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(54) **GROWTH FACTOR ANALOGS**

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(57) **ABSTRACT**

The invention provides synthetic heparin-binding growth factor analogs of formulas I or II as given in the specification, having two peptide chains branched from a dipeptide branch moiety composed of at least one and preferably two trifunctional amino acid residues, which peptide chain or chains bind a heparin-binding growth factor receptor. The synthetic heparin-binding growth factor analogs are useful as pharmaceutical agents, soluble biologics or as surface coatings for medical devices.

GROWTH FACTOR ANALOGS

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application is a divisional application of U.S. patent application Ser. No. 11/051,292, entitled "Growth Factor Analogs," filed on Feb. 4, 2005. This application also claims the benefit of the filing of U.S. Provisional Patent Application Ser. No. 60/542,272, entitled "Growth Factor Analogs", filed on Feb. 4, 2004, and the specification thereof is incorporated herein by reference.

BACKGROUND OF THE INVENTION

[0002] 1. Field of the Invention (Technical Field)

[0003] The invention relates to the field of synthetic peptides and analogs of heparin-binding growth factors. The invention further relates to the clinical uses of such analogs as soluble drugs and as coatings for medical devices.

[0004] 2. Description of Related Art

[0005] Note that the following discussion refers to a number of publications by author(s) and year of publication, and that due to recent publication dates certain publications are not to be considered as prior art vis-a-vis the present invention. Discussion of such publications herein is given for more complete background and is not to be construed as an admission that such publications are prior art for patentability determination purposes.

[0006] The heparin-binding growth factors (HBGFs) constitute a large class of growth factors that includes the 23 fibroblast growth factors identified to date (FGFs 1-23), HBBM (heparin-binding brain mitogen), HB-GAF (heparin-binding growth associated factor), HB-EGF (heparin-binding EGF-like factor) HB-GAM (heparin-binding growth associated molecule), TGF-α (transforming growth factor-α), TGF-βs (transforming growth factor-βs), PDGF (platelet-derived growth factor), EGF (epidermal growth factor), VEGF (vascular endothelial growth factor), IGF-1 (insulin-like growth factor-1), IGF-2 (insulin-like growth factor-2), HGF (hepatocyte growth factor), IL-1 (interleukin-1), IL-2 (interleukin-2), IFN-α (interferon-α), IFN-γ (interferon-γ), TNF-α (tumor necrosis factor-α), SDGF (Schwannoma-derived growth factor) and the many other growth factors, cytokines, lymphokines and chemokines that have an affinity for heparin.

[0007] Peptides from natural HBGFs that bind heparin-binding growth factor receptors have been identified. See for example Ray et al., Proc. Natl. Acad. Sci. USA 94:7047-7052 (1997). These authors demonstrated that two amino acid sequences from FGF-2 are sufficient to block the mitogenic activity of FGF-2 on neural progenitor cells. The first peptide is a ten amino acid sequence, from amino acids 65-74, the second peptide extends from amino acids 115-129.

[0008] In an alternative approach, an artificial peptide that binds a heparin-binding growth factor receptor was identified by a phage display method. Ballinger et al., Nature BioTechnology 17:1199-1204 (1999) used this technique to isolate a 28 amino acid peptide called C19, which binds FGF-2 receptors, but by itself fails to stimulate biological activity. The peptide has no amino acid sequence identity with any known FGF.

[0009] HBGFs useful in prevention or therapy of a wide range of diseases and disorders may be purified from natural sources or produced by recombinant DNA methods, however, such preparations are expensive and generally difficult to prepare.

[0010] Some efforts have been made to generate heparin-binding growth factor analogs. For example, natural PDGF occurs as an A chain and a B chain arranged in head-to-head (AA or BB) homodimers, or (AB or BA) heterodimers. Thus, U.S. Pat. No. 6,350,731 to Jehanli et al. discloses PDGF analogs in which two synthetic PDGF receptor-binding domains are covalently linked through a polyglycine or an N-(4-carboxy-cyclohexylmethyl)-maleimide (SMCC) chain to mimic the natural active polypeptide dimer.

[0011] U.S. Pat. No. 6,235,716 to Ben-Sasson discloses analogs of angiogenic factors. The analogs are branched multivalent ligands that include two or more angiogenic homology regions connected by a multilinker backbone.

[0012] Other workers have described compositions that include homologs and analogs of fibroblast growth factors (FGFs). See for example U.S. Pat. No. 5,679,673 to Lappi and Baird; U.S. Pat. No. 5,989,866 to Deisher et al. and U.S. Pat. No. 6,294,359 to Fiddes et al. These disclosures relate to FGF homologs or analogs that are either conjugated to a toxic moiety and are targeted to the FGF receptor-bearing cells; or are homologs or analogs that modulate the biological pathways through the signal transduced by the FGF receptor upon binding by the FGF homolog or analog.

[0013] A series of patent applications to Kochendoerfer et al. disclose polymer-modified proteins, including synthetic chemokines and erythropoiesis stimulating proteins. See, for example, International Publications WO 02/04105, WO 02/19963 and WO 02/20033. These include chemically ligated peptide segments of a polypeptide chain of a synthetic erythropoiesis protein, such that a polypeptide chain results, with a water soluble polymer attached at one or more glycosylation sites on the protein. These applications also disclose synthetic chemokines, which are also polymer modified, and are asserted to be antagonists. Other erythropoietin mimetics are known, such as those disclosed in U.S. Pat. Nos. 5,773,569 and 5,830,851 to Wrighton et al.

[0014] International Publication WO 00/18921 to Ballinger and Kavanaugh discloses a composition consisting of fusion proteins having FGF receptor affinity linked to an "oligomerization domain", either directly or through a linking group. The oligomerization domain ranges in length from about 20 to 300 residues, and includes constructs such as transcription factors, Fc portions of IgG, leucine zippers and the like. The oligomerization domains disclosed are homodimeric domains, wherein a single FGF receptor affinity fusion protein is linked to a single domain, such as a leucine zipper, which in turn is linked to a similar molecule by means of cysteine residues at both the amino and carboxy termini of the leucine zippers, such that two parallel leucine zippers, each with a single FGF receptor affinity fusion protein, are cross-linked by means of disulfide bonds. It is also disclosed that fusion proteins may include a heparin binding domain, such as the use of jun as a multimerization domain, which is asserted to be a heparin binding domain. Thus the compositions disclosed by Ballinger and Kavanaugh are all composed of a single receptor-binding sequence covalently attached to an oligomerization domain, whereby two or more similar oligomerization domains, each with a single receptor-binding sequence, are conjoined by means of either an association

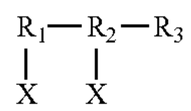
provided by the oligomerization domain, or alternatively, are chemically cross-linked to provide for the covalent bonding of the individual components.

[0015] The above described homologs, analogs, conjugates or ligands each include a receptor-binding domain. However, none of these or other known heparin-binding growth factor analogs provide the advantages described herein below. There is still a need for new peptide analogs of HBGFs, particularly for those that function as agonists. In particular, there is still a need for cost-effective synthetic peptide agonists of heparin-binding growth factor receptors, particularly synthetic heparin-binding growth factor agonists.

[0016] This application is related to U.S. patent application Ser. No. 10/644,703, entitled Synthetic Heparin-Binding Growth Factor Analogs, filed on Aug. 19, 2003, and to U.S. patent application Ser. No. 10/224,268, entitled Synthetic Heparin-Binding Growth Factor Analogs, filed on Aug. 20, 2002, and the specification thereof of each is incorporated herein by reference.

BRIEF SUMMARY OF THE INVENTION

[0017] In one embodiment, the invention provides a heparin-binding growth factor (HBGF) analog of formula I:



I

[0018] wherein:

[0019] each X is a peptide chain that (i) has a minimum of three amino acid residues, (ii) has a maximum of about fifty amino acid residues, and (iii) binds a heparin-binding growth factor receptor (HBGFR);

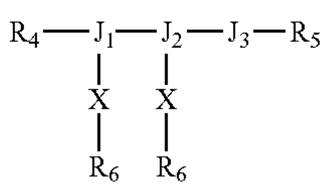
[0020] R_1 is an amino acid residue, wherein X is covalently bonded through the N-terminus of R_1 or through a side chain of R_1;

[0021] R_2 is a trifunctional alpha amino acid residue, wherein X is covalently bonded through a side chain of R_2; and

[0022] R_3 is a chain comprising a chain from 7 to about 50 atoms covalently bonded to R_2.

[0023] In the compound of formula I, X is preferably a synthetic peptide chain. R_3 can be a chain that (i) comprises a chain of a minimum of about 9 and a maximum of about 50 atoms, and (ii) is not found in the natural ligand of the heparin-binding growth factor receptor (HBGFR) which X binds. In one embodiment, R_1 is a trifunctional amino acid residue, where X is covalently bonded through a side chain of R_1.

[0024] In another embodiment, the invention provides an HBGF analog of formula II:



II

[0025] wherein:

[0026] R_4 and R_6 are each independently NH_2, an acyl group with a linear or branched C_1 to C_{17} alkyl, aryl, heteroaryl, alkene, alkenyl or aralkyl chain including an N-terminus NH_2, NH_3^+, or NH group or a corresponding acylated derivative, or is amino acid, a dipeptide or a tripeptide with an N-terminus NH_2, NH_3^+, or NH group or a corresponding acylated derivative;

[0027] R_5 is —OH, NH_2, NH—R_6, or is an amino acid, a dipeptide or a tripeptide with a C-terminus —OH, NH_2, or NH—R_7;

[0028] R_7 is an aliphatic C_1 to C_{17} chain;

[0029] each X is a peptide chain that (i) has a minimum of three amino acid residues, (ii) has a maximum of about fifty amino acid residues, and (iii) binds a heparin-binding growth factor receptor (HBGFR);

[0030] J_1 and J_2 are each independently a trifunctional alpha amino acid residue, wherein each X is covalently bonded through a side chain of J_1 or J_2;

[0031] J_3 is a chain comprising a chain from 0 to about 50 atoms covalently bonded to J_2;

[0032] n is 0 or 1, wherein when n=1 the synthetic peptide chains X are identical.

[0033] In a preferred embodiment of formula I, X and J_3 are each synthetic peptide chains. The resulting compound of formula I is preferably a synthetic heparin-binding growth factor analog.

[0034] In the formula of formula I, J_3 preferably includes a chain with a minimum of about 9 and a maximum of about 50 atoms, where J_3 is not found in the natural ligand of the heparin-binding growth factor receptor (HBGFR) which X binds.

[0035] Binding of the heparin-binding growth factor analog of formula II to a heparin-binding growth factor receptor can initiate a signal by the heparin-binding growth factor receptor or alternatively can block signaling by the heparin-binding growth factor receptor.

[0036] In a preferred embodiment of formula II, J_1 and J_2 are diamine amino acid residues, including without limitation a 2,3 diamino propionyl amino acid residue, lysine, 2,4 diamino butylic acid or ornithine. The covalent bond between each X chain and J_1 and J_2 is preferably an amide or peptide bond, but may be a disulfide, thioether, Schiff base, reduced Schiff base, imide, secondary amine, carbonyl, urea, hydrazone or oxime bond. Thus in one embodiment of formula II, the side chains of J_1 and J_2 include reactive carboxyl groups.

[0037] In the heparin-binding growth factor analog of formula I, the peptide chains X have a minimum of approximately five amino acid residues, alternatively a minimum of approximately nine amino acid residues, and yet in another alternative a maximum of approximately thirty three amino acid residues.

[0038] The peptide chain X in one embodiment includes an amino acid sequence found in a heparin-binding growth factor, which heparin-binding growth factor can be a hormone, a cytokine, a lymphokine, a chemokine or an interleukin. Thus X can include an amino acid sequence found in any of FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, FGF-15, FGF-16, FGF-17, FGF-18, FGF-19, FGF-20, FGF-21, FGF-22, FGF-23, HBBM (heparin-binding brain mitogen), HBGAF (heparin-binding growth associated factor), HB-EGF (heparin-binding EGF-like factor) HB-GAM (heparin-binding growth associated molecule, also known as pleiotrophin, PTN, HARP), TGF-α (transforming growth factor-α), TGF-βs (transforming growth factor-βs), VEGF (vascular endothelial growth factor), EGF (epidermal growth factor), IGF-1 (insulin-like growth factor-1), IGF-2 (insulin-like growth fac-

tor-2), PDGF (platelet derived growth factor), RANTES, SDF-1, secreted frizzled-related protein-1 (SFRP-1), small inducible cytokine A3 (SCYA3), inducible cytokine subfamily A member 20 (SCYA20), inducible cytokine subfamily B member 14 (SCYB14), inducible cytokine subfamily D member 1 (SCYD1), stromal cell-derived factor-1 (SDF-1), thrombospondins 1, 2, 3 and 4 (THBS1-4), platelet factor 4 (PF4), lens epithelium-derived growth factor (LEDGF), midikine (MK), macrophage inflammatory protein (MIP-1), moesin (MSN), hepatocyte growth factor (HGF, also called SF), placental growth factor, IL-1 (interleukin-1), IL-2 (interleukin-2), IL-3 (interleukin-3), IL-6 (interleukin-6), IL-7 (interleukin-7), IL-10 (interleukin-10), IL-12 (interleukin-12), IFN-α (interferon-α), IFN-γ (interferon-γ), TNF-α (tumor necrosis factor-α), SDGF (Schwannoma-derived growth factor), nerve growth factor, neurite growth-promoting factor 2 (NEGF2), neurotrophin, BMP-2 (bone morphogenic protein 2), OP-1 (osteogenic protein 1, also called BMP-7), keratinocyte growth factor (KGF), interferon-γ inducible protein-20, RANTES, and HIV-tat-transactivating factor, amphiregulin (AREG), angio-associated migratory cell protein (AAMP), angiostatin, betacellulin (BTC), connective tissue growth factor (CTGF), cysteine-rich angiogenic inducer 61 (CYCR61), endostatin, fractalkine/neuroactin, glial derived neurotrophic factor (GDNF), GRO2, hepatoma-derived growth factor (HDGF), and granulocyte-macrophage colony stimulating factor (GMCSF). In one particularly preferred embodiment, X includes an amino acid sequence found in a fibroblast growth factor (FGF). Alternatively, the peptide chain X can include an amino acid sequence not found in the natural heparin-binding growth factor receptor ligand.

[0039] In a preferred embodiment, the heparin-binding growth factor analog of formula II binds an FGF receptor. In any embodiment, the heparin-binding growth factor analog of formula II may be an agonist of the heparin-binding growth factor receptor, an antagonist of the heparin-binding growth factor receptor, a positive modulator of the biological response to a heparin-binding growth factor or a negative modulator of the biological response to a heparin-binding growth factor.

[0040] In an alternative embodiment of the heparin-binding growth factor analog of formula II the peptide chains X are cross-linked or cyclized. Such peptide chains X may be cross-linked or cyclized by at least one disulfide, peptide, or thioether bond.

[0041] In one embodiment of the heparin-binding growth factor analog of formula II, J_3 comprises between one and about thirty-three ethylene glycol units. Alternatively, J_3 can include a branched or unbranched, saturated or unsaturated alkyl chain of between one and about twenty carbon atoms. In yet another embodiment, J_3 comprises $[NH_2—(CH_2)_pCO]_q$ wherein p is from 1 to about 10 and q is from 1 to about 20. In yet another embodiment, J_3 includes a peptide sequence of from one to about 16 Gly residues.

[0042] The invention further provides a pharmaceutical composition that is the heparin-binding growth factor analog of formula II or a pharmaceutically acceptable salt thereof and a pharmaceutical carrier.

[0043] The invention further provides a method for stimulating growth factor receptor signaling in a cell, the method including contacting the cell with an effective amount of a heparin-binding growth factor analog of formula II. The signaling can stimulate proliferation of the cell, which cell may be part of a mammal.

[0044] The invention further provides a method for delivering an active heparin-binding growth factor analog to a mammal, the method including providing a medical device coated on the surface thereof via non-covalent bonds with a synthetic heparin-binding growth factor analog of formula II, and placing the medical device onto a surface of, or implanting the medical device into, the mammal. The medical device may be a suture, graft material, wound covering, nerve guide, bone wax, aneurysm coil, embolization particle, microbead, stent, dental implant, or bone prosthesis, a tissue scaffold or a controlled release drug delivery device. The surface of the medical device may be stainless steel, titanium, platinum, tungsten, ceramics, polyurethane, polytetrafluoroethylene, extended polytetrafluoroethylene, polycarbonate, polyester, polypropylene, polyethylene, polystyrene, polyvinyl chloride, polyamide, polyacrylate, polyurethane, polyvinyl alcohol, polycaprolactone, polyactide, polyglycolide, polysiloxanes, natural rubbers, artificial rubbers, block polymers, or copolymers of block polymers.

[0045] Other objects, advantages and novel features, and further scope of applicability of the present invention will be set forth in part in the detailed description to follow, taken in conjunction with the accompanying drawings, and in part will become apparent to those skilled in the art upon examination of the following, or may be learned by practice of the invention. The objects and advantages of the invention may be realized and attained by means of the instrumentalities and combinations particularly pointed out in the appended claims.

DETAILED DESCRIPTION OF THE INVENTION

[0046] Each synthetic HBGF analog of the invention is an analog of a particular HBGF that binds to one or more of the receptors bound by the particular HBGF. The synthetic HBGF analog may be an analog of a hormone, a cytokine, a lymphokine, a chemokine or an interleukin.

[0047] In one aspect the synthetic HBGF analog of the present invention is a molecule of any one of formula I or II. HBGFs include any growth factor that binds selectively to heparin. For example, the HBGF can be any of the known FGFs (FGF-1 to FGF-23), HBBM (heparin-binding brain mitogen), HB-GAF (heparin-binding growth associated factor), HB-EGF (heparin-binding EGF-like factor) HB-GAM (heparin-binding growth associated molecule, also known as pleiotrophin, PTN, HARP), TGF-α (transforming growth factor-α), TGF-βs (transforming growth factor-βs), VEGF (vascular endothelial growth factor), EGF (epidermal growth factor), IGF-1 (insulin-like growth factor-1), IGF-2 (insulin-like growth factor-2), PDGF (platelet derived growth factor), RANTES, SDF-1, secreted frizzled-related protein-1 (SFRP-1), small inducible cytokine A3 (SCYA3), inducible cytokine subfamily A member 20 (SCYA20), inducible cytokine subfamily B member 14 (SCYB14), inducible cytokine subfamily D member 1 (SCYD1), stromal cell-derived factor-1 (SDF-1), thrombospondins 1, 2, 3 and 4 (THBS1-4), platelet factor 4 (PF4), lens epithelium-derived growth factor (LEDGF), midikine (MK), macrophage inflammatory protein (MIP-1), moesin (MSN), hepatocyte growth factor (HGF, also called SF), placental growth factor, IL-1 (interleukin-1), IL-2 (interleukin-2), IL-3 (interleukin-3), IL-6 (interleukin-6), IL-7 (interleukin-7), IL-10 (interleukin-10), IL-12 (interleukin-12), IFN-α (interferon-α), IFN-γ (interferon-γ), TNF-α (tumor necrosis factor-α), SDGF (Schwannoma-derived growth factor), nerve growth factor, neurite growth-

promoting factor 2 (NEGF2), neurotrophin, BMP-2 (bone morphogenic protein 2), OP-1 (osteogenic protein 1, also called BMP-7), keratinocyte growth factor (KGF), interferon-γ inducible protein-20, RANTES, and HIV-tat-transactivating factor, amphiregulin (AREG), angio-associated migratory cell protein (AAMP), angiostatin, betacellulin (BTC), connective tissue growth factor (CTGF), cysteine-rich angiogenic inducer 61 (CYCR61), endostatin, fractalkine/neuroactin, or glial derived neurotrophic factor (GDNF), GRO2, hepatoma-derived growth factor (HDGF), granulocyte-macrophage colony stimulating factor (GMCSF), and the many growth factors, cytokines, interleukins and chemokines that have an affinity for heparin.

[0048] The amino acid sequences of many of these and other HBGFs are available from the National Library of Medicine Protein Database which can be accessed through the internet site www.ncbi.nlm.nih.gov/entrez. These HBGF amino acid sequences on the foregoing internet site are hereby incorporated by reference. The use of synthetic HBGF analogs incorporating the amino acid sequences of the receptor binding domains from these and other HBGFs is specifically contemplated in the present invention.

[0049] In particular embodiments of the present invention, the synthetic HBGF analog of the present invention consists essentially of the molecule of any one of formula I or II, i.e. the molecule of any one of formula I or II is the major active component in the synthetic HBGF analog composition.

[0050] In other particular embodiments, the synthetic HBGF analog of the present invention consists entirely of the molecule of any one of formula I or II, i.e. the molecule of any one of formula I or II is the only component in the synthetic HBGF analog composition.

[0051] The Heparin-Binding Growth Factors of Formulas I and II

[0052] The region X of the synthetic HBGF analogs of formulas I and II include amino acid residues, and optionally the regions R_3 and J_3 include amino acid residues. An amino acid residue is defined as —NHRCO—, where R can be hydrogen or any organic group. The amino acids can be D-amino acids or L-amino acids. Additionally, the amino acids can be α-amino acids, β-amino acids, γ-amino acids, or δ-amino acids and so on, depending on the length of the carbon chain of the amino acid.

[0053] The amino acids of the X, R_3 and J_3 component regions of the synthetic HBGF analogs of the invention can include any of the twenty amino acids found naturally in proteins, i.e. alanine (ala, A), arginine (Arg, R), asparagine (Asn, N), aspartic acid (Asp, D), cysteine (Cys, C), glutamic acid (Glu, E), glutamine (Gln, Q), glycine (Gly, G), histidine (His, H), isoleucine, (Iie, I), leucine (Leu, L), lysine (Lys, K), methionine (Met, M), phenylalanine (Phe, F), proline (Pro, P), serine (Ser, S), threonine (Thr, T), tryptophan (Trp, W), tyrosine (Tyr, Y), and valine (Val, V).

[0054] Furthermore, the amino acids of the X, R_3 and J_3 component regions of the synthetic HBGF analogs of the invention can include any of the naturally occurring amino acids not found naturally in proteins, e.g. β-alanine, betaine (N,N,N-trimethylglycine), homoserine, homocysteine, γ-amino butyric acid, ornithine, and citrulline.

[0055] Additionally, the amino acids of the X, R_3 and J_3 component regions of the synthetic HBGF analogs of the invention can include any of the non-biological amino acids, i.e. those not normally found in living systems, such as for instance, a straight chain amino-carboxylic acid not found in nature. Examples of straight chain amino-carboxylic acids not found in nature include 6-aminohexanoic acid, 7-aminoheptanoic acid, 9-aminononanoic acid and the like.

[0056] The molecule includes two X regions that are identical in amino acid sequence. The molecule is thus a branched chain that may also optionally be constrained by cross-links between the two X regions as described below, or may be cyclicized as described below. In this embodiment, each HBGF analog of the present invention can bind two HBGFRs and induce receptor dimerization. Advantageously, the dimerization in turn potentiates enhanced receptor signaling activity of the HBGFRs.

[0057] In the constructs of formula I, one X region is covalently linked through an amino acid R_1, which is in turn covalently linked to a second amino acid, R_2, which is a trifunctional alpha amino acid, and preferably a diamine amino acid. R_1 is linked to one amino group of R_2. The second X region is covalently linked to R_2 through a second reactive group of R_2, such as the second amino group of a diamine amino acid. R_2 is then covalently linked through its carboxy terminus to the R_3 region of the synthetic HBGF analog. Similarly, in formula II one X region is covalently linked through a reactive side chain of an amino acid J_1, which is in turn covalently linked to a second amino acid, J_2, both J_1 and J_2 constituting trifunctional amino acids, preferably diamine amino acids. The second X region is covalently linked to J_2 through a second reactive group of J_2, such as the second amino group of a diamine amino acid. J_2 is then covalently linked through its carboxy terminus to the J_3 region of the synthetic HBGF analog.

[0058] The amino acid R_1 of formula I can be any of the amino acids described above. R_2 of formula I, and J_1 and J_2 of formula II, can be any trifunctional amino acid residue, preferably a trifunctional alpha amino acid residue. In a preferred embodiment, the trifunctional amino acid residue is a diamine amino acid, such as for instance lysine or ornithine, or any other amino acid having two amino groups.

[0059] The region X of formulas I and II of the synthetic HBGF analogs of the present invention is a synthetic peptide chain that binds an HBGFR. Region X can, for example, have any amino acid sequence that binds an HBGFR, and can include amino acid sequences that are identical to a portion of the amino acid sequence of a HBGF. Alternatively, X can have an amino acid sequence homologous rather than identical to the amino acid sequence of an HBGF. The particular HBGFR bound by the synthetic HBGF analog of the invention may or may not be the cognate receptor of the original HBGF, i.e. the synthetic HBGF analog may additionally or solely bind to the receptor of a different HBGF.

[0060] The term 'homologous', as used herein refers to peptides that differ in amino acid sequence at one or more amino acid positions when the sequences are aligned. For example, the amino acid sequences of two homologous peptides can differ only by one amino acid residue within the aligned amino acid sequences of five to ten amino acids. Alternatively, two homologous peptides of ten to fifteen amino acids can differ by no more than two amino acid residues when aligned. In another alternative, two homologous peptides of fifteen to twenty or more amino acids can differ by up to three amino acid residues when aligned. For longer peptides, homologous peptides can differ by up to approximately 5%, 10%, 20% or 25% of the amino acid residues when the amino acid sequences of the two peptide homologs are aligned.

[0061] Particularly useful amino acid sequences as X regions of formulas I and II include homologs of fragments of naturally occurring HBGFs that differ from the amino acid sequences of natural growth factor in only one or two or a very few positions. Such sequences preferably include conservative changes, where the original amino acid is replaced with an amino acid of a similar character according to well known principles; for example, the replacement of a non-polar amino acid such as alanine with valine, leucine, isoleucine or proline; or the substitution of one acidic or basic amino acid with another of the same acidic or basic character.

[0062] In another alternative, the X region of the synthetic HBGF analog can include an amino acid sequence that shows no detectable homology to the amino acid sequence of any HBGF. Peptides or growth factor analogs useful as components of the X region of the synthetic analogs of the present invention, that have little or no amino acid sequence homology with the cognate growth factor and yet bind HBGFRs may be obtained by any of a wide range of methods, including for instance, selection by phage display. See as an example: Sidhu et al. Phage display for selection of novel binding peptides. Methods Enzymol. 328:333-63 (2000).

[0063] The X region of the synthetic HBGF analogs of the invention can have any length that includes an amino acid sequence that effectively binds an HBGFR. Preferably, the X regions of the synthetic HBGF analogs have a minimum length of at least approximately three amino acid residues. More preferably, the X regions of the synthetic HBGF analogs have a minimum length of at least approximately six amino acid residues. Most preferably the X regions of the synthetic HBGF analogs have a minimum length of at least approximately ten amino acid residues. The X regions of the synthetic HBGF analogs of the invention preferably also have a maximum length of up to approximately fifty amino acid residues, more preferably a maximum length of up to approximately forty amino acid residues, and most preferably a maximum length of up to approximately thirty amino acid residues.

[0064] In one embodiment of the synthetic HBGF analogs that include two X regions, the X regions are covalently cross-linked. Suitable cross links can be formed by S—S bridges of cysteines linking the two X regions. Alternatively, the cross link can be conveniently formed during simultaneous and parallel peptide synthesis of the X region amino acids chains by incorporating a lanthionine (thio-dialanine) residue to link the two identical X chains at alanine residues that are covalently bonded together by a thioether bond. In another method the two X region amino acid chains can be cross-linked by introducing a cross-linking agent, such as a dicarboxylic acid, e.g. suberic acid (octanedioic acid), or the like, thereby introducing a hydrocarbon bridge between the two identical X regions having a free amino, hydroxyl or thiol group. The cross-linked X regions can constitute a cyclic peptide, such as where the terminal amino acids of X are cross-linked through reactive side chains or the terminal groups, optionally with a bridge or other link.

[0065] In the synthetic HBGF analogs of the present invention, in one preferred embodiment the R_3 and J_3 region of formulas I and II is a chain that is sufficiently hydrophobic to non-covalently bind the HBGF analog to a polystyrene or polycaprolactone surface, or the like. In addition, the R_3 and J_3 regions may bind to other hydrophobic surfaces, particularly the hydrophobic surfaces formed from materials used in medical devices. Such surfaces are typically hydrophobic surfaces. Examples of suitable surfaces include but are not limited to those formed from hydrophobic polymers such as polycarbonate, polyester, polypropylene, polyethylene, polystyrene, polytetrafluoroethylene, expanded polytetrafluoroethylene, polyvinyl chloride, polyamide, polyacrylate, polyurethane, polyvinyl alcohol, polyurethane, poly ethyl vinyl acetate, poly(butyl methacrylate), poly(ethylene-co-vinyl acetate), poiycaprolactone, polylactide, polyglycolide and copolymers of any two or more of the foregoing; siloxanes such as 2,4,6,8-tetramethylcyclotetrasiloxane; natural and artificial rubbers; glass; and metals including stainless steel, titanium, platinum, and nitinol. Preferably, the binding of the HBGF analogs to the hydrophobic surface is of sufficient quantity to be detected by an analytical method such as an enzyme-linked immunoassay or a biological assay.

[0066] The R_3 and J_3 regions of formulas I and II can include a chain of atoms or a combination of atoms that form a chain. Typically, the chains are chains of carbon atoms, that may also optionally include oxygen, nitrogen or sulfur atoms, such as for example chains of atoms formed from amino acids (e.g. amino acids found in proteins, as listed above; naturally occurring amino acids not found in proteins, such as ornithