Innovation, Sciences et Développement économique Canada
Office de la Propriété Intellectuelle du Canada

Innovation, Science and Economic Development Canada
Canadian Intellectual Property Office

CA 2777717 C 2021/05/25

(11)(21) 2 777 717

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(86) Date de dépôt PCT/PCT Filing Date: 2010/10/15
(87) Date publication PCT/PCT Publication Date: 2011/04/21
(45) Date de délivrance/Issue Date: 2021/05/25
(85) Entrée phase nationale/National Entry: 2012/04/13
(86) N° demande PCT/PCT Application No.: US 2010/052852
(87) N° publication PCT/PCT Publication No.: 2011/047267
(30) Priorité/Priority: 2009/10/15 (US61/252,074)

(51) Cl.Int./Int.Cl. *C12N 15/18* (2006.01), *A61K 38/18* (2006.01), *A61P 3/10* (2006.01), *C07K 14/50* (2006.01), *C07K 16/22* (2006.01), *C12N 15/09* (2006.01), *C12N 15/12* (2006.01)

(72) Inventeur/Inventor:
SONODA, JUNICHIRO, US

(73) Propriétaire/Owner:
GENENTECH, INC., US

(74) Agent: SMART & BIGGAR LLP

(54) Titre : FACTEURS DE CROISSANCE CHIMERIQUES DES FIBROBLASTES AVEC SPECIFICITE DE RECEPTEUR MODIFIEE

(54) Title: CHIMERIC FIBROBLAST GROWTH FACTORS WITH ALTERED RECEPTOR SPECIFICITY

(57) Abrégé/Abstract:
The present invention is directed to novel chimeric fibroblast growth factor (FGF) polypeptides, novel DNA encoding chimeric FGF polypeptides, and to the recombinant production of chimeric FGF polypeptides, and to methods, compositions and assays utilizing chimeric FGF polypeptides for the therapeutic treatment of metabolic -related disorders and other conditions, and for producing pharmaceutically active compositions including chimeric FGF polypeptides, the compositions having therapeutic and pharmacologic properties including those associated with the treatment of metabolic -related disorders and other conditions.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 April 2011 (21.04.2011)

PCT

(10) International Publication Number
WO 2011/047267 A1

(51) International Patent Classification:
C12N 15/09 (2006.01) *A61K 38/24* (2006.01)

(21) International Application Number:
PCT/US2010/052852

(22) International Filing Date:
15 October 2010 (15.10.2010)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/252,074 15 October 2009 (15.10.2009) US

(71) Applicant *(for all designated States except US)*: **GENENTECH, INC.** [US/US]; 1 Dna Way, South San Francisco, CA 94080 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **SONODA, Junichiro** [JP/US]; 29 Creekside Lane, San Mateo, CA 94401 (US).

(74) Agents: **YONKER, Stephanie** et al.; Morrison & Foerster LLP, 755 Page Mill Road, Palo Alto, CA 94304-1018 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

WO 2011/047267 A1

(54) Title: CHIMERIC FIBROBLAST GROWTH FACTORS WITH ALTERED RECEPTOR SPECIFICITY

(57) Abstract: The present invention is directed to novel chimeric fibroblast growth factor (FGF) polypeptides, novel DNA encoding chimeric FGF polypeptides, and to the recombinant production of chimeric FGF polypeptides, and to methods, compositions and assays utilizing chimeric FGF polypeptides for the therapeutic treatment of metabolic -related disorders and other conditions, and for producing pharmaceutically active compositions including chimeric FGF polypeptides, the compositions having therapeutic and pharmacologic properties including those associated with the treatment of metabolic -related disorders and other conditions.

CHIMERIC FIBROBLAST GROWTH FACTORS WITH ALTERED RECEPTOR SPECIFICITY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority benefit to United States Provisional Application Ser. No. 61/252,074, filed October 15, 2009.

FIELD OF THE INVENTION

[0002] The present invention relates generally to novel polypeptides designated herein as chimeric fibroblast growth factor (FGF) polypeptides, novel DNA encoding chimeric FGF polypeptides, and to the recombinant production of chimeric FGF polypeptides, and to methods, compositions and assays utilizing chimeric FGF polypeptides for the therapeutic treatment of metabolic-related disorders and other conditions, and for producing pharmaceutically active compositions including chimeric FGF polypeptides, the compositions having therapeutic and pharmacologic properties including those associated with the treatment of metabolic-related disorders and other conditions.

BACKGROUND OF THE INVENTION

[0003] The family of fibroblast growth factors (FGF) includes the FGF19 subfamily, which consists of human FGF21, FGF23 and FGF19 and mouse FGF15. Unlike other members of the FGF family, which typically act on their tissue of origin in a paracrine manner, members of the FGF19 subfamily act on specific distal tissues in an endocrine manner. The effects of FGF family members are the result of their heparin-dependent binding to one or more members of the FGF receptor tyrosine kinase (FGFR) family. This family of receptors includes four members each having a tyrosine kinase domain, FGFR1, FGFR2, FGFR3 and FGFR4, as well as two splice variants each of FGFR1, FGFR2 and FGFR3. These splice variants, which occur in exon 3 of FGFR1, FGFR2 and FGFR3, are designated as "b" and "c" variants (*i.e.*, FGFR1b, FGFR2b, FGFR3c, FGFR1c, FGFR2c and FGFR3c, which are also known as FGFR1(III)b, FGFR2(III)b, FGFR3(III)c, FGFR1(III)c, FGFR2(III)c and FGFR3(III)c, respectively).

[0004] Members of the FGF19 subfamily have been implicated in regulating a variety of tissue-specific metabolic processes in mammals. Of particular interest is FGF19, which has been shown to target and have effects on both adipocytes and hepatocytes. For example, mice treated with recombinant human FGF19 (rhFGF19), despite being on a high-fat diet, show increased metabolic rates, increased lipid oxidation, a lower respiratory quotient and weight loss. Moreover, such mice showed lower serum levels of leptin, insulin, cholesterol and triglycerides, and normal levels of blood glucose despite the high-fat diet and without appetite diminishment. Obese mice

that lacked leptin but included a FGF19 transgene showed weight loss, lowered cholesterol and triglycerides, and did not develop diabetes. Obese, diabetic mice that lack leptin, when injected with rhFGF19, showed reversal of their metabolic characteristics in the form of weight loss and lowered blood glucose. (Fu, L. et al., *Endocrinology* 145(6), 2594-2603 (2004); Tomlinson, E. et al., *Endocrinology* 143(5), 1741-1747 (2002)).

[0005] Another member of the FGF19 subfamily, FGF21, is expressed primarily by the liver and has metabolic effects similar to that of FGF19, such as increased metabolism via its effects on adipose tissue, weight loss, lowered blood glucose levels, and resistance to obesity and diabetes. (Kharitonenkov, A. et al., *J Clin Invest* 115(6), 1627-1635 (2005)). FGF21-transgenic mice were also resistant to diet-induced obesity. Moreover, in diabetic rodent models, FGF21 administration lowered blood glucose and triglyceride levels.

[0006] FGF21 has been also shown to have a role in regulating the growth hormone (GH) pathway. The anabolic effects of GH are mediated by insulin-like growth factor 1 (IGF-1), which is primarily produced by the liver. GH induces IGF-1 transcription, thereby increasing its circulating levels, via activation of the Janus kinase 2 (JAK2) by the GH receptor. Activated JAK2 phosphorylates members of the signal transducers and activators of transcription (STAT) family which, when phosphorylated, undergo nuclear translocation and bind to regulatory elements of target genes, including those of IGF-1. In particular, STAT5, in its phosphorylated form, has been shown to have a prominent role in this response.

[0007] The effects of GH on IGF-1 levels appear to be countered by starvation or fasting – conditions that result in lower levels of IGF-1 transcription and circulating IGF-1. (Thissen, J.P. et al., *Endocr. Rev.* 15, 80–101 (1994)). These effects on IGF-1 may be due to reduced levels of phosphorylated STAT5. In particular, fasted rats injected with GH have lower levels of hepatic phosphorylated STAT5 than non-fasted rats. (Beauloye, V. et al., *Endocrinology* 143, 792–800 (2002)). FGF21, which is induced in the liver under starvation or fasting conditions, may mediate this effect. FGF21-transgenic mice have been shown to have lowered levels of IGF-1 and phosphorylated STAT5. (Inagaki, T. et al., *Cell Metabolism* 8, 77-83 (2008)).

[0008] The metabolic effects of FGF19 and FGF21 are effected via their binding to the FGFR1c, FGFR2c and FGFR3c receptors, of which the binding to FGFR1c and FGFR2c are the most significant. Furthermore, binding of FGF19 and FGF21 to these receptors require the co-receptor Klotho-beta. Despite the prevalence of these FGFR receptors, the metabolic effects of FGF19 and FGF21 are made adipocyte-specific due to this requirement for the Klotho-beta co-receptor, which has tissue-specific localization.

[0009] FGF19 has also been shown to have effects that are distinct from FGF21. For example, FGF19 has been shown to regulate bile production by the liver via its liver-specific effects. In

response to postprandial bile-production, FGF19 negatively regulates bile production by repressing transcription of the cholesterol 7-alpha-hydroxylase gene (CYP7A1), a rate limiting enzyme in the synthesis of bile acids, and by stimulating the filling of the gall bladder. In addition, FGF19 appears to have liver mitogenic effects that are not observed with respect to FGF21. For example, FGF19 transgenic mice develop hepatic adenocarcinoma due to increased proliferation and dysplasia of hepatocytes, and rhFGF19-treated mice exhibit hepatocyte proliferation of hepatocytes. (Nicholes, K. et al., *Am J Pathol* 160, 2295–2307 (2000).)

[0010] These additional activities of FGF19 appear to be mediated via its binding to FGFR4. FGF19 can bind FGFR4 in both a Klotho-beta-dependent and –independent manner. Although FGF21 has also been shown to bind FGFR4 in a Klotho-beta-dependent manner, no efficient signaling results from the binding of FGF21 to FGFR4.

[0011] There is a need to develop new therapies for the treatment of metabolic-related disorders such as diabetes, obesity, high blood sugar, and other related disorders. There is also a need to develop new therapies for such metabolic-related disorders in which the undesired growth or proliferation potential (*e.g.*., tumorigenic potential) of such a therapy is eliminated or reduced. There is also a need to develop new therapies for such metabolic-related disorders in which the potential for growth hormone resistance of such a therapy is eliminated or reduced.

BRIEF SUMMARY OF THE INVENTION

[0012] In a first aspect, the present invention provides a chimeric human fibroblast growth factor 19 (hFGF19) polypeptide. In some embodiments of the present invention, the sequence of the chimeric polypeptide includes a C-terminal portion that includes a C-terminal portion of the hFGF19 polypeptide sequence, and an N-terminal portion that includes an N-terminal portion of the hFGF21 polypeptide sequence. In certain embodiments, the C-terminal portion of the hFGF19 polypeptide sequence is from about 45 to about 185 residues in length and the N-terminal portion of the hFGF21 polypeptide sequence is from about 7 to about 140 residues in length.

[0013] In another embodiment of the present invention, a chimeric hFGF19 polypeptide is provided in which the sequence of the polypeptide includes a C-terminal portion that includes a C-terminal portion of the hFGF21 polypeptide sequence, and an N-terminal portion that includes an N-terminal portion of the hFGF19 polypeptide sequence. In some embodiments, the C-terminal portion of the hFGF21 polypeptide sequence is from about 8 to about 145 residues in length, and the N-terminal portion of the hFGF19 polypeptide sequence is from about 45 to about 175 residues in length.

[0014] In another embodiment of the present invention, a chimeric hFGF19 polypeptide is provided in which the sequence of the chimeric polypeptide includes a first polypeptide sequence having at least a certain sequence identity to the sequence of hFGF19 polypeptide, and wherein a

portion of the first polypeptide sequence is substituted with a portion of a second polypeptide sequence, the second polypeptide sequence having at least a certain sequence identity to the sequence of hFGF21 polypeptide, such that the substituted portion of the first polypeptide sequence is from about 3 to about 185 residues in length.

[0015] In some embodiments of the present invention, a chimeric hFGF19 polypeptide is provided in which the sequence of the chimeric polypeptide includes a first polypeptide sequence having at least a certain sequence identity to the sequence of hFGF19 polypeptide, and wherein a portion of the first polypeptide sequence is substituted with more than one portion of a second polypeptide sequence, the second polypeptide sequence having at least a certain sequence identity to the sequence of hFGF21 polypeptide. In some embodiments, the chimeric hFGF19 polypeptide comprises a substitution of the ß1-ß2 loop of the first polypeptide, a substitution of the ß10-ß12 segment of the first polypeptide, and/or a substitution of the five residues WGDPI of the first polypeptide with the ß1-ß2 loop of the second polypeptide, the ß10-ß12 segment of the second polypeptide, and/or the corresponding sequence GQV of the second polypeptide.

[0016] In certain embodiments of the present invention, the chimeric hFGF19 polypeptide comprises the sequence
HPIPDSSPLLQFGGQVRQRYLYTSGPHGLSSCFLRIRADGVVDCARGQSAHSLLEIKAVAL
RTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLS
SAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGL
EAVRSPSFEK (SEQ ID NO:5).

[0017] In certain embodiments, the chimeric hFGF19 polypeptide of the present invention is fused to a second polypeptide, the second polypeptide is the Fc portion of an immunoglobulin, an analog of the Fc portion of an immunoglobulin and one or more fragments of the Fc portion of an immunoglobulin. In certain embodiments, the immunoglobulin is selected from the group consisting of: IgG-1, IgG-2, IgG-3, IgG-4, IgA-1, IgA-2, IgE, IgD and IgM. In some embodiments, the Fc portion is human or humanized. In some embodiments, the C-terminus of the chimeric hFGF19 polypeptide is fused to the N-terminus of the second polypeptide. In some embodiments, the C-terminus of the chimeric hFGF19 polypeptide is fused to the N-terminus of the second polypeptide via a linker, the linker is selected from the group consisting of: a $[Gly]_n$ linker, a $[Gly_3Ser]$m linker and a $[Gly_4Ser]$m linker, wherein n is an integer from 1-30 and m is an integer from 1-6.

[0018] The present invention includes chimeric hFGF19 polypeptides that have a physiological half-life that is at least or about the same as native hFGF19. The present invention includes chimeric hFGF19 polypeptides that have a physiological half-life that is at least or about the same as native hFGF21.

[0019] In certain embodiments, the chimeric hFGF19 polypeptide does not substantially activate FGFR4 in either a Klotho-beta independent or Klotho-beta dependent manner. In certain embodiments, the chimeric hFGF19 polypeptide activates FGFR1c in a Klotho-beta dependent manner.

[0020] In certain embodiments, the chimeric hFGF19 polypeptide when administered to an individual does not reduce the lean mass of the individual. In certain embodiments, the chimeric hFGF19 polypeptide when administered to an individual does not substantially reduce the lean mass of the individual. In certain embodiments, the chimeric hFGF19 polypeptide when administered to an individual does not reduce the bone density of the individual. In certain embodiments, the chimeric hFGF19 polypeptide when administered to an individual does not substantially reduce the bone density of the individual. In certain embodiments, the chimeric hFGF19 polypeptide when administered to an individual does not reduce the cardiac capacity of the individual. In certain embodiments, the chimeric hFGF19 polypeptide when administered to an individual does not substantially reduce the cardiac capacity of the individual.

[0021] In certain embodiments, the chimeric hFGF19 polypeptide does not reduce or does not substantially reduce the amount of phosphorylated STAT5 polypeptide in vivo. In certain embodiments, the chimeric hFGF19 polypeptide when administered to an individual does not reduce or does not substantially reduce the amount of phosphorylated STAT5 polypeptide in the individual. In certain embodiments when the chimeric hFGF19 polypeptide is administered to an individual, the amount of phosphorylated STAT5 polypeptide is reduced in the individual but this amount of phosphorylated STAT5 polypeptide is greater than the amount of phosphorylated STAT5 polypeptide upon administration of native hFGF21 to the individual. In certain embodiments when the chimeric hFGF19 polypeptide is administered to an individual, the amount of phosphorylated STAT5 polypeptide is any of: from 100% to 5%, from 100% to 10%, from 100% to 20%, from 100% to 30%, from 100% to 40%, from 100% to 50%, from 100% to 60%, from 100% to 70%, from 100% to 80%, from 100% to 90% or from 100% to 95%, of the amount of phosphorylated STAT5 polypeptide in the individual without such administration. In certain embodiments when the chimeric hFGF19 polypeptide is administered to an individual, the reduction in the amount of phosphorylated STAT5 polypeptide is less than reduction in the amount of phosphorylated STAT5 polypeptide upon administration of native hFGF21. For example, the reduction of phosphorylated STAT5 polypeptide when the chimeric hFGF19 polypeptide is administered to an individual is by any of: from 0% to 5%, from 0% to 10%, from 0% to 20%, from 0% to 30%, from 0% to 40%, from 0% to 50%, from 0% to 60%, from 0% to 70%, from 0% to 80%, from 0% to 90% or from 0% to 95%, of the reduction in the amount of phosphorylated STAT5 polypeptide upon administration of native hFGF21.

[0022] In certain embodiments, the chimeric hFGF19 polypeptide does not reduce or does not substantially reduce the amount of circulating insulin-like growth factor 1 (IGF-1) in vivo. In certain embodiments, the chimeric hFGF19 polypeptide when administered to an individual does not reduce or does not substantially reduce the amount of circulating IGF-1 in the individual. In certain embodiments when the chimeric hFGF19 polypeptide is administered to an individual, the amount of circulating IGF-1 is reduced but this amount of circulating IGF-1 is greater than the amount of circulating IGF-1 upon administration of native hFGF21 to the individual. In certain embodiments when the chimeric hFGF19 polypeptide is administered to an individual, the amount of circulating IGF-1 is any of: from 100% to 5%, from 100% to 10%, from 100% to 20%, from 100% to 30%, from 100% to 40%, from 100% to 50%, from 100% to 60%, from 100% to 70%, from 100% to 80%, from 100% to 90% or from 100% to 95%, of the amount of circulating IGF-1 in the individual without such administration. In certain embodiments when the chimeric hFGF19 polypeptide is administered to an individual, the reduction in the amount of circulating IGF-1 is less than reduction in the amount of circulating IGF-1 upon administration of native hFGF21. For example, the reduction of circulating IGF-1 when the chimeric hFGF19 polypeptide is administered to an individual is by any of: from 0% to 5%, from 0% to 10%, from 0% to 20%, from 0% to 30%, from 0% to 40%, from 0% to 50%, from 0% to 60%, from 0% to 70%, from 0% to 80%, from 0% to 90% or from 0% to 95%, of the reduction in the amount of circulating IGF-1 upon administration of native hFGF21.

[0023] In another aspect, the present invention provides a pharmaceutical composition comprising a therapeutically effective amount of a chimeric hFGF19 polypeptide of the present invention; and an acceptable pharmaceutical carrier.

[0024] In another aspect, the present invention provides methods of treating an individual exhibiting one or more of obesity, type 1 diabetes, type 2 diabetes, high blood glucose, metabolic syndrome, atherosclerosis, hypercholesterolemia, stroke, osteoporosis, osteoarthritis, degenerative joint disease, muscle atrophy, sarcopenia, decreased lean body mass, baldness, wrinkles, increased fatigue, decreased stamina, decreased cardiac function, immune system dysfunction, cancer, Parkinson's disease, senile dementia, Alzheimer's disease and decreased cognitive function, the method comprising administering to the individual a therapeutically effective amount of the pharmaceutical composition of the present invention.

[0025] In another aspect, the present invention provides a method of lowering the blood glucose of an individual in need of such treatment, the method comprising administering to the individual a therapeutically effective amount of the pharmaceutical composition of the present invention.

[0026] In another aspect, the present invention provides an isolated nucleic acid molecule comprising DNA having at least an 80%, at least a 90%, at least a 95% or at least a 99% sequence identity to a DNA molecule encoding a polypeptide having amino acid residues from about 1 to about 190 of SEQ ID NO:5, or the complement thereof.

[0027] In another aspect, the present invention provides a isolated nucleic acid molecule comprising the sequence:

CACCCCATCCCTGACTCCAGTCCTCTCCTGCAATTCGGGGGCCAAGTCCGGCAGCGG
TACCTCTACACCTCCGGCCCCCACGGGCTCTCCAGCTGCTTCCTGCGCATCCGTGCCG
ACGGCGTCGTGGACTGCGCGCGGGGCCAGAGCGCGCACAGTTTGCTGGAGATCAAG
GCAGTCGCTCTGCGGACCGTGGCCATCAAGGGCGTGCACAGCGTGCGGTACCTCTGC
ATGGGCGCCGACGGCAAGATGCAGGGGCTGCTTCAGTACTCGGAGGAAGACTGTGC
TTTCGAGGAGGAGATCCGCCCAGATGGCTACAATGTGTACCGATCCGAGAAGCACCG
CCTCCCGGTCTCCCTGAGCAGTGCCAAACAGCGGCAGCTGTACAAGAACAGAGGCTT
TCTTCCACTCTCTCATTTCCTGCCCATGCTGCCCATGGTCCCAGAGGAGCCTGAGGAC
CTCAGGGGCCACTTGGAATCTGACATGTTCTCTTCGCCCCTGGAGACCGACAGCATG
GACCCATTTGGGCTTGTCACCGGACTGGAGGCCGTGAGGAGTCCCAGCTTTGAGAAG

(SEQ ID NO:7), or a portion thereof.

[0028] In certain embodiments, a isolated nucleic acid of the present invention further comprises a sequence encoding the amino acid residues corresponding to a Fc portion of an immunoglobulin.

[0029] In another aspect, the present invention provides an expression system comprising the nucleic acid molecule of the present invention. In another aspect, the present invention provides a host cell comprising an expression system or nucleic acid of the present invention.

[0030] In another aspect, the present invention provides an isolated polypeptide encoded by a nucleic acid molecule of the present invention.

[0031] In another aspect, the present invention provides a process for producing an isolated polypeptide comprising culturing a host cell of the present invention under conditions suitable for expression of the encoded polypeptide and recovering the encoded polypeptide from the cell culture. In another aspect, the present invention provides an isolated polypeptide produced by a process of the present invention.

[0031A] The present invention as claimed relates to:

- a chimeric FGF19 polypeptide comprising an amino acid sequence that has at least 95% identity to the amino acid sequence of SEQ ID NO:74 or 241;

- a chimeric FGF19 polypeptide comprising an N-terminal portion of FGF21 and a C-terminal portion of FGF19, wherein the C-terminal portion of the chimeric FGF19 polypeptide comprises an amino acid sequence that has at least 95% identity to the amino acid sequence of SEQ ID NO:1;

- a pharmaceutical composition comprising: (a) a therapeutically effective amount of the chimeric FGF19 polypeptide as described herein; and (b) an acceptable pharmaceutical carrier, wherein the pharmaceutical composition is for treating one or more of obesity, type 1 diabetes, type 2 diabetes, high blood glucose, metabolic syndrome, atherosclerosis, hypercholesterolemia, stroke, osteoporosis, osteoarthritis, degenerative joint disease, muscle atrophy, sarcopenia, decreased lean body mass, baldness, decreased cardiac function, immune system dysfunction, cancer, Parkinson's disease, senile dementia, Alzheimer's disease or decreased cognitive function;

- an isolated nucleic acid molecule comprising a DNA molecule encoding a polypeptide comprising an amino acid sequence that has at least 95% identity to the amino acid sequence of SEQ ID NO:74 or 241; and

- an isolated nucleic acid molecule comprising a DNA molecule encoding a polypeptide comprising an amino acid sequence that has at least 90% identity to the amino acid sequence of SEQ ID NO:5.

BRIEF DESCRIPTION OF THE DRAWINGS

[0032] FIG. 1 shows the amino acid sequence of human FGF19 polypeptide (SEQ ID NO:1) and human pre-FGF19 polypeptide (SEQ ID NO:3);

[0033] FIG. 2 shows the amino acid sequence of human FGF21 polypeptide (SEQ ID NO:2) and human pre-FGF21 polypeptide (SEQ ID NO:4);

[0034] FIG. 3 shows exemplary results of a receptor binding assay using chimeric FGF19 polypeptides of the present invention;

[0035] FIG. 4 shows exemplary results of a receptor binding assay using chimeric FGF19 polypeptides of the present invention;

[0036] FIG. 5 shows exemplary results of a liver-specific gene expression assay using chimeric FGF19 polypeptides of the present invention;

[0037] FIG. 6 shows exemplary results of a adipocyte-specific gene expression assay using chimeric FGF19 polypeptides of the present invention;

[0038] FIG. 7 shows exemplary results of a blood glucose lowering assay using chimeric FGF19 polypeptides of the present invention;

[0039] FIG. 8 shows exemplary results of a glucose tolerance test assay using chimeric FGF19 polypeptides of the present invention;

[0040] FIG. 9 shows exemplary results of an activity assay using immunoglobulin Fc fusions of chimeric FGF19 polypeptides of the present invention; and

[0041] FIG. 10-14 shows exemplary results of a receptor specificity assay using chimeric FGF19 polypeptides of the present invention.

[0042] FIG. 15 shows exemplary results of using a chimeric FGF19 polypeptide of the present invention on the levels of phosphorylated-Stat5 protein.

[0043] FIG. 16 shows exemplary results of using a chimeric FGF19 polypeptide of the present invention on the total metabolic activity of cells.

[0044] Figure 17 shows that FGFR4 was required for bile acid ("BA") regulation but not for improvement in glucose tolerance by FGF19. Figure 17A shows glucose level of FGF19 or PBS treated wildtype ("WT") and FGFR4 knockout ("KO") mice in glucose tolerance test. *$p<0.05$. **$p<0.01$. p value for area under the curve (AUC) was $p<0.02$ (WT) and $p<0.005$ (KO). N=6~8. Figure 17B shows various metabolic parameters (body weight (g), liver/BW ratio (%), serum insulin (ng/mL), serum beta-hydroxybutyrate ("BHB") (mg/L), serum lactate (mg/dl), and serum triglyceride (mg/dl)) in FGF19 or PBS treated WT and FGFR4 KO mice at euthanasia on day 7. Mice were euthanized and the serum was prepared after 3hr fast. N=6~8. Figure 17C shows serum BA composition analysis in FGF19 or PBS treated WT and FGFR4 KO mice. Only major BA species are shown. CA: cholic acid, DCA: deoxycholic acid, MCA: muricholic acid, T-: taurineconjugated. Figure 17D shows relative expression of various hepatic genes (Egr-1, c-Fos, AFP, Cyp7a1, Cyp8b, Cyp27a1, Cyp7b, and GK) in FGF19 or PBS treated WT and FGFR4 KO mice determined by real-time qPCR. N=6~8. p values for Figures 17B-17D are: <0.05, **<0.005 (PBS vs FGF19), #<0.05, ##<0.005 (WT vs FGFR4KO).

[0045] Figure 18 shows identification of FGF19 variants with reduced FGFR4 activity. Figure 18A shows the relative firefly luciferase activity normalized by renilla luciferase activity (shown as relative luciferase unit ("RLU")) from a GAL-Elk1 luciferase assay using rat L6 cells transfected with KLB and FGFR1c or FGFR4 and incubated with media containing increasing concentrations of FGF19 (O) or FGF21(▲). Figure 18B shows drawings (to scale) of FGF19 (top), FGF21 (bottom), and various chimeric proteins with amino acid composition at left. Based on the results of GAL-Elk1 assays shown in Fig. 18C, each chimera was classified into class (I), (II) or (III) as indicated at right. Chimeras which did not exhibit an equivalent FGFR1c activity to FGF21 or FGF19 when conditioned medium was used are not shown here. Figure 18C shows the activation of FGFR1c or FGFR4 in a GAL-Elk-1 assay using L6 cells cotransfected with KLB and/or FGFR (FGFR1c or FGFR4) and incubated with conditioned medium from 293 cells transiently transfected with various FGF constructs (*see* Figure 18B for amino acid compositions for FGF constructs used). The results are shown as a fold induction over control media conditioned with mock transfected cells. Figure 18D shows the fold induction for FGFR activation in a GAL-Elk1 luciferase assay using rat L6 cells transfected with FGFR1c, FGFR4 + KLB, or FGFR4 and incubated with media containing increasing concentrations of purified FGF19 (O) or FGF19v (▼) (the construct #4 in Figs. 18B and 18C). Figure 18E shows solid phase binding assay results for FGF19 and FGF19v to FGFR4 fused to Fc fragment. The schematic diagram for the experiments is shown at right. Figure 18F shows that the anti-FGF19 antibody used in Fig. 18E recognized FGF19 and FGF19v at indistinguishable affinity (control ELISA experiment). The schematic diagram for the experiments is shown at right.

[0046] Figure 19 shows the RLU in a GAL-Elk1 luciferase assay in rat L6 cells transfected with KLB and FGFR2c or FGFR3c and incubated with media containing increasing concentrations of FGF19 (O) or FGF21(▲). L6 cells were cotransfected with expression vectors for KLB and the indicated FGFR together with GAL-Elk1, SV40-Renilla Luciferase, and Gal-responsive luciferase reporter. Transfected cells were incubated with media containing increasing concentrations of FGF19 or FGF21 for 6 hours before luciferase assays. Transcriptional activation was assessed by the relative luciferase activity normalized by Renilla luciferase activity and expressed as relative luciferase unit (RLU). This figure shows that FGF21 and FGF19 activated FGFR2c and FGFR3c in the presence of KLB.

[0047] Figure 20 shows the activation of FGFR1c or FGFR4 in a GAL-Elk-1 assay using L6 cells cotransfected with KLB and/or FGFR (FGFR1c or FGFR4) and incubated with conditioned medium from 293 cells transiently transfected with various FGF constructs shown at X-axis (see Figure 18B for amino acid compositions of FGF constructs used). The results are shown as a fold

induction over control media conditioned with mock transfected cells. The numbering indicated at X-axis corresponds to the numbering of the construct shown in Fig. 18B.

[0048] Figure 21 shows the effects of FGF19v in chow-fed mice. Figure 21A shows relative expression of various genes (c-Fos, Egr-1, GK, SHP, and Cyp7a1) in FVB mice injected (via tail vein) with FGF21, FGF19, or FGF19v at 1mg/kg or PBS control. p values: *<0.05, **<0.01, ***<0.001 (vs PBS). At 4 hours post-injection, hepatic mRNA was prepared from each mouse and subjected to real-time qPCR analysis for the indicated genes. Figure 21B shows relative expression of various genes (c-Fos, Egr-1, GK, SHP, and Cyp7a1) in WT or FGFR4 KO (KO) mice (N=5-7) i.p. injected with FGF21 or FGF19 at 1mg/kg or PBS control. Overnight fasted mice were i.p. injected with FGF protein or PBS control. At 4 hours post-injection, hepatic mRNA was prepared from each mouse and subjected to real-time qPCR analysis for the indicated genes. p values: *<0.05, **<0.01, ***<0.001. Figure 21C shows the proliferation of HepG2 cells treated with FGF21, FGF19, or FGF19v at various concentrations (10 ng/mL, 60 ng/mL, and 200 ng/mL) measured by the fluorescent intensity (x 10^5) in an anchorage independent cell growth assay. Proliferation of HepG2 cells in soft agar was estimated based on conversion of Resazurin (Alamer Blue), a non-fluorescent indicator dye, to resorufin. Figure 21D shows fold change of $BrdU^+$ hepatocytes in FVB mice infused with FGF19 or FGF19v. N=6, *p<0.01, ***p<5E- 5 (vs PBS), ##p<0.0002 (vs FGF19). FVB mice were implanted with an osmotic pump to continuously infuse indicated FGF protein at 1ng/hr (~0.8mg/kg/day) (day 0). The mice also received daily injection of 1mg/kg/day FGF protein (q.d.) and 30mg/kg/day BrdU (b.i.d.) starting day 1. On day 7, livers were dissected out and subjected to anti-BrdU staining. The results are shown as a fold induction over mock treated animals for the number of BrdU positive hepatocytes per area analyzed. Figure 21E shows representative images for the study shown in Figure 21C. Figure 21F shows relative expression of various genes (c-Fos, Egr-1, AFP, GK, Cyp7a1 and Cyp8b) in mice used in Figures 21D and 21E. N=6. *p<0.05, **p<0.005, ***p<0.001 (vs PBS), #p<0.05, ##p<0.005 (vs FGF19).

[0049] Figure 22 shows that FGF19v and FGF21 had similar metabolic effects and ameliorate hyperglycemia in ob/ob mice. 11-week-old ob/ob mice were subcutaneously implanted with an osmotic pump to infuse 1ng/hr FGF protein (0.4mg/kg/day) or PBS control (N=7). Figure 22A shows body weight (g) and blood glucose (mg/dl) in ob/ob mice infused by osmotic pump with 1ng/hr FGF21 or FGF19v (0.4mg/kg/day) or PBS control (N=7). The osmotic pump was implanted on day 0. Figure 22B shows blood glucose levels (mg/dl) in ob/ob mice infused with FGF21, FGF19v or PBS control at random fed condition and after overnight fast. Figure 22C shows serum non-esterified fatty acids ("NEFA") levels in ob/ob mice infused with FGF21, FGF19v or PBS control on day 8. Figure 22D shows glucose tolerance test results conducted in

ob/ob mice infused with FGF21, FGF19v or PBS control on day 6. Mice were overnight fasted and i.p. injected with 1g/kg glucose at t=0. Figure 22E shows organ/body weight ratio (%) in ob/ob mice infused with FGF21, FGF19v or PBS control on day 8. Figure 22F shows relative expression of various genes (AFP, IGFBP2, SCD-1, Cyp7A, Cyp8B, UCP-1, MCAD, and SREBP-1c) from various tissues (liver, brown adipose tissue ("BAT"), and white adipose tissue ("WAT")) in ob/ob mice infused with FGF21, FGF19v or PBS control determined by qPCR. p values: *<0.05, **<0.005, ***<0.0005 (vs PBS control).

DETAILED DESCRIPTION OF THE INVENTION

[0050] The present invention provides novel chimeric FGF19 polypeptides. In some embodiments of the present invention, a chimeric FGF19 polypeptide sequence includes a portion of a FGF19 polypeptide sequence and a portion of a FGF21 polypeptide sequence. In certain preferred embodiments, the FGF19 polypeptide is processed human FGF19 (hFGF19) polypeptide whose sequence is defined in SEQ ID NO:1. In certain preferred embodiments, the FGF21 polypeptide is processed human FGF21 (hFGF21) polypeptide whose sequence is defined in SEQ ID NO:2. In another aspect, the present invention provides novel chimeric FGF19 polypeptides that are further fused to an immunoglobulin domain, such as the Fc domain.

[0051] In another aspect, the present invention provides novel chimeric FGF19 polypeptides that have altered receptor specificity. In certain preferred embodiments, a chimeric FGF19 polypeptide of the present invention does not substantially activate FGFR4 in a Klotho-beta-dependent or -independent manner. In certain embodiments, a chimeric FGF19 polypeptide of the present invention does activate at least one of FGFR1c, FGFR2c or FGFR3c in a Klotho-beta-dependent manner.

[0052] In some embodiments, a chimeric FGF19 polypeptide of the present invention may have one or more of the following advantageous features: the polypeptide does not substantially induce hepatocyte proliferation in an individual upon administration, the polypeptide does not substantially induce growth hormone resistance in an individual upon administration, the polypeptide does not include a residue that is polymorphic in the population, the polypeptide has an *in vivo* physiological half-life that is at least or about the same as native FGF19 polypeptide (such as native hFGF19 polypeptide), the polypeptide has an *in vivo* physiological half-life that is at least or about the same as native FGF21 polypeptide (such as native hFGF21 polypeptide), the polypeptide does not substantially reduce lean mass in an individual upon administration, the polypeptide does not substantially reduce bone density in an individual upon administration, and the polypeptide does not reduce substantially cardiac capacity in an individual upon administration.

[0053] Further advantageous features of a chimeric FGF19 polypeptide of the present invention may include one or more of following: the polypeptide reduces the blood glucose in an individual in an individual in need of such treatment; the polypeptide activates at least one of FGFR1c, FGFR2c or FGFR3c in a Klotho-beta-dependent manner; the polypeptide does not substantially activate FGFR4 in a Klotho-beta-dependent manner; the polypeptide does not substantially activate FGFR4 in a Klotho-beta-independent manner; the polypeptide does not reduce or does not substantially reduce the amount of phosphorylated STAT5 polypeptide in an individual upon administration; the polypeptide when administered to an individual the amount of phosphorylated STAT5 polypeptide in the individual is reduced but this amount of phosphorylated STAT5 polypeptide is greater than the amount of phosphorylated STAT5 polypeptide upon administration of native hFGF21 to the individual; ; the polypeptide when administered to an individual the reduction in the amount of phosphorylated STAT5 polypeptide in the individual is less than reduction in the amount of phosphorylated STAT5 polypeptide upon administration of native hFGF21 in the individual; the polypeptide does not reduce or does not substantially reduce the amount of circulating IGF-1 polypeptide in an individual upon administration; the polypeptide when administered to an individual the amount of circulating IGF-1 in the individual is reduced but this amount of circulating IGF-1 is greater than the amount of circulating IGF-1upon administration of native hFGF21 to the individual; ; the polypeptide when administered to an individual the reduction in the amount of circulating IGF-1in the individual is less than reduction in the amount of circulating IGF-1 upon administration of native hFGF21 in the individual; and the polypeptide does not reduce or does not substantially reduce the amount of circulating IGF-1 polypeptide in an individual having a normal or supranormal amount of GH.

[0054] In certain embodiments, a chimeric FGF19 polypeptide of the present invention has the advantageous features of activating at least one of FGFR1c, FGFR2c or FGFR3c in a Klotho-beta-dependent manner, and does not substantially activate FGFR4 in a Klotho-beta-dependent or -independent manner. In certain embodiments, a chimeric FGF19 polypeptide of the present invention has the advantageous features of activating at least one of FGFR1c, FGFR2c or FGFR3c in a Klotho-beta-dependent manner, does not substantially activate FGFR4 in a Klotho-beta-dependent or -independent manner, and reduces the amount of phosphorylated STAT5 polypeptide in an individual but this amount of phosphorylated STAT5 polypeptide is greater than the amount of phosphorylated STAT5 polypeptide upon administration of native hFGF21 to the individual. In certain embodiments, a chimeric FGF19 polypeptide of the present invention has the advantageous features of activating at least one of FGFR1c, FGFR2c or FGFR3c in a Klotho-beta-dependent manner, and does not substantially activate FGFR4 in a Klotho-beta-

dependent or -independent manner, and does not include a residue that is polymorphic in the population. In certain embodiments, a chimeric FGF19 polypeptide of the present invention has the advantageous features of activating at least one of FGFR1c, FGFR2c or FGFR3c in a Klotho-beta-dependent manner, does not substantially activate FGFR4 in a Klotho-beta-dependent or -independent manner, reduces the amount of phosphorylated STAT5 polypeptide in an individual but this amount of phosphorylated STAT5 polypeptide is greater than the amount of phosphorylated STAT5 polypeptide upon administration of native hFGF21 to the individual and does not include a residue that is polymorphic in the population,.

[0055] In another aspect, the present invention provides novel chimeric FGF19 polypeptides that have altered receptor specificity. In certain preferred embodiments, a chimeric FGF19 polypeptide of the present invention does not substantially activate FGFR4 in a Klotho-beta-dependent or -independent manner. In certain embodiments, a chimeric FGF19 polypeptide of the present invention does activate at least one of FGFR1c, FGFR2c or FGFR3c in a Klotho-beta-dependent manner.

[0056] In another aspect, the chimeric FGF19 polypeptide does not effect growth hormone resistance activity in an individual compared to the growth hormone resistance effected by native FGF21. In another aspect, the chimeric FGF19 polypeptide does not effect substantial growth hormone resistance activity in an individual compared to the growth hormone resistance effected by native FGF21. In certain embodiments, the chimeric FGF19 polypeptide does not reduce or does not substantially reduce the amount of phosphorylated STAT5 polypeptide in an individual. In certain embodiments when the chimeric hFGF19 polypeptide is administered to an individual, the amount of phosphorylated STAT5 polypeptide is reduced in the individual but this amount of phosphorylated STAT5 polypeptide is greater than the amount of phosphorylated STAT5 polypeptide upon administration of native hFGF21 to the individual. In certain embodiments when the chimeric hFGF19 polypeptide is administered to an individual, the reduction in the amount of phosphorylated STAT5 polypeptide is less than reduction in the amount of phosphorylated STAT5 polypeptide upon administration of native hFGF21. In certain embodiments, the chimeric FGF19 polypeptide does not reduce or does not substantially reduce the amount of circulating insulin-like growth factor 1 (IGF-1). In certain embodiments when the chimeric hFGF19 polypeptide is administered to an individual, the amount of circulating IGF-1 is reduced but this amount of circulating IGF-1 is greater than the amount of circulating IGF-1 upon administration of native hFGF21 to the individual. In certain embodiments when the chimeric hFGF19 polypeptide is administered to an individual, the reduction in the amount of circulating IGF-1 is less than reduction in the amount of circulating IGF-1 upon administration of native hFGF21.

[0057] In another aspect, the present invention provides novel isolated nucleic acid molecules having a sequence that encodes a chimeric FGF19 polypeptide of the present invention, novel expression systems that include a nucleic acid molecule of the present invention, and host cells that include a inventive nucleic acid molecule or an inventive expression system.

[0058] In another aspect, the present invention includes antibodies that can specifically bind a chimeric FGF19 polypeptide of the present invention.

[0059] In another aspect, the present invention provides pharmaceutical compositions that include a chimeric FGF19 polypeptide of the present invention and a pharmaceutically-acceptable carrier.

[0060] In another aspect, the present invention provides methods of treating an individual for a metabolic-related disorder by administering a chimeric FGF19 polypeptide of the present invention, or a suitable pharmaceutical formulation thereof. In another aspect, the present invention provides methods for effecting at least one or more of the following effects in an individual: lowering blood glucose, reducing obesity, increasing metabolic rate, increasing lipid oxidation, reducing weight, lower serum levels of glucose, leptin, insulin, cholesterol and/or triglycerides, treating diabetes, and other metabolic effects, wherein such effects are by administering to the individual a therapeutic amount of an inventive chimeric FGF19 polypeptide or pharmaceutical formulation thereof.

I. Chimeric FGF19 Polypeptides With N-Terminal FGF21 Polypeptide Sequences

[0061] In an aspect of the present invention, a chimeric FGF19 polypeptide sequence includes a C-terminal portion and an N-terminal portion. The N-terminal portion of the chimeric FGF19 polypeptide sequence includes an N-terminal portion of a FGF21 polypeptide sequence and the C-terminal portion of the chimeric FGF19 polypeptide sequence includes a C-terminal portion of a FGF19 polypeptide sequence. In some embodiments of the foregoing, the C-terminal portion of a FGF19 polypeptide sequence and the N-terminal portion of the chimeric FGF21 polypeptide sequence are contiguously joined. In some embodiments of the foregoing, the C-terminal portion of a FGF19 polypeptide sequence and the N-terminal portion of the chimeric FGF21 polypeptide sequence are contiguously joined by overlapping the 1, 2, 3 or more residues in common between the two portions. In some alternative embodiments, the C-terminal portion of a FGF19 polypeptide sequence and the N-terminal portion of the chimeric FGF21 polypeptide sequence have an intervening spacer of 1, 2, 3, 4, 5 or more amino residues.

