of SEQ ID NO: 13.

19. The TβRII fusion polypeptide of any one of claims 1 to 18, wherein the first amino acid sequence comprises an insertion corresponding to SEQ ID NO: 18 between the residues corresponding to positions 117 and 118 of SEQ ID NO: 47.

20. The TβRII fusion polypeptide of any one of claims 1 to 19, wherein the heterologous portion comprises one or more polypeptide portions that enhance one or more of: in vivo stability, in vivo half life, uptake/administration, tissue localization or distribution, formation of protein complexes, and/or purification.

21. The TβRII fusion polypeptide of any one of claims 1 to 20, wherein the heterologous portion comprises a polypeptide portion selected from: an immunoglobulin Fc domain and a serum albumin.

22. The TβRII fusion polypeptide of claim 21, wherein the immunoglobulin Fc domain is joined to the TβRII polypeptide by a linker.

23. The TβRII fusion polypeptide of any one of claims 1 to 22, wherein the polypeptide includes one or more modified amino acid residues selected from: a glycosylated amino acid, a PEGylated amino acid, a farnesylated amino acid, an acetylated amino acid, a biotinylated amino acid, an amino acid conjugated to a lipid moiety, and an amino acid conjugated to an organic derivatizing agent.

24. The TβRII fusion polypeptide of claim 23, wherein the polypeptide is glycosylated.

25. A TβRII fusion polypeptide comprising a first amino acid sequence consisting of a portion of the extracellular domain of TβRII that comprises an amino acid sequence that is at least 95% identical to an amino acid sequence selected from SEQ ID NOs: 7-17 and 47-49, and a second heterologous portion.

26. A TβRII fusion polypeptide comprising a first amino acid sequence consisting of a portion of the extracellular domain of TβRII that comprises an amino acid sequence that is at least 97% identical to an amino acid sequence selected from SEQ ID NOs: 7-17 and 47-49, and a second heterologous portion.

27. A TβRII fusion polypeptide comprising a first amino acid sequence consisting of a portion of the extracellular domain of TβRII that comprises an amino acid sequence that is at least 98% identical to an amino acid sequence selected from SEQ ID NOs: 7-17 and 47-49, and a second heterologous portion.

28. A TβRII fusion polypeptide comprising a first amino acid sequence consisting of a portion of the extracellular domain of TβRII that comprises an amino acid sequence that is at least 99% identical to an amino acid sequence selected from SEQ ID NOs: 7-17 and 47-49, and a second heterologous portion.

29. A TβRII fusion polypeptide comprising a first amino acid sequence consisting of a portion of the extracellular domain of TβRII that comprises an amino acid sequence is an amino acid sequence selected from SEQ ID NOs: 7-17 and 47-49 and a second heterologous portion.

30. A polypeptide comprising an amino acid sequence that is at least 95% identical to an amino acid sequence selected from SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 53, 54, 55, 56, 57, 58, 59, 60, 61, and 62.

31. A polypeptide comprising an amino acid sequence that is at least 97% identical to an amino acid sequence selected from SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 53, 54, 55, 56, 57, 58, 59, 60, 61, and 62.

32. A polypeptide comprising an amino acid sequence that is at least 98% identical to an amino acid sequence selected from SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 53, 54, 55, 56, 57, 58, 59, 60, 61, and 62.

33. A polypeptide comprising an amino acid sequence that is at least 99% identical to an amino acid sequence selected from SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37, 39, 41,43, 53, 54, 55, 56, 57, 58, 59, 60, 61, and 62.

34. A polypeptide comprising an amino acid sequence selected from SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 53, 54, 55, 56, 57, 58, 59, 60, 61, and 62.

35. A TβRII polypeptide comprising an amino acid sequence encoded by a nucleic acid that hybridizes under stringent conditions to a complement of a nucleotide sequence selected from SEQ ID NOs: 26, 28, 30, 32, 34, 36, 38, 40, 42 and 44.

36. The polypeptide of any one of claims 1 to 35, wherein the polypeptide binds human GDF15 with an equilibrium dissociation constant (K_D) less than 1 x 10^{-8} M.

37. The polypeptide of any one of claims 1 to 36, wherein the polypeptide has a glycosylation pattern characteristic of expression of the polypeptide in CHO cells.

38. A homodimer comprising two polypeptides as defined in any of claims 1 to 37.

39. An isolated polynucleotide comprising a coding sequence for the polypeptide of any one of claims 1 to 37.

40. A recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide of claim 39.

41. A cell transformed with an isolated polynucleotide of claim 35 or a recombinant polynucleotide of claim 40.

42. The cell of claim 41, wherein the cell is a mammalian cell.

43. The cell of claim 42, wherein the cell is a CHO cell or a human cell.

44. A pharmaceutical preparation comprising the polypeptide of any of claims 1 to 37 or the homodimer of claim 38 and a pharmaceutically acceptable excipient.

45. A method of modulating the response of a cell to a TGFβ superfamily member, the method comprising exposing the cell to a polypeptide of any one of claims 1 to 37 or the homodimer of claim 38.

46. A method of treating a disease or condition associated with a TGFβ superfamily member in a patient in need thereof, the method comprising administering to the patient an effective amount of a polypeptide of any of claims 1 to 37 or the homodimer of claim 38.

47. The method of claim 46, wherein the TGFβ superfamily member is TGFβ1, TGFβ3 or GDF15.

48. The method of claim 46 or 47, wherein the disease or condition is a cancer.

49. The method of claim 48, wherein the cancer is selected from stomach cancer, intestinal cancer, skin cancer, breast cancer, melanoma, bone cancer and thyroid cancer.

50. The method of claim 46 or 47, wherein the disease or condition is a fibrotic or sclerotic disease or disorder.

51. The method of claim 50, wherein the fibrotic or sclerotic disease or disorder is selected from scleroderma, atherosclerosis, liver fibrosis, diffuse systemic sclerosis, glomerulonephritis, neural scarring, dermal scarring, radiation-induced fibrosis, hepatic fibrosis, and myelofibrosis.

52. The method of claim 46 or 47, wherein the disease or condition is heart disease.

53. The method of claim 46 or 47, wherein the disease or condition is selected from hereditary hemorrhagic telangiectasia (HHT), Marfan syndrome, Loeys-Dietz syndrome, familial thoracic aortic aneurysm syndrome, arterial tortuosity syndrome, pre-eclampsia, atherosclerosis, restenosis, and hypertrophic cardiomyopathy/congestive heart failure.

54. An antibody, or antigen binding fragment thereof, that binds to GDF15 and blocks the interaction between GDF15 and TβRII.

55. A GDF15 polypeptide comprising the amino acid sequence of SEQ ID NO: 1 or a fragment thereof that binds TβRII, wherein the GDF15 polypeptide is at least 95% pure, with respect to protein contaminants.

56. A GDF15 polypeptide comprising the amino acid sequence of SEQ ID NO: 1 or a fragment thereof that binds to a polypeptide of any one of claims 1 to 37, wherein the GDF15 polypeptide is at least 95% pure, with respect to protein contaminants.

57. The GDF15 polypeptide of claim 55, wherein the GDF15 polypeptide binds TβRII with an equilibrium dissociation constant (K_D) of no greater than 10^{-8} M.

58. The GDF15 polypeptide of claim 56, wherein the GDF15 polypeptide binds to a polypeptide of any one of claims 1 to 37 with an equilibrium dissociation constant (K_D) of no greater than 10^{-8} M.

59. The GDF15 polypeptide of any of claims 55 to 58, wherein the polypeptide is produced by expression in CHO cells.

60. A method of concentrating or purifying GDF15, comprising contacting a sample containing GDF15 with a polypeptide of any of claims 1 to 37.

61. A polypeptide that comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO:50.

62. A polypeptide of claim 61 that comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:50.

63. A polypeptide of claim 61 that comprises an amino acid sequence that is at least 99% identical to the amino acid sequence of SEQ ID NO:50.

64. A polypeptide of claim 61 that comprises an amino acid sequence that is identical to the amino acid sequence of SEQ ID NO:50.

65. A polypeptide of claim 61 that consists of an amino acid sequence that is identical to the amino acid sequence of SEQ ID NO:50.

66. A nucleic acid that encodes a polypeptide of any of claims 61-65.

67. A nucleic acid comprising the nucleic acid sequence of SEQ ID NO: 51.

68. A polypeptide produced by expressing the nucleic acid of claim 67 in a mammalian cell.

69. The polypeptide of claim 68, wherein the mammalian cell is a Chinese Hamster Ovary cell (CHO cell).

```
  1  MPGQELRTVN GSQMLLVLLV LSWLPHGGAL SLAEASRASF PGPSELHSED
 51  SRFRELRKRY EDLLTRLRAN QSWEDSNTDL VPAPAVRILT PEVRLGSGGH
101  LHLRISRAAL PEGLPEASRL HRALFRLSPT ASRSWDVTRP LRRQLSLARP
151  QAPALHLRLS PPPSQSDQLL AESSSARPQL ELHLRPQAAR GRRRARARNG
201  DHCPLGPGRC CRLHTVRASL EDLGWADWVL SPREVQVTMC IGACPSQFRA
251  ANMHAQIKTS LHRLKPDTVP APCCVPASYN PMVLIQKTDT GVSLQTYDDL
301  LAKDCHCI    (SEQ ID NO: 1)
```

FIGURE 1

```
  1   ATGCCCGGGC AAGAACTCAG GACGGTGAAT GGCTCTCAGA TGCTCCTGGT
 51   GTTGCTGGTG CTCTCGTGGC TGCCGCATGG GGGCGCCCTG TCTCTGGCCG
101   AGGCGAGCCG CGCAAGTTTC CCGGGACCCT CAGAGTTGCA CTCCGAAGAC
151   TCCAGATTCC GAGAGTTGCG GAAACGCTAC GAGGACCTGC TAACCAGGCT
201   GCGGGCCAAC CAGAGCTGGG AAGATTCGAA CACCGACCTC GTCCCGGCCC
251   CTGCAGTCCG GATACTCACG CCAGAAGTGC GGCTGGGATC CGGCGGCCAC
301   CTGCACCTGC GTATCTCTCG GGCCGCCCTT CCCGAGGGGC TCCCCGAGGC
351   CTCCCGCCTT CACCGGGCTC TGTTCCGGCT GTCCCCGACG GCGTCAAGGT
401   CGTGGGACGT GACACGACCG CTGCGGCGTC AGCTCAGCCT TGCAAGACCC
451   CAGGCACCCG CGCTGCACCT GCGACTGTCG CCGCCGCCGT CGCAGTCGGA
501   CCAACTGCTG GCAGAATCTT CGTCCGCACG GCCCCAGCTG GAGTTGCACT
551   TGCGGCCGCA AGCCGCCAGG GGGCGCCGCA GAGCGCGTGC GCGCAACGGG
601   GACCACTGTC CGCTCGGGCC CGGGCGTTGC TGCCGTCTGC ACACGGTCCG
651   CGCGTCGCTG GAAGACCTGG GCTGGGCCGA TTGGGTGCTG TCGCCACGGG
701   AGGTGCAAGT GACCATGTGC ATCGGCGCGT GCCCGAGCCA GTTCCGGGCG
751   GCAAACATGC ACGCGCAGAT CAAGACGAGC CTGCACCGCC TGAAGCCCGA
801   CACGGTGCCA GCGCCCTGCT GCGTGCCCGC CAGCTACAAT CCCATGGTGC
851   TCATTCAAAA GACCGACACC GGGGTGTCAC TCCAGACCTA TGATGACTTG
901   TTAGCCAAAG ACTGCCACTG CATA          (SEQ ID NO: 2)
```

FIGURE 2

```
  1   MAPPALQAQP PGGSQLRFLL FLLLLLLLLS WPSQGDALAM PEQRPSGPES
 51   QLNADELRGR FQDLLSRLHA NQSREDSNSE PSPDPAVRIL SPEVRLGSHG
101   QLLLRVNRAS LSQGLPEAYR VHRALLLLTP TARPWDITRP LKRALSLRGP
151   RAPALRLRLT PPPDLAMLPS GGTQLELRLR VAAGRGRRSA HAHPRDSCPL
201   GPGRCCHLET VQATLEDLGW SDWVLSPRQL QLSMCVGECP HLYRSANTHA
251   QIKARLHGLQ PDKVPAPCCV PSSYTPVVLM HRTDSGVSLQ TYDDLVARGC
301   HCA        (SEQ ID NO: 3)
```

FIGURE 3

```
  1  ATGGCCCCGC CCGCGCTCCA GGCCCAGCCT CCAGGCGGCT CTCAACTGAG
 51  GTTCCTGCTG TTCCTGCTGC TGTTGCTGCT GCTGCTGTCA TGGCCATCGC
101  AGGGGGACGC CCTGGCAATG CCTGAACAGC GACCCTCCGG CCCTGAGTCC
151  CAACTCAACG CCGACGAGCT ACGGGGTCGC TTCCAGGACC TGCTGAGCCG
201  GCTGCATGCC AACCAGAGCC GAGAGGACTC GAACTCAGAA CCAAGTCCTG
251  ACCCAGCTGT CCGGATACTC AGTCCAGAGG TGAGATTGGG GTCCCACGGC
301  CAGCTGCTAC TCCGCGTCAA CCGGGCGTCG CTGAGTCAGG GTCTCCCCGA
351  AGCCTACCGC GTGCACCGAG CGCTGCTCCT GCTGACGCCG ACGGCCCGCC
401  CCTGGGACAT CACTAGGCCC CTGAAGCGTG CGCTCAGCCT CCGGGGACCC
451  CGTGCTCCCG CATTACGCCT GCGCCTGACG CCGCCTCCGG ACCTGGCTAT
501  GCTGCCCTCT GGCGGCACGC AGCTGGAACT GCGCTTACGG GTAGCCGCCG
551  GCAGGGGGCG CCGAAGCGCG CATGCGCACC CAAGAGACTC GTGCCCACTG
601  GGTCCAGGGC GCTGCTGTCA CTTGGAGACT GTGCAGGCAA CTCTTGAAGA
651  CTTGGGCTGG AGCGACTGGG TGCTGTCCCC GCGCCAGCTG CAGCTGAGCA
701  TGTGCGTGGG CGAGTGTCCC CACCTGTATC GCTCCGCGAA CACGCATGCG
751  CAGATCAAAG CACGCCTGCA TGGCCTGCAG CCTGACAAGG TGCCTGCCCC
801  GTGCTGTGTC CCCTCCAGCT ACACCCCGGT GGTTCTTATG CACAGGACAG
851  ACAGTCGTGT GTCACTGCAG ACTTATGATG ACCTGGTGGC CCGGGGCTGC
901  CACTGCGCT          (SEQ ID NO: 4)
```

FIGURE 4

```
  1  mgrgllrglw plhivlwtri astipphvqk svnndmivtd nngavkfpql
 51  ckfcdvrfst cdnqkscmsn csitsicekp qevcvavwrk ndenitletv
101  chdpklpyhd filedaaspk cimkekkkpg etffmcscss decndniifs
151  eeyntsnpdl llvifqvtgi sllpplgvai sviiifycyr vnrqqklsst
201  wetgktrklm efsehcaiil eddrsdisst canninhnte llpieldtlv
251  gkgrfaevyk aklkqntseq fetvavkifp yeeyaswkte kdifsdinlk
301  henilqflta eerktelgkq ywlitafhak gnlqeyltrh viswedlrkl
351  gsslargiah lhsdhtpcgr pkmpivhrdl kssnilvknd ltcclcdfgl
401  slrldptlsv ddlansgqvg tarymapevl esrmnlenve sfkqtdvysm
451  alvlwemtsr cnavgevkdy eppfgskvre hpcvesmkdn vlrdrgrpei
501  psfwlnhqgi qmvcetltec wdhdpearlt aqcvaerfse lehldrlsgr
551  scseekiped gslnttk                (SEQ ID NO: 5)
```

FIGURE 5

```
  1  mgrgllrglw plhivlwtri astipphvqk sdvemeaqkd eiicpscnrt
 51  ahplrhinnd mivtdnngav kfpqlckfcd vrfstcdnqk scmsncsits
101  icekpqevcv avwrkndeni tletvchdpk lpyhdfiled aaspkcimke
151  kkkpgetffm cscssdecnd niifseeynt snpdlllvif qvtgisllpp
201  lgvaisviii fycyrvnrqq klsstwetgk trklmefseh caiileddrs
251  disstcanni nhntellpie ldtlvgkgrf aevykaklkq ntseqfetva
301  vkifpyeeya swktekdifs dinlkhenil qfltaeerkt elgkqywlit
351  afhakgnlqe yltrhviswe dlrklgssla rgiahlhsdh tpcgrpkmpi
401  vhrdlkssni lvkndltccl cdfglslrld ptlsvddlan sgqvgtarym
451  apevlesrmn lenvesfkqt dvysmalvlw emtsrcnavg evkdyeppfg
501  skvrehpcve smkdnvlrdr grpeipsfwl nhqgiqmvce tltecwdhdp
551  earltaqcva erfselehld rlsgrscsee kipedgslnt tk
```

(SEQ ID NO: 6)

FIGURE 6

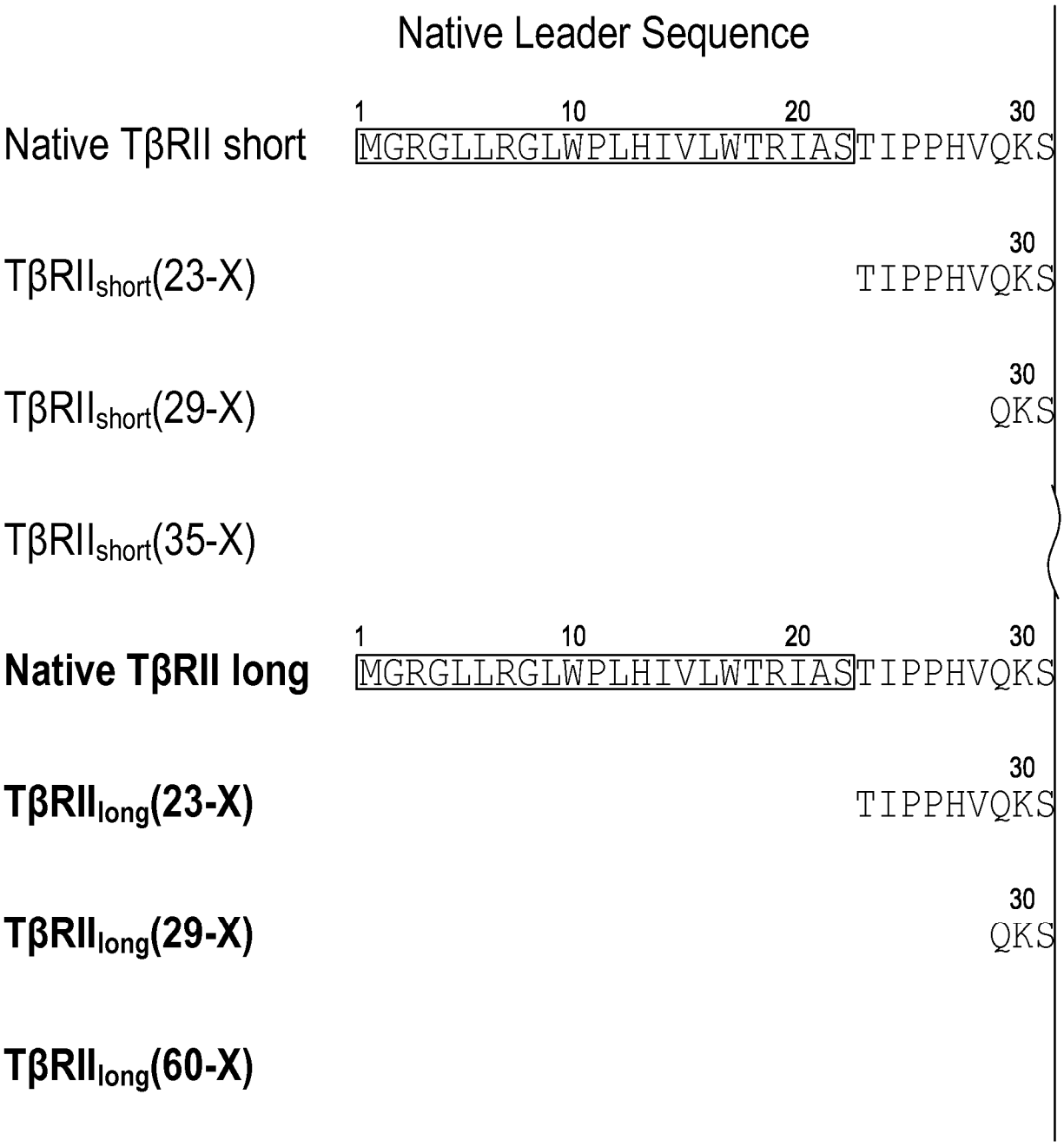
Native Leader Sequence
Native TβRII short
1 10 20 30
MGRGLLRGLWPLHIVLWTRIASTIPPHVQKS
TβRII$_{short}$(23-X)
30
TIPPHVQKS
TβRII$_{short}$(29-X)
30
QKS
TβRII$_{short}$(35-X)
Native TβRII long
1 10 20 30
MGRGLLRGLWPLHIVLWTRIASTIPPHVQKS
TβRII$_{long}$(23-X)
30
TIPPHVQKS
TβRII$_{long}$(29-X)
30
QKS
TβRII$_{long}$(60-X)

FIGURE 7

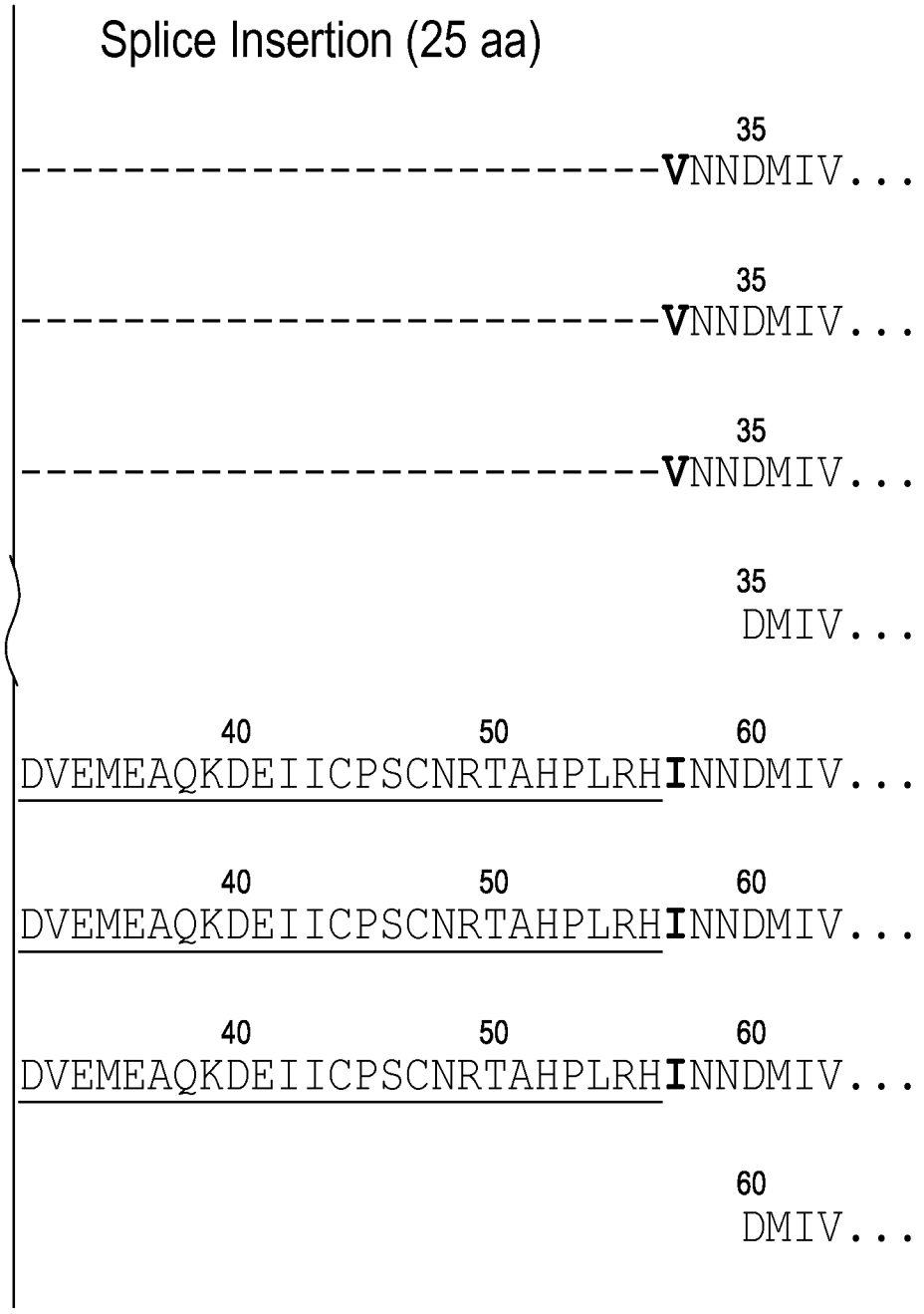

FIGURE 7 (continued)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/052130

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
the subject matter listed in Rule 39 on which, under Article 17(2)(a)(i), an international search is not required to be carried out, including

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box for Details

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/052130

A. CLASSIFICATION OF SUBJECT MATTER

C07K 19/00 (2006.01) C07K 14/475 (2006.01) C07K 16/22 (2006.01) A61K 38/16 (2006.01) A61P 9/10 (2006.01) A61P 35/00 (2006.01)

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)

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Databases EPODOC, MEDLINE, NPL, WPI, XPMISC, XPOAC and XPTK searched with keywords GDF15 (and synonyms) and TGFβRII (and synonyms)

Databases EPODOC and MEDLINE searched with inventors' names

GenomeQuest: all protein databases searched with SEQ ID NO:1, and with a consensus sequence derived from the Fc region and amino acids 35-153 of SEQ ID NO: 5

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 September 2014	30 September 2014
Name and mailing address of the ISA/AU	**Authorised officer**
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au	Andrew Bryce AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +61 2 6283 3132

INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. **PCT/US2014/052130**
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/109789 A2 (THE JOHNS HOPKINS UNIVERSITY) 09 November 2011 See SEQ ID NOs: 7, 12 and 13; paragraphs [0047], [0050], [0051], [0198] and [0208]; Figures 7, 10, 11 and 55	1-7, 20-36, 39-41, 44-49, 61, 62, 66
X	WO 2010/003118 A1 (TRUBION PHARMACEUTICALS, INC.) 07 January 2010 See SEQ ID NOs: 737 and 738; Paragraphs [0003], [0031], [00224]; Claims 13 and 14	1, 8-11, 23-28, 30, 35, 36, 39-41, 44-53, 61, 66
X	WO 2013/000234 A1 (HUABO BIOPHARM CO., LTD) 03 January 2013 See SEQ ID NO: 8; Page 17; Page 18 paragraph 2; Page 4, paragraphs 6 and 7; Examples 1 and 2; Figures 3 and 4B	25-29, 36-38, 42-51, 61, 66
X	WO 1998/048024 A1 (BIOGEN, INC.) 29 October 1998 See SEQ ID NO: 9, Claims 4 and 16-20	25-32, 35, 36, 44-47, 50, 51, 61-69
X	WO 2013/012648 A1 (EMORY UNIVERSITY) 24 January 2013 See SEQ ID NO: 1; Claim 21	54-59
X	CN 101852804 A (INSTITUTE OF PATHOGEN BIOLOGY, CHINESE ACADEMY OF MEDICAL SCIENCES) 06 October 2010 & Derwent Abstract Accession No. 2010-P08192 See SEQ ID NO: 1; Title; Abstract	54-59

INTERNATIONAL SEARCH REPORT	International application No. PCT/US2014/052130

Supplemental Box

Continuation of: **Box III**
This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

This Authority has found that there are different inventions based on the following features that separate the claims into distinct groups:

- Claims 1-53 and 60-69 are directed to fusion proteins that comprise a TGFβRII extracellular domain (ECD) fused to a heterologous protein (such as an immunoglobulin Fc domain), and to nucleic acids that encode these fusion proteins and therapeutic uses of these fusion proteins.
- Claims 54-59 relate to the GDF15 protein, and to antibodies that bind to GDF15.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art. When there is no special technical feature common to all the claimed inventions there is no unity of invention.

In the above groups of claims, the identified features may have the potential to make a contribution over the prior art but are not common to all the claimed inventions and therefore cannot provide the required technical relationship. Therefore there is no special technical feature common to all the claimed inventions and the requirements for unity of invention are consequently not satisfied *a priori*.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2014/052130**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
WO 2011/109789 A2	09 November 2011	CA 2791383 A1	09 Sep 2011
		EP 2542590 A2	09 Jan 2013
		JP 2013521311 A	10 Jun 2013
		US 2013039911 A1	14 Feb 2013
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		EP 2310508 A1	20 Apr 2011
		JP 2011526794 A	20 Oct 2011
		KR 20110044992 A	03 May 2011
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WO 1998/048024 A1	29 October 1998	AU 737106 B2	09 Aug 2001
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		EP 0975771 B1	11 Jul 2007
		IS 5217 A	15 Oct 1999
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		NO 994998 A	20 Dec 1999
		NZ 500284 A	28 Sep 2001
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		US 2005203022 A1	15 Sep 2005
WO 2013/012648 A1	24 January 2013	None	
CN 101852804 A	06 October 2010	CN 101852804 B	12 Jun 2013

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2014/052130**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**

End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

(19)中华人民共和国国家知识产权局

(12)发明专利申请

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(43)申请公布日 2016.06.08

(21)申请号 201480057870.0

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(54)发明名称

TGF-β 受体 II 型变体及其用途

(57)摘要

在某些方面,本公开内容涉及可用于选择性地拮抗 TβRII 配体的包含 TβRII 多肽胞外域的截短的配体结合部分的多肽。本公开内容进一步提供用于治疗或预防 TGFβ 相关病症的组合物和方法。

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1   MPGQELRTVN GSQMLLVLLV LSWLPHGGAL SLAEASRASF PGPSELHSED
51  SRFRELRKRY EDLLTRLRAN QSWEDSNTDL VPAPAVRILT PEVRLGSGGH
101 LHLRISRAAL PEGLPEASRL HRALFRLSPT ASRSWDVTRP LRRQLSLARP
151 QAPALHLRLS PPPSQSDQLL AESSSARPQL ELHLRPQAAR GRRRARARNG
201 DHCPLGPGRC CRLHTVRASL EDLGWADWVL SPREVQVTMC IGACPSQFRA
251 ANMHAQIKTS LHRLKPDTVP APCCVPASYN PMVLIQKTDT GVSLQTYDDL
301 LAKDCHCI    (SEQ ID NO: 1)
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1．一种包含来自TβRII胞外域的第一氨基酸序列和异源氨基酸序列的TβRII融合多肽，其中所述第一氨基酸序列由与以下序列有至少80%同一性的氨基酸序列组成：

a）始于SEQ ID NO: 5的23-35位的任一个和止于SEQ ID NO: 5的153-159位的任一个的序列，或

b）始于SEQ ID NO: 6的23-60位的任一个和止于SEQ ID NO: 6的178-184位的任一个的序列。

2．权利要求1的TβRII融合多肽，其中所述第一氨基酸序列由始于SEQ ID NO: 5的23位和止于SEQ ID NO: 5的159位的序列组成。

3．权利要求1的TβRII融合多肽，其中所述第一氨基酸序列由始于SEQ ID NO: 5的29位和止于SEQ ID NO: 5的159位的序列组成。

4．权利要求1的TβRII融合多肽，其中所述第一氨基酸序列由始于SEQ ID NO: 5的35位和止于SEQ ID NO: 5的159位的序列组成。

5．权利要求1的TβRII融合多肽，其中所述第一氨基酸序列由始于SEQ ID NO: 5的23位和止于SEQ ID NO: 5的153位的序列组成。

6．权利要求1的TβRII融合多肽，其中所述第一氨基酸序列由始于SEQ ID NO: 5的29位和止于SEQ ID NO: 5的153位的序列组成。

7．权利要求1的TβRII融合多肽，其中所述第一氨基酸序列由始于SEQ ID NO: 5的35位和止于SEQ ID NO: 5的153位的序列组成。

8．权利要求1的TβRII融合多肽，其中所述第一氨基酸序列由始于SEQ ID NO: 6的23位和止于SEQ ID NO: 6的184位的序列组成。

9．权利要求1的TβRII融合多肽，其中所述第一氨基酸序列由始于SEQ ID NO: 6的29位和止于SEQ ID NO: 6的184位的序列组成。

10．权利要求1的TβRII融合多肽，其中所述第一氨基酸序列由始于SEQ ID NO: 6的23位和止于SEQ ID NO: 6的178位的序列组成。

11．权利要求1的TβRII融合多肽，其中所述第一氨基酸序列由始于SEQ ID NO: 6的29位和止于SEQ ID NO: 6的178位的序列组成。

12．权利要求1-11中任一项的TβRII融合多肽，其中所述第一氨基酸序列由在相当于SEQ ID NO: 47的36位的位置处具有D和/或在相当于SEQ ID NO: 47的76位的位置处具有K的序列组成。

13．一种TβRII融合多肽，其包含与SEQ ID NO: 7或SEQ ID NO: 13的序列有至少80%同一性的第一氨基酸序列或其活性片段和第二异源部分，其中所述第一氨基酸序列在相当于SEQ ID NO: 47的36位的位置处具有D和/或在相当于SEQ ID NO: 47的76位的位置处具有K。

14．权利要求13的TβRII融合多肽，其中所述第一氨基酸序列包含相当于SEQ ID NO: 7的氨基酸1-12的1-12个氨基酸或相当于SEQ ID NO: 13的氨基酸1-37的1-37个氨基酸的N端截短。

15．权利要求13或14的TβRII融合多肽，其中所述第一氨基酸序列包含相当于SEQ ID NO: 7或SEQ ID NO: 13的氨基酸1-6的6个氨基酸的N端截短。

16．权利要求13或14的TβRII融合多肽，其中所述第一氨基酸序列包含相当于SEQ ID

NO: 7的氨基酸1-12的12个氨基酸或相当于SEQ ID NO: 13的氨基酸1-37的37个氨基酸的N端截短。

17. 权利要求13-16中任一项的TβRII融合多肽,其中所述第一氨基酸序列包含相当于SEQ ID NO: 7的氨基酸137-132或SEQ ID NO: 13的氨基酸162-157的1-6个氨基酸的C端截短。

18. 权利要求13-17中任一项的TβRII融合多肽,其中所述第一氨基酸序列包含相当于SEQ ID NO: 7的氨基酸132-137或SEQ ID NO: 13的氨基酸157-162的6个氨基酸的C端截短。

19. 权利要求1-18中任一项的TβRII融合多肽,其中所述第一氨基酸序列包含在相当于SEQ ID NO: 47的117和118位的残基之间的相当于SEQ ID NO: 18的插入。

20.权利要求1-19中任一项的TβRII融合多肽,其中所述异源部分包含提高以下一种或多种的一个或多个多肽部分:体内稳定性、体内半寿期、摄取/给予、组织定位或分布、蛋白质复合体的形成和/或纯化。

21.权利要求1-20中任一项的TβRII融合多肽,其中所述异源部分包含选自以下的多肽部分:免疫球蛋白Fc结构域和血清白蛋白。

22.权利要求21的TβRII融合多肽,其中所述免疫球蛋白Fc结构域通过接头与TβRII多肽连接。

23.权利要求1-22中任一项的TβRII融合多肽,其中所述多肽包括选自以下的一个或多个修饰的氨基酸残基:糖基化氨基酸、聚乙二醇化氨基酸、法尼基化氨基酸、乙酰化氨基酸、生物素化氨基酸、与脂质部分缀合的氨基酸和与有机衍生剂缀合的氨基酸。

24.权利要求23的TβRII融合多肽,其中所述多肽被糖基化。

25. 一种TβRII融合多肽,其包含由包含与选自SEQ ID NO: 7-17和47-49的氨基酸序列有至少95%同一性的氨基酸序列的TβRII胞外域的一部分组成的第一氨基酸序列和第二异源部分。

26. 一种TβRII融合多肽,其包含由包含与选自SEQ ID NO: 7-17和47-49的氨基酸序列有至少97%同一性的氨基酸序列的TβRII胞外域的一部分组成的第一氨基酸序列和第二异源部分。

27. 一种TβRII融合多肽,其包含由包含与选自SEQ ID NO: 7-17和47-49的氨基酸序列有至少98%同一性的氨基酸序列的TβRII胞外域的一部分组成的第一氨基酸序列和第二异源部分。

28. 一种TβRII融合多肽,其包含由包含与选自SEQ ID NO: 7-17和47-49的氨基酸序列有至少99%同一性的氨基酸序列的TβRII胞外域的一部分组成的第一氨基酸序列和第二异源部分。

29. 一种TβRII融合多肽,其包含由包含是选自SEQ ID NO: 7-17和47-49的氨基酸序列的氨基酸序列的TβRII胞外域的一部分组成的第一氨基酸序列和第二异源部分。

30. 一种多肽,其包含与选自SEQ ID NO: 25、27、29、31、33、35、37、39、41、43、53、54、55、56、57、58、59、60、61和62的氨基酸序列有至少95%同一性的氨基酸序列。

31. 一种多肽,其包含与选自SEQ ID NO: 25、27、29、31、33、35、37、39、41、43、53、54、55、56、57、58、59、60、61和62的氨基酸序列有至少97%同一性的氨基酸序列。

32. 一种多肽，其包含与选自SEQ ID NO: 25、27、29、31、33、35、37、39、41、43、53、54、55、56、57、58、59、60、61和62的氨基酸序列有至少98%同一性的氨基酸序列。

33. 一种多肽，其包含与选自SEQ ID NO: 25、27、29、31、33、35、37、39、41、43、53、54、55、56、57、58、59、60、61和62的氨基酸序列有至少99%同一性的氨基酸序列。

34. 一种多肽，其包含选自SEQ ID NO: 25、27、29、31、33、35、37、39、41、43、53、54、55、56、57、58、59、60、61和62的氨基酸序列。

35. 一种TβRII多肽，其包含由在严格条件下与选自SEQ ID NO: 26、28、30、32、34、36、38、40、42和44的核苷酸序列的互补序列杂交的核酸编码的氨基酸序列。

36. 权利要求1-35中任一项的多肽，其中所述多肽以小于1 x 10^{-8} M的平衡解离常数(K_D)结合人GDF15。

37.权利要求1-36中任一项的多肽，其中所述多肽具有CHO细胞中多肽表达特有的糖基化形式。

38.一种同二聚体，其包含两个权利要求1-37中任一项限定的多肽。

39.一种分离的多核苷酸，其包含权利要求1-37中任一项的多肽的编码序列。

40.一种重组多核苷酸，其包含与权利要求39的多核苷酸有效连接的启动子序列。

41.一种细胞，其用权利要求35的分离的多核苷酸或权利要求40的重组多核苷酸转化。

42.权利要求41的细胞，其中所述细胞是哺乳动物细胞。

43.权利要求42的细胞，其中所述细胞是CHO细胞或人细胞。

44.一种药物制剂，其包含权利要求1-37中任一项的多肽或权利要求38的同二聚体和药学上可接受的赋形剂。

45.一种调节细胞对TGFβ超家族成员的应答的方法，所述方法包括使细胞暴露于权利要求1-37中任一项的多肽或权利要求38的同二聚体中。

46.一种治疗有需要的患者的与TGFβ超家族成员有关的疾病或病况的方法，所述方法包括给予患者有效量的权利要求1-37中任一项的多肽或权利要求38的同二聚体。

47.权利要求46的方法，其中所述TGFβ超家族成员是TGFβ1、TGFβ3或GDF15。

48.权利要求46或47的方法，其中所述疾病或病况是癌症。

49.权利要求48的方法，其中所述癌症选自胃癌、肠癌、皮肤癌、乳腺癌、黑素瘤、骨癌和甲状腺癌。

50.权利要求46或47的方法，其中所述疾病或病况是纤维化或硬化疾病或病症。

51.权利要求50的方法，其中所述纤维化或硬化疾病或病症选自硬皮病、动脉粥样硬化、肝纤维化、弥漫性系统性硬化、肾小球性肾炎、神经瘢痕形成、皮肤瘢痕形成、放射诱导的纤维化、肝纤维化和骨髓纤维化。

52.权利要求46或47的方法，其中所述疾病或病况是心脏病。

53.权利要求46或47的方法，其中所述疾病或病况选自遗传性出血性毛细血管扩张症(HHT)、马方综合征、Loeys-Dietz综合征、家族性胸主动脉瘤综合征、动脉迂曲综合征、先兆子痫、动脉粥样硬化、再狭窄和肥厚型心肌病/充血性心力衰竭。

54.一种与GDF15结合并阻断GDF15和TβRII之间的相互作用的抗体或其抗原结合片段。

55. 一种包含SEQ ID NO: 1的氨基酸序列或其结合TβRII的片段的GDF15多肽，其中所述GDF15多肽相对于蛋白质污染物为至少95%纯的。

56. 一种包含SEQ ID NO: 1的氨基酸序列或其与权利要求1-37中任一项的多肽结合的片段的GDF15多肽，其中所述GDF15多肽相对于蛋白质污染物是至少95%纯的。

57. 权利要求55的GDF15多肽，其中所述GDF15多肽以不大于10^{-8} M的平衡解离常数(K_D)结合TβRII。

58. 权利要求56的GDF15多肽，其中所述GDF15多肽以不大于10^{-8} M的平衡解离常数(K_D)与权利要求1-37中任一项的多肽结合。

59.权利要求55-58中任一项的GDF15多肽，其中所述多肽通过在CHO细胞中表达产生。

60.一种浓缩或纯化GDF15的方法，所述方法包括使含有GDF15的样品与权利要求1-37中任一项的多肽接触。

61. 一种多肽，其包含与SEQ ID NO:50的氨基酸序列有至少90%同一性的氨基酸序列。

62. 权利要求61的多肽，其包含与SEQ ID NO:50的氨基酸序列有至少95%同一性的氨基酸序列。

63. 权利要求61的多肽，其包含与SEQ ID NO:50的氨基酸序列有至少99%同一性的氨基酸序列。

64. 权利要求61的多肽，其包含与SEQ ID NO:50的氨基酸序列相同的氨基酸序列。

65. 权利要求61的多肽，其由与SEQ ID NO:50的氨基酸序列相同的氨基酸序列组成。

66.一种核酸，其编码权利要求61-65中任一项的多肽。

67. 一种核酸，其包含SEQ ID NO: 51的核酸序列。

68.一种通过在哺乳动物细胞中表达权利要求67的核酸产生的多肽。

69.权利要求68的多肽，其中所述哺乳动物细胞是中国仓鼠卵巢细胞(CHO细胞)。

TGF-β受体II型变体及其用途

[0001] 相关申请的交叉引用

本申请要求2013年8月22日提交的美国临时申请61/868,713、2013年11月19日提交的美国临时申请61/906,270和2013年11月20日提交的美国临时申请61/906,849的优先权。优先权申请的公开内容通过引用以其整体结合到本文中。

[0002] 发明背景

转化生长因子-β (TGFβ)超家族的成员是参与例如增殖、分化、凋亡、运动、胞外基质产生、组织重建、血管生成、免疫应答、细胞粘附等基本细胞功能的多效细胞因子,并且还在像慢性炎性病况和癌症那样不同的疾病状态的病理生理学中起关键作用。TGFβ超家族的成员被归类为主要的家族分组,其包括TGFβ、骨形态生成蛋白(BMP)、成骨蛋白(OP)、生长和分化因子(GDF)、抑制素/激活素、mullerian抑制物质(MIS)和神经胶质衍生神经营养因子(GDNF)。

[0003] TGFβ超家族成员通过诱导特异性I型和II型丝氨酸/苏氨酸激酶受体异聚复合体的形成而将其信号跨质膜转导,所述复合体进而激活特定亚类的SMAD蛋白(一些抑制性,一些刺激性)。SMAD分子化合物将信号传送入核,在核中它们与其它蛋白质联合指导转录反应。

[0004] 功能失常的TGFβ超家族信号转导与几种临床病症有关,包括癌症、纤维化、骨病、糖尿病性肾病以及慢性血管病,例如动脉粥样硬化。

[0005] 因此,本公开内容的一个目标是提供用于调节TGFβ超家族信号转导的组合物和方法。

[0006] 发明概述

在某种程度上,本公开内容提供TβRII多肽和所述TβRII多肽作为GDF15、TGFβ1或TGFβ3的选择性拮抗剂的用途。如本文所述,包含有或没有其它突变的TβRII胞外域(ECD)的部分或全部的多肽,以不同的亲和力结合和/或抑制GDF15、TGFβ1或TGFβ3。因此,在某些方面,本公开内容提供TβRII多肽用于选择性抑制TGFβ超家族相关病症。

[0007] 在某些方面,本公开内容提供在TβRII的胞外域中包含突变和/或截短的多肽。在某些方面,本公开内容提供包含来自TβRII胞外域的第一氨基酸序列和异源氨基酸序列的TβRII融合多肽,其中第一氨基酸序列包含与a)始于SEQ ID NO: 5的23-35位的任一个和止于SEQ ID NO: 5的153-159位的任一个的序列或b)始于SEQ ID NO: 6的23-60位的任一个和止于SEQ ID NO: 6的178-184位的任一个的序列有至少80%、至少85%、至少90%、至少95%、至少96%、至少97%、至少98%、至少99%同一性或相同的氨基酸序列或由其组成。

[0008] 在某些方面,本公开内容提供包含与人IgG2的Fc结构域的至少一部分融合的TβRII的野生型或改变和/或截短的胞外域的多肽。因此在某些方面,本公开内容提供包含来自TβRII胞外域的第一氨基酸序列和异源氨基酸序列的TβRII融合多肽,其中第一氨基酸序列包含与a)始于SEQ ID NO: 5的23-35位的任一个和止于SEQ ID NO: 5的153-159位的任一个的序列或b)始于SEQ ID NO: 6的23-60位的任一个和止于SEQ ID NO: 6的178-184位的任一个的序列有至少80%、至少85%、至少90%、至少95%、至少96%、至少97%、至少98%、至少

99%同一性或相同的氨基酸序列或由其组成，且其中所述多肽含有第二多肽序列，第二多肽序列包含至少人IgG2的恒定结构域，且可任选包含与SEQ ID NO: 19有至少80%、至少85%、至少90%、至少95%、至少96%、至少97%、至少98%、至少99%同一性的氨基酸序列或由其组成，且其中接头任选位于第一多肽和第二多肽之间。一个实例以SEQ ID NO:50提供，并由SEQ ID NO:51的核酸序列编码。在某些实施方案中，本公开内容提供具有包含与SEQ ID NO:50的氨基酸序列有至少80%、85%、90%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列或由其组成的氨基酸序列的多肽。在某些实施方案中，本公开内容提供由包含与SEQ ID NO:51的核酸序列有至少80%、85%、90%、95%、96%、97%、98%、99%或100%同一性的核酸序列或由其组成的核酸序列编码的多肽。

[0009] 在一些实施方案中，第一氨基酸序列包含始于SEQ ID NO: 5的23位和止于SEQ ID NO: 5的159位的序列或由其组成。在一些实施方案中，第一氨基酸序列包含始于SEQ ID NO: 5的29位和止于SEQ ID NO: 5的159位的序列或由其组成。在一些实施方案中，第一氨基酸序列包含始于SEQ ID NO: 5的35位和止于SEQ ID NO: 5的159位的序列或由其组成。在一些实施方案中，第一氨基酸序列包含始于SEQ ID NO: 5的23位和止于SEQ ID NO: 5的153位的序列或由其组成。在一些实施方案中，第一氨基酸序列包含始于SEQ ID NO: 5的29位和止于SEQ ID NO: 5的153位的序列或由其组成。在一些实施方案中，第一氨基酸序列包含始于SEQ ID NO: 5的35位和止于SEQ ID NO: 5的153位的序列或由其组成。

[0010] 在一些实施方案中，第一氨基酸序列包含始于SEQ ID NO: 6的23位和止于SEQ ID NO: 6的184位的序列或由其组成。在一些实施方案中，第一氨基酸序列包含始于SEQ ID NO: 6的29位和止于SEQ ID NO: 6的184位的序列或由其组成。在一些实施方案中，第一氨基酸序列包含始于SEQ ID NO: 6的23位和止于SEQ ID NO: 6的178位的序列或由其组成。在一些实施方案中，第一氨基酸序列包含始于SEQ ID NO: 6的29位和止于SEQ ID NO: 6的178位的序列或由其组成。

[0011] 在一些实施方案中，第一氨基酸序列包含在相当于SEQ ID NO: 47的36位的位置处具有D和/或在相当于SEQ ID NO: 47的76位的位置处具有K的序列或由其组成。

[0012] 在某些方面，本公开内容提供包含与SEQ ID NO: 7或SEQ ID NO: 13的序列有至少80%、至少85%、至少90%、至少95%、至少96%、至少97%、至少98%、至少99%同一性或相同的第一氨基酸序列或其活性片段和第二异源部分的TβRII融合多肽，其中第一氨基酸序列在相当于SEQ ID NO: 47的36位的位置处具有D和/或在相当于SEQ ID NO: 47的76位的位置处具有K。

[0013] 在一些实施方案中，第一氨基酸序列包含相当于SEQ ID NO: 7的氨基酸1-12的1-12个氨基酸或相当于SEQ ID NO: 13的氨基酸1-37的1-37个氨基酸的N端截短。在一些实施方案中，第一氨基酸序列包含相当于SEQ ID NO: 7或SEQ ID NO: 13的氨基酸1-6的6个氨基酸的N端截短。在一些实施方案中，第一氨基酸序列包含相当于SEQ ID NO: 7的氨基酸1-12的12个氨基酸或相当于SEQ ID NO: 13的氨基酸1-37的37个氨基酸的N端截短。在一些实施方案中，第一氨基酸序列包含相当于SEQ ID NO: 7的氨基酸137-132或SEQ ID NO: 13的氨基酸162-157的1-6个氨基酸的C端截短。在一些实施方案中，第一氨基酸序列包含相当于SEQ ID NO: 7的氨基酸132-137或SEQ ID NO: 13的氨基酸157-162的6个氨基酸的C端截短。在一些实施方案中，第一氨基酸序列包含在相当于SEQ ID NO: 47的117和118位的残基

之间的相当于SEQ ID NO: 18的插入。

[0014] 在一些实施方案中，异源部分包含提高以下一种或多种的一个或多个多肽部分：体内稳定性、体内半寿期、摄取/给予、组织定位或分布、蛋白质复合体的形成和/或纯化。在一些实施方案中，异源部分包含选自以下的多肽部分：免疫球蛋白Fc结构域和血清白蛋白。在又一个实施方案中，免疫球蛋白Fc结构域通过接头与TβRII多肽连接。

[0015] 在一些实施方案中，多肽包括选自以下的一个或多个修饰的氨基酸残基：糖基化氨基酸、聚乙二醇化氨基酸、法尼基化氨基酸、乙酰化氨基酸、生物素化氨基酸、与脂质部分缀合的氨基酸和与有机衍生剂缀合的氨基酸。在一些实施方案中，多肽被糖基化。

[0016] 在某些方面，本公开内容提供TβRII融合多肽，其包含由包含与选自SEQ ID NO: 7-17和47-49的氨基酸序列有至少80%、至少85%、至少90%或至少95%同一性的氨基酸序列的TβRII胞外域的一部分组成的第一氨基酸序列和第二异源部分。在某些方面，本公开内容提供TβRII融合多肽，其包含由包含与选自SEQ ID NO: 7-17和47-49的氨基酸序列有至少96%同一性的氨基酸序列的TβRII胞外域的一部分组成的第一氨基酸序列和第二异源部分。在某些方面，本公开内容提供TβRII融合多肽，其包含由包含与选自SEQ ID NO: 7-17和47-49的氨基酸序列有至少97%同一性的氨基酸序列的TβRII胞外域的一部分组成的第一氨基酸序列和第二异源部分。在某些方面，本公开内容提供TβRII融合多肽，其包含由包含与选自SEQ ID NO: 7-17和47-49的氨基酸序列有至少98%同一性的氨基酸序列的TβRII胞外域的一部分组成的第一氨基酸序列和第二异源部分。在某些方面，本公开内容提供TβRII融合多肽，其包含由包含与选自SEQ ID NO: 7-17和47-49的氨基酸序列有至少99%同一性的氨基酸序列的TβRII胞外域的一部分组成的第一氨基酸序列和第二异源部分。在某些方面，本公开内容提供TβRII融合多肽，其包含由包含是选自SEQ ID NO: 7-17和47-49的氨基酸序列的氨基酸序列的TβRII胞外域的一部分组成的第一氨基酸序列和第二异源部分。

[0017] 在某些方面，本公开内容提供包含与选自SEQ ID NO: 25、27、29、31、33、35、37、39、41和43的氨基酸序列有至少80%、至少85%、至少90%或至少95%同一性的氨基酸序列或其前导序列除去的部分或由其组成的多肽，例如，包含与选自SEQ ID NO: 53、54、55、56、57、58、59、60、61和62的氨基酸序列有至少80%、至少85%、至少90%或至少95%同一性的氨基酸序列或由其组成的多肽。在某些方面，本公开内容提供包含与选自SEQ ID NO: 25、27、29、31、33、35、37、39、41和43的氨基酸序列有至少96%同一性的氨基酸序列或其前导序列除去的部分或由其组成的多肽，例如，包含与选自SEQ ID NO: 53、54、55、56、57、58、59、60、61和62的氨基酸序列有至少96%同一性的氨基酸序列或由其组成的多肽。在某些方面，本公开内容提供包含选自SEQ ID NO: 25、27、29、31、33、35、37、39、41和43的氨基酸序列有至少97%同一性的氨基酸序列或其前导序列除去的部分或由其组成的多肽，例如，包含与选自SEQ ID NO: 53、54、55、56、57、58、59、60、61和62的氨基酸序列有至少97%同一性的氨基酸序列或由其组成的多肽。在某些方面，本公开内容提供包含与选自SEQ ID NO: 25、27、29、31、33、35、37、39、41和43的氨基酸序列有至少98%同一性的氨基酸序列或其前导序列除去的部分或由其组成的多肽，例如，包含与选自SEQ ID NO: 53、54、55、56、57、58、59、60、61和62的氨基酸序列有至少98%同一性的氨基酸序列或由其组成的多肽。在某些方面，本公开内容提供包含与选自SEQ ID NO: 25、27、29、31、33、35、37、39、41和43的氨基酸序列有至少99%同一性的氨基酸序列或其前导序列除去的部分或由其组成的多肽，例如，包含与选自SEQ ID NO:

53、54、55、56、57、58、59、60、61和62的氨基酸序列有至少99%同一性的氨基酸序列或由其组成的多肽。在某些方面，本公开内容提供包含选自SEQ ID NO: 25、27、29、31、33、35、37、39、41和43的氨基酸序列或其前导序列除去的部分或由其组成的多肽，例如，包含选自SEQ ID NO: 53、54、55、56、57、58、59、60、61和62的氨基酸序列或由其组成的多肽。

[0018] 在某些方面，本公开内容提供包含由在严格条件下与选自SEQ ID NO: 26、28、30、32、34、36、38、40、42和44的核苷酸序列的互补序列杂交的核酸编码的氨基酸序列的TβRII多肽。

[0019] 在前面提到的每一个中，可选择不包括全长TβRII ECD的TβRII多肽。TβRII多肽可用作单体蛋白质或呈二聚形式。TβRII多肽还可与第二多肽部分融合以提供改进的性质，例如延长半寿期或更容易生产或纯化。融合可以是直接的，或可将接头插入TβRII多肽和任何其它部分之间。接头可以是结构化的或非结构化的，可由1、2、3、4、5、10、15、20、30、50个或更多个氨基酸组成，任选相对不含二级结构。

[0020] 在一些实施方案中，本公开内容的TβRII多肽以小于1 x 10^{-8} M的平衡解离常数(K_D)结合人GDF15。

[0021] 在一些实施方案中，本公开内容的TβRII多肽具有CHO细胞中多肽表达特有的糖基化形式。

[0022] 在一些实施方案中，本公开内容提供包含本公开内容的2个TβRII多肽的同二聚体。

[0023] 在一些实施方案中，本公开内容提供包含本公开内容的TβRII多肽的编码序列的分离的多核苷酸。在一些实施方案中，本公开内容提供包含与分离的多核苷酸有效连接的启动子序列的重组多核苷酸。在一些实施方案中，本公开内容提供用本公开内容的分离的多核苷酸或重组多核苷酸转化的细胞。在一些实施方案中，细胞是哺乳动物细胞。在一些实施方案中，细胞是CHO细胞或人细胞。在一些实施方案中，细胞是HEK-293细胞。

[0024] 在某些方面，本公开内容提供包含本公开内容的TβRII多肽或同二聚体和药学上可接受的赋形剂的药物制剂。

[0025] 在某些方面，本公开内容提供调节细胞对TGFβ超家族成员的应答的方法，所述方法包括使细胞暴露于本公开内容的TβRII多肽或同二聚体中。

[0026] 在某些方面，本公开内容提供治疗有需要的患者的与TGFβ超家族成员有关的疾病或病况的方法，所述方法包括给予患者有效量的本公开内容的TβRII多肽或同二聚体。在一些实施方案中，TGFβ超家族成员是TGFβ1、TGFβ3或GDF15。

[0027] 在一些实施方案中，疾病或病况是癌症。在一些实施方案中，癌症选自胃癌、肠癌、皮肤癌、乳腺癌、黑素瘤、骨癌和甲状腺癌。

[0028] 在一些实施方案中，疾病或病况是纤维化或硬化疾病或病症。在一些实施方案中，纤维化或硬化疾病或病症选自硬皮病、动脉粥样硬化、肝纤维化、弥漫性系统性硬化、肾小球性肾炎、神经瘢痕形成(neural scarring)、皮肤瘢痕形成、放射诱导的纤维化、肝纤维化和骨髓纤维化。

[0029] 在一些实施方案中，疾病或病况是心脏病。

[0030] 在一些实施方案中，疾病或病况选自遗传性出血性毛细血管扩张症(HHT)、马方综合征(Marfan syndrome)、Loeys-Dietz综合征、家族性胸主动脉瘤综合征、动脉迂曲综合

征、先兆子痫、动脉粥样硬化、再狭窄和肥厚型心肌病/充血性心力衰竭。

[0031] 在某些方面，本公开内容提供与GDF15结合并阻断GDF15和TβRII之间的相互作用的抗体或其抗原结合片段。

[0032] 在某些方面，本公开内容提供包含SEQ ID NO: 1的氨基酸序列或其结合TβRII的片段的GDF15多肽，其中相对于蛋白质污染物，GDF15多肽为至少95%、至少96%、至少97%、至少98%或至少99%纯的。

[0033] 在某些方面，本公开内容提供包含SEQ ID NO: 1的氨基酸序列或其结合本公开内容的TβRII多肽的片段的GDF15多肽，其中相对于蛋白质污染物，GDF15多肽为至少95%、至少96%、至少97%、至少98%或至少99%纯的。

[0034] 在一些实施方案中，GDF15多肽以不大于10^{-8} M的平衡解离常数(K_D)结合TβRII。在一些实施方案中，GDF15多肽以不大于10^{-8} M的平衡解离常数(K_D)结合本公开内容的TβRII多肽。

[0035] 在一些实施方案中，GDF15多肽通过在CHO细胞中表达产生。

[0036] 在某些方面，本公开内容提供浓缩或纯化GDF15的方法，所述方法包括使含有GDF15的样品与本公开内容的TβRII多肽接触。

[0037] 附图简述

图1显示人GDF15的天然前体的氨基酸序列(NCBI参考序列:NP_004855.2)。实心下划线表示成熟的GDF15 (残基197-308)，具有通过测序测定的N端。虚线下划线表示前导序列(残基1-29)。

[0038] 图2显示编码人GDF15的天然前体的核苷酸序列。实心下划线表示编码成熟GDF15的序列(核苷酸589-924)，虚线下划线表示编码前导序列的序列(核苷酸1-87)。用于破坏NM_004864.2的SfoI位点的沉默突变(G456A)用双下划线标出。

[0039] 图3显示鼠GDF15的天然前体的氨基酸序列(NP_035949.2)。实心下划线表示成熟的GDF15 (残基192-303)，具有通过测序测定的N端。虚线下划线表示前导序列(残基1-30)。

[0040] 图4显示编码鼠GDF15的天然前体的核苷酸序列(来源于NM_011819.2)。实心下划线表示编码成熟GDF15的序列(核苷酸574-909)，虚线下划线表示编码前导序列的序列(核苷酸1-90)。

[0041] 图5显示人TGFβ受体II型(hTβRII)的B (短)同种型的天然前体的氨基酸序列(NP_003233.4)。实心下划线表示成熟胞外域(ECD) (残基23-159)，双下划线表示A (长)同种型中被置换的缬氨酸。虚线下划线表示前导序列(残基1-22)。

[0042] 图6显示人TβRII的A (长)同种型的天然前体的氨基酸序列(NP_001020018.1)。实心下划线表示成熟的ECD (残基23-184)，双下划线表示剪接产生的异亮氨酸取代。虚线下划线表示前导序列(残基1-22)。

[0043] 图7表示$hT\beta RII_{短}$截短及其$hT\beta RII_{长}$对应物的N端比对。存在于$hT\beta RII_{长}$截短的25个氨基酸插入用下划线标出。注意剪接过程引起侧接短同种型的插入位点的缬氨酸被长同种型中的异亮氨酸置换。用方框标出的序列表示前导序列。

[0044] 发明详述

1. 概述

本文所述蛋白质是人的形式，除非另有说明。蛋白质的NCBI索引如下:人TβRII同种型A

(hTβRII长),NP_001020018.1;人TβRII同种型B (hTβRII短),NP_003233.4;人GDF15,NP_004855.2;鼠GDF15,NP_035949.2。图1-6中给出人和小鼠的天然TβRII和GDF15蛋白的序列。

[0045] TGFβ超家族含有多种生长因子,其具有共同的序列元件和结构基序。已知这些蛋白质在脊椎动物和无脊椎动物两者中对多种细胞类型发挥生物作用。该超家族的成员在图式形成和组织特化的胚胎发育期间发挥重要功能,可影响各种分化过程,包括脂肪形成、肌发生、软骨发生、心脏发生、血细胞生成、神经发生和上皮细胞分化。通过操控TGFβ家族成员的活性,常常可引起生物体的重大生理改变。例如Piedmontese和Belgian Blue牛品种在GDF8 (亦称肌肉生长抑制素)基因中携带引起肌肉质量明显增加的功能缺失突变。Grobet等, Nat Genet. 1997, 17(1):71-4。类似地,在人中,GDF8的无活性等位基因与肌肉质量增加和据报道的异常强度有关。Schuelke等,N Engl J Med 2004,350:2682-8。

[0046] TGF-β信号受I型(例如TβRI)和II型(例如TβRII)丝氨酸/苏氨酸激酶受体的异聚复合体介导,所述受体在配体刺激时使下游SMAD蛋白磷酸化并激活下游SMAD蛋白(Massagué,2000,Nat. Rev. Mol. Cell Biol. 1:169-178)。这些I型和II型受体是跨膜蛋白,由具有富半胱氨酸区的配体结合胞外域、跨膜结构域和具有预测的丝氨酸/苏氨酸特异性的胞质结构域组成。I型受体是信号转导所必需的;II型受体是结合配体和表达I型受体所必需的。I型和II型受体在配体结合后形成稳定的复合体,导致I型受体被II型受体磷酸化。TGFβ具有3种哺乳动物同种型TGFβ1、TGFβ2和TGFβ3,各自在体内具有截然不同的功能。TGFβ与TβRII的结合是引发导致SMAD2磷酸化的TGFβ信号转导途径活化和活化SMAD2/SMAD4复合体转运至核中以调节基因表达的关键步骤。

[0047] 生长分化因子15 (GDF15)是TGFβ家族的成员。与含有特有的半胱氨酸结基序的TGFβ超家族的其它配体一样,合成具有较大前域(prodomain)的成熟的GDF15 (Harrison等, Growth Factors 29:174, 2011;Shi等, Nature 474:343, 2011),该前域通过在典型的RXXR位点被弗林蛋白酶样蛋白酶切割除去以产生成熟的二聚GDF15。GDF15在文献中被描述为巨噬细胞抑制细胞因子-1 (MIC-1)、胎盘骨形态发生蛋白(PLAB)、胎盘转化生长因子β(PTGFβ)、前列腺衍生因子(PDF)和非甾体抗炎激活基因-1 (NAG-1),反映了这种蛋白质所隐含的不同功能。GDF15与几种生理和病理情况有关。例如,GDF15在胎盘中高表达,并且是维持妊娠所必需的。GDF15浓度还在患有前列腺癌、结肠直肠癌或胰腺癌以及神经胶质瘤的患者血清中明显增加。未表明GDF15在生物化学上直接与任何受体结合或相互作用。本公开内容部分涉及TGFβ II型受体TβRII以高亲和力与GDF15结合且是GDF15的功能受体的发现。本文证实TβRII融合多肽和含有TβRII的配体结合部分的其它多肽抑制GDF15诱导的基因活化。GDF15信号转导的有效抑制提供TβRII是GDF15的功能II型受体的证据,开启了用于在该信号转导途径中的治疗性干预的新手段。因此,在某种程度上,本公开内容鉴定了GDF15多肽的生理学的高亲和力受体。

[0048] 出乎意料的是,本文表明可溶性TβRII多肽对GDF15具有高特异性、高亲和力结合。TβRII是已知的TGFβ的II型受体,并以高亲和力与TGFβ1和TGFβ3结合。人TβRII以通过胞外域(ECD)的可变剪接产生的至少2种同种型(A (长)和B (短))天然存在(图6和5和SEQ ID NO: 6和5)。长同种型具有25个氨基酸插入,剪接过程引起侧接短同种型的插入位点的缬氨酸被长同种型中的异亮氨酸置换。可溶性受体胞外域可起清除剂或配体陷阱(ligand trap)的作用以抑制配体-受体相互作用。配体陷阱(例如掺入天然TβRII胞外结构域(胞外

域)的可溶性TβRII-Fc融合蛋白)可起针对TβRII配体(包括TGFβ1、TGFβ3和根据本文公开的研究结果GDF15)的泛抑制剂(pan-inhibitor)的作用。虽然在一些治疗背景下,这种较广泛的配体结合和信号抑制可能是有利的,但在其它背景下,更大选择性的分子可能较好。十分需要显示选择性配体结合特征的配体陷阱,例如TβRII胞外域多肽。本公开内容涉及以下出乎意料的发现,即包含TβRII的胞外域的截短部分和/或胞外域内的突变的多肽对由GDF15、TGFβ1或TGFβ3引起的细胞信号转导具有不同的抑制作用。在某种程度上,本公开内容提供由TβRII胞外域的一系列突变和/或截短产生、显示完全不同于天然TβRII胞外域的不同的配体结合特征的配体陷阱。本文公开的变体TβRII多肽提供相对于天然全长胞外域的有利性质,并且体内可用于选择性地抑制由不同TβRII配体介导的途径。

[0049] 因此,在某些方面,本公开内容提供TβRII多肽作为GDF15、TGFβ1或TGFβ3的拮抗剂用于治疗各种GDF15-、TGFβ1-或TGFβ3-相关病症。虽然不希望受任何特殊作用机制的束缚,但预期所述多肽通过与GDF15、TGFβ1或TGFβ3结合并抑制这些配体形成三元信号转导复合体的能力起作用。

[0050] 用于本说明书的术语一般具有其在本领域、在本发明背景内和在使用各术语的特定背景下的普通含义。下面或本说明书的其它部分论述了某些术语,以在描述本发明的组合物和方法和如何制备和使用它们时对实施者提供额外指导。从使用术语的特定背景来看,术语的任何应用的范围或含义都将是显而易见的。

[0051] 2. TβRII多肽

天然存在的TβRII蛋白是跨膜蛋白,其中蛋白质的一部分位于细胞外(胞外部分),蛋白质的一部分位于细胞内(胞内部分)。本公开内容的方面包括包含TβRII的胞外域内的突变和/或胞外域的截短部分的变体TβRII多肽。如上所述,人TβRII以通过胞外域(ECD)的可变剪接产生的至少2种同种型(A (长)和B (短))天然存在(图6和5和SEQ ID NO: 6和5)。相当于SEQ ID NO: 5的残基23-159的SEQ ID NO: 7,表示TβRII的短同种型天然全长胞外域。相当于SEQ ID NO: 6的残基23-184的SEQ ID NO: 13,表示TβRII长同种型的天然全长胞外域。除非另有说明,否则有关基于TβRII短和长同种型的变体的氨基酸位置编号分别是指天然前体SEQ ID NO: 5和SEQ ID NO:6的相应位置。

[0052] 在某些实施方案中,本公开内容提供变体TβRII多肽。本公开内容的TβRII多肽可结合并抑制TGFβ超家族成员的功能,所述成员例如但不限于GDF15、TGFβ1或TGFβ3。TβRII多肽可包括由与天然存在的TβRII多肽的截短的ECD结构域(其C端出现在SEQ ID NO: 5的氨基酸153-159的任一个上)有至少80%同一性、任选至少85%、90%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列组成或包含所述氨基酸序列的多肽。TβRII多肽可包括由与天然存在的TβRII多肽的截短的ECD结构域(其C端出现在SEQ ID NO: 6的氨基酸178-184的任一个处)有至少80%同一性、任选至少85%、90%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列组成或包含所述氨基酸序列的多肽。任选TβRII多肽不包括来自由SEQ ID NO: 5的氨基酸160-567组成的序列或来自由SEQ ID NO: 6的氨基酸185-592组成的序列的大于5个连续氨基酸或大于10、20、30、40、50、52、60、70、80、90、100、150或200或更多个连续氨基酸。未加工的TβRII多肽可包括或不包括任何信号序列以及信号序列N端的任何序列。如本文所述,成熟的(加工的) TβRII多肽的N端可出现在SEQ ID NO: 5的氨基酸23-35或SEQ ID NO: 6的23-60的任一个处。成熟TβRII多肽的实例包括但不限于SEQ ID NO: 5的氨基酸23-159

(SEQ ID NO: 7所示)、SEQ ID NO: 5的氨基酸29-159 (SEQ ID NO: 9所示)、SEQ ID NO: 5的氨基酸35-159 (SEQ ID NO: 10所示)、SEQ ID NO: 5的氨基酸23-153 (SEQ ID NO: 11所示)、SEQ ID NO: 5的氨基酸29-153 (SEQ ID NO: 48所示)、SEQ ID NO: 5的氨基酸35-153 (SEQ ID NO: 47所示)、SEQ ID NO: 6的氨基酸23-184 (SEQ ID NO: 13所示)、SEQ ID NO: 6的氨基酸29-184 (SEQ ID NO: 15所示)、SEQ ID NO: 6的氨基酸60-184 (SEQ ID NO: 10所示)、SEQ ID NO: 6的氨基酸23-178 (SEQ ID NO: 16所示)、SEQ ID NO: 6的氨基酸29-178 (SEQ ID NO: 49所示)和SEQ ID NO: 6的氨基酸60-178 (SEQ ID NO: 47所示)。同样地,TβRII多肽可包含由SEQ ID NO: 30的核苷酸73-465、SEQ ID NO: 34的核苷酸73-447、SEQ ID NO: 38的核苷酸73-465、SEQ ID NO: 38的核苷酸91-465或SEQ ID NO: 38的核苷酸109-465或其沉默变体或在严格杂交条件(所述条件通常是本领域已知的,但可例如包括在50% v/v甲酰胺、5x SSC、2% w/v封闭剂、0.1% N-月桂酰肌氨酸和0.3% SDS中在65℃下杂交过夜,并在例如5x SSC中在约65℃下洗涤)下与其互补序列杂交的核酸编码的多肽。本领域技术人员应了解,基于TβRII的长同种型的相应变体可包括编码25个氨基酸插入以及插入的C端的侧翼位置处的保守Val-Ile取代的核苷酸序列。TβRII多肽因此可包括分离的TβRII多肽的胞外部分,包括短和长同种型两者、其变体(包括例如在相当于SEQ ID NO: 5的氨基酸23-159或SEQ ID NO: 6的氨基酸23-184的序列中包含不大于2、3、4、5、10、15、20、25、30或35个氨基酸取代的变体)、其片段和包含前述的任一个的融合蛋白,但在各种情况下,优选前述TβRII多肽的任一个将保持对GDF15、TGFβ1或TGFβ3中的至少一种的基本亲和力。一般而言,可将TβRII多肽设计为在生物学相关温度、pH水平和摩尔渗透压浓度下可溶于水溶液中。

[0053] 在一些实施方案中,本公开内容的变体TβRII多肽包含胞外域中赋予改变的配体结合特征的一个或多个突变。TβRII多肽可在氨基酸序列中包括相对于天然存在的TβRII多肽的相应部分的1、2、5个或更多个变化。在一些实施方案中,突变导致相当于SEQ ID NO: 5的70位的位置处的取代、插入或缺失。在一些实施方案中,突变导致相当于SEQ ID NO: 5的110位的位置处的取代、插入或缺失。实例包括但不限于分别相当于SEQ ID NO: 5的70和110位的位置处的N至D取代或D至K取代。所述变体TβRII多肽的实例包括但不限于SEQ ID NO: 8、SEQ ID NO:14、SEQ ID NO: 12和SEQ ID NO: 17所示序列。TβRII多肽可包含由SEQ ID NO: 26的核苷酸73-483、SEQ ID NO: 42的核苷酸73-465或其沉默变体或在严格杂交条件下与其互补序列杂交的核酸编码的多肽或其部分。

[0054] 在一些实施方案中,本公开内容的变体TβRII多肽还包含位于人TβRII ECD的C端附近的谷氨酸残基对(SEQ ID NO: 5的151和152位或SEQ ID NO: 6的176和177位)之间的36个氨基酸(SEQ ID NO: 18)的插入,如天然存在于人TβRII同种型C中的一样(Konrad等,BMC Genomics 8:318,2007)。

[0055] 本公开内容进一步证实可对TβRII多肽进行修饰以选择性地拮抗TβRII配体。此处提供的数据表明,包含TβRII多肽的较短的N端和C端截短的变体的Fc融合蛋白显示对通过GDF15、TGFβ1和TGFβ3介导的细胞信号转导的不同的抑制作用。具体地说,发现与TβRII短同种型的全长胞外域相比,始于SEQ ID NO: 5的氨基酸29或35并分别携带胞外域的6或12个氨基酸N端截短的N端截短变体最有效地抑制GDF15,最不有效地抑制TGFβ3,而抑制TGFβ1至中等程度。止于SEQ ID NO: 5的氨基酸153并携带胞外域的6个氨基酸C端截短的C端截短变

体对配体结合没有实质作用，因此可与全长形式互换使用。发现相当于SEQ ID NO: 5的70位的位置处的N至D取代有效抑制TGFβ3、对GDF15具有中等作用，并对TGFβ1具有可忽略的作用。N70残基表示可能的糖基化位点。此外，发现与TβRII短同种型的全长胞外域相比，包含相当于SEQ ID NO: 5的110位的位置处的D至K取代的Fc融合蛋白最有效地抑制GDF15、最不有效地抑制TGFβ1、抑制TGFβ3至中等程度。110位附近的区域与已知的TβRII配体TGFβ1、TGFβ2和TGFβ3的选择性无关。因此，料想不到的是，预期含有ECD的突变例如但不限于N70D和D110K（残基的编号相当于SEQ ID NO: 5的编号）和/或始于氨基酸29和35之间和/或止于氨基酸153和氨基酸159之间的TβRII多肽均有活性，并且对于不同的配体均显示大不相同的抑制效能。可能需要使用这些截短变体形式的任一个，这取决于临床或实验背景。

[0056] 在某些实施方案中，TβRII多肽与GDF15结合，且TβRII多肽不显示与TGFβ1或TGFβ3实质结合。在某些实施方案中，TβRII多肽与TGFβ1结合，且TβRII多肽不显示与GDF15或TGFβ3实质结合。在某些实施方案中，TβRII多肽与TGFβ3结合，且TβRII多肽不显示与GDF15或TGFβ1实质结合。可在溶液中或在表面等离子体共振系统(例如Biacore™系统)中使用纯化的蛋白质评价结合。

[0057] 在某些实施方案中，TβRII多肽抑制GDF15细胞信号转导，且TβRII多肽对TGFβ1或TGFβ3具有中等或有限的抑制作用。在某些实施方案中，TβRII多肽抑制TGFβ1细胞信号转导，TβRII多肽对GDF15或TGFβ3具有中等或有限的抑制作用。在某些实施方案中，TβRII多肽抑制TGFβ3细胞信号转导，且TβRII多肽对GDF15或TGFβ1具有中等或有限的抑制作用。对细胞信号转导的抑制作用可通过本领域已知方法评价。

[0058] 总之，TβRII多肽的活性部分可包含SEQ ID NO: 5的氨基酸序列23-153、23-154、23-155、23-156、23-157或23-158，以及始于SEQ ID NO: 5的氨基酸24-35的任一个的这些序列的变体。同样地，TβRII多肽的活性部分可包含SEQ ID NO: 6的氨基酸序列23-178、23-179、23-180、23-181、23-182或23-183以及始于SEQ ID NO: 6的氨基酸24-60的任一个的这些序列的变体。示例性的TβRII多肽包含SEQ ID NO: 5的氨基酸序列29-159、35-159、23-153、29-153和35-153或SEQ ID NO: 6的氨基酸序列29-184、60-184、23-178、29-178和60-178。还考虑了这些范围内的变体，特别是与SEQ ID NO: 5或SEQ ID NO: 6的相应部分有至少80%、85%、90%、95%或99%同一性的变体。可选择不包括由SEQ ID NO:5的氨基酸160-567或SEQ ID NO:6的氨基酸185-592组成的序列的TβRII多肽。

[0059] 如上所述，本公开内容提供与天然存在的TβRII多肽共有规定程度的序列同一性或相似性的TβRII多肽。为了测定2个氨基酸序列的百分比同一性，对序列进行比对用于最佳比较目的(例如，可在第一和第二氨基酸或核酸序列的一个或两个中引入空位用于最佳比对，并可忽略非同源序列用于比较目的)。然后比较相应氨基酸位置处的氨基酸残基。当第一序列的一个位置被与第二序列的相应位置相同的氨基酸残基占据时，则在该位置处的分子是相同的(本文所用氨基酸“同一性”等同于氨基酸“同源性”)。考虑需要引入用于2个序列的最佳比对的空位的数目和各空位的长度，2个序列间的百分比同一性是序列所共有的相同位置数的函数。

[0060] 序列的比较和2个序列的百分比同一性和相似性的测定可应用数学算法完成(Computational Molecular Biology, Lesk, A. M.编辑, Oxford University Press, New York, 1988;Biocomputing: Informatics and Genome Projects, Smith, D. W.编

辑, Academic Press, New York, 1993;Computer Analysis of Sequence Data, Part 1, Griffin, A. M.和Griffin, H. G.编辑, Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987;以及Sequence Analysis Primer, Gribskov, M.和Devereux, J.编辑, M Stockton Press, New York, 1991)。

[0061] 在一个实施方案中,应用整合到GCG软件包的GAP程序(可获自http://www.gcg.com)中的Needleman和Wunsch (J Mol. Biol. (48):444-453 (1970))算法,测定2个氨基酸序列间的百分比同一性。在一个具体的实施方案中,在GAP程序中使用下列参数:Blosum 62矩阵或PAM250矩阵,16、14、12、10、8、6或4的空位权重和1、2、3、4、5或6的长度权重。在又一个实施方案中,2个核苷酸序列间的百分比同一性应用GCG软件包的GAP程序测定(Devereux, J.等, Nucleic Acids Res. 12(1):387 (1984)) (可获自http://www.gcg.com)。示例性的参数包括使用NWSgapdna.CMP矩阵和40、50、60、70或80的空位权重和1、2、3、4、5或6的长度权重。除非另有说明,否则2个氨基酸序列间的百分比同一性使用Blosum 62矩阵、10的空位权重和3的长度权重,应用GAP程序测定,并且如果所述算法无法计算所需的百分比同一性,则应选择本文公开的合适的备选方法。

[0062] 在另一个实施方案中,应用整合到ALIGN程序(2.0版)中的E. Myers和W. Miller的算法(CABIOS, 4:11-17 (1989)),使用PAM120权重残基表、12的空位长度罚分和4的空位罚分,测定2个氨基酸序列间的百分比同一性。

[0063] 测定2个氨基酸序列间的最佳总体比对的另一个实施方案可应用基于Brutlag等(Comp. App. Biosci., 6:237-245 (1990))的算法的FASTDB计算机程序测定。在序列比对中,查询序列和主题序列均为氨基酸序列。所述全局序列比对的结果以百分比同一性为单位提供。在一个实施方案中,氨基酸序列同一性应用基于Brutlag等(Comp. App. Biosci., 6:237-245 (1990))的算法的FASTDB计算机程序进行。在一个具体的实施方案中,用于计算氨基酸比对的百分比同一性和相似性的参数包括:矩阵=PAM 150,k-元组=2,错配罚分=1,连接罚分(Joining Penalty)=20,随机组长度(Randomization Group Length)=0,截止点分数=1,空位罚分=5和空位大小罚分=0.05。

[0064] TβRII多肽可另包括N端处的不同前导序列的任一个。所述序列可允许肽表达并靶向真核系统中的分泌途径。参见例如Ernst等,美国专利号5,082,783 (1992)。或者,可使用天然TβRII信号序列以实现从细胞中排出。可能的前导序列包括天然前导序列、组织纤溶酶原激活物(TPA)和蜜蜂蜂毒肽(分别为SEQ ID NO. 22-24)。掺入TPA前导序列的TβRII-Fc融合蛋白的实例包括SEQ ID NO: 25、27、29、31、33、35、37、39、41和43。信号肽的加工可随所选择的前导序列、所用细胞类型和培养条件连同其它变量而变化,因此成熟的TβRII多肽的实际N端起始位点可在N端或C端方向移动1、2、3、4或5个氨基酸。TβRII-Fc融合蛋白的实例包括SEQ NO: 25、27、29、31、33、35、37、39、41、43、53、54、55、56、57、58、59、60、61和62,如本文下划线标示的TβRII多肽部分所示(参见实施例)。本领域技术人员应了解,基于TβRII的长同种型的相应变体可包括25个氨基酸插入以及插入的C端的侧翼位置处的保守Val-Ile取代。

[0065] 在某些实施方案中,本公开内容考虑TβRII多肽的特定突变以改变多肽的糖基化。可选择这类突变,使得引入或剔除一个或多个糖基化位点,例如O-联糖基化位点或N-联糖

基化位点。天冬酰胺连接的糖基化识别位点一般包含被合适的细胞糖基化酶特异性识别的三肽序列,即天冬酰胺-X-苏氨酸(或天冬酰胺-X-丝氨酸)(其中"X"为任何氨基酸)。还可通过添加一个或多个丝氨酸或苏氨酸残基至野生型TβRII多肽的序列,或者用一个或多个丝氨酸或苏氨酸残基取代野生型TβRII多肽的序列,来进行改变(对于O-联糖基化位点)。在糖基化识别位点第一或第三氨基酸位置的一个或两个上的各种氨基酸取代或缺失(和/或在第二位置上的氨基酸缺失)在修饰三肽序列上导致非糖基化。在TβRII多肽上增加糖部分的数目的另一种方法是将糖苷与TβRII多肽化学偶联或酶促偶联。根据所采用的偶联方式,可将糖连接至:(a)精氨酸和组氨酸;(b)游离羧基;(c)游离巯基,例如半胱氨酸的巯基;(d)游离羟基,例如丝氨酸、苏氨酸或羟脯氨酸的羟基;(e)芳族残基,例如苯丙氨酸、酪氨酸或色氨酸的芳族残基;或(f)谷氨酰胺的酰胺基。这些方法参见1987年9月11日公布的WO 87/05330以及Aplin和Wriston (1981) CRC Crit. Rev. Biochem., 第259-306页,通过引用结合到本文中。可用化学方法和/或酶方法实现TβRII多肽上存在的一个或多个糖部分的脱去。化学去糖基化可包括例如将TβRII多肽暴露于化合物三氟甲烷磺酸或等同化合物中。这种处理导致除连接糖(N-乙酰氨基葡萄糖或N-乙酰半乳糖胺)以外的大部分或所有糖被切割,同时保持氨基酸序列完整。化学去糖基化另参见Hakimuddin等(1987) Arch. Biochem. Biophys. 259:52和Edge等(1981) Anal. Biochem. 118:131。可按Thotakura等(1987) Meth. Enzymol. 138:350所述,通过使用各种内切糖苷酶和外切糖苷酶完成TβRII多肽上的糖部分的酶促切割。适当时,可根据所采用的表达系统类型,调整TβRII多肽的序列,因为哺乳动物、酵母、昆虫和植物细胞均可引入可受所述肽的氨基酸序列影响的不同糖基化模式。一般而言,用于人的TβRII多肽可在提供适当糖基化的哺乳动物细胞系(例如HEK293或CHO细胞系)中表达,虽然预期其它哺乳动物表达细胞系、具有工程改造的糖基化酶的酵母细胞系以及昆虫细胞也是有用的。

[0066] 本公开内容还考虑产生突变体、特别是数套TβRII多肽的组合突变体以及截短突变体的方法;组合突变体库尤其可用于鉴定功能变体序列。筛选这类组合文库的目的可以是产生例如可用作激动剂或拮抗剂或者总之具有新活性的TβRII多肽变体。下面提供各种筛选测定法,这类测定法可用来评价变体。例如,可针对与TβRII配体结合的能力筛选TβRII多肽变体,以防止TβRII配体与TβRII多肽结合或干扰由TβRII配体引起的信号转导。还可在基于细胞的测定法或体内测定法中,特别是实施例公开的任何测定法中,测试TβRII多肽或其变体的活性。

[0067] 可以产生相对于包含天然存在的TβRII多肽的胞外域的TβRII多肽具有选择性或通常提高的效能的组合来源的变体。同样地,诱变可引起血清半寿期显著不同于相应的野生型TβRII多肽的变体。例如,可使改变的蛋白质对蛋白水解降解或导致天然TβRII多肽破坏或以别的方式消除或失活的其它过程更稳定或更不稳定。可使用这类变体和编码变体的基因,通过调节TβRII多肽的半寿期来改变TβRII多肽水平。例如,短的半寿期可引起较短暂的生物作用,并可允许较严格地控制患者内的重组TβRII多肽水平。在Fc融合蛋白中,可在接头(如有的话)和/或Fc部分中进行突变以改变蛋白质的半寿期。

[0068] 可通过编码多肽文库的基因的简并文库产生组合文库,所述多肽各自包含可能的TβRII多肽序列的至少一部分。例如,可以酶的方法将合成寡核苷酸的混合物与基因序列连接,使得可能的TβRII多肽核苷酸序列的简并组可作为个别多肽表达,或者作为一组较大的

融合蛋白表达(例如用于噬菌体展示)。

[0069] 存在许多可从简并寡核苷酸序列产生可能的TβRII多肽变体的文库的方法。简并基因序列的化学合成可在自动DNA合成仪中进行,合成基因然后与用于表达的合适载体连接。简并寡核苷酸的合成是本领域众所周知的(参见例如Narang,SA (1983) Tetrahedron 39:3;Itakura等(1981) Recombinant DNA, Proc. 3rd Cleveland Sympos. Macromolecules, 主编AG Walton,Amsterdam: Elsevier, 第273-289页;Itakura等(1984) Annu. Rev. Biochem. 53:323;Itakura等(1984) Science 198:1056;Ike等(1983) Nucleic Acid Res. 11:477)。这类技术已应用于其它蛋白质的定向进化中(参见例如Scott等(1990) Science 249:386-390;Roberts等(1992) PNAS USA 89:2429-2433;Devlin等(1990) Science 249:404-406;Cwirla等(1990) PNAS USA 87: 6378-6382;以及美国专利号5,223,409、5,198,346和5,096,815)。

[0070] 或者,可利用其它诱变形式产生组合文库。例如,可采用以下诱变,通过筛选从文库中产生和分离TβRII多肽变体:例如丙氨酸扫描诱变等(Ruf等(1994) Biochemistry 33: 1565-1572;Wang等(1994) J. Biol. Chem. 269:3095-3099;Balint等(1993) Gene 137: 109-118;Grodberg等(1993) Eur. J. Biochem. 218:597-601;Nagashima等(1993) J. Biol. Chem. 268:2888-2892;Lowman等(1991) Biochemistry 30:10832-10838;以及Cunningham等(1989) Science 244:1081-1085);通过接头扫描诱变(Gustin等(1993) Virology 193:653-660;Brown等(1992) Mol. Cell Biol. 12:2644-2652;McKnight等(1982) Science 232:316);通过饱和诱变(Meyers等(1986) Science 232:613);通过PCR诱变(Leung等(1989) Method Cell Mol Biol 1:11-19);或者通过随机诱变,包括化学诱变等(Miller等(1992) A Short Course in Bacterial Genetics,CSHL Press,Cold Spring Harbor,NY;以及Greener等(1994) Strategies in Mol Biol 7:32-34)。接头扫描诱变,特别在组合背景下,是用于鉴定截短(生物活性)形式的TβRII多肽的有吸引力的方法。

[0071] 本领域已知用于筛选通过点突变和截短制备的组合文库的基因产物和在这方面筛选具有某种性质的基因产物的cDNA文库的各种技术。这类技术一般可适用于快速筛选由TβRII多肽的组合诱变所产生的基因文库。最广泛采用的筛选大型基因文库的技术通常包括将基因文库克隆至可复制的表达载体中,用所得载体文库转化合适的细胞,并在其中所需活性的检测有利于相对容易地分离编码其产物被检测的基因的载体的条件下,表达组合基因。优选的测定法包括TβRII配体结合测定法和配体介导的细胞信号转导测定法。

[0072] 在某些实施方案中,除天然存在于TβRII多肽中的任何修饰以外,本公开内容的TβRII多肽还可包含翻译后修饰。这类修饰包括但不限于乙酰化、羧化、糖基化、磷酸化、脂化、聚乙二醇化(聚乙二醇)和酰化。因此,修饰的TβRII多肽可含有非氨基酸成分,例如聚乙二醇、脂质、单糖或多糖和磷酸酯。可按本文有关其它TβRII多肽变体方面所述,测试这类非氨基酸成分对TβRII多肽的功能性的作用。如果TβRII多肽在细胞中通过切割TβRII多肽的新生形式产生,则翻译后加工对于蛋白质的正确折叠和/或功能也可能十分重要。对于这类翻译后活性,不同的细胞(例如CHO、HeLa、MDCK、293、WI38、NIH-3T3或HEK293)具有特殊的细胞机器和特有机制,可选择不同的细胞以确保TβRII多肽的正确修饰和加工。

[0073] 在某些方面,TβRII多肽的功能变体或修饰形式包括具有TβRII多肽的至少一部分

和一个或多个融合结构域的融合蛋白。这类融合结构域的众所周知的实例包括但不限于聚组氨酸、Glu-Glu、谷胱甘肽S转移酶(GST)、硫氧还蛋白、A蛋白、G蛋白、免疫球蛋白重链恒定区(Fc)、麦芽糖结合蛋白(MBP)或人血清白蛋白。可选择融合结构域以提供所需要的性质。例如,一些融合结构域特别可用于通过亲和层析法分离融合蛋白。为了亲和纯化的目的,使用用于亲和层析法的相关基质,例如谷胱甘肽缀合树脂、淀粉酶缀合树脂和镍缀合树脂或钴缀合树脂。这类基质的许多种可以"试剂盒"形式获得,例如Pharmacia GST纯化系统和可与(HIS_6)融合配偶体一起使用的QIAexpress™系统(Qiagen)。作为另一个实例,可选择融合结构域以促进TβRII多肽的检测。这类检测结构域的实例包括各种荧光蛋白(例如GFP)以及"表位标签",其通常是短肽序列,可获得其特异性抗体。易获得其特异性单克隆抗体的众所周知的表位标签包括FLAG、流感病毒血凝素(HA)和c-myc标签。在一些情况下,融合结构域具有蛋白酶切割位点(例如因子Xa或凝血酶的切割位点),其允许相关蛋白酶部分消化融合蛋白,从而从中释放重组蛋白。然后,可通过随后的层析分离,从融合结构域分离出释放的蛋白质。在某些优选的实施方案中,TβRII多肽与体内稳定TβRII多肽的结构域("稳定剂"结构域)融合。所谓"稳定"意指延长血清半寿期的任何事物,不论这是否是因为破坏减少、肾清除率降低或其它药代动力学作用。已知与免疫球蛋白的Fc部分的融合物赋予多种蛋白质所需要的药代动力学性质。同样,与人血清白蛋白的融合物可赋予所需要的性质。可选择的融合结构域的其它类型包括多聚化(例如二聚化、四聚化)结构域和功能结构域。

[0074] 作为具体实例,本公开内容提供包含与3个Fc结构域序列之一(例如SEQ ID NO:19、20和21)融合的TβRII多肽的变体的融合蛋白。任选Fc结构域在例如Asp-265、Lys-322和Asn-434等残基(按照相应的全长IgG编码)处具有一个或多个突变。在某些情况下,相对于野生型Fc结构域,具有这些突变的一个或多个(例如Asp-265突变)的突变型Fc结构域与Fcγ受体结合的能力降低。在其它情况下,相对于野生型Fc结构域,具有这些突变的一个或多个(例如Asn-434突变)的突变型Fc结构域与MHC I类相关Fc-受体(FcRN)结合的能力提高。

[0075] 要了解,融合蛋白的不同成分可以与所需功能性一致的任何方式排列。例如,可将TβRII多肽置于异源结构域的C端,或者,可将异源结构域置于TβRII多肽的C端。TβRII多肽结构域和异源结构域在融合蛋白中不必邻接,并且任一结构域的C端或N端或在结构域之间可包括其它结构域或氨基酸序列。

[0076] 本文所用术语"免疫球蛋白Fc结构域"或简称"Fc"要理解为是指免疫球蛋白链恒定区、优选免疫球蛋白重链恒定区的羧基端部分或其部分。例如,免疫球蛋白Fc区可包含1)CH1结构域、CH2结构域和CH3结构域,2) CH1结构域和CH2结构域,3) CH1结构域和CH3结构域,4) CH2结构域和CH3结构域,或5)两个或更多个结构域和免疫球蛋白铰链区的组合。在一个优选的实施方案中,免疫球蛋白Fc区包含至少免疫球蛋白铰链区CH2结构域和CH3结构域,并优选缺乏CH1结构域。

[0077] 在一个实施方案中,重链恒定区来源于其中的免疫球蛋白类别为IgG (Igγ) (γ亚类1、2、3或4)。可使用免疫球蛋白的其它类别IgA (Igα)、IgD (Igδ)、IgE (Igε)和IgM (Igμ)。合适免疫球蛋白重链恒定区的选择详细论述于美国专利号5,541,087和5,726,044。为获得特定结果的来自某些免疫球蛋白类别和亚类的特定的免疫球蛋白重链恒定区序列的选择被视为在本领域的技术水平内。编码免疫球蛋白Fc区的DNA构建体的部分优选包含铰链结构域的至少一部分,优选Fc γ的CH_3结构域或IgA、IgD、IgE或IgM任一种的同源结构

域的至少一部分。

[0078] 此外，预期免疫球蛋白重链恒定区内氨基酸的取代或缺失可用于实施本文公开的方法和组合物。一个实例可为将氨基酸取代引入上部CH2区以产生对Fc受体的亲和力降低的Fc变体(Cole等(1997) J. Immunol. 159:3613)。

[0079] 在某些实施方案中，本公开内容可获得TβRII多肽的分离和/或纯化形式，其自其它蛋白质和/或其它TβRII多肽类分离或以别的方式基本不含(例如至少80%、90%、95%、96%、97%、98%或99%没有)其它蛋白质和/或其它TβRII多肽类。TβRII多肽一般可通过从重组核酸表达来产生。

[0080] 在某些实施方案中，本公开内容包括包含TβRII蛋白胞外部分的编码序列的编码可溶性TβRII多肽的核酸。在进一步的实施方案中，本公开内容还涉及包含这类核酸的宿主细胞。宿主细胞可以是任何原核细胞或真核细胞。例如，本公开内容的多肽可在细菌细胞(例如大肠杆菌(E. coli))、昆虫细胞(例如使用状病毒表达系统)、酵母或哺乳动物细胞中表达。其它合适的宿主细胞是本领域技术人员已知的。因此，本公开内容的一些实施方案还涉及产生TβRII多肽的方法。

[0081] 3. 编码TβRII多肽的核酸

在某些方面，本公开内容提供编码本文公开的TβRII多肽(包括片段、功能变体和融合蛋白)的任一种的分离核酸和/或重组核酸。SEQ ID NO: 26、28、30、32、34、36、38、40、42和44编码与IgG2 Fc或N端截短IgG1 Fc结构域融合的TβRII胞外域的变体。主题核酸可为单链或双链的。这类核酸可以是DNA或RNA分子。这些核酸可用于例如用于制备TβRII多肽的方法中或可用作直接的治疗剂(例如在反义物、RNAi或基因疗法方法中)。

[0082] 在某些方面，要进一步了解，编码TβRII多肽的主题核酸包括作为SEQ ID NO: 26、28、30、32、34、36、38、40、42和44的变体的核酸。变体核苷酸序列包括因一个或多个核苷酸取代、添加或缺失而不同的序列，例如等位基因变体。

[0083] 在某些实施方案中，本公开内容提供与SEQ ID NO: 26、28、30、32、34、36、38、40、42和44有至少80%、85%、90%、95%、96%、97%、98%、99%或100%同一性的分离核酸序列或重组核酸序列。本领域普通技术人员应认识到，与SEQ ID NO: 26、28、30、32、34、36、38、40、42和44互补的核酸序列和SEQ ID NO: 26、28、30、32、34、36、38、40、42和44的变体也落入本公开内容的范围内。在其它实施方案中，本公开内容的核酸序列可以是分离的、重组的和/或与异源核苷酸序列融合或在DNA文库中。

[0084] 在其它实施方案中，本公开内容的核酸还包括在高严格条件下与SEQ ID NO: 26、28、30、32、34、36、38、40、42和44中规定的核苷酸序列或SEQ ID NO: 26、28、30、32、34、36、38、40、42和44的互补序列或其片段杂交的核苷酸序列。如上所述，本领域普通技术人员容易了解，可以改变促进DNA杂交的合适的严格性条件。例如，可在6.0 x氯化钠/柠檬酸钠(SSC)下于约45℃进行杂交，接着2.0 x SSC于50℃洗涤。例如，可从约2.0 x SSC于50℃的低严格性到约0.2 x SSC于50℃的高严格性，选择洗涤步骤的盐浓度。另外，可从室温(约22℃)下的低严格性条件提高洗涤步骤的温度到约65℃下的高严格性条件。温度和盐两者均可改变，或者可保持温度或盐浓度恒定，而改变另一个变量。在一些实施方案中，本公开内容提供在6 x SSC于室温的低严格性条件下杂交接着2 x SSC于室温洗涤的核酸。

[0085] 由于遗传密码的简并性所致不同于SEQ ID NO: 26、28、30、32、34、36、38、40、42和

44所示核酸的分离核酸也在本公开内容的范围内。例如，多种氨基酸用不只一个三联体指定。指定同一氨基酸的密码子或同义密码子(例如CAU和CAC是组氨酸的同义密码子)，可导致不影响蛋白质的氨基酸序列的“沉默”突变。然而，预期在哺乳动物细胞中可存在确实引起主题蛋白质氨基酸序列改变的DNA序列多态性。本领域技术人员认识到，由于天然等位基因变化所致，在给定物种的个体之间，这些变化可存在编码特定蛋白质的核酸的一个或多个核苷酸(多至约3-5%的核苷酸)中。任何和所有这类核苷酸变化和所得氨基酸多态性也在本公开内容的范围内。

[0086] 本领域技术人员应认识到，基于TβRII的长同种型的相应变体可包括编码25个氨基酸插入以及插入C端的侧翼位置处的保守Val-Ile取代的核苷酸序列。还应认识到，基于TβRII的长(A)或短(B)同种型的相应变体可包括包含在天然存在的TβRII同种型C所述相同位置处编码36个氨基酸插入(SEQ ID NO: 18)的108个核苷酸的插入的变体核苷酸序列(参见实例)。

[0087] 在某些实施方案中，在表达构建体中，本公开内容的重组核酸可与一个或多个调节核苷酸序列有效连接。调节核苷酸序列一般可适于用于表达的宿主细胞。本领域已知用于各种宿主细胞的合适表达载体和合适调节序列的许多类型。所述一个或多个调节核苷酸序列通常可包括但不限于启动子序列、前导序列或信号序列、核糖体结合位点、转录起始序列和终止序列、翻译起始序列和终止序列及增强子或激活物序列。本公开内容考虑本领域已知的组成型启动子或诱导型启动子。启动子可以是天然存在的启动子或将不止一种启动子的元件组合的杂合启动子。表达构建体可存在于细胞的附加体(例如质粒)上，或者表达构建体可插入染色体中。在一个优选的实施方案中，表达载体含有选择标记基因以供转化宿主细胞的选择。选择标记基因是本领域众所周知的，并可随所采用的宿主细胞而改变。

[0088] 在本文公开的某些方面，在包含编码TβRII多肽并与至少一个调节序列有效连接的核苷酸序列的表达载体中提供主题核酸。调节序列是本领域公认的，并被选择来指导表达TβRII多肽。因此，术语调节序列包括启动子、增强子和其它表达调控元件。示例性的调节序列描述于Goeddel;Gene Expression Technology: Methods in Enzymology, Academic Press, San Diego, CA (1990)。例如，当与DNA序列有效连接时控制DNA序列表达的各种表达调控序列的任一种，可被用于这些载体以表达编码TβRII多肽的DNA序列。这类有用的表达调控序列包括例如SV40的早期启动子和晚期启动子、tet启动子、腺病毒或巨细胞病毒立即早期启动子、RSV启动子、lac系统、trp系统、TAC或TRC系统、其表达受T7 RNA聚合酶指导的T7启动子、噬菌体λ的主要操纵基因和启动子区、fd外壳蛋白的调控区、3-磷酸甘油酸激酶或其它糖酵解酶的启动子、酸性磷酸酶启动子(例如Pho5)、酵母α-交配因子启动子、杆状病毒系统的多角体(polyhedron)启动子和已知控制原核细胞或真核细胞或其病毒的基因表达的其它序列及其各种组合。应当了解，表达载体的设计可取决于以下这类因素，例如待转化的宿主细胞的选择和/或欲表达的蛋白质类型。此外，还应考虑载体拷贝数、控制该拷贝数的能力和由该载体编码的任何其它蛋白质(例如抗生素标记)的表达。

[0089] 可通过将克隆的基因或其部分连接至适于在原核细胞、真核细胞(酵母、禽、昆虫或哺乳动物)或两者中表达的载体中来产生本公开内容所包括的重组核酸。用于产生重组TβRII多肽的表达载体包括质粒和其它载体。例如，合适的载体包括用于在原核细胞(例如大肠杆菌)中表达的以下类型的质粒：pBR322衍生质粒、pEMBL衍生质粒、pEX衍生质粒、pBTac

衍生质粒和pUC衍生质粒。

[0090] 一些哺乳动物表达载体既含有利于载体在细菌中繁殖的原核序列，又含有在真核细胞中表达的一个或多个真核转录单位。pcDNAI/amp、pcDNAI/neo、pRc/CMV、pSV2gpt、pSV2neo、pSV2-dhfr、pTk2、pRSVneo、pMSG、pSVT7、pko-neo和pHyg衍生载体是适于转染真核细胞的哺乳动物表达载体的实例。这些载体的一些用来源于细菌质粒(例如pBR322)的序列修饰，以利于在原核细胞和真核细胞两者中复制和进行药物抗性选择。或者，可使用诸如牛乳头瘤病毒(BPV-1)或埃巴病毒(pHEBo、pREP衍生物和p205)等病毒衍生物在真核细胞中瞬时表达蛋白质。其它病毒(包括反转录病毒)表达系统的实例可参见下面基因疗法递送系统的描述。用于质粒制备和宿主生物转化中的各种方法是本领域众所周知的。对于原核和真核细胞两者的其它合适的表达系统以及通用重组方法，参见MolecularCloning A Laboratory Manual, 第3版, 编辑Sambrook, Fritsch和Maniatis (Cold Spring Harbor Laboratory Press, 2001)。在某些情况下，可能需要通过利用杆状病毒表达系统来表达重组多肽。这类杆状病毒表达系统的实例包括pVL衍生载体(例如pVL1392、pVL1393和pVL941)、pAcUW衍生载体(例如pAcUW1)和pBlueBac衍生载体(例如含β-gal的pBlueBac III)。

[0091] 在某些实施方案中，可设计在CHO细胞中产生主题TβRII多肽的载体，例如Pcmv-Script载体(Stratagene,La Jolla,Calif.)、pcDNA4载体(Invitrogen,Carlsbad,Calif.)和pCI-neo载体(Promega,Madison,Wisc.)。在一个优选的实施方案中，可设计在HEK-293细胞中产生主题TβRII多肽的载体。显然，可使用主题基因构建体在于培养基中繁殖的细胞中使主题TβRII多肽表达，例如产生蛋白质(包括融合蛋白或变体蛋白)以便纯化。

[0092] 本公开内容还涉及用包括一个或多个主题TβRII多肽的编码序列(例如SEQ ID NO: 26、28、30、32、34、36、38、40、42或44)的重组基因转染的宿主细胞。宿主细胞可以是任何原核或真核细胞。例如，本文公开的TβRII多肽可在细菌细胞(例如大肠杆菌)、昆虫细胞(例如使用杆状病毒表达系统)、酵母或哺乳动物细胞中表达。其它合适的宿主细胞是本领域技术人员已知的。

[0093] 因此，本公开内容进一步涉及产生主题TβRII多肽的方法。例如，可将用编码TβRII多肽的表达载体转染的宿主细胞在允许TβRII多肽表达发生的合适条件下培养。可使TβRII多肽分泌并从细胞和含有TβRII多肽的培养基的混合物中分离出来。或者，可使TβRII多肽保持在胞质或在膜部分中，然后收获、裂解细胞并分离蛋白质。细胞培养物包括宿主细胞和培养基。用于细胞培养的合适培养基是本领域众所周知的。可采用本领域已知的用于纯化蛋白质的技术，包括离子交换层析法、凝胶过滤层析法、超滤法、电泳、使用对TβRII多肽的特定表位有特异性的抗体进行的免疫亲和纯化以及用结合与TβRII多肽融合的结构域的物质进行的亲和纯化(例如A蛋白柱可用来纯化TβRII-Fc融合物)，将主题TβRII多肽从细胞培养基、宿主细胞或两者中分离出来。在一个优选的实施方案中，TβRII多肽是含有促进其纯化的结构域的融合蛋白。例如，通过一系列的柱层析步骤达到纯化，所述步骤包括例如任何顺序的下列三种或更多种方法:A蛋白层析法、Q琼脂糖凝胶层析法、苯基琼脂糖凝胶层析法、大小排阻层析法和阳离子交换层析法。纯化可用病毒过滤和缓冲液更换来完成。

[0094] 在另一个实施方案中，编码纯化前导序列(例如重组TβRII多肽的所需部分的N端上的聚-(His)/肠激酶切割位点序列)的融合基因，可允许使用Ni^{2+}金属树脂通过亲和层析

法对表达的融合蛋白进行纯化。然后，纯化前导序列随后可通过肠激酶处理除去，得到纯化的TβRII多肽(例如参见Hochuli等(1987) J. Chromatography 411:177;以及Janknecht等,PNAS USA 88:8972)。

[0095] 制备融合基因的技术是众所周知的。基本上，编码不同多肽序列的各种DNA片段的连接按照常规技术进行，采用用于连接的平端或交错端，限制性内切酶消化以提供合适末端，适当时补平黏性末端，碱性磷酸酶处理以避免不需要的连接，并进行酶促连接。在另一个实施方案中，融合基因可通过常规技术合成，包括自动DNA合成仪。或者，可使用在两个连续基因片段之间产生互补突出端的锚定引物进行基因片段的PCR扩增，所述基因片段随后可退火得到嵌合基因序列(参见例如Current Protocols in Molecular Biology，编辑Ausubel等,John Wiley & Sons:1992)。

[0096] 作为TβRII、TGFβ1、TGFβ3和GDF15拮抗剂的核酸化合物类别的实例包括反义核酸、RNAi构建体和催化性核酸构建体。核酸化合物可以是单链或双链的。双链化合物还可包括其中链的一条或另一条是单链的突出端区或非互补区。单链化合物可包括自我互补区，意指化合物形成具有双螺旋结构区的所谓“发夹”或“茎-环”结构。核酸化合物可包含以下核苷酸序列，其与由不超过全长TβRII核酸序列或配体核酸序列的1000个、不超过500个、不超过250个、不超过100个或不超过50、35、30、25、22、20或18个核苷酸组成的区域互补。互补区优选为至少8个核苷酸，任选至少10或至少15个核苷酸，例如介于15和25个核苷酸之间。互补区可落入靶转录物的内含子、编码序列或非编码序列中，例如编码序列部分。核酸化合物的长度一般可为约8-约500个核苷酸或碱基对，例如约14-约50个核苷酸。核酸可以是DNA(特别对于用作反义物)、RNA或RNA:DNA杂合体。任一条链可包括DNA和RNA的混合物以及不容易归类为DNA或RNA的修饰形式。同样，双链化合物可以是DNA:DNA、DNA:RNA或RNA:RNA，任一条链还可包括DNA和RNA的混合物，以及不易归类为DNA或RNA的修饰形式。核酸化合物可包括各种修饰的任一种，包括骨架(天然核酸中的糖-磷酸酯部分，包括核苷酸间键)或碱基部分(天然核酸的嘌呤或嘧啶部分)的一种或多种修饰。反义核酸化合物的长度可优选为约15-约30个核苷酸，并且通常含有一种或多种修饰以改进特性，例如在血清、细胞或化合物很可能被递送至的部位(例如在口服递送化合物的情况下为胃，而对吸入化合物而言为肺)中的稳定性。在RNAi构建体的情况下，与靶转录物互补的链一般是RNA或其修饰物。另一条链可以是RNA、DNA或任何其它变异物。双链或单链“发夹” RNAi构建体的双链体部分的长度可优选为18-40个核苷酸，任选长度为约21-23个核苷酸，只要它用作切酶底物即可。催化性核酸或酶促核酸(enzymatic nucleic acid)可以是核酶或DNA酶，并且还可含有修饰形式。当在生理条件下和在其中无义或有义对照几乎没有作用或者没有作用的浓度下与细胞接触时，核酸化合物可抑制靶标表达达约50%、75%、90%或更高。测试核酸化合物的作用的优选浓度为1、5和10微摩尔。也可测试核酸化合物对例如血管生成的作用。

[0097] 4. Fc-融合蛋白的变化

本申请还提供具有改造的Fc区或变体Fc区的TβRII-Fc融合蛋白。这类抗体与Fc融合蛋白可用于例如调节效应子功能，例如依赖抗原的细胞毒性(ADCC)和依赖补体的细胞毒性(CDC)。此外，修饰可改进抗体与Fc融合蛋白的稳定性。通过将合适的核苷酸变化引入DNA或通过肽合成来制备抗体与Fc融合蛋白的氨基酸序列变体。这类变体包括例如本文公开的抗体与Fc融合蛋白的氨基酸序列内残基的缺失和/或插入和/或取代。进行缺失、插入和取代

的任何组合以得到最终构建体，条件是最终构建体具有所需特性。氨基酸变化也可改变抗体与Fc融合蛋白的翻译后加工，例如改变糖基化位点的数目或位置。

[0098] 可通过将变化引入氨基酸序列，包括但不限于Bluestone等(参见WO 94/28027和WO 98/47531；另参见Xu等, 2000 Cell Immunol 200;16-26)描述的Ala-Ala突变，来产生效应子功能降低的抗体与Fc融合蛋白。因此，在某些实施方案中，具有恒定区内的突变(包括Ala-Ala突变)的本公开内容的抗体与Fc融合蛋白可用来降低或消除效应子功能。按照这些实施方案，抗体与Fc融合蛋白可包含在234位至丙氨酸的突变或在235位至丙氨酸的突变或其组合。在一个实施方案中，抗体或Fc融合蛋白包含IgG4构架，其中Ala-Ala突变可描述在234位从苯丙氨酸到丙氨酸的突变和/或在235位从亮氨酸到丙氨酸的突变。在另一个实施方案中，抗体或Fc融合蛋白包含IgG1构架，其中Ala-Ala突变可描述在234位从亮氨酸到丙氨酸的突变和/或在235位从亮氨酸到丙氨酸的突变。抗体或Fc融合蛋白可备选或另外携带其它突变，包括CH2结构域的点突变K322A (Hezareh等, 2001 J Virol. 75: 12161-8)。

[0099] 在具体的实施方案中，可对抗体或Fc融合蛋白进行修饰以增强或抑制依赖补体的细胞毒性(CDC)。可通过将一个或多个氨基酸取代、插入或缺失引入Fc区，来实现受调节的CDC活性(参见例如美国专利号6,194,551)。备选或此外，可将半胱氨酸残基引入Fc区，从而允许在该区形成链间二硫键。由此产生的同二聚抗体可具有改进或降低的内化能力和/或增强或减弱的补体介导的细胞杀死。参见Caron等, J. Exp Med. 176:1191-1195 (1992)和Shopes, B. J. Immunol. 148:2918-2922 (1992)；WO99/51642；Duncan和Winter Nature 322: 738-40 (1988)；美国专利号5,648,260；美国专利号5,624,821和WO94/29351。

[0100] 5. GDF15-TβRII信号转导

本公开内容部分涉及TGFβ II型受体(TβRII)以高亲和力与GDF15结合的发现。迄今为止，未表明GDF15在生物化学上直接与受体结合或相互作用。不充分或不适当的配体纯化可能是市购可获得的GDF15失活的可能原因。本文公开了证实TβRII结合活性的示例性GDF15多肽和制备和纯化这类多肽的方法。来自人和小鼠的天然前体GDF15蛋白和核苷酸的序列见图1-4。成熟的人GDF15从SEQ ID NO: 1的残基197延伸至308。同样地，成熟的小鼠GDF15从SEQ ID NO: 3的残基192延伸至303。在某些实施方案中，本公开内容使得可获得从其它蛋白质和/或其它GDF15多肽类分离或以别的方式基本不含(例如至少80%、90%、95%、96%、97%、98%或99%没有)其它蛋白质和/或其它GDF15多肽类的GDF15多肽或其片段的分离和/或纯化形式。本公开内容的GDF15多肽以高亲和力与TβRII结合。可在溶液中或在表面等离子体共振系统(例如Biacore™系统)中，使用纯化的蛋白质评价结合。对于TβRII多肽，GDF15多肽可具有约10^{-6}、10^{-7}、10^{-8}、10^{-9} M或更小的亲和力(解离常数)。优选按照本文所述方法分离和纯化本公开内容的GDF15多肽。GDF15多肽一般通过从重组核酸表达来产生。

[0101] GDF15多肽可包括由与SEQ ID NO: 1或SEQ ID NO: 3的GDF15多肽有至少80%同一性、任选至少85%、90%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列组成或包含所述氨基酸序列的多肽或其功能片段。GDF15多肽可包括由与包含SEQ ID NO: 1的残基197-308或SEQ ID NO: 3的残基192-303的GDF15多肽有至少80%同一性、任选至少85%、90%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列组成或包含所述氨基酸序列的多肽或其功能片段。未加工的GDF15多肽可包括或不包括任何信号序列以及信号序列N端的任何序列。

GDF15多肽可包括SEQ ID NO: 1或SEQ ID NO: 3或其分别相当于SEQ ID NO: 1的197-308或SEQ ID NO: 3的残基19-303的部分的变体(包括在SEQ ID NO: 1或SEQ ID NO: 3的序列中包含例如不大于2、3、4、5、10、15、20、25、30或35个氨基酸取代的变体)、其片段和包含前述的任一个的融合蛋白,但在各种情况下,优选前述GDF15多肽的任一个对TβRII多肽可具有基本亲和力。

[0102] 在某些实施方案中,除天然存在于GDF15多肽中的任何修饰以外,本公开内容的GDF15多肽还可包含翻译后修饰。这类修饰包括但不限于乙酰化、羧化、糖基化、磷酸化、脂化、聚乙二醇化(聚乙二醇)和酰化。因此,修饰的GDF15多肽可含有非氨基酸成分,例如聚乙二醇、脂质、单糖或多糖和磷酸酯。可按本文有关其它GDF15多肽方面所述,测试这类非氨基酸成分对GDF15多肽的功能性的作用。如果GDF15多肽在细胞中通过切割GDF15多肽的新生形式产生,则翻译后加工对于蛋白质的正确折叠和/或功能也可能十分重要。对于这类翻译后活性,不同的细胞(例如CHO、HeLa、MDCK、293、WI38、NIH-3T3或HEK-293)具有特殊的细胞机器和特有机制,可选择不同的细胞以确保GDF15多肽的正确修饰和加工。

[0103] 在某些实施方案中,本公开内容包括编码前体和成熟GDF15多肽的核酸。在其它实施方案中,本公开内容还涉及包含这类核酸的宿主细胞。宿主细胞可以是任何原核或真核细胞。例如,本公开内容的多肽可在细菌细胞(例如大肠杆菌)、昆虫细胞(例如使用杆状病毒表达系统)、酵母或哺乳动物细胞中表达。其它合适的宿主细胞是本领域技术人员已知的。因此,本公开内容的一些实施方案还涉及产生GDF15多肽的方法。

[0104] 在某些方面,本公开内容提供编码本文公开的GDF15多肽(包括片段、功能变体和融合蛋白)的任一种的分离和/或重组核酸。主题核酸可以是单链或双链的。这类核酸可以是DNA或RNA分子。这些核酸可用于例如用于制备GDF15多肽的方法中或用作直接的治疗剂(例如在反义物、RNAi或基因疗法方法)。

[0105] 在某些方面,编码GDF15多肽的主题核酸要进一步理解为包括作为SEQ ID NO: 1或SEQ ID NO: 3的变体的核酸。变体核苷酸序列包括因一个或多个核苷酸取代、添加或缺失而不同的序列,例如等位基因变体。

[0106] 在某些实施方案中,本公开内容提供与SEQ ID NO: 1或SEQ ID NO: 3有至少80%、85%、90%、95%、96%、97%、98%、99%或100%同一性的分离核酸序列或重组核酸序列。本领域普通技术人员应认识到,与SEQ ID NO: 1或SEQ ID NO: 3互补的核酸序列和SEQ ID NO: 1或SEQ ID NO: 3的变体也落入本公开内容的范围内。在其它实施方案中,本公开内容的核酸序列可以是分离的、重组的和/或与异源核苷酸序列融合,或在DNA文库中。

[0107] 在其它实施方案中,本公开内容的核酸还包括在高严格条件下与SEQ ID NO: 1或SEQ ID NO: 3规定的核苷酸序列、SEQ ID NO: 1或SEQ ID NO: 3的互补序列或其片段杂交的核苷酸序列。如上所述,本领域普通技术人员容易了解,可以改变促进DNA杂交的合适的严格性条件。例如,可在6.0 x氯化钠/柠檬酸钠(SSC)中于约45℃进行杂交,接着2.0 x SSC于50℃洗涤。例如,可从约2.0 x SSC于50℃的低严格性到约0.2 x SSC于50℃的高严格性,选择洗涤步骤的盐浓度。另外,可从室温(约22℃)下的低严格性条件提高洗涤步骤的温度到约65℃下的高严格性条件。温度和盐两者均可改变,或者可保持温度或盐浓度恒定,而改变另一个变量。在一些实施方案中,本公开内容提供在6 x SSC于室温的低严格性条件下杂交接着2 x SSC于室温洗涤的核酸。

[0108] 由于遗传密码的简并性所致不同于SEQ ID NO: 1或SEQ ID NO: 3所示核酸的分离核酸也在本公开内容的范围内。例如，多种氨基酸用不只一个三联体指定。指定同一氨基酸的密码子或同义密码子(例如CAU和CAC是组氨酸的同义密码子)，可导致不影响蛋白质的氨基酸序列的“沉默”突变。然而，预期在哺乳动物细胞中可存在确实引起主题蛋白质氨基酸序列改变的DNA序列多态性。本领域技术人员应认识到，由于天然等位基因变化所致，在给定物种的个体之间，这些变化可存在编码特定蛋白质的核酸的一个或多个核苷酸(多至约3-5%的核苷酸)中。任何和所有这类核苷酸变化和所得氨基酸多态性也在本公开内容的范围内。

[0109] 在某些实施方案中，在表达构建体中，本公开内容的重组核酸可与一个或多个调节核苷酸序列有效连接。调节核苷酸序列一般可适于用于表达的宿主细胞。本领域已知用于各种宿主细胞的合适表达载体和合适调节序列的许多类型。所述一个或多个调节核苷酸序列通常可包括但不限于启动子序列、前导序列或信号序列、核糖体结合位点、转录起始序列和终止序列、翻译起始序列和终止序列及增强子或激活物序列。本公开内容考虑本领域已知的组成型启动子或诱导型启动子。启动子可以是天然存在的启动子或将不止一种启动子的元件组合的杂合启动子。表达构建体可存在于细胞的附加体(例如质粒)上，或者表达构建体可插入染色体中。在一个优选的实施方案中，表达载体含有选择标记基因以供转化宿主细胞的选择。选择标记基因是本领域众所周知的，并可随所采用的宿主细胞而改变。

[0110] 在本文公开的某些方面，在包含编码GDF15多肽并与至少一个调节序列有效连接的核苷酸序列的表达载体中提供主题核酸。调节序列是本领域公认的，并被选择来指导表达GDF15多肽。因此，术语调节序列包括启动子、增强子和其它表达调控元件。示例性的调节序列描述于Goeddel;Gene Expression Technology: Methods in Enzymology, Academic Press, San Diego, CA (1990)。例如，当与DNA序列有效连接时控制DNA序列表达的各种表达调控序列的任一种，可被用于这些载体以表达编码GDF15多肽的DNA序列。这类有用的表达调控序列包括例如SV40的早期启动子和晚期启动子、tet启动子、腺病毒或巨细胞病毒立即早期启动子、RSV启动子、lac系统、trp系统、TAC或TRC系统、其表达受T7 RNA聚合酶指导的T7启动子、噬菌体λ的主要操纵基因和启动子区、fd外壳蛋白的调控区、3-磷酸甘油酸激酶或其它糖酵解酶的启动子、酸性磷酸酶启动子(例如Pho5)、酵母α-交配因子启动子、杆状病毒系统的多角体启动子和已知控制原核细胞或真核细胞或其病毒的基因表达的其它序列及其各种组合。应当了解，表达载体的设计可取决于以下这类因素，例如待转化的宿主细胞的选择和/或欲表达的蛋白质类型。此外，还应考虑载体拷贝数、控制该拷贝数的能力和由该载体编码的任何其它蛋白质(例如抗生素标记)的表达。

[0111] 可通过将克隆的基因或其部分连接至适于在原核细胞、真核细胞(酵母、禽、昆虫或哺乳动物)或两者中表达的载体中来产生本公开内容所包括的重组核酸。用于产生重组GDF15多肽的表达载体包括质粒和其它载体。例如，合适的载体包括用于在原核细胞(例如大肠杆菌)中表达的以下类型的质粒：pBR322衍生质粒、pEMBL衍生质粒、pEX衍生质粒、pBTac衍生质粒和pUC衍生质粒。

[0112] 一些哺乳动物表达载体既含有利于载体在细菌中繁殖的原核序列，又含有在真核细胞中表达的一个或多个真核转录单位。pcDNAI/amp、pcDNAI/neo、pRc/CMV、pSV2gpt、pSV2neo、pSV2-dhfr、pTk2、pRSVneo、pMSG、pSVT7、pko-neo和pHyg衍生载体是适于转染真核

细胞的哺乳动物表达载体的实例。这些载体的一些用来源于细菌质粒(例如pBR322)的序列修饰,以利于在原核细胞和真核细胞两者中复制和进行药物抗性选择。或者,可使用诸如牛乳头瘤病毒(BPV-1)或埃巴病毒(pHEBo、pREP衍生物和p205)等病毒衍生物在真核细胞中瞬时表达蛋白质。其它病毒(包括反转录病毒)表达系统的实例可参见下面基因疗法递送系统的描述。用于质粒制备和宿主生物转化中的各种方法是本领域众所周知的。对于原核和真核细胞两者的其它合适的表达系统以及通用重组方法,参见MolecularCloning A Laboratory Manual, 第3版, 编辑Sambrook, Fritsch和Maniatis (Cold Spring Harbor Laboratory Press, 2001)。在某些情况下,可能需要通过利用杆状病毒表达系统来表达重组多肽。这类杆状病毒表达系统的实例包括pVL衍生载体(例如pVL1392、pVL1393和pVL941)、pAcUW衍生载体(例如pAcUW1)和pBlueBac衍生载体(例如含β-gal的pBlueBac III)。

[0113] 在一个优选的实施方案中,可设计在CHO细胞中产生主题TβRII多肽的载体,例如Pcmv-Script载体(Stratagene,La Jolla,Calif.)、pcDNA4载体(Invitrogen,Carlsbad,Calif.)和pCI-neo载体(Promega,Madison,Wisc.)和UCOE™衍生载体(Millipore)。显然,可使用主题基因构建体在于培养基中繁殖的细胞中使主题GDF15多肽表达,例如产生蛋白质(包括融合蛋白或变体蛋白)以便纯化。

[0114] 本公开内容还涉及包括用一个或多个主题GDF15多肽的编码序列(例如SEQ ID NO: 1或SEQ ID NO:3)的重组基因转染的宿主细胞。宿主细胞可以是任何原核或真核细胞。例如,本文公开的GDF15多肽可在细菌细胞(例如大肠杆菌)、昆虫细胞(例如使用杆状病毒表达系统)、酵母或哺乳动物细胞中表达。其它合适的宿主细胞是本领域技术人员已知的。在一个优选的实施方案中,本文公开的GDF15多肽在CHO细胞中表达。

[0115] 因此,本发明进一步涉及产生主题GDF15多肽的方法。例如,可将用编码GDF15多肽的表达载体转染的宿主细胞在允许GDF15多肽表达发生的合适条件下培养。可使GDF15多肽分泌并从细胞和含有GDF15多肽的培养基的混合物中分离出来。或者,可使TβRII多肽保持在胞质或在膜部分中,然后收获、裂解细胞并分离蛋白质。细胞培养物包括宿主细胞和培养基。用于细胞培养的合适培养基是本领域众所周知的。可采用本领域已知的用于纯化蛋白质的技术,包括离子交换层析法、凝胶过滤层析法、超滤法、电泳、使用对GDF15多肽的特定表位有特异性的抗体进行的免疫亲和纯化以及用结合与GDF15多肽融合的结构域的物质进行的亲和纯化(例如A蛋白柱可用来纯化GDF15-Fc融合物),将主题GDF15多肽从细胞培养基、宿主细胞或两者中分离出来。在一个优选的实施方案中,GDF15多肽是含有促进其纯化的结构域的融合蛋白。例如,通过一系列的柱层析步骤达到纯化,所述步骤包括例如任何顺序的下列三种或更多种方法:A蛋白层析法、Q琼脂糖凝胶层析法、苯基琼脂糖凝胶层析法、大小排阻层析法和阳离子交换层析法。纯化可用病毒过滤和缓冲液更换来完成。

[0116] 在一个优选的实施方案中,采用一系列阳离子交换柱层析步骤,将主题GDF15多肽从培养基中纯化。用于阳离子交换柱的材料的实例可以是具有例如羧甲基(CM)、磺乙基(SE)、磺丙基(SP)、磷酸基(P)和磺酸基(S)等取代基的树脂。用于阳离子交换柱层析法的材料的实例包括SP Sepharose™ Fast Flow、Q Sepharose™ Fast Flow、DEAE Sepharose™ Fast Flow、Capto™ S、Capto™ DEAE (GE Healthcare)、S HyperCel™ (Pall)、TOYOPEARL GigaCap S-650 (TOSOH)或弱阳离子交换体例如羧甲基。优选SP Sepharose™

Fast Flow和Q Sepharose™ Fast Flow。

[0117] 为了开始纯化，需要时，可调节稳定表达GDF15多肽的宿主细胞的条件培养基的参数，例如pH、离子强度和温度。在一些实施方案中，冲洗层析柱，在与含多肽的上清液接触之前用一种或多种溶液平衡。这类溶液可包括例如缓冲液(例如介于1-500 mM、25-100 mM、15-30 mM之间或20 mM的例如Tris、MES、HEPES、组氨酸、磷酸盐或乙酸钠)和/或盐(例如介于0-2 M、1-2 M或500 mM-1M之间的例如NaCl、$NaPO_4$、乙酸钠或$CaCl_2$)。平衡溶液的pH的范围一般为3.5-10（例如介于pH 3.5-6、4.0-5.5、4.5-4.8之间或4.7)。在使柱与含多肽的流体接触后，可洗涤结合的柱。洗涤溶液可包括缓冲液(例如介于1-500 mM、25-100 mM、15-30 mM之间或20 mM的例如Tris、MES、HEPES、组氨酸、磷酸盐或乙酸钠)和/或盐(例如介于0-2 M、1-2 M、100 mM-1M或100 mM-500 mM之间的例如NaCl、$NaPO_4$、乙酸钠或$CaCl_2$)和/或添加剂(例如胍、脲、蔗糖、精氨酸或精氨酸衍生物)和/或溶剂(例如乙醇、乙腈或聚乙二醇)。洗涤溶液一般具有介于3.5和10之间的pH（例如介于4.5和8.0之间的pH)。可采用pH、盐类型、盐浓度、溶剂类型、溶剂浓度、置换剂类型、置换剂浓度或其组合的步进或梯度变化，将多肽从柱中洗脱。一般而言，为了将多肽从柱中洗脱，使培养基与洗脱缓冲液接触。在一些实施方案中，洗脱缓冲液含有缓冲液(例如10-100 mM、25-75 mM或50 mM的例如HEPES或Tris)和/或含有盐(例如0-2 M、例如10-100 mM的例如NaCl或$CaCl_2$)。在一些实施方案中，洗脱缓冲液可含有甘氨酸、乙酸或柠檬酸(例如20-250 mM或150 mM)。洗脱缓冲液还可含有乙酸(例如20 mM-约50 mM)、添加剂(例如1-10 M、2-8 M或6 M的例如胍、脲或蔗糖)和/或溶剂(例如乙醇、乙腈、聚乙二醇，例如1-10%溶剂、例如5%溶剂)。洗脱缓冲液的pH的范围可为约5.0-约10.0。在一些实施方案中，可改变(例如逐步改变) pH以产生梯度洗脱。在一些实施方案中，洗脱缓冲液的pH为约8.0。在一些实施方案中，进行一系列柱层析步骤。

[0118] 本文提供的数据表明，TβRII多肽用作GDF15信号转导的拮抗剂。虽然可溶性TβRII多肽、特别是TβRII-Fc是优选的拮抗剂，但是预期其它类型的GDF15拮抗剂是有用的，包括抑制GDF15或TβRII产生的抗GDF15抗体、抗TβRII抗体、反义物、RNAi或核酶核酸和GDF15或TβRII的其它抑制剂，特别是破坏GDF15-TβRII结合的抑制剂。

[0119] 与GDF15多肽起特异性反应且与GDF15多肽结合使得与其竞争结合(竞争性地结合) TβRII多肽或以别的方式抑制GDF15介导的信号转导的抗体可用作GDF15多肽活性的拮抗剂。同样地，与TβRII多肽有特异性反应且破坏GDF15结合的抗体可用作拮抗剂。

[0120] 可通过标准方案，使用来源于GDF15多肽或TβRII多肽的免疫原，制备抗蛋白质/抗肽抗血清或单克隆抗体(参见例如Antibodies: A Laboratory Manual, Harlow和Lane编辑(Cold Spring Harbor Press: 1988))。哺乳动物，例如小鼠、仓鼠或兔可用免疫原形式的GDF15多肽、能够诱导抗体应答的抗原片段或融合蛋白免疫接种。赋以蛋白质或肽免疫原性的技术包括与载体缀合或本领域众所周知的其它技术。可在佐剂存在下给予GDF15或TβRII多肽的免疫原性部分。可通过检测血浆或血清中的抗体效价监测免疫接种的过程。可应用标准ELISA或其它免疫测定法用免疫原作为抗原评价抗体水平。

[0121] 在动物用GDF15多肽的抗原制备物进行免疫接种后，可获得抗血清，如有需要，可从血清中分离出多克隆抗体。为了产生单克隆抗体，可从经免疫接种的动物中收获产抗体细胞(淋巴细胞)，并通过标准体细胞融合方法与永生化细胞(例如骨髓瘤细胞)融合，得到杂交瘤细胞。这类技术是本领域众所周知的，包括例如杂交瘤技术(最初由Kohler和

Milstein,(1975) Nature,256:495-497开发);人B细胞杂交瘤技术(Kozbar等(1983) Immunology Today, 4: 72);以及产生人单克隆抗体的EBV-杂交瘤技术(Cole等(1985) Monoclonal Antibodies and Cancer herapy, Alan R. Liss, Inc. 第77-96页)。可按免疫化学法筛选杂交瘤细胞以产生与GDF15多肽特异性反应的抗体,并从包含这类杂交瘤细胞的培养物中分离单克隆抗体。

[0122] 本文使用的术语"抗体"旨在包括其同样与主题多肽特异性反应的片段。可采用常规技术使抗体片段化,并以与上述用于完整抗体同样的方法针对效用筛选片段。例如,可通过用胃蛋白酶处理抗体得到$F(ab)_2$片段。可处理所得$F(ab)_2$片段,以将二硫桥还原得到Fab片段。本发明的抗体还旨在包括双特异性、单链、嵌合、人源化和完整人分子,其具有由抗体的至少一个CDR区提供的对TβRII或GDF15多肽的亲和力。抗体还可包含与之连接并且能够检出的标记(例如标记可以是放射性同位素、荧光化合物、酶或酶辅因子)。

[0123] 在某些实施方案中,抗体是重组抗体,该术语包括部分通过分子生物学技术产生的任何抗体,包括CDR移植抗体或嵌合抗体、人抗体或从文库选择抗体结构域装配的其它抗体、单链抗体和单一结构域抗体(例如人V_H蛋白或骆驼(camelid) V_{HH}蛋白)。在某些实施方案中,本发明的抗体是单克隆抗体,而在某些实施方案中,本发明可获得产生新抗体的方法。例如,产生与GDF15多肽或TβRII多肽特异性结合的单克隆抗体的方法,可包括给予小鼠一定量的有效刺激可检测免疫应答的包含抗原多肽的免疫原性组合物,由小鼠得到产抗体细胞(例如得自脾的细胞),并将产抗体细胞与骨髓瘤细胞融合,得到产抗体杂交瘤,测试产抗体杂交瘤以鉴定产生与抗原特异性结合的单克隆抗体的杂交瘤。一旦得到所述杂交瘤,便可将杂交瘤在细胞培养物中增殖,任选在杂交瘤衍生细胞产生与抗原特异性结合的单克隆抗体的培养条件下增殖。可从细胞培养物中纯化出单克隆抗体。

[0124] 正如本领域通常理解的一样,用于提及抗体的形容词"与……特异性反应的"意指抗体在目标抗原(例如GDF15多肽)与其它非目标抗原之间有足够选择性,使得所述抗体至少可用于在特定的生物样品类型中检测目标抗原的存在情况。在某些使用抗体的方法例如治疗应用中,在结合中可能需要较高程度的特异性。单克隆抗体通常具有有效区分所需抗原和交叉反应多肽的较大趋势(与多克隆抗体相比)。影响抗体:抗原相互作用的特异性的一个特征是抗体对抗原的亲和力。虽然可在不同亲和力范围内达到所需特异性,但是一般优选的抗体具有约10^{-6}、10^{-7}、10^{-8}、10^{-9}或更小的亲和力(解离常数)。假定在GDF15和TβRII之间有高亲和力,则预期中和抗GDF15或抗TβRII抗体一般可具有10^{-9}或更低的解离常数。

[0125] 另外,用于筛选抗体以鉴定所需要的抗体的技术可影响所得抗体的性质。例如,如果抗体被用于在溶液中结合抗原,则可能需要测试溶液结合。可获得用于测定抗体和抗原之间的相互作用以鉴定特别需要的抗体的各种不同技术。这类技术包括ELISA、表面等离子体共振结合测定法(例如Biacore™结合测定法,Biacore AB,Uppsala,Sweden)、夹心测定法(例如顺磁珠系统,IGEN International,Inc.,Gaithersburg,Maryland)、蛋白质印迹、免疫沉淀测定法和免疫组织化学法。

[0126] 作为GDF15或TβRII拮抗剂的核酸化合物类别的实例包括反义核酸、RNAi构建体和催化性核酸构建体。核酸化合物可以是单链或双链的。双链化合物还可包括其中链的一条或另一条是单链的突出端区或非互补区。单链化合物可包括自我互补区,意指化合物形成具有双螺旋结构区的所谓"发夹"或"茎-环"结构。核酸化合物可包含以下核苷酸序列,其与

由不超过全长GDF15核酸序列或TβRII核酸序列的1000个、不超过500个、不超过250个、不超过100个或不超过50、35、30、25、22、20或18个核苷酸组成的区域互补。互补区优选为至少8个核苷酸，任选至少10或至少15个核苷酸，例如15-25个核苷酸。互补区可落入靶转录物的内含子、编码序列或非编码序列中，例如编码序列部分。核酸化合物的长度一般可为约8-约500个核苷酸或碱基对，例如约14-约50个核苷酸。核酸可以是DNA（特别对于用作反义物）、RNA或RNA:DNA杂合体。任一条链可包括DNA和RNA的混合物以及不容易归类为DNA或RNA的修饰形式。同样，双链化合物可以是DNA:DNA、DNA:RNA或RNA:RNA，任一条链还可包括DNA和RNA的混合物，以及不易归类为DNA或RNA的修饰形式。核酸化合物可包括各种修饰的任一种，包括骨架(天然核酸中的糖-磷酸酯部分，包括核苷酸间键)或碱基部分(天然核酸的嘌呤或嘧啶部分)的一种或多种修饰。反义核酸化合物的长度可优选为约15-约30个核苷酸，并且通常含有一种或多种修饰以改进特性，例如在血清、细胞或化合物很可能被递送至的部位(例如在口服递送化合物的情况下为胃，而对吸入化合物而言为肺)中的稳定性。在RNAi构建体的情况下，与靶转录物互补的链一般是RNA或其修饰物。另一条链可以是RNA、DNA或任何其它变异物。双链或单链“发夹”RNAi构建体的双链体部分的长度可优选为18-40个核苷酸，任选长度为约21-23个核苷酸，只要它用作切酶底物即可。催化性核酸或酶促核酸可以是核酶或DNA酶，并且还可含有修饰形式。当在生理条件下和在其中无义或有义对照几乎没有作用或者没有作用的浓度下与细胞接触时，核酸化合物可抑制靶标表达达约50%、75%、90%或更高。测试核酸化合物的作用的优选浓度为1、5和10微摩尔。

[0127] 6．筛选测定法

在某些方面，本发明涉及TβRII多肽(例如可溶性TβRII多肽)和GDF15多肽鉴定作为GDF15-TβRII信号转导途径的激动剂或拮抗剂的化合物(物质)的用途。可对通过这种筛选法鉴定的化合物进行测试以评价其体外调节GDF15信号转导活性的能力。任选还可在动物模型中对这些化合物进行测试以评价其体内调节组织生长的能力。

[0128] 有许多针对通过靶向GDF15和TβRII多肽调节组织生长筛选治疗剂的方法。在某些实施方案中，可进行高通量的化合物筛选以鉴定干扰GDF15或TβRII介导的细胞信号转导的物质。在某些实施方案中，进行所述测定法以筛选和鉴定特异性抑制或降低TβRII多肽与GDF15结合的化合物。或者，所述测定法可用于鉴定增强TβRII多肽与GDF15结合的化合物。在又一个实施方案中，可通过其与GDF15或TβRII多肽相互作用的能力来鉴定化合物。

[0129] 各种测定形式便可满足需要，然而根据本公开内容，本领域的普通技术人员仍应了解未在本文明确描述的测定形式。如本文所述，可通过任何组合化学方法产生本发明的试验化合物(物质)。或者，主题化合物可以是体内或体外合成的天然存在的生物分子。待测定其起组织生长调节剂作用的能力的化合物(物质)可通过例如细菌、酵母、植物或其它生物产生(例如天然产物)、以化学法产生(例如小分子，包括肽模拟物)或以重组法产生。本发明考虑的试验化合物包括非肽基有机分子、肽、多肽、肽模拟物、糖、激素和核酸分子。在一个具体的实施方案中，试验物质是有机小分子，分子量小于约2,000道尔顿。

[0130] 本发明的试验化合物可以单一的分散实体提供，或者以较复杂的文库提供，例如通过组合化学制备的文库。这些文库可包含例如醇、烷基卤化物、胺、酰胺、酯、醛、醚和有机化合物的其它类别。向试验系统中提供的试验化合物可呈分离形式或作为化合物的混合物，尤其在最初的筛选步骤中。任选化合物可任选用其它化合物衍生化并具有利于化合物

分离的衍生化基团。衍生化基团的非限制实例包括生物素、荧光素、洋地黄毒苷(digoxygenin)、绿色荧光蛋白、同位素、聚组氨酸、磁珠、谷胱甘肽S转移酶(GST)、可光活化的交联剂或其任何组合。

[0131] 在测试化合物文库和天然提取物的许多药物筛选程序中,需要高通量测定法以便在给定时间内使研究的化合物的数目最大化。在无细胞系统(例如可用纯化的或半纯化的蛋白质衍生)中进行的测定法,常常优选作为"初步"筛选,因为可产生无细胞系统以允许快速显现和相对容易地检测由试验化合物介导的分子靶标中的改变。此外,在体外系统中一般可忽略试验化合物的细胞毒性或生物利用度的作用,测定法反而主要集中在药物对分子靶标的作用,如可表现在TβRII多肽和GDF15之间结合亲和力的改变。

[0132] 仅举例来说,在本发明的示例性筛选测定法中,将目标化合物与通常能够与GDF15结合的分离和纯化的TβRII多肽接触。然后向化合物和TβRII多肽的混合物中加入含有TβRII配体的组合物。TβRII/GDF15复合体的检测和定量测定提供用于测定化合物抑制(或增强)在TβRII多肽和GDF15之间形成复合体的功效的手段。可通过从使用不同浓度的试验化合物所得到的数据绘制剂量反应曲线,来评价化合物的功效。此外,还可进行对照测定,以提供比较基线。例如,在对照测定中,将分离和纯化的GDF15加入含有TβRII多肽的组合物中,在缺乏试验化合物时,定量测定TβRII/GDF15复合体的形成。应了解,一般可以改变可使反应物混合的顺序,并且可以同时混合。此外,可使用细胞提取物和裂解物替换经纯化的蛋白质以提供合适的无细胞测定系统。

[0133] 可通过各种技术,检测TβRII多肽和GDF15之间的复合体形成。例如,可使用例如可检测标记的蛋白质,例如放射性标记(例如^{32}P、^{35}S、^{14}C或^{3}H)、荧光标记(例如FITC)或酶标记的TβRII多肽或GDF15,通过免疫测定或通过层析检测,定量测定复合体形成的调节。

[0134] 在某些实施方案中,本发明考虑在直接或间接测定TβRII多肽与其结合蛋白间的相互作用的程度中,采用荧光偏振测定法和荧光共振能量转移(FRET)测定法。另外,其它检测方式,例如基于光波导(PCT公布号WO 96/26432和美国专利号5,677,196)、表面等离子体共振(SPR)、表面电荷传感器和表面力传感器的检测方式都适于本发明的许多实施方案。

[0135] 此外,本发明考虑采用相互作用陷阱测定法(interaction trap assay),亦称"双杂合测定法(two hybrid assay)",鉴定干扰或增强TβRII多肽与其结合蛋白间的相互作用的物质。参见例如美国专利号5,283,317;Zervos等(1993) Cell 72:223-232;Madura等(1993) J Biol Chem 268:12046-12054;Bartel等(1993) Biotechniques 14:920-924;以及Iwabuchi等(1993) Oncogene 8:1693-1696)。在一个具体的实施方案中,本发明考虑采用反相双杂交系统(reverse two hybrid system)鉴定离解TβRII多肽与其结合蛋白间的相互作用的化合物(例如小分子或肽)。参见例如Vidal和Legrain,(1999) Nucleic Acids Res 27:919-29;Vidal和Legrain,(1999) Trends Biotechnol 17:374-81;以及美国专利号5,525,490、5,955,280和5,965,368。

[0136] 在某些实施方案中,通过其与本发明的TβRII或GDF15多肽相互作用的能力来鉴定主题化合物。化合物和TβRII或GDF15多肽间的相互作用可以是共价的或非共价的。例如,可采用体外生化方法在蛋白质水平上鉴定这类相互作用,包括光致交联、放射性同位素标记配体结合和亲和层析法(Jakoby WB等,1974,Methods in Enzymology 46:1)。在某些情况下,可在基于机制的测定法中筛选化合物,例如检测与GDF15或TβRII多肽结合的化合物的

测定法。这可包括固相或液相结合事件。或者，可将编码GDF15或TβRII多肽的基因与报道系统(例如β-半乳糖苷酶、萤光素酶或绿色荧光蛋白)一起转染至细胞中，优选通过高通量筛选针对文库进行筛选或用文库的各个成员进行筛选。可采用基于其它机制的结合测定法，例如检测自由能变化的结合测定法。结合测定法可用固定于孔、珠粒或芯片或被固定化抗体俘获或被毛细管电泳分离的靶标进行。一般可采用比色法或荧光法或表面等离子体共振检测结合的化合物。

[0137] 在某些方面，本发明提供用于调节(刺激或抑制) GDF15介导的细胞信号转导的方法和物质。因此，可在体外或体内在完整细胞或组织中测定所鉴定的任何化合物，以证实其调节GDF15信号转导的能力。可将本领域已知的各种方法用于此目的。

[0138] 7．示例性治疗应用

本文使用的“预防”病症或病况的治疗药是指以下化合物，其在统计样本中，与未治疗对照样品相比，减少治疗样品中的病症或病况的发生，或者与未治疗对照样品相比，延缓病症或病况的一种或多种症状发作或者降低病症或病况的一种或多种症状的严重程度。本文使用的术语“治疗”包括病况一旦确诊便改善或根除病况。在任一情况下，可通过医师给出的诊断和给予治疗剂的预期结果来分辨预防或治疗。

[0139] 本公开内容提供通过给予受试者有效量的TβRII多肽(包括所述TβRII多肽的TβRII-Fc融合蛋白或核酸拮抗剂(例如反义物或siRNA)，下文统称为“治疗剂”)治疗或预防与TGFβ超家族成员相关的疾病或病况的方法。在一些实施方案中，疾病或病况与GDF15、TGFβ1或TGFβ3信号转导失调有关。还提供用于治疗某些心血管疾病或血管疾病的方法和组合物。另外，本公开内容提供用于治疗或预防癌症的方法和组合物。另外，本公开内容提供用于治疗或预防纤维化病症和病况的方法和组合物。

[0140] 具体地说，本公开内容的多肽治疗剂可用于治疗或预防慢性血管或心血管疾病。这类的示例性病症包括但不限于心脏病(包括心肌病、心肌梗塞、心绞痛和心瓣膜疾病)；肾病(包括慢性肾小球炎症、糖尿病性肾衰竭和狼疮相关肾炎)；与动脉粥样硬化或其它类型的动脉硬化相关病症(包括中风、脑出血、蛛网膜下腔出血、心绞痛和肾动脉硬化)；血栓性病症(包括脑血栓形成、血栓性肠坏死)；糖尿病并发症(包括糖尿病相关肾病、白内障、糖尿病相关肾病、糖尿病相关神经病、糖尿病相关坏疽和糖尿病相关慢性感染)；血管炎性病症(系统性红斑狼疮、关节风湿病、关节动脉炎、大细胞动脉炎、川崎病、大动脉炎、Churg-Strauss综合征和诺赫-舍恩莱因紫癜(Henoch-Schoenlein purpura))；糖尿病血管病变和心脏病症例如先天性心脏病、心肌病(例如扩张型、肥厚型、限制性心肌病)和充血性心力衰竭。示例性病症还包括但不限于遗传性出血性毛细血管扩张症(HHT)、马方综合征、Loeys-Dietz综合征、家族性胸主动脉瘤综合征、动脉迂曲综合征、先兆子痫和再狭窄。

[0141] 可单独或与可用于治疗TGFβ相关心血管病症和/或病况的一种或多种药剂或治疗方式(例如治疗剂)组合将TβRII多肽给予受试者。在某些实施方案中，第二药剂或治疗方式选自以下一种或多种：血管成形术、β阻断剂、抗高血压药、强心药、抗血栓药、血管扩张药、激素拮抗剂、内皮素拮抗剂、钙通道阻断剂、磷酸二酯酶抑制剂、血管紧张素2型拮抗剂和/或细胞因子阻断剂/抑制剂。

[0142] 具体地说，本公开内容的多肽治疗剂可用于治疗或预防癌症(肿瘤)。术语“癌症”和“癌症的”是指或描述其特征通常为细胞生长/增殖不受控制的哺乳动物生理学病况。癌

症或肿瘤性病症的实例包括但不限于癌、淋巴瘤、胚细胞瘤、肉瘤和白血病。这类癌症的更具体的实例包括鳞状上皮细胞癌、腹膜癌、肝细胞癌、胃肠癌、胰腺癌、成胶质细胞瘤、宫颈癌、卵巢癌、肝癌、膀胱癌、肝细胞瘤、乳腺癌、结肠癌、结肠直肠癌、子宫内膜癌或子宫癌、唾腺癌、肾癌、前列腺癌、阴道癌、甲状腺癌、肝癌、胃癌、肠癌、皮肤癌、骨癌、胃癌、黑素瘤和各种类型的头颈癌，包括鳞状上皮细胞头颈癌。肿瘤性病症和相关病况的其它实例包括食管癌、泡膜细胞瘤、卵巢男胚瘤、子宫内膜增生、子宫内膜异位症、纤维肉瘤、绒毛膜癌、鼻咽癌、喉癌、肝胚细胞瘤、卡波西肉瘤(Kaposi's sarcoma)、皮肤癌、血管瘤、海绵状血管瘤、成血管细胞瘤、成视网膜细胞瘤、星形细胞瘤、成胶质细胞瘤、神经鞘瘤、少突神经胶质瘤、成神经管细胞瘤、成神经细胞瘤、横纹肌肉瘤、骨源性肉瘤、平滑肌肉瘤、尿道癌、维尔姆斯瘤(Wilm's tumor)、肾细胞癌、前列腺癌、斑痣性错构瘤病相关异常血管增生和梅格斯综合征(Meigs' syndrome)。特别适于用本文所述治疗剂治疗的癌症的特征可在于以下一种或多种:癌症具有在肿瘤或血清中可检测的高TβRII水平;GDF15、TGFβ1或TGFβ3表达水平或生物活性升高;是转移性的或有变成转移性的风险，或其任何组合。

[0143] 在这类方法的某些实施方案中，可在一起(同时)或在不同的时间(序贯)给予一种或多种多肽治疗剂。另外，可与用于治疗癌症或抑制血管生成的另一类型的化合物一起给予多肽治疗剂。

[0144] 在某些实施方案中，本公开内容的主题方法可单独使用。或者，主题方法可与针对增殖性病症(例如肿瘤)的治疗或预防的其它常规抗癌治疗方法联用。例如，这类方法可用于预防性癌症预防、手术后癌症复发和转移预防，并可用作其它常规癌症疗法的辅助剂。本公开内容认识到可通过使用主题多肽治疗剂提高常规癌症疗法(例如化学疗法、放射疗法、光线疗法、免疫疗法和手术)的疗效。

[0145] 已表明一系列常规化合物具有抗瘤或抗癌活性。已在化学疗法中将这些化合物用作药剂，以缩小实体瘤、防止转移和进一步生长或减少白血病或骨髓恶性肿瘤中恶性细胞的数目。虽然化学疗法在治疗各种类型的恶性肿瘤中是有效的，但是许多抗瘤化合物引起不期望的副作用。已表明，当将两种或更多种不同的治疗组合时，治疗可协同起作用，并允许降低各种治疗的剂量，从而降低因较高剂量的各种化合物发挥的有害副作用。在其它情况下，对治疗不应的恶性肿瘤可对两种或更多种不同的治疗的组合疗法起反应。

[0146] 当本文公开的治疗剂与另一种常规抗肿瘤药组合同时或序贯给予时，这类治疗剂可提高抗肿瘤药的治疗作用或克服细胞对所述抗肿瘤药的抗性。这允许降低抗肿瘤药的剂量，从而降低不期望的副作用或恢复抗性细胞中抗肿瘤药的疗效。

[0147] 按照本公开内容，本文所述多肽治疗剂可与用于治疗疾病的其它组合物和方法联用。例如，可用与TβRII多肽组合的手术、放射或化学疗法按常规治疗肿瘤，然后可将TβRII多肽随后给予患者以延长微转移的蜇伏并稳定任何残留的原发性肿瘤。

[0148] 在本发明的某些方面，可用于与TβRII多肽的组合肿瘤疗法的其它治疗剂包括其它癌症疗法:例如手术、细胞毒性剂、包括辐射或给予放射性物质的放射治疗、化学治疗剂、抗激素药、生长抑制药、抗瘤组合物和用本文所列和本领域已知的抗癌药治疗或其组合。

[0149] 本文所用术语"细胞毒性剂"是指抑制或防止细胞的功能和/或引起细胞破坏的物质。该术语旨在包括放射性同位素(例如At^{211}、I^{131}、I^{125}、Y^{90}、Re^{186}、Re^{188}、Sm^{153}、Bi^{212}、P^{32}和Lu的放射性同位素)、化学治疗剂，例如甲氨蝶呤、阿霉素、长春花属生物碱(长春新碱、长春

碱、依托泊苷)、多柔比星、美法仑、丝裂霉素C、苯丁酸氮芥、柔红霉素或其它嵌入剂、酶和其片段(例如溶核酶)、抗生素和毒素(例如小分子毒素)或细菌、真菌、植物或动物源的酶促活性毒素,包括其片段和/或变体,以及下文公开的各种抗肿瘤剂或抗癌剂。下面描述了其它细胞毒性剂。杀肿瘤剂致使肿瘤细胞遭破坏。

[0150] “化学治疗剂”是可用于治疗癌症的化合物。化学治疗剂的实例包括烷化剂,例如塞替派和CYTOXAN® 环磷酰胺;烷基磺酸酯,例如白消安、英丙舒凡和哌泊舒凡;吖丙啶类,例如苯佐替派(benzodopa)、卡波醌、美妥替哌(meturedopa)和乌瑞替派(uredopa);乙烯亚胺和methylamelamines,包括六甲蜜胺、曲他胺、三乙烯磷酰胺(trietylenephosphoramide)、三乙烯硫代磷酰胺和三羟甲蜜胺;acetogenins (尤其是bullatacin和bullatacinone);δ-9-四氢大麻酚(屈大麻酚、MARINOL®);β-lapachone;拉帕醇;秋水仙碱;桦木酸;喜树碱(包括合成类似物托泊替康(HYCAMTIN®)、CPT-11 (伊立替康、CAMPTOSAR®)、乙酰基喜树碱、东莨菪亭和9-氨基喜树碱);苔藓抑素;callystatin;CC-1065 (包括其阿多来新、卡折来新和比折来新合成类似物);鬼臼毒素;鬼臼酸;替尼泊苷;念珠藻环肽(特别是念珠藻环肽1和念珠藻环肽8);多拉司他汀;duocarmycin (包括合成类似物、KW-2189和CB1-TM1);艾榴素;pancratistatin;匍枝珊瑚醇;海绵素;氮芥,例如苯丁酸氮芥、萘氮芥、环磷酰胺、雌莫司汀、异环磷酰胺、氮芥、盐酸氧氮芥、美法仑、新氮芥、苯芥胆甾醇(phenesterine)、泼尼莫司汀、曲磷胺、乌拉莫司汀;亚硝基脲,例如卡莫司汀、氯脲菌素、福莫司汀、洛莫司汀、尼莫司汀和雷莫司汀;抗生素,例如烯二炔类抗生素(例如卡奇霉素、特别是卡奇霉素γ1I和卡奇霉素ωI1 (参见例如Agnew, Chem Intl. Ed. Engl., 33: 183-186 (1994));烯二炔蒽环类抗生素,包括烯二炔蒽环类抗生素A;埃斯波霉素;以及新制癌菌素发色团和相关色蛋白烯二炔抗生素发色团、阿克拉霉素、放线菌素、authramycin、偶氮丝氨酸、博来霉素、放线菌素C、carabicin、去甲柔红霉素、嗜癌霉素、chromomycinis、放线菌素D、柔红霉素、地托比星、6-重氮基-5-氧代-L-正亮氨酸、ADRIAMYCIN® 多柔比星(包括吗啉代-多柔比星、氰基吗啉代-多柔比星、2-吡咯啉子基(pyrrolino)-多柔比星和去氧多柔比星)、表柔比星、依索比星、伊达比星、马赛罗霉素、丝裂霉素(例如丝裂霉素C)、麦考酚酸、诺拉霉素、橄榄霉素、培洛霉素、紫菜霉素、嘌罗霉素、三铁阿霉素、罗多比星、链黑霉素、链佐星、杀结核菌素、乌苯美司、净司他丁、佐柔比星;抗代谢物,例如甲氨蝶呤和5-氟尿嘧啶(5-FU);叶酸类似物,例如二甲叶酸、甲氨蝶呤、蝶罗呤、三甲曲沙;嘌呤类似物,例如氟达拉滨、6-巯基嘌呤、thiamiprine、硫鸟嘌呤;嘧啶类似物,例如安西他滨、阿扎胞苷、6-氮尿苷、卡莫氟、阿糖胞苷、二脱氧尿苷、去氧氟尿苷、依诺他滨、氟尿苷;雄激素,例如卡鲁睾酮、屈他雄酮丙酸酯、环硫雄醇、美雄烷、睾内酯;抗肾上腺素药(anti-adrenals),例如氨鲁米特、米托坦、曲洛司坦;叶酸补充剂,例如亚叶酸;醋葡醛内酯;醛磷酰胺糖苷;氨基酮戊酸;恩尿嘧啶;安吖啶;bestrabucil;比生群;依达曲沙;秋水仙胺;地吖醌;elfornithine;依利醋铵;埃坡霉素;依托格鲁;硝酸镓;羟基脲;香菇多糖;氯尼达明;美坦生类化合物,例如美登素和安丝菌素;米托胍腙;米托蒽醌;莫哌达醇(mopidanmol);尼曲吖啶(nitraerine);喷司他丁;phenamet;吡柔比星;洛索蒽醌;2-乙基酰肼;丙卡巴肼;PSK® 多糖复合体(JHS Natural Products, Eugene, OR);雷佐生;利索新;西佐喃;锗螺胺;细交链孢菌酮酸;三亚胺醌;2,2',2″-三氯三乙胺;单端孢菌霉素(尤其T-2毒素、verracurin A、杆孢菌素A和anguidine);乌拉坦;长春地辛(ELDISINE®、

FILDESIN®);达卡巴嗪;甘露莫司汀;二溴甘露醇;二溴卫矛醇;哌泊溴烷;gacytosine;阿拉伯糖苷C ("Ara-C");塞替派;紫杉醇类,例如TAXOL®紫杉醇(Bristol-Myers Squibb Oncology, Princeton, N.J.)、ABRAXANE™ Cremophor-free、紫杉醇的白蛋白工程化纳米粒制剂(American Pharmaceutical Partners, Schaumberg, Illinois)和TAXOTERE® doxetaxel (Rhône-Poulenc Rorer, Antony, France);苯丁酸氮芥;吉西他滨(GEMZAR®);6-硫鸟嘌呤;巯基嘌呤;甲氨蝶呤;铂类似物,例如顺铂和卡铂;长春碱(VELBAN®);铂;依托泊苷(VP-16);异环磷酰胺;米托蒽醌;长春新碱(ONCOVIN®);奥沙利铂;leucovovin;长春瑞滨(NAVELBINE®);诺安托;依达曲沙;道诺霉素;氨基蝶呤;伊班膦酸盐;拓扑异构酶抑制剂RFS 2000;二氟甲基鸟氨酸(DMFO);类视黄醇,例如视黄酸;卡培他滨(XELODA®);上述任一种的药学上可接受的盐、酸或衍生物;以及上述两种或更多种的组合,例如CHOP,一个环磷酰胺、多柔比星、长春新碱和泼尼松龙组合疗法的缩略语,以及FOLFOX,一个与5-FU和leucovovin组合的奥沙利铂(ELOXATIN™)治疗方案的缩略语。

[0151] 该定义中还包括起调节、降低、阻断或抑制可促进癌生长的激素的作用,且常常呈系统或全身治疗形式的抗激素药。它们可以是激素本身。实例包括抗雌激素和选择性雌激素受体调节剂(SERM),包括例如他莫昔芬(包括NOLVADEX®他莫昔芬)、EVISTA®雷洛昔芬、屈洛昔芬、4-羟基他莫昔芬、曲沃昔芬、keoxifene、LY1 17018、奥那司酮和FARESTON®托瑞米芬;抗黄体酮;雌激素受体下调剂(ERD);起抑制或中断卵巢作用的作用剂,例如促性腺素释放素(LHRH)激动剂,例如LUPRON®和ELIGARD®醋酸亮丙立德、醋酸戈舍瑞林、醋酸布舍瑞林和曲普瑞林(tripterelin);其它抗雄激素,例如氟他胺、尼鲁米特和比卡鲁胺;以及抑制芳香酶(其调节肾上腺的雌激素产生)的芳香酶抑制剂,例如4(5)-咪唑、氨鲁米特、MEGASE®醋酸甲地孕酮、AROMASIN®依西美坦、福美坦、法倔唑、RIVIS OR®伏氯唑、FEMARA®来曲唑和ARIMIDEX®阿那曲唑。另外,化学治疗剂的这类定义包括二膦酸盐,例如氯膦酸盐(例如BONEFOS®或OSTAC®)、DIDROC AL®依替膦酸、NE-58095、ZOMET A®唑来膦酸/唑来膦酸盐、FOSAMAX®阿伦膦酸盐、AREDIA®帕米膦酸盐、SKELID®替鲁膦酸盐或ACTONEL®利塞膦酸盐;以及曲沙他滨(一种1,3-二氧杂环戊烷核苷胞嘧啶类似物);反义寡核苷酸,特别是抑制参与异常细胞增殖的信号转导途径的基因表达的反义寡核苷酸,例如PKC-α、Raf、H-Ras和表皮生长因子受体(EGF-R);疫苗,例如THERATOPE®疫苗和基因疗法疫苗,例如ALLOVECTIN®疫苗、LEUVECTIN®疫苗和VAXID®疫苗;LURTOTECAN®拓扑异构酶1抑制剂;ABARELIX® rmRH;托西拉帕替尼(一种ErbB-2和EGFR双重酪氨酸激酶小分子抑制剂,亦称GW572016)和上述任一种的药学上可接受的盐、酸或衍生物。

[0152] "生长抑制剂"当在本文使用时是指体外或体内抑制细胞生长的化合物或组合物。因此,生长抑制剂可以是显著降低S期中细胞的百分比的生长抑制剂。生长抑制药的实例包括阻断细胞周期过程(处于S期以外的位置)的抑制剂,例如引起G1停滞和M期停滞的抑制剂。经典的M期阻断剂包括长春花属生物碱(长春新碱和长春碱)、紫杉烷类和拓扑异构酶II抑制剂,例如多柔比星、表柔比星、柔红霉素、依托泊苷和博来霉素。阻滞G1的那些抑制剂也可波及到S期停滞,例如DNA烷化剂,例如他莫昔芬、泼尼松、达卡巴嗪、氮芥、顺铂、甲氨蝶呤、5-氟尿嘧啶和ara-C。更多的信息可参见The Molecular Basis of Cancer, Mendelsohn和Israel编辑, 第1章, Murakami等标题为"Cell cycle regulation, oncogenes, and antineoplastic drugs ("细胞周期调节、癌基因和抗肿瘤药)"(WB

Saunders: Philadelphia, 1995)，尤其第13页。紫杉烷类(紫杉醇和多西他赛)是均来源于紫杉树的抗癌药。来源于欧洲紫杉的多西他赛(TAXOTERE®，Rhone -Poulenc Rorer)是紫杉醇(TAXOL®，Bristol-Myers Squibb)的半合成类似物。紫杉醇和多西他赛促进从微管蛋白二聚体装配成微管，并通过防止解聚稳定微管，这导致在细胞中抑制有丝分裂。

[0153] 在另外其它的实施方案中，TβRII多肽可用于治疗或预防纤维化。本文所用术语“纤维化”是指器官或组织的细胞中过量纤维性结缔组织的异常形成或发生。虽然与纤维化有关的过程可作为正常组织形成或修复的一部分发生，但这些过程的失调可导致细胞组成改变和过量结缔组织沉积，其逐步损害组织或器官功能。纤维组织的形成可产生于修复性或反应性过程。纤维化病症或病况包括但不限于与血管疾病例如心脏病、脑病和外周血管病相关的纤维增殖性病症，以及与组织和器官系统包括心脏、皮肤、肾、腹膜、肠和肝相关的纤维增殖性病症(如公开于例如Wynn，2004，Nat Rev 4:583-594，通过引用结合到本文中)。可治疗的示例性病症包括但不限于肾纤维化，包括与损伤/纤维化有关的肾病，例如与糖尿病有关的慢性肾病(例如糖尿病性肾病)、狼疮、硬皮病、肾小球肾炎、局灶性节段性肾小球硬化和IgA肾病；肠纤维化，例如硬皮病和放射诱导的肠纤维化；肝纤维化，例如肝硬化、酒精性肝纤维化、胆管损伤、原发性胆汁性肝硬化、感染性或病毒性肝纤维化、先天性肝纤维化和自身免疫性肝炎；以及其它纤维化病况，例如囊性纤维化、心内膜心肌纤维化、纵隔纤维化、结节病、硬皮病、脊髓损伤/纤维化、骨髓纤维化、血管再狭窄、动脉粥样硬化、注射性纤维化(这可作为肌内注射的并发症发生，尤其在儿童中)、心内膜心肌纤维化、腹膜后纤维化和肾源性系统性纤维化。

[0154] 本文所用术语“纤维化病症”、“纤维化病况”和“纤维化疾病”可互换使用，是指以纤维化为特征的病症、病况或疾病。纤维化病症的实例包括但不限于硬化病症(例如硬皮病、动脉粥样硬化、弥漫性系统性硬化)、血管纤维化、胰腺纤维化、肝纤维化(例如肝硬化)、肾纤维化、肌骨骼纤维化、心脏纤维化(例如心内膜心肌纤维化、特发性心肌病)、皮肤纤维化(例如硬皮病、创伤后、手术皮肤瘢痕形成、瘢痕瘤和皮肤瘢痕瘤形成)、眼纤维化(例如青光眼、眼硬化症、结膜和角膜瘢痕形成和翼状胬肉)、骨髓纤维化、进行性系统性硬化病(PSS)、慢性移植物抗宿主病、佩罗尼病、膀胱镜检查后尿道狭窄、特发性和药物诱导性腹膜后纤维化、纵隔纤维化、增生性纤维化、增生性纤维变性、杜普伊特伦病(Dupuytren's disease)、狭窄、神经瘢痕形成、皮肤瘢痕形成和放射诱导性纤维化。

[0155] 如本文所用，抑制细胞的纤维化反应包括但不限于抑制以下细胞的纤维化反应：肝(或肝组织)内的一种或多种细胞；肾(或肾组织)内的一种或多种细胞；肌肉组织内的一种或多种细胞；心脏(或心脏组织)内的一种或多种细胞；胰腺内的一种或多种细胞；皮肤内一种或多种细胞；骨内的一种或多种细胞、血管系统内的一种或多种细胞、一种或多种干细胞或眼内的一种或多种细胞。

[0156] 本发明考虑与一种或多种其它治疗方式组合的TβRII多肽的应用。因此，除TβRII多肽的应用以外，还可将用于治疗纤维化病症的一种或多种“标准”疗法给予受试者。例如，可将TβRII多肽与细胞毒素、免疫抑制剂、放射性毒性剂和/或治疗性抗体组合(即共同)给予。本发明考虑的具体的共治疗剂包括但不限于类固醇类(例如皮质甾类，例如泼尼松)、免疫抑制药和/或抗炎药(例如γ-干扰素、环磷酰胺、硫唑嘌呤、甲氨蝶呤、青霉胺、环孢菌素、秋水仙碱、抗胸腺细胞球蛋白、麦考酚酸酯和羟氯喹)、细胞毒性药物、钙通道阻断剂(例如

硝苯地平)、血管紧张素转化酶抑制剂(ACE)抑制剂、对-氨基苯甲酸(PABA)、二甲亚砜、转化生长因子β (TGFβ)抑制剂、白介素-5 (IL-5)抑制剂和泛胱天蛋白酶抑制剂。

[0157] 可与TβRII多肽联用的其它抗纤维化药包括但不限于凝集素(如描述于例如美国专利号:7,026,283,其完整内容通过引用结合到本文中),以及Wynn等(2007,J Clin Invest 117:524-529,其完整内容通过引用结合到本文中)描述的抗纤维化药。例如,其它抗纤维化药和疗法包括但不限于各种抗炎药/免疫抑制药/细胞毒性药(包括秋水仙碱、硫唑嘌呤、环磷酰胺、泼尼松、沙利度胺、己酮可可碱和茶碱)、TGFβ信号转导调节剂(包括松弛素、SMAD7、HGF和BMP7以及TGFβ1、TβRI、TβRII、EGR-I和CTGF抑制剂)、细胞因子和细胞因子受体拮抗剂(IL-1β、IL-5、IL-6、IL-13、IL-21、IL-4R、IL-13Rα1、GM-CSF、TNF-α、制癌蛋白M、W1SP-I和PDGF的抑制剂)、细胞因子和趋化因子(IFN-γ、IFN-α/β、IL-12、IL-10、HGF、CXCL10和CXCL11)、趋化因子拮抗剂(CXCL1、CXCL2、CXCL12、CCL2、CCL3、CCL6、CCL17和CCL18的抑制剂)、趋化因子受体拮抗剂(CCR2、CCR3、CCR5、CCR7、CXCR2和CXCR4的抑制剂)、TLR拮抗剂(TLR3、TLR4和TLR9的抑制剂)、血管生成拮抗剂(VEGF特异性抗体和腺苷脱氨酶替代疗法)、抗高血压药(ANG 11、ACE和醛固酮的β阻断剂和抑制剂)、血管活性物质(ET-1受体拮抗剂和bosetan)、合成和加工胶原的酶的抑制剂(脯氨酰羟化酶抑制剂)、B细胞拮抗剂(利妥昔单抗)、整联蛋白/粘附分子拮抗剂(阻断α1β1和αvβ6整联蛋白的分子以及整联蛋白相关激酶抑制剂和ICAM-I和VCAM-I的特异性抗体)、靶向成肌纤维细胞的促凋亡药物、MMP抑制剂(MMP2、MMP9和MMP12的抑制剂)和TlMP抑制剂(TIMP-1的特异性抗体)。

[0158] 可在同一制剂中或分别给予TβRII多肽和共治疗剂或协同疗法。在分别给药的情况下,可在共治疗剂或协同疗法之前、之后或与之同时给予TβRII多肽。一种药剂可先于或后于另一种药剂给药数分钟到数周的间隔。在其中两种或更多种不同种类的治疗剂分别施于受试者的实施方案中,通常会确保每次递送的时间之间的可观的一段时间不届满,使得这些不同种类的药剂仍能够对靶组织或细胞发挥有利的联合作用。

[0159] 8. 药物组合物

可将本文所述治疗剂(例如TβRII多肽)配制成药物组合物。可以常规方式使用一种或多种生理学上可接受的载体或赋形剂配制按照本公开内容使用的药物组合物。这类制剂一般将是基本无热原的,与大部分管理机构的要求相容。

[0160] 在某些实施方案中,本公开内容的治疗方法包括全身或者以植入物或装置局部给予组合物。当给药时,用于本公开内容的治疗组合物呈无致热原的生理学上可接受的形式。还可任选包含在上述组合物中的TβRII信号转导拮抗剂以外的治疗上有用的物质,在本文公开的方法中可与主题化合物(例如TβRII多肽)同时或序贯给予。

[0161] 通常可胃肠外(特别是静脉内或皮下)给予本文公开的蛋白质治疗剂。适于胃肠外给予的药物组合物可包含一种或多种TβRII多肽以及一种或多种药学上可接受的无菌等渗水性溶液或非水性溶液、分散体、混悬液或乳液或者无菌粉剂(所述粉剂可在临用前重构成无菌注射用溶液或分散体),其可含有抗氧化剂、缓冲剂、抑菌剂、使制剂与预期接受者血液等渗的溶质或者助悬剂或增稠剂。可用于本公开内容的药物组合物的合适水性载体和非水性载体的实例包括水、乙醇、多元醇(例如甘油、丙二醇、聚乙二醇等)及其合适的混合物、植物油(例如橄榄油)和注射用有机酯(例如油酸乙酯)。可通过例如使用包衣材料(例如卵磷脂),在分散体的情况下通过保持所需要的粒径,以及通过使用表面活性剂,来保持适当的

流动性。

[0162] 如有需要，组合物和制剂可以可装有一个或多个含有活性成分的单位剂型的药包或分配装置提供。药包可例如包含金属箔或塑料薄片，例如泡罩包装。药包或分配装置可附有给药说明书。

[0163] 另外，组合物可以递送至靶组织部位的形式装入胶囊或注射。在某些实施方案中，本发明的组合物可包含基质，其能够将一种或多种治疗化合物(例如TβRII多肽)递送到靶组织部位，为发育组织提供结构，并且最好能够被吸收进体内。例如，基质可提供慢释的TβRII多肽。这类基质可由目前用于其它植入医学应用的材料形成。

[0164] 基质材料的选择取决于生物相容性、生物降解能力、机械性能、美容外观和界面性质。主题组合物的具体应用将界定适当的制剂。组合物可能的基质可以是生物可降解的和化学成分确定的硫酸钙、磷酸三钙、羟磷灰石、聚乳酸和聚酐。其它可能的材料是生物可降解的和生物学上明确限定的，例如骨或真皮胶原。其它基质由纯的蛋白质或胞外基质组分组成。其它可能的基质是非生物可降解的和化学上确定的，例如烧结羟磷灰石、生物玻璃、铝酸盐或其它陶瓷。基质可由任何上述类型的材料的组合组成，例如聚乳酸和羟磷灰石或胶原和磷酸三钙。生物陶瓷可在组成方面改变，例如呈钙-铝酸盐-磷酸盐，并进行加工以改变孔径、颗粒大小、颗粒形状和生物降解能力。

[0165] 在某些实施方案中，本发明的方法可以例如以下形式口服给予：胶囊剂、扁囊剂、丸剂、片剂、锭剂(使用调味基料，一般为蔗糖和阿拉伯胶或西黄蓍胶)、散剂、颗粒剂，或者作为水性液体或非水性液体中的溶液剂或混悬剂、或作为水包油或油包水液体乳剂、或作为酏剂或糖浆剂、或作为软锭剂(使用惰性基料，例如明胶和甘油或蔗糖和阿拉伯胶)和/或作为漱口剂等，各自含有预定量的物质作为活性成分。药剂还可作为大丸剂、药糖剂或糊剂给予。

[0166] 在口服给药的固体剂型(胶囊剂、片剂、丸剂、糖衣丸、散剂、颗粒剂等)中，可将一种或多种本发明的治疗化合物与一种或多种药学上可接受的载体例如柠檬酸钠或磷酸二钙和/或以下的任一种混合：(1)填充剂或增充剂，例如淀粉、乳糖、蔗糖、葡萄糖、甘露醇和/或硅酸；(2)粘合剂，例如羧甲基纤维素、藻酸盐、明胶、聚乙烯吡咯烷酮、蔗糖和/或阿拉伯胶；(3)湿润剂，例如甘油；(4)崩解剂，例如琼脂、碳酸钙、马铃薯或木薯淀粉、藻酸、某些硅酸盐和碳酸钠；(5)溶液阻滞剂，例如石蜡；(6)吸收促进剂，例如季铵化合物；(7)润湿剂，例如鲸蜡醇和单硬脂酸甘油酯；(8)吸收剂，例如高岭土和膨润土；(9)润滑剂，例如滑石粉、硬脂酸钙、硬脂酸镁、固态聚乙二醇、十二烷基硫酸钠及其混合物；和(10)着色剂。在胶囊剂、片剂和丸剂的情况下，药物组合物还可包含缓冲剂。在使用诸如乳糖以及高分子量聚乙二醇等赋形剂的软充填和硬充填明胶胶囊剂中，相似类型的固体组合物也可用作填充剂。

[0167] 用于口服给药的液体剂型包括药学上可接受的乳剂、微乳剂、溶液剂、混悬剂、糖浆剂和酏剂。除活性成分以外，液体剂型可含有常用于本领域的惰性稀释剂，例如水或其它溶剂、增溶剂和乳化剂，例如乙醇、异丙醇、碳酸乙酯、乙酸乙酯、苯甲醇、苯甲酸苄酯、丙二醇、1,3-丁二醇、油(具体地说为棉籽油、花生油、玉米油、胚芽油、橄榄油、蓖麻油和芝麻油)、甘油、四氢糠醇、聚乙二醇和失水山梨糖醇的脂肪酸酯及其混合物。除惰性稀释剂以外，口服组合物还可包含辅料，例如润湿剂、乳化剂和助悬剂、甜味剂、矫味剂、着色剂、芳香剂和防腐剂。

[0168] 除活性化合物以外，混悬剂可含有助悬剂，例如乙氧基化异十八醇、聚氧乙烯山梨糖醇和失水山梨糖醇酯、微晶纤维素、偏氢氧化铝(aluminum metahydroxide)、膨润土、琼脂和西黄蓍胶及其混合物。

[0169] 本发明的组合物还可含有辅料，例如防腐剂、润湿剂、乳化剂和分散剂。可通过加入多种抗细菌剂和抗真菌剂，例如对羟基苯甲酸酯、三氯叔丁醇、苯酚、山梨酸等，来确保防止微生物的作用。组合物中还可能需要包含等渗剂(例如糖、氯化钠等)。另外，可通过加入延迟吸收的物质(例如单硬脂酸铝和明胶)，使可注射药物形式的吸收延长。

[0170] 要了解，主治医师在考虑修正本发明的主题化合物(例如TβRII多肽)的作用的各种因素后，可确定剂量方案。各种因素包括但不限于患者的年龄、性别和饮食、疾病严重程度、给药时间和其它临床因素。任选剂量可随用于重构的基质类型和组合物中化合物的类型而改变。向最终组合物中加入其它已知的生长因子也可影响剂量。可通过定期评价骨生长和/或修复，例如X射线(包括DEXA)、组织形态学测定和四环素标记，来监测进程。

[0171] 在某些实施方案中，本发明还提供用于体内产生TβRII多肽的基因疗法。这类疗法可通过将TβRII多核苷酸序列引入受累于上文所列病症的细胞或组织中，来达到其治疗效果。可使用重组表达载体(例如嵌合病毒或胶体分散系统)实现TβRII多核苷酸序列的递送。优选的TβRII多核苷酸序列的治疗递送是使用靶向脂质体。

[0172] 可用于本文教导的基因疗法的各种病毒载体包括腺病毒、疱疹病毒、痘苗病毒或优选RNA病毒(例如反转录病毒)。优选反转录病毒载体是鼠或禽反转录病毒的衍生物。可插入单一外源基因的反转录病毒载体的实例包括但不限于：莫洛尼鼠白血病病毒(MoMuLV)、Harvey鼠肉瘤病毒(HaMuSV)、鼠乳腺肿瘤病毒(MuMTV)和劳斯肉瘤病毒(Rous sarcoma virus, RSV)。许多其它的反转录病毒载体可掺入多个基因。所有的这些载体可转移或整合选择标记的基因，使得可鉴定和产生转导细胞。可通过连接例如糖、糖脂或蛋白质，使反转录病毒载体具有靶标特异性。通过使用抗体来完成优选的打靶。本领域的技术人员应认识到，可将特异性多核苷酸序列插入反转录病毒基因组或与病毒包膜连接，以供含有TβRII多核苷酸的反转录病毒载体的靶标特异性递送。在一个优选的实施方案中，载体靶定骨或软骨。

[0173] 或者，可通过常规磷酸钙转染，将组织培养细胞用编码反转录病毒结构基因gag、pol和env的质粒直接转染。然后将这些细胞用含有目标基因的载体质粒转染。所得细胞释放反转录病毒载体到培养基中。

[0174] TβRII多核苷酸的另一种靶定递送系统是胶体分散系统。胶体分散系统包括大分子复合体、纳米囊、微球、珠粒和脂质型系统，其包括水包油乳液、微团、混合微团和脂质体。优选的本发明的胶体系统是脂质体。脂质体是可体外和体内用作递送载体的人工膜囊泡。RNA、DNA和完整病毒体可包封在水性内部，并以生物活性形式递送至细胞(参见例如Fraley等, Trends Biochem. Sci., 6:77,1981)。使用脂质体载体的有效基因转移方法是本领域已知的，参见例如Mannino等, Biotechniques, 6:682, 1988。脂质体的组成一般是磷脂的组合，一般与类固醇(尤其是胆固醇)组合。也可以使用其它磷脂或其它脂质。脂质体的物理特性取决于pH、离子强度和二价阳离子的存在情况。

[0175] 可用于产生脂质体的脂质的实例包括磷脂酰化合物，例如磷脂酰甘油、磷脂酰胆碱、磷脂酰丝氨酸、磷脂酰乙醇胺、鞘脂、脑苷脂和神经节苷脂。说明性的磷脂包括蛋黄磷脂

酰胆碱(egg phosphatidylcholine)、二棕榈酰磷脂酰胆碱和二硬脂酰磷脂酰胆碱。根据例如器官特异性、细胞特异性和细胞器特异性靶定脂质体也是可行的，并且是本领域已知的。

[0176] 本公开内容提供可以改变成包括酸和碱以调节pH以及缓冲剂以保持pH在窄范围内的的制剂。

[0177] 实例

现对本发明进行了大致描述，通过参照下列实施例可更容易地理解本发明，包括了所述实施例仅为了说明本发明的某些实施方案的目的，并无意限制本发明。

[0178] 实施例1. 生物活性GDF15的产生

GDF15 (亦称巨噬细胞抑制细胞因子-1)在生物化学上未显示直接与任何受体结合或相互作用。申请人首次尝试使用市购可获得的在哺乳动物CHO细胞中产生的人GDF15 (R&D Systems)，鉴定对GDF15具有高亲和力结合的天然受体但未成功。与含有特有的半胱氨酸结基序的TGFβ超家族中的其它配体一样，合成具有较大前域的成熟GDF15 (Harrison等, Growth Factors 29:174, 2011;Shi等, Nature 474:343, 2011)，所述前域通过在典型的RXXR位点被弗林蛋白酶样蛋白酶切割除去，产生成熟的二聚GDF15。因为不充分或不适当的配体纯化可能是市购可获得的GDF15失活的可能原因，所以申请人测试了用于GDF15的不同的纯化方法。

[0179] CHO细胞中GDF15的稳定表达

申请人使用CHO细胞表达人GDF15 (hGDF15)和鼠GDF15 (mGDF15)用于进一步研究。hGDF15的天然前体的氨基酸序列见图1，相应的核苷酸序列(与天然序列相比具有沉默的单一核苷酸取代)见图2。mGDF15前体的天然氨基酸和核苷酸序列分别见图3和图4。对于在CHO细胞中表达，将编码人或鼠GDF15前体的基于UCOE™的构建体稳定转染至CHO-PACE细胞系。以10 nM、20 nM和50 nM的甲氨蝶呤水平选择克隆，然后合并形成集落的任何克隆(每甲氨蝶呤浓度一个或两个)。不进行基因扩增，因为难以扩增UCOE™库同时保持表达的稳定性。鉴定高表达库，并用于产生hGDF15和mGDF15，而不是稀释克隆。

[0180] 人GDF15的纯化

为了开始纯化，稳定表达hGDF15的CHO细胞的条件培养基用乙酸调节至pH 4.7。在环境温度下温育培养基10分钟后，通过离心除去沉淀。将上清液用0.8 μm一次性过滤器过滤。将SP Sepharose™ Fast Flow柱(GE Healthcare)用缓冲液A (20 mM乙酸钠，pH 4.7)和B (20 mM乙酸钠、1M NaCl，pH 4.7)平衡。以100 cm/小时进行上样。柱用20% B (200 mM NaCl)洗涤直到无更多蛋白质从柱中洗脱出，然后反冲洗至0% B以除去任何残留的盐。蛋白质用50 mM Tris、6M脲，pH 8.0 (Tris+脲合并物)洗脱直到无更多蛋白质从柱中洗脱出，接着用50 mM Tris、6M脲、1M NaCl，pH 8.0 (Tris+脲+盐合并物)洗脱。将各合并物在4℃下在50 mM 4-吗啉乙磺酸(MES，pH 6.5)中透析过夜。

[0181] 根据蛋白质印迹分析，使存在于Tris+脲+盐合并物中的GDF15降解，因此弃去该合并物。将Tris+脲合并物上样到之前用缓冲液A (50 mM MES，pH 6.5)和B (50 mM MES、1M NaCl，pH 6.5)平衡的Q Sepharose™ Fast Flow柱(GE Healthcare)中。收集流出液，将柱用10% B (100 mM NaCl)洗涤，接着以120 cm/小时用5个柱体积的10-50% B梯度(100-500 mM NaCl)洗涤。在通过蛋白质印迹评价流出液和洗涤流分后，主要在流出液中发现蛋白质。将流出液注入含缓冲液A (水/0.1% TFA)和B (乙腈/0.1% TFA)的与HPLC连接的反相制备

型C4柱(Vydac)中。在1小时内以4.5 mL/分钟的25-40% B梯度产生最佳分离。所收集的流分通过SDS-PAGE凝胶(Sypro Ruby)和蛋白质印迹评价以选择用于在离心蒸发器中浓缩的流分。

[0182] 鼠GDF15的纯化

条件培养基的pH用乙酸调节至 pH 4.7。在环境温度下温育培养基10分钟后,通过离心除去沉淀。将上清液用0.8 μm一次性过滤器过滤。将SP Sepharose™ Fast Flow柱(GE Healthcare)用缓冲液A (20 mM乙酸钠,pH 4.7)和B (20 mM乙酸钠、1M NaCl,pH 4.7)平衡。以100-150 cm/小时进行上样,将柱用缓冲液A洗涤直到无更多蛋白质从柱中洗脱出。以60% B (600 mM NaCl)进行洗涤3-4个柱体积,接着用100% B (1M NaCl)洗涤3-4个柱体积。继续用50 mM Tris、6M脲,pH 8.0洗脱以除去仍与树脂结合的任何蛋白质。

[0183] 通过蛋白质印迹分析SP-柱流分的非还原样品。虽然大部分蛋白质存在于Tris洗脱流分中,但之前的实验表明,存在于这些流分中的mGDF15基本上是无活性的,因此不用于进一步纯化。相反,用存在于100% B洗脱(盐-洗脱合并物)的蛋白质继续纯化。将该合并物注入与HPLC连接的反相制备型C4柱(Vydac)中。缓冲液A为水/0.1% TFA,缓冲液B为乙腈/0.1% TFA。在1小时内以4.5 mL/分钟以25-40% B梯度洗脱蛋白质。在通过SDS-PAGE凝胶(Sypro Ruby)和蛋白质印迹评价反相柱流分后,合并含有纯mGDF15的流分,并在离心蒸发器中浓缩。

[0184] hGDF15和mGDF15的身份各自通过N端测序证实。两种类型的纯化的GDF15在两个不同的细胞系中刺激SMAD2/3磷酸化,从而提供配体活性的证实。

[0185] 实施例2. 以高亲和力结合GDF15的TGFβ超家族受体的鉴定

一旦获得有活性的GDF15蛋白,则针对与按实施例1所述产生和纯化的人或鼠GDF15的结合筛选包含TGFβ超家族受体的受体-Fc融合蛋白。这些融合蛋白掺入IgG1 Fc结构域,购自R&D Systems或内部产生。在5种II型受体(TGFβ受体II型、激活素受体II型A、激活素受体IIB型、BMP受体II型和MIS受体II型)中,仅TGFβ受体II型(TβRII)显示与GDF15的可检测结合(k_a = 2.92 x 10^5 $M^{-1}s^{-1}$;k_d = 0.001 s^{-1}),通过用俘获的受体-Fc融合蛋白的表面等离子体共振测定。hGDF15在37℃下以9.56 nM的平衡解离常数(K_D)与俘获的hTβRII-Fc结合。7种I型受体(ALK1、ALK2、ALK3、ALK4、ALK5、ALK6和ALK7)中无一显示与GDF15可检测的结合(mGDF15在20 nM或200 nM下)。

[0186] 人TβRII以通过胞外域(ECD)的可变剪接产生的至少2种同种型(A (长)和B (短))天然存在(图5、6)。用于上述筛选的hTβRII-hG1Fc融合蛋白(R&D Systems)掺入野生型TβRII$_{短}$同种型。在跟踪分析中,通过表面等离子体共振发现mGDF15与掺入野生型TβRII$_{长}$同种型(R&D Systems)的融合蛋白结合的亲和力与掺入TβRII$_{短}$同种型的融合蛋白的亲和力非常类似(K_D在37℃下分别为2.7 nM和4.8 nM)。有关GDF15结合观察到这些短和长的同种型大致相等,申请人然后产生了受体-Fc融合蛋白,其由通过最小接头在其C端与人IgG2 Fc结构域融合的hTβRII$_{短}$的野生型ECD(SEQ ID NO: 7)组成。除非另有说明,否则有关基于TβRII短和长同种型的变体的氨基酸位置编号分别是指天然前体SEQ ID NO: 5和SEQ ID NO: 6的相应位置。

[0187] 鉴于TβRII与GDF15的高亲和力结合,我们测试了TβRII是否可用作GDF15的抑制剂。在用含有CAGA-12启动子构建体的报道基因转染的A549细胞中测试了融合蛋白hTβRII$_{短}$

(23-159)-hG2Fc，发现其在这类细胞中以0.15-0.5 nM的IC_{50}抑制hGDF15诱导的基因活化。GDF15信号转导被hTβRII短ECD有效抑制提供了TβRII是GDF15的高亲和力受体的额外证据。即使GDF15在无细胞条件下不显示与ALK5的可检测结合，但与对照处理相比，在A549细胞(一种人肺上皮细胞系)中通过siRNA方法抑制内源ALK5 mRNA显著降低mGDF15介导的信号转导。相比之下，在A549细胞中，通过siRNA方法抑制其它I型受体(ALK2、ALK3、ALK4和ALK7)无法改变GDF15介导的信号转导。该结果表明，GDF15三重信号转导复合体包括ALK5 (TGFβ受体I型)作为其I型受体，因此提供TβRII作为GDF15的功能II型受体的确切证据。

[0188] 实施例3. 受体融合蛋白变体的产生

TβRII ECD变体

因为TβRII还以高亲和力与TGFβ1和TGFβ3结合，所以天然TβRII-Fc融合蛋白影响这些配体以及GDF15的信号转导。虽然在一些治疗背景下这种较广泛的配体结合可能是有利的，但在其它背景下，更大选择性的分子可能较好。因此，申请人通过产生包含人TβRII ECD的变体的融合蛋白寻找对GDF15选择性提高或降低的多肽。下面所示野生型hTβRII短(23-159)序列(SEQ ID NO: 7)用作下列5种受体ECD变体(SEQ ID NO: 8-12)的基础。使野生型hTβRII短(23-159)与IgG2的Fc部分融合，得到新的基础Fc融合物构建体。参见下面的SEQ ID NO. 50、51和52。

```
  1  TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSNCS
 51  ITSICEKPQE VCVAVWRKND ENITLETVCH DPKLPYHDFI LEDAASPKCI
101  MKEKKKPGET FFMCSCSSDE CNDNIIFSEE YNTSNPD (SEQ ID NO: 7)
```

[0189] (1) 下面显示hTβRII短(23-159/D110K)氨基酸序列(SEQ ID NO: 8)，其中取代的残基用下划线标出。

```
  1  TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSNCS
 51  ITSICEKPQE VCVAVWRKND ENITLETVCH DPKLPYHKFI LEDAASPKCI
101  MKEKKKPGET FFMCSCSSDE CNDNIIFSEE YNTSNPD (SEQ ID NO: 8)
```

[0190] (2) 下面显示N端截短的hTβRII短(29-159)氨基酸序列(SEQ ID NO: 9)。

```
  1  QKSVNNDMIV TDNNGAVKFP QLCKFCDVRF STCDNQKSCM SNCSITSICE
 51  KPQEVCVAVW RKNDENITLE TVCHDPKLPY HDFILEDAAS PKCIMKEKKK
101  PGETFFMCSC SSDECNDNII FSEEYNTSNP D   (SEQ ID NO: 9)
```

[0191] (3) 下面显示N端截短的hTβRII短(35-159)氨基酸序列(SEQ ID NO: 10)。

```
  1  DMIVTDNNGA VKFPQLCKFC DVRFSTCDNQ KSCMSNCSIT SICEKPQEVC
 51  VAVWRKNDEN ITLETVCHDP KLPYHDFILE DAASPKCIMK EKKKPGETFF
101  MCSCSSDECN DNIIFSEEYN TSNPD     (SEQ ID NO: 10)
```

[0192] (4) 下面显示C端截短的hTβRII短(23-153)氨基酸序列(SEQ ID NO: 11)。

```
  1  TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSNCS
 51  ITSICEKPQE VCVAVWRKND ENITLETVCH DPKLPYHDFI LEDAASPKCI
101  MKEKKKPGET FFMCSCSSDE CNDNIIFSEE Y  (SEQ ID NO: 11)
```

[0193] (5) 下面显示C端截短的hTβRII短(23-153/N70D)氨基酸序列(SEQ ID NO: 12),其中取代的残基用下划线标出。

```
  1  TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSDCS
 51  ITSICEKPQE VCVAVWRKND ENITLETVCH DPKLPYHDFI LEDAASPKCI
101  MKEKKKPGET FFMCSCSSDE CNDNIIFSEE Y   (SEQ ID NO: 12)
```

[0194] 申请人还预期基于以下所示野生型hTβRII长(23-184)序列(SEQ ID NO: 13)的5种相应的变体(SEQ ID NO: 14-17),其中25个氨基酸插入用下划线标出。注意剪接导致插入的C端的侧翼位置处的保守氨基酸取代(Val→Ile)。几种hTβRII短变体及其hTβRII长对应物的序列关系见图7。

```
  1  TIPPHVQKSD VEMEAQKDEI ICPSCNRTAH PLRHINNDMI VTDNNGAVKF
 51  PQLCKFCDVR FSTCDNQKSC MSNCSITSIC EKPQEVCVAV WRKNDENITL
101  ETVCHDPKLP YHDFILEDAA SPKCIMKEKK KPGETFFMCS CSSDECNDNI
151  IFSEEYNTSN PD   (SEQ ID NO: 13)
```

[0195] (1) 下面显示hTβRII长(23-184/D135K)氨基酸序列(SEQ ID NO: 14),其中取代的残基用双下划线表示。

```
  1  TIPPHVQKSD VEMEAQKDEI ICPSCNRTAH PLRHINNDMI VTDNNGAVKF
 51  PQLCKFCDVR FSTCDNQKSC MSNCSITSIC EKPQEVCVAV WRKNDENITL
101  ETVCHDPKLP YHKFILEDAA SPKCIMKEKK KPGETFFMCS CSSDECNDNI
151  IFSEEYNTSN PD   (SEQ ID NO: 14)
```

[0196] (2) 下面显示N端截短的hTβRII长(29-184)氨基酸序列(SEQ ID NO: 15)。

```
  1  QKSDVEMEAQ KDEIICPSCN RTAHPLRHIN NDMIVTDNNG AVKFPQLCKF
 51  CDVRFSTCDN QKSCMSNCSI TSICEKPQEV CVAVWRKNDE NITLETVCHD
101  PKLPYHDFIL EDAASPKCIM KEKKKPGETF FMCSCSSDEC NDNIIFSEEY
151  NTSNPD    (SEQ ID NO: 15)
```

[0197] (3) 下面显示N端截短的hTβRII长(60-184)氨基酸序列(与SEQ ID NO: 10相同)。

```
  1  DMIVTDNNGA VKFPQLCKFC DVRFSTCDNQ KSCMSNCSIT SICEKPQEVC
```

[0198]

```
 51  VAVWRKNDEN ITLETVCHDP KLPYHDFILE DAASPKCIMK EKKKPGETFF
101  MCSCSSDECN DNIIFSEEYN TSNPD
```

(与SEQ ID NO: 10相同)

(4) 下面显示C端截短的hTβRII长(23-178)氨基酸序列(SEQ ID NO: 16)。

```
  1  TIPPHVQKSD VEMEAQKDEI ICPSCNRTAH PLRHINNDMI VTDNNGAVKF
 51  PQLCKFCDVR FSTCDNQKSC MSNCSITSIC EKPQEVCVAV WRKNDENITL
101  ETVCHDPKLP YHDFILEDAA SPKCIMKEKK KPGETFFMCS CSSDECNDNI
151  IFSEEY    (SEQ ID NO: 16)
```

[0199] (5) 下面显示C端截短的hTβRII长(23-178/N95D)氨基酸序列(SEQ ID NO: 17),其

中取代的残基用双下划线表示。

```
  1  TIPPHVQKSD VEMEAQKDEI ICPSCNRTAH PLRHINNDMI VTDNNGAVKF
 51  PQLCKFCDVR FSTCDNQKSC MSDCSITSIC EKPQEVCVAV WRKNDENITL
101  ETVCHDPKLP YHDFILEDAA SPKCIMKEKK KPGETFFMCS CSSDECNDNI
151  IFSEEY     (SEQ ID NO: 17)
```

[0200] 其它TβRII ECD变体包括：

(A) 下面显示N端和C端截短的hTβRII短(35-153)或hTβRII长(60-178)氨基酸序列(SEQ ID NO: 47)。

```
  1  DMIVTDNNGA VKFPQLCKFC DVRFSTCDNQ KSCMSNCSIT SICEKPQEVC
 51  VAVWRKNDEN ITLETVCHDP KLPYHDFILE DAASPKCIMK EKKKPGETFF
101  MCSCSSDECN DNIIFSEEY      (SEQ ID NO: 47)
```

[0201] (B) 下面显示N端和C端截短的hTβRII短(29-153)氨基酸序列(SEQ ID NO: 48)。

```
  1  QKSVNNDMIV TDNNGAVKFP QLCKFCDVRF STCDNQKSCM SNCSITSICE
 51  KPQEVCVAVW RKNDENITLE TVCHDPKLPY HDFILEDAAS PKCIMKEKKK
101  PGETFFMCSC SSDECNDNII FSEEY    (SEQ ID NO: 48)
```

[0202] (C) 下面显示N端和C端截短的hTβRII长(29-178)氨基酸序列(SEQ ID NO: 49)。

```
  1  QKSDVEMEAQ KDEIICPSCN RTAHPLRHIN NDMIVTDNNG AVKFPQLCKF
 51  CDVRFSTCDN QKSCMSNCSI TSICEKPQEV CVAVWRKNDE NITLETVCHD
101  PKLPYHDFIL EDAASPKCIM KEKKKPGETF FMCSCSSDEC NDNIIFSEEY
     (SEQ ID NO: 49)
```

[0203] 像天然存在于hTβRII同种型C (Konrad等,BMC Genomics 8:318,2007)中一样,可将上述变体的任一个(SEQ ID NO: 8-12、14-17和47-49)在位于hTβRII ECD的C端附近的谷氨酸残基对(SEQ ID NO: 5的151和152位或SEQ ID NO: 6的176和177位)之间掺入36个氨基酸的插入序列(SEQ ID NO: 18)。

```
GRCKIRHIGS NNRLQRSTCQ NTGWESAHVM KTPGFR (SEQ ID NO: 18)
```

[0204] 例如,下面表示hTβRII短(29-159)变体(SEQ ID NO: 9)的侧接任选插入位点的配对谷氨酸残基(下划线)。

```
  1  QKSVNNDMIV TDNNGAVKFP QLCKFCDVRF STCDNQKSCM SNCSITSICE
 51  KPQEVCVAVW RKNDENITLE TVCHDPKLPY HDFILEDAAS PKCIMKEKKK
101  PGETFFMCSC SSDECNDNII FSEEYNTSNP D    (SEQ ID NO: 9)
```

[0205] Fc结构域变体

产生hTβRII-hFc融合蛋白,其中上述5种hTβRII短变体各自在其C端(通过最小接头)与人IgG2 Fc结构域融合,其具有下列氨基酸序列(SEQ ID NO: 19):

```
  1  VECPPCPAPP VAGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVQ
 51  FNWYVDGVEV HNAKTKPREE QFNSTFRVVS VLTVVHQDWL NGKEYKCKVS
101  NKGLPAPIEK TISKTKGQPR EPQVYTLPPS REEMTKNQVS LTCLVKGFYP
151  SDIAVEWESN GQPENNYKTT PPMLDSDGSF FLYSKLTVDK SRWQQGNVFS
201  CSVMHEALHN HYTQKSLSLS PGK        (SEQ ID NO: 19)
```

申请人预期包含备选Fc结构域(包括全长人IgG1 Fc (hG1Fc) (SEQ ID NO: 20,见下)和N端截短的人IgG1 Fc (hG1Fc短) (SEQ ID NO: 21,见下))的hTβRII-hFc融合蛋白。任选可连接与Fc结构域无关的多肽以替换Fc结构域。

```
  1  GGPKSCDKTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV
 51  DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS VLTVLHQDWL
101  NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS REEMTKNQVS
151  LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK
201  SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK    (SEQ ID NO: 20)
```

```
  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ VSLTCLVKGF
151  YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
201  FSCSVMHEAL HNHYTQKSLS LSPGK    (SEQ ID NO: 21)
```

[0206] 前导序列变体

考虑了下列3个前导序列:

(1) 天然:MGRGLLRGLWPLHIVLWTRIAS (SEQ ID NO: 22)

(2) 组织纤溶酶原激活物(TPA):MDAMKRGLCCVLLLCGAVFVSP (SEQ ID NO: 23)

(3)蜜蜂蜂毒肽(HBML):MKFLVNVALVFMVVYISYIYA (SEQ ID NO: 24)

hTβRII-hFc融合蛋白的表达

将选择的hTβRII-hFc蛋白变体掺入TPA前导序列,并具有SEQ ID NO: 25、29、33、37和41所示未加工的氨基酸序列(参见实施例5)。这些变体相应的核苷酸序列为SEQ ID NO: 26、30、34、38和42。使所选择的各含G2Fc结构域(SEQ ID NO: 19)的hTβRII-hFc变体在HEK-293细胞中表达,通过过滤和A蛋白层析法从条件培养基中纯化。通过SDS-PAGE和蛋白质印迹分析评价用于报道基因测定法的样品的纯度。

[0207] 申请人预期具有SEQ ID NO: 27、31、35、39和43所示未加工的氨基酸序列的其它hTβRII-hFc蛋白变体和SEQ ID NO: 28、32、36、40和44所示相应的核苷酸序列。

[0208] 野生型短构建体hTβRII短(23-159)-hG2Fc的氨基酸序列(SEQ ID NO: 50显示如下。

TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSNCS
ITSICEKPQE VCVAVWRKND ENITLETVCH DPKLPYHDFI LEDAASPKCI
MKEKKKPGET FFMCSCSSDE CNDNIIFSEE YNTSNPDTGG GVECPPCPAP
PVAGPSVFLF PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV QFNWYVDGVE
VHNAKTKPRE EQFNSTFRVV SVLTVVHQDW LNGKEYKCKV SNKGLPAPIE
KTISKTKGQP REPQVYTLPP SREEMTKNQV SLTCLVKGFY PSDIAVEWES
NGQPENNYKT TPPMLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH
NHYTQKSLSL SPGK (SEQ ID NO: 50)

[0209] 该蛋白质从如下所示包括TPA前导序列的构建体(SEQ ID NO:52)表达。虚线下划线表示前导序列,实心下划线表示接头。

1 MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSVNNDMIV TDNNGAVKFP
51 QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAVW RKNDENITLE
101 TVCHDPKLPY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
151 FSEEYNTSNP DTGGGVECPP CPAPPVAGPS VFLFPPKPKD TLMISRTPEV
201 TCVVVDVSHE DPEVQFNWYV DGVEVHNAKT KPREEQFNST FRVVSVLTVV
251 HQDWLNGKEY KCKVSNKGLP APIEKTISKT KGQPREPQVY TLPPSREEMT
301 KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPMLD SDGSFFLYSK
351 LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK
(SEQ ID NO: 52)

[0210] 编码SEQ ID NO:52的核酸序列显示如下:

1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
51 AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG
101 TTAATAACGA CATGATAGTC ACTGACAACA ACGGTGCAGT CAAGTTTCCA
151 CAACTGTGTA AATTTTGTGA TGTGAGATTT TCCACCTGTG ACAACCAGAA
201 ATCCTGCATG AGCAACTGCA GCATCACCTC CATCTGTGAG AAGCCACAGG
251 AAGTCTGTGT GGCTGTATGG AGAAAGAATG ACGAGAACAT AACACTAGAG
301 ACAGTTTGCC ATGACCCCAA GCTCCCCTAC CATGACTTTA TTCTGGAAGA

```
 351  TGCTGCTTCT CCAAAGTGCA TTATGAAGGA AAAAAAAAAG CCTGGTGAGA
 401  CTTTCTTCAT GTGTTCCTGT AGCTCTGATG AGTGCAATGA CAACATCATC
 451  TTCTCAGAAG AATATAACAC CAGCAATCCT GACACCGGTG GTGGAGTCGA
 501  GTGCCCACCG TGCCCAGCAC CACCTGTGGC AGGACCGTCA GTCTTCCTCT
 551  TCCCCCCAAA ACCCAAGGAC ACCCTCATGA TCTCCCGGAC CCCTGAGGTC
 601  ACGTGCGTGG TGGTGGACGT GAGCCACGAA GACCCCGAGG TCCAGTTCAA
 651  CTGGTACGTG GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCACGGG
 701  AGGAGCAGTT CAACAGCACG TTCCGTGTGG TCAGCGTCCT CACCGTCGTG
 751  CACCAGGACT GGCTGAACGG CAAGGAGTAC AAGTGCAAGG TCTCCAACAA
 801  AGGCCTCCCA GCCCCCATCG AGAAAACCAT CTCCAAAACC AAAGGGCAGC
 851  CCCGAGAACC ACAGGTGTAC ACCCTGCCCC CATCCCGGGA GGAGATGACC
 901  AAGAACCAGG TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT ACCCCAGCGA
 951  CATCGCCGTG GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACAAGA
1001  CCACACCTCC CATGCTGGAC TCCGACGGCT CCTTCTTCCT CTACAGCAAG
1051  CTCACCGTGG ACAAGAGCAG GTGGCAGCAG GGGAACGTCT TCTCATGCTC
1101  CGTGATGCAT GAGGCTCTGC ACAACCACTA CACGCAGAAG AGCCTCTCCC
1151  TGTCTCCGGG TAAA                    (SEQ ID NO: 51)
```

实施例4. 在基于细胞的测定法中通过受体融合蛋白变体的差异配体抑制

采用A549细胞中的报道基因测定法测定hTβRII-hFc变体抑制GDF15、TGFβ1、TGFβ2和TGFβ3的活性的能力。该测定法基于pGL3(CAGA)12报道质粒(Dennler等, 1998, EMBO 17: 3091-3100)以及Renilla报道质粒(pRLCMV)转染的人肺癌细胞系以控制转染效率。CAGA基序存在于TGFβ-反应基因的启动子(例如PAI-1)中,使得该载体通用于通过SMAD2和SMAD3的因子信号转导。

[0211] 在测定的第一天,以6.5x10^{4}个细胞/孔将A549细胞(ATCC®:CCL-185™)分布在48孔板中。在第二天,使含有10 μg pGL3(CAGA)12、100ng pRLCMV、30 μl X-tremeGENE 9 (Roche Applied Science)和970 μl OptiMEM (Invitrogen)的溶液预温育30分钟,然后加入补充0.1% BSA的Eagle最小必需培养基(EMEM,ATCC®)中,将其加入铺板细胞(500 μl/孔)中用于在室温下温育过夜。在第三天,除去培养基,使细胞与如下所述制备的配体和抑制剂的混合物一起在37℃下温育过夜。

[0212] 在48孔板上在200 μl体积的测定缓冲液(EMEM + 0.1 % BSA)中制备连续稀释的试验品。加入含有试验配体的等体积的测定缓冲液,得到等于之前测定的EC50的最终配体浓度。人GDF15和鼠GDF15在内部产生(见上文),而人TGFβ1、人TGFβ2和人TGFβ3获自PeproTech。使试验溶液在37℃下温育30分钟,然后将250 μl混合物加入所有孔中。一式两份测定各浓度的试验品。在与试验溶液温育过夜后,将细胞用磷酸缓冲盐水漂洗,然后用被动裂解缓冲液(Promega E1941)裂解,并在-70℃下保存过夜。在第4和最后一天,在轻轻振荡的同时使板升温至室温。将细胞裂解物一式两份转移到化学发光板(96孔)上,在发光计中用来自Dual-Luciferase Reporter Assay系统(Promega E1980)的试剂分析以确定归一化萤光素酶活性。

[0213] 采用该测定法针对通过TβRII配体对细胞信号转导的可能的抑制作用筛选受体融合蛋白变体。与之前有关野生型TβRII短-Fc和TβRII长-Fc的报告(del Re等,J Biol Chem 279:22765,2004)一致,受测变体甚至在高浓度下无一能够抑制TGFβ2。然而hTβRII-hFc变体料想不到地显示由GDF15、TGFβ1和TGFβ3介导的细胞信号转导的差异抑制。与野生型TβRII短(23-159)-G2Fc相比,TβRII短(23-159/D110K)-G2Fc变体显示GDF15的有效抑制,但丧失TGFβ1的抑制,且TGFβ3抑制大大降低(~50倍)(参见下表)。第110位位于TβRII的"钩"区(Radaev等,J Biol Chem 285:14806,2010),但并未表明在公认的TβRII配体TGFβ1、TGFβ2和TGFβ3之间提供选择性。因此,该变体显示差异配体抑制的特征,其中GDF15最有效地被抑制,TGFβ1最不有效地被抑制,且TGFβ3被抑制至中等程度。

构建体		IC_{50} (nM)		
		mGDF15 (35 ng/ml)	hTGFβ1 (640 pg/ml)	hTGFβ3 (270 pg/ml)
全长野生型 ECD	TβRII 短(23-159)-G2Fc	~0.12	1.73	0.14
具有 D110K 取代的全长 ECD	TβRII 短(23-159/D110K)-G2Fc	~0.7	ND (> 73.6)	~6.9

ND，未测定

[0214] 在第2个实验中,将具有N端截短的TβRII ECD的变体的效能与全长野生型TβRII ECD的进行比较。如下表所示,与TβRII短(23-159)-G2Fc (野生型)相比,TβRII短(29-159)-G2Fc和TβRII短(35-159)-G2Fc显示抑制TGFβ3的能力大大减弱,但抑制GDF15的能力未减弱(N'Δ6)或仅略减弱(N'Δ12)。与野生型相比,N端截短对TGFβ1的抑制的影响在量级上是中等的。因此,这2种变体显示差异配体抑制的特征,其中GDF15最有效地被抑制,TGFβ3最不有效地被抑制,TGFβ1被抑制至中等程度。

构建体		IC_{50} (nM)		
		hGDF15 (70 或 112 ng/ml)	hTGFβ1 (640 pg/ml)	hTGFβ3 (270 pg/ml)
全长野生型 ECD	TβRII 短(23-159)-G2Fc	0.14 – 0.53	0.52	0.37
N'Δ6 ECD	TβRII 短(29-159)-G2Fc	0.40	2.05	ND (> 7.5)
N'Δ12 ECD	TβRII 短(35-159)-G2Fc	0.92	2.51	ND (> 7.5)

ND，未测定

[0215] 在第3个实验中,我们测定了C端截短的TβRII ECD中的N70D取代对效能的作用。该天冬氨酸残基表示可能的糖基化位点。如下表所示,TβRII短(23-153/N70D)-G2Fc显示与TβRII短(23-153)-G2Fc相比,抑制TGFβ1的能力大大减弱,而抑制TGFβ3的能力几乎未减弱。与TβRII短(23-153)-G2Fc和野生型两者相比,N70D取代对GDF15抑制的作用在量级上是中等的。因此,具有N70D取代的C端截短变体显示差异配体抑制的特征,其中TGFβ3最有效地被抑制,TGFβ1最不有效地被抑制,GDF15被抑制至中等程度。

构建体		IC_{50} (nM)		
		hGDF15 (70 ng/ml)	hTGFβ1 (640 pg/ml)	hTGFβ3 (270 pg/ml)
全长野生型 ECD	TβRII 短(23-159)-G2Fc	0.14	ND	ND
C'Δ6 ECD	TβRII 短(23-153)-G2Fc	0.18	2.62	0.14
具有 N70D 取代的 C'Δ6 ECD	TβRII 短(23-153/N70D)-G2Fc	2.42	17.7	0.28

[0216] 总起来,这些结果表明,申请人产生了显示极不同的配体结合特征的TβRII ECD的截短物和突变物。值得注意地,该实证显示适当表达和纯化的GDF15与TβRII直接相互作用,并可被包含TβRII ECD变体的融合蛋白差异性抑制。这些变体的活性特征可概括于下表。

配体选择性的概括				
构建体		配体抑制的程度		
		有效	中等	可忽略
全长野生型 ECD	TβRII 短(23-159)-G2Fc	GDF15 TGFβ1 TGFβ3	---	TGFβ2
具有 D110K 取代的全长 ECD	TβRII 短(23-159/D110K)-G2Fc	GDF15	TGFβ3	TGFβ1 TGFβ2
N'Δ6 ECD	TβRII 短(29-159)-G2Fc	GDF15	TGFβ1	TGFβ2 TGFβ3
N'Δ12 ECD	TβRII 短(35-159)-G2Fc	GDF15	TGFβ1	TGFβ2 TGFβ3
具有N70D取代的 C'Δ6 ECD	TβRII 短(23-153/N70D)-G2Fc	TGFβ3	GDF15	TGFβ1 TGFβ2

[0217] 我们预测,这些$TβRII_{短}$ECD变体的$TβRII_{长}$ECD对应物将显示类似的配体选择性。另外,C'Δ6截短的ECD(例如对于$TβRII_{短}$和$TβRII_{长}$同种型分别为SEQ ID NO: 11和16)可用作其中引入突变和N端截短的$TβRII_{短}$或$TβRII_{长}$的基础序列。

[0218] 实施例5. 示例性hTβRII-hFc核酸和蛋白质

该实施例概述了可用于按照本文提供的方法在HEK-293或CHO细胞中表达TβRII构建体以提供从细胞培养物中分离的蛋白质的核酸构建体。在各种情况下,可使从细胞培养物中分离的成熟蛋白质除去前导序列(以下各序列中的虚线下划线)。

[0219] 第1项显示$hTβRII_{短}$(23-159/D110K)-hG2Fc的氨基酸序列(SEQ ID NO: 25)。双下划线表示D110K取代。虚线下划线表示前导序列,实心下划线表示接头。

1 MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSVNNDMIV TDNNGAVKFP

```
 51  QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAVW RKNDENITLE
101  TVCHDPKLPY HKFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
151  FSEEYNTSNP DTGGGVECPP CPAPPVAGPS VFLFPPKPKD TLMISRTPEV
201  TCVVVDVSHE DPEVQFNWYV DGVEVHNAKT KPREEQFNST FRVVSVLTVV
251  HQDWLNGKEY KCKVSNKGLP APIEKTISKT KGQPREPQVY TLPPSREEMT
301  KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPMLD SDGSFFLYSK
351  LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK
     (SEQ ID NO: 25)
```

[0220] 第2项显示编码hTβRII短(23-159/D110K)-hG2Fc的核苷酸序列(SEQ ID NO: 26)。双下划线表示D110K取代。虚线下划线表示前导序列,实心下划线表示接头。

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG
 101  TTAATAACGA CATGATAGTC ACTGACAACA ACGGTGCAGT CAAGTTTCCA
 151  CAACTGTGTA AATTTTGTGA TGTGAGATTT TCCACCTGTG ACAACCAGAA
 201  ATCCTGCATG AGCAACTGCA GCATCACCTC CATCTGTGAG AAGCCACAGG
 251  AAGTCTGTGT GGCTGTATGG AGAAAGAATG ACGAGAACAT AACACTAGAG
 301  ACAGTTTGCC ATGACCCCAA GCTCCCCTAC CATAAGTTTA TTCTGGAAGA
 351  TGCTGCTTCT CCAAAGTGCA TTATGAAGGA AAAAAAAAAG CCTGGTGAGA
 401  CTTTCTTCAT GTGTTCCTGT AGCTCTGATG AGTGCAATGA CAACATCATC
 451  TTCTCAGAAG AATATAACAC CAGCAATCCT GACACCGGTG GTGGAGTCGA
 501  GTGCCCACCG TGCCCAGCAC CACCTGTGGC AGGACCGTCA GTCTTCCTCT
 551  TCCCCCCAAA ACCCAAGGAC ACCCTCATGA TCTCCCGGAC CCCTGAGGTC
 601  ACGTGCGTGG TGGTGGACGT GAGCCACGAA GACCCCGAGG TCCAGTTCAA
 651  CTGGTACGTG GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCACGGG
 701  AGGAGCAGTT CAACAGCACG TTCCGTGTGG TCAGCGTCCT CACCGTCGTG
 751  CACCAGGACT GGCTGAACGG CAAGGAGTAC AAGTGCAAGG TCTCCAACAA
 801  AGGCCTCCCA GCCCCCATCG AGAAAACCAT CTCCAAAACC AAAGGGCAGC
 851  CCCGAGAACC ACAGGTGTAC ACCCTGCCCC CATCCCGGGA GGAGATGACC
 901  AAGAACCAGG TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT ACCCCAGCGA
 951  CATCGCCGTG GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACAAGA
1001  CCACACCTCC CATGCTGGAC TCCGACGGCT CCTTCTTCCT CTACAGCAAG
1051  CTCACCGTGG ACAAGAGCAG GTGGCAGCAG GGGAACGTCT TCTCATGCTC
1101  CGTGATGCAT GAGGCTCTGC ACAACCACTA CACGCAGAAG AGCCTCTCCC
1151  TGTCTCCGGG TAAA                    (SEQ ID NO: 26)
```

[0221] 第3项显示hTβRII短(23-159/D110K)-hG1Fc短的氨基酸序列(SEQ ID NO: 27)。双下划线表示D110K取代。虚线下划线表示前导序列,实心下划线表示接头。

```
  1  MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSVNNDMIV TDNNGAVKFP
 51  QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAVW RKNDENITLE
101  TVCHDPKLPY HKFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
151  FSEEYNTSNP DTGGGTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP
201  EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT
251  VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE
301  MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY
351  SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK
     (SEQ ID NO: 27)
```

[0222] 第4项显示编码hTβRII短(23-159/D110K)-hG1Fc短的核苷酸序列(SEQ ID NO: 28)。双下划线表示D110K取代。虚线下划线表示前导序列,实心下划线表示接头。

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG
 101  TTAATAACGA CATGATAGTC ACTGACAACA ACGGTGCAGT CAAGTTTCCA
 151  CAACTGTGTA AATTTTGTGA TGTGAGATTT TCCACCTGTG ACAACCAGAA
 201  ATCCTGCATG AGCAACTGCA GCATCACCTC CATCTGTGAG AAGCCACAGG
 251  AAGTCTGTGT GGCTGTATGG AGAAAGAATG ACGAGAACAT AACACTAGAG
 301  ACAGTTTGCC ATGACCCCAA GCTCCCCTAC CATAAGTTTA TTCTGGAAGA
 351  TGCTGCTTCT CCAAAGTGCA TTATGAAGGA AAAAAAAAAG CCTGGTGAGA
 401  CTTTCTTCAT GTGTTCCTGT AGCTCTGATG AGTGCAATGA CAACATCATC
 451  TTCTCAGAAG AATATAACAC CAGCAATCCT GACACCGGTG GTGGAACTCA
 501  CACATGCCCA CCGTGCCCAG CACCTGAACT CCTGGGGGGA CCGTCAGTCT
 551  TCCTCTTCCC CCCAAAACCC AAGGACACCC TCATGATCTC CCGGACCCCT
 601  GAGGTCACAT GCGTGGTGGT GGACGTGAGC CACGAAGACC CTGAGGTCAA
 651  GTTCAACTGG TACGTGGACG GCGTGGAGGT GCATAATGCC AAGACAAAGC
 701  CGCGGGAGGA GCAGTACAAC AGCACGTACC GTGTGGTCAG CGTCCTCACC
 751  GTCCTGCACC AGGACTGGCT GAATGGCAAG GAGTACAAGT GCAAGGTCTC
 801  CAACAAAGCC CTCCCAGCCC CCATCGAGAA AACCATCTCC AAAGCCAAAG
 851  GGCAGCCCCG AGAACCACAG GTGTACACCC TGCCCCCATC CCGGGAGGAG
 901  ATGACCAAGA ACCAGGTCAG CCTGACCTGC CTGGTCAAAG GCTTCTATCC
 951  CAGCGACATC GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG GAGAACAACT
1001  ACAAGACCAC GCCTCCCGTG CTGGACTCCG ACGGCTCCTT CTTCCTCTAT
1051  AGCAAGCTCA CCGTGGACAA GAGCAGGTGG CAGCAGGGGA ACGTCTTCTC
1101  ATGCTCCGTG ATGCATGAGG CTCTGCACAA CCACTACACG CAGAAGAGCC
1151  TCTCCCTGTC CCCGGGTAAA          (SEQ ID NO: 28)
```

[0223] 第5项显示hTβRII短(29-159)-hG2Fc的氨基酸序列(SEQ ID NO: 29)。虚线下划线表示前导序列,实心下划线表示接头。

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAQKSVNN DMIVTDNNGA VKFPQLCKFC
 51  DVRFSTCDNQ KSCMSNCSIT SICEKPQEVC VAVWRKNDEN ITLETVCHDP
101  KLPYHDFILE DAASPKCIMK EKKKPGETFF MCSCSSDECN DNIIFSEEYN
151  TSNPDTGGGV ECPPCPAPPV AGPSVFLFPP KPKDTLMISR TPEVTCVVVD
201  VSHEDPEVQF NWYVDGVEVH NAKTKPREEQ FNSTFRVVSV LTVVHQDWLN
251  GKEYKCKVSN KGLPAPIEKT ISKTKGQPRE PQVYTLPPSR EEMTKNQVSL
301  TCLVKGFYPS DIAVEWESNG QPENNYKTTP PMLDSDGSFF LYSKLTVDKS
351  RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK    (SEQ ID NO: 29)
```

[0224] 第6项显示编码hTβRII短(29-159)-hG2Fc的核苷酸序列(SEQ ID NO: 30)。虚线下划线表示前导序列,实心下划线表示接头。

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCCAGAAGTC GGTTAATAAC GACATGATAG
 101  TCACTGACAA CAACGGTGCA GTCAAGTTTC CACAACTGTG TAAATTTTGT
 151  GATGTGAGAT TTTCCACCTG TGACAACCAG AAATCCTGCA TGAGCAACTG
 201  CAGCATCACC TCCATCTGTG AGAAGCCACA GGAAGTCTGT GTGGCTGTAT
 251  GGAGAAAGAA TGACGAGAAC ATAACACTAG AGACAGTTTG CCATGACCCC
 301  AAGCTCCCCT ACCATGACTT TATTCTGGAA GATGCTGCTT CTCCAAAGTG
 351  CATTATGAAG GAAAAAAAAA AGCCTGGTGA GACTTTCTTC ATGTGTTCCT
 401  GTAGCTCTGA TGAGTGCAAT GACAACATCA TCTTCTCAGA AGAATATAAC
 451  ACCAGCAATC CTGACACCGG TGGTGGAGTC GAGTGCCCAC CGTGCCCAGC
 501  ACCACCTGTG GCAGGACCGT CAGTCTTCCT CTTCCCCCCA AAACCCAAGG
 551  ACACCCTCAT GATCTCCCGG ACCCCTGAGG TCACGTGCGT GGTGGTGGAC
 601  GTGAGCCACG AAGACCCCGA GGTCCAGTTC AACTGGTACG TGGACGGCGT
 651  GGAGGTGCAT AATGCCAAGA CAAAGCCACG GGAGGAGCAG TTCAACAGCA
 701  CGTTCCGTGT GGTCAGCGTC CTCACCGTCG TGCACCAGGA CTGGCTGAAC
 751  GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC AAAGGCCTCC CAGCCCCCAT
 801  CGAGAAAACC ATCTCCAAAA CCAAAGGGCA GCCCCGAGAA CCACAGGTGT
 851  ACACCCTGCC CCCATCCCGG GAGGAGATGA CCAAGAACCA GGTCAGCCTG
 901  ACCTGCCTGG TCAAAGGCTT CTACCCCAGC GACATCGCCG TGGAGTGGGA
```

```
 951  GAGCAATGGG CAGCCGGAGA ACAACTACAA GACCACACCT CCCATGCTGG
1001  ACTCCGACGG CTCCTTCTTC CTCTACAGCA AGCTCACCGT GGACAAGAGC
1051  AGGTGGCAGC AGGGGAACGT CTTCTCATGC TCCGTGATGC ATGAGGCTCT
1101  GCACAACCAC TACACGCAGA AGAGCCTCTC CCTGTCTCCG GGTAAA
      (SEQ ID NO: 30)
```

[0225] 第7项显示hTβRII短(29-159)-hG1Fc短的氨基酸序列(SEQ ID NO: 31)。虚线下划线表示前导序列,实心下划线表示接头。

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAQKSVNN DMIVTDNNGA VKFPQLCKFC
 51  DVRFSTCDNQ KSCMSNCSIT SICEKPQEVC VAVWRKNDEN ITLETVCHDP
101  KLPYHDFILE DAASPKCIMK EKKKPGETFF MCSCSSDECN DNIIFSEEYN
151  TSNPDTGGGT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV
201  VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW
251  LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SREEMTKNQV
301  SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD
351  KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK   (SEQ ID NO: 31)
```

[0226] 第8项显示编码hTβRII短(29-159)-hG1Fc短的核苷酸序列(SEQ ID NO: 32)。虚线下划线表示前导序列,实心下划线表示接头。

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCCAGAAGTC GGTTAATAAC GACATGATAG
 101  TCACTGACAA CAACGGTGCA GTCAAGTTTC CACAACTGTG TAAATTTTGT
 151  GATGTGAGAT TTTCCACCTG TGACAACCAG AAATCCTGCA TGAGCAACTG
 201  CAGCATCACC TCCATCTGTG AGAAGCCACA GGAAGTCTGT GTGGCTGTAT
 251  GGAGAAAGAA TGACGAGAAC ATAACACTAG AGACAGTTTG CCATGACCCC
 301  AAGCTCCCCT ACCATGACTT TATTCTGGAA GATGCTGCTT CTCCAAAGTG
 351  CATTATGAAG GAAAAAAAAA AGCCTGGTGA GACTTTCTTC ATGTGTTCCT
 401  GTAGCTCTGA TGAGTGCAAT GACAACATCA TCTTCTCAGA AGAATATAAC
 451  ACCAGCAATC CTGACACCGG TGGTGGAACT CACACATGCC CACCGTGCCC
 501  AGCACCTGAA CTCCTGGGGG GACCGTCAGT CTTCCTCTTC CCCCCAAAAC
 551  CCAAGGACAC CCTCATGATC TCCCGGACCC CTGAGGTCAC ATGCGTGGTG
 601  GTGGACGTGA GCCACGAAGA CCCTGAGGTC AAGTTCAACT GGTACGTGGA
 651  CGGCGTGGAG GTGCATAATG CCAAGACAAA GCCGCGGGAG GAGCAGTACA
 701  ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG
 751  CTGAATGGCA AGGAGTACAA GTGCAAGGTC TCCAACAAAG CCCTCCCAGC
 801  CCCCATCGAG AAAACCATCT CCAAAGCCAA AGGGCAGCCC CGAGAACCAC
```

```
 851  AGGTGTACAC CCTGCCCCCA TCCCGGGAGG AGATGACCAA GAACCAGGTC
 901  AGCCTGACCT GCCTGGTCAA AGGCTTCTAT CCCAGCGACA TCGCCGTGGA
 951  GTGGGAGAGC AATGGGCAGC CGGAGAACAA CTACAAGACC ACGCCTCCCG
1001  TGCTGGACTC CGACGGCTCC TTCTTCCTCT ATAGCAAGCT CACCGTGGAC
1051  AAGAGCAGGT GGCAGCAGGG GAACGTCTTC TCATGCTCCG TGATGCATGA
1101  GGCTCTGCAC AACCACTACA CGCAGAAGAG CCTCTCCCTG TCCCCGGGTA
1151  AA      (SEQ ID NO: 32)
```

[0227] 第9项显示hTβRII短(35-159)-hG2Fc的氨基酸序列(SEQ ID NO: 33)。虚线下划线表示前导序列,实心下划线表示接头。

```
   1  MDAMKRGLCC VLLLCGAVFV SPGADMIVTD NNGAVKFPQL CKFCDVRFST
  51  CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD
 101  FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPDT
 151  GGGVECPPCP APPVAGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP
 201  EVQFNWYVDG VEVHNAKTKP REEQFNSTFR VVSVLTVVHQ DWLNGKEYKC
 251  KVSNKGLPAP IEKTISKTKG QPREPQVYTL PPSREEMTKN QVSLTCLVKG
 301  FYPSDIAVEW ESNGQPENNY KTTPPMLDSD GSFFLYSKLT VDKSRWQQGN
 351  VFSCSVMHEA LHNHYTQKSL SLSPGK      (SEQ ID NO: 33)
```

[0228] 第10项显示编码hTβRII短(35-159)-hG2Fc的核苷酸序列(SEQ ID NO: 34)。虚线下划线表示前导序列,实心下划线表示接头。

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCGACATGAT AGTCACTGAC AACAACGGTG
 101  CAGTCAAGTT TCCACAACTG TGTAAATTTT GTGATGTGAG ATTTTCCACC
 151  TGTGACAACC AGAAATCCTG CATGAGCAAC TGCAGCATCA CCTCCATCTG
 201  TGAGAAGCCA CAGGAAGTCT GTGTGGCTGT ATGGAGAAAG AATGACGAGA
 251  ACATAACACT AGAGACAGTT TGCCATGACC CCAAGCTCCC CTACCATGAC
 301  TTTATTCTGG AAGATGCTGC TTCTCCAAAG TGCATTATGA AGGAAAAAAA
 351  AAAGCCTGGT GAGACTTTCT TCATGTGTTC CTGTAGCTCT GATGAGTGCA
 401  ATGACAACAT CATCTTCTCA GAAGAATATA ACACCAGCAA TCCTGACACC
 451  GGTGGTGGAG TCGAGTGCCC ACCGTGCCCA GCACCACCTG TGGCAGGACC
 501  GTCAGTCTTC CTCTTCCCCC CAAAACCCAA GGACACCCTC ATGATCTCCC
 551  GGACCCCTGA GGTCACGTGC GTGGTGGTGG ACGTGAGCCA CGAAGACCCC
 601  GAGGTCCAGT TCAACTGGTA CGTGGACGGC GTGGAGGTGC ATAATGCCAA
 651  GACAAAGCCA CGGGAGGAGC AGTTCAACAG CACGTTCCGT GTGGTCAGCG
 701  TCCTCACCGT CGTGCACCAG GACTGGCTGA ACGGCAAGGA GTACAAGTGC
 751  AAGGTCTCCA ACAAAGGCCT CCCAGCCCCC ATCGAGAAAA CCATCTCCAA
```

```
 801  AACCAAAGGG CAGCCCCGAG AACCACAGGT GTACACCCTG CCCCCATCCC
 851  GGGAGGAGAT GACCAAGAAC CAGGTCAGCC TGACCTGCCT GGTCAAAGGC
 901  TTCTACCCCA GCGACATCGC CGTGGAGTGG GAGAGCAATG GGCAGCCGGA
 951  GAACAACTAC AAGACCACAC CTCCCATGCT GGACTCCGAC GGCTCCTTCT
1001  TCCTCTACAG CAAGCTCACC GTGGACAAGA GCAGGTGGCA GCAGGGGAAC
1051  GTCTTCTCAT GCTCCGTGAT GCATGAGGCT CTGCACAACC ACTACACGCA
1101  GAAGAGCCTC TCCCTGTCTC CGGGTAAA      (SEQ ID NO: 34)
```

[0229] 第11项显示hTβRII短(35-159)-hG1Fc短的氨基酸序列(SEQ ID NO: 35)。虚线下划线表示前导序列,实心下划线表示接头。

```
  1  MDAMKRGLCC VLLLCGAVFV SPGADMIVTD NNGAVKFPQL CKFCDVRFST
 51  CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD
101  FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPDT
151  GGGTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE
201  DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY
251  KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSREEMT KNQVSLTCLV
301  KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ
351  GNVFSCSVMH EALHNHYTQK SLSLSPGK        (SEQ ID NO: 35)
```

[0230] 第12项显示编码hTβRII短(35-159)-hG1Fc短的核苷酸序列(SEQ ID NO: 36)。虚线下划线表示前导序列,实心下划线表示接头。

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCGACATGAT AGTCACTGAC AACAACGGTG
 101  CAGTCAAGTT TCCACAACTG TGTAAATTTT GTGATGTGAG ATTTTCCACC
 151  TGTGACAACC AGAAATCCTG CATGAGCAAC TGCAGCATCA CCTCCATCTG
 201  TGAGAAGCCA CAGGAAGTCT GTGTGGCTGT ATGGAGAAAG AATGACGAGA
 251  ACATAACACT AGAGACAGTT TGCCATGACC CCAAGCTCCC CTACCATGAC
 301  TTTATTCTGG AAGATGCTGC TTCTCCAAAG TGCATTATGA AGGAAAAAAA
 351  AAAGCCTGGT GAGACTTTCT TCATGTGTTC CTGTAGCTCT GATGAGTGCA
 401  ATGACAACAT CATCTTCTCA GAAGAATATA ACACCAGCAA TCCTGACACC
 451  GGTGGTGGAA CTCACACATG CCCACCGTGC CCAGCACCTG AACTCCTGGG
 501  GGGACCGTCA GTCTTCCTCT TCCCCCCAAA ACCCAAGGAC ACCCTCATGA
 551  TCTCCCGGAC CCCTGAGGTC ACATGCGTGG TGGTGGACGT GAGCCACGAA
 601  GACCCTGAGG TCAAGTTCAA CTGGTACGTG GACGGCGTGG AGGTGCATAA
 651  TGCCAAGACA AAGCCGCGGG AGGAGCAGTA CAACAGCACG TACCGTGTGG
 701  TCAGCGTCCT CACCGTCCTG CACCAGGACT GGCTGAATGG CAAGGAGTAC
 751  AAGTGCAAGG TCTCCAACAA AGCCCTCCCA GCCCCCATCG AGAAAACCAT
```

```
 801  CTCCAAAGCC AAAGGGCAGC CCCGAGAACC ACAGGTGTAC ACCCTGCCCC
 851  CATCCCGGGA GGAGATGACC AAGAACCAGG TCAGCCTGAC CTGCCTGGTC
 901  AAAGGCTTCT ATCCCAGCGA CATCGCCGTG GAGTGGGAGA GCAATGGGCA
 951  GCCGGAGAAC AACTACAAGA CCACGCCTCC CGTGCTGGAC TCCGACGGCT
1001  CCTTCTTCCT CTATAGCAAG CTCACCGTGG ACAAGAGCAG GTGGCAGCAG
1051  GGGAACGTCT TCTCATGCTC CGTGATGCAT GAGGCTCTGC ACAACCACTA
1101  CACGCAGAAG AGCCTCTCCC TGTCCCCGGG TAAA
      (SEQ ID NO: 36)
```

[0231] 第13项显示hTβRII短(23-153)-hG2Fc的氨基酸序列(SEQ ID NO: 37)。虚线下划线表示前导序列,实心下划线表示接头。

```
  1  MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSVNNDMIV TDNNGAVKFP
 51  QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAVW RKNDENITLE
101  TVCHDPKLPY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
151  FSEEYTGGGV ECPPCPAPPV AGPSVFLFPP KPKDTLMISR TPEVTCVVVD
201  VSHEDPEVQF NWYVDGVEVH NAKTKPREEQ FNSTFRVVSV LTVVHQDWLN
251  GKEYKCKVSN KGLPAPIEKT ISKTKGQPRE PQVYTLPPSR EEMTKNQVSL
301  TCLVKGFYPS DIAVEWESNG QPENNYKTTP PMLDSDGSFF LYSKLTVDKS
351  RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK    (SEQ ID NO: 37)
```

[0232] 第14项显示编码hTβRII短(23-153)-hG2Fc的核苷酸序列(SEQ ID NO: 38)。虚线下划线表示前导序列,实心下划线表示接头。

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG
 101  TTAATAACGA CATGATAGTC ACTGACAACA ACGGTGCAGT CAAGTTTCCA
 151  CAACTGTGTA AATTTTGTGA TGTGAGATTT TCCACCTGTG ACAACCAGAA
 201  ATCCTGCATG AGCAACTGCA GCATCACCTC CATCTGTGAG AAGCCACAGG
 251  AAGTCTGTGT GGCTGTATGG AGAAAGAATG ACGAGAACAT AACACTAGAG
 301  ACAGTTTGCC ATGACCCCAA GCTCCCCTAC CATGACTTTA TTCTGGAAGA
 351  TGCTGCTTCT CCAAAGTGCA TTATGAAGGA AAAAAAAAAG CCTGGTGAGA
 401  CTTTCTTCAT GTGTTCCTGT AGCTCTGATG AGTGCAATGA CAACATCATC
 451  TTCTCAGAAG AATATACCGG TGGTGGAGTC GAGTGCCCAC CGTGCCCAGC
 501  ACCACCTGTG GCAGGACCGT CAGTCTTCCT CTTCCCCCCA AAACCCAAGG
 551  ACACCCTCAT GATCTCCCGG ACCCCTGAGG TCACGTGCGT GGTGGTGGAC
 601  GTGAGCCACG AAGACCCCGA GGTCCAGTTC AACTGGTACG TGGACGGCGT
 651  GGAGGTGCAT AATGCCAAGA CAAAGCCACG GGAGGAGCAG TTCAACAGCA
 701  CGTTCCGTGT GGTCAGCGTC CTCACCGTCG TGCACCAGGA CTGGCTGAAC
```

```
 751  GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC AAAGGCCTCC CAGCCCCCAT
 801  CGAGAAAACC ATCTCCAAAA CCAAAGGGCA GCCCCGAGAA CCACAGGTGT
 851  ACACCCTGCC CCCATCCCGG GAGGAGATGA CCAAGAACCA GGTCAGCCTG
 901  ACCTGCCTGG TCAAAGGCTT CTACCCCAGC GACATCGCCG TGGAGTGGGA
 951  GAGCAATGGG CAGCCGGAGA ACAACTACAA GACCACACCT CCCATGCTGG
1001  ACTCCGACGG CTCCTTCTTC CTCTACAGCA AGCTCACCGT GGACAAGAGC
1051  AGGTGGCAGC AGGGGAACGT CTTCTCATGC TCCGTGATGC ATGAGGCTCT
1101  GCACAACCAC TACACGCAGA AGAGCCTCTC CCTGTCTCCG GGTAAA
      (SEQ ID NO: 38)
```

[0233] 第15项显示hTβRII短(23-153)-hG1Fc短的氨基酸序列(SEQ ID NO: 39)。虚线下划线表示前导序列,实心下划线表示接头。

```
   1  MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSVNNDMIV TDNNGAVKFP
  51  QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAVW RKNDENITLE
 101  TVCHDPKLPY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
 151  FSEEYTGGGT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV
 201  VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW
 251  LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SREEMTKNQV
 301  SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD
 351  KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK     (SEQ ID NO: 39)
```

[0234] 第16项显示编码hTβRII短(23-153)-hG1Fc短的核苷酸序列(SEQ ID NO: 40)。虚线下划线表示前导序列,实心下划线表示接头。

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG
 101  TTAATAACGA CATGATAGTC ACTGACAACA ACGGTGCAGT CAAGTTTCCA
 151  CAACTGTGTA AATTTTGTGA TGTGAGATTT TCCACCTGTG ACAACCAGAA
 201  ATCCTGCATG AGCAACTGCA GCATCACCTC CATCTGTGAG AAGCCACAGG
 251  AAGTCTGTGT GGCTGTATGG AGAAAGAATG ACGAGAACAT AACACTAGAG
 301  ACAGTTTGCC ATGACCCCAA GCTCCCCTAC CATGACTTTA TTCTGGAAGA
 351  TGCTGCTTCT CCAAAGTGCA TTATGAAGGA AAAAAAAAAG CCTGGTGAGA
 401  CTTTCTTCAT GTGTTCCTGT AGCTCTGATG AGTGCAATGA CAACATCATC
 451  TTCTCAGAAG AATATACCGG TGGTGGAACT CACACATGCC CACCGTGCCC
 501  AGCACCTGAA CTCCTGGGGG GACCGTCAGT CTTCCTCTTC CCCCCAAAAC
 551  CCAAGGACAC CCTCATGATC TCCCGGACCC CTGAGGTCAC ATGCGTGGTG
 601  GTGGACGTGA GCCACGAAGA CCCTGAGGTC AAGTTCAACT GGTACGTGGA
 651  CGGCGTGGAG GTGCATAATG CCAAGACAAA GCCGCGGGAG GAGCAGTACA
```

```
 701  ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG
 751  CTGAATGGCA AGGAGTACAA GTGCAAGGTC TCCAACAAAG CCCTCCCAGC
 801  CCCCATCGAG AAAACCATCT CCAAAGCCAA AGGGCAGCCC CGAGAACCAC
 851  AGGTGTACAC CCTGCCCCCA TCCCGGGAGG AGATGACCAA GAACCAGGTC
 901  AGCCTGACCT GCCTGGTCAA AGGCTTCTAT CCCAGCGACA TCGCCGTGGA
 951  GTGGGAGAGC AATGGGCAGC CGGAGAACAA CTACAAGACC ACGCCTCCCG
1001  TGCTGGACTC CGACGGCTCC TTCTTCCTCT ATAGCAAGCT CACCGTGGAC
1051  AAGAGCAGGT GGCAGCAGGG GAACGTCTTC TCATGCTCCG TGATGCATGA
1101  GGCTCTGCAC AACCACTACA CGCAGAAGAG CCTCTCCCTG TCCCCGGGTA
1151  AA      (SEQ ID NO: 40)
```

[0235] 第17项显示hTβRII短(23-153/N70D)-hG2Fc的氨基酸序列(SEQ ID NO: 41)。双下划线表示N70D取代。虚线下划线表示前导序列,实心下划线表示接头。

```
  1  MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSVNNDMIV TDNNGAVKFP
 51  QLCKFCDVRF STCDNQKSCM SDCSITSICE KPQEVCVAVW RKNDENITLE
101  TVCHDPKLPY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
151  FSEEYTGGGV ECPPCPAPPV AGPSVFLFPP KPKDTLMISR TPEVTCVVVD
201  VSHEDPEVQF NWYVDGVEVH NAKTKPREEQ FNSTFRVVSV LTVVHQDWLN
251  GKEYKCKVSN KGLPAPIEKT ISKTKGQPRE PQVYTLPPSR EEMTKNQVSL
301  TCLVKGFYPS DIAVEWESNG QPENNYKTTP PMLDSDGSFF LYSKLTVDKS
351  RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK     (SEQ ID NO: 41)
```

[0236] 第18项显示编码hTβRII短(23-153/N70D)-hG2Fc的核苷酸序列(SEQ ID NO: 42)。双下划线表示N70D取代。虚线下划线表示前导序列,实心下划线表示接头。

```
  1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
 51  AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG
101  TTAATAACGA CATGATAGTC ACTGACAACA ACGGTGCAGT CAAGTTTCCA
151  CAACTGTGTA AATTTTGTGA TGTGAGATTT TCCACCTGTG ACAACCAGAA
201  ATCCTGCATG AGCGACTGCA GCATCACCTC CATCTGTGAG AAGCCACAGG
251  AAGTCTGTGT GGCTGTATGG AGAAAGAATG ACGAGAACAT AACACTAGAG
301  ACAGTTTGCC ATGACCCCAA GCTCCCCTAC CATGACTTTA TTCTGGAAGA
351  TGCTGCTTCT CCAAAGTGCA TTATGAAGGA AAAAAAAAAG CCTGGTGAGA
401  CTTTCTTCAT GTGTTCCTGT AGCTCTGATG AGTGCAATGA CAACATCATC
451  TTCTCAGAAG AATATACCGG TGGTGGAGTC GAGTGCCCAC CGTGCCCAGC
501  ACCACCTGTG GCAGGACCGT CAGTCTTCCT CTTCCCCCCA AAACCCAAGG
```

```
 551  ACACCCTCAT GATCTCCCGG ACCCCTGAGG TCACGTGCGT GGTGGTGGAC
 601  GTGAGCCACG AAGACCCCGA GGTCCAGTTC AACTGGTACG TGGACGGCGT
 651  GGAGGTGCAT AATGCCAAGA CAAAGCCACG GGAGGAGCAG TTCAACAGCA
 701  CGTTCCGTGT GGTCAGCGTC CTCACCGTCG TGCACCAGGA CTGGCTGAAC
 751  GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC AAAGGCCTCC CAGCCCCCAT
 801  CGAGAAAACC ATCTCCAAAA CCAAAGGGCA GCCCCGAGAA CCACAGGTGT
 851  ACACCCTGCC CCCATCCCGG GAGGAGATGA CCAAGAACCA GGTCAGCCTG
 901  ACCTGCCTGG TCAAAGGCTT CTACCCCAGC GACATCGCCG TGGAGTGGGA
 951  GAGCAATGGG CAGCCGGAGA ACAACTACAA GACCACACCT CCCATGCTGG
1001  ACTCCGACGG CTCCTTCTTC CTCTACAGCA AGCTCACCGT GGACAAGAGC
1051  AGGTGGCAGC AGGGGAACGT CTTCTCATGC TCCGTGATGC ATGAGGCTCT
1101  GCACAACCAC TACACGCAGA AGAGCCTCTC CCTGTCTCCG GGTAAA
      (SEQ ID NO: 42)
```

[0237] 第19项显示hTβRII短(23-153/N70D)-hG1Fc短的氨基酸序列(SEQ ID NO: 43)。双下划线表示N70D取代。虚线下划线表示前导序列,实心下划线表示接头。

```
  1  MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSVNNDMIV TDNNGAVKFP
 51  QLCKFCDVRF STCDNQKSCM SDCSITSICE KPQEVCVAVW RKNDENITLE
101  TVCHDPKLPY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
151  FSEEYTGGGT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV
201  VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW
251  LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SREEMTKNQV
301  SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD
351  KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK     (SEQ ID NO: 43)
```

[0238] 第20项显示编码hTβRII短(23-153/N70D)-hG1Fc短的核苷酸序列(SEQ ID NO: 44)。双下划线表示N70D取代。虚线下划线表示前导序列,实心下划线表示接头。

```
  1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
 51  AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG
101  TTAATAACGA CATGATAGTC ACTGACAACA ACGGTGCAGT CAAGTTTCCA
151  CAACTGTGTA AATTTTGTGA TGTGAGATTT TCCACCTGTG ACAACCAGAA
201  ATCCTGCATG AGCGACTGCA GCATCACCTC CATCTGTGAG AAGCCACAGG
251  AAGTCTGTGT GGCTGTATGG AGAAAGAATG ACGAGAACAT AACACTAGAG
301  ACAGTTTGCC ATGACCCCAA GCTCCCCTAC CATGACTTTA TTCTGGAAGA
351  TGCTGCTTCT CCAAAGTGCA TTATGAAGGA AAAAAAAAAG CCTGGTGAGA
```

```
 401  CTTTCTTCAT GTGTTCCTGT AGCTCTGATG AGTGCAATGA CAACATCATC
 451  TTCTCAGAAG AATATACCGG TGGTGGAACT CACACATGCC CACCGTGCCC
 501  AGCACCTGAA CTCCTGGGGG GACCGTCAGT CTTCCTCTTC CCCCCAAAAC
 551  CCAAGGACAC CCTCATGATC TCCCGGACCC CTGAGGTCAC ATGCGTGGTG
 601  GTGGACGTGA GCCACGAAGA CCCTGAGGTC AAGTTCAACT GGTACGTGGA
 651  CGGCGTGGAG GTGCATAATG CCAAGACAAA GCCGCGGGAG GAGCAGTACA
 701  ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG
 751  CTGAATGGCA AGGAGTACAA GTGCAAGGTC TCCAACAAAG CCCTCCCAGC
 801  CCCCATCGAG AAAACCATCT CCAAAGCCAA AGGGCAGCCC CGAGAACCAC
 851  AGGTGTACAC CCTGCCCCCA TCCCGGGAGG AGATGACCAA GAACCAGGTC
 901  AGCCTGACCT GCCTGGTCAA AGGCTTCTAT CCCAGCGACA TCGCCGTGGA
 951  GTGGGAGAGC AATGGGCAGC CGGAGAACAA CTACAAGACC ACGCCTCCCG
1001  TGCTGGACTC CGACGGCTCC TTCTTCCTCT ATAGCAAGCT CACCGTGGAC
1051  AAGAGCAGGT GGCAGCAGGG GAACGTCTTC TCATGCTCCG TGATGCATGA
1101  GGCTCTGCAC AACCACTACA CGCAGAAGAG CCTCTCCCTG TCCCCGGGTA
1151  AA          (SEQ ID NO: 44)
```

[0239] 第21项显示hTβRII短(23-159/D110K)-hG2Fc的成熟氨基酸序列(即无前导序列)(SEQ ID NO: 53)。双下划线表示D110K取代。单下划线表示接头。

```
                                TIPPHV QKSVNNDMIV TDNNGAVKFP
QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAVW RKNDENITLE
TVCHDPKLPY HKFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
FSEEYNTSNP DTGGGVECPP CPAPPVAGPS VFLFPPKPKD TLMISRTPEV
TCVVVDVSHE DPEVQFNWYV DGVEVHNAKT KPREEQFNST FRVVSVLTVV
HQDWLNGKEY KCKVSNKGLP APIEKTISKT KGQPREPQVY TLPPSREEMT
KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPMLD SDGSFFLYSK
LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK
(SEQ ID NO:53)
```

[0240] 第22项显示hTβRII短(23-159/D110K)-hG1Fc短的成熟氨基酸序列(即无前导序列)(SEQ ID NO: 54)。双下划线表示D110K取代。单下划线表示接头。

```
                                TIPPHV QKSVNNDMIV TDNNGAVKFP
QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAVW RKNDENITLE
TVCHDPKLPY HKFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
```

FSEEYNTSNP DTGGGTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP
EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT
VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE
MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY
SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK (SEQ ID
NO: 54)

[0241] 第23项显示hTβRII短(29-159)-hG2Fc的成熟氨基酸序列(即无前导序列)(SEQ ID NO: 55)。单下划线表示接头。

QKSVNN DMIVTDNNGA VKFPQLCKFC
DVRFSTCDNQ KSCMSNCSIT SICEKPQEVC VAVWRKNDEN ITLETVCHDP
KLPYHDFILE DAASPKCIMK EKKKPGETFF MCSCSSDECN DNIIFSEEYN
TSNPDTGGGV ECPPCPAPPV AGPSVFLFPP KPKDTLMISR TPEVTCVVVD
VSHEDPEVQF NWYVDGVEVH NAKTKPREEQ FNSTFRVVSV LTVVHQDWLN
GKEYKCKVSN KGLPAPIEKT ISKTKGQPRE PQVYTLPPSR EEMTKNQVSL
TCLVKGFYPS DIAVEWESNG QPENNYKTTP PMLDSDGSFF LYSKLTVDKS
RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK (SEQ ID NO: 55)

[0242] 第24项显示hTβRII短(29-159)-hG1Fc短的成熟氨基酸序列(即无前导序列)(SEQ ID NO: 56)。单下划线表示接头。

QKSVNN DMIVTDNNGA VKFPQLCKFC
DVRFSTCDNQ KSCMSNCSIT SICEKPQEVC VAVWRKNDEN ITLETVCHDP
KLPYHDFILE DAASPKCIMK EKKKPGETFF MCSCSSDECN DNIIFSEEYN
TSNPDTGGGT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV
VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW
LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SREEMTKNQV
SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD
KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK (SEQ ID NO: 56)

[0243] 第25项显示hTβRII短(35-159)-hG2Fc的成熟氨基酸序列(即无前导序列)(SEQ ID NO: 57)。单下划线表示接头。

DMIVTD NNGAVKFPQL CKFCDVRFST
CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD
FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPDT

GGGVECPPCP APPVAGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP
EVQFNWYVDG VEVHNAKTKP REEQFNSTFR VVSVLTVVHQ DWLNGKEYKC
KVSNKGLPAP IEKTISKTKG QPREPQVYTL PPSREEMTKN QVSLTCLVKG
FYPSDIAVEW ESNGQPENNY KTTPPMLDSD GSFFLYSKLT VDKSRWQQGN
VFSCSVMHEA LHNHYTQKSL SLSPGK (SEQ ID NO: 57)

[0244] 第26项显示hTβRII短(35-159)-hG1Fc短的成熟氨基酸序列(即无前导序列)(SEQ ID NO: 58)。单下划线表示接头。

DMIVTD NNGAVKFPQL CKFCDVRFST
CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD
FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPDT
GGGTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE
DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY
KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSREEMT KNQVSLTCLV
KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ
GNVFSCSVMH EALHNHYTQK SLSLSPGK (SEQ ID NO: 58)

[0245] 第27项显示hTβRII短(23-153)-hG2Fc的成熟氨基酸序列(即无前导序列)(SEQ ID NO: 59)。单下划线表示接头。

TIPPHV QKSVNNDMIV TDNNGAVKFP
QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAVW RKNDENITLE
TVCHDPKLPY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
FSEEYTGGGV ECPPCPAPPV AGPSVFLFPP KPKDTLMISR TPEVTCVVVD
VSHEDPEVQF NWYVDGVEVH NAKTKPREEQ FNSTFRVVSV LTVVHQDWLN
GKEYKCKVSN KGLPAPIEKT ISKTKGQPRE PQVYTLPPSR EEMTKNQVSL
TCLVKGFYPS DIAVEWESNG QPENNYKTTP PMLDSDGSFF LYSKLTVDKS
RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK (SEQ ID NO: 59)

[0246] 第28项显示hTβRII短(23-153)-hG1Fc短的成熟氨基酸序列(即无前导序列)(SEQ ID NO: 60)。单下划线表示接头。

TIPPHV QKSVNNDMIV TDNNGAVKFP
QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAVW RKNDENITLE
TVCHDPKLPY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
FSEEYTGGGT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV
VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW

LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SREEMTKNQV
SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD
KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK (SEQ ID NO: 60)

[0247] 第29项显示hTβRII短(23-153/N70D)-hG2Fc的成熟氨基酸序列(即无前导序列)(SEQ ID NO: 61)。双下划线表示N70D取代。单下划线表示接头。

TIPPHV QKSVNNDMIV TDNNGAVKFP
QLCKFCDVRF STCDNQKSCM SDCSITSICE KPQEVCVAVW RKNDENITLE
TVCHDPKLPY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
FSEEYTGGGV ECPPCPAPPV AGPSVFLFPP KPKDTLMISR TPEVTCVVVD
VSHEDPEVQF NWYVDGVEVH NAKTKPREEQ FNSTFRVVSV LTVVHQDWLN
GKEYKCKVSN KGLPAPIEKT ISKTKGQPRE PQVYTLPPSR EEMTKNQVSL
TCLVKGFYPS DIAVEWESNG QPENNYKTTP PMLDSDGSFF LYSKLTVDKS
RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK (SEQ ID NO: 61)

[0248] 第30项显示hTβRII短(23-153/N70D)-hG1Fc短的成熟氨基酸序列(即无前导序列)(SEQ ID NO: 62)。双下划线表示N70D取代。单下划线表示接头。

TIPPHV QKSVNNDMIV TDNNGAVKFP
QLCKFCDVRF STCDNQKSCM SDCSITSICE KPQEVCVAVW RKNDENITLE
TVCHDPKLPY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
FSEEYTGGGT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV
VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW
LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SREEMTKNQV
SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD
KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK (SEQ ID NO: 62)

[0249] 通过引用结合

本文提及的所有出版物和专利均通过引用以其整体结合到本文中,就像每个独立出版物或专利具体而单独指明通过引用结合一样。

[0250] 虽然论述了主题的具体实施方案,但上述说明书是说明性的而非限制性的。当回顾本说明书和随附权利要求书时,许多变动对本领域技术人员而言将变得显而易见。应参照权利要求书及其等同内容的整个范围和说明书及这类变化来确定本发明的整个范围。

```
  1  MPGQELRTVN GSQMLLVLLV LSWLPHGGAL SLAEASRASF PGPSELHSED
 51  SRFRELRKRY EDLLTRLRAN QSWEDSNTDL VPAPAVRILT PEVRLGSGGH
101  LHLRISRAAL PEGLPEASRL HRALFRLSPT ASRSWDVTRP LRRQLSLARP
151  QAPALHLRLS PPPSQSDQLL AESSSARPQL ELHLRPQAAR GRRRARARNG
201  DHCPLGPGRC CRLHTVRASL EDLGWADWVL SPREVQVTMC IGACPSQFRA
251  ANMHAQIKTS LHRLKPDTVP APCCVPASYN PMVLIQKTDT GVSLQTYDDL
301  LAKDCHCI     (SEQ ID NO: 1)
```

图 1

```
   1  ATGCCCGGGC AAGAACTCAG GACGGTGAAT GGCTCTCAGA TGCTCCTGGT
  51  GTTGCTGGTG CTCTCGTGGC TGCCGCATGG GGGCGCCCTG TCTCTGGCCG
 101  AGGCGAGCCG CGCAAGTTTC CCGGGACCCT CAGAGTTGCA CTCCGAAGAC
 151  TCCAGATTCC GAGAGTTGCG GAAACGCTAC GAGGACCTGC TAACCAGGCT
 201  GCGGGCCAAC CAGAGCTGGG AAGATTCGAA CACCGACCTC GTCCCGGCCC
 251  CTGCAGTCCG GATACTCACG CCAGAAGTGC GGCTGGGATC CGGCGGCCAC
 301  CTGCACCTGC GTATCTCTCG GGCCGCCCTT CCCGAGGGGC TCCCCGAGGC
 351  CTCCCGCCTT CACCGGGCTC TGTTCCGGCT GTCCCCGACG GCGTCAAGGT
 401  CGTGGGACGT GACACGACCG CTGCGGCGTC AGCTCAGCCT TGCAAGACCC
 451  CAGGCACCCG CGCTGCACCT GCGACTGTCG CCGCCGCCGT CGCAGTCGGA
 501  CCAACTGCTG GCAGAATCTT CGTCCGCACG GCCCCAGCTG GAGTTGCACT
 551  TGCGGCCGCA AGCCGCCAGG GGGCGCCGCA GAGCGCGTGC GCGCAACGGG
 601  GACCACTGTC CGCTCGGGCC CGGGCGTTGC TGCCGTCTGC ACACGGTCCG
 651  CGCGTCGCTG GAAGACCTGG GCTGGGCCGA TTGGGTGCTG TCGCCACGGG
 701  AGGTGCAAGT GACCATGTGC ATCGGCGCGT GCCCGAGCCA GTTCCGGGCG
 751  GCAAACATGC ACGCGCAGAT CAAGACGAGC CTGCACCGCC TGAAGCCCGA
 801  CACGGTGCCA GCGCCCTGCT GCGTGCCCGC CAGCTACAAT CCCATGGTGC
 851  TCATTCAAAA GACCGACACC GGGGTGTCAC TCCAGACCTA TGATGACTTG
 901  TTAGCCAAAG ACTGCCACTG CATA        (SEQ ID NO: 2)
```

图 2

```
  1  MAPPALQAQP PGGSQLRFLL FLLLLLLLLS WPSQGDALAM PEQRPSGPES
 51  QLNADELRGR FQDLLSRLHA NQSREDSNSE PSPDPAVRIL SPEVRLGSHG
101  QLLLRVNRAS LSQGLPEAYR VHRALLLLTP TARPWDITRP LKRALSLRGP
151  RAPALRLRLT PPPDLAMLPS GGTQLELRLR VAAGRGRRSA HAHPRDSCPL
201  GPGRCCHLET VQATLEDLGW SDWVLSPRQL QLSMCVGECP HLYRSANTHA
251  QIKARLHGLQ PDKVPAPCCV PSSYTPVVLM HRTDSGVSLQ TYDDLVARGC
301  HCA        (SEQ ID NO: 3)
```

图 3

```
  1  ATGGCCCCGC CCGCGCTCCA GGCCCAGCCT CCAGGCGGCT CTCAACTGAG
 51  GTTCCTGCTG TTCCTGCTGC TGTTGCTGCT GCTGCTGTCA TGGCCATCGC
101  AGGGGGACGC CCTGGCAATG CCTGAACAGC GACCCTCCGG CCCTGAGTCC
151  CAACTCAACG CCGACGAGCT ACGGGGTCGC TTCCAGGACC TGCTGAGCCG
201  GCTGCATGCC AACCAGAGCC GAGAGGACTC GAACTCAGAA CCAAGTCCTG
251  ACCCAGCTGT CCGGATACTC AGTCCAGAGG TGAGATTGGG GTCCCACGGC
301  CAGCTGCTAC TCCGCGTCAA CCGGGCGTCG CTGAGTCAGG GTCTCCCCGA
351  AGCCTACCGC GTGCACCGAG CGCTGCTCCT GCTGACGCCG ACGGCCCGCC
401  CCTGGGACAT CACTAGGCCC CTGAAGCGTG CGCTCAGCCT CCGGGGACCC
451  CGTGCTCCCG CATTACGCCT GCGCCTGACG CCGCCTCCGG ACCTGGCTAT
501  GCTGCCCTCT GGCGGCACGC AGCTGGAACT GCGCTTACGG GTAGCCGCCG
551  GCAGGGGGCG CCGAAGCGCG CATGCGCACC CAAGAGACTC GTGCCCACTG
601  GGTCCAGGGC GCTGCTGTCA CTTGGAGACT GTGCAGGCAA CTCTTGAAGA
651  CTTGGGCTGG AGCGACTGGG TGCTGTCCCC GCGCCAGCTG CAGCTGAGCA
701  TGTGCGTGGG CGAGTGTCCC CACCTGTATC GCTCCGCGAA CACGCATGCG
751  CAGATCAAAG CACGCCTGCA TGGCCTGCAG CCTGACAAGG TGCCTGCCCC
801  GTGCTGTGTC CCCTCCAGCT ACACCCCGGT GGTTCTTATG CACAGGACAG
851  ACAGTGGTGT GTCACTGCAG ACTTATGATG ACCTGGTGGC CCGGGGCTGC
901  CACTGCGCT          (SEQ ID NO: 4)
```

图 4

```
  1  mgrgllrglw plhivlwtri astipphvqk svnndmivtd nngavkfpql
 51  ckfcdvrfst cdnqkscmsn csitsicekp qevcvavwrk ndenitletv
101  chdpklpyhd filedaaspk cimkekkkpg etffmcscss decndniifs
151  eeyntsnpdl llvifqvtgi sllpplgvai sviiifycyr vnrqqklsst
201  wetgktrklm efsehcaiil eddrsdisst canninhnte llpieldtlv
251  gkgrfaevyk aklkqntseq fetvavkifp yeeyaswkte kdifsdinlk
301  henilqflta eerktelgkq ywlitafhak gnlqeyltrh viswedlrkl
351  gsslargiah lhsdhtpcgr pkmpivhrdl kssnilvknd ltcclcdfgl
401  slrldptlsv ddlansgqvg tarymapevl esrmnlenve sfkqtdvysm
451  alvlwemtsr cnavgevkdy eppfgskvre hpcvesmkdn vlrdrgrpei
501  psfwlnhqgi qmvcetltec wdhdpearlt aqcvaerfse lehldrlsgr
551  scseekiped gslnttk                (SEQ ID NO: 5)
```

图 5

```
  1  mgrgllrglw plhivlwtri astipphvqk sdvemeaqkd eiicpscnrt
 51  ahplrhinnd mivtdnngav kfpqlckfcd vrfstcdnqk scmsncsits
101  icekpqevcv avwrkndeni tletvchdpk lpyhdfiled aaspkcimke
151  kkkpgetffm cscssdecnd niifseeynt snpdlllvif qvtgisllpp
201  lgvaisviii fycyrvnrqq klsstwetgk trklmefseh caiileddrs
251  disstcanni nhntellpie ldtlvgkgrf aevykaklkq ntseqfetva
301  vkifpyeeya swktekdifs dinlkhenil qfltaeerkt elgkqywlit
351  afhakgnlqe yltrhviswe dlrklgssla rgiahlhsdh tpcgrpkmpi
401  vhrdlkssni lvkndltccl cdfglslrld ptlsvddlan sgqvgtarym
451  apevlesrmn lenvesfkqt dvysmalvlw emtsrcnavg evkdyeppfg
501  skvrehpcve smkdnvlrdr grpeipsfwl nhqgiqmvce tltecwdhdp
551  earltaqcva erfselehld rlsgrscsee kipedgslnt tk
     (SEQ ID NO: 6)
```

图 6

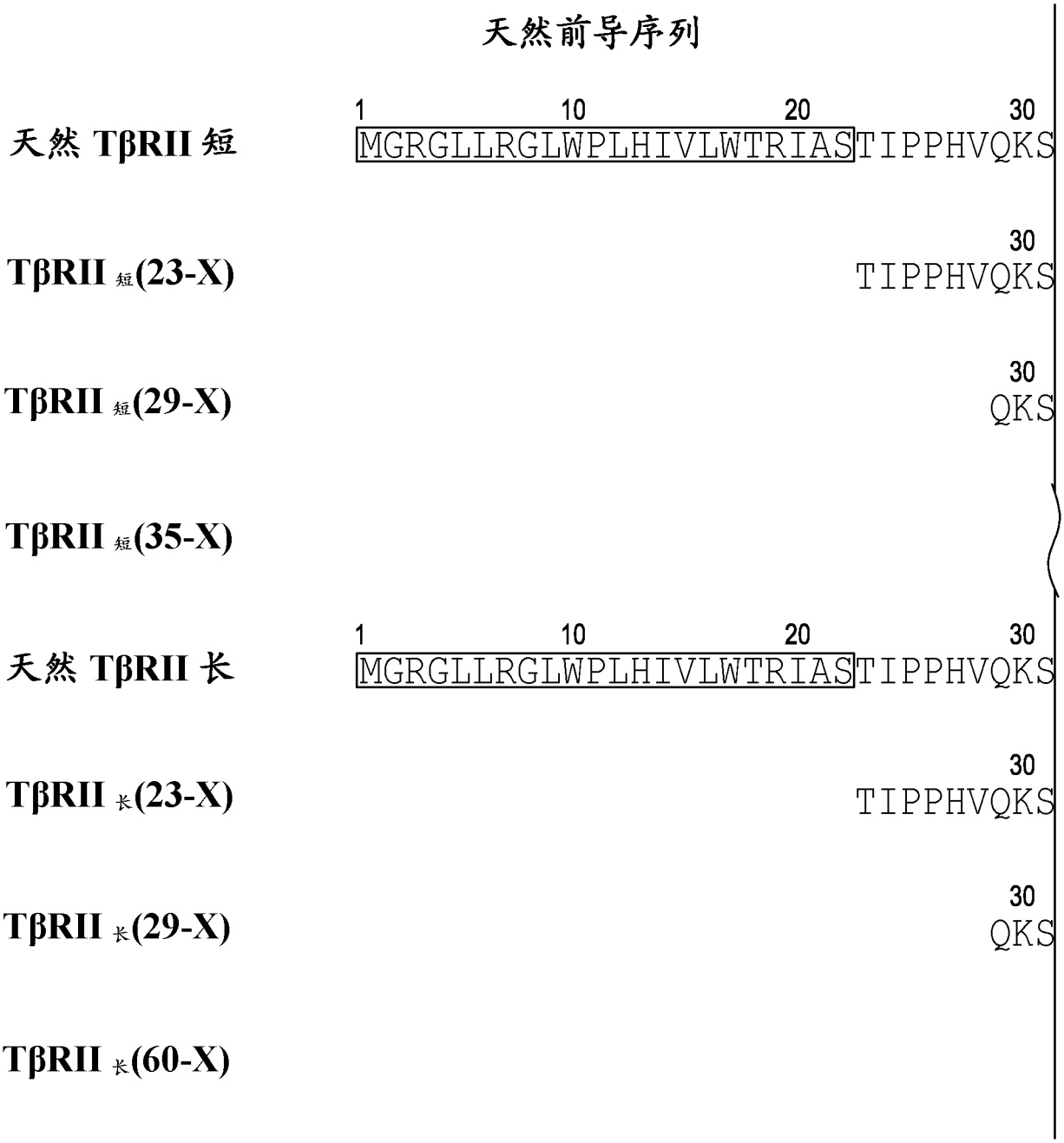
天然前导序列
1
10
20
30
天然 TβRII 短
MGRGLLRGLWPLHIVLWTRIASTIPPHVQKS
TβRII 短(23-X)
TIPPHVQKS
TβRII 短(29-X)
QKS
TβRII 短(35-X)
天然 TβRII 长
MGRGLLRGLWPLHIVLWTRIASTIPPHVQKS
TβRII 长(23-X)
TIPPHVQKS
TβRII 长(29-X)
QKS
TβRII 长(60-X)

图 7

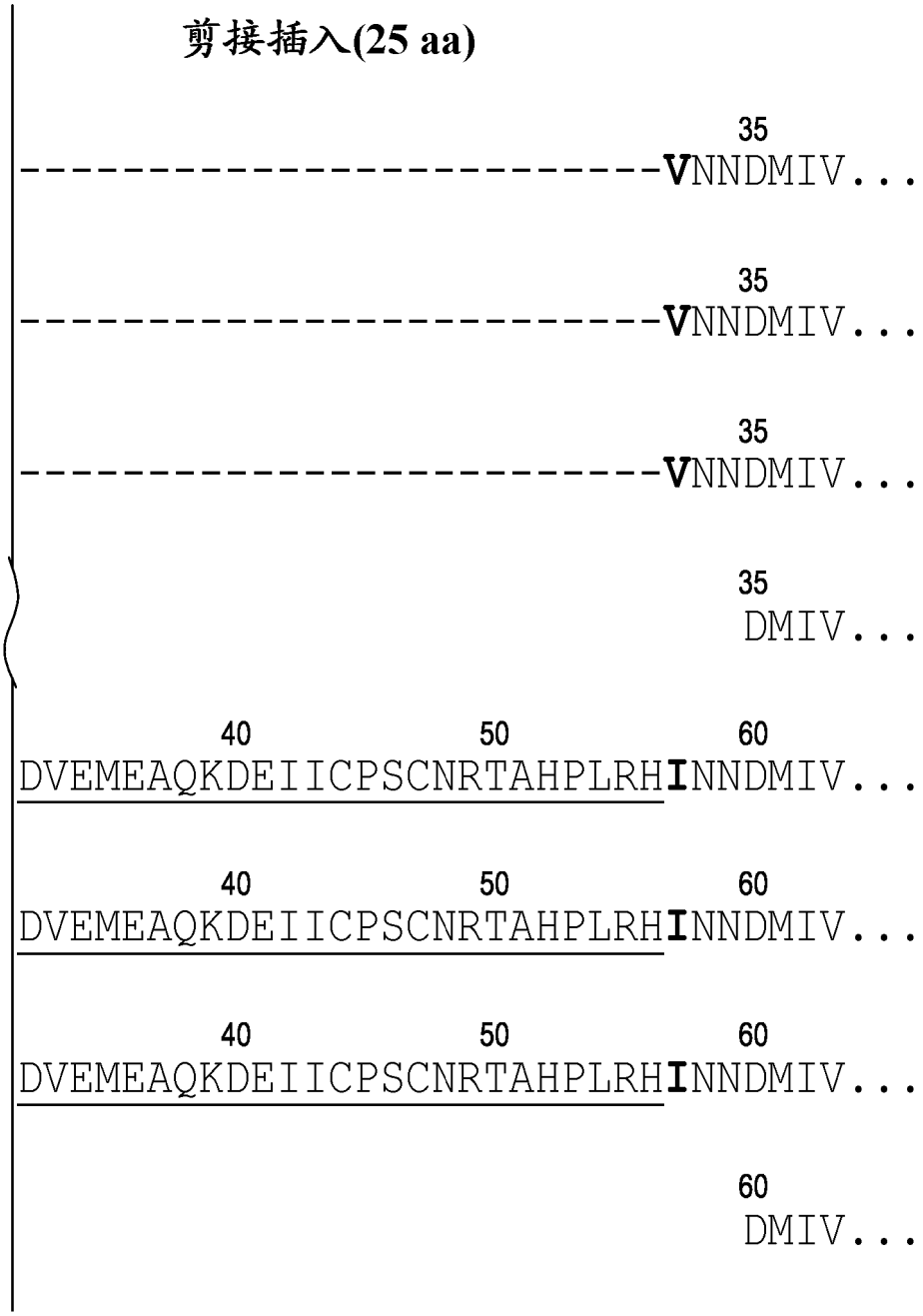
剪接插入(25 aa)
35
----------VNNDMIV...
35
----------VNNDMIV...
35
----------VNNDMIV...
35
DMIV...
40 50 60
DVEMEAQKDEIICPSCNRTAHPLRHINNDMIV...
40 50 60
DVEMEAQKDEIICPSCNRTAHPLRHINNDMIV...
40 50 60
DVEMEAQKDEIICPSCNRTAHPLRHINNDMIV...
60
DMIV...

图 7（续）