[0062] In certain preferred embodiments, the FGF19 polypeptide is human FGF19 (hFGF19) polypeptide whose sequence is defined in SEQ ID NO:1 (Fig. 1). In some embodiments, the C-terminal portion of the hFGF19 polypeptide sequence is from about 45 to about 185 residues in length, expressly including the sequence lengths of the hFGF19 C-terminal portions shown in

Table 1. In some embodiments, the C-terminal portion of the chimeric FGF19 polypeptide sequence includes a C-terminal portion of a polypeptide sequence that has at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or at least 99% amino acid sequence identity to the hFGF19 polypeptide sequence. In some embodiments, the FGF19 polypeptide is pre-processed human FGF19 (hFGF19) polypeptide, which includes its signal sequence and whose sequence is defined in SEQ ID NO:3 (Fig. 1).

[0063] In certain preferred embodiments, the FGF21 polypeptide is human FGF21 (hFGF21) polypeptide whose sequence is defined in SEQ ID NO:2 (Fig. 2). In some embodiments, the N-terminal portion of the hFGF21 polypeptide sequence is from about 7 to about 140 residue in length, expressly including the sequence lengths of the hFGF19 N-terminal portions shown in Table 2. In some embodiments, the N-terminal portion of the chimeric FGF19 polypeptide sequence includes a N-terminal portion of a polypeptide sequence that has at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or at least 99% amino acid sequence identity to the hFGF21 polypeptide sequence. In some embodiments, the FGF21 polypeptide is pre-processed human FGF21 (hFGF21) polypeptide, which includes its signal sequence and whose sequence is defined in SEQ ID NO:4 (Fig. 2).

[0064] As used herein, a C-terminal portion, an N-terminal portion, a substituted portion or a substituting portion of a polypeptide sequence, such as that of the hFGF19 or hFGF21 polypeptide sequences, has a first position and a final position. These positions correspond to positions in the polypeptide sequence from which the portion is referenced. Thus, the sequence of a defined portion is the contiguous sequence of amino acids that begins at or about the position in the polypeptide sequence that corresponds to the first position, and ends at or about the position in the polypeptide sequence that corresponds to the final position. In some embodiments, the final position of a C-terminal portion of a polypeptide corresponds to or about the final residue of the polypeptide. In some embodiments, the first position of an N-terminal portion of a polypeptide corresponds to or about the first residue of the polypeptide.

[0065] Examples of C-terminal portions of hFGF19 polypeptide sequence referred to in the present invention include, without limitation, those that have a first position that correspond to or about any one of positions 10, 11, 25, 26, 27, 28, 30, 33, 35, 37, 40, 41, 42, 43, 44, 45, 52, 53, 54, 56, 57, 58, 59, 72, 73, 74, 79, 80, 81, 143, 144, 145 or 146 of SEQ ID NO:1. Each exemplary C-terminal portion also has a final position that corresponds to or about position 194 of SEQ ID NO:1. Table 1 shows the polypeptide sequences of exemplary C-terminal portions of hFGF19. Other analogous portions of hFGF19 are also contemplated herein.

Table 1: Exemplary C-Terminal Portions of hFGF19 Polypeptide Sequence

Name	Amino Acid Sequence (N-C)	SEQ ID NO
hFGF19 –C10	PHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQSAHSLLEIKA VALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAFEEEIRPDGYNVYR SEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMF SSPLETDSMDPFGLVTGLEAVRSPSFEK	8
hFGF19 –C11	HVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQSAHSLLEIKAV ALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAFEEEIRPDGYNVYRSE KHRLPVSLSSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSS PL ETDSMDPFGLVTGLEAVRSPSFEK	9
hFGF19 –C25	LYTSGPHGLSSCFLRIRADGVVDCARGQSAHSLLEIKAVALRTVAIKGVHSVR YLCMGADGKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQR QLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVT GLEAVRSPSFEK	10
hFGF19 –C26	YTSGPHGLSSCFLRIRADGVVDCARGQSAHSLLEIKAVALRTVAIKGVHSVRY LCMGADGKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQ LYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPL ETDSMDPFGLVTGLEAVRSPSFEK	11
hFGF19 –C27	TSGPHGLSSCFLRIRADGVVDCARGQSAHSLLEIKAVALRTVAIKGVHSVRYL CMGADGKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQ LYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPL ETDSMDPFGLVTGLEAVRSPSFEK	12
hFGF19 –C28	SGPHGLSSCFLRIRADGVVDCARGQSAHSLLEIKAVALRTVAIKGVHSVRYLC MGADGKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQ LYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPL ETDSMDPFGLVTGLEAVRSPSFEK	13
hFGF19 –C30	PHGLSSCFLRIRADGVVDCARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCM GADGKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYK NRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEA VRSPSFEK	14
hFGF19 –C33	LSSCFLRIRADGVVDCARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGAD GKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRG FLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSP SFEK	15

Name	Amino Acid Sequence (N-C)	SEQ ID NO
hFGF19 –C35	SCFLRIRADGVVDCARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGK MQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLP LSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFE K	16
hFGF19 –C37	FLRIRADGVVDCARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKM QGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPL SHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFE K	17
hFGF19 –C40	IRADGVVDCARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGL LQYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHF LPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEK	18
hFGF19 –C41	RADGVVDCARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLL QYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFL PMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEK	19
hFGF19 –C42	ADGVVDCARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQ YSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLP MLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEK	20
hFGF19 –C43	DGVVDCARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQY SEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLPM LPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEK	21
hFGF19 –C44	GVVDCARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYS EEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLPML PMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEK	22
hFGF19 –C45	VVDCARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSE EDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLPMLP MVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEK	23
hFGF19 –C52	QSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAFEEE IRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPED LRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEK	24
hFGF19 –C53	SAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAFEEEI RPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDL RGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEK	25

Name	Amino Acid Sequence (N-C)	SEQ ID NO
hFGF19 –C54	AHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAFEEEIR PDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLR GHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEK	26
hFGF19 –C56	SLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAFEEEIRPD GYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRG HLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEK	27
hFGF19 –C57	LLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAFEEEIRPDG YNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHL ESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEK	28
hFGF19 –C58	LEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAFEEEIRPDGY NVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLE SDMFSSPLETDSMDPFGLVTGLEAVRSPSFEK	29
hFGF19 –C59	EIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAFEEEIRPDGYN VYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLES DMFSSPLETDSMDPFGLVTGLEAVRSPSFEK	30
hFGF19 –C72	GVHSVRYLCMGADGKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLPVS LSSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPL ETDSMDPFGLVTGLEAVRSPSFEK	31
hFGF19 –C73	VHSVRYLCMGADGKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLS SAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPL ETDSMDPFGLVTGLEAVRSPSFEK	32
hFGF19 –C74	HSVRYLCMGADGKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSS AKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPL ETDSMDPFGLVTGLEAVRSPSFEK	33
hFGF19 –C79	LCMGADGKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQ LYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPL ETDSMDPFGLVTGLEAVRSPSFEK	34
hFGF19 –C80	CMGADGKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQ LYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTG LEAVRSPSFEK	35
hFGF19 –C81	MGADGKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQ LYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTG	36

Name	Amino Acid Sequence (N-C)	SEQ ID NO
	LEAVRSPSFEK	
hFGF19–C143	FLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEK	37
hFGF19–C144	LPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEK	38
hFGF19–C145	PMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEK	39
hFGF19–C146	MLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEK	40

[0066] Examples of N-terminal portions of hFGF21 polypeptide sequence referred to in the present invention include ,without limitation, those that have a final position that correspond to or about any one of positions 7, 8, 20, 21, 22, 23, 25, 27, 29, 31, 34, 35, 36, 37, 38, 39, 46, 47, 48, 50, 51, 52, 53, 66, 67, 68, 73, 74, 75, 135, 136, 137 and 138 of SEQ ID NO:2. Each exemplary N-terminal portion also has a first position that corresponds to or about position 1 of SEQ ID NO:2. Table 2 shows a list of exemplary N-terminal portions of hFGF21. Other analogous portions of hFGF21 are also contemplated herein.

Table 2: Exemplary N-Terminal Portions of hFGF21 Polypeptide Sequence

Name	Amino Acid Sequence (N-C)	SEQ ID NO
hFGF21–N7	HPIPDSS	41
hFGF21–N8	HPIPDSSP	42
hFGF21–N20	HPIPDSSPLLQFGGQVRQRY	43
hFGF21–N21	HPIPDSSPLLQFGGQVRQRYL	44
hFGF21–N22	HPIPDSSPLLQFGGQVRQRYLY	45
hFGF21–N23	HPIPDSSPLLQFGGQVRQRYLYT	46
hFGF21–N25	HPIPDSSPLLQFGGQVRQRYLYTDD	47
hFGF21–N27	HPIPDSSPLLQFGGQVRQRYLYTDDAQ	48
hFGF21–N29	HPIPDSSPLLQFGGQVRQRYLYTDDAQQT	49
hFGF21–N31	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEA	50
hFGF21–N34	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLE	51

Name	Amino Acid Sequence (N-C)	SEQ ID NO
hFGF21–N35	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEI	52
hFGF21–N36	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIR	53
hFGF21–N37	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIRE	54
hFGF21–N38	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIRED	55
hFGF21–N39	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDG	56
hFGF21–N46	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAAD	57
hFGF21–N47	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQ	58
hFGF21–N48	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQS	59
hFGF21–N50	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQS PE	60
hFGF21–N51	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQS PES	61
hFGF21–N52	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQS PESL	62
hFGF21–N53	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQS PESLL	63
hFGF21–N66	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQS PESLLQLKALKPGVIQIL	64
hFGF21–N67	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQS PESLLQLKALKPGVIQILG	65
hFGF21–N68	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQS PESLLQLKALKPGVIQILGV	66
hFGF21–N73	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQS PESLLQLKALKPGVIQILGVKTSRF	67
hFGF21–N74	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQS PESLLQLKALKPGVIQILGVKTSRFL	68
hFGF21–N75	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQS PESLLQLKALKPGVIQILGVKTSRFLC	69
hFGF21–N135	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQS PESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEACSFRE LLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPAR	70

Name	Amino Acid Sequence (N-C)	SEQ ID NO
hFGF21–N136	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQS PESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEACSFRE LLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARF	71
hFGF21–N137	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQS PESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEACSFRE LLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFL	72
hFGF21–N138	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQS PESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEACSFRE LLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLP	73

[0067] It is intended that a C-terminal, N-terminal or any other portion of a polypeptide sequence defined herein may independently and optionally include one to five or more additional or fewer residues at the defined first or final position. For example, a C-terminal portion of a polypeptide having a first position at or about residue 100 may, independently, (i) optionally include 1, 2, 3, 4, 5 or more additional residues N-terminal to the residue at position 100, (ii) optionally include 1, 2, 3, 4, 5 or more additional residues C-terminal to the final residue, (iii) optionally begin at a position 1, 2, 3, 4, 5 or more residues C-terminal to the residue at position 100 or (iv) optionally end at a position 1, 2, 3, 4, 5 or more residues N-terminal to the final residue. If present, one or more of the additional residues may or may not be the same as the residues at the corresponding position in the polypeptide.

[0068] In some embodiments of a chimeric FGF19 polypeptide of the present invention, the N-terminal portion of its sequence includes a sequence that is selected from the N-terminal portions of the hFGF21 polypeptide sequence listed in Table 2, and the C-terminal portion of its sequence includes a sequence that is selected from among the C-terminal portions of the hFGF19 polypeptide sequence listed in Table 1. In some embodiments, the selected hFGF21 N-terminal portion and the selected hFGF19 C-terminal portion are selected independently with respect to each other. In some embodiments, the hFGF21 N-terminal sequence portion and the hFGF19 C-terminal sequence portion are selected such that the C-terminus of the N-terminal sequence portion and the N-terminus end of the C-terminal sequence portion have at least 1, at least 2 or at least 3 or more residues in common. In some embodiments, the sequence of the chimeric FGF19 polypeptide comprises a sequence in which the C-terminal portion of a FGF19 polypeptide sequence and the N-terminal portion of the chimeric FGF21 polypeptide sequence are contiguous

without intervening amino acids therebetween. In some embodiments of the foregoing, the sequence of the chimeric FGF19 polypeptide comprises a sequence in which the C-terminal portion of a FGF19 polypeptide sequence and the N-terminal portion of the chimeric FGF21 polypeptide sequence are contiguously joined by overlapping the 1, 2, 3 or more residues in common between the two portions. In some alternative embodiments, the sequence of the chimeric FGF19 polypeptide comprises a sequence in which includes the C-terminal portion of a FGF19 polypeptide sequence and the N-terminal portion of the chimeric FGF21 polypeptide sequence, and further includes an intervening spacer therebetween of 1, 2, 3, 4, 5 or more amino residues.

[0069] Exemplary sequences of chimeric FGF19 polypeptides of the present invention are shown in Table 3, wherein its N-terminal portion includes an N-terminal portion of a hFGF21 polypeptide sequence and its C-terminal portion includes a C-terminal portion of a hFGF19 polypeptide sequence.

Table 3: Exemplary Chimeric FGF19 Polypeptide Sequences

Name	Amino Acid Sequence (N-C)	SEQ ID NO
cFGF21/19-1	**HPIPDSS** PHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQSA HSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCA FEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLPM LPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFE K	74
cFGF21/19-2	**HPIPDSSPLLQFGGQVRQRY** LYTSGPHGLSSCFLRIRADGVVDCARGQSAHSLLEIKAVALRTVAIK GVHSVRYLCMGADGKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEK HRLPVSLSSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLE SDMFSSPL ETDSMDPFGLVTGLEAVRSPSFEK	5
cFGF21/19-3	**HPIPDSSPLLQFGGQVRQRYLYTDD** PHGLSSCFLRIRADGVVDCARGQSAHSLLEIKAVALRTVAIKGVHS VRYLCMGADGKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLP VSLSSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMF SSPLETDSMDPFGLVTGLEAVRSPSFEK	75
cFGF21/19-4	**HPIPDSSPLLQFGGQVRQRYLYTDDAQ** LSSCFLRIRADGVVDCARGQSAHSLLEIKAVALRTVAIKGVHSVRY LCMGADGKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSL	76

Name	Amino Acid Sequence (N-C)	SEQ ID NO
	SSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSP LETDSMDPFGLVTGLEAVRSPSFEK	
cFGF21/19-5	**HPIPDSSPLLQFGGQVRQRYLYTDDAQQT** SCFLRIRADGVVDCARGQSAHSLLEIKAVALRTVAIKGVHSVRYLC MGADGKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSS AKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLE TDSMDPFGLVTGLEAVRSPSFEK	77
cFGF21/19-6	**HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEA** FLRIRADGVVDCARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCM GADGKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAK QRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETD SMDPFGLVTGLEAVRSPSFEK	78
cFGF21/19-7	**HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLE** IRADGVVDCARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGAD GKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQ LYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMD PFGLVTGLEAVRSPSFEK	79
cFGF21/19-8	**HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIRE** DGVVDCARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGK MQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLY KNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPF GLVTGLEAVRSPSFEK	80
cFGF21/19-9	**HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGG AAD** QSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEE DCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSH FLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVR SPSFEK	81
cFGF21/19-10	**HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGG AADQSPE** SLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAF EEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLPM	82

Name	Amino Acid Sequence (N-C)	SEQ ID NO
	LPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEK	
cFGF21/19-11	**HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESLLQLKALKPGVIQIL**GVHSVRYLCMGADGKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPL ETDSMDPFGLVTGLEAVRSPSFEK	83
cFGF21/19-12	**HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESLLQLKALKPGVIQILGVKTSRF**LCMGADGKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEK	84
cFGF21/19-13	**HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPAR**FLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEK	85

II. Chimeric FGF19 Polypeptides With C-Terminal FGF21 Polypeptide Sequences

[0070] In a second aspect of the present invention, a chimeric FGF19 polypeptide sequence includes a C-terminal portion and an N-terminal portion. The N-terminal portion of the chimeric FGF19 polypeptide sequence includes a N-terminal portion of a FGF19 polypeptide sequence and the C-terminal portion of the chimeric FGF19 polypeptide sequence includes an C-terminal portion of a FGF21 polypeptide sequence. In some embodiments of the foregoing, the C-terminal portion of a FGF21 polypeptide sequence and the N-terminal portion of the chimeric FGF19 polypeptide sequence are contiguously joined. In some embodiments of the foregoing, the C-terminal portion of a FGF21 polypeptide sequence and the N-terminal portion of the chimeric FGF19 polypeptide sequence are contiguously joined by overlapping the 1, 2, 3 or more residues in common between the two portions. In some alternative embodiments, the C-terminal portion of a FGF21 polypeptide sequence and the N-terminal portion of the chimeric FGF19 polypeptide sequence have an intervening spacer of 1, 2, 3, 4, 5 or more amino residues.

[0071] In certain preferred embodiments, the FGF19 polypeptide is human FGF19 (hFGF19) polypeptide whose sequence is defined in SEQ ID NO:1. In some embodiments, the N-terminal portion of the hFGF19 polypeptide sequence is from about 45 to about 175 residues in length, expressly including the sequence lengths of the hFGF19 N-terminal portions shown in Table 4. In some embodiments, the N-terminal portion of the chimeric FGF19 polypeptide sequence includes a N-terminal portion of a polypeptide sequence that has at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or at least 99% amino acid sequence identity to the hFGF19 polypeptide sequence.

[0072] In certain preferred embodiments, the FGF21 polypeptide is human FGF21 (hFGF21) polypeptide whose sequence is defined in SEQ ID NO:2. In some embodiments, the C-terminal portion of the hFGF21 polypeptide sequence is from about 8 to about 145 residues in length, expressly including the sequence lengths of the hFGF19 C-terminal portions shown in Table 5. In some embodiments, the C-terminal portion of the chimeric FGF21 polypeptide sequence includes a C-terminal portion of a polypeptide sequence that has at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or at least 99% amino acid sequence identity to the hFGF21 polypeptide sequence.

[0073] Examples of N-terminal portions of hFGF19 polypeptide sequence referred to in the present invention include, without limitation, those that have a final position that correspond to or about any one of positions 9, 10, 24, 25, 26, 27, 39, 40, 41, 42, 43, 44, 51, 52, 53, 55, 56, 57, 58, 71, 72, 73, 78, 79, 80, 142, 143, 144 and 145 of SEQ ID NO:1. Each exemplary N-terminal portion also has a final position that corresponds to or about position 1 of SEQ ID NO:1. Table 4 shows the polypeptide sequences of exemplary N-terminal portions of hFGF19. Other analogous portions of hFGF19 are also contemplated herein.

Table 4: Exemplary N-Terminal Portions of hFGF19 Polypeptide Sequence

Name	Amino Acid Sequence (N-C)	SEQ ID NO
hFGF19 –N9	RPLAFSDAG	86
hFGF19 –N10	RPLAFSDAGP	87
hFGF19 –N24	RPLAFSDAGPHVHYGWGDPIRLRH	88
hFGF19 –N25	RPLAFSDAGPHVHYGWGDPIRLRHL	89

Name	Amino Acid Sequence (N-C)	SEQ ID NO
hFGF19 –N26	RPLAFSDAGPHVHYGWGDPIRLRHLY	90
hFGF19 –N27	RPLAFSDAGPHVHYGWGDPIRLRHLYT	91
hFGF19 –N39	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLR	92
hFGF19 –N40	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRI	93
hFGF19 –N41	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIR	94
hFGF19 –N42	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRA	95
hFGF19 –N43	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRAD	96
hFGF19 –N44	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADG	97
hFGF19 –N51	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARG	98
hFGF19 –N52	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQ	99
hFGF19 –N53	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQ S	100
hFGF19 –N55	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQ SAH	101
hFGF19 –N56	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQ SAHS	102
hFGF19 –N57	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQ SAHSL	103
hFGF19 –N58	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQ SAHSLL	104
hFGF19 –N71	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQ SAHSLLEIKAVALRTVAIK	105

Name	Amino Acid Sequence (N-C)	SEQ ID NO
hFGF19 –N72	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQ SAHSLLEIKAVALRTVAIKG	106
hFGF19 –N73	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQ SAHSLLEIKAVALRTVAIKGV	107
hFGF19 –N78	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQ SAHSLLEIKAVALRTVAIKGVHSVRY	108
hFGF19 –N79	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQ SAHSLLEIKAVALRTVAIKGVHSVRYL	109
hFGF19 –N80	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQ SAHSLLEIKAVALRTVAIKGVHSVRYLC	110
hFGF19 –N142	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQ SAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAFEEEI RPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSH	111
hFGF19 –N143	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQ SAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAFEEEI RPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHF	112
hFGF19 –N144	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQ SAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAFEEEI RPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFL	113
hFGF19 –N145	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQ SAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAFEEEI RPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLP	114

[0074] Examples of C-terminal portions of hFGF21 polypeptide sequence referred to in the present invention include ,without limitation, those that have a final position that correspond to or about any one of positions 8, 9, 21, 22, 23, 24, 35, 36, 37, 38, 39, 40, 47, 48, 49, 51, 52, 53, 54, 67, 68, 69, 146, 147, 148 and 149 of SEQ ID NO:2. Each exemplary C-terminal portion also has a final position that corresponds to or about position 181 of SEQ ID NO:2. Table 5 shows a list of exemplary C-terminal portions of hFGF21. Other analogous portions of hFGF21 are also contemplated herein.

Table 5: Exemplary C-Terminal Portions of hFGF21 Polypeptide Sequence

Name	Amino Acid Sequence (N-C)	SEQ ID NO
hFGF21–C8	PLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESL LQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEACSFRELL LEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPA LPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYAS	115
hFGF21–C9	LLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESLL QLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEACSFRELLL EDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPAL PEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYAS	116
hFGF21–C21	LYTDDAQQTEAHLEIREDGTVGGAADQSPESLLQLKALKPGVIQIL GVKTSRFLCQRPDGALYGSLHFDPEACSFRELLLEDGYNVYQSEAH GLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPALPEPPGILAPQPPDV GSSDPLSMVGPSQGRSPSYAS	117
hFGF21–C22	YTDDAQQTEAHLEIREDGTVGGAADQSPESLLQLKALKPGVIQILG VKTSRFLCQRPDGALYGSLHFDPEACSFRELLLEDGYNVYQSEAHG LPLHLPGNKSPHRDPAPRGPARFLPLPGLPPALPEPPGILAPQPPDVG SSDPLSMVGPSQGRSPSYAS	118
hFGF21–C23	TDDAQQTEAHLEIREDGTVGGAADQSPESLLQLKALKPGVIQILGV KTSRFLCQRPDGALYGSLHFDPEACSFRELLLEDGYNVYQSEAHGL PLHLPGNKSPHRDPAPRGPARFLPLPGLPPALPEPPGILAPQPPDVGS SDPLSMVGPSQGRSPSYAS	119
hFGF21–C24	DDAQQTEAHLEIREDGTVGGAADQSPESLLQLKALKPGVIQILGVK TSRFLCQRPDGALYGSLHFDPEACSFRELLLEDGYNVYQSEAHGLP LHLPGNKSPHRDPAPRGPARFLPLPGLPPALPEPPGILAPQPPDVGSS DPLSMVGPSQGRSPSYAS	120
hFGF21–C35	IREDGTVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQRPDGA LYGSLHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRD PAPRGPARFLPLPGLPPALPEPPGILAPQPPDVGSSDPLSMVGPSQGR SPSYAS	121
hFGF21–C36	REDGTVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQRPDGA LYGSLHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRD PAPRGPARFLPLPGLPPALPEPPGILAPQPPDVGSSDPLSMVGPSQGR SPSYAS	122

Name	Amino Acid Sequence (N-C)	SEQ ID NO
hFGF21–C37	EDGTVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQRPDGAL YGSLHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDP APRGPARFLPLPGLPPALPEPPGILAPQPPDVGSSDPLSMVGPSQGRS PSYAS	123
hFGF21–C38	DGTVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQRPDGALY GSLHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPA PRGPARFLPLPGLPPALPEPPGILAPQPPDVGSSDPLSMVGPSQGRSP SYAS	124
hFGF21–C39	GTVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYG SLHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAP RGPARFLPLPGLPPALPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPS YAS	125
hFGF21–C40	TVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGS LHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPR GPARFLPLPGLPPALPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSY AS	126
hFGF21–C47	QSPESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEAC SFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLP GLPPALPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYAS	127
hFGF21–C48	SPESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEACS FRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLP GLPPALPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYAS	128
hFGF21–C49	PESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEACSF RELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPG LPPALPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYAS	129
hFGF21–C51	SLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEACSFRE LLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLP PALPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYAS	130
hFGF21–C52	LLQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEACSFREL LLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPP ALPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYAS	131
hFGF21–C53	LQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEACSFRELL	132

Name	Amino Acid Sequence (N-C)	SEQ ID NO
	LEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPA LPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYAS	
hFGF21–C54	QLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEACSFRELLL EDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPAL PEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYAS	133
hFGF21–C67	GVKTSRFLCQRPDGALYGSLHFDPEACSFRELLLEDGYNVYQSEAH GLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPALPEPPGILAPQPPDV GSSDPLSMVGPSQGRSPSYAS	134
hFGF21–C68	VKTSRFLCQRPDGALYGSLHFDPEACSFRELLLEDGYNVYQSEAHG LPLHLPGNKSPHRDPAPRGPARFLPLPGLPPALPEPPGILAPQPPDVG SSDPLSMVGPSQGRSPSYAS	135
hFGF21–C69	KTSRFLCQRPDGALYGSLHFDPEACSFRELLLEDGYNVYQSEAHGL PLHLPGNKSPHRDPAPRGPARFLPLPGLPPALPEPPGILAPQPPDVGS SDPLSMVGPSQGRSPSYAS	136
hFGF21–C74	LCQRPDGALYGSLHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLP GNKSPHRDPAPRGPARFLPLPGLPPALPEPPGILAPQPPDVGSSDPLS MVGPSQGRSPSYAS	137
hFGF21–C75	CQRPDGALYGSLHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLPG NKSPHRDPAPRGPARFLPLPGLPPALPEPPGILAPQPPDVGSSDPLSM VGPSQGRSPSYAS	138
hFGF21–C76	QRPDGALYGSLHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLPGN KSPHRDPAPRGPARFLPLPGLPPALPEPPGILAPQPPDVGSSDPLSMV GPSQGRSPSYAS	139
hFGF21–C146	FLPLPGLPPALPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYAS	140
hFGF21–C147	LPLPGLPPALPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYAS	141
hFGF21–C148	PLPGLPPALPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYAS	142
hFGF21–C149	LPGLPPALPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYAS	143

[0075] In some embodiments of a chimeric FGF19 polypeptide of the present invention, the N-terminal portion of its sequence includes a sequence that is selected from the hFGF19 polypeptide sequence portions listed in Table 4, and the C-terminal portion of its sequence includes a sequence that is selected from among the hFGF21 polypeptide sequence portions listed in Table 5. In some embodiments, the selected hFGF19 N-terminal portion and the selected hFGF21 C-

terminal portion are selected independently with respect to each other. In some embodiments, the hFGF19 N-terminal sequence portion and the hFGF21 C-terminal sequence portion are selected such that the C-terminus of the N-terminal sequence portion and the N-terminus end of the C-terminal sequence portion have at least 1, at least 2 or at least 3 or more residues in common. In some embodiments, the sequence of the chimeric FGF19 polypeptide comprises a sequence in which the N-terminal portion of a FGF19 polypeptide sequence and the C-terminal portion of the chimeric FGF21 polypeptide sequence are contiguous without intervening amino acids therebetween. In some embodiments, the sequence of the chimeric FGF19 polypeptide comprises a sequence in which the N-terminal portion of a FGF19 polypeptide sequence and the C-terminal portion of the chimeric FGF21 polypeptide sequence are contiguously joined by overlapping the 1, 2, 3 or more residues in common between the two portions. In some alternative embodiments, the sequence of the chimeric FGF19 polypeptide comprises a sequence in which includes the N-terminal portion of a FGF19 polypeptide sequence and the C-terminal portion of the chimeric FGF21 polypeptide sequence, and further includes an intervening spacer therebetween of 1, 2, 3, 4, 5 or more amino residues.

[0076] Exemplary sequences of chimeric FGF19 polypeptides of the present invention are shown in Table 6, wherein its N-terminal portion includes an N-terminal portion of a hFGF19 polypeptide sequence and its C-terminal portion includes a C-terminal portion of a hFGF21 polypeptide sequence.

Table 6: Exemplary Chimeric FGF19 Polypeptide Sequences

Name	Amino Acid Sequence (N-C)	SEQ ID NO
cFGF19/21-1	**RPLAFSDAG** PLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESL LQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEACSFRELL LEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPA LPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYAS	144
cFGF19/21-2	**RPLAFSDAGPHVHYGWGDPIRLRH** LYTDDAQQTEAHLEIREDGTVGGAADQSPESLLQLKALKPGVIQIL GVKTSRFLCQRPDGALYGSLHFDPEACSFRELLLEDGYNVYQSEAH GLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPALPEPPGILAPQPPDV GSSDPLSMVGPSQGRSPSYAS	145
cFGF19/21-3	**RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLR** IREDGTVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQRPDGA LYGSLHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRD	146

Name	Amino Acid Sequence (N-C)	SEQ ID NO
	PAPRGPARFLPLPGLPPALPEPPGILAPQPPDVGSSDPLSMVGPSQGR SPSYAS	
cFGF19/21-4	**RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRA** DGTVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQRPDGALY GSLHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPA PRGPARFLPLPGLPPALPEPPGILAPQPPDVGSSDPLSMVGPSQGRSP SYAS	147
cFGF19/21-5	**RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADG VVDCARG** QSPESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEAC SFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLP GLPPALPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYAS	148
cFGF19/21-6	**RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADG VVDCARGQSAH** SLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEACSFRE LLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLP PALPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYAS	149
cFGF19/21-7	**RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADG VVDCARGQSAHSLLEIKAVALRTVAIK** GVKTSRFLCQRPDGALYGSLHFDPEACSFRELLLEDGYNVYQSEAH GLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPALPEPPGILAPQPPDV GSSDPLSMVGPSQGRSPSYAS	150
cFGF19/21-8	**RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADG VVDCARGQSAHSLLEIKAVALRTVAIKGVHSVRY** LCQRPDGALYGSLHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLP GNKSPHRDPAPRGPARFLPLPGLPPALPEPPGILAPQPPDVGSSDPLS MVGPSQGRSPSYAS	151
cFGF19/21-9	**RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADG VVDCARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGK MQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQR QLYKNRGFLPLSH** FLPLPGLPPALPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYAS	152

Name	Amino Acid Sequence (N-C)	SEQ ID NO
cFGF19/21-10 (Construct 8 shown in Figure 18B)	**RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADG VVDCARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGK MQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQR QLYKNRGFLPLSHFLP** LPGLPPALPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYAS	271
cFGF19/21-11 (Construct 9 shown in Figure 18B)	**RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADG VVDCARGQSAHSLLEIKAVALRTVAIKGV** KTSRFLCQRPDGALYGSLHFDPEACSFRELLLEDGYNVYQSE AHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPALPEPPGILAPQPP DVGSSDPLSMVGPSQGRSPSYAS	272
cFGF19/21-12 (Construct 10 shown in Figure 18B)	**RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADG VVDCARGQSAHSLL** QLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEACSFRELLL EDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPAL PEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYAS	273
cFGF19/21-13 (Construct 11 shown in Figure 18B)	**RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADG VVDCARGQS** PESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEACSF RELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPG LPPALPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYAS	274
cFGF19/21-14 (Construct 12 shown in Figure 18B)	**RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADG** TVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGS LHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPR GPARFLPLPGLPPALPEPPGILAPQPPDVGSSDPL SMVGPSQGRSPSYAS	275
cFGF19/21-15 (Construct 13 shown in Figure 18B)	**RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIR** EDGTVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQRPDGAL YGSLHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDP APRGPARFLPLPGLPPALPEPPGILAPQPPDVGSSDPLSMVGPSQGRS PSYAS	276
cFGF19/21-16 (Construct 14 shown in Figure 18B)	**RPLAFSDAGPHVHYGWGDPIRLRHLYT** DDAQQTEAHLEIREDGTVGGAADQSPESLLQLKALKPGVIQILGVK TSRFLCQRPDGALYGSLHFDPEACSFRELLLEDGYNVY QSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPALPEPPGILAP QPPDVGSSDPLSMVGPSQGRSPSYAS	277
cFGF19/21-17 (Construct 15 shown in Figure 18B)	**RPLAFSDAGP** LLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESLL QLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEACSFRELLL EDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPAL PEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYAS	278

III. Chimeric FGF19 Polypeptides With Substituting FGF21 Polypeptide Sequences

[0077] In a third aspect of the present invention, a chimeric FGF19 polypeptide sequence includes a first polypeptide sequence in which a portion of first polypeptide sequence is substituted with a portion of a second polypeptide sequence. In preferred embodiments, the first polypeptide is human FGF19 (hFGF19) polypeptide whose sequence is defined in SEQ ID NO:1. In some embodiments, the first polypeptide sequence is a polypeptide sequence that has at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or at least 99% amino acid sequence identity to the hFGF19 polypeptide sequence.

[0078] In certain preferred embodiments, the second polypeptide is human FGF21 (hFGF21) polypeptide whose sequence is defined in SEQ ID NO:2. In some embodiments, the FGF21 polypeptide sequence is a polypeptide sequence that has at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or at least 99% amino acid sequence identity to the hFGF21 polypeptide sequence.

[0079] In some embodiments, the sequence of the chimeric FGF19 polypeptide includes a hFGF19 polypeptide sequence in which the portion to be substituted is from a group that include, without limitation, portions that have (i) a first position that corresponds to or about any one of positions 1, 10, 11, 17, 18, 21, 22, 25, 26, 27, 28, 40, 41, 42, 43, 44, 45, 52, 53, 54, 56, 57, 58, 59, 63, 72, 73, 74, 79, 80, 81, 143, 144, 145 and 146 in SEQ ID NO:1, and (ii) a final position that corresponds to or about any one of positions 9, 10, 24, 25, 26, 27, 29, 31, 32, 34, 36, 39, 40, 41, 42, 43, 44, 51, 52, 53, 55, 56, 57, 58, 66, 71, 72, 73, 78, 79, 80, 142, 143, 144, 145 and 194 in SEQ ID NO:1, such that the final position is C-terminal to the first position and the positions are selected independently. Table 7 shows a list of exemplary portions of the hFGF19 polypeptide sequence that are to be substituted for a portion of the hFGF21 polypeptide sequence.

Table 7: Exemplary Substituted Portions of the hFGF19 Polypeptide Sequence

Name	Amino Acid Sequence (N-C)	SEQ ID NO
hFGF19–S10-27	PHVHYGWGDPIRLRHLYT	153
hFGF19–S10-29	PHVHYGWGDPIRLRHLYTSG	154
hFGF19–S10-31	PHVHYGWGDPIRLRHLYTSGPH	155
hFGF19–S10-32	PHVHYGWGDPIRLRHLYTSGPHG	156

Name	Amino Acid Sequence (N-C)	SEQ ID NO
hFGF19–S10-34	PHVHYGWGDPIRLRHLYTSGPHGLS	157
hFGF19–S10-36	PHVHYGWGDPIRLRHLYTSGPHGLSSC	158
hFGF19–S10-41	PHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIR	159
hFGF19–S10-44	PHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADG	160
hFGF19–S10-52	PHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQS	161
hFGF19–S10-58	PHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQSAHSLL	162
hFGF19–S10-73	PHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQSAHSLLEIKAVALRTVAIKGV	163
hFGF19–S17-27	GDPIRLRHLYT	164
hFGF19–S17-29	GDPIRLRHLYTSG	165
hFGF19–S17-31	GDPIRLRHLYTSGPH	166
hFGF19–S17-32	GDPIRLRHLYTSGPHG	167
hFGF19–S17-34	GDPIRLRHLYTSGPHGLS	168
hFGF19–S17-36	GDPIRLRHLYTSGPHGLSSC	169
hFGF19–S21-27	RLRHLYT	170
hFGF19–S21-29	RLRHLYTSG	171
hFGF19–S21-	RLRHLYTSGPH	172

Name	Amino Acid Sequence (N-C)	SEQ ID NO
31		
hFGF19–S21-32	RLRHLYTSGPHG	173
hFGF19–S21-34	RLRHLYTSGPHGLS	174
hFGF19–S21-36	RLRHLYTSGPHGLSSC	175
hFGF19–S25-41	LYTSGPHGLSSCFLRIR	176
hFGF19–S25-44	LYTSGPHGLSSCFLRIRADG	177
hFGF19–S25-52	LYTSGPHGLSSCFLRIRADGVVDCARGQS	178
hFGF19–S25-58	LYTSGPHGLSSCFLRIRADGVVDCARGQSAHSLL	179
hFGF19–S25-73	LYTSGPHGLSSCFLRIRADGVVDCARGQSAHSLLEIKAVALRTVAIKGV	180
hFGF19–S63-66	VALR	181

[0080] The substituted portion of the hFGF19 polypeptide sequence is substituted with a portion of the hFGF21 polypeptide sequence. Exemplary substituting portions of the hFGF21 polypeptide sequence include, without limitation, portions that have (i) a first position that corresponds to or about any one of positions 1, 8, 9, 13, 14, 17, 18, 21, 22, 23, 24, 35, 36, 37, 38, 39, 40, 47, 48, 49, 51, 52, 53, 54, 58, 67, 68, 69, 74, 75, 76, 136, 137, 138 and 139 in SEQ ID NO:2, and (ii) a final position that corresponds to or about any one of positions 7, 8, 20, 21, 22, 23, 24, 25, 27, 29, 31, 34, 35, 36, 37, 38, 39, 46, 47, 48, 50, 51, 52, 53, 61, 66, 67, 68, 73, 74, 75, 135, 136, 137, 138 and 181 in SEQ ID NO:2, such that the final position is C-terminal to the first position and the positions are selected independently. Table 8 shows a list of exemplary portions of hFGF21 polypeptide sequence that are to substitute for a portion of the hFGF19 polypeptide sequence.

Table 8: Exemplary Substituting Portions From hFGF21 Polypeptide Sequence

Name	Amino Acid Sequence (N-C)	SEQ ID NO
hFGF21–S8-23	PLLQFGGQVRQRYLYT	182
hFGF21–S8-25	PLLQFGGQVRQRYLYTDD	183
hFGF21–S8-27	PLLQFGGQVRQRYLYTDDAQ	184
hFGF21–S8-29	PLLQFGGQVRQRYLYTDDAQQT	185
hFGF21–S8-31	PLLQFGGQVRQRYLYTDDAQQTEA	186
hFGF21–S8-36	PLLQFGGQVRQRYLYTDDAQQTEAHLEIR	187
hFGF21–S8-39	PLLQFGGQVRQRYLYTDDAQQTEAHLEIREDG	188
hFGF21–S8-48	PLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQS	189
hFGF21–S8-53	PLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESLL	190
hFGF21–S8-68	PLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESLLQL KALKPGV	191
hFGF21–S13-23	GGQVRQRYLYT	192
hFGF21–S13-25	GGQVRQRYLYTDD	193
hFGF21–S13-27	GGQVRQRYLYTDDAQ	194
hFGF21–S13-29	GGQVRQRYLYTDDAQQT	195
hFGF21–S13-31	GGQVRQRYLYTDDAQQTEA	196
hFGF21–S17-23	RQRYLYT	197
hFGF21–S17-25	RQRYLYTDD	198
hFGF21–S17-27	RQRYLYTDDAQ	199
hFGF21–S17-29	RQRYLYTDDAQQT	200
hFGF21–S17-	RQRYLYTDDAQQTEA	201

Name	Amino Acid Sequence (N-C)	SEQ ID NO
31		
hFGF21–S21-36	LYTDDAQQTEAHLEIR	202
hFGF21–S21-39	LYTDDAQQTEAHLEIREDG	203
hFGF21–S21-48	LYTDDAQQTEAHLEIREDGTVGGAADQS	204
hFGF21–S21-53	LYTDDAQQTEAHLEIREDGTVGGAADQSPESLL	205
hFGF21–S21-68	LYTDDAQQTEAHLEIREDGTVGGAADQSPESLLQLKALKPGV	206
hFGF21–S58-62	LKPG	207

[0081] In some embodiments, a chimeric FGF19 polypeptide includes the polypeptide sequence of hFGF19 with a portion of its sequence, such as a sequence selected from the hFGF19 polypeptide sequence portions listed in Table 7, substituted with a portion of the hFGF21 polypeptide sequence, such as the sequence portions listed in Table 8. In some embodiments, the selected hFGF19 N-terminal portion and the selected hFGF21 C-terminal portion are selected independently with respect to each other. In some embodiments, the hFGF19 portion to be substituted includes the N-terminal residue of the hFGF19 polypeptide. In some embodiments, the hFGF19 portion to be substituted includes the C-terminal residue of the hFGF19 polypeptide. In some embodiments, the substituting hFGF21 portion includes the N-terminal residue of the hFGF21 polypeptide. In some embodiments, the substituting hFGF21 portion includes the C-terminal residue of the hFGF21 polypeptide. In some embodiments, the hFGF19 portion to be substituted includes the N-terminal residue of the hFGF19 polypeptide, and the substituting hFGF21 portion also includes the N-terminal residue of the hFGF21 polypeptide. In some embodiments, the hFGF19 portion to be substituted includes the C-terminal residue of the hFGF19 polypeptide, and the substituting hFGF21 portion also includes the C-terminal residue of the hFGF21 polypeptide.

[0082] In some embodiments, the hFGF19 sequence portion and the hFGF21 sequence portion are selected such that at least one of their respective corresponding ends (*e.g.*., the N-terminal end

of the hFGF19 portion and the N-terminal end of the hFGF21 portion, the C-terminal end of the hFGF19 portion and the C-terminal end of the hFGF21 portion, or both) have at least 1, at least 2 or at least 3 or more residues in common at said corresponding ends. In some embodiments, the sequence of the chimeric FGF19 polypeptide comprises a sequence in which the substituting portion of the hFGF21 polypeptide sequence is contiguous with the remaining hFGF19 polypeptide sequence by overlapping the 1, 2, 3 or more residues in common between the two portions.

[0083] In some embodiments, the sequence of the chimeric FGF19 polypeptide comprises a sequence in which the portion of the hFGF21 polypeptide sequence is substituted in the hFGF19 polypeptide sequence such that the hFGF19 and hFGF21 polypeptide sequences are contiguous and without additional, intervening amino acids therebetween. In some alternative embodiments, the sequence of the chimeric FGF19 polypeptide comprises a sequence in which the portion of the hFGF21 polypeptide sequence is substituted in the hFGF19 polypeptide sequence such that the chimeric FGF19 polypeptide sequence further includes an intervening spacer therebetween of 1, 2, 3, 4, 5 or more amino residues between the hFGF19 and hFGF21 sequences.

[0084] Exemplary sequences of chimeric FGF19 polypeptides of the present invention are shown in Table 9, in which a portion of hFGF19 is substituted with a portion of hFGF21.

Table 9: Exemplary Chimeric FGF19 Polypeptide Sequences

Name	Amino Acid Sequence (N-C)	SEQ ID NO
cFGF19/21/19-1	RPLAFSDAGPLLQFGGQVRQRYLYTSGPHGLSSCFLRIRADGVVDCA RGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYS EEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLS HFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVR SPSFEK	208
cFGF19/21/19-2	RPLAFSDAGPLLQFGGQVRQRYLYTDDPHGLSSCFLRIRADGVVDC ARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQY SEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPL SHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAV RSPSFEK	209
cFGF19/21/19-3	RPLAFSDAGPLLQFGGQVRQRYLYTDDAQGLSSCFLRIRADGVVDC ARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQY SEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPL SHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAV	210

Name	Amino Acid Sequence (N-C)	SEQ ID NO
	RSPSFEK	
cFGF19/21/19-4	RPLAFSDAGPLLQFGGQVRQRYLYTDDAQLSSCFLRIRADGVVDCA RGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYS EEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLS HFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVR SPSFEK	211
cFGF19/21/19-5	RPLAFSDAGPLLQFGGQVRQRYLYTDDAQQTSCFLRIRADGVVDCA RGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYS EEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLS HFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVR SPSFEK	212
cFGF19/21/19-6	RPLAFSDAGPLLQFGGQVRQRYLYTDDAQQTEAFLRIRADGVVDCA RGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYS EEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLS HFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVR SPSFEK	213
cFGF19/21/19-7	RPLAFSDAGPLLQFGGQVRQRYLYTDDAQQTEAHLEIRADGVVDCA RGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYS EEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLS HFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVR SPSFEK	214
cFGF19/21/19-8	RPLAFSDAGPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGVVDCA RGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYS EEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLS HFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVR SPSFEK	215
cFGF19/21/19-9	RPLAFSDAGPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGA ADQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYS EEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLS HFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVR SPSFEK	216

Name	Amino Acid Sequence (N-C)	SEQ ID NO
cFGF19/21/19-10	RPLAFSDAGPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGA ADQSPESLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSE EDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSH FLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRS PSFEK	217
cFGF19/21/19-11	RPLAFSDAGPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGA ADQSPESLLQLKALKPGVIQILGVHSVRYLCMGADGKMQGLLQYSE EDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSH FLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRS PSFEK	218
cFGF19/21/19-12	RPLAFSDAGPHVHYGWGGQVRQRYLYTSGPHGLSSCFLRIRADGVV DCARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLL QYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFL PLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLE AVRSPSFEK	219
cFGF19/21/19-13	RPLAFSDAGPHVHYGWGGQVRQRYLYTDDPHGLSSCFLRIRADGVV DCARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLL QYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFL PLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLE AVRSPSFEK	220
cFGF19/21/19-14	RPLAFSDAGPHVHYGWGGQVRQRYLYTDDAQGLSSCFLRIRADGV VDCARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGL LQYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGF LPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGL EAVRSPSFEK	221
cFGF19/21/19-15	RPLAFSDAGPHVHYGWGGQVRQRYLYTDDAQLSSCFLRIRADGVV DCARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLL QYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFL PLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLE AVRSPSFEK	222
cFGF19/21/19-	RPLAFSDAGPHVHYGWGGQVRQRYLYTDDAQQTSCFLRIRADGVV	223

Name	Amino Acid Sequence (N-C)	SEQ ID NO
16	DCARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLL QYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFL PLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLE AVRSPSFEK	
cFGF19/21/19-17	RPLAFSDAGPHVHYGWGGQVRQRYLYTDDAQQTEAFLRIRADGVV DCARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLL QYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFL PLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLE AVRSPSFEK	224
cFGF19/21/19-18	RPLAFSDAGPHVHYGWGDPIRQRYLYTSGPHGLSSCFLRIRADGVVD CARGQSAIISLLEIKAVALRTVAIKGVIISVRYLCMGADGKMQGLLQ YSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLP LSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEA VRSPSFEK	225
cFGF19/21/19-19	RPLAFSDAGPHVHYGWGDPIRQRYLYTDDPHGLSSCFLRIRADGVV DCARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLL QYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFL PLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLE AVRSPSFEK	226
cFGF19/21/19-20	RPLAFSDAGPHVHYGWGDPIRQRYLYTDDAQGLSSCFLRIRADGVV DCARGQSAIISLLEIKAVALRTVAIKGVIISVRYLCMGADGKMQGLL QYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFL PLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLE AVRSPSFEK	227
cFGF19/21/19-21	RPLAFSDAGPHVHYGWGDPIRQRYLYTDDAQLSSCFLRIRADGVVD CARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQ YSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLP LSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEA VRSPSFEK	228
cFGF19/21/19-22	RPLAFSDAGPHVHYGWGDPIRQRYLYTDDAQQTSCFLRIRADGVVD CARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQ	229

Name	Amino Acid Sequence (N-C)	SEQ ID NO
	YSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLP LSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEA VRSPSFEK	
cFGF19/21/19-23	RPLAFSDAGPHVHYGWGDPIRQRYLYTDDAQQTEAFLRIRADGVVD CARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQ YSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLP LSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEA VRSPSFEK	230
cFGF19/21/19-24	RPLAFSDAGPHVHYGWGDPIRLRHLYTDDAQQTEAHLEIRADGVVD CARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQ YSEEDCAFEEEIRPDGYNVYRSEKIIRLPVSLSSAKQRQLYKNRGFLP LSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEA VRSPSFEK	231
cFGF19/21/19-25	RPLAFSDAGPHVHYGWGDPIRLRHLYTDDAQQTEAHLEIREDGVVD CARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQ YSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLP LSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEA VRSPSFEK	232
cFGF19/21/19-26	RPLAFSDAGPHVHYGWGDPIRLRHLYTDDAQQTEAHLEIREDGTVG GAADQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQ YSEEDCAFEEEIRPDGYNVYRSEKIIRLPVSLSSAKQRQLYKNRGFLP LSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEA VRSPSFEK	233
cFGF19/21/19-27	RPLAFSDAGPHVHYGWGDPIRLRHLYTDDAQQTEAHLEIREDGTVG GAADQSPESLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQ YSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLP LSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEA VRSPSFEK	234
cFGF19/21/19-28	RPLAFSDAGPHVHYGWGDPIRLRHLYTDDAQQTEAHLEIREDGTVG GAADQSPESLLQLKALKPGVIQILGVHSVRYLCMGADGKMQGLLQY SEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPL	235

Name	Amino Acid Sequence (N-C)	SEQ ID NO
	SHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAV RSPSFEK	
cFGF19/21/19-29	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVD CARGQSAHSLLEIKALKPGTVAIKGVHSVRYLCMGADGKMQGLLQ YSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLP LSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEA VRSPSFEK	236

[0085] In some embodiments of any of the chimeric hFGF19, the chimeric hFGF19 polypeptide includes a first polypeptide sequence having at least a certain sequence identity to the sequence of hFGF19 polypeptide, and wherein a portion of the first polypeptide sequence is substituted with more than one portion of a second polypeptide sequence, the second polypeptide sequence having at least a certain sequence identity to the sequence of hFGF21 polypeptide. In some embodiments, the chimeric hFGF19 polypeptide further comprises a substitution of the ß1-ß2 loop of the first polypeptide, a substitution of the ß10-ß12 segment of the first polypeptide, and/or a substitution of the five residues WGDPI (SEQ ID NO:287) of the first polypeptide with the ß1-ß2 loop of the second polypeptide, the ß10-ß12 segment of the second polypeptide, and/or the corresponding sequence GQV of the second polypeptide. In some embodiments, the chimeric hFGF19 polypeptide further comprises a substitution of the ß1-ß2 loop (amino acid residues 50-57 of FGF19 (SGPHGLSS (SEQ ID NO:288)) of FGF19 with the ß1-ß2 loop (amino acid residues 51-57 of FGF21 (DDAQQTE (SEQ ID NO:289)) of FGF21. In some embodiments, the chimeric hFGF19 polypeptide further comprises a substitution of the ß10-ß12 segment (amino acid residues 146-162 of FGF19 (SSAKQRQLYKNRGFLPL (SEQ ID NO:290)) of FGF19 with the ß10-ß12 segment (amino acid residues 147-161 of FGF21 (PGNKSPHRDPAPRGP (SEQ ID NO:291)) of FGF21. In some embodiments, the chimeric hFGF19 polypeptide further comprises a substitution of amino acid residues 38-42 (WGDPI (SEQ ID NO:287)) of FGF19 with amino acid residues 41-43 (GQV) of FGF21.

[0086] Chimeric FGF19 polypeptides of the present invention, and particularly pharmaceutically active compositions thereof and methods of using said chimeric FGF19 polypeptides in therapeutic treatment of one or more of the diseases, conditions, etc. listed or described herein or known in the art have certain advantages over the use of either native FGF19 (*e.g.*., hFGF19) or native FGF21 (*e.g.*., hFGF21).

[0087] In some embodiments, chimeric FGF19 polypeptides may be less immunogenic than one or both of their native parental FGFs. A native FGF19 and/or FGF21 (such as hFGF19 or hFGF21) may be present in the population in more than one allelic variation, wherein there is at least one amino acid residue that is different between the allelic forms. For example, hFGF21 is known to have a polymorphism at position 146 in the mature form, where this is residue can be leucine (as in Fig. 2 and SEQ ID NO:2) or proline in different alleles. Such polymorphism may limit the usefulness of native hFGF21 as a therapeutic composition. For example, administering a FGF19 polypeptide to an individual, wherein the individual's endogenous FGF19 has a different sequence than the administered FGF19, may result in an immune response by the individual to the administered hFGF21. Thus, in some embodiments, a chimeric FGF19 polypeptide of the present invention may include a portion of the hFGF21 polypeptide sequence and a portion of the hFGF19 polypeptide sequence, wherein both portions include only portions of the respective polypeptide sequences that are non-polymorphic. This can be accomplished by, for example, substituting a polymorphic sequence portion of one FGF with the analogous, non-polymorphic portion of the other FGF polypeptide. For example, a chimeric FGF19 polypeptide of the present invention, such as cFGF21/19-2 (*cf.* Table 3) that includes a portion of hFGF21 but does not include position 146 lacks the polymorphism at this position. In this manner, chimeric FGF19 polypeptides of the present invention may be advantageously less immunogenic, and thus may be advantageously more suitable for administration in a wide range of individuals.

[0088] In some embodiments, chimeric FGF19 polypeptides may be less tumorigenic than one or both of their corresponding native FGFs. In particular, chimeric FGF19 polypeptides may be less tumorigenic than native hFGF19. Native hFGF19, as discussed hereinabove, demonstrates potential tumorigenic activity via its binding to FGFR4. This tumorigenic activity appears separable from hFGF19's metabolic effects, which, like hFGF21, are effected via Klotho-beta-dependent binding to FGFR1c, FGFR2c and/or FGFR3c. In some embodiments, chimeric FGF19 polypeptides of the present invention include a portion of the hFGF19 polypeptide sequence that does not include the FGFR4-effector motif, and are instead substituted with a corresponding sequence from hFGF21. In some embodiments, chimeric FGF19 polypeptides of the present invention no longer substantially binds to and/or substantially activates FGFR4. In some embodiments, chimeric FGF19 polypeptides of the present invention no longer substantially binds to and/or substantially activates a receptor, such as FGFR4, in a Klotho-beta-independent manner. In this manner, chimeric FGF19 polypeptides of the present invention may be advantageously less tumorigenic, and thus may be advantageously more suitable for administration in a wide range of individuals.

[0089] In some embodiments, chimeric FGF19 polypeptides may not effect growth hormone (GH) resistance, or demonstrate substantially less GH resistance activity, than one or both of their corresponding native FGFs. In some embodiments, chimeric FGF19 polypeptides may have less GH resistance activity than native FGF21, such as native hFGF21. GH normally has growth and metabolic effects that are mediated by insulin-like growth factor 1 (IGF-1). The binding of GH to its receptor results in activation of Janus kinase 2 (JAK2), which then phosphorylates the STAT5 protein. Phosphorylated STAT5 is translocated to the nucleus and binds to gene regulatory response elements that promote IGF-1 expression.

[0090] GH's effects can be blunted in individuals by increased levels of FGF21, or by prolonged starvation or fasting, which also increases levels of FGF21. The effects of GH resistance in individuals include energy conservation, increased torpor, decreased body temperature, decreased physical activity, growth inhibition, loss of lean mass, and induction of ketone body synthesis. Native hFGF21 effects GH resistance by, for example, reducing the level of IGF-1 that is normally induced by GH. Without being bound by theory, this GH resistance activity of hFGF21 may be mediated by its ability to reduce the amount of phosphorylated STAT5 polypeptide and, as a result, reduce the translocation of the phosphorylated STAT5 to the nucleus and thus reduce the expression of IGF-1, thereby resisting the effects of GH.

[0091] In some embodiments, chimeric FGF19 polypeptides of the present invention do not reduce or do not substantially reduce the amount of phosphorylated STAT5 polypeptide. In this manner, chimeric FGF19 polypeptides of the present invention may demonstrate less or substantially no GH resistance activity, and thus may be advantageously more suitable for administration in a wide range of individuals.

[0092] In some embodiments, chimeric FGF19 polypeptides do not substantially promote anchorage-independent growth of cells. In some embodiments, chimeric FGF19 polypeptides may not substantially promote increased metabolic activity and/or the proliferation of cells in an environment requiring anchorage-independent growth. In some embodiments, chimeric FGF19 polypeptides may promote anchorage-independent growth of cells to an extent that is less than the corresponding anchorage-independent growth promotion of native FGF19. In some embodiments, chimeric FGF19 polypeptides may promote increased metabolic activity and/or the proliferation of cells in an environment requiring anchorage-independent growth to an extent that is less than the corresponding effect of native FGF19. As anchorage-independent growth is one of the defining characteristics of transformed cells, such chimerical FGF19 polypeptides of the present invention that do not promote anchorage-independent growth of cells, or do not substantially promote increased metabolic activity and/or the proliferation of cells in an environment requiring anchorage-independent growth, may be less able to promote or increase

differentially the growth and/or metabolic activity of transformed cells, and thus may be advantageously more suitable for administration in a wide range of individuals.

IV. Definitions

[0093] The terms "FGF19 polypeptide", "FGF19 protein" and "FGF19" when used herein encompass a polypeptide having an amino acid sequence that is the same as the native sequence of a member of the fibroblast growth factor 19 family. Members of such family include the 194-amino acid sequence of human FGF19 (hFGF19) as provided by SEQ ID NO:1 and in FIG. 1. An FGF19 polypeptide can be isolated from nature or can be produced by recombinant and/or synthetic means. An FGF19 polypeptide specifically encompasses naturally-occurring truncated or secreted forms, naturally-occurring variant forms (*e.g.*., alternatively spliced forms) and naturally-occurring allelic variants of the FGF19. An FGF19 polypeptide also specifically encompasses both unprocessed and processed forms of FGF19 such as, for example, the 216-amino acid sequence of the pre-human FGF19 polypeptide as provided by SEQ ID NO:3 and in FIG.1.

[0094] The terms "FGF21 polypeptide", "FGF21 protein" and "FGF21" when used herein encompass a polypeptide having an amino acid sequence that is the same as the native sequence of a member of the fibroblast growth factor 21 family. Members of such family include the 181-amino acid sequence of human FGF21 (hFGF21) as provided by SEQ ID NO:2 and in FIG. 2. An FGF21 polypeptide can be isolated from nature or can be produced by recombinant and/or synthetic means. An FGF21 polypeptide specifically encompasses naturally-occurring truncated or secreted forms, naturally-occurring variant forms (*e.g.*., alternatively spliced forms) and naturally-occurring allelic variants of the FGF21. An FGF21 polypeptide also specifically encompasses both unprocessed and processed forms of FGF21 such as, for example, the 209-amino acid sequence of the pre-human FGF21 polypeptide as provided by SEQ ID NO:4 and in FIG.2..

[0095] The terms "FGF polypeptide" and "FGF protein" when used herein encompass a polypeptide having the native sequence of a member of the FGF family, such as human FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, FGF7, FGF8, FGF9, FGF10, FGF11, FGF12, FGF13, FGF14, FGF16, FGF17, FGF18, FGF19, FGF20, FGF21, FGF22, FGF23, and mammalian homologues thereof. A native sequence of a given FGF polypeptide can be isolated from nature or can be produced by recombinant and/or synthetic means. The native sequence of a given FGF specifically encompasses naturally-occurring truncated or secreted forms, naturally-occurring variant forms (*e.g.*., alternatively spliced forms), naturally-occurring allelic variants thereof, and both unprocessed and processed forms of FGF.

[0096] The terms "chimeric polypeptide" and "chimeric protein" when used herein encompass a polypeptide having a sequence that includes at least a portion of a full-length sequence of first polypeptide sequence and at least a portion of a full-length sequence of a second polypeptide sequence, wherein the first and second polypeptides are different polypeptides. A chimeric polypeptide also encompasses polypeptides that include two or more non-contiguous portions derived from the same polypeptide. A chimeric polypeptide also encompasses polypeptides having at least one substitution, wherein the chimeric polypeptide includes a first polypeptide sequence in which a portion of the first polypeptide sequence has been substituted by a portion of a second polypeptide sequence.

[0097] The term "portion," when used herein with respect to a given polypeptide sequence, refers to a contiguous length of the given polypeptide's sequence that is shorter than the given polypeptide's full-length sequence. A portion of a given polypeptide may be defined by its first position and its final position, in which the first and final positions each correspond to a position in the sequence of the given polypeptide, wherein the sequence position corresponding to the first position is situated N-terminal to the sequence position corresponding to the final position, and whereby the sequence of the portion is the contiguous sequence of amino acids in the given polypeptide that begins at the sequence position corresponding to the first position and ending at the sequence position corresponding to the final position. A portion may also be defined by reference to a position in the given polypeptide sequence and a length of residues relative to the referenced position, whereby the sequence of the portion is a contiguous sequence of amino acids in the given polypeptide that has the defined length and that is located in the given polypeptide in reference to the defined position.

[0098] The term "N-terminal portion" of a given polypeptide sequence is a contiguous length of the given polypeptide sequence that begins at or near the N-terminal residue of the given polypeptide sequence. An N-terminal portion of the given polypeptide can be defined by a length. Similarly, the term "C-terminal portion" of a given polypeptide sequence is a contiguous length of the given polypeptide sequence that ends at or near the C-terminal residue of the given polypeptide sequence. An C-terminal portion of the given polypeptide can be defined by a length.

[0099] The terms "chimeric FGF polypeptide" and "chimeric FGF protein" when used herein encompass a polypeptide having a sequence that includes at least a portion of a first FGF polypeptide sequence and a portion of a second FGF polypeptide sequence, wherein the first and the second FGF polypeptides are different from each other. A chimeric FGF polypeptide also encompasses polypeptides that include two or more non-contiguous portions derived from the same FGF polypeptide. A chimeric FGF polypeptide also encompasses polypeptides having at least one substitution, wherein the chimeric FGF polypeptide includes a first FGF polypeptide

sequence in which a portion of the first FGF polypeptide sequence has been substituted by a portion of a second FGF polypeptide sequence.

[0100] The terms "chimeric FGF19 polypeptide" and "chimeric FGF19 protein" when used herein encompass a chimeric FGF polypeptide having a sequence that includes at least a portion of a FGF19 polypeptide sequence and a portion of a second polypeptide sequence. For example, a chimeric FGF19 polypeptide encompasses polypeptides in which the second polypeptide sequence is a FGF21 polypeptide sequence.

[0101] A chimeric FGF19 polypeptide also encompasses polypeptides that include two or more non-contiguous portions derived from a FGF19 polypeptide sequence. A chimeric FGF19 polypeptide also encompasses polypeptides having at least one substitution, wherein the chimeric FGF19 polypeptide sequence includes a FGF19 polypeptide sequence in which a portion of the FGF19 polypeptide sequence has been substituted by a portion of a second polypeptide sequence. In such cases, a chimeric FGF19 polypeptide expressly encompasses polypeptides in which the substituting portion is a portion of a FGF21 polypeptide sequence.

[0102] A chimeric FGF19 polypeptide also encompasses a polypeptide whose sequence consists only of portions derived from either a FGF19 polypeptide sequence or a second polypeptide sequence, such as a FGF21 polypeptide sequence. Unless otherwise stated, a chimeric FGF19 polypeptide is not limited to, nor does it imply unless otherwise indicated, the respective order or locations of the FGF19 polypeptide sequence with respect to any other sequence portions within the chimeric FGF19 polypeptide sequence.

[0103] "Percent (%) amino acid sequence identity" with respect to a given polypeptide sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, ALIGN-2 or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared.

[0104] For purposes herein, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows: 100 times the fraction X/Y where X is the number of amino acid residues scored as identical matches by a given

sequence alignment program in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A.

[0105] "Percent (%) nucleic acid sequence identity" with respect to a polypeptide-encoding nucleic acid sequences identified herein is defined as the percentage of nucleotides in a candidate sequence that are identical with the nucleotides in the polypeptide-encoding nucleic acid sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, ALIGN-2 or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared.

[0106] For purposes-herein, the % nucleic acid sequence identity of a given nucleic acid sequence C to, with, or against a given nucleic acid sequence D (which can alternatively be phrased as a given nucleic acid sequence C that has or comprises a certain % nucleic acid sequence identity to, with, or against a given nucleic acid sequence D) is calculated as follows: 100 times the fraction W/Z where W is the number of nucleotides scored as identical matches by a given sequence alignment program in that program's alignment of C and D, and where Z is the total number of nucleotides in D. It will be appreciated that where the length of nucleic acid sequence C is not equal to the length of nucleic acid sequence D, the % nucleic acid sequence identity of C to D will not equal the % nucleic acid sequence identity of D to C.

[0107] "Isolated," when used to describe the various polypeptides disclosed herein, means polypeptide that has been identified and separated and/or recovered from a component of its natural environment. Preferably, the isolated polypeptide is free of association with all components with which it is naturally associated. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be purified (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (2) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Isolated polypeptide includes polypeptide *in situ* within recombinant cells, since at least one component of the polypeptide's

natural environment will not be present. Ordinarily, however, isolated polypeptide will be prepared by at least one purification step.

[0108] An "isolated" nucleic acid molecule encoding a polypeptide is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the polypeptide-encoding nucleic acid. Preferably, the isolated nucleic is free of association with all components with which it is naturally associated. An isolated polypeptide-encoding nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from the polypeptide-encoding nucleic acid molecule as it exists in natural cells. However, an isolated nucleic acid molecule encoding a polypeptide includes polypeptide-encoding nucleic acid molecules contained in cells that ordinarily express polypeptide where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

[0109] The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

[0110] Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

[0111] The term "antibody" is used in the broadest sense and specifically covers, for example, single monoclonal antibodies (including agonist, antagonist, and neutralizing antibodies), antibody compositions with polyepitopic specificity, single chain antibodies, and fragments of antibodies (see below). The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts.

[0112] With regard to the binding of an polypeptide, antibody, oligopeptide or other organic molecule to a target molecule or cognate receptor, the term "specific binding" or "specifically binds to" or is "specific for" a particular polypeptide or an epitope on a particular polypeptide target or cognate receptor means binding that is measurably different from a non-specific interaction. Specific binding can be measured, for example, by determining binding of a molecule compared to binding of a control molecule, which generally is a molecule of similar structure that does not have binding activity. For example, specific binding can be determined by competition with a control molecule that is similar to the target, for example, an excess of non-labeled target. In this case, specific binding is indicated if the binding of the labeled target to a probe is competitively inhibited by excess unlabeled target. The term "specific binding" or "specifically binds to" or is "specific for" a particular polypeptide or an epitope on a particular polypeptide target or cognate receptor as used herein can be exhibited, for example, by a molecule having a Kd for the target of at least about 10^{-4} M, alternatively at least about 10^{-5} M, alternatively at least about 10^{-6} M, alternatively at least about 10^{-7} M, alternatively at least about 10^{-8} M, alternatively at least about 10^{-9} M, alternatively at least about 10^{-10} M, alternatively at least about 10^{-11} M, alternatively at least about 10^{-12} M, or greater. In one embodiment, the term "specific binding" refers to binding where a molecule binds to a particular polypeptide or epitope on a particular polypeptide or cognate receptor without substantially binding to any other polypeptide or polypeptide epitope or receptor.

[0113] "Stringency" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer probes require higher temperatures for proper annealing, while shorter probes need lower temperatures. Hybridization generally depends on the ability of denatured DNA to reanneal when complementary strands are present in an environment below their melting temperature. The higher the degree of desired homology between the probe and hybridizable sequence, the higher the relative temperature which can be used. As a result, it follows that higher relative temperatures would tend to make the reaction conditions more stringent, while lower temperatures less so. For additional details and explanation of stringency of hybridization reactions, see Ausubel et al., *Current Protocols in Molecular Biology*, Wiley Interscience Publishers, (1995).

[0114] "Stringent conditions" or "high stringency conditions", as defined herein, may be identified by those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50° C.; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50

mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42° C.; or (3) employ 50% formamide, 5xSSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5x Denhardt's solution, sonicated salmon sperm DNA (50 μg/ml), 0.1% SDS, and 10% dextran sulfate at 42° C., with washes at 42° C. in 0.2xSSC (sodium chloride/sodium citrate) and 50% formamide at 55° C., followed by a high-stringency wash consisting of 0.1xSSC containing EDTA at 55° C.

[0115] "Moderately stringent conditions" may be identified as described by Sambrook et al., *Molecular Cloning: A Laboratory Manual,* New York: Cold Spring Harbor Press, 1989, and include the use of washing solution and hybridization conditions (*e.g.*., temperature, ionic strength and % SDS) less stringent that those described above. An example of moderately stringent conditions is overnight incubation at 37° C. in a solution comprising: 20% formamide, 5xSSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 mg/ml denatured sheared salmon sperm DNA, followed by washing the filters in 1xSSC at about 37-50° C. The skilled artisan will recognize how to adjust the temperature, ionic strength, etc. as necessary to accommodate factors such as probe length and the like.

[0116] The term "epitope tagged" when used herein refers to a polypeptide fused to a "tag polypeptide". The tag polypeptide has enough residues to provide an epitope against which an antibody can be made, yet is short enough such that it does not interfere with activity of the polypeptide to which it is fused. The tag polypeptide preferably also is fairly unique so that the antibody does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually between about 8 and 50 amino acid residues (preferably, between about 10 and 20 amino acid residues).

[0117] As used herein, the term "immunoadhesin" designates antibody-like molecules which combine the binding specificity of a heterologous protein (an "adhesin") with the effector functions of immunoglobulin constant domains. Structurally, the immunoadhesins comprise a fusion of an amino acid sequence with the desired binding specificity which is other than the antigen recognition and binding site of an antibody (*i.e.*, is "heterologous"), and an immunoglobulin constant domain sequence. The adhesin part of an immunoadhesin molecule typically is a contiguous amino acid sequence comprising at least the binding site of a receptor or a ligand. The immunoglobulin constant domain sequence in the immunoadhesin may be obtained from any immunoglobulin, such as IgG-1, IgG-2, IgG-3, or IgG-4 subtypes, IgA (including IgA-1 and IgA-2), IgE, IgD or IgM.

[0118] "Active" or "activity" for the purposes herein refers to chimeric FGF19 polypeptide which retains at least one biological and/or immunological activity of native or naturally-

occurring FGF19 polypeptide and/or FGF21 polypeptide, particularly native or naturally-occurring hFGF19 polypeptide and/or hFGF21 polypeptide. "Biological" activity refers to a biological function (either inhibitory, stimulatory or cooperative) caused by a native or naturally-occurring FGF19 polypeptide and/or FGF21 polypeptide other than the ability to induce the production of an antibody against an antigenic epitope possessed by a native or naturally-occurring FGF19 polypeptide and/or FGF21 polypeptide. "Biological" activity may also refer to a cellular or biochemical function of native or naturally-occurring FGF19 polypeptide and/or FGF21 polypeptide, such as the ability to bind to one or more of its respective cognate receptors. "Immunological" activity refers to the ability to induce the production of an antibody against an antigenic epitope possessed by a native or naturally-occurring FGF19 polypeptide and/or FGF21 polypeptide. A preferred biological activity includes any one or more of the following exemplary activities: increases metabolism (or metabolic rate) in an individual, decreases body weight of an individual, decreases adiposity in an individual, decreases glucose uptake into adipocytes, increases leptin release from adipocytes, decreases triglycerides in an individual, decreases free fatty acids in an individual, Klotho-beta-dependent binding to a cognate FGF receptor, and Klotho-beta-independent binding to a cognate FGF receptor. It is understood that some of the activities of FGF19 and/or FGF21 polypeptides are directly induced by the polypeptide and some are indirectly induced, however, each are the result of the presence of FGF19 and/or FGF21 polypeptide and would not otherwise have the result in the absence of the polypeptide.

[0119] The term "antagonist" is used in the broadest sense, and includes any molecule that partially or fully blocks, inhibits, or neutralizes a biological activity of a native or chimeric polypeptide disclosed herein. In a similar manner, the term "agonist" is used in the broadest sense and includes any molecule that mimics a biological activity of a native or chimeric polypeptide disclosed herein. Suitable agonist or antagonist molecules specifically include agonist or antagonist antibodies or antibody fragments, fragments or amino acid sequence variants of native polypeptides, peptides, small organic molecules, etc. Methods for identifying agonists or antagonists of a polypeptide may comprise contacting a polypeptide with a candidate agonist or antagonist molecule and measuring a detectable change in one or more biological activities normally associated with the polypeptide.

[0120] As used herein, "treatment" or "treating" is an approach for obtaining beneficial or desired results including clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, one or more of the following: decreasing one or more symptoms resulting from the disease, diminishing the extent of the disease, stabilizing the disease (*e.g.*., preventing or delaying the worsening of the disease), delay or slowing the

progression of the disease, ameliorating the disease state, decreasing the dose of one or more other medications required to treat the disease, and/or increasing the quality of life.

[0121] As used herein, "delaying" the progression means to defer, hinder, slow, retard, stabilize, and/or postpone development of the disease. This delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated.

[0122] In some embodiments, the methods of treatment described herein ameliorate (*e.g.*., reduce incidence of, reduce duration of, reduce or lessen severity of) of one or more symptoms of the disease.

[0123] A "symptom" is any morbid phenomenon or departure from the normal in structure, function, or sensation, experienced by the individual.

[0124] An "effective amount" of a polypeptide, antibody, agonist, or antagonist thereof as disclosed herein is an amount sufficient to carry out a specifically stated purpose. An "effective amount" may be determined empirically and in a routine manner, in relation to the stated purpose.

[0125] The term "therapeutically effective amount" refers to an amount of an antibody, polypeptide, or other drug effective to "treat" a disease or disorder in an individual or mammal. See the definition herein of "treating".

[0126] "Chronic" administration refers to administration of the agent(s) in a continuous mode as opposed to an acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time. "Intermittent" administration is treatment that is not consecutively done without interruption, but rather is cyclic in nature.

[0127] "Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, cats, cattle, horses, sheep, pigs, goats, rabbits, etc. Preferably, the mammal is human.

[0128] "Individual" is any mammal, preferably a human.

[0129] "Obesity" refers to a condition whereby a mammal has a Body Mass Index (BMI), which is calculated as weight (kg) per height^2 (meters), of at least 25.9. Conventionally, those persons with normal weight have a BMI of 19.9 to less than 25.9. The obesity herein may be due to any cause, whether genetic or environmental. Examples of disorders that may result in obesity or be the cause of obesity include overeating and bulimia, polycystic ovarian disease, craniopharyngioma, the Prader-Willi Syndrome, Frohlich's syndrome, Type II diabetes, GH-deficient individuals, normal variant short stature, Turner's syndrome, and other pathological conditions showing reduced metabolic activity or a decrease in resting energy expenditure as a percentage of total fat-free mass, *e.g.*., children with acute lymphoblastic leukemia.

[0130] "Conditions related to obesity" refer to conditions which are the result of or which are exasperated by obesity, such as, but not limited to dermatological disorders such as infections,

varicose veins, Acanthosis nigricans, and eczema, exercise intolerance, diabetes mellitus, insulin resistance, hypertension, hypercholesterolemia, cholelithiasis, osteoarthritis, orthopedic injury, thromboembolic disease, cancer, and coronary (or cardiovascular) heart disease, particular those cardiovascular conditions associated with high triglycerides and free fatty acids in an individual.

[0131] Administration "in combination with" or "in conjunction with" one or more further therapeutic agents includes simultaneous, concurrent, consecutive and sequential administration in any order.

[0132] "Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™.

[0133] Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. The Fab fragment consists of an entire L chain along with the variable region domain of the H chain (V_H), and the first constant domain of one heavy chain (C_{H1}). Each Fab fragment is monovalent with respect to antigen binding, *i.e.*, it has a single antigen-binding site. Pepsin treatment of an antibody yields a single large $F(ab')_2$ fragment which roughly corresponds to two disulfide linked Fab fragments having divalent antigen-binding activity and is still capable of cross-linking antigen. Fab' fragments differ from Fab fragments by having additional few residues at the carboxy terminus of the C_{H1} domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0134] "Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the VH-VL dimer.

Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0135] The "Fc" fragment comprises the carboxy-terminal portions of both H chains held together by disulfides. The effector functions of antibodies are determined by sequences in the Fc region, which region is also the part recognized by Fc receptors (FcR) found on certain types of cells.

[0136] The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab fragments differ from Fab' fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region.

[0137] The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains.

[0138] Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2.

[0139] "Single-chain Fv" or "sFv" antibody fragments comprise the VH and VL domains of antibody, wherein these domains are present in a single polypeptide chain. Preferably, the Fv polypeptide further comprises a polypeptide linker between the VH and VL domains which enables the sFv to form the desired structure for antigen binding. For a review of sFv, see Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

[0140] The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) in the same polypeptide chain (VH-VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP Patent Publication 0404097; PCT International Patent Publication WO 93/11161; and Hollinger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993).

[0141] An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the

antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

[0142] The word "label" when used herein refers to a detectable compound or composition which is conjugated directly or indirectly to the antibody so as to generate a "labeled" antibody. The label may be detectable by itself (*e.g.*. radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable.

[0143] By "solid phase" is meant a non-aqueous matrix to which the antibody of the present invention can adhere. Examples of solid phases encompassed herein include those formed partially or entirely of glass (*e.g.*., controlled pore glass), polysaccharides (*e.g.*., agarose), polyacrylamides, polystyrene, polyvinyl alcohol and silicones. In certain embodiments, depending on the context, the solid phase can comprise the well of an assay plate; in others it is a purification column (*e.g.*., an affinity chromatography column). This term also includes a discontinuous solid phase of discrete particles, such as those described in U.S. Pat. No. 4,275,149.

[0144] A "liposome" is a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug (such as a chimeric FGF19 polypeptide or antibody thereto) to a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

[0145] Reference to "about" a value or parameter herein includes (and describes) variations that are directed to that value or parameter per se. For example, description referring to "about X" includes description of "X".'

[0146] As used herein and in the appended claims, the singular forms "a," "or," and "the" include plural referents unless the context clearly dictates otherwise. It is understood that aspects and variations of the invention described herein include "consisting" and/or "consisting essentially of" aspects and variations.

V. Chimeric FGF19 Variants

[0147] In addition to the chimeric FGF19 polypeptides described herein, it is contemplated that chimeric FGF19 variant polypeptides (or "chimeric FGF19 variants") can be prepared. Chimeric FGF19 variants can be prepared by introducing appropriate nucleotide changes into a DNA encoding a chimeric or native FGF19 or FGF21 polypeptide, and/or by synthesis of the desired chimeric FGF19 variant. Those skilled in the art will appreciate that amino acid changes may alter post-translational processes of the chimeric FGF19 variant, such as changing the number or position of glycosylation sites or altering the membrane anchoring characteristics.

[0148] Variations in chimeric FGF19 polypeptides of the present invention or in various domains thereof can be made, for example, using any of the techniques and guidelines for conservative and non-conservative mutations set forth, for instance, in U.S. Pat. No. 5,364,934. Variations may be a substitution, deletion or insertion of one or more codons encoding the chimeric FGF19 polypeptide that results in a change in the amino acid sequence of the chimeric FGF19 polypeptide. The variations may be with respect to one or more codons encoding the chimeric FGF19 polypeptide that is derived from native FGF19 or FGF21 polypeptide. Optionally the variation is by substitution of at least one amino acid with any other amino acid in one or more of the domains of the chimeric FGF19 polypeptide. Guidance in determining which amino acid residue may be inserted, substituted or deleted without adversely affecting the desired activity may be found by comparing the sequence of the chimeric FGF19 polypeptide with that of homologous known protein molecules and minimizing the number of amino acid sequence changes made in regions of high homology. Amino acid substitutions can be the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with a serine, *i.e.*, conservative amino acid replacements. Insertions or deletions may optionally be in the range of about 1 to 5 amino acids. The variation allowed may be determined by systematically making insertions, deletions or substitutions of amino acids in the sequence and testing the resulting variants for activity exhibited by the full-length or mature native sequence.

[0149] Chimeric FGF19 polypeptide fragments ("or chimeric FGF19 fragments") are provided herein. Such fragments may be truncated at the N-terminus or C-terminus, or may lack internal residues, for example, when compared with a full length native protein. Certain fragments lack amino acid residues that are not essential for a desired biological activity of the chimeric FGF19 polypeptide.

[0150] Chimeric FGF19 fragments may be prepared by any of a number of conventional techniques. Desired peptide fragments may be chemically synthesized. An alternative approach involves generating chimeric FGF19 fragments by enzymatic digestion, *e.g.*., by treating the protein with an enzyme known to cleave proteins at sites defined by particular amino acid

residues, or by digesting the DNA with suitable restriction enzymes and isolating the desired fragment. Yet another suitable technique involves isolating and amplifying a DNA fragment encoding a desired polypeptide fragment, by polymerase chain reaction (PCR). Oligonucleotides that define the desired termini of the DNA fragment are employed at the 5' and 3' primers in the PCR. Preferably, chimeric FGF19 polypeptide fragments share at least one biological and/or immunological activity with a native FGF19 polypeptide, such as hFGF19 polypeptide shown in FIG. 1 (SEQ ID NO:1) or the native FGF21 polypeptide, such as hFGF21 shown in FIG. 2 (SEQ ID NO:2).

[0151] In particular embodiments, conservative substitutions of interest are shown in Table 10 under the heading of preferred substitutions. If such substitutions result in a change in biological activity, then more substantial changes, denominated exemplary substitutions in Table 10, or as further described below in reference to amino acid classes, are introduced and the products screened.

Table 10: Preferred Amino Acid Residue Substitutions

Original Residue	**Exemplary Substitutions**	**Preferred Substitutions**
Ala (A)	val; leu; ile	val
Arg (R)	lys; gln; asn	lys
Asn (N)	gln; his; lys; arg	gln
Asp (D)	glu	glu
Cys (C)	ser	ser
Gln (Q)	asn	asn
Glu (E)	asp	asp
Gly (G)	pro; ala	ala
His (H)	asn; gln; lys; arg	arg
Ile (I)	leu; val; met; ala; phe; norleucine	leu
Leu (L)	norleucine; ile; val; met; ala; phe	ile
Lys (K)	arg; gln; asn	arg
Met (M)	leu; phe; ile	leu
Phe (F)	leu; val; ile; ala; tyr	leu
Pro (P)	ala	ala
Ser (S)	thr	thr
Thr (T)	ser	ser
Trp (W)	tyr; phe	tyr

Tyr (Y)	trp; phe; thr; ser	phe
Val (V)	ile; leu; met; phe; ala; norleucine	leu

[0152] Substantial modifications in function or immunological identity of the chimeric FGF19 polypeptide are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

(1) hydrophobic: norleucine, met, ala, val, leu, ile;

(2) neutral hydrophilic: cys, ser, thr;

(3) acidic: asp, glu;

(4) basic: asn, gln, his, lys, arg;

(5) residues that influence chain orientation: gly, pro; and

(6) aromatic: trp, tyr, phe.

[0153] Non-conservative substitutions will entail exchanging a member of one of these classes for another class. Such substituted residues also may be introduced into the conservative substitution sites or, more preferably, into the remaining (non-conserved) sites.

[0154] The variations can be made using methods known in the art such as oligonucleotide-mediated (site-directed) mutagenesis, alanine scanning, and PCR mutagenesis. Site-directed mutagenesis [Carter et al., Nucl. Acids Res., 13:4331 (1986); Zoller et al., Nucl. Acids Res., 10:6487 (1987)], cassette mutagenesis [Wells et al., Gene, 34:315 (1985)], restriction selection mutagenesis [Wells et al., Philos. Trans. R. Soc. London SerA, 317:415 (1986)] or other known techniques can be performed on the cloned DNA to produce the DNA encoding the chimeric FGF19 variant polypeptide.

[0155] Scanning amino acid analysis can also be employed to identify one or more amino acids along a contiguous sequence. Among the preferred scanning amino acids are relatively small, neutral amino acids. Such amino acids include alanine, glycine, serine, and cysteine. Alanine is typically a preferred scanning amino acid among this group because it eliminates the side-chain beyond the beta-carbon and is less likely to alter the main-chain conformation of the variant [Cunningham and Wells, Science, 244: 1081-1085 (1989)]. Alanine is also typically preferred because it is the most common amino acid. Further, it is frequently found in both buried and exposed positions [Creighton, The Proteins, (W.H. Freeman & Co., N.Y.); Chothia, J. Mol. Biol., 150:1 (1976)]. If alanine substitution does not yield adequate amounts of variant, an isoteric amino acid can be used.

VI. Modifications of Chimeric FGF19

[0156] Covalent modifications of chimeric FGF19 polypeptide are included within the scope of this invention. One type of covalent modification includes reacting targeted amino acid residues of a chimeric FGF19 polypeptide with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues of the chimeric FGF19 polypeptide. Derivatization with bifunctional agents is useful, for instance, for cross-linking chimeric FGF19 polypeptide to a water-insoluble support matrix or surface for use in the method for purifying antibodies, and vice-versa. Commonly used cross-linking agents include, *e.g.*., 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis(succinimidylpropionate), bifunctional maleimides such as bis-N-maleimido-1,8-octane and agents such as methyl-3-[(p-azidophenyl)dithio]propioimidate.

[0157] Other modifications include deamidation of glutaminyl and asparaginyl residues to the corresponding glutamyl and aspartyl residues, respectively, hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the α-amino groups of lysine, arginine, and histidine side chains [T. E. Creighton, Proteins: Structure and Molecular Properties, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)], acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

[0158] Another type of covalent modification of the chimeric FGF19 polypeptide included within the scope of this invention comprises altering the glycosylation pattern of the polypeptide. "Altering the native glycosylation pattern" is intended for purposes herein to mean deleting one or more carbohydrate moieties found in the corresponding native FGF19 polypeptide and/or FGF21 polypeptide sequence (either by removing the underlying glycosylation site or by deleting the glycosylation by chemical and/or enzymatic means), and/or adding one or more glycosylation sites that are not present in the native FGF19 polypeptide and/or FGF21 polypeptide sequence. In addition, the phrase includes qualitative changes in the glycosylation of the native proteins, involving a change in the nature and proportions of the various carbohydrate moieties present.

[0159] Addition of glycosylation sites to the chimeric FGF19 polypeptide may be accomplished by altering the amino acid sequence. The alteration may be made, for example, by the addition of, or substitution by, one or more serine or threonine residues to the chimeric FGF19 polypeptide (for O-linked glycosylation sites). The chimeric FGF19 polypeptide amino acid sequence may optionally be altered through changes at the DNA level, particularly by mutating the DNA encoding the chimeric FGF19 polypeptide at preselected bases such that codons are generated that will translate into the desired amino acids.

[0160] Another means of increasing the number of carbohydrate moieties on the chimeric FGF19 polypeptide is by chemical or enzymatic coupling of glycosides to the polypeptide. Such methods are described in the art, *e.g.*., in WO 87/05330 published 11 Sep. 1987, and in Aplin and Wriston, CRC Crit. Rev. Biochem., pp. 259-306 (1981).

[0161] Removal of carbohydrate moieties present on the chimeric FGF19 polypeptide may be accomplished chemically or enzymatically or by mutational substitution of codons encoding for amino acid residues that serve as targets for glycosylation. Chemical deglycosylation techniques are known in the art and described, for instance, by Hakimuddin, et al., Arch. Biochem. Biophys., 259:52 (1987) and by Edge et al., Anal. Biochem., 118131 (1981). Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura et al., Meth. Enzymol., 138:350 (1987).

[0162] Another type of covalent modification of chimeric FGF19 polypeptide comprises linking the chimeric FGF19 polypeptide to one of a variety of nonproteinaceous polymers, *e.g.*., polyethylene glycol (PEG), polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Pat. No. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

[0163] The chimeric FGF19 polypeptide of the present invention may also be modified by fusing the chimeric FGF19 polypeptide fused to another, heterologous polypeptide or amino acid sequence.

[0164] In one embodiment, such a chimeric molecule comprises a fusion of the chimeric FGF19 polypeptide with a tag polypeptide which provides an epitope to which an anti-tag antibody can selectively bind. The epitope tag is generally placed at the amino- or carboxyl-terminus of the chimeric FGF19 polypeptide. The presence of such epitope-tagged forms of the chimeric FGF19 polypeptide can be detected using an antibody against the tag polypeptide. Also, provision of the epitope tag enables the chimeric FGF19 polypeptide to be readily purified by affinity purification using an anti-tag antibody or another type of affinity matrix that binds to the epitope tag. Various tag polypeptides and their respective antibodies are well known in the art. Examples include poly-histidine (poly-his) or poly-histidine-glycine (poly-his-gly) tags; the flu HA tag polypeptide and its antibody 12CA5 [Field et al., Mol. Cell. Biol., 8:2159-2165 (1988)]; the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto [Evan et al., Molecular and Cellular Biology, 5:3610-3616 (1985)]; and the Herpes Simplex virus glycoprotein D (gD) tag and its anti body [Paborsky et al., Protein Engineering, 3(6):547-553 (1990)]. Other tag polypeptides include the Flag-peptide [Hopp et al., BioTechnology, 6: 1204-1210 (1988)]; the KT3 epitope peptide [Martin et al., Science, 255:192-194 (1992)]; an α-tubulin epitope peptide [Skinner et al., J. Biol. Chem., 266:15163-15166 (1991)]; and the T7 gene 10 protein peptide tag [Lutz-Freyermuth et al., Proc. Natl. Acad. Sci. USA, 87:6393-6397 (1990)].

[0165] In an alternative embodiment, a polypeptide of the present invention may comprise a fusion of a chimeric FGF19 polypeptide with an immunoglobulin or a particular region of an immunoglobulin. For a bivalent form of the chimeric molecule (also referred to as an "immunoadhesin"), such a fusion could be to the Fc portion of an immunoglobulin, an analog of the Fc portion of an immunoglobulin and one or more fragments of the Fc portion of an immunoglobulin. In some embodiments, the immunoglobulin is selected from the group consisting of: IgG-1, IgG-2, IgG-3, IgG-4, IgA-1, IgA-2, IgE, IgD and IgM. In some embodiments, the Fc portion is human or humanized.

[0166] In some embodiments, the C-terminus of the chimeric FGF19 polypeptide and the N-terminus of the Fc portion are fused. In some embodiments, the N-terminus of the chimeric FGF19 polypeptide and the C-terminus of the Fc portion are fused. In some embodiments, the immunoglobulin fusion includes the hinge, CH2 and CH3, or the hinge, CH1, CH2 and CH3 regions of an IgG1 molecule. For the production of immunoglobulin fusions see also U.S. Pat. No. 5,428,130 issued Jun. 27, 1995. In some embodiments, the C-terminus of the chimeric FGF19 polypeptide is fused to the N-terminus of the Fc portion via a linker, the linker is selected from the group consisting of: a $[Gly]_n$ linker, a $[Gly_3Ser]_m$ linker and a $[Gly_4Ser]_m$ linker, wherein n is an integer from 1-30 and m is an integer from 1-6.

VII. Uses and Methods Using Chimeric FGF19 Polypeptides

[0167] The chimeric FGF19 polypeptides and modulators thereof described herein may also be employed as therapeutic agents. The chimeric FGF19 polypeptides and modulators thereof of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the chimeric FGF19 polypeptides hereof is combined in admixture with a pharmaceutically acceptable carrier vehicle. Therapeutic formulations are prepared for storage by mixing the active ingredient having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone, amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, PLURONICS™ or PEG.

[0168] The formulations to be used for in vivo administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes, prior to or following lyophilization and reconstitution.

[0169] Therapeutic compositions herein generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

[0170] The route of administration is in accord with known methods, *e.g.*. injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial or intralesional routes, topical administration, or by sustained release systems.

[0171] Dosages and desired drug concentrations of pharmaceutical compositions of the present invention may vary depending on the particular use envisioned. The determination of the appropriate dosage or route of administration is well within the skill of an ordinary physician. Animal experiments provide reliable guidance for the determination of effective doses for human therapy. Interspecies scaling of effective doses can be performed following the principles laid down by Mordenti, J. and Chappell, W. "The use of interspecies scaling in toxicokinetics" In Toxicokinetics and New Drug Development, Yacobi et al., Eds., Pergamon Press, New York 1989, pp. 42-96.

[0172] When *in vivo* administration of a chimeric FGF19 polypeptide or agonist or antagonist thereof is employed, normal dosage amounts may vary from about 10 ng/kg to up to 100 mg/kg of mammal body weight or more per day, preferably about 1 μg/kg/day to 10 mg/kg/day, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature; see, for example, U.S. Pat. No. 4,657,760; 5,206,344; or 5,225,212. It is anticipated that different formulations will be effective for different treatment compounds and different disorders, that administration targeting one organ or tissue, for example, may necessitate delivery in a manner different from that to another organ or tissue.

[0173] Where sustained-release administration of a chimeric FGF19 polypeptide or modulator is desired in a formulation with release characteristics suitable for the treatment of any disease or disorder requiring administration of the chimeric FGF19 polypeptide or modulator, microencapsulation is contemplated. Microencapsulation of recombinant proteins for sustained release has been successfully performed with human growth hormone (rhGH), interferon-(rhIFN-), interleukin-2, and MN rgp120. Johnson et al., Nat. Med., 2:795-799 (1996); Yasuda, Biomed. Ther., 27:1221-1223 (1993); Hora et al., Bio/Technology 8:755-758 (1990); Cleland, "Design and Production of Single Immunization Vaccines Using Polylactide Polyglycolide Microsphere Systems," in Vaccine Design: The Subunit and Adjuvant Approach, Powell and

Newman, eds, (Plenum Press: New York, 1995), pp. 439-462; WO 97/03692, WO 96/40072, WO 96/07399; and U.S. Pat. No. 5,654,010.

[0174] The sustained-release formulations of these proteins were developed using poly-lactic-coglycolic acid (PLGA) polymer due to its biocompatibility and wide range of biodegradable properties. The degradation products of PLGA, lactic and glycolic acids, can be cleared quickly within the human body. Moreover, the degradability of this polymer can be adjusted from months to years depending on its molecular weight and composition. Lewis. "Controlled release of bioactive agents from lactide/glycolide polymer," in: M. Chasin and R. Langer (Eds.), Biodegradable Polymers as Drug Delivery Systems (Marcel Dekker: New York, 1990), pp. 1-41.

[0175] The therapeutic agents and compositions comprising chimeric FGF19 polypeptide provided herein can be used in a number of applications. The applications include treating an individual with obesity or a condition associated with obesity. In one aspect, chimeric FGF19 polypeptide is administered to an individual in need thereof in an amount effective to treat the condition. Preferably, the condition is one which requires at least one of the following to be treated: decrease in blood glucose, an increase in metabolism, a decrease in body weight, a decrease in body fat, a decrease in triglycerides, a decrease in free fatty acids, an increase in glucose release from adipocytes and/or an increase in leptin release from adipocytes. Each of these parameters can be measured by standard methods, for example, by measuring oxygen consumption to determine metabolic rate, using scales to determine weight, and measuring size to determine fat. Moreover, the presence and amount of triglycerides, free fatty acids, glucose and leptin can be determined by standard methods. The applications include treating an individual with one or more of type 1 diabetes, type 2 diabetes, high blood glucose, metabolic syndrome, atherosclerosis, hypercholesterolemia, stroke, osteoporosis, osteoarthritis, degenerative joint disease, muscle atrophy, sarcopenia, decreased lean body mass, baldness, wrinkles, increased fatigue, decreased stamina, decreased cardiac function, immune system dysfunction, cancer, Parkinson's disease, senile dementia, Alzheimer's disease and decreased cognitive function.

[0176] Chimeric FGF19 polypeptide and compositions comprising chimeric FGF19 polypeptide are preferably used in vivo. However, as discussed below, administration can be in vitro such as in the methods described below for screening for modulators of chimeric FGF19 polypeptide. Although, it is understood that modulators of chimeric FGF19 polypeptide can also be identified by the use of animal models and samples from individuals.

[0177] The present invention also includes aspects in which a chimeric FGF19 polypeptide of the present invention or a pharmaceutical composition thereof is administered to an individual in combination with a second agent, wherein the second agent is preferably a pharmacological agent. In some embodiments, the chimeric FGF19 polypeptide of the present invention or a

pharmaceutical composition thereof is administered in a therapeutically effect amount in combination with a therapeutically effective amount of the second agent. In some embodiments, the chimeric FGF19 polypeptide or pharmaceutical composition thereof is administered in conjunction with the second agent, *i.e.*, the respective periods of administration are part of a single administrative regimen. In some embodiments, the chimeric FGF19 polypeptide or pharmaceutical composition thereof and the second agent are administered concurrently, *i.e.*, the respective periods of administration overlap each other. In some embodiments, the chimeric FGF19 polypeptide or pharmaceutical composition thereof and the second agent are administered non-concurrently, *i.e.*, the respective periods of administration do not overlap each other. In some embodiments, the chimeric FGF19 polypeptide or pharmaceutical composition thereof and the second agent are administered sequentially, *i.e.*, the chimeric FGF19 polypeptide or pharmaceutical composition thereof is administered prior to and/or after the administration of the second agent. In some embodiments, the chimeric FGF19 polypeptide or pharmaceutical composition thereof and the second agent are administered simultaneously as separate compositions. In some embodiments, the chimeric FGF19 polypeptide or pharmaceutical composition thereof and the second agent are administered simultaneously as part of the same compositions.

[0178] In some embodiments, the second agent is different chimeric FGF19 polypeptide of the present invention. In some embodiments, the second agent is an anti-inflammatory agent, an anti-diabetic agent, and /or cholesterol-lowering drug of the "statin" class. In some embodiments, the second active agent is insulin. In some embodiments, the insulin is rapid acting, short acting, regular acting, intermediate acting, or long acting insulin. In some embodiments, the insulin is and/or comprises Humalog, Lispro, Novolog, Apidra, Humulin, Aspart, regular insulin, NPH, Lente, Ultralente, Lantus, Glargine, Levemir, or Detemir. In some embodiments, the second active agent is a statin. In some embodiments, the statin is and/or comprises Atorvastatin (*e.g.*., Lipitor or Torvast), Cerivastatin (*e.g.*., Lipobay or Baycol), Fluvastatin (*e.g.*., Lescol or Lescol), Lovastatin (*e.g.*., Mevacor, Altocor, or Altoprev) Mevastatin, Pitavastatin (*e.g.*., Livalo or Pitava), Pravastatin (*e.g.*., Pravachol, Selektine, or Lipostat) Rosuvastatin (*e.g.*., Crestor), Simvastatin (*e.g.*., Zocor or Lipex), Vytorin, Advicor, Besylate Caduet or Simcor.

[0179] In another aspect of the present invention, a chimeric FGF19 polypeptide of the present invention or a pharmaceutical composition thereof is administered to an individual in combination with a second therapy performed on the individual, wherein the second therapy comprises a surgery. In some embodiments, the chimeric FGF19 polypeptide of the present invention or a pharmaceutical composition thereof is administered in a therapeutically effect amount in combination with the second therapy. In some embodiments, the chimeric FGF19

polypeptide or pharmaceutical composition thereof is administered in conjunction with the second therapy, *i.e.*, the administration and the therapy are part of a single administrative regimen. In some embodiments, the chimeric FGF19 polypeptide or pharmaceutical composition thereof is administered concurrently with the second therapy, *i.e.*, the respective periods of administration and therapy overlap each other. In some embodiments, the chimeric FGF19 polypeptide or pharmaceutical composition thereof and the second agent is administered non-concurrently with the second therapy, *i.e.*, the respective periods of administration and therapy do not overlap each other. In some embodiments, the chimeric FGF19 polypeptide or pharmaceutical composition thereof and the second agent is administered sequentially with the second therapy, *i.e.*, the chimeric FGF19 polypeptide or pharmaceutical composition thereof is administered prior to and/or after the second therapy. In some embodiments, the chimeric FGF19 polypeptide or pharmaceutical composition thereof and the second agent is administered simultaneously with the second therapy.

[0180] The chimeric FGF19 polypeptides described herein may also be employed as molecular weight markers for protein electrophoresis purposes.

[0181] The chimeric FGF19 polypeptides and nucleic acid molecules of the present invention may also be used for tissue typing, wherein the chimeric FGF19 polypeptides of the present invention may be differentially expressed in one tissue as compared to another. Chimeric FGF19 polypeptide nucleic acid molecules will find use for generating probes for PCR, Northern analysis, Southern analysis and Western analysis.

[0182] Chimeric FGF19 polypeptides of the present invention which bind to another protein (example, one of the FGFRs), the chimeric FGF19 polypeptide can be used in assays to identify the other proteins or molecules involved in the binding interaction. By such methods, inhibitors of the receptor/ligand binding interaction can be identified. Proteins involved in such binding interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction. Also, the chimeric FGF19 polypeptide can be used to isolate correlative ligand(s). Screening assays can be designed to find lead compounds that mimic the biological activity of a native FGF19, native FGF21, chimeric FGF19 polypeptide, or a receptor for FGF19 and/or FGF21. Such screening assays will include assays amenable to high-throughput screening of chemical libraries, making them particularly suitable for identifying small molecule drug candidates. Small molecules contemplated include synthetic organic or inorganic compounds. The assays can be performed in a variety of formats, including protein-protein binding assays, biochemical screening assays, immunoassays and cell based assays, which are well characterized in the art.

[0183] As an alternative approach for receptor identification, labeled chimeric FGF19 polypeptide can be photoaffinity-linked with cell membrane or extract preparations that express the receptor molecule. Cross-linked material is resolved by PAGE and exposed to X-ray film. The labeled complex containing the receptor can be excised, resolved into peptide fragments, and subjected to protein micro-sequencing. The amino acid sequence obtained from micro-sequencing would be used to design a set of degenerate oligonucleotide probes to screen a cDNA library to identify the gene encoding the putative receptor.

[0184] In one embodiment herein where competitive binding assays are performed, FGFR1c, FGFR2c, FGFR3c and/or FGFR4 or an antibody to chimeric FGF19 polypeptide is used as a competitor.

VIII. Antibodies to Chimeric FGF19 Polypeptide

1. Polyclonal Antibodies

[0185] The anti- chimeric FGF19 polypeptide antibodies may comprise polyclonal antibodies. Methods of preparing polyclonal antibodies are known to the skilled artisan. Polyclonal antibodies can be raised in a mammal, for example, by one or more injections of an immunizing agent and, if desired, an adjuvant. Typically, the immunizing agent and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections. The immunizing agent may include the chimeric FGF19 polypeptide or a fusion protein thereof. It may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. Examples of adjuvants which may be employed include Freund's complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate). The immunization protocol may be selected by one skilled in the art without undue experimentation.

2. Monoclonal Antibodies

[0186] The anti- chimeric FGF19 polypeptide antibodies may, alternatively, be monoclonal antibodies. Monoclonal antibodies may be prepared using hybridoma methods, such as those described by Kohler and Milstein, Nature, 256:495 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal, is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized in vitro. In preferred embodiments, the anti-chimeric FGF19 polypeptide antibody specifically bind the polypeptide of the present invention. In more preferred embodiments, the specifically-binding antibody does not bind native FGF19 polypeptide or native FGF21 polypeptide.

[0187] The immunizing agent will typically include the chimeric FGF19 polypeptide or a fusion protein thereof. Generally, either peripheral blood lymphocytes ("PBLs") are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell [Goding, Monoclonal Antibodies: Principles and Practice, Academic Press, (1986) pp. 59-103]. Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells may be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

[0188] Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, Calif. and the American Type Culture Collection, Manassas, Va. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies [Kozbor, J. Immunol., 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, Marcel Dekker, Inc., New York, (1987) pp. 51-63].

[0189] The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against chimeric FGF19 polypeptide. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson and Pollard, Anal. Biochem., 107:220 (1980).

[0190] After the desired hybridoma cells are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods [Goding, supra]. Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells may be grown in vivo as ascites in a mammal.

[0191] The monoclonal antibodies secreted by the subclones may be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such

as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

[0192] The monoclonal antibodies may also be made by recombinant DNA methods, such as those described in U.S. Pat. No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (*e.g.*., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences [U.S. Pat. No. 4,816,567; Morrison et al., supra] or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

[0193] The antibodies may be monovalent antibodies. Methods for preparing monovalent antibodies are well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain crosslinking. Alternatively, the relevant cysteine residues are substituted with another amino acid residue or are deleted so as to prevent crosslinking.

[0194] In vitro methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly, Fab fragments, can be accomplished using routine techniques known in the art.

3. Human and Humanized Antibodies

[0195] The anti- chimeric FGF19 polypeptide antibodies of the invention may further comprise humanized antibodies or human antibodies. Humanized forms of non-human (*e.g.*., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human

species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin [Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol., 2:593-596 (1992)].

[0196] Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers [Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-327 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988)], by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

[0197] Human antibodies can also be produced using various techniques known in the art, including phage display libraries [Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol. 222:581 (1991)]. The techniques of Cole et al. and Boerner et al. are also available for the preparation of human monoclonal antibodies (Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985) and Boerner et al., J. Immunol., 147(1):86-95 (1991)]. Similarly, human antibodies can be made by introducing of human immunoglobulin loci into transgenic animals, *e.g.*., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Pat. Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks et al., Bio/Technology 10, 779-783 (1992);

Lonberg et al., Nature 368 856-859 (1994); Morrison, Nature 368, 812-13 (1994); Fishwild et al., Nature Biotechnology 14, 845-51 (1996); Neuberger, Nature Biotechnology 14, 826 (1996); Lonberg and Huszar, Intern. Rev. Immunol. 13 65-93 (1995).

4. Bispecific Antibodies

[0198] Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for the FGF19, the other one is for any other antigen, and preferably for a cell-surface protein or receptor or receptor subunit.

[0199] Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities [Milstein and Cuello, Nature, 305:537-539 (1983)]. Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker et al., EMBO J., 10:3655-3659 (1991).

[0200] Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. For further details of generating bispecific antibodies see, for example, Suresh et al., Methods in Enzymology, 121:210 (1986).

[0201] According to another approach described in WO 96/27011, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the CH3 region of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (*e.g.*. tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (*e.g.*. alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

[0202] Bispecific antibodies can be prepared as full length antibodies or antibody fragments (*e.g.*. $F(ab')_2$ bispecific antibodies). Techniques for generating bispecific antibodies from antibody fragments have been described in the literature. For example, bispecific antibodies can be prepared can be prepared using chemical linkage. Brennan et al., Science 229:81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate $F(ab')_2$ fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

[0203] Fab' fragments may be directly recovered from E. coli and chemically coupled to form bispecific antibodies. Shalaby et al., J. Exp. Med. 175:217-225 (1992) describe the production of a fully humanized bispecific antibody $F(ab')_2$ molecule. Each Fab' fragment was separately secreted from E. coli and subjected to directed chemical coupling in vitro to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

[0204] Various technique for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny et al., J. Immunol. 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger et al., Proc. Natl. Acad. Sci. USA 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_L and V_H domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See, Gruber et al., J. Immunol. 152:5368 (1994). Antibodies with more than two valencies are

contemplated. For example, trispecific antibodies can be prepared. Tutt et al., J. Immunol. 147:60 (1991).

[0205] Exemplary bispecific antibodies may bind to two different epitopes on a given FGF19 polypeptide herein. Alternatively, an anti- chimeric FGF19 polypeptide polypeptide arm may be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (*e.g.*. CD2, CD3, CD28, or B7), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) so as to focus cellular defense mechanisms to the cell expressing the particular FGF19 polypeptide. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express a particular FGF19 polypeptide. These antibodies possess a FGF19-binding arm and an arm which binds a cytotoxic agent or a radionuclide chelator, such as EOTUBE, DPTA, DOTA, or TETA. Another bispecific antibody of interest binds the FGF19 polypeptide and further binds tissue factor (TF).

5. Heteroconjugate Antibodies

[0206] Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells [U.S. Pat. No. 4,676,980], and for treatment of HIV infection [WO 91/00360; WO 92/200373; EP 03089]. It is contemplated that the antibodies may be prepared in vitro using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Pat. No. 4,676,980.

6. Effector Function Engineering

[0207] It may be desirable to modify the antibody of the invention with respect to effector function, so as to enhance, *e.g.*., the effectiveness of the antibody. For example, cysteine residue(s) may be introduced into the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron et al., J. Exp Med., 176: 1191-1195 (1992) and Shopes, J. Immunol., 148: 2918-2922 (1992). In one example, homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff et al. Cancer Research, 53: 2560-2565 (1993). Alternatively, an antibody can be engineered that has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson et al., Anti-Cancer Drug Design, 3: 219-230 (1989).

7. Immunoconjugates

[0208] The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (*e.g.*., an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (*i.e.*, a radioconjugate).

[0209] Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Enzymatically active toxins and fragments thereof that can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria, officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. A variety of radionuclides are available for the production of radioconjugated antibodies. Examples include ^{212}Bi, ^{131}I, ^{131}In, ^{90}Y, and ^{186}Re.

[0210] Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutareldehyde), bis-azido compounds (such as bis(p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science, 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026.

[0211] In another embodiment, the antibody may be conjugated to a "receptor" (such streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the individual, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (*e.g.*., avidin) that is conjugated to a cytotoxic agent (*e.g.*., a radionucleotide).

8. Immunoliposomes

[0212] The antibodies disclosed herein may also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein et al., *Proc. Natl. Acad. Sci. USA*, 82: 3688 (1985); Hwang et al., *Proc. Natl. Acad. Sci.*

USA, 77: 4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Pat. No. 5,013,556.

[0213] Particularly useful liposomes can be generated by the reverse-phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin et al., J. Biol. Chem., 257: 286-288 (1982) via a disulfide-interchange reaction.

9. Pharmaceutical Compositions of Antibodies

[0214] Antibodies specifically binding a FGF19 polypeptide identified herein, as well as other molecules identified by the screening assays disclosed hereinbefore, can be administered for the treatment of various disorders in the form of pharmaceutical compositions.

[0215] If the FGF19 polypeptide is intracellular and whole antibodies are used as inhibitors, internalizing antibodies are preferred. However, lipofections or liposomes can also be used to deliver the antibody, or an antibody fragment, into cells. Where antibody fragments are used, the smallest inhibitory fragment that specifically binds to the binding domain of the target protein is preferred. For example, based upon the variable-region sequences of an antibody, peptide molecules can be designed that retain the ability to bind the target protein sequence. Such peptides can be synthesized chemically and/or produced by recombinant DNA technology. See, *e.g.*., Marasco et al., Proc. Natl. Acad. Sci. USA, 90: 7889-7893 (1993). The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Alternatively, or in addition, the composition may comprise an agent that enhances its function, such as, for example, a cytotoxic agent, cytokine, chemotherapeutic agent, or growth-inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

[0216] The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles, and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences, supra.

[0217] The formulations to be used for in vivo administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

[0218] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.*., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37° C., resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S--S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

G. Uses for Antibodies

[0219] The anti- chimeric FGF19 polypeptide antibodies of the invention have various utilities. For example, anti-FGF19 antibodies may be used in diagnostic assays for chimeric FGF19 polypeptide, *e.g.*., detecting its expression in specific cells, tissues, or serum. Various diagnostic assay techniques known in the art may be used, such as competitive binding assays, direct or indirect sandwich assays and immunoprecipitation assays conducted in either heterogeneous or homogeneous phases [Zola, Monoclonal Antibodies: A Manual of Techniques, CRC Press, Inc. (1987) pp. 147-158]. The antibodies used in the diagnostic assays can be labeled with a detectable moiety. The detectable moiety should be capable of producing, either directly or indirectly, a detectable signal. For example, the detectable moiety may be a radioisotope, such as ^{3}H, ^{14}C, ^{32}P, ^{35}S, or ^{125}I, a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin, or an enzyme, such as alkaline phosphatase, beta-galactosidase or horseradish peroxidase. Any method known in the art for conjugating the antibody to the detectable moiety may be employed, including those methods described by Hunter et al., Nature, 144:945 (1962); David et al., Biochemistry, 13: 1014 (1974); Pain et al., J. Immunol. Meth., 40:219 (1981); and Nygren, J. Histochem. and Cytochem., 30:407 (1982).

[0220] Anti-chimeric FGF19 polypeptide antibodies also are useful for the affinity purification of chimeric FGF19 polypeptide from recombinant cell culture or natural sources. In this process, the antibodies against chimeric FGF19 polypeptide are immobilized on a suitable support, such a Sephadex resin or filter paper, using methods well known in the art. The immobilized antibody then is contacted with a sample containing the chimeric FGF19 polypeptide to be purified, and thereafter the support is washed with a suitable solvent that will remove substantially all the material in the sample except the chimeric FGF19 polypeptide, which is bound to the immobilized antibody. Finally, the support is washed with another suitable solvent that will release the chimeric FGF19 polypeptide from the antibody.

IX. Preparation of Chimeric FGF19 Polypeptide

[0221] The description below relates primarily to production of chimeric FGF19 polypeptide by culturing cells transformed or transfected with a vector containing nucleic acid encoding chimeric FGF19 polypeptide. It is, of course, contemplated that alternative methods, which are well known in the art, may be employed to prepare chimeric FGF19 polypeptide. For instance, the chimeric FGF19 polypeptide, or portions thereof, may be produced by direct peptide synthesis using solid-phase techniques [see, *e.g.*., Stewart et al., Solid-Phase Peptide Synthesis, W.H. Freeman Co., San Francisco, Calif. (1969); Merrifield, J. Am. Chem. Soc., 85:2149-2154 (1963)]. In vitro protein synthesis may be performed using manual techniques or by automation; Automated synthesis may be accomplished, for instance, using an Applied Biosystems Peptide Synthesizer (Foster City, Calif.) using manufacturer's instructions. Various portions of the chimeric FGF19 polypeptide may be chemically synthesized separately and combined using chemical or enzymatic methods to produce the full-length chimeric FGF19 polypeptide.

1. Isolation of DNA Encoding Chimeric FGF19 Polypeptide

[0222] cDNA fragments encoding chimeric FGF polypeptides of the present invention can be generated by using PCR methodology using cDNA encoding at least a portion of native FGF19 polypeptide and at least a portion of native FGF21 polypeptide as templates. For example, in one instance, a cDNA fragment encoding an N-terminal portion of FGF21 polypeptide and a cDNA fragment encoding a C-terminal portion of FGF19 polypeptide are separately amplified and purified by a standard procedure, such as by using PCR followed by agarose gel electrophoresis. Primer sequences are designed such that there is an 18 nucleotide overlap at the 3' end of the FGF21 cDNA fragment and 5' end of the FGF19 cDNA fragment. A second amplification using PCR is conducted using a mixture of the two cDNA fragments as templates, resulting in a cDNA that encodes a chimeric polypeptide of the FGF21 fragment and FGF19 fragment. The resulting cDNA fragment is digested with appropriate restriction enzymes, purified by agarose gel electrophoresis, and cloned into plasmid vector pRK5.sm (a pUC based plasmid vector

containing CMV promoter for mammalian expression) using standard procedures. The sequence of the resulting plasmid was confirmed by the Sanger DNA sequencing method.

[0223] DNA encoding chimeric FGF19 polypeptide may be obtained from a cDNA library prepared from tissue believed to possess the FGF19 and/or FGF21 mRNA and to express it at a detectable level. Accordingly, human FGF19 and/or FGF21 DNA can be conveniently obtained from a cDNA library prepared from human tissue, such as described in the Examples. The FGF19- and/or FGF21-encoding gene may also be obtained from a genomic library or by known synthetic procedures (*e.g.*., automated nucleic acid synthesis).

[0224] Libraries can be screened with probes (such as antibodies to the FGF19 and/or FGF21 or oligonucleotides of at least about 20-80 bases) designed to identify the gene of interest or the protein encoded by it. Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures, such as described in Sambrook et al., Molecular Cloning: A Laboratory Manual (New York: Cold Spring Harbor Laboratory Press, 1989). An alternative means to isolate the gene encoding FGF19 is to use PCR methodology [Sambrook et al., supra; Dieffenbach et al., PCR Primer: A Laboratory Manual (Cold Spring Harbor Laboratory Press, 1995)].

[0225] The Examples below describe techniques for screening a cDNA library. The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized. The oligonucleotide is preferably labeled such that it can be detected upon hybridization to DNA in the library being screened. Methods of labeling are well known in the art, and include the use of radiolabels like ^{32}P-labeled ATP, biotinylation or enzyme labeling. Hybridization conditions, including moderate stringency and high stringency, are provided in Sambrook et al., supra.

[0226] Sequences identified in such library screening methods can be compared and aligned to other known sequences deposited and available in public databases such as GenBank or other private sequence databases. Sequence identity (at either the amino acid or nucleotide level) within defined regions of the molecule or across the full-length sequence can be determined using methods known in the art and as described herein.

[0227] Nucleic acid having protein coding sequence may be obtained by screening selected cDNA or genomic libraries using the deduced amino acid sequence disclosed herein for the first time, and, if necessary, using conventional primer extension procedures as described in Sambrook et al., supra, to detect precursors and processing intermediates of mRNA that may not have been reverse-transcribed into cDNA.

2. Selection and Transformation of Host Cells

[0228] Host cells are transfected or transformed with expression or cloning vectors described herein for chimeric FGF19 polypeptide production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. The culture conditions, such as media, temperature, pH and the like, can be selected by the skilled artisan without undue experimentation. In general, principles, protocols, and practical techniques for maximizing the productivity of cell cultures can be found in Mammalian Cell Biotechnology: a Practical Approach, M. Butler, ed. (IRL Press, 1991) and Sambrook et al., supra.

[0229] Methods of eukaryotic cell transfection and prokaryotic cell transformation are known to the ordinarily skilled artisan, for example, $CaCl_2$, $CaPO_4$, liposome-mediated and electroporation. Depending on the host cell used, transformation is performed using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in Sambrook et al., supra, or electroporation is generally used for prokaryotes. Infection with Agrobacterium tumefaciens is used for transformation of certain plant cells, as described by Shaw et al., Gene, 23:315 (1983) and WO 89/05859 published 29 Jun. 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, Virology, 52:456-457 (1978) can be employed. General aspects of mammalian cell host system transfections have been described in U.S. Pat. No. 4,399,216. Transformations into yeast are typically carried out according to the method of Van Solingen et al., J. Bact., 130:946 (1977) and Hsiao et al., Proc. Natl. Acad. Sci. (USA), 76:3829 (1979). However, other methods for introducing DNA into cells, such as by nuclear microinjection, electroporation, bacterial protoplast fusion with intact cells, or polycations, *e.g.*., polybrene, polyornithine, may also be used. For various techniques for transforming mammalian cells, see Keown et al., Methods in Enzymology, 185:527-537 (1990) and Mansour et al., Nature, 336:348-352 (1988).

[0230] Suitable host cells for cloning or expressing the DNA in the vectors herein include prokaryote, yeast, or higher eukaryote cells. Suitable prokaryotes include but are not limited to eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as E. coli. Various E. coli strains are publicly available, such as E. coli K12 strain MM294 (ATCC 31,446); E. coli X11776 (ATCC 31,537); E. coli strain W3110 (ATCC 27,325) and K5772 (ATCC 53,635). Other suitable prokaryotic host cells include Enterobacteriaceae such as Escherichia, *e.g.*., E. coli, Enterobacter, Erwinia, Klebsiella, Proteus, Salmonella, *e.g.*., Salmonella typhimurium, Serratia, *e.g.*., Serratia marcescans, and Shigella, as well as Bacilli such as B. subtilis and B. licheniformis (*e.g.*., B. licheniformis 41P disclosed in DD 266,710 published 12 Apr. 1989), Pseudomonas such as P. aeruginosa, and Streptomyces. These examples are illustrative rather than limiting. Strain W3110 is one particularly preferred host or parent host

because it is a common host strain for recombinant DNA product fermentations. Preferably, the host cell secretes minimal amounts of proteolytic enzymes. For example, strain W3110 may be modified to effect a genetic mutation in the genes encoding proteins endogenous to the host, with examples of such hosts including E. coli W3110 strain 1A2, which has the complete genotype tonA; E. coli W3110 strain 9E4, which has the complete genotype tonA ptr3; E. coli W3110 strain 27C7 (ATCC 55,244), which has the complete genotype tonA ptr3 phoA E15 (argF-lac)169 degP ompT kan^r; E. coli W3110 strain 37D6, which has the complete genotype tonA ptr3 phoA E15 (argF-lac)169 degP ompT rbs7 ilvG kan^r; E. coli W3110 strain 40B4, which is strain 37D6 with a non-kanamycin resistant degP deletion mutation; and an E. coli strain having mutant periplasmic protease disclosed in U.S. Pat. No. 4,946,783 issued 7 Aug. 1990. Alternatively, in vitro methods of cloning, *e.g.*., PCR or other nucleic acid polymerase reactions, are suitable.

[0231] In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for FGF19-encoding vectors. Saccharomyces cerevisiae is a commonly used lower eukaryotic host microorganism. Others include Schizosaccharomyces pombe (Beach and Nurse, Nature, 290: 140 [1981]; EP 139,383 published 2 May 1985); Kluyveromyces hosts (U.S. Pat. No. 4,943,529; Fleer et al., Bio/Technology, 9:968-975 (1991)) such as, *e.g.*., K. lactis (MW98-8C, CBS683, CBS4574; Louvencourt et al., J. Bacteriol., 154(2):737-742 [1983]), K. fragilis (ATCC 12,424), K. bulgaricus (ATCC 16,045), K. wickeramii (ATCC 24,178), K. waltii (ATCC 56,500), K. drosophilarum (ATCC 36,906; Van den Berg et al., Bio/Technology, 8:135 (1990)), K. thermotolerans, and K. marxianus; yarrowia (EP 402,226); Pichia pastoris (EP 183,070; Sreekrishna et al., J. Basic Microbiol., 28:265-278 [1988]); Candida; Trichoderma reesia (EP 244,234); Neurospora crassa (Case et al., Proc. Natl. Acad. Sci. USA, 76:5259-5263 [1979]); Schwanniomyces such as Schwanniomyces occidentalis (EP 394,538 published 31 Oct. 1990); and filamentous fungi such as, *e.g.*., Neurospora, Penicillium, Tolypocladium (WO 91/00357 published 10 Jan. 1991), and Aspergillus hosts such as A. nidulans (Ballance et al., Biochem. Biophys. Res. Commun., 112:284-289 [1983]; Tilburn et al., Gene, 26:205-221 [1983]; Yelton et al., Proc. Natl. Acad. Sci. USA, 81: 1470-1474 [1984]) and A. niger (Kelly and Hynes, EMBO J., 4:475-479 [1985]). Methylotropic yeasts are suitable herein and include, but are not limited to, yeast capable of growth on methanol selected from the genera consisting of Hansenula, Candida, Kloeckera, Pichia, Saccharomyces, Torulopsis, and Rhodotorula. A list of specific species that are exemplary of this class of yeasts may be found in C. Anthony, The Biochemistry of Methylotrophs, 269 (1982).

[0232] Suitable host cells for the expression of glycosylated chimeric FGF19 polypeptide are derived from multicellular organisms. Examples of invertebrate cells include insect cells such as Drosophila S2 and Spodoptera Sf9, as well as plant cells. Examples of useful mammalian host

cell lines include Chinese hamster ovary (CHO) and COS cells. More specific examples include monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., J. Gen Virol., 36:59 (1977)); Chinese hamster ovary cells/-DHFR (CHO, Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, 77:4216 (1980)); mouse sertoli cells (TM4, Mather, Biol. Reprod., 23:243-251 (1980)); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); and mouse mammary tumor (MMT 060562, ATCC CCL51). The selection of the appropriate host cell is deemed to be within the skill in the art.

3. Selection and Use of a Replicable Vector

[0233] The nucleic acid (*e.g.*., cDNA or genomic DNA) encoding chimeric FGF19 polypeptide may be inserted into a replicable vector for cloning (amplification of the DNA) or for expression. Various vectors are publicly available. The vector may, for example, be in the form of a plasmid, cosmid, viral particle, or phage. The appropriate nucleic acid sequence may be inserted into the vector by a variety of procedures. In general, DNA is inserted into an appropriate restriction endonuclease site(s) using techniques known in the art. Vector components generally include, but are not limited to, one or more of a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence. Construction of suitable vectors containing one or more of these components employs standard ligation techniques which are known to the skilled artisan.

[0234] The chimeric FGF19 polypeptide may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which may be a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the chimeric FGF19 polypeptide-encoding DNA that is inserted into the vector. The signal sequence may be the original signal sequence of FGF19 or FGF21, such as hFGF19 or hFGF21. Thus, in such embodiments, a chimeric FGF19 polypeptide of the present invention may include at least an N-terminal portion of pre-hFGF19, such as at least residues 1-22 of SEQ ID NO:3. In such embodiments, a chimeric FGF19 polypeptide of the present invention may include at least an N-terminal portion of pre-hFGF1, such as at least residues 1-28 of SEQ ID NO:4.

[0235] The signal sequence may be a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the signal sequence may be, *e.g.*., the yeast invertase leader, alpha factor leader (including Saccharomyces and Kluyveromyces α-factor leaders, the latter described in U.S. Pat. No. 5,010,182), or acid phosphatase leader, the C. albicans glucoamylase leader (EP 362,179 published 4 Apr. 1990), or the signal described in WO 90/13646 published 15 Nov. 1990. In

mammalian cell expression, mammalian signal sequences may be used to direct secretion of the protein, such as signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders.

[0236] Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2μ plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells.

[0237] Expression and cloning vectors will typically contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, *e.g.*., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, *e.g.*., the gene encoding D-alanine racemase for Bacilli.

[0238] An example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the FGF19-encoding nucleic acid, such as DHFR or thymidine kinase. An appropriate host cell when wild-type DHFR is employed is the CHO cell line deficient in DHFR activity, prepared and propagated as described by Urlaub et al., Proc. Natl. Acad. Sci. USA, 77:4216 (1980). A suitable selection gene for use in yeast is the trp1 gene present in the yeast plasmid YRp7 [Stinchcomb et al., Nature, 282:39 (1979); Kingsman et al., Gene, 7:141 (1979); Tschemper et al., Gene, 10: 157 (1980)]. The trp1 gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1 [Jones, Genetics, 85:12 (1977)].

[0239] Expression and cloning vectors usually contain a promoter operably linked to the FGF19-encoding nucleic acid sequence to direct mRNA synthesis. Promoters recognized by a variety of potential host cells are well known. Promoters suitable for use with prokaryotic hosts include the β-lactamase and lactose promoter systems [Chang et al., Nature, 275:615 (1978); Goeddel et al., Nature, 281:544 (1979)], alkaline phosphatase, a tryptophan (trp) promoter system [Goeddel, Nucleic Acids Res., 8:4057 (1980); EP 36,776], and hybrid promoters such as the tac promoter [deBoer et al., Proc. Natl. Acad. Sci. USA, 80:21-25 (1983)]. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding FGF19.

[0240] Examples of suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase [Hitzeman et al., J. Biol. Chem., 255:2073 (1980)] or other glycolytic enzymes [Hess et al., J. Adv. Enzyme Reg., 7:149 (1968); Holland, Biochemistry, 17:4900 (1978)], such as enolase, glyceraldehyde-3-phosphate dehydrogenase,

hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

[0241] Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate-dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in EP 73,657.

[0242] Transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 Jul. 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40 (SV40), from heterologous mammalian promoters, *e.g.*., the actin promoter or an immunoglobulin promoter, and from heat-shock promoters, provided such promoters are compatible with the host cell systems.

[0243] Transcription of a DNA encoding the chimeric FGF19 polypeptide by higher eukaryotes may be increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp, that act on a promoter to increase its transcription. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. The enhancer may be spliced into the vector at a position 5' or 3' to the chimeric FGF19 polypeptide coding sequence, but is preferably located at a site 5' from the promoter.

[0244] Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding chimeric FGF19 polypeptide.

[0245] Still other methods, vectors, and host cells suitable for adaptation to the synthesis of chimeric FGF19 polypeptide in recombinant vertebrate cell culture are described in Gething et

al., Nature, 293:620-625 (1981); Mantei et al., Nature, 281:40-46 (1979); EP 117,060; and EP 117,058.

4. Detecting Gene Amplification/Expression

[0246] Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA [Thomas, Proc. Natl. Acad. Sci. USA, 77:5201-5205 (1980)], dot blotting (DNA analysis), or in situ hybridization, using an appropriately labeled probe, based on the sequences provided herein. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

[0247] Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of cells or tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal. Conveniently, the antibodies may be prepared against a chimeric FGF19 polypeptide or against a synthetic peptide based on the DNA sequences provided herein or against exogenous sequence fused to DNA encoding chimeric FGF19 polypeptide and encoding a specific antibody epitope.

5. Purification of Polypeptide

[0248] Forms of chimeric FGF19 polypeptide may be recovered from culture medium or from host cell lysates. If membrane-bound, it can be released from the membrane using a suitable detergent solution (*e.g.*. Triton-X 100) or by enzymatic cleavage. Cells employed in expression of chimeric FGF19 polypeptide can be disrupted by various physical or chemical means, such as freeze-thaw cycling, sonication, mechanical disruption, or cell lysing agents.

[0249] It may be desired to purify chimeric FGF19 polypeptide from recombinant cell proteins or polypeptides. The following procedures are exemplary of suitable purification procedures: by fractionation on an ion-exchange, column; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75; protein A Sepharose columns to remove contaminants such as IgG; and metal chelating columns to bind epitope-tagged forms of the chimeric FGF19 polypeptide. Various methods of protein purification may be employed and such methods are known in the art and described for example in Deutscher, Methods in Enzymology, 182 (1990); Scopes, Protein Purification: Principles and

Practice, Springer-Verlag, New York (1982). The purification step(s) selected will depend, for example, on the nature of the production process used and the particular chimeric FGF19 polypeptide produced.

X. Nucleic Acids Encoding Chimeric FGF19 Polypeptides and Their Uses

[0250] The present invention includes in another aspect nucleotide sequences (or their complement) that encode chimeric FGF19 polypeptides (or "chimeric FGF19 nucleic acids") of the present invention. Chimeric FGF19 nucleic acids of the present invention have various applications in the art of molecular biology, including uses as hybridization probes, in chromosome and gene mapping and in the generation of anti-sense RNA and DNA. Chimeric FGF19 nucleic acids will also be useful for the preparation of chimeric FGF19 polypeptides by the recombinant techniques described herein.

[0251] In some embodiments, chimeric FGF19 polypeptides may include one or more epitope tags. In some embodiments, an epitope tag is positioned at the N-terminus of the chimeric FGF19 polypeptide. In some embodiments, an epitope tag is positioned at the C-terminus of the chimeric FGF19 polypeptide. In some embodiments, an epitope tag is positioned at the N-terminus of the chimeric FGF19 polypeptide.

[0252] In some embodiments, chimeric FGF19 polypeptides may include one or more epitope tags. In some embodiments, an epitope tag is positioned at the N-terminus of the chimeric FGF19 polypeptide. In some embodiments, an epitope tag is positioned at the C-terminus of the chimeric FGF19 polypeptide. In some embodiments, an epitope tag is positioned at the N-terminus of the chimeric FGF19 polypeptide. In some embodiments, the epitope tag comprises the amino acid sequence DYKDDDDK (SEQ ID NO:279).

[0253] In an exemplary embodiment, a chimeric FGF19 nucleic acid of the present invention includes the sequence:

CACCCCATCCCTGACTCCAGTCCTCTCCTGCAATTCGGGGGCCAAGTCCGGCAGCGG
TACCTCTACACCTCCGGCCCCCACGGGCTCTCCAGCTGCTTCCTGCGCATCCGTGCCG
ACGGCGTCGTGGACTGCGCGCGGGGCCAGAGCGCGCACAGTTTGCTGGAGATCAAG
GCAGTCGCTCTGCGGACCGTGGCCATCAAGGGCGTGCACAGCGTGCGGTACCTCTGC
ATGGGCGCCGACGGCAAGATGCAGGGGCTGCTTCAGTACTCGGAGGAAGACTGTGC
TTTCGAGGAGGAGATCCGCCCAGATGGCTACAATGTGTACCGATCCGAGAAGCACCG
CCTCCCGGTCTCCCTGAGCAGTGCCAAACAGCGGCAGCTGTACAAGAACAGAGGCTT
TCTTCCACTCTCTCATTTCCTGCCCATGCTGCCCATGGTCCCAGAGGAGCCTGAGGAC
CTCAGGGGCCACTTGGAATCTGACATGTTCTCTTCGCCCCTGGAGACCGACAGCATG
GACCCATTTGGGCTTGTCACCGGACTGGAGGCCGTGAGGAGTCCCAGCTTTGAGAAG

(SEQ ID NO:7). This exemplary nucleic acid sequence encodes a polypeptide having an amino

acid sequence that corresponds to the chimeric FGF19 polypeptide cFGF21/19-2, as shown in Table 3.

[0254] In another exemplary embodiment, a chimeric FGF19 nucleic acid of the present invention includes the sequence:

ATGGACTCGGACGAGACCGGGTTCGAGCACTCAGGgCTGTGGGTTTCTGTGCTGGCT
GGTCTTCTGCTGGGAGCCTGCCAGGCACACCCCATCCCTGACTCCAGTCCTCTCCTGC
AATTCGGGGGCCAAGTCCGGCAGCGGTACCTCTACACCTCCGGCCCCCACGGGCTCT
CCAGCTGCTTCCTGCGCATCCGTGCCGACGGCGTCGTGGACTGCGCGCGGGGCCAGA
GCGCGCACAGTTTGCTGGAGATCAAGGCAGTCGCTCTGCGGACCGTGGCCATCAAGG
GCGTGCACAGCGTGCGGTACCTCTGCATGGGCGCCGACGGCAAGATGCAGGGGCTG
CTTCAGTACTCGGAGGAAGACTGTGCTTTCGAGGAGGAGATCCGCCCAGATGGCTAC
AATGTGTACCGATCCGAGAAGCACCGCCTCCCGGTCTCCCTGAGCAGTGCCAAACAG
CGGCAGCTGTACAAGAACAGAGGCTTTCTTCCACTCTCTCATTTCCTGCCCATGCTGC
CCATGGTCCCAGAGGAGCCTGAGGACCTCAGGGGCCACTTGGAATCTGACATGTTCT
CTTCGCCCCTGGAGACCGACAGCATGGACCCATTTGGGCTTGTCACCGGACTGGAGG
CCGTGAGGAGTCCCAGCTTTGAGAAGGACTACAAAGACGATGACGACAAGTGA (SEQ ID NO:281). This exemplary nucleic acid sequence encodes a polypeptide having an amino acid sequence that includes the sequence of the chimeric FGF19 polypeptide cFGF21/19-2, as shown in Table 3. The polypeptide also includes a C-terminal epitope tag DYKDDDK (SEQ ID NO:280) and the N-terminal native signal sequence of hFGF21 polypeptide (MDSDETGFEHSGLWVSVLAGLLLGACQA; SEQ ID NO:282).

[0255] In another exemplary embodiment, a chimeric FGF19 nucleic acid of the present invention includes the sequence:

ATGCGGAGCGGGTGTGTGGTGGTCCACGTATGGATCCTGGCCGGCCTCTGGCTGGCC
GTGGCCGGGCGCCCCCTCGCCTTCTCGGACGCGGGGCCCCACGTGCACTACGGCTGG
GGCGACCCCATCCGCCTGCGGCACCTGTACACAGATGATGCCCAGCAGACAGAAGC
CCACCTGGAGATCAGGGAGGATGGGACGGTGGGGGGCGCTGCTGACCAGAGCCCCG
AAAGTCTCCTGCAGCTGAAAGCCTTGAAGCCGGGAGTTATTCAAATCTTGGGAGTCA
AGACATCCAGGTTCCTGTGCCAGCGGCCAGATGGGGCCCTGTATGGATCGCTCCACT
TTGACCCTGAGGCCTGCAGCTTCCGGGAGCTGCTTCTTGAGGACGGATACAATGTTT
ACCAGTCCGAAGCCCACGGCCTCCCGCTGCACCTGCCAGGGAACAAGTCCCCACACC
GGGACCCTGCACCCCGAGGACCAGCTCGCTTCCTGCCACTACCAGGCCTGCCCCCCG
CACTCCCGGAGCCACCCGGAATCCTGGCCCCCCAGCCCCCCGATGTGGGCTCCTCGG
ACCCTCTGAGCATGGTGGGACCTTCCCAGGGCCGAAGCCCCAGCTACGCTTCCGACT
ACAAGGACGACGATGACAAGTGA (SEQ ID NO:283). This exemplary nucleic acid

sequence encodes a polypeptide having an amino acid sequence that includes the sequence of the chimeric FGF19 polypeptide cFGF19/21-2, as shown in Table 6. The polypeptide also includes a C-terminal epitope tag DYKDDDK (SEQ ID NO:280) and the N-terminal native signal sequence of hFGF19 polypeptide (MRSGCVVVHVWILAGLWLAVAG; SEQ ID NO:284).

[0256] In another exemplary embodiment, a chimeric FGF19 nucleic acid of the present invention includes the sequence:

ATGGAcTCGGACGAGACCGGGTTCGAGCACTCAGGGCTGTGGGTTTCTGTGCTGGCT
GGTCTTCTGCTGGGAGCCTGCCAGGCACACCCCATCCCTGACTCCAGTCCTCTCCTGC
AATTCGGGGGCCAAGTCCGGCAGCGGTACCTCTACACAGATGATGCCCAGCAGACA
GAAGCCCACCTGGAGATCAGGGAGGATGGGACGGTGGGGGGCGCTGCTGACCAGAG
CCCCGAAAGTCTCCTGCAGCTGAAAGCCTTGAAGCCGGGAGTTATTCAAATCTTGGG
AGTCAAGACATCCAGGTTCCTGTGCCAGCGGCCAGATGGGGCCCTGTATGGATCGCT
CCACTTTGACCCTGAGGCCTGCAGCTTCCGGGAGCTGCTTCTTGAGGACGGATACAA
TGTTTACCAGTCCGAAGCCCACGGCCTCCCGCTGCACCTGCCAGGGAACAAGTCCCC
ACACCGGGACCCTGCACCCCGAGGACCAGCTCGCTTCCTTCCACTCTCTCATTTCCTG
CCCATGCTGCCCATGGTCCCAGAGGAGCCTGAGGACCTCAGGGGCCACTTGGAATCT
GACATGTTCTCTTCGCCCCTGGAGACCGACAGCATGGACCCATTTGGGCTTGTCACC
GGACTGGAGGCCGTGAGGAGTCCCAGCTTTGAGAAGGACTACAAAGACGATGACGA
CAAGTGA (SEQ ID NO:285). This exemplary nucleic acid sequence encodes a polypeptide having an amino acid sequence that includes the sequence of the chimeric FGF19 polypeptide cFGF21/19-13, as shown in Table 5. The polypeptide also includes a C-terminal epitope tag DYKDDDK (SEQ ID NO:280) and the N-terminal native signal sequence of hFGF21 polypeptide (MDSDETGFEHSGLWVSVLAGLLLGACQA; SEQ ID NO:282).

[0257] The full-length native nucleic acid sequence of hFGF19 gene (SEQ ID NO:5), the full-length native nucleic acid sequence of FGF21 gene (SEQ ID NO:6), the full-length native nucleic acid sequence of a chimeric FGF19 polypeptide of the present invention, or portions thereof of any of the foregoing, may be used as hybridization probes for detecting or screening for nucleic acids that encode chimeric FGF19 polypeptides of the present invention. Optionally, the length of the probes will be about 20 to about 50 bases. By way of example, a screening method will comprise isolating the coding region of the FGF19 gene using the known DNA sequence to synthesize a selected probe of about 40 bases. Hybridization probes may be labeled by a variety of labels, including radionucleotides such as ^{32}P or ^{35}S, or enzymatic labels such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems. Labeled probes having a sequence complementary to that of the FGF19 gene of the present invention can be used to screen libraries of human cDNA, genomic DNA or mRNA to determine which members of such

libraries the probe hybridizes to. Hybridization techniques are described in further detail in the Examples below.

[0258] Any EST sequences disclosed in the present application may similarly be employed as probes, using the methods disclosed herein.

[0259] Other useful fragments of the chimeric FGF19 nucleic acids include antisense or sense oligonucleotides comprising a singe-stranded nucleic acid sequence (either RNA or DNA) capable of binding to target chimeric FGF19 mRNA (sense) or chimeric FGF19 DNA (antisense) sequences. Antisense or sense oligonucleotides, according to the present invention, comprise a fragment of the coding region of chimeric FGF19 DNA. Such a fragment generally comprises at least about 14 nucleotides, preferably from about 14 to 30 nucleotides. The ability to derive an antisense or a sense oligonucleotide, based upon a cDNA sequence encoding a given protein is described in, for example, Stein and Cohen (Cancer Res. 48:2659, 1988) and van der Krol et al. (BioTechniques 6:958, 1988).

[0260] Binding of antisense or sense oligonucleotides to target nucleic acid sequences results in the formation of duplexes that block transcription or translation of the target sequence by one of several means, including enhanced degradation of the duplexes, premature termination of transcription or translation, or by other means. The antisense oligonucleotides thus may be used to block expression of chimeric FGF19 polypeptides. Antisense or sense oligonucleotides further comprise oligonucleotides having modified sugar-phosphodiester backbones (or other sugar linkages, such as those described in WO 91/06629) and wherein such sugar linkages are resistant to endogenous nucleases. Such oligonucleotides with resistant sugar linkages are stable in vivo (*i.e.*, capable of resisting enzymatic degradation) but retain sequence specificity to be able to bind to target nucleotide sequences.

[0261] Other examples of sense or antisense oligonucleotides include those oligonucleotides which are covalently linked to organic moieties, such as those described in WO 90/10048, and other moieties that increases affinity of the oligonucleotide for a target nucleic acid sequence, such as poly-(L-lysine). Further still, intercalating agents, such as ellipticine, and alkylating agents or metal complexes may be attached to sense or antisense oligonucleotides to modify binding specificities of the antisense or sense oligonucleotide for the target nucleotide sequence.

[0262] Antisense or sense oligonucleotides may be introduced into a cell containing the target nucleic acid sequence by any gene transfer method, including, for example, $CaPO_4$-mediated DNA transfection; electroporation, or by using gene transfer vectors such as Epstein-Barr virus. In a preferred procedure, an antisense or sense oligonucleotide is inserted into a suitable retroviral vector. A cell containing the target nucleic acid sequence is contacted with the recombinant retroviral vector, either in vivo or ex vivo. Suitable retroviral vectors include, but are not limited

to, those derived from the murine retrovirus M-MuLV, N2 (a retrovirus derived from M-MuLV), or the double copy vectors designated DCT5A, DCT5B and DCT5C (see WO 90/13641).

[0263] Sense or antisense oligonucleotides also may be introduced into a cell containing the target nucleotide sequence by formation of a conjugate with a ligand binding molecule, as described in WO 91/04753. Suitable ligand binding molecules include, but are not limited to, cell surface receptors, growth factors, other cytokines, or other ligands that bind to cell surface receptors. Preferably, conjugation of the ligand binding molecule does not substantially interfere with the ability of the ligand binding molecule to bind to its corresponding molecule or receptor, or block entry of the sense or antisense oligonucleotide or its conjugated version into the cell.

[0264] Alternatively, a sense or an antisense oligonucleotide may be introduced into a cell containing the target nucleic acid sequence by formation of an oligonucleotide-lipid complex, as described in WO 90/10448. The sense or antisense oligonucleotide-lipid complex is preferably dissociated within the cell by an endogenous lipase.

[0265] The probes may also be employed in PCR techniques to generate a pool of sequences for identification of closely related chimeric FGF19 coding sequences.

[0266] Nucleic acid encoding the chimeric FGF19 polypeptide may also be used in gene therapy. In gene therapy applications, genes are introduced into cells in order to achieve in vivo synthesis of a therapeutically effective genetic product, for example for replacement of a defective gene. "Gene therapy" includes both conventional gene therapy where a lasting effect is achieved by a single treatment, and the administration of gene therapeutic agents, which involves the one time or repeated administration of a therapeutically effective DNA or mRNA. Antisense RNAs and DNAs can be used as therapeutic agents for blocking the expression of certain genes in vivo. It has already been shown that short antisense oligonucleotides can be imported into cells where they act as inhibitors, despite their low intracellular concentrations caused by their restricted uptake by the cell membrane. (Zamecnik et al., Proc. Natl. Acad. Sci. USA 83:4143-4146 [1986]). The oligonucleotides can be modified to enhance their uptake, *e.g.*. by substituting their negatively charged phosphodiester groups by uncharged groups.

[0267] There are a variety of techniques available for introducing nucleic acids into viable cells. The techniques vary depending upon whether the nucleic acid is transferred into cultured cells in vitro, or in vivo in the cells of the intended host. Techniques suitable for the transfer of nucleic acid into mammalian cells in vitro include the use of liposomes, electroporation, microinjection, cell fusion, DEAE-dextran, the calcium phosphate precipitation method, etc. The currently preferred in vivo gene transfer techniques include transfection with viral (typically retroviral) vectors and viral coat protein-liposome mediated transfection (Dzau et al., Trends in Biotechnology 11, 205-210 [1993]). In some situations it is desirable to provide the nucleic acid

source with an agent that targets the target cells, such as an antibody specific for a cell surface membrane protein or the target cell, a ligand for a receptor on the target cell, etc. Where liposomes are employed, proteins which bind to a cell surface membrane protein associated with endocytosis may be used for targeting and/or to facilitate uptake, *e.g.*. capsid proteins or fragments thereof tropic for a particular cell type, antibodies for proteins which undergo internalization in cycling, proteins that target intracellular localization and enhance intracellular half-life. The technique of receptor-mediated endocytosis is described, for example, by Wu et al., J. Biol. Chem. 262, 4429-4432 (1987); and Wagner et al., Proc. Natl. Acad. Sci. USA 87, 3410-3414 (1990). For review of gene marking and gene therapy protocols see Anderson et al., Science 256, 808-813 (1992).

X. Articles of Manufacture

[0268] In another embodiment of the invention, an article of manufacture containing materials useful for treating metabolic-related disorders, conditions or symptoms as described above is provided. Preferably, the article of manufacture comprises:(a) a container comprising a composition comprising a chimeric FGF19 polypeptide described herein and a pharmaceutically acceptable carrier or diluent within the container; and (b) a package insert with instructions for administering the composition to an individual suffering from or exhibiting the metabolic-related disorders, conditions or symptoms.

[0269] In some embodiments, the individual has a metabolic-related disorders, conditions or symptoms. In some embodiments, the individual is at risk for developing a metabolic-related disorder, condition or symptom. In some embodiments, the individual has one or more characteristics selected from the group consisting of (a) waist circumference of about 102 cm or more in men and about 88 cm or more in women, (b) fasting triglycerides of about 150 mg/dL or more, (c) a fasting glucose of about 95 mg/dL or higher, and (d) high levels of oxidized LDL. In some embodiments, the individual further has inflammation associated with diabetes. In some embodiments, the individual has a blood glucose level of about 95 mg/dL or higher after an overnight fast. In some embodiments, the individual has a blood glucose level of about 126 mg/dL or higher after an overnight fast. In some embodiments, the individual has a blood glucose level of about 140 mg/dL after a two-hour oral glucose tolerance test. In some embodiments, the individual has a blood glucose level of about 200 mg/dL after a two-hour oral glucose tolerance test. In some embodiments, the individual has pre-diabetes. In some embodiments, the individual has diabetes. In some embodiments, the diabetes is selected from the group consisting of type-I diabetes, type-II diabetes, and gestational diabetes. In some embodiments, the diabetes is type-II diabetes.

[0270] The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds or contains a composition that is effective for treating the multiple sclerosis and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is the chimeric FGF19 polypeptide. The label or package insert indicates that the composition is used for treating metabolic-related disorders, conditions or symptoms in an individual suffering therefrom with specific guidance regarding dosing amounts and intervals of antibody and any other drug being provided. The article of manufacture may further comprise a second container comprising a pharmaceutically acceptable diluent buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. The article of manufacture may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[0271] Optionally, the article of manufacture herein further comprises a container comprising a second agent other than the polypeptide for treatment and further comprising instructions on treating the mammal with such agent. In some embodiments, the second agent is an anti-inflammatory agent, an anti-diabetic agent, and /or cholesterol-lowering drug of the "statin" class. In some embodiments, the second active agent is insulin. In some embodiments, the insulin is rapid acting, short acting, regular acting, intermediate acting, or long acting insulin. In some embodiments, the insulin is and/or comprises Humalog, Lispro, Novolog, Apidra, Humulin, Aspart, regular insulin, NPH, Lente, Ultralente, Lantus, Glargine, Levemir, or Detemir. In some embodiments, the second active agent is a statin. In some embodiments, the statin is and/or comprises Atorvastatin (*e.g.*., Lipitor or Torvast), Cerivastatin (*e.g.*., Lipobay or Baycol), Fluvastatin (*e.g.*., Lescol or Lescol), Lovastatin (*e.g.*., Mevacor, Altocor, or Altoprev) Mevastatin, Pitavastatin (*e.g.*., Livalo or Pitava), Pravastatin (*e.g.*., Pravachol, Selektine, or Lipostat) Rosuvastatin (*e.g.*., Crestor), Simvastatin (*e.g.*., Zocor or Lipex), Vytorin, Advicor, Besylate Caduet or Simcor.

[0272] A "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, contraindications, other therapeutic products to be combined with the packaged product, and/or warnings concerning the use of such therapeutic products, etc.

[0273] The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

[0274]

EXAMPLES

[0275] Commercially available reagents referred to in the examples were used according to manufacturer's instructions unless otherwise indicated. The source of the cells identified in the following examples, and throughout the specification, by ATCC accession numbers is the American Type Culture Collection, Manassas, VA. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37 °C under 5% CO_2, unless otherwise noted.

Example 1: KLB-Independent FGFR Binding Activity of Chimeric and Native FGF Polypeptides

[0276] The *in vitro* FGF receptor-binding activity of a chimeric FGF polypeptide of the present invention was measured using an enzyme-linked immunosorbent assay (ELISA). Referring to Fig. 3A (top), a schematic diagram of the ELISA for measuring FGF receptor (FGFR) *in vitro* binding activity and its corresponding control are depicted.

[0277] Monoclonal antibodies specific for human IgG-Fc fragment (Jackson ImmunoResearch, West Grove, PA, USA) were immobilized in the wells of Maxisorp™ flat-bottom 96-well plates (Nunc, Thermo Fisher Scientific, Rochester, New York) by overnight incubation with 100 µl per well of 2µg/ml antibody solution. Each well was then incubated with either 1µg/ml of FGFR4-Fc (a recombinant polypeptide comprising a human FGFR4 extracellular domain fused to a human IgG1 Fc fragment; catalog no. 685-FR-050, R&D Systems, Inc., Minneapolis, Minnesota) or 1 µg/ml FGFR1c-Fc (a recombinant polypeptide comprising a human FGFR1c extracellular domain fused to a human IgG1 Fc fragment; catalog no. 658-FR-050, R&D Systems).

[0278] The surface-immobilized FGFR4-Fc or FGFR1c-Fc polypeptides were incubated for 1 hr with native human FGF19-Flag polypeptide with a C-terminal epitope tag (See FGF19-Flag in Table 10, SEQ ID NO:237) at concentrations of 1 µg/mL, 0.4 µg/mL, 0.16 µg/mL, 0.064 µg/mL, 0.0256 µg/mL, 0.004096 mg/mL or 0.0016384 µg/mL, each with 2 µg/mL heparin, to allow binding of the FGF19 polypeptide to the receptor domain. Similarly, the surface-immobilized FGFR4-Fc or FGFR1c-Fc polypeptides were incubated for 1 hr with a chimeric FGF19 polypeptide with a C-terminal epitope tag (See cFGF21/19-2/Flag in Table 10; SEQ ID NO:242) at concentrations of 1 µg/mL, 0.4 µg/mL, 0.16 µg/mL, 0.064 µg/mL, 0.0256 µg/mL, 0.004096 µg/mL or 0.0016384 mg/mL, each with 2 µg/mL heparin, to allow binding of the chimeric FGF19 polypeptide to the receptor domain. Following incubation, the amount of native or chimeric FGF19 polypeptide bound to the receptor domain at a given FGF19 polypeptide concentration was determined using biotinylated anti-human FGF19 polyclonal antibody (catalog no. BAF969, R&D Systems), streptavidin-horseradish peroxidase (HRP) (catalog no. RPN1231V, Amersham

Biosciences, Pittsburgh, Pennsylvania) and 3, 3', 5, 5'-tetramethylbenzidine substrate (catalog no. TMBE-1000, Moss, Inc., Pasadena, Maryland), and by measuring concentration of the HRP-dependent product by its absorption at 450 nm. Control ELISA experiments were performed to demonstrate that the native and chimeric FGF19 polypeptides are recognized by the anti-human FGF19 polyclonal antibody with an equivalent efficiency (data not shown).

[0279] Referring to Fig. 3A, the results of the *in vitro* FGFR binding assay to surface-immobilized FGFR4-Fc or FGFR1c-Fc polypeptides showed that native human FGF19 polypeptide bound to FGFR4-Fc in a concentration-dependent, Klotho-beta-independent manner, but did not appreciably bind to FGFR1c-Fc. Klotho-beta-independent binding of chimeric FGFR19 polypeptide (cFGF21/19-2/Flag; SEQ ID NO:242) to either FGFR4-Fc or FGFR1c-Fc was not detected.

[0280] Referring to Fig. 3B, a schematic diagram of an assay for FGFR activation is depicted. In this assay, transiently-transfected L6 cells express an FGF receptor, such as human FGFR1c or human FGFR4, on their cell surfaces. Effective binding of a ligand to the FGF receptor can result in activation of an endogenous MAP kinase pathway, which can result in phosphorylation of a chimeric transcriptional activator having an Elk-1 activation domain and a GAL4 DNA-binding domain. The phosphorylated transcriptional activator can activate expression of a reporter gene under control of a suitable upstream activation sequence (UAS), such as the yeast GAL4 UAS. The reporter gene may encode an enzyme such as a luciferase enzyme, particularly a firefly luciferase enzyme. The L6 cells may be further transfected with a constitutively-expressed *Renilla* luciferase, which can serve as a normalization control for the inducible firefly luciferase.

[0281] In this assay, rat L6 myoblasts in a 96-well plate were transiently-transfected with an expression vector encoding a human FGFR4 polypeptide, an expression vector encoding a GAL4-Elk-1 transcriptional activator (catalog no. 219005, pFA2-Elk1, Stratagene, La Jolla, California), an expression vector encoding a firefly luciferase reporter gene under control of the yeast GAL4 upstream activator (catalog no. 219050, pFR-luc, Stratagene). A vector for the constitutive expression of *Renilla* luciferase (catalog no. E2231, pRL-SV40, Promega, Madison, Wisconsin) was also transfected into the cells. Transfections were performed using FuGENE HD Transfection Reagent (catalog no. 04 709 705 001, Roche Applied Science, Indianapolis, Indiana) in accordance with the manufacturer's instructions.

[0282] The transfected L6 cells were cultured overnight in DMEM (prepared from Cellgro 50-013-PC, Mediatech, Inc., Manassas, Virginia) containing 10% FBS (catalog no. F2442, Sigma-Aldrich, St. Louis, Missouri). The cells were then washed and cultured for an additional 6 hours in an enriched serum-free medium derived from the F12/DME 50:50 blend containing 25 mg/L porcine heparin and a given concentration of the FGF19 polypeptide. The FGF19 polypeptides

that were assayed were native FGF19-Flag polypeptide (See FGF19-Flag in Table 10; SEQ ID NO:237), native FGF21-His polypeptide (See FGF21-His in Table 10; SEQ ID NO:238) and a chimeric FGF19-Flag polypeptide (See cFGF21/19-2/Flag in Table 10; SEQ ID NO:242). The cells were incubated with the polypeptide at concentrations of 10 µg/mL, 1666.7 ng/mL, 277.8 ng/mL, 46.3 ng/mL, 7.7 ng/mL, 1.3 ng/mL, 0.21 ng/mL, 0.036 ng/mL, 0.0060 ng/mL, 0.00099 ng/mL, 0.00017 ng/mL or 0.000028 ng/mL. The cells were then lysed with PLB reagent (catalog no. E1941, Promega) and luciferase activity in each well was determined using Dual-Glo Luciferase Assay System (catalog no. E2940, Promega) and EnVision Multilabel Reader (catalog no. 2103, PerkinElmer, Waltham, Massachusetts) in accordance with the respective manufacturers' instructions. Each firefly luciferase activity was normalized to the co-expressed *Renilla* luciferase activity, and each sample condition was performed in triplicate.

Table 10: Polypeptide Sequences

Name	Amino Acid Sequence (N-C)	SEQ ID NO
FGF19-Flag	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDC ARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSE EDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFL PMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGL VTGLEAVRSPSFEKDYKDDDDK	237
FGF21-HIis	HHHHHHHHHIPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGG AADQSPESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEA CSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLP GLPPALPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYAS	238
FGF21-FlagC	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQS PESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEACSFRE LLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPA LPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYASDYKDDDDK	239
FGF21-FlagN	KDYKDDDDKLEHPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIRE DGTVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGS LHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGP ARFLPLPGLPPALPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYAS	240
cFGF21/19-1/Flag	HPIPDSSPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARG QSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDC	241

Name	Amino Acid Sequence (N-C)	SEQ ID NO
	AFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLPML PMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEKD YKDDDDK	
cFGF21/19-2/Flag	HPIPDSSPLLQFGGQVRQRYLYTSGPHGLSSCFLRIRADGVVDCARGQS AHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAF EEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLPMLP MVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEKDY KDDDDK	242
cFGF21/19-3/Flag	HPIPDSSPLLQFGGQVRQRYLYTDDPHGLSSCFLRIRADGVVDCARGQ SAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCA FEEEIRPDGYNVYRSEKIIRLPVSLSSAKQRQLYKNRGFLPLSIIFLPMLP MVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEKDY KDDDDK	243
cFGF21/19-4/Flag	HPIPDSSPLLQFGGQVRQRYLYTDDAQLSSCFLRIRADGVVDCARGQS AHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAF EEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLPMLP MVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEKDY KDDDDK	244
cFGF21/19-5/Flag	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTSCFLRIRADGVVDCARGQS AHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAF EEEIRPDGYNVYRSEKIIRLPVSLSSAKQRQLYKNRGFLPLSIIFLPMLP MVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEKDY KDDDDK	245
cFGF21/19-6/Flag	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAFLRIRADGVVDCARGQS AHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAF EEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLPMLP MVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEKDY KDDDDK	246
cFGF21/19-7/Flag	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIRADGVVDCARGQS AHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAF EEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLPMLP	247

Name	Amino Acid Sequence (N-C)	SEQ ID NO
	MVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEKDY KDDDDK	
cFGF21/19-8/Flag	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGVVDCARGQS AHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAF EEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLPMLP MVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEKDY KDDDDK	248
cFGF21/19-9/Flag	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAAD QSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDC AFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLPML PMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEKD YKDDDDK	249
cFGF21/19-10/Flag	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQS PESLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAF EEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLPMLP MVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEKDY KDDDDK	250
cFGF21/19-11/Flag	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQS PESLLQLKALKPGVIQILGVHSVRYLCMGADGKMQGLLQYSEEDCAFE EEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLPMLPM VPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEKDYK DDDDK	251
cFGF21/19-12/Flag	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQS PESLLQLKALKPGVIQILGVKTSRFLCMGADGKMQGLLQYSEEDCAFE EEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLPMLPM VPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEKDYK DDDDK	252
cFGF21/19-13/Flag	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQS PESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEACSFRE LLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPMLPMVPE EPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEKDYKDDD	253

Name	Amino Acid Sequence (N-C)	SEQ ID NO
	DK	
cFGF19/21-1/Flag	RPLAFSDAGPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAA DQSPESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEACS FRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLP PALPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYASDYKDDDDK	254
cFGF19/21-2/Flag	RPLAFSDAGPHVHYGWGDPIRLRHLYTDDAQQTEAHLEIREDGTVGG AADQSPESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEA CSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLP GLPPALPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYASDYKDDDDK	255
cFGF19/21-3/Flag	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIREDGTVGG AADQSPESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEA CSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLP GLPPALPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYASDYKDDDDK	256
cFGF19/21-4/Flag	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGTVGG AADQSPESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEA CSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLP GLPPALPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYASDYKDDDDK	257
cFGF19/21-5/Flag	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDC ARGQSPESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEA CSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLP GLPPALPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYASDYKDDDDK	258
cFGF19/21-6/Flag	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDC ARGQSAHSLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPE ACSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPL PGLPPALPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYASDYKDDDD K	259
cFGF19/21-7/Flag	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDC ARGQSAHSLLEIKAVALRTVAIKGVKTSRFLCQRPDGALYGSLHFDPE ACSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPL PGLPPALPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYASDYKDDDD	260

Name	Amino Acid Sequence (N-C)	SEQ ID NO
	K	
cFGF19/21-8/Flag	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDC ARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCQRPDGALYGSLHFDPE ACSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPL PGLPPALPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYASDYKDDDD K	261
cFGF19/21-9/Flag	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDC ARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSE EDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFL PLPGLPPALPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYASDYKDD DDK	262
cFGF19/21/19-1/Flag	RPLAFSDAGPLLQFGGQVRQRYLYTSGPHGLSSCFLRIRADGVVDCAR GQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEED CAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLP MLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFE KDYKDDDDK	263
cFGF19/21/19-2/Flag	RPLAFSDAGPLLQFGGQVRQRYLYTDDPHGLSSCFLRIRADGVVDCAR GQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEED CAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLP MLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFE KDYKDDDDK	264
cFGF19/21/19-3/Flag	RPLAFSDAGPLLQFGGQVRQRYLYTDDAQGLSSCFLRIRADGVVDCAR GQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEED CAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLP MLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFE KDYKDDDDK	265
cFGF19/21/19-4/Flag	RPLAFSDAGPLLQFGGQVRQRYLYTDDAQLSSCFLRIRADGVVDCARG QSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDC AFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLPML PMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEKD	266

Name	Amino Acid Sequence (N-C)	SEQ ID NO
	YKDDDDK	
cFGF19/21/19-5/Flag	RPLAFSDAGPLLQFGGQVRQRYLYTDDAQQTSCFLRIRADGVVDCAR GQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEED CAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLP MLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFE KDYKDDDDK	267
cFGF19/21/19-6/Flag	RPLAFSDAGPLLQFGGQVRQRYLYTDDAQQTEAFLRIRADGVVDCAR GQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEED CAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLP MLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFE KDYKDDDDK	268
cFGF19/21/19-29/Flag	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDC ARGQSAHSLLEIKALKPGTVAIKGVHSVRYLCMGADGKMQGLLQYSE EDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFL PMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFE KDYKDDDDK	269

[0283] Referring again to Fig. 3B, each normalized luciferase activity is shown as an average and standard error of the mean of the three replicas. The results show that L6 cells expressing FGFR4 but not Klotho-beta, when treated with native FGF19 polypeptide, show dose-dependent activation of luciferase activity, whereas neither native FGF21 polypeptide nor chimeric FGF19 polypeptide showed such activity.

Example 2: KLB-Dependent FGFR4 Binding Activity of Chimeric and Native FGF Polypeptides

[0284] In this assay, rat L6 myoblasts in a 96-well plate were transiently-transfected with an expression vector encoding either human FGFR4 polypeptide (based on NCBI Reference Sequence: NM_002011.3) or human FGFR1c polypeptide (based on NCBI Reference Sequence: NM_015850.3), an expression vector encoding Klotho-beta (KLB) polypeptide (based on NCBI Reference Sequence: NM_175737.3 fused to a C-terminal LEDYKDDDDK epitope sequence), an expression vector encoding a GAL4-Elk-1 transcriptional activator (pFA2-Elk1, Stratagene), and an expression vector encoding a firefly luciferase reporter gene under control of the yeast GAL4 upstream activator (pFR-luc, Stratagene). A vector for the constitutive expression of *Renilla* luciferase (pRL-SV40, Promega) was also transfected into the cells. Transfections were

performed using FuGENE HD Transfection Reagent (Roche Applied Science) in accordance with the manufacturer's instructions.

[0285] The transfected L6 cells were cultured overnight in DMEM containing 10% FBS, as above. The cells were then washed and cultured for an additional 6 hours in serum-free medium containing 25 mg/L porcine heparin and a given concentration of a FGF polypeptide. The FGF polypeptides that were assayed were native human FGF19-Flag polypeptide (See FGF19-Flag in Table 10, SEQ ID NO:237), native human FGF21-His polypeptide (See FGF21-His in Table 10,SEQ ID NO:238) and a chimeric FGF19-Flag polypeptide (See cFGF21/19-2/Flag in Table 10; SEQ ID NO:242). The cells were incubated with the FGF19 polypeptide at concentrations of 500 ng/mL, 83.3 ng/mL, 13.9 ng/mL, 2.3 ng/mL, 0.39 ng/mL, 0.064 ng/mL or 0.011 ng/mL. The cells were incubated with the chimeric FGF19 polypeptide at concentrations of 2667 ng/mL, 444.4 ng/mL, 74.1 ng/mL,12.3 ng/mL, 2.06 ng/mL, 0.34 ng/mL or 0.057 ng/mL The cells were then lysed with PLB reagent (Promega) and luciferase activity in each well was determined using Dual-Glo Luciferase Assay System (Promega) and EnVision Multilabel Reader (PerkinElmer) in accordance with the respective manufacturers' instructions. Each firefly luciferase activity was normalized to the co-expressed *Renilla* luciferase activity, and each sample condition was performed in triplicate.

[0286] Referring to Fig. 4, each normalized luciferase activity is shown as an average and standard error of the mean of the three replicas. The results show that native FGF19 polypeptide and chimeric FGF19 polypeptide show similar dose-dependent activation of luciferase in the presence of KLB and FGFR1c, with an EC_{50} of 34.3 ng/mL and 22.7 ng/mL, respectively. In cells transformed with FGFR4 and KLB, the dose-dependent activation of luciferase activity by chimeric FGF19 polypeptide was significantly lower than that of native FGF19, with an EC_{50} of 269 ng/mL and 2.6 ng/mL, respectively. The selectivity of each FGF19 polypeptide for FGFR1c and FGFR4 was estimated based on the EC_{50} values calculated using Prism 5 software (GraphPad Software, La Jolla, California). In this example, the chimeric FGF19 polypeptide showed a higher relative selectivity for FGFR1c over FGFR4 (based on the respective calculated EC_{50} values) than the corresponding relative selectivity of native FGF19 polypeptide.

Example 3: Induction of Liver-Specific Genes by Chimeric and Native FGF Polypeptides

[0287] In this example, FVB mice were fasted overnight. A sample group (n = 5 or 6) of fasted mice was injected via tail vein with native human FGF19-Flag polypeptide (FGF19-Flag in Table 10, native human FGF21-His polypeptide (FGF21-His in Table 10), chimeric FGF19-Flag polypeptide (cFGF21/19-2/Flag in Table 10; SEQ ID NO:242) or phosphate-buffer saline (PBS) vehicle control. The polypeptides were provided in PBS at a dosage of 1 mg/kg. At 4 hours post-injection, liver tissue was harvested from each mouse and snap-frozen in liquid nitrogen. Total

tissue RNA was isolated from the harvested liver tissue using Qiazol (catalog no. 79306, Qiagen, Germantown, Maryland) and used as a template for cDNA synthesis (Quantitect Reverse Transcription Kit, catalog no. 205311, Qiagen). Following standard protocols for quantitative real-time PCR, the cDNA was quantified using SYBR Green dye (catalog no. 11760500, Invitrogen, Carlsbad, California) and 7900HT Fast Real-Time PCR System (Applied BioSystems, Inc., Foster City, California), with 36B4 gene as a standard. Referring to Fig. 5, the results show that levels of Egr-1 and cFos mRNA were highest in the sample injected with native FGF19 polypeptide, whereas the levels of Egr-1 and cFos mRNA were either absent or significantly lower in the samples injected with native FGF21 polypeptide or the chimeric FGF19 polypeptide. The relative levels of SHP mRNA or Cyp7A1 mRNA were comparable between the samples that were injected with the polypeptides. Levels with p values of < 0.05, < 0.01 and < 0.0005 are indicated with "*", "**" and "***", respectively.

Example 4: Induction of Adipocyte-Specific Genes by Chimeric and Native FGF Polypeptides

[0288] The example was performed as described in Example 3, with brown adipose tissue (BAT) and white adipose tissue (WAT) harvested at 4 hours post-injection and snap-frozen in liquid nitrogen. Referring to Fig. 6, the results show that levels of Egr-1 mRNA in WAT and UCP-3 mRNA in BAT, neither of which expresses detectable FGFR4, were similarly regulated by the FGF polypeptides used. Levels with p values of < 0.05, < 0.01 and < 0.001 are indicated with "*", "**" and "***", respectively.

Example 5: Reduction of Blood Glucose In Diabetic Obese Mice by Chimeric FGF19 and Native FGF21 Polypeptides

[0289] In this example, 11-week-old *ob/ob* mice (stock#000632, The Jackson Laboratory, Bar Harbor, Maine) were subcutaneously implanted with an osmotic pump (catalog no. 2001, Alzet, Cupertino, California) containing 200 µL of native human FGF21 polypeptide (FGF21-FlagN in Table 10; 1mg/mL in PBS), chimeric FGF19 polypeptide (cFGF21/19-2/Flag in Table 10; SEQ ID NO:242) (1mg/mL in PBS) or vehicle control (PBS). Each sample group consisted of nine (9) mice. The osmotic pump was configured to provide polypeptide at a rate of ~ 0.4 mg/kg/day.

[0290] Referring to Fig. 7A, the body weight and random-fed blood glucose level of each mouse was measured beginning three (3) days prior to pump implantation until five (5) days post-implantation at the indicated time points. Blood glucose was measured using One Touch 2 Ultra Blood glucose monitoring system (LifeScan, Milpitas, California). Fig. 7A shows the average body weight and blood glucose level for each sample group (levels with p values of < 0.05, < 0.001 and $< 5 \times 10^{-7}$ are indicated with "*", "**" and "***", respectively). On day 5, the mice were fasted overnight and the fasting blood glucose was measured in the next morning. Referring to Fig. 7B, the blood glucose levels for each mouse at days 5 and 6 (overnight fasted) are shown

(levels with p values of < 0.002, < 0.0005 and < 5 x 10^{-10} are indicated with "*", "**" and "***", respectively). The results show that both native human FGF21 polypeptide and the chimeric FGF19 polypeptide reduced blood glucose to similar levels in these mice.

Example 6: Intraperitoneal Glucose Tolerance In Diabetic Obese Mice by Chimeric FGF19 and Native FGF21Polypeptide

[0291] The mice from Example 5 were injected intraperitoneally with bolus glucose in PBS (1 g/kg) following the overnight fasting on day 6 to test glucose tolerance. The bolus injection occurred at the point corresponding to time = 0 in Fig. 8A. Subsequent to the bolus injection, blood glucose levels for each mouse were measured at the indicated time points, with the average blood glucose level for each sample group shown in Fig. 8A. Referring to Fig. 8B, the area under the curve (AUC) between t = 0 and 120 min during the glucose tolerance test (GTT) for each animal was plotted. The *p* values for the sample injected with native human FGF21 polypeptide or the chimeric FGF19 polypeptide compared to the PBS control were both < 0.001 according to student t-test. The results show that both native human FGF21 polypeptide and the chimeric FGF19 polypeptide showed similar glucose tolerance in these fasted mice.

Example 7: Activity of Native and Chimeric FGF – Fc Fusion Polypeptides

[0292] In this example, conditioned media containing a FGF-Fc fusion polypeptide is harvested from cells transfected with the corresponding expression vector. HEK293S cells were transiently transfected with an expression vector encoding native human FGF19 polypeptide fused to the N-terminus of human IgG1-Fc fragment via a 21-amino acid linker GGGGSGGGGSDYKDDDDKGRAQVT (SEQ ID NO:286), native human FGF21 polypeptide fused to the N-terminus of human IgG1-Fc fragment via a 4-amino acid linker GGGG, or human chimeric FGF19 polypeptide (cFGF21/19-2) fused to the N-terminus of human IgG1-Fc fragment via a 4-amino acid linker GGGS. Mock-transfected cells were used as a control. The cells were cultured overnight in DMEM containing 10% FBS, as above. The cells were then washed and cultured in an enriched serum-free medium derived from the F12/DME 50:50 blend for two (2) days. From each sample, conditioned medium was harvested. Equal volumes (6.5 µL) of each conditioned medium from each sample was used for immunoblot analysis using antibodies specific for the human IgG-Fc fragment. The immunoblot results are shown in Fig. 9B, which shows the presence of an Fc fragment-containing polypeptide with the expected molecular weight in the conditioned media that were harvested from the cells transformed with FGF19-Fc, FGF21-Fc and cFGF21/19-2-Fc fusion.

[0293] In this example, the conditioned media were used to demonstrate the activity of the FGF-Fc fusion polypeptides. HEK293S cells in a 96-well plate were transiently-transfected with an expression vector encoding a GAL4-Elk-1 transcriptional activator (pFA2-Elk1, Stratagene),

and an expression vector encoding a firefly luciferase reporter gene under control of the yeast GAL4 upstream activator (pFR-luc, Stratagene). In some experiments, cells were also transfected with an expression vector encoding Klotho-beta (KLB) polypeptide. A vector for the constitutive expression of *Renilla* luciferase (pRL-SV40, Promega) was also transfected into the cells. Transfections were performed using FuGENE HD Transfection Reagent (Roche Applied Science) in accordance with the manufacturer's instructions.

[0294] The transfected cells were cultured overnight in DMEM containing 10% FBS, as above. The cells were then washed and cultured for an additional 6 hours in a medium made from 1 part of the conditioned medium diluted with 3 parts of an enriched serum-free medium derived from the F12/DME 50:50 blend, the final medium containing 25 mg/L porcine heparin. The cells were then lysed with PLB reagent (catalog no. E1941, Promega) and luciferase activity in each well was determined using Dual-Glo Luciferase Assay System (Promega) and EnVision Multilabel Reader (PerkinElmer) in accordance with the respective manufacturers' instructions. Each firefly luciferase activity was normalized to the co-expressed *Renilla* luciferase activity, and each sample condition was performed in triplicate.

[0295] Referring to Fig. 9A, each normalized luciferase activity is shown as an average and standard error of the mean of the three replicas. The results show that the luciferase activity of the transformed HEK293S cells, with the presence of KLB, can be activated by native human FGF19-Fc polypeptide or the chimeric FGF19-Fc polypeptide in a manner similar to their respective non-Fc-fusion analogues. However, in contrast to the corresponding non-Fc-fusion analogue of native FGF21 polypeptide, the FGF21-Fc fusion polypeptide showed a substantially lower activation of firefly luciferase even with the presence of KLB.

Example 8: Receptor Specificity of Native and Chimeric FGF Polypeptides

[0296] In this example, rat L6 myoblasts in a 48-well plate were transiently-transfected with an expression vector encoding either human FGFR4 polypeptide, human FGFR1c polypeptide or a vector control. Also transfected in each cell sample were an expression vector encoding Klotho-beta (KLB) polypeptide, an expression vector encoding a GAL4-Elk-1 transcriptional activator (pFA2-Elk1, Stratagene), an expression vector encoding a firefly luciferase reporter gene under control of the yeast GAL4 upstream activator (pFR-luc, Stratagene), and vector for the constitutive expression of *Renilla* luciferase (pRL-SV40, Promega). Transfections were performed using FuGENE HD Transfection Reagent (Roche Applied Science) in accordance with the manufacturer's instructions.

[0297] The transfected L6 cells were cultured overnight in DMEM containing 10% FBS, as above. The cells were then washed and cultured for an additional 6 hours in serum-free conditioned medium (each conditioned medium was produced and harvested in accordance with

Example 7, diluted for use with an equal volume of serum-free medium) containing 25 mg/L porcine heparin. The conditioned media contained either a vector control (group A in Fig. 10) (pUC-derived vector containing CMV promoter:); native human FGF21-FlagC polypeptide (FGF21-FlagC in Table 10; group B in Fig. 10); native human FGF19-Flag polypeptide (FGF19-Flag in Table 10; group C in Fig. 10); a first chimeric FGF19 polypeptide having a N-terminal sequence derived from native human FGF21 (group D in Fig. 10) (cFGF21/19-13/Flag in Table 10,
HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESLLQLKALKP GVIQILGVKTSRFLCQRPDGALYGSLHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLPG NKSPHRDPAPRGPARFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAV RSPSFEKDYKDDDDK; SEQ ID NO:253); a second chimeric FGF19 polypeptide having a N-terminal sequence derived from native human FGF21 (group E in Fig. 10) (cFGF21/19-2/Flag in Table 10;
HPIPDSSPLLQFGGQVRQRYLYTSGPHGLSSCFLRIRADGVVDCARGQSAHSLLEIKAVAL RTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLS SAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGL EAVRSPSFEKDYKDDDDK; SEQ ID NO:242) or a chimeric FGF19 polypeptide having a N-terminal sequence derived from native human FGF19 (group F in Fig. 10) (cFGF19/21-2/Flag in Table 10;
RPLAFSDAGPHVHYGWGDPIRLRHLYTDDAQQTEAHLEIREDGTVGGAADQSPESLLQL KALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEACSFRELLLEDGYNVYQSEAHGLP LHLPGNKSPHRDPAPRGPARFLPLPGLPPALPEPPGILAPQPPDVGSSDPLSMVGPSQGRSP SYASDYKDDDDK; SEQ ID NO:255). The cells were then lysed with PLB reagent (Promega) and luciferase activity in each well was determined using Dual-Glo Luciferase Assay System (Promega) and EnVision Multilabel Reader (PerkinElmer) in accordance with the respective manufacturers' instructions. Each firefly luciferase activity was normalized to the co-expressed *Renilla* luciferase activity, and each sample condition was performed in triplicate.

[0298] Referring to Fig. 10, each normalized luciferase activity is shown as a fold induction over the correspondingly-transfected cells in sample group A, which were incubated in control conditioned medium derived from cells transfected with vector control. Each fold induction is shown as an average and standard error of the mean of the three replicas. The results show that fold induction of normalized luciferase activity in L6 cells expressing FGFR1c was comparable between the non-control samples. However, the fold induction in L6 cells expressing FGFR4 were significantly higher in cells incubated with native FGF19 than those cells incubated with either native FGF21 or chimeric FGF19 polypeptides.

Example 9: Activity of Chimeric FGF19 Polypeptides – Part 1

[0299] In this example, chimeric FGF19 polypeptides having N-terminal domains derived from native human FGF19 polypeptide were assayed for activity. All assayed polypeptides also contained a C-terminal Flag epitope tag. HEK293S cells were transiently-transfected with an expression vector encoding Klotho-beta (KLB) polypeptide, an expression vector encoding a GAL4-Elk-1 transcriptional activator (pFA2-Elk1, Stratagene), an expression vector encoding a firefly luciferase reporter gene under control of the yeast GAL4 upstream activator (pFR-luc, Stratagene), and vector for the constitutive expression of *Renilla* luciferase (pRL-SV40, Promega). Rat L6 myoblasts were transiently-transfected with an expression vector encoding either human FGFR4 polypeptide or human FGFR1c polypeptide, an expression vector encoding Klotho-beta (KLB) polypeptide, an expression vector encoding a GAL4-Elk-1 transcriptional activator (pFA2-Elk1, Stratagene), an expression vector encoding a firefly luciferase reporter gene under control of the yeast GAL4 upstream activator (pFR-luc, Stratagene), and vector for the constitutive expression of *Renilla* luciferase (pRL-SV40, Promega). Transfections were performed using FuGENE HD Transfection Reagent (Roche Applied Science) in accordance with the manufacturer's instructions.

[0300] The transfected HEK293S and L6 cells were cultured overnight in DMEM containing 10% FBS, as above. The cells were then washed and cultured for an additional 6 hours in serum-free conditioned medium (each conditioned medium was produced and harvested in accordance with Example 7, diluted for use with an equal volume of serum-free medium) containing 25 mg/L porcine heparin. The conditioned media were harvested from cells transfected with native human FGF21-FlagC polypeptide (A in Fig. 11), chimeric FGF19 polypeptide cFGF19/21-1/Flag (cFGF19/21-1/Flag in Table 10; B in Fig. 11), chimeric FGF19 polypeptide cFGF19/21-2/Flag (cFGF19/21-2/Flag in Table 10; C in Fig. 11), chimeric FGF19 polypeptide cFGF19/21-3/Flag (cFGF19/21-3/Flag in Table 10; D in Fig. 11), chimeric FGF19 polypeptide cFGF19/21-4/Flag (cFGF19/21-4/Flag in Table 10; E in Fig. 11), chimeric FGF19 polypeptide cFGF19/21-5/Flag (cFGF19/21-5/Flag in Table 10; F in Fig. 11), chimeric FGF19 polypeptide cFGF19/21-6/Flag (cFGF19/21-6/Flag in Table 10; G in Fig. 11), chimeric FGF19 polypeptide cFGF19/21-7/Flag (cFGF19/21-7/Flag in Table 10; H in Fig. 11), chimeric FGF19 polypeptide cFGF19/21-8/Flag (cFGF19/21-8/Flag in Table 10; I in Fig. 11), chimeric FGF19 polypeptide cFGF19/21-9/Flag (cFGF19/21-9/Flag in Table 10; J in Fig. 11), chimeric FGF19 polypeptide cFGF19/21/19-29/Flag (cFGF19/21/19-29/Flag in Table 10; K in Fig. 11), or native FGF19-Flag polypeptide (L in Fig. 11).

[0301] Referring to Fig. 11, the firefly luciferase activity for each sample was normalized to the co-expressed *Renilla* luciferase activity, and each sample condition was performed in

triplicate. The normalized luciferase activity was compared to the activity for native human FGF19-Flag polypeptide, where "+" indicates substantially equivalent activity to that of native human FGF19-Fc fusion polypeptide, "+/-" indicates intermediate activity, and "—" indicates no detectable activity. Conditioned media that showed no detectable or intermediate activity in HEK293S cells were not tested in L6 cells.

Example 10: Activity of Chimeric FGF19 Polypeptides – Part 2

[0302] In this example, chimeric FGF19 polypeptides having N-terminal domains derived from native human FGF21 polypeptide were assayed for activity. All assayed polypeptides also contained a C-terminal Flag epitope tag. The assay was performed as described in Example 9. The conditioned media were harvested from cells transfected with native human FGF19-Flag polypeptide (FGF19-Flag in Table 10; A in Fig. 12), chimeric FGF19 polypeptide cFGF21/19-1/Flag (cFGF21/19-1/Flag in Table 10; B in Fig. 12), chimeric FGF19 polypeptide cFGF21/19-2/Flag (cFGF21/19-2/Flag in Table 10; C in Fig. 12), chimeric FGF19 polypeptide cFGF21/19-7/Flag (cFGF21/19-7/Flag in Table 10; D in Fig. 12), chimeric FGF19 polypeptide cFGF21/19-8/Flag (cFGF21/19-8/Flag in Table 10; E in Fig. 12), chimeric FGF19 polypeptide cFGF21/19-9/Flag (cFGF21/19-9/Flag in Table 10; F in Fig. 12), chimeric FGF19 polypeptide cFGF21/19-10/Flag (cFGF21/19-10/Flag in Table 10; G in Fig. 12), chimeric FGF19 polypeptide cFGF21/19-11/Flag (cFGF21/19-11/Flag in Table 10; H in Fig. 12), chimeric FGF19 polypeptide cFGF21/19-12/Flag (cFGF21/19-12/Flag in Table 10; I in Fig. 12), chimeric FGF19 polypeptide cFGF21/19-13/Flag (cFGF21/19-13/Flag in Table 10; J in Fig. 12), or native FGF21-FlagC polypeptide (FGF21-FlagC in Table 10; K in Fig. 12).

[0303] Referring to Fig. 12, the firefly luciferase activity for each sample was normalized to the co-expressed *Renilla* luciferase activity, and each sample condition was performed in triplicate. The normalized luciferase activity was compared to the activity for native human FGF19-Fc fusion polypeptide, where "+" indicates substantially equivalent activity to that of native human FGF19-Fc fusion polypeptide, "+/-" indicates intermediate activity, and "—" indicates no detectable activity. Conditioned media that showed no detectable or intermediate activity in HEK293S cells were not tested in L6 cells.

Example 11: Activity of Chimeric FGF19 Polypeptides – Part 3

[0304] In this example, chimeric FGF19 polypeptides having N-terminal domains derived from native human FGF21 polypeptide were assayed for activity. All assayed polypeptides also contained a C-terminal Flag epitope tag. The assay was performed as described in Example 9, except only the transfected HEK293S cells and the FGFR4-transfected L6 cells were used in the assay. The conditioned media were harvested from cells transfected with native human FGF21-FlagC polypeptide (FGF21-FlagC in Table 10; A in Fig. 13), native human FGF19-Flag

polypeptide (FGF19-Flag in Table 10; B in Fig. 13), chimeric FGF19 polypeptide cFGF21/19-1/Flag (cFGF21/19-1/Flag in Table 10; C in Fig. 13), chimeric FGF19 polypeptide cFGF21/19-2/Flag (cFGF21/19-2/Flag in Table 10; D in Fig. 13), chimeric FGF19 polypeptide cFGF21/19-3/Flag (cFGF21/19-3/Flag in Table 10; E in Fig. 13), chimeric FGF19 polypeptide cFGF21/19-4/Flag (cFGF21/19-4/Flag in Table 10; F in Fig. 13), chimeric FGF19 polypeptide cFGF21/19-5/Flag (cFGF21/19-5/Flag in Table 10; G in Fig. 13), or chimeric FGF19 polypeptide cFGF21/19-6/Flag (cFGF21/19-6/Flag in Table 10; G in Fig. 13).

[0305] Referring to Fig. 13, the firefly luciferase activity for each sample was normalized to the co-expressed *Renilla* luciferase activity, and each sample condition was performed in triplicate. The normalized luciferase activity was compared to the activity for native human FGF19-Flag polypeptide, where "+" indicates substantially equivalent activity to that of native human FGF19-Flagn polypeptide, "+/-" indicates intermediate activity, and "—" indicates no detectable activity. Conditioned media that showed no detectable or intermediate activity in HEK293S cells were not tested in L6 cells.

[0306] Fig. 13 further shows a proposed alignment of the respective amino acid sequences of N-terminal portions of the assayed polypeptides. Selected amino acid residues which correspond to the conserved LYT and LxxIxxG motifs in each polypeptide are indicated in the alignment by outlined boxes.

Example 12: Activity of Chimeric FGF19 Polypeptides – Part 4

[0307] In this example, chimeric FGF19 polypeptides having N-terminal and internal domains derived from native human FGF21 polypeptide were assayed for activity. All assayed polypeptides also contained a C-terminal Flag epitope tag. The assay was performed as described in Example 9. The conditioned media were harvested from cells transfected with native human FGF21-FlagC polypeptide (as indicated in Fig. 14), native human FGF19-Flag polypeptide (as indicated in Fig. 14), chimeric FGF19 polypeptide cFGF21/19-2/Flag (cFGF21/19-2/Flag in Table 10; A in Fig. 14), chimeric FGF19 polypeptide cFGF21/19-3/Flag (cFGF21/19-3/Flag in Table 10; B in Fig. 14), chimeric FGF19 polypeptide cFGF21/19-4/Flag (cFGF21/19-4/Flag in Table 10; C in Fig. 14), chimeric FGF19 polypeptide cFGF21/19-5/Flag (cFGF21/19-5/Flag in Table 10; D in Fig. 14), chimeric FGF19 polypeptide cFGF21/19-6/Flag (cFGF21/19-6/Flag in Table 10; E in Fig. 14), chimeric FGF19 polypeptide cFGF19/21/19-1/Flag (cFGF19/21/19-1/Flag in Table 10; F in Fig. 14), chimeric FGF19 polypeptide cFGF19/21/19-2/Flag (cFGF19/21/19-2/Flag in Table 10; G in Fig. 14), chimeric FGF19 polypeptide cFGF19/21/19-3/Flag (cFGF19/21/19-3/Flag in Table 10; H in Fig. 14), chimeric FGF19 polypeptide cFGF19/21/19-4/Flag (cFGF19/21/19-4/Flag in Table 10; I in Fig. 14), chimeric FGF19 polypeptide cFGF19/21/19-5/Flag (cFGF19/21/19-5/Flag in Table 10; J in Fig. 14), chimeric

FGF19 polypeptide cFGF19/21/19-6/Flag (cFGF19/21/19-6/Flag in Table 10; K in Fig. 14), or chimeric FGF19 polypeptide cFGF19/21-1/Flag (cFGF19/21-1/Flag in Table 10; L in Fig. 14).

[0308] Referring to Fig. 14, the firefly luciferase activity for each sample was normalized to the co-expressed *Renilla* luciferase activity, and each sample condition was performed in triplicate. The normalized luciferase activity was compared to the activity for native human FGF19-Flag polypeptide, where "+" indicates substantially equivalent activity to that of native human FGF19-Flag polypeptide, "+/—" indicates intermediate activity, and "—" indicates no detectable activity. Conditioned media that showed no detectable or intermediate activity in HEK293S cells were not tested in L6 cells.

[0309] Fig. 14 further shows a proposed alignment of the respective amino acid sequences of N-terminal portions of the assayed polypeptides. Selected amino acid residues which correspond to the conserved LYT and LxxIxxG motifs in each polypeptide are indicated in the alignment by outlined boxes.

Example 13: Reduced STAT5 Dephosphorylation By Chimeric FGF19 Polypeptides

[0310] In this example, a chimeric FGF19 polypeptide of the present invention was tested for its effect on STAT5 dephosphorylation. Five-week old male C57BL/6J mice (about 18 to 19 grams each) were subcutaneously injected in duplicate with native human FGF21-His polypeptide (catalog no. 2539-FG-025/CF, R&D Systems, Inc., Minneapolis, Minnesota), chimeric FGF19-Flag polypeptide (cFGF21/19-2/Flag in Table 10; SEQ ID NO:242) or phosphate-buffered saline (PBS) carrier control. The polypeptides were solubilized in PBS and provided at a dosage of 1 mg/kg (about 20 μg polypeptide per injection) twice daily for two days. On the following morning of the third day, the mice were intraperitoneally injected with a fifth and final 1 mg/kg dose of respective polypeptide or control, and sacrificed 2 hours later. The liver was recovered from each mouse and nuclear extract was prepared from the liver using Nuclear Extraction Kit (catalog no. 10009277, Cayman Chemical, Ann Arbor, Michigan). For each nuclear extract sample, 22.5 μg of protein was resolved by SDS polyacrylamide gel electrophoresis and analyzed by immunoblotting using an antibody specific for the Stat5 protein that is phosphorylated at Tyr694 (catalog no. 9314, Cell Signaling Technology, Danvers, Massachusetts). A non-specific band ("NS"), which was used a loading control, is shown to be detectable at approximately the same amounts in each lane. Similar results were observed (data not shown) regarding the levels of Try694-phosphorylated-Stat5 with another monoclonal antibody specific for the Tyr694-phosphorylated-Stat5 (catalog no. 9359, Cell Signaling Technology).

[0311] Referring to Fig. 15, the results show Tyr694-phosphorylated Stat5 protein was not detectable in the mice that were injected with native human FGF21 polypeptide. However, mice

that were injected with the chimeric FGF19 polypeptide showed significant levels of the phosphorylated Stat5 protein.

Example 14: Reduced Promotion of Anchorage-Independent Growth By Chimeric FGF19 Polypeptides

[0312] In this example, a chimeric FGF19 polypeptide of the present invention was tested for its effect on anchorage-dependent growth of human hepatoma HepG2 cells, which express KLB and FGFR4. A 96-well-plate was filled with 50 µL per well of molten base agar (DMEM, 0.5% agarose and 10% FBS). After the base agar had solidified, about 670 HepG2 cells suspended in 50 µL molten top agar solution (DMEM, 0.35% agarose and 10% FBS) were added to the base agar in each well, and allowed to solidify.

[0313] Following solidification of the cell suspension, 20 µL of growth medium (DMEM and 10% FBS) was added to each well on designated day zero (0). For a given experimental sample, the growth medium further included either native human FGF19-Flag polypeptide, native human FGF21-His polypeptide (catalog no. 2539-FG-025/CF, R&D Systems, Inc., Minneapolis, Minnesota), chimeric FGF19 flag-tagged polypeptide (cFGF21/19-2/Flag; SEQ ID NO:242), or no FGF polypeptide as a control. The polypeptide concentration in the growth medium that was added on day zero was either 120 ng/mL or 1200 ng/mL, so that the final concentration in each well becomes 20 ng/mL or 200 ng/mL, respectively. On each of subsequent days 2, 4, 6 and 8, an further 20 µL of growth medium was added to each well, wherein the further added growth medium to a given well contained the same FGF polypeptide as in previous applications to that well, but with one-sixth the concentration of FGF polypeptide (*i.e.* 20 ng/mL or 200 ng/mL) as that of the day zero amount. A subset of the sample wells were also treated with G418 protein synthesis inhibitor to provide a background fluorescence signal.

[0314] On day 9, 10 µL AlamarBlue reagent (catalog no. DAL1100, Invitrogen) was added to each sample well and the plate was further incubated for 5 hrs to assay the total metabolic activity in each well. The resulting fluorescent intensity was measured using EnVision Multilabel Reader (PerkinElmer). Five (5) replicas of each sample were tested.

[0315] Referring to Fig. 16, the results are shown as fluorescent intensity above background and represent the average and standard deviation of the five (5) replicas. The results show that the total metabolic activity, as a proposed indicator of anchorage-independent growth of the cells, was promoted by the addition of native human FGF19 polypeptide, but such activity was reduced with the addition of native human FGF21 or the chimeric FGF19 polypeptides (p values < 0.05 compared to mock treated samples according to student t-test).

Example 15: FGF19 regulates cell proliferation, glucose and bile acid metabolism via FGFR4-dependent and independent pathways

[0316] To investigate the requirement for FGFR4 in mediating FGF19 activity by using Fgfr4 deficient mice as well as a protein variant of FGF19, which is specifically impaired in its ability to activate FGFR4.

Materials and Methods

[0317] **Expression of recombinant FGF protein.** Amino acid sequences of FGF19, FGF21, and chimeras were constructed, the drawings of the chimera constructs made are shown in Figure 18B). The constructs with the numbering of 1-17 shown in Figure 18B correspond to constructs comprising the amino acid sequences of SEQ ID NO:1, SEQ ID NO:270 (RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQSAHSLLE IKALKPGTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHR LPVSLSSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPF GLVTGLEAVRSPSFEK), SEQ ID NO:74, SEQ ID NO:5, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:271, SEQ ID NO:272, SEQ ID NO:273, SEQ ID NO:274, SEQ ID NO:275, SEQ ID NO:276, SEQ ID NO:277, SEQ ID NO:278, SEQ ID NO:85, and SEQ ID NO:2, respectively. All the contructs shown in Figure 18B also included signal sequences at the N-terminal end (cleaved upon secretion) and the flag tag (DYKDDDDK (SEQ ID NO:279)) at the C-terminal end.

[0318] Unless otherwise noted, recombinant human FGF21, FGF19 and variants produced in transiently transfected CHO cell and purified to homogeneity in PBS were used for experiments. For some experiments, *E. coli* derived FGF21 (2539-FG/CF, R&D systems) were used. All the purified proteins were tested for the activity by cell based GAL-Elk1 assays prior to application for other assay. For experiments in Fig 18B, 18C, and 20, FGF proteins were expressed in transiently transfected HEK293 cells and fresh conditioned serum-free medium was used for assays without purification.

[0319] **Luciferase assay.** All the cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37°C under 5% CO2. Rat L6 myoblasts in a 96-well plate were transiently-transfected with expression vectors encoding Renilla luciferase (pRL-SV40, Promega), human KLB, appropriate human FGFR, GAL4-Elk-1 transcriptional activator (pFA2-Elk1, Stratagene), and firefly luciferase reporter driven GAL4 binding sites (pFR-luc, Stratagene), using FuGENE HD Transfection Reagent (Roche Applied Science). On the next day, the transfected cells were cultured for an additional 6-8 hours in serum free media containing 25 mg/L porcine heparin (Sigma) and FGF protein at a various

concentrations. The cells were then lysed with PLB reagent (Promega) and luciferase activity in each well was determined using Dual-Glo Luciferase Assay System (Promega) and EnVision Multilabel Reader (PerkinElmer). Firefly luciferase activity was normalized to the co-expressed Renilla luciferase activity, and was shown as an average and standard error of the mean of the three replicas.

[0320] Anchorage independent cell proliferation assay. A 96-well-plate was filled with 50 μL/well of 0.5% molten agarose in growth media. After the base agarose had solidified, about 670 HepG2 cells suspended in 50 μL top molten agarose solution (0.35% agarose in growth media) were added to the base agar in each well, and allowed to solidify. Following solidification, 20 μL of growth medium containing appropriate amount of FGF protein was added to each well on designated day 0. On each of subsequent days 2, 4, 6 and 8, a further 20 μL of growth medium with appropriate amount of FGF protein was added to each well. A subset of the sample wells was also treated with protein synthesis inhibitor Geneticin (invitrogen) to provide a background fluorescence signal. On day 9, 10 μL AlamarBlue reagent (Invitrogen) was added to each sample well and the plate was further incubated for 5 hrs. The resulting fluorescent intensity was measured using EnVision Multilabel Reader (PerkinElmer) and used as an indication of the total metabolic activity in each well. Five replicas of each sample were tested.

[0321] FGFR/ligand binding assay. FGFR-binding activity of FGF19 and FGF19v were measured as described in Desnoyers et al., *Oncogene* 27(1):85-97 (2008) using biotinylated anti-FGF19 antibody (BAF969, R&D systems) in the presence of 2 μg/mL heparin. Control ELSA experiments were performed using anti-FGF19 antibody (AF969, R&D systems) and biotinylated anti-FGF19 antibody (BAF969, R&D systems) to confirm that the antibody reacts to FGF19 and FGF19v in an indistinguishable manner.

[0322] Mouse Studies. Mice were maintained in a pathogen-free animal facility at 21□C under standard 12 hr light/12 hr dark cycle with access to chow (a standard rodent chow (Labdiet 5010, 12.7% calories from fat) or a high fat, high carbohydrate diet (Harlan Teklad TD.03584, 58.4% calories from fat) and water ad libitum. Male mice were used for all the experiments. FGFR4 KO mice in C57BL/6 background were previously described Weinstein et al., *Development* 1998 125(18):3615-23 (1998). C57BL/6 mice, ob/ob mice in C57BL/6 background and FVB/NJ mice were purchased from Jackson Laboratory. For continuous infusion of FGF protein, an osmotic pump (Alzet 2001) was subcutaneously implanted. For glucose tolerance test, glucose levels were measured using One Touch Ultra glucometer. Statistics were performed by Student's t test. Values were presented as means+/-SEMs. BrdU staining was carried out as described as {Nicholes, 2002 #79} and BrdU positive hepatocytes were counted by using the

Ariol automated image analysis system. All animal studies were performed under Genentech's Institutional Animal Care and Use Committee approved protocols.

[0323] **Serum analysis.** Total cholesterol, triglyceride, ß-hydroxybutylate (BHB), lactate Thermo DMA) and nonesterified fatty acid (Roche) were determined by using enzymatic reactions. Serum insulin levels were determined by ELISA (crystal chem). BA composition was determined by liquid chromatography-mass spectrometry analysis as previously described Stedman et al., *J Biol Chem.* 279(12):11336-43 (2004).

[0324] **Gene Expression Analysis.** Tissue RNAs were isolated by using QIAzol reagent (Qiagen). cDNA was synthesize with the Quantitect Reverse Transcription Kit (Qiagen). For real time qPCR, samples were run in triplicate in the ABI Prism 7900HT (Applied Biosystems) by using SYBR green universal mix (Invitrogen) or by Taqman universal mix (Roche) and normalized by levels of 36B4. Pre-designed Quantitect primers for GK, SHP, Cyp8b1, IGFBP2, and AFP were obtained from Qiagen and all other primers were designed using primer express software (Applied Biosystems).

A. FGFR4 regulates serum bile acids, but not improvement of glucose tolerance by recombinant FGF19

[0325] In order to determine which of the metabolic effects elicited by FGF19 are mediated by FGFR4, HFD-fed WT or Fgfr4 KO mice were treated with recombinant FGF19 or vehicle control, and metabolic phenotypes and gene expression were studied. To achieve sustained exposure to FGF19, 12 to 15 weeks old FGFR4 WT and KO Mice on high fat diet for 6 weeks were implanted with an osmotic pump to continuously infuse FGF19 at 1ng/hr. This achieved an average FGF19 serum concentration of 26 ng/ml, as determined by ELISA, which is about 50- to 250-fold higher than circulating FGF19 concentrations in humans. On day 6, overnight fasted mice were subjected to glucose tolerance test with i.p. injection of glucose at 1g/kg. FGF19 infusion improved glucose tolerance to a similar extent both in WT and Fgfr4 KO mice (Fig. 17A), indicating that FGFR4 is dispensable for improvement in glucose tolerance in HFD-fed mice. Continuous infusion of FGF19 did not induce significant weight loss, thus the improvement glucose tolerance was independent of body weight. By day 7, FGF19 reduced liver weight and serum insulin as well as increasing ketone body (BHB) formation in both WT and Fgfr4 KO mice (Fig. 17B). FGF19 also reduced serum lactate and triglycerides in WT but not Fgfr4 KO mice (Fig. 17B), even though the latter exhibited reduced lactate and triglyceride levels prior to treatment.

[0326] To evaluate changes in BA metabolism, serum BA composition was determined by liquid chromatography-mass spectrometry (Fig. 17C). FGF19 infusion reduced free and taurine conjugated cholic acid (CA) and the CA-derived secondary bile acid deoxycholic acid in WT

mice, while having minimal effect on CDCA (CDCA) metabolites. This finding is consistent with a shift of BA synthesis to the alternative (acidic) pathway, bypassing FGF19-suppressed Cyp7a1 and proceeding though Cyp7b1 (Fig. 17D). Correspondingly, loss of Fgfr4 increased basal levels of CA and its metabolites while reducing muricholic acids (hydroxylated metabolites of CDCA), indicating that FGFR4 is not only important as a regulator of bile acid synthesis, but is also a determinant of the ratio of CA to CDCA production. To determine the role of FGFR4 in regulation of hepatic gene expression, a range of hepatic mRNAs by QPCR was examined (Fig. 17D). FGF19 infusion induced expression of cell proliferation markers such as Egr-1, c-Fos, and AFP, and suppressed expression of Cyp7a1 in WT but not in Fgfr4 KO mice. In contrast, FGF19 suppressed Cyp8b1 and glucokinase (GK) in both WT and Fgfr4 KO mice, while basal expression of Cyp8b1 and Cyp27a1 levels were much higher in Fgfr4 KO compared to WT mice. Cyp8b1 is obligatory for the synthesis of cholic, but not CDCA, thus the observed changes in Cyp8b1 expression contribute to the altered balance between CA and CDCA metabolites (muricholic acids) in Fgfr4 KO mice (Fig. 17C and D). Taken together, our findings reveal that FGFR4 is a regulator of BA synthesis and impacts on hepatocyte proliferation, but not required for the regulation of glucose utilization, insulin sensitivity, and ketone body production by FGF19.

B. Identification of FGF19 variants with a specific reduction in FGFR4 activation.

[0327] In order to quantitatively evaluate specific activation of FGFRs by FGF19, an FGF-responsive GAL-Elk1 luciferase reporter assay was introduced into rat L6 cells. In this assay, effective binding of a ligand to FGFR results in activation of an endogenous MAP kinase pathway, leading to activation of a chimeric transcriptional activator comprising of an Elk-1 activation domain and a GAL4 DNAbinding domain. L6 cells lack functional FGFR or KLB and are only responsive to FGF19 or FGF21 when cotransfected with cognate receptors. L6 cells were cotransfected with expression vectors for KLB and FGFR (FGFG1c or FGFR4) together with GAL-Elk1, SV40-renilla Luciferase, and Gal-responsive firefly luciferase reporter. Transfected cells were incubated with media containing increasing concentrations of FGF19 or FGF21 for 6 hours before luciferase assays. The results from the luciferase assay show that FGF19 and FGF21 activated FGFR1c, 2c and 3c in the presence of KLB, with similar potency and efficacy (Fig. 18A and 19). In contrast, FGF19, but not FGF21, efficiently activated FGFR4, even in the presence of KLB (Fig. 18A). To map the signals required for FGFR4 activation, a number of chimeric constructs between FGF19 and FGF21 were generated using conserved residues to form junctions (Fig. 18B). Sequences are discussed above in the Materials and Methods section entitled Expression of recombinant FGF protein. Each FGF construct was expressed in transiently transfected HEK293 cells and the culture supernatants containing secreted chimeric FGF proteins

were tested for activation of FGFR1c and/or FGFR4 in KLB-expressing L6 cells using the GAL-Elk1 reporter assay. Based on the activity of FGFR1c and FGFR4, the chimeric constructs were classified into 4 classes: high FGFR1c and FGFR4 activity (Class I, FGF19-like); high FGFR1c activity and low, but detectable FGFR4 activity (Class II); high FGFR1c activity without detectable FGFR4activity (Class III, FGF21-like) and very low or undetectable FGFR1c and FGFR4 activity due to poor expression (Class IV) (I-III, Fig. 18C and 20; IV not shown). This mapping indicated that the N-terminal 39 amino acids of FGF19 are sufficient to confer some FGFR4 activity when transferred to FGF21. In addition, the N-terminal 24 amino acids and the C-terminal 49 amino acids of FGF19 are necessary for full FGFR4 activity, but are not sufficient to confer FGFR4 activity when transferred to FGF21. Thus multiple signals at both the N-terminus and C-terminus of FGF19 contribute to FGFR4 activation.

[0328] One chimeric construct classified as a class II molecule, consisting of amino acids 1-20 of FGF21 and 25-194 of FGF19 (> 90% identical to FGF19), was selected for large scale synthesis in CHO cells and this varient is referred to as "FGF19v",. When compared with FGF19 using the luciferase reporter assay, FGF19v protein exhibited a similar dose-dependent activity to FGF19 in L6 cells cotransfected with KLB and FGFR1c (Fig 18D). However, FGF19v activity was significantly diminished in L6 cells cotransfected with either FGFR4 alone or a combination of FGFR4 and KLB (Fig. 18D), FGF19 having been previously shown to directly bind to FGFR4 even in the absence of KLB. FGF19, but not FGF19v, exhibited dose-dependent binding activity to FGFR4 (Fig. 18E and F).

C. FGFR4 mediates hepatocyte proliferation in vitro and in vivo

[0329] Activity of FGF19v was further tested in vivo in comparison with FGF19 and FGF21 by intravenously injection into overnight fasted FVB mice. Livers were harvested at 4 hours post injection and hepatic mRNA expression was determined by QPCR. Genes that were acutely induced by FGF19 but not by FGF21, such as Egr-1 and c-Fos, were not efficiently induced by FGF19v, consistent with the reduced FGFR4 activity of FGF19v (Fig. 21A). FGF19v had similar activity to FGF19 or FGF21 on genes were co-regulated by FGF19 and FGF21, such as GK. Using Fgfr4 KO mice, FGFR4 contributes to the regulation of Egr-1 and c-Fos, but not GK, by FGF19 (Fig. 21B). Unexpectedly, FGF21 (as well as FGF19 and FGF19v) altered expression of SHP and Cyp7a1 (Fig. 21A), which were proposed to be major targets for FGFR4-dependent regulation by FGF19. Alterations in SHP and Cyp7a1 by FGF19 and FGF21 were observed even in Fgfr4 KO mice, indicating that with this acutetreatment, both endocrine FGFs can modulate expression of these genes through an FGFR4-independent pathway (Fig. 21B).

[0330] FGF19 increased anchorage-independent proliferation of HepG2 cells in soft agar, and this effect was much less apparent for FGF19v or FGF21 proteins (Fig. 21C). To see whether

FGF19v also exhibited reduced ability to induce hepatocyte proliferation in vivo, mice were infused with FGF19, FGF19v (1 ng/h) or vehicle control by osmotic minipump. In addition, 1 mg/kg/day of FGF protein was injected intraperitoneally daily for 7 days to the same mice to achieve high peak exposures. To capture intermittent proliferative events, BrdU solution (30mg/kg) was injected twice daily for total of 13 times. Hepatocyte proliferation was determined by measuring BrdU positive hepatocytes in liver harvested on day 7. As previously reported, FGF19 treatment resulted in a dramatic increase in BrdU incorporation; however, this response was significantly blunted for FGF19v (Fig. 21D and E). Hepatic mRNA for Egr-1, c-Fos, and the hepatocyte proliferation marker AFP were all dramatically induced by FGF19 and these inductions were largely absent for FGF19v, while regulation of GK, Cyp7a1 and Cyp8b1 did not differ between FGF19 and FGF19v (Fig. 21F).

D. FGFR4 is not required for amelioration of hyperglycemia in ob/ob mice by FGF19.

[0331] The in vitro and in vivo results described above raised the question as to whether FGF19v, a variant of FGF19 with reduced FGFR4 activity and proliferative potential, could improve hyperglycemia in diabetic animals as FGF21 does. FGF21, FGF19v (1ng/h) or vehicle control were continuously infused subcutaneously into ob/ob mice using osmotic minipumps. While infusion did not significantly affect body weight (Fig. 22A), both FGF21 and FGF19v dramatically reduced blood glucose levels in both random fed and fasted mice (Fig. 22A and B), reduced circulating free fatty acid levels (Fig. 22C), and improved glucose tolerance (Fig. 22D).

[0332] To visualize hepatocyte proliferation, animals were injected with BrdU 4 hours prior to sacrifice on day 7. Neither FGF21 nor FGF19v increased hepatic BrdU incorporation (not shown), rather gross liver weight was significantly reduced (Fig. 22E) and no significant change in hepatic expression of AFP mRNA was observed (Fig. 22F). Taken together, FGF19v can improve the metabolic status of obese mice without induction of hepatocyte proliferation.

[0333] A number of genes were indentied which exhibited commonly altered expression in ob/ob mice treated with FGF21 and FGF19v. In the liver, both proteins induced IGFBP2 (a recently demonstrated anti-diabetic protein), and suppressed stearoyl-Coenzyme A desaturase 1 (SCD-1; a lipogenic gene) and Cyp8b1 (the determinant of the balance between CA and CDCA production). In addition, they both induced UCP-1 (adaptive thermogenesis), SCD-1 and Medium-Chain Acyl-CoA Dehydrogenase (MCAD; mitochondrial fatty acid oxidation) in brown adipose tissue, and SREBP-1c (lipogenic transcription factor) in white adipose tissue (Fig. 22F). Thus, actions in multiple tissues could mediate the anti-diabetic effects of FGF21 and FGF19v acting through a FGFR4 independent mechanism.

[0334] By examining individual serum BA, recombinant FGF19, acting through Fgfr4, was demonstrated to suppress Cyp7a1 causing bile acid synthesis to proceed by the Cyp7a1-

independant alternate (acidic) pathway leading to the production of CDCA at the expense of CA. Cyp8b1 expression increased several-fold in Fgfr4 knockout mice and that FGF19 treatment suppresses Cyp8b1, an obligatory enzymatic step for CA synthesis. FGFR4 was a determinant of the ratio of CDCA to CAproduction, through negative regulation of both Cyp7a1 and Cyp8b1. FGFR4 activation shifts BA production towards CDCA, while its abrogation leads to CA formation. In addition, FGF19 increased hepatic AFP expression in an Fgfr4 dependent manner. FGF19 improved glucose tolerance in HFD-fed FGFR4 KO mice (Fig. 17) and FGF19v, a protein specifically impaired for FGFR4 binding and activation, ameliorates hyperglycemia in ob/ob mice (Fig. 18 and 21-22). In addition to the effects in insulin resistance and glucose metabolism, FGF19 increases serum BIIB levels even in FGFR4 KO mice (Fig. 17), like FGF21. Both FGF19 and FGF21 can bind and activate FGFR1c, FGFR2c, and FGFR3c in the presence of KLB. Thus FGFR1c, FGFR2c, or FGFR3c, in cooperation with KLB, may mediate the common metabolic effects of FGF19 and FGF21.

SEQUENCE LISTING IN ELECTRONIC FORM

In accordance with Section 111(1) of the Patent Rules, this description contains a sequence listing in electronic form in ASCII text format (file: 69790-118 Seq 14-06-12 v1.txt).

A copy of the sequence listing in electronic form is available from the Canadian Intellectual Property Office.

CLAIMS:

1. A chimeric FGF19 polypeptide comprising an amino acid sequence that has at least 95% identity to the amino acid sequence of SEQ ID NO:74 or 241.

2. The chimeric FGF19 polypeptide of claim 1, wherein the chimeric FGF19 polypeptide comprises an amino acid sequence that has at least 95% identity to the amino acid sequence of SEQ ID NO:74.

3. The chimeric FGF19 polypeptide of claim 2, wherein the chimeric FGF19 polypeptide comprises the amino acid sequence of SEQ ID NO:74.

4. The chimeric FGF19 polypeptide of claim 1, wherein the chimeric FGF19 polypeptide comprises an amino acid sequence that has at least 95% identity to the amino acid sequence of SEQ ID NO:241.

5. The chimeric FGF19 polypeptide of claim 4, wherein the chimeric FGF19 polypeptide comprises the amino acid sequence of SEQ ID NO:241.

6. A chimeric FGF19 polypeptide comprising an N-terminal portion of FGF21 and a C-terminal portion of FGF19, wherein the C-terminal portion of the chimeric FGF19 polypeptide comprises an amino acid sequence that has at least 95% identity to the amino acid sequence of SEQ ID NO:1.

7. The chimeric FGF19 polypeptide of any one of claims 1-6, wherein the chimeric FGF19 polypeptide is fused to a second polypeptide, and wherein the second polypeptide is selected from the group consisting of: the Fc portion of an immunoglobulin and one or more fragments of the Fc portion of an immunoglobulin.

8. The chimeric FGF19 polypeptide of claim 7, wherein the immunoglobulin is selected from the group consisting of: IgG-1, IgG-2, IgG-3, IgG-4, IgA-1, IgA-2, IgE, IgD and IgM.

9. The chimeric FGF19 polypeptide of claim 7 or 8, wherein the Fc portion is human or humanized.

Date Reçue/Date Received 2020-10-22

10. The chimeric FGF19 polypeptide of any one of claims 7-9, wherein the C-terminus of the chimeric FGF19 polypeptide is fused to the N-terminus of the second polypeptide.

11. The chimeric FGF19 polypeptide of claim 10, wherein the C-terminus of the chimeric FGF19 polypeptide is fused to the N-terminus of the second polypeptide via a linker, the linker is selected from the group consisting of: a [Gly]n linker, a [Gly3Ser]m linker and a [Gly4Ser]m linker, wherein n is an integer from 1-30 and m is an integer from 1-6.

12. A pharmaceutical composition comprising:

(a) a therapeutically effective amount of the chimeric FGF19 polypeptide of any one of claims 1-11; and

(b) an acceptable pharmaceutical carrier,

wherein the pharmaceutical composition is for treating one or more of obesity, type 1 diabetes, type 2 diabetes, high blood glucose, metabolic syndrome, atherosclerosis, hypercholesterolemia, stroke, osteoporosis, osteoarthritis, degenerative joint disease, muscle atrophy, sarcopenia, decreased lean body mass, baldness, decreased cardiac function, immune system dysfunction, cancer, Parkinson's disease, senile dementia, Alzheimer's disease or decreased cognitive function.

13. An isolated nucleic acid molecule comprising a DNA molecule encoding a polypeptide comprising an amino acid sequence that has at least 95% identity to the amino acid sequence of SEQ ID NO:74 or 241.

14. An isolated nucleic acid molecule comprising a DNA molecule encoding a polypeptide comprising an amino acid sequence that has at least 90% identity to the amino acid sequence of SEQ ID NO:5.

15. The isolated nucleic acid of claim 13 or 14 wherein the encoded polypeptide further comprises the amino acid residues corresponding to the Fc portion of an immunoglobulin.

16. An expression vector comprising the nucleic acid molecule of any one of claims 13-15.

17. A host cell comprising the expression vector of claim 16.

Date Reçue/Date Received 2020-10-22

18. A host cell comprising the nucleic acid molecule of any one of claims 13-15.

19. A process for producing an isolated polypeptide comprising: culturing the host cell of claim 17 or 18 under conditions suitable for expression of the encoded polypeptide; and recovering the encoded polypeptide from the cell culture.

20. An isolated polypeptide produced by the process of claim 19.

Date Reçue/Date Received 2020-10-22

FIG. 1

SEQ ID NO: 1 - hFGF19 (194 aa)

```
  1    RPLAF SDAGP HVHYG WGDPI RLRHL YTSGP HGLSS CFLRI RADGV VDCAR
 51    GQSAH SLLEI KAVAL RTVAI KGVHS VRYLC MGADG KMQGL LQYSE EDCAF
101    EEEIR PDGYN VYRSE KHRLP VSLSS AKQRQ LYKNR GFLPL SHFLP MLPMV
151    PEEPE DLRGH LESDM FSSPL ETDSM DPFGL VTGLE AVRSP SFEK
```

SEQ ID NO: 3 - Pre-hFGF19 (216 aa)

```
  1    MRSGC VVVHV WILAG LWLAV AG
 23    RPLAF SDAGP HVHYG WGDPI RLRHL YTSGP HGLSS CFLRI RADGV VDCAR
 74    GQSAH SLLEI KAVAL RTVAI KGVHS VRYLC MGADG KMQGL LQYSE EDCAF
123    EEEIR PDGYN VYRSE KHRLP VSLSS AKQRQ LYKNR GFLPL SHFLP MLPMV
174    PEEPE DLRGH LESDM FSSPL ETDSM DPFGL VTGLE AVRSP SFEK
```

FIG. 2

SEQ ID NO: 2 - hFGF21 (181 aa)

```
  1 HPIPD SSPLL QFGGQ VRQRY LYTDD AQQTE AHLEI REDGT VGGAA DQSPE
 51 SLLQL KALKP GVIQI LGVKT SRFLC QRPDG ALYGS LHFDP EACSF RELLL
101 EDGYN VYQSE AHGLP LHLPG NKSPH RDPAP RGPAR FLPLP GLPPA LPEPP
151 GILAP QPPDV GSSDP LSMVG PSQGR SPSYA S
```

SEQ ID NO: 4 - Pre-hFGF21 (209 aa)

```
  1 MDSDE TGFEH SGLWV SVLAG LLLGA CQA
 29 HPIPD SSPLL QFGGQ VRQRY LYTDD AQQTE AHLEI REDGT VGGAA DQSPE
 79 SLLQL KALKP GVIQI LGVKT SRFLC QRPDG ALYGS LHFDP EACSF RELLL
129 EDGYN VYQSE AHGLP LHLPG NKSPH RDPAP RGPAR FLPLP GLPPA LPEPP
179 GILAP QPPDV GSSDP LSMVG PSQGR SPSYA S
```

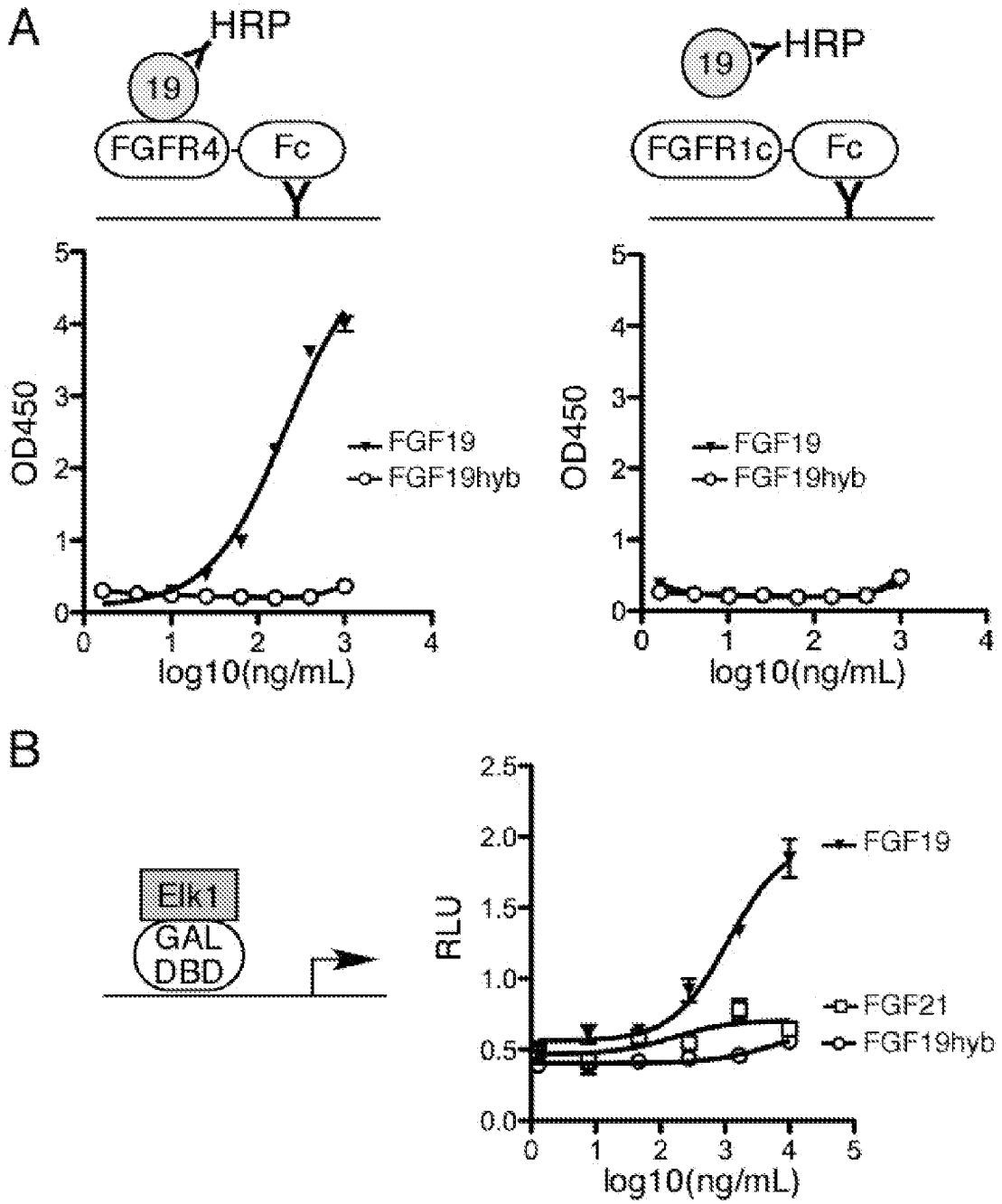

A
HRP
19
FGFR4
Fc
OD450
log10(ng/mL)
FGF19
FGF19hyb
19
HRP
FGFR1c
Fc
OD450
log10(ng/mL)
FGF19
FGF19hyb
B
Elk1
GAL
DBD
RLU
log10(ng/mL)
FGF19
FGF21
FGF19hyb

FIG. 3

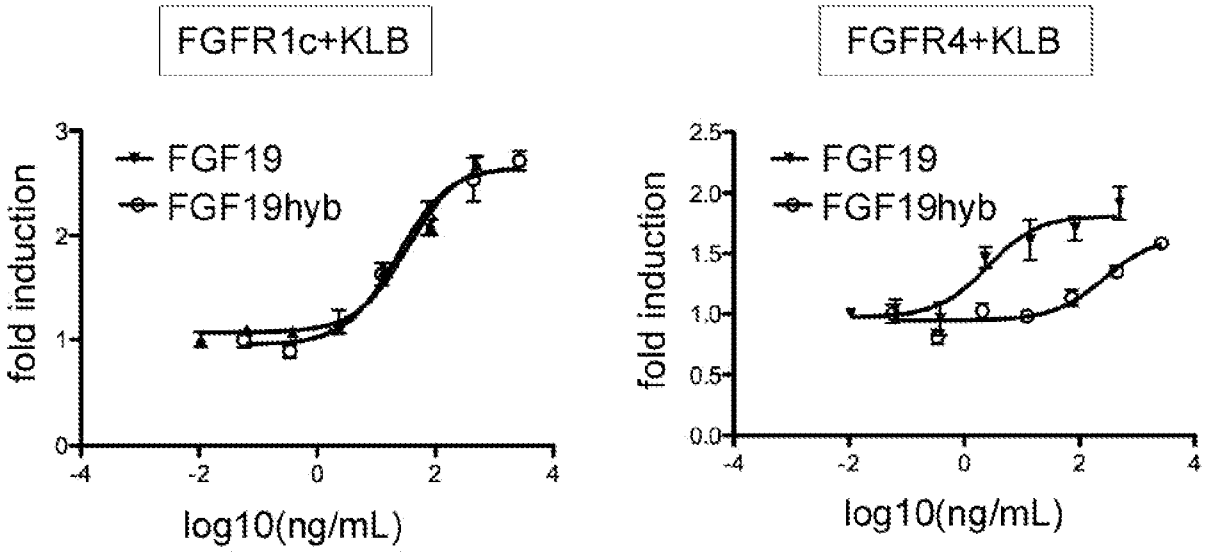

FGFR1c+KLB
FGF19
FGF19hyb
fold induction
log10(ng/mL)
FGFR4+KLB
FGF19
FGF19hyb
fold induction
log10(ng/mL)

FIG. 4

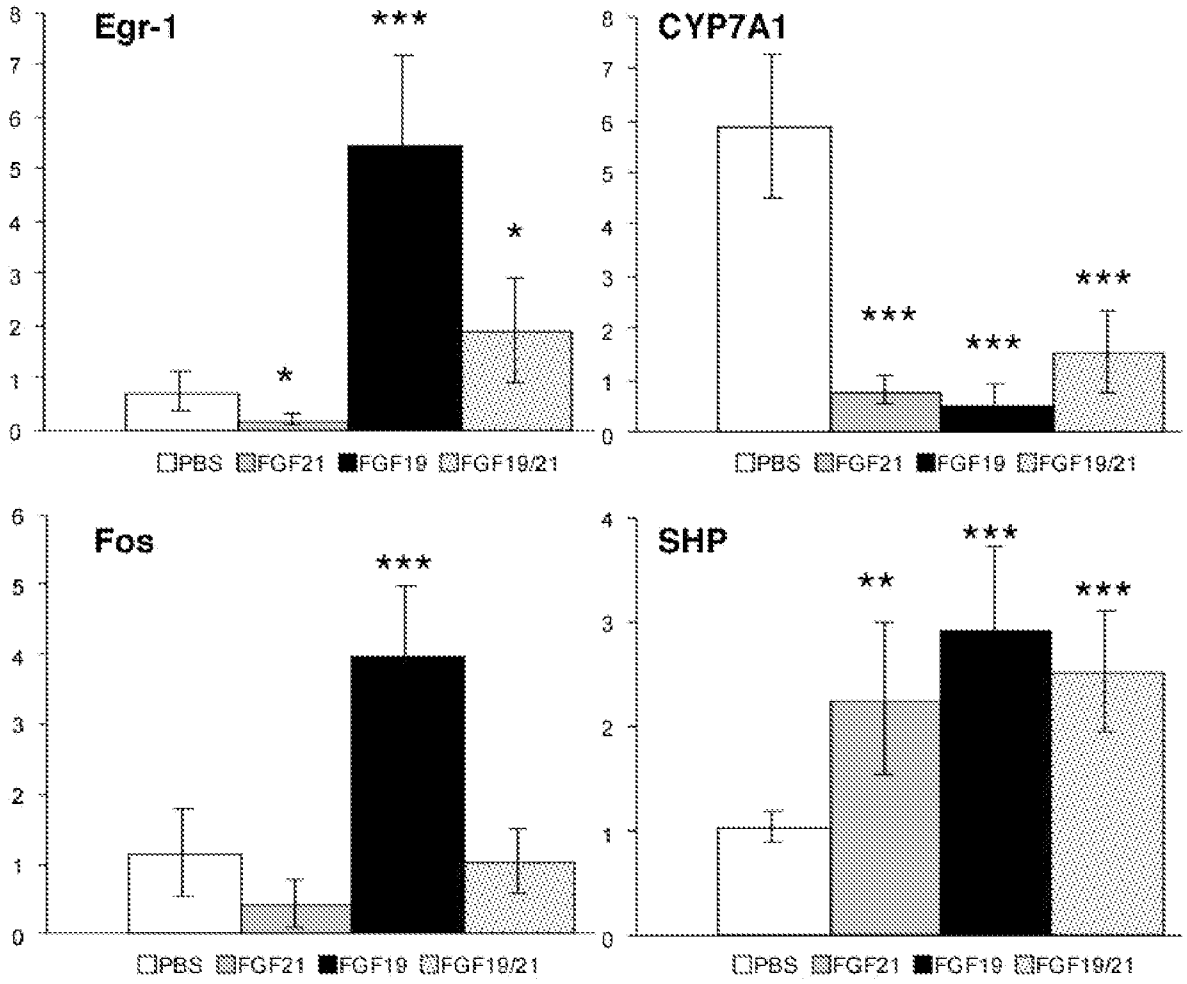


FIG. 5

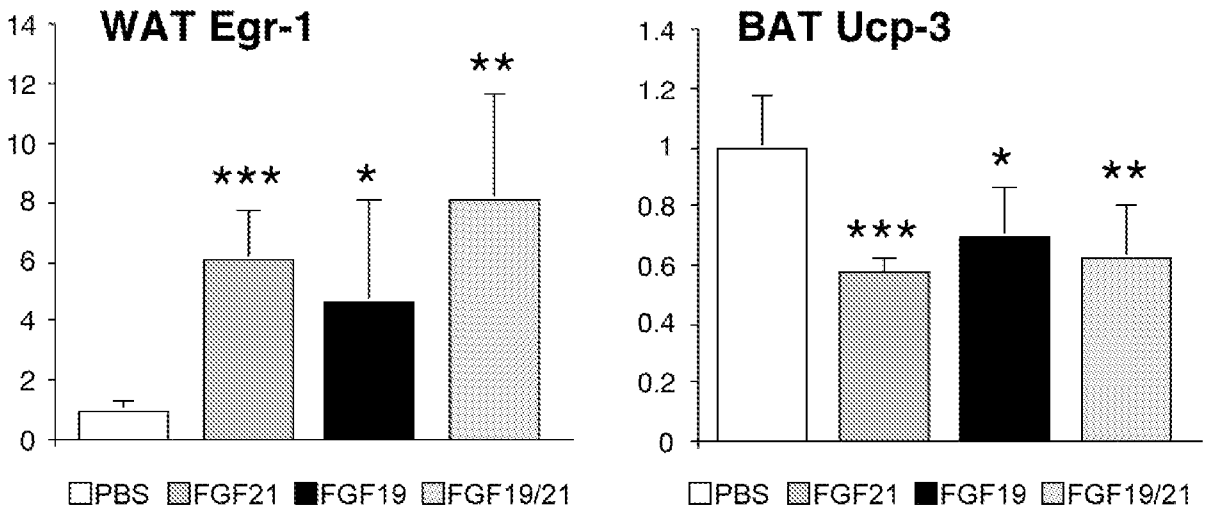

WAT Egr-1
14
12
10
8
6
4
2
0
***
*
**
PBS FGF21 FGF19 FGF19/21
BAT Ucp-3
1.4
1.2
1
0.8
0.6
0.4
0.2
0
***
*
**
PBS FGF21 FGF19 FGF19/21

FIG. 6

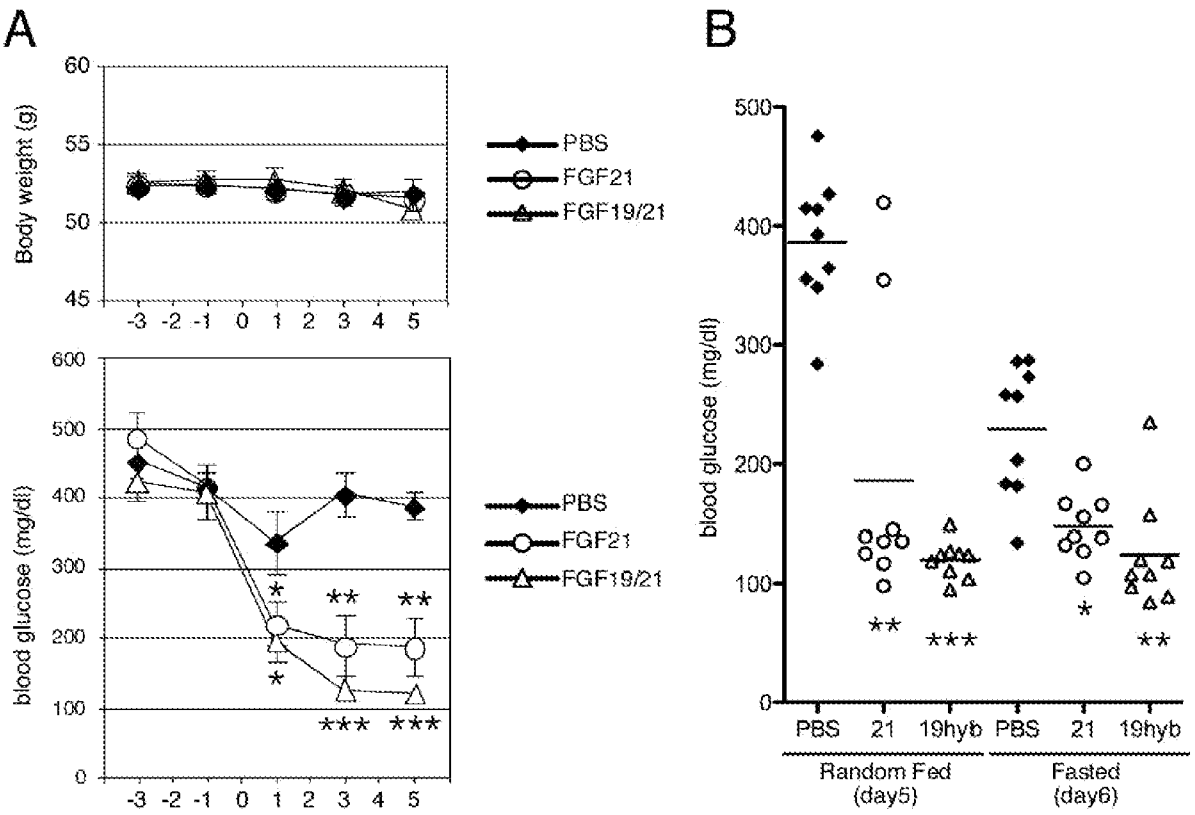

A
Body weight (g)
60
55
50
45
-3 -2 -1 0 1 2 3 4 5
PBS
FGF21
FGF19/21
blood glucose (mg/dl)
600
500
400
300
200
100
0
-3 -2 -1 0 1 2 3 4 5
*
**
**
*
***
***
PBS
FGF21
FGF19/21
B
blood glucose (mg/dl)
500
400
300
200
100
0
**
***
*
**
PBS
21
19hyb
PBS
21
19hyb
Random Fed
(day5)
Fasted
(day6)

FIG. 7

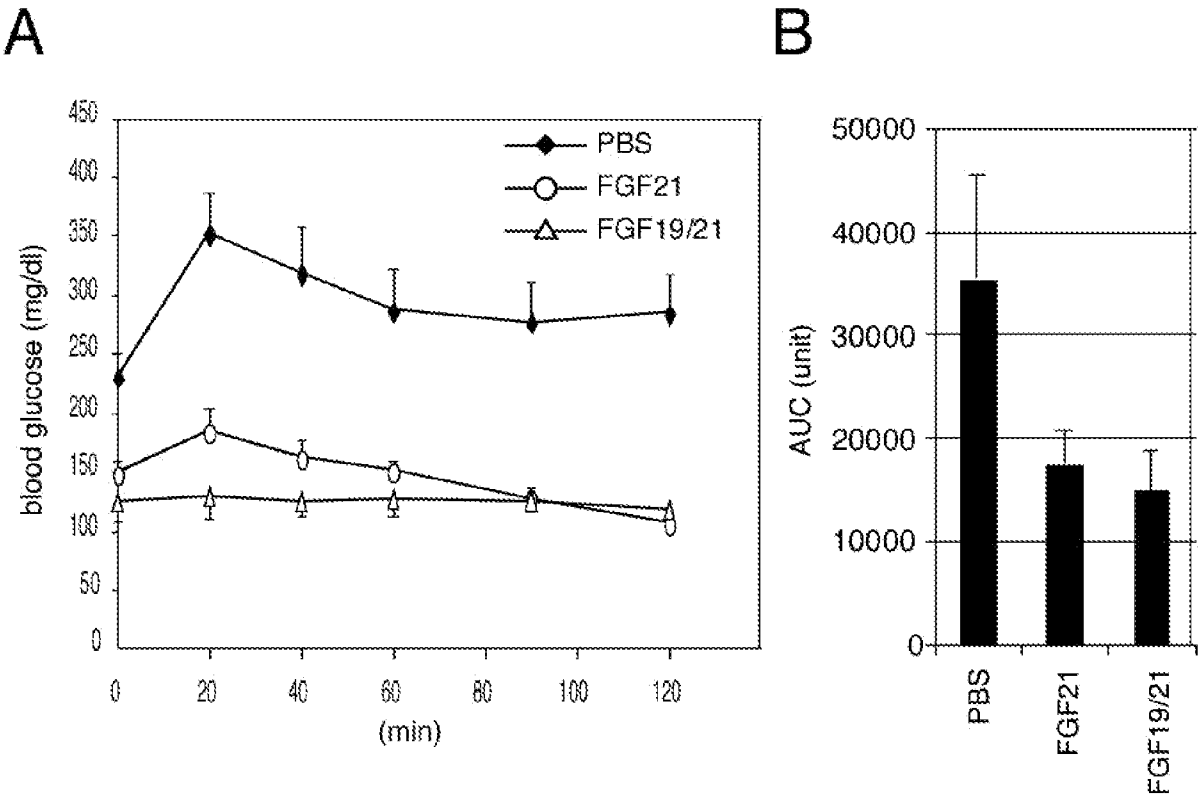

A
B
PBS
FGF21
FGF19/21
blood glucose (mg/dl)
(min)
AUC (unit)
PBS
FGF21
FGF19/21

FIG. 8

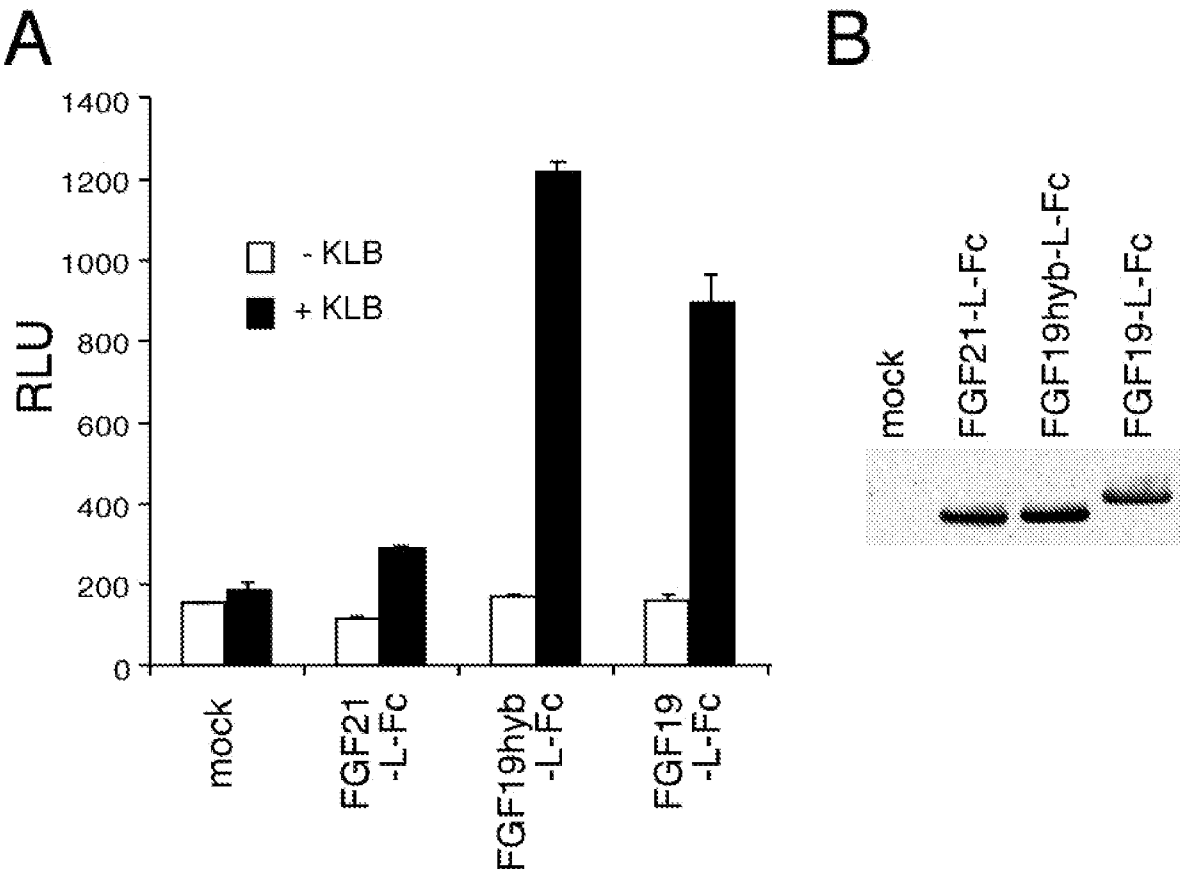

A
B
1400
1200
1000
800
600
400
200
0
RLU
- KLB
+ KLB
mock
FGF21 -L-Fc
FGF19hyb -L-Fc
FGF19 -L-Fc
mock
FGF21-L-Fc
FGF19hyb-L-Fc
FGF19-L-Fc

FIG. 9

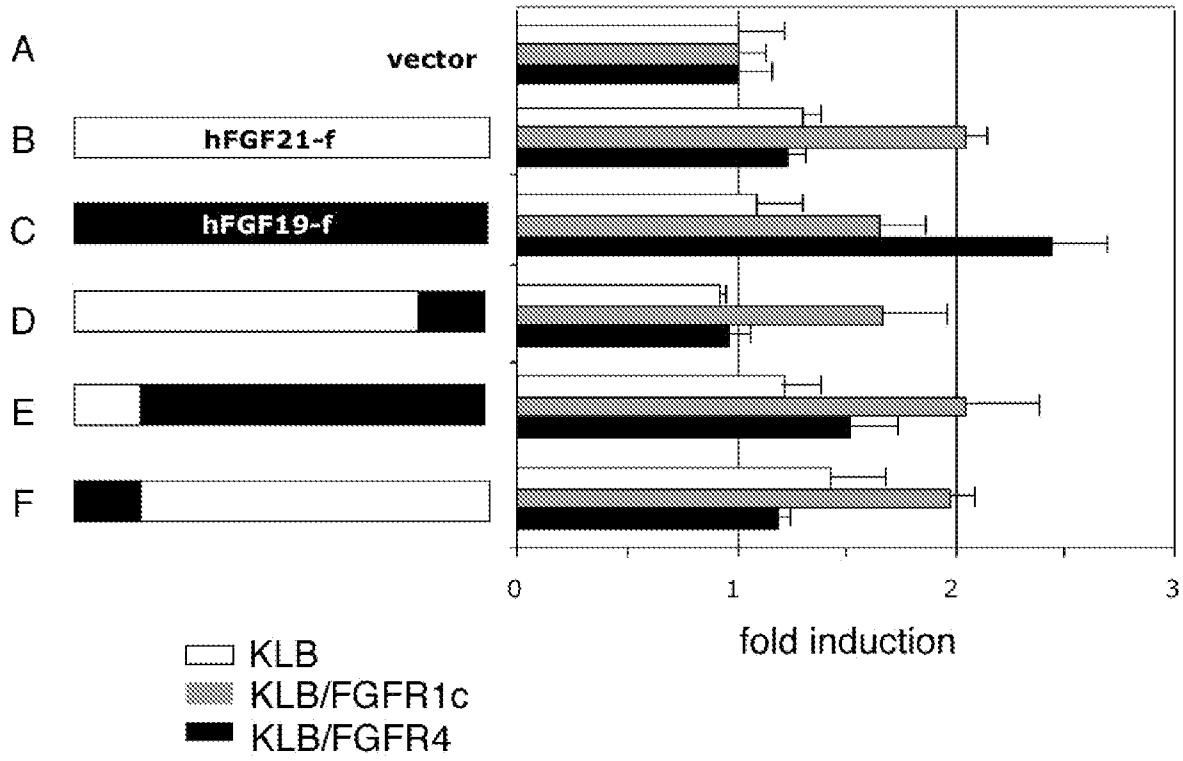

A
vector
B
hFGF21-f
C
hFGF19-f
D
E
F
0
1
2
3
fold induction
KLB
KLB/FGFR1c
KLB/FGFR4

FIG. 10

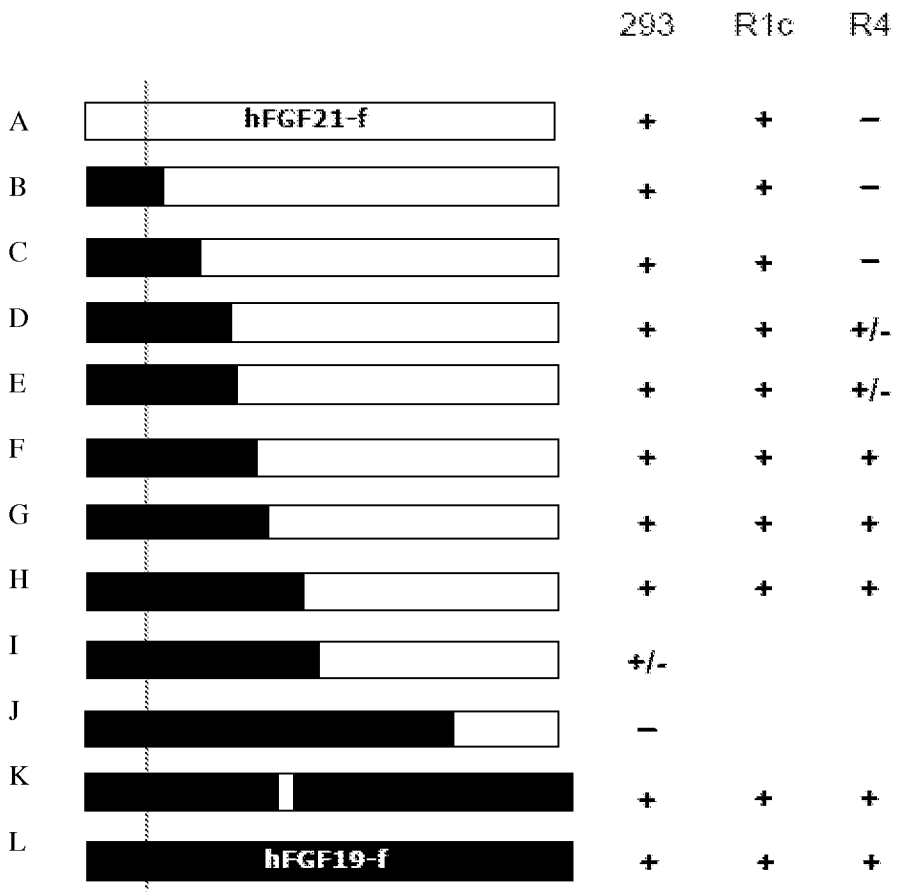


FIG. 11

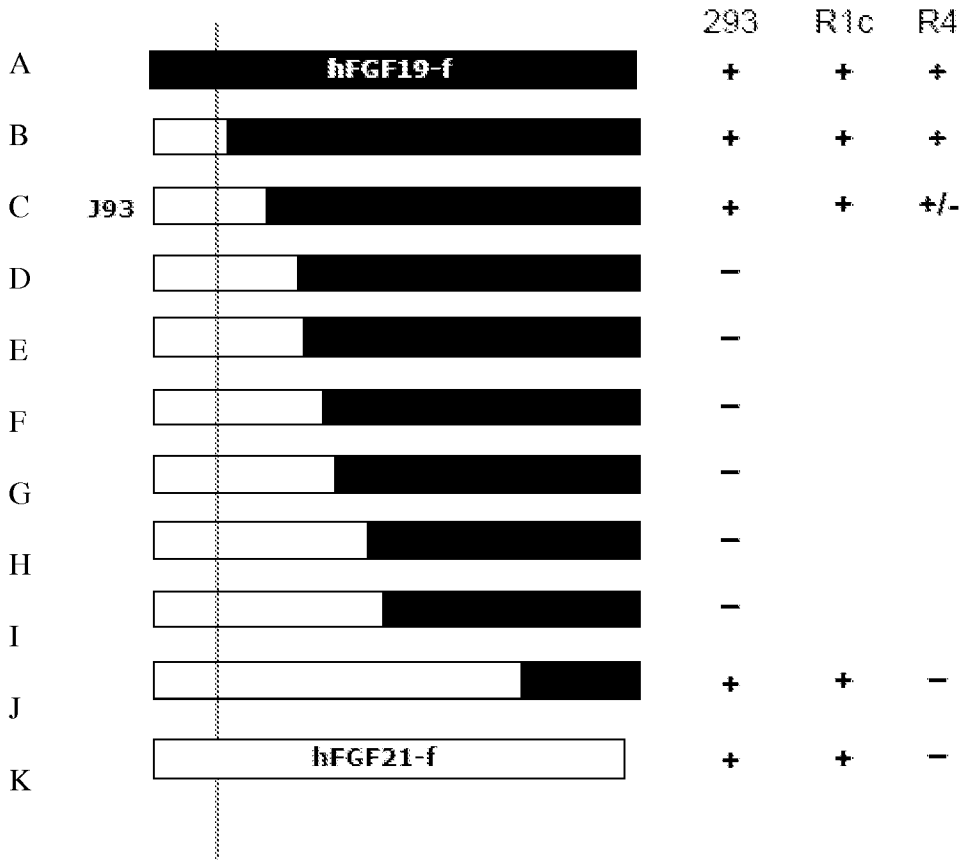


FIG. 12

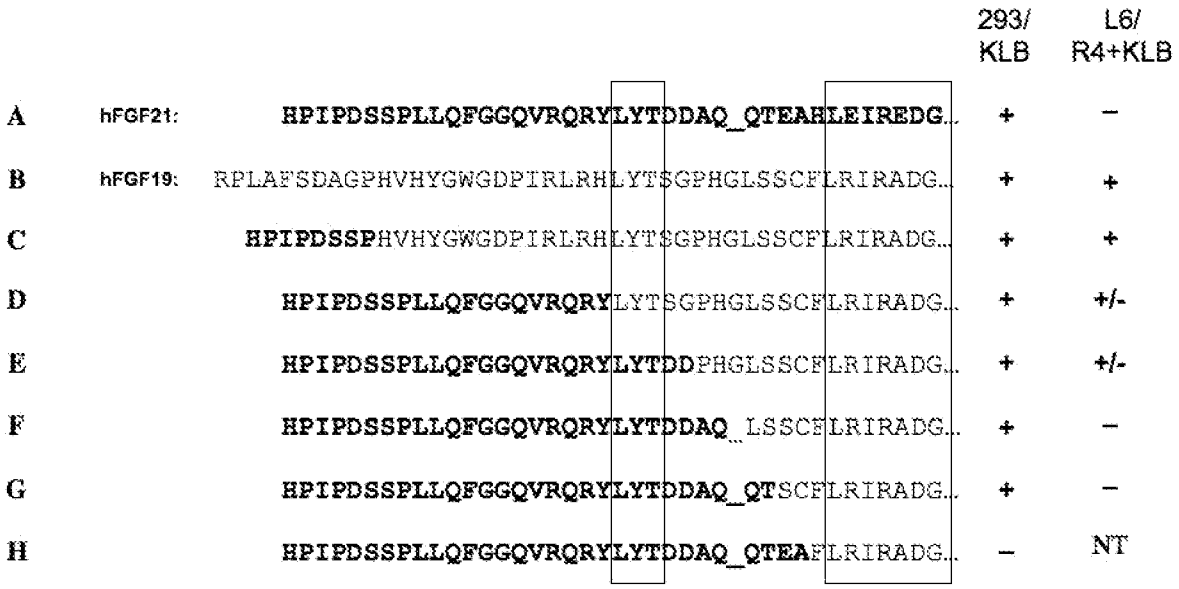

293/ KLB
L6/ R4+KLB
A hFGF21: HPIPDSSPLLQFGGQVRQRYLYTDDAQ_QTEAHLEIREDG... + −
B hFGF19: RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADG... + +
C HPIPDSSPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADG... + +
D HPIPDSSPLLQFGGQVRQRYLYTSGPHGLSSCFLRIRADG... + +/-
E HPIPDSSPLLQFGGQVRQRYLYTDDPHGLSSCFLRIRADG... + +/-
F HPIPDSSPLLQFGGQVRQRYLYTDDAQ__LSSCFLRIRADG... + −
G HPIPDSSPLLQFGGQVRQRYLYTDDAQ_QTSCFLRIRADG... + −
H HPIPDSSPLLQFGGQVRQRYLYTDDAQ_QTEAFLRIRADG... − NT

FIG. 13

		293/KLB	L6/KLB+ R1c	L6/KLB+ R4
hFGF21:	**HPIPDSSPLLQFGGQVRQRYLYTDDAQ_QTEAHLEIREDG**...	+	+	−
hFGF19:	RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADG...	+	+	+
A	**HPIPDSSPLLQFGGQVRQRY**LYTSGPHGLSSCFLRIRADG...	+	+	+/-
B	**HPIPDSSPLLQFGGQVRQRYLYTDD**PHGLSSCFLRIRADG...	+	+	+/-
C	**HPIPDSSPLLQFGGQVRQRYLYTDDAQ_**LSSCFLRIRADG...	+	+	−
D	**HPIPDSSPLLQFGGQVRQRYLYTDDAQ_QT**SCFLRIRADG...	+	+	−
E	**HPIPDSSPLLQFGGQVRQRYLYTDDAQ_QTEA**FLRIRADG...	−	NT	NT
F	RPLAFSDAGP**LLQFGGQVRQRY**LYTSGPHGLSSCFLRIRADG...	+	+	+/-
G	RPLAFSDAGP**LLQFGGQVRQRYLYTDD**PHGLSSCFLRIRADG...	+/-	NT	NT
H	RPLAFSDAGP**LLQFGGQVRQRYLYTDDAQ**GLSSCFLRIRADG...	−	NT	NT
I	RPLAFSDAGP**LLQFGGQVRQRYLYTDDAQ_**LSSCFLRIRADG...	−	NT	NT
J	RPLAFSDAGP**LLQFGGQVRQRYLYTDDAQ_QT**SCFLRIRADG...	−	NT	NT
K	RPLAFSDAGP**LLQFGGQVRQRYLYTDDAQ_QTEA**FLRIRADG...	−	NT	NT
L	RPLAFSDAGP**LLQFGGQVRQRYLYTDDAQ_QTEAHLEIREDG**...	+	+	−

FIG. 14

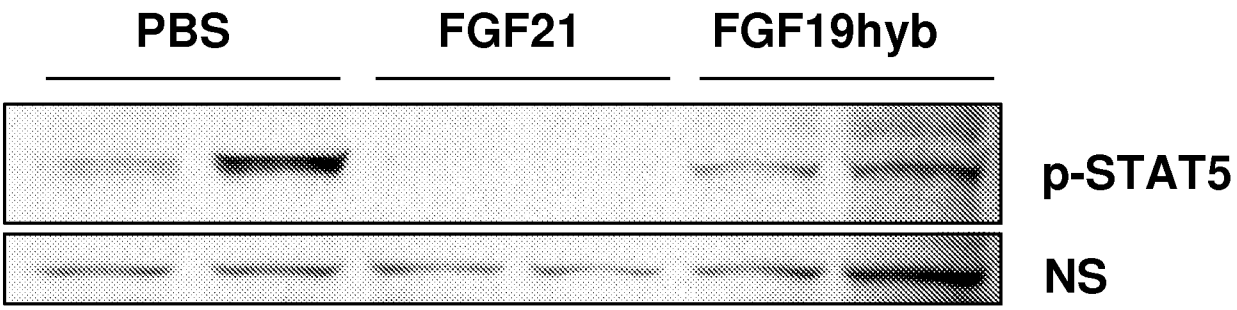


FIG. 15

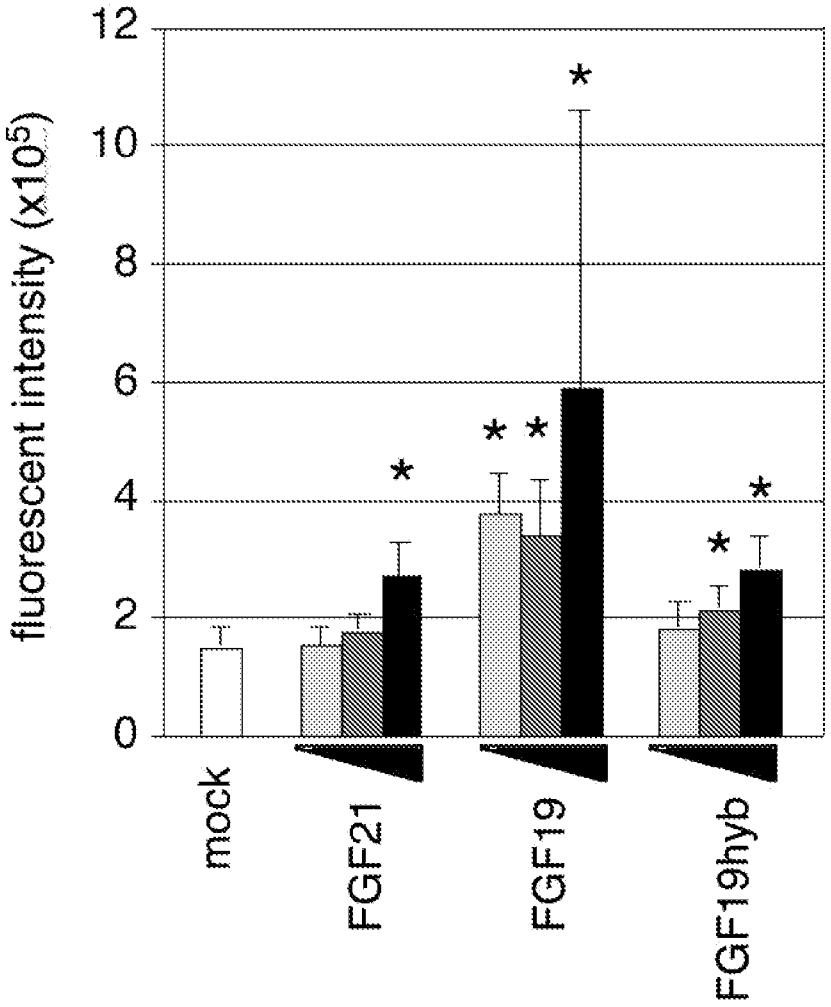

fluorescent intensity (x10^5)
12
10
8
6
4
2
0
*
* *
*
* *
mock
FGF21
FGF19
FGF19hyb

FIG. 16

FIG. 17

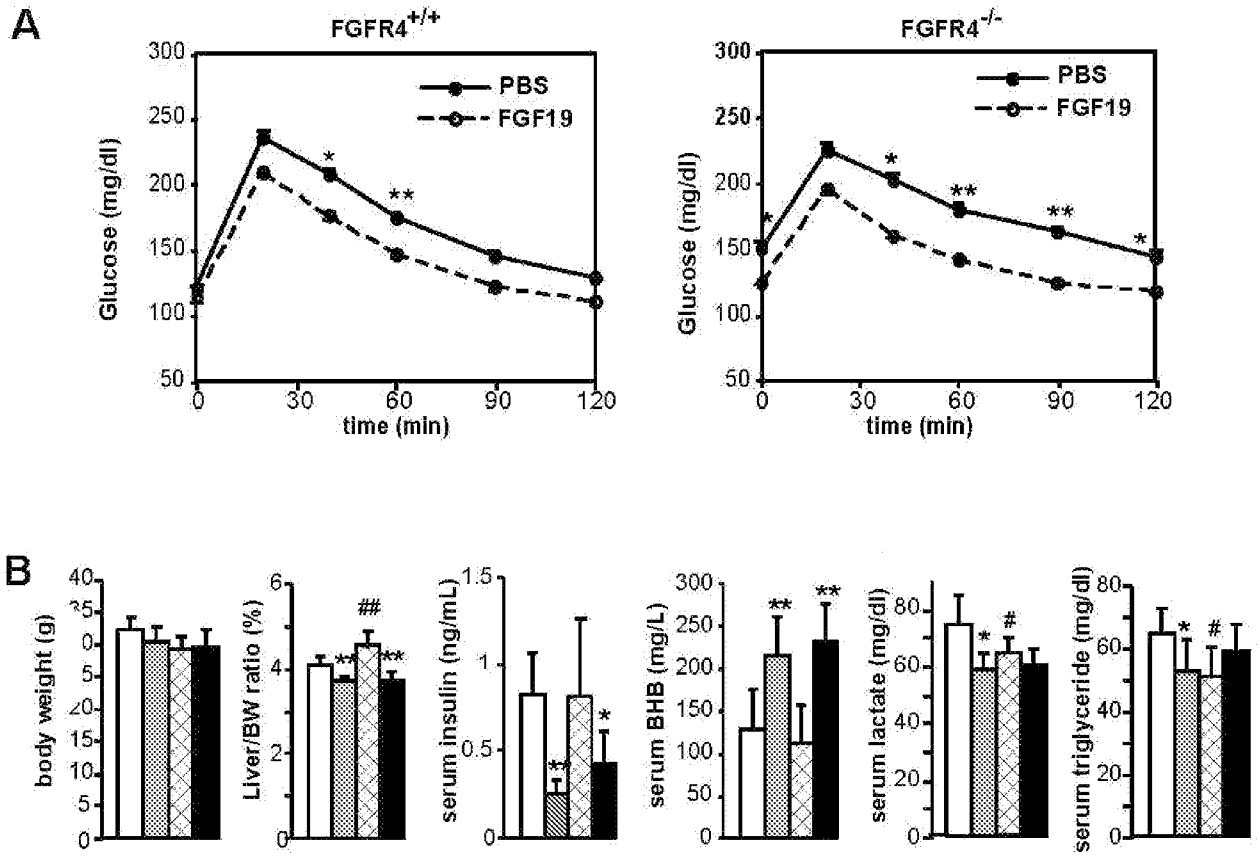

A
FGFR4+/+
FGFR4-/-
PBS
FGF19
Glucose (mg/dl)
time (min)
B
body weight (g)
Liver/BW ratio (%)
serum insulin (ng/mL)
serum BHB (mg/L)
serum lactate (mg/dl)
serum triglyceride (mg/dl)

FIG. 17

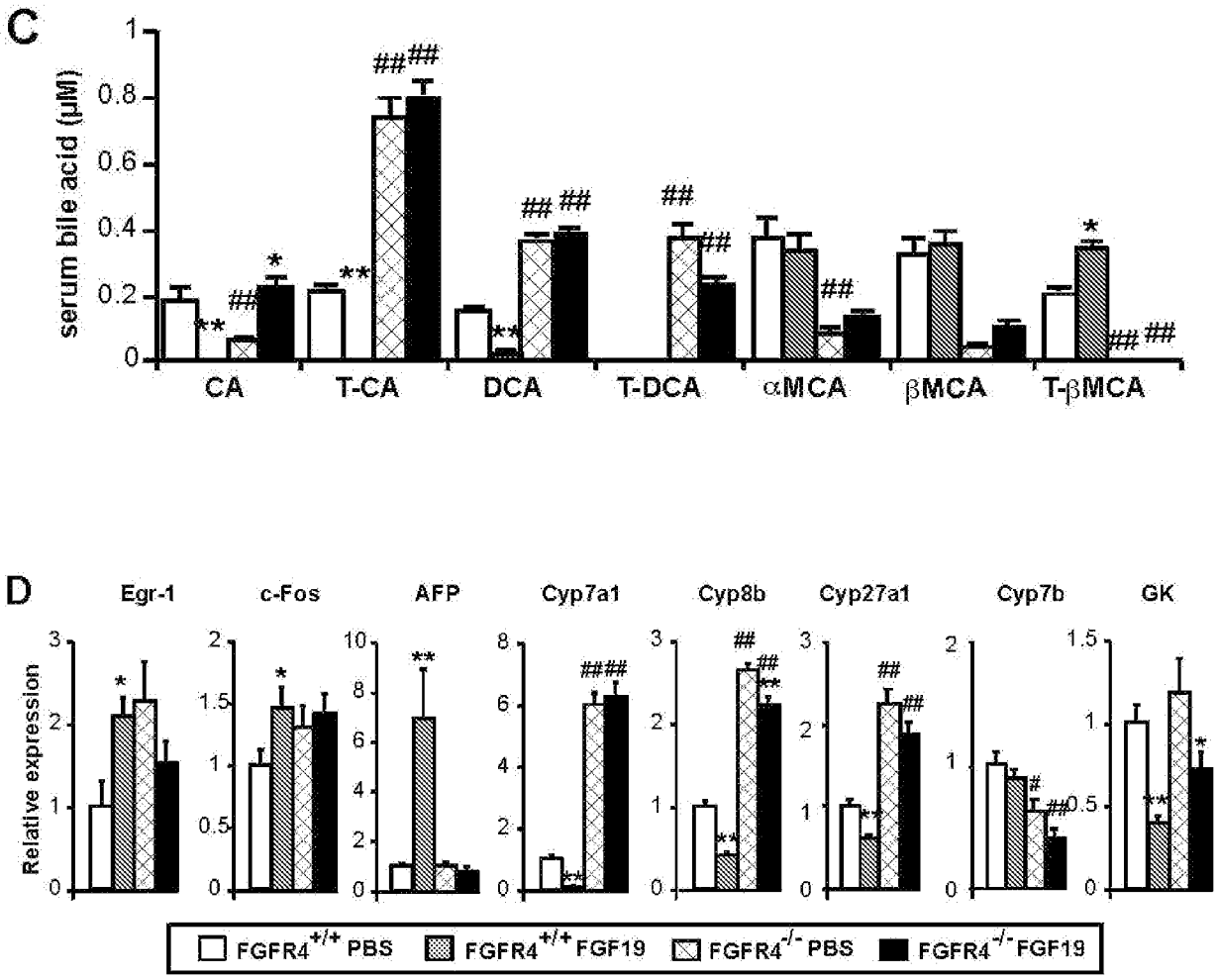

C
serum bile acid (μM)
CA
T-CA
DCA
T-DCA
αMCA
βMCA
T-βMCA
D
Egr-1
c-Fos
AFP
Cyp7a1
Cyp8b
Cyp27a1
Cyp7b
GK
Relative expression
FGFR4+/+ PBS
FGFR4+/+ FGF19
FGFR4-/- PBS
FGFR4-/- FGF19

FIG. 18

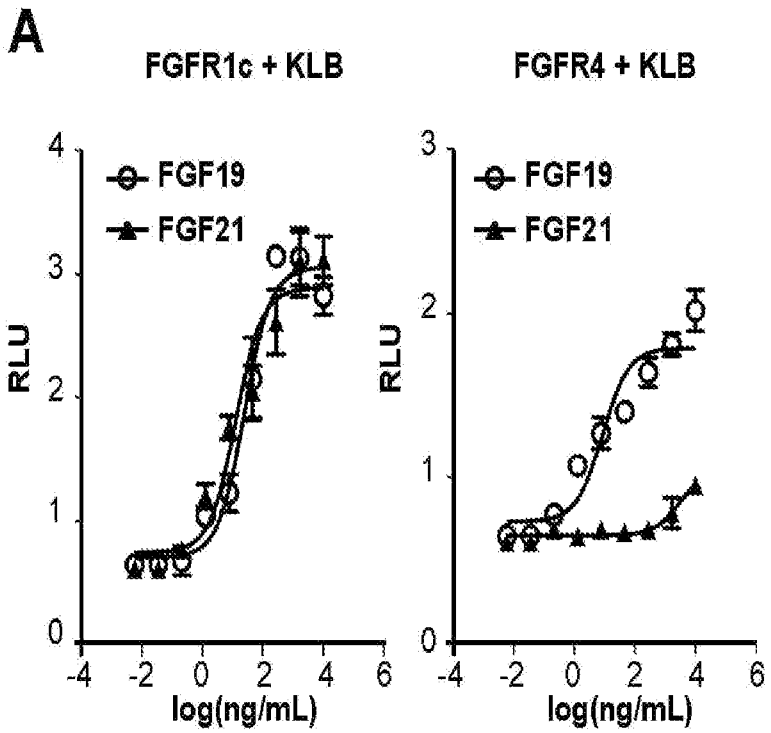
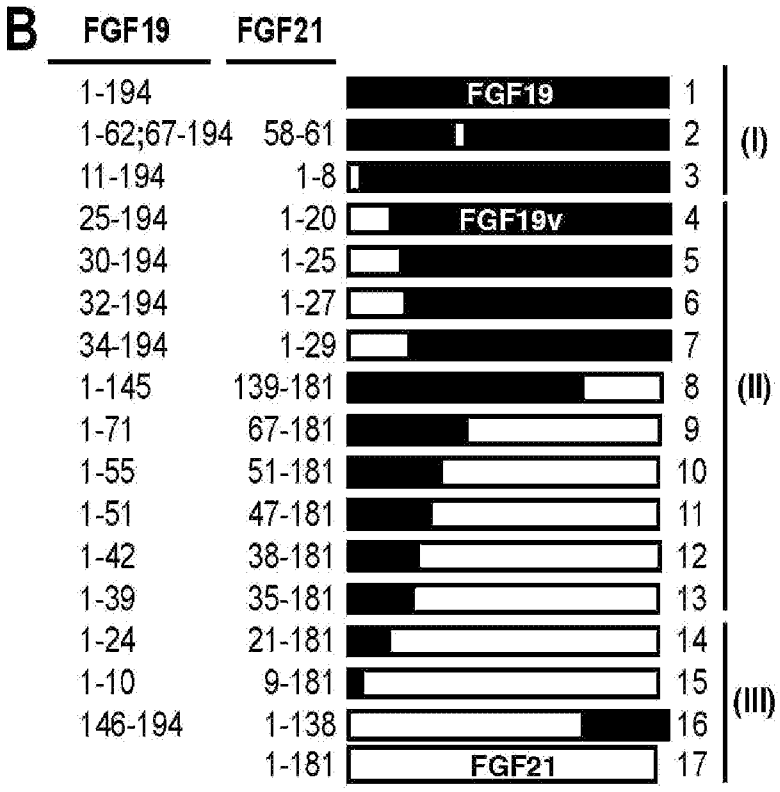


FIG. 18


B
FGF19
FGF21
1-194
1-62;67-194 58-61
11-194 1-8
25-194 1-20
30-194 1-25
32-194 1-27
34-194 1-29
1-145 139-181
1-71 67-181
1-55 51-181
1-51 47-181
1-42 38-181
1-39 35-181
1-24 21-181
1-10 9-181
146-194 1-138
1-181
FGF19
FGF19v
FGF21
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
(I)
(II)
(III)

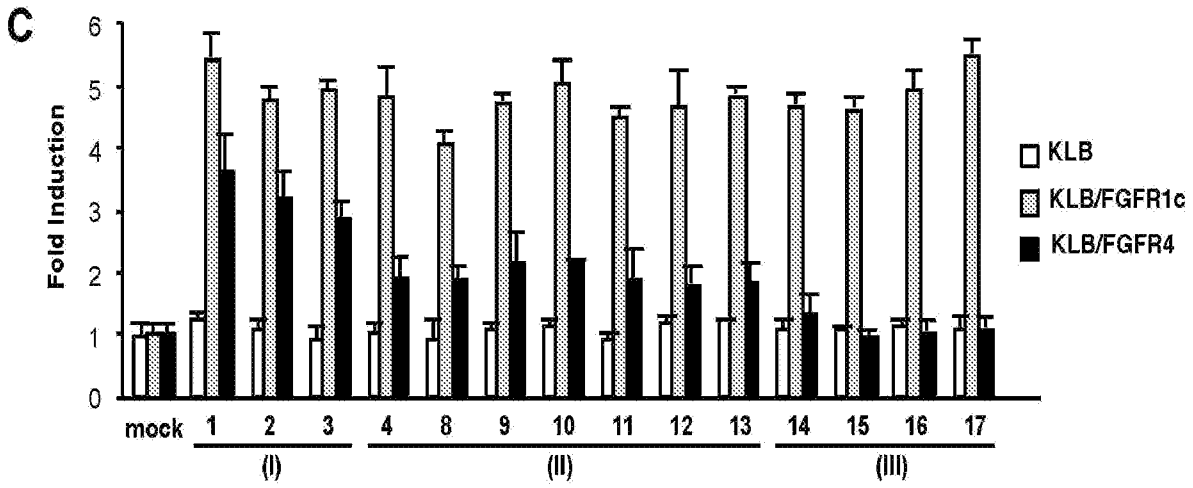

C
Fold Induction
0
1
2
3
4
5
6
mock
1
2
3
4
8
9
10
11
12
13
14
15
16
17
(I)
(II)
(III)
KLB
KLB/FGFR1c
KLB/FGFR4

FIG. 18

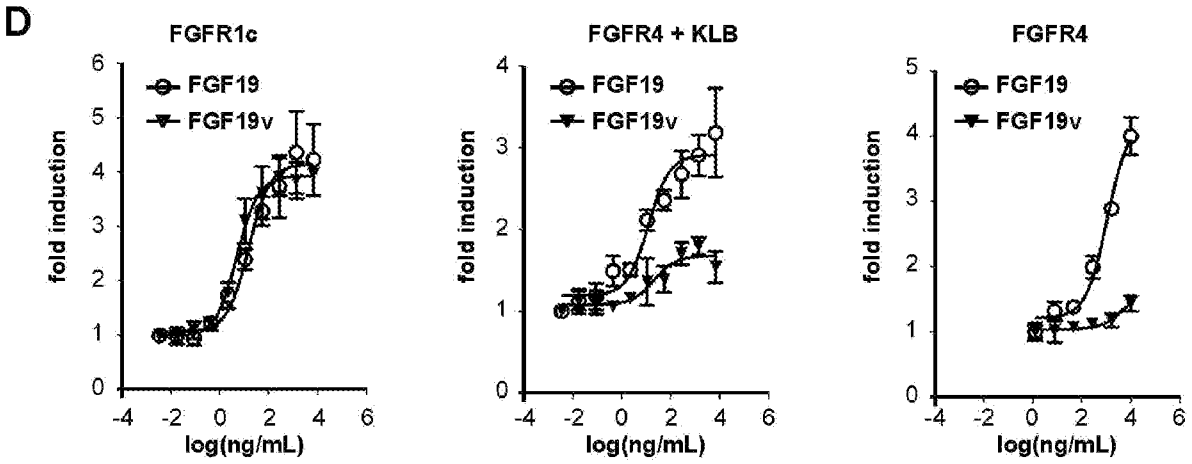

D
FGFR1c
FGF19
FGF19v
fold induction
log(ng/mL)
FGFR4 + KLB
FGF19
FGF19v
fold induction
log(ng/mL)
FGFR4
FGF19
FGF19v
fold induction
log(ng/mL)

FIG. 18

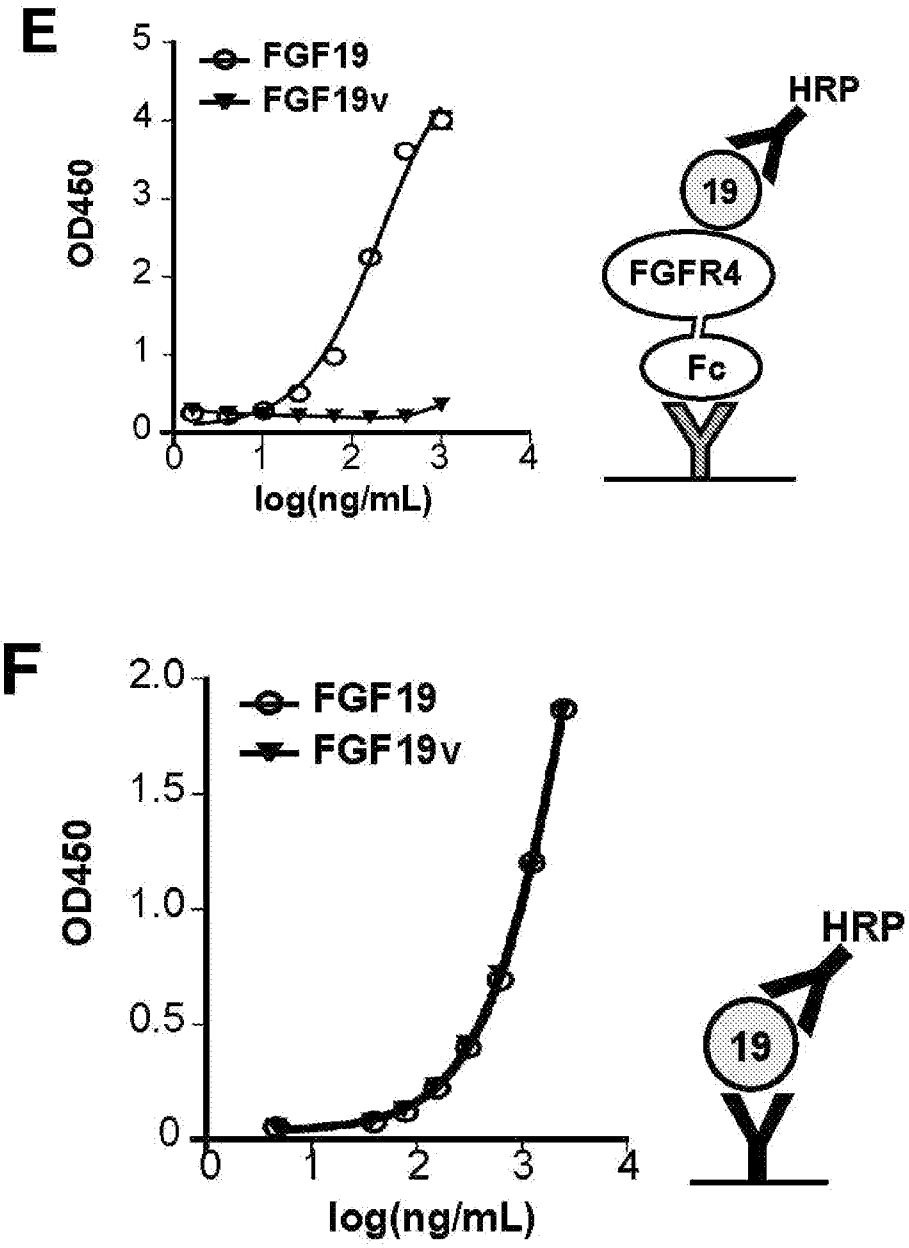

E
FGF19
FGF19v
OD450
log(ng/mL)
HRP
19
FGFR4
Fc
F
FGF19
FGF19v
OD450
log(ng/mL)
HRP
19

FIG. 19

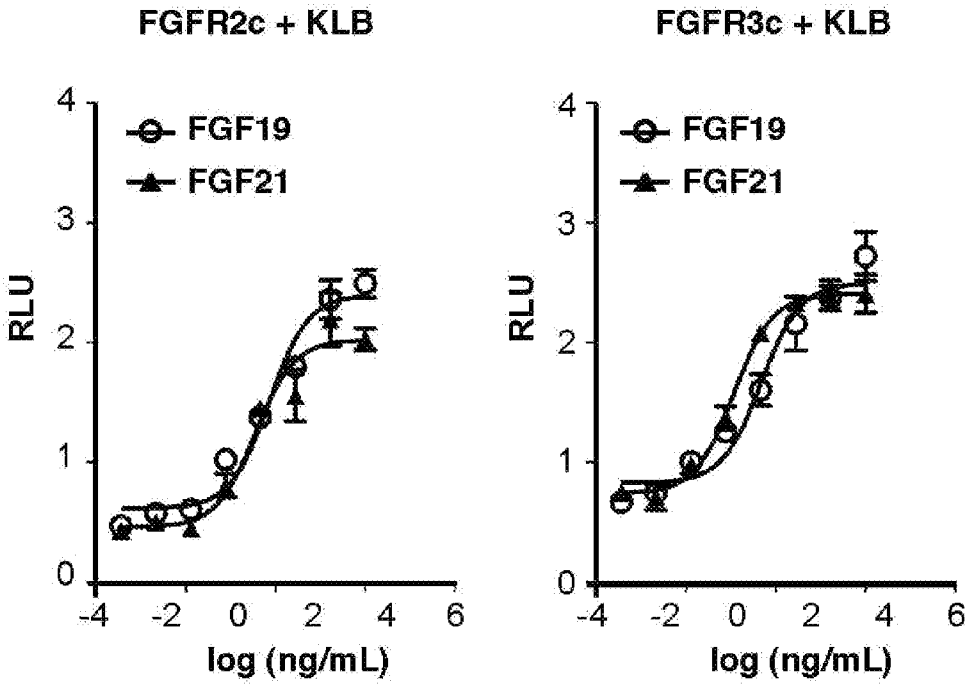

FGFR2c + KLB
FGFR3c + KLB
FGF19
FGF21
RLU
log (ng/mL)
0
1
2
3
4
-4
-2
0
2
4
6

FIG. 20

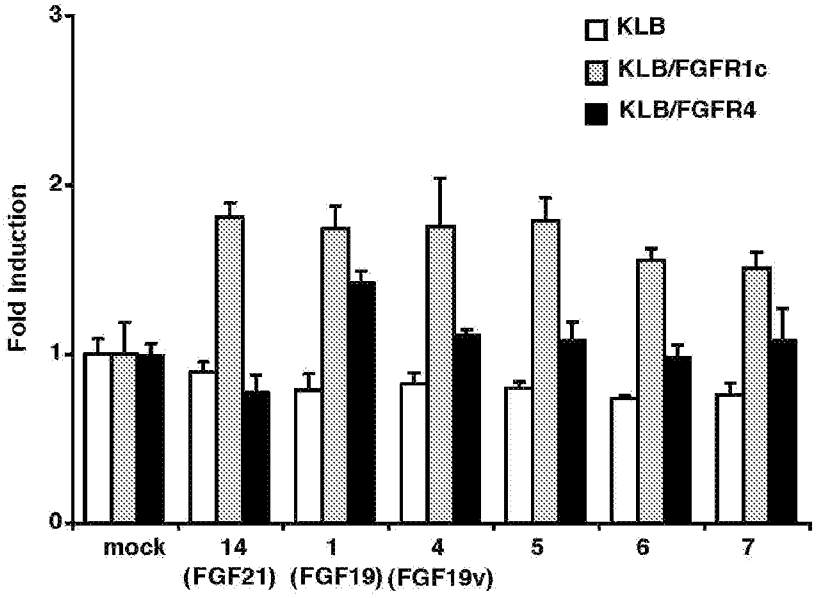

KLB
KLB/FGFR1c
KLB/FGFR4
Fold Induction
0
1
2
3
mock
14
(FGF21)
1
(FGF19)
4
(FGF19v)
5
6
7

FIG. 21

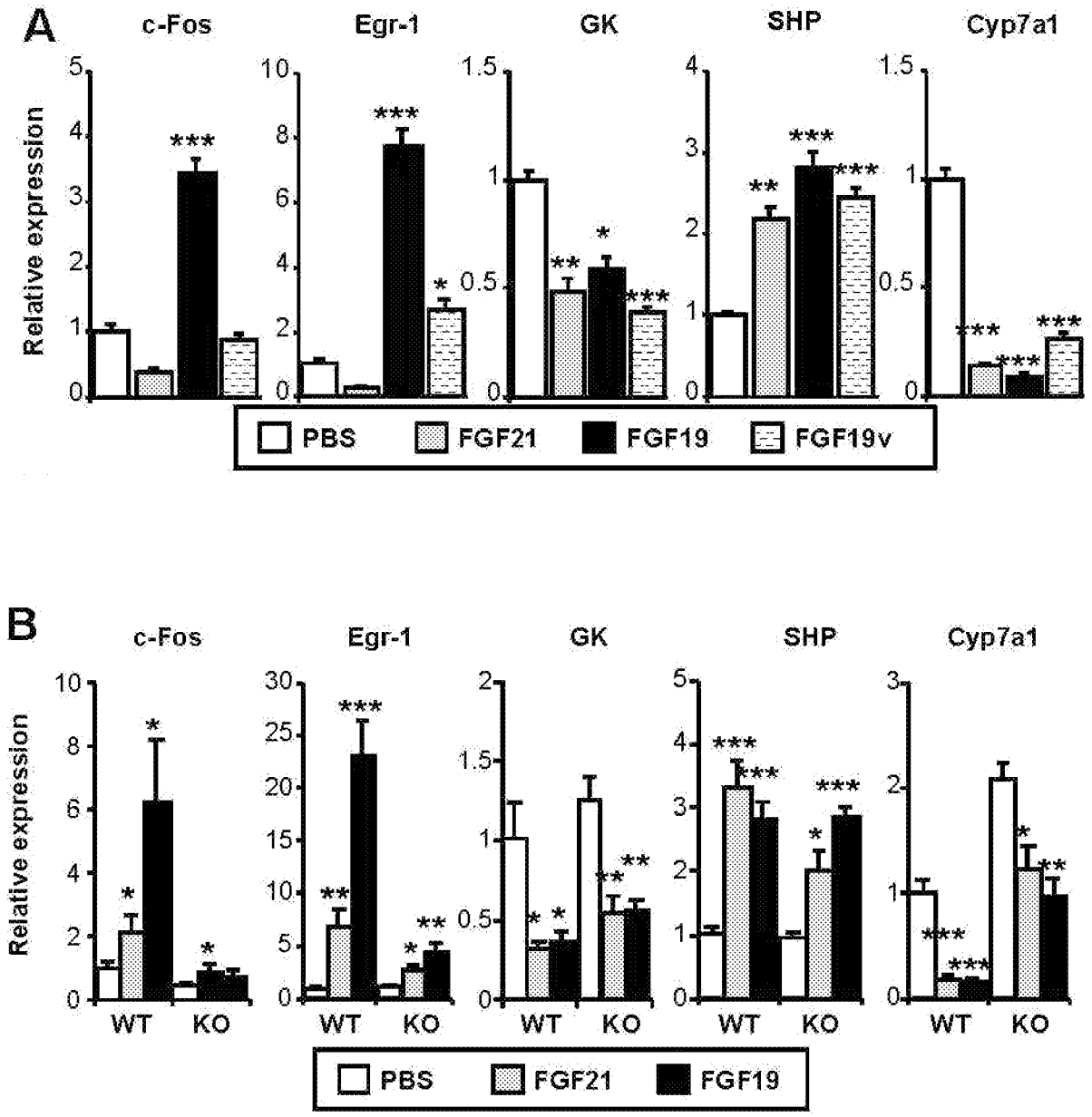

A
c-Fos
Egr-1
GK
SHP
Cyp7a1
Relative expression
PBS
FGF21
FGF19
FGF19v
B
c-Fos
Egr-1
GK
SHP
Cyp7a1
Relative expression
WT
KO
PBS
FGF21
FGF19

FIG. 21

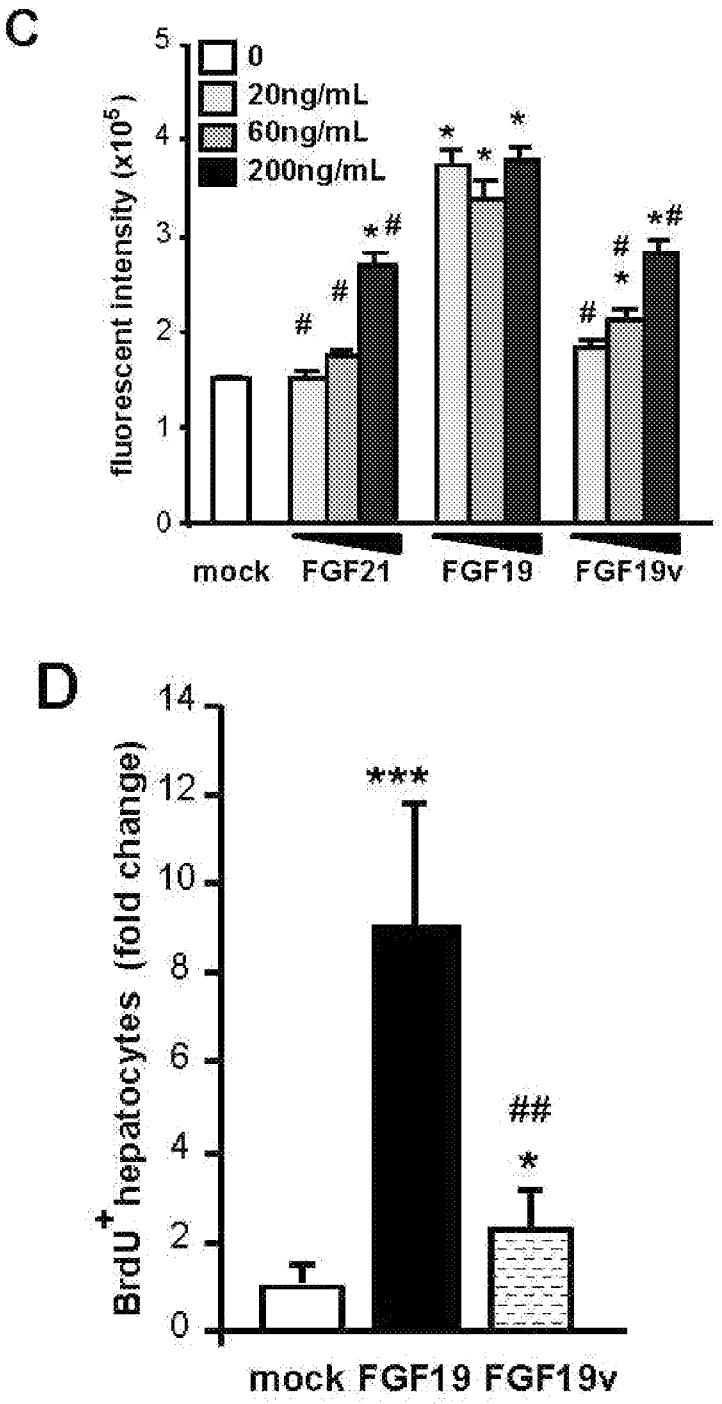

C
fluorescent intensity (x10^5)
0
20ng/mL
60ng/mL
200ng/mL
mock
FGF21
FGF19
FGF19v
D
BrdU+ hepatocytes (fold change)
mock FGF19 FGF19v

FIG. 21

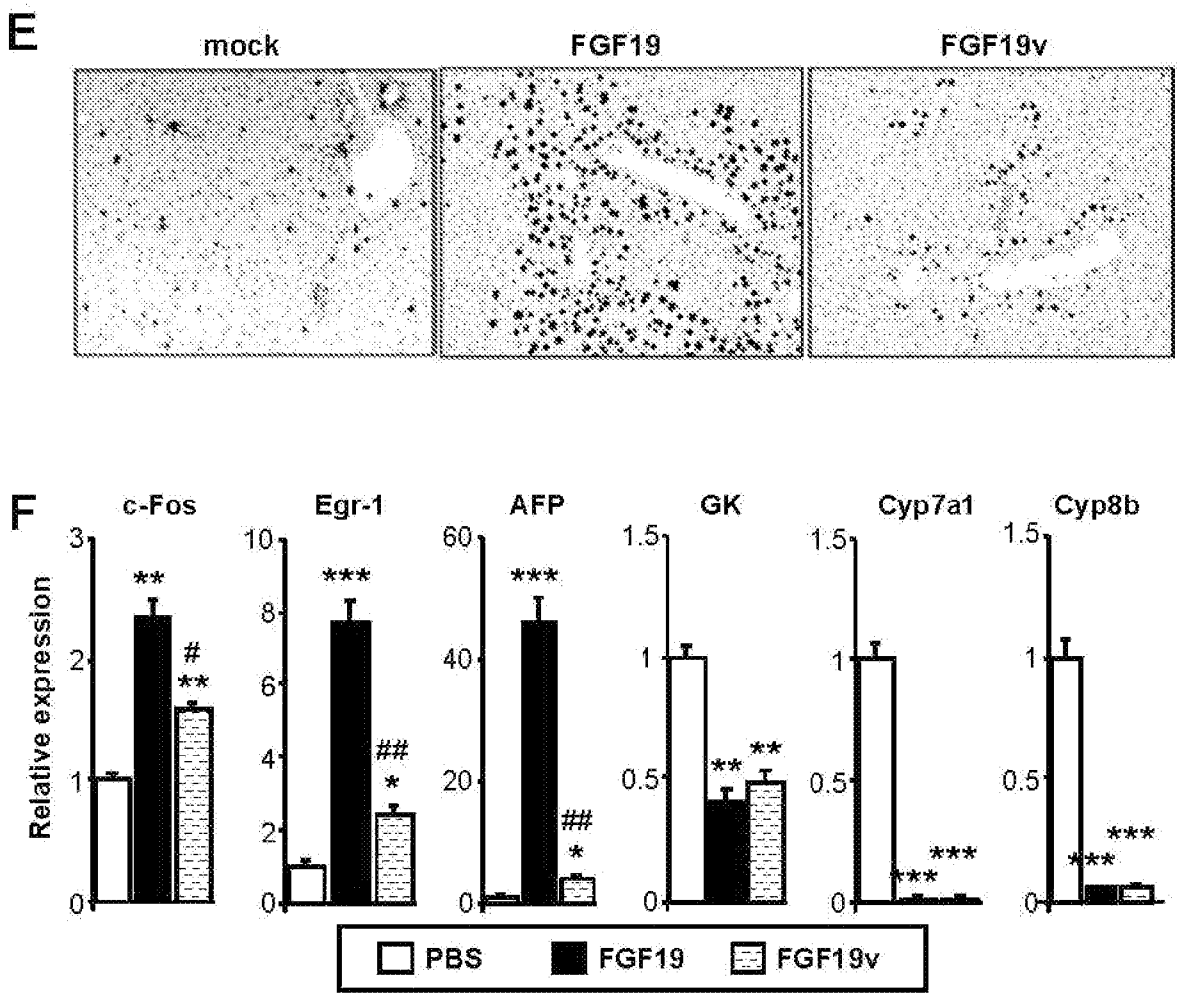

E
mock
FGF19
FGF19v
F
c-Fos
Egr-1
AFP
GK
Cyp7a1
Cyp8b
Relative expression
PBS
FGF19
FGF19v

FIG. 22

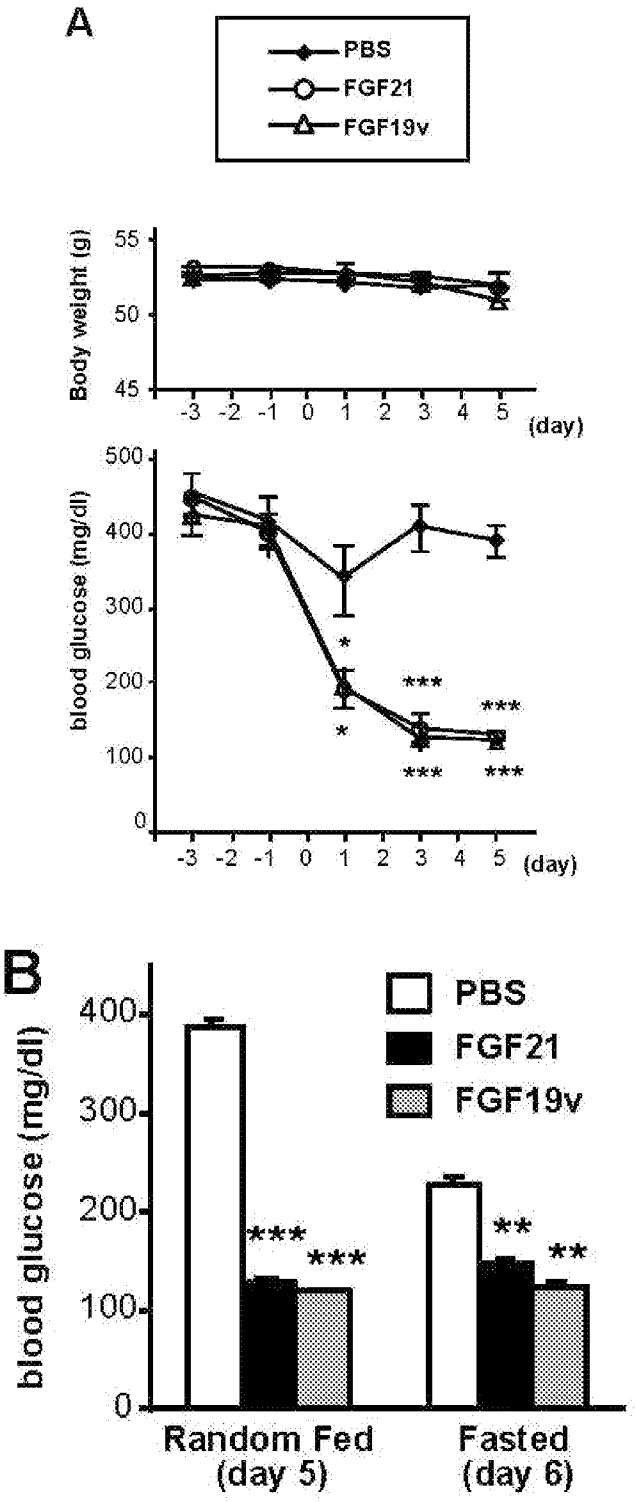

A
PBS
FGF21
FGF19v
Body weight (g)
55
50
45
-3 -2 -1 0 1 2 3 4 5
(day)
blood glucose (mg/dl)
500
400
300
200
100
0
*
***
***
*
***
***
-3 -2 -1 0 1 2 3 4 5
(day)
B
blood glucose (mg/dl)
400
300
200
100
0
PBS
FGF21
FGF19v
***
***
**
**
Random Fed
(day 5)
Fasted
(day 6)

FIG. 22

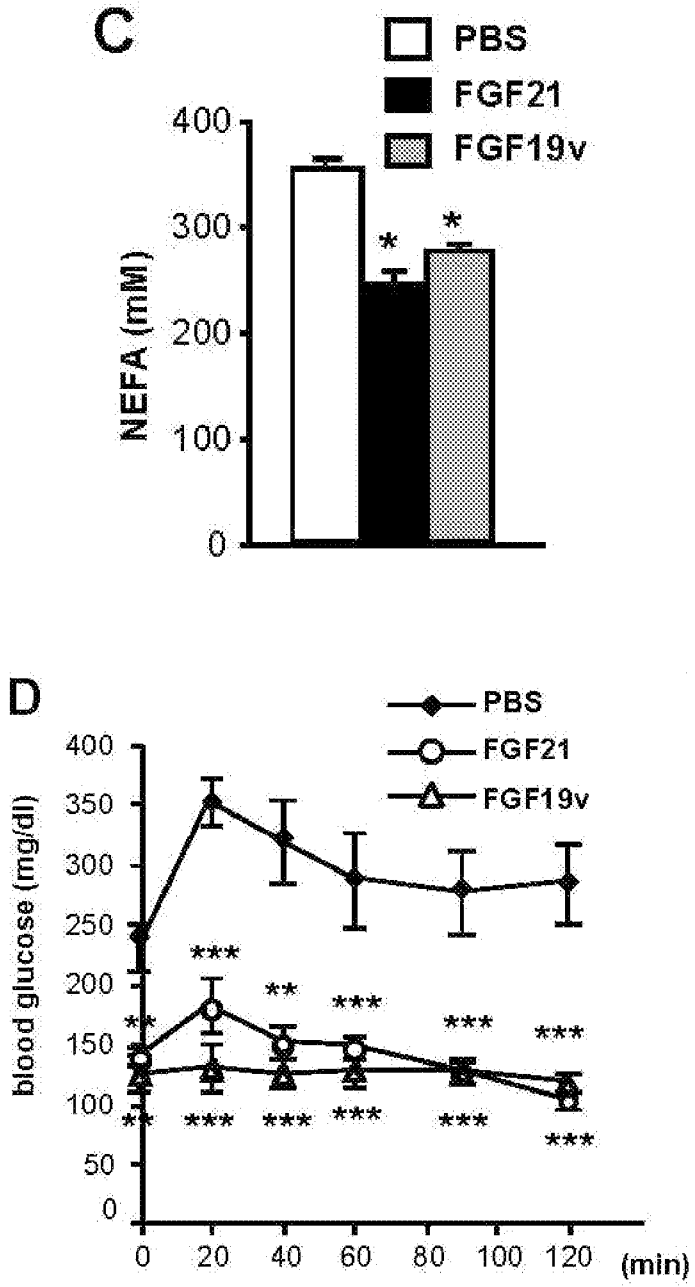
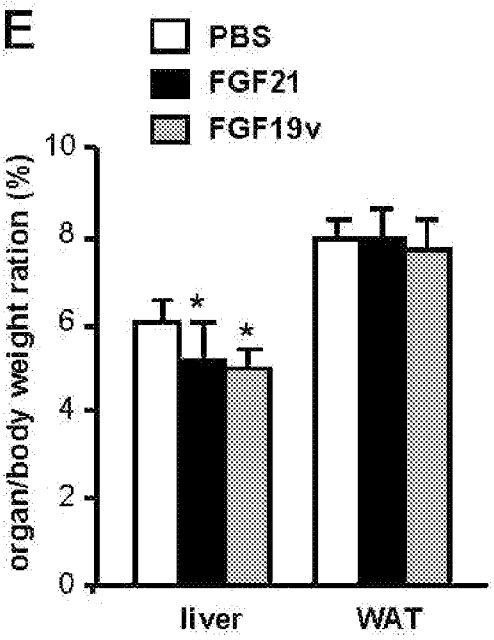
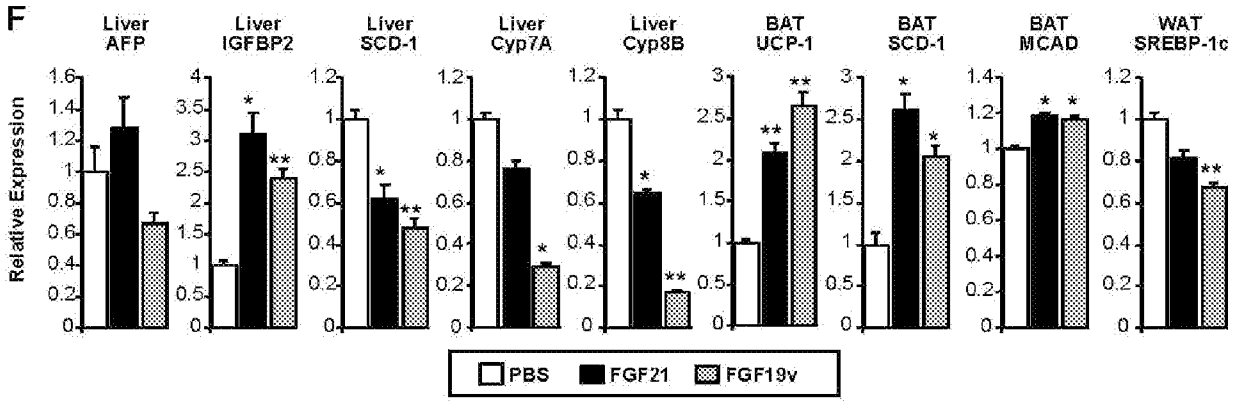

C
PBS
FGF21
FGF19v
NEFA (mM)
400
300
200
100
0
*
*
D
PBS
FGF21
FGF19v
blood glucose (mg/dl)
400
350
300
250
200
150
100
50
0
0
20
40
60
80
100
120
(min)
***
**
***
**
***
***
**
***
***
***
***
***

FIG. 22


E
PBS
FGF21
FGF19v
organ/body weight ration (%)
10
8
6
4
2
0
*
*
liver
WAT


F
Liver AFP
Liver IGFBP2
Liver SCD-1
Liver Cyp7A
Liver Cyp8B
BAT UCP-1
BAT SCD-1
BAT MCAD
WAT SREBP-1c
Relative Expression
PBS
FGF21
FGF19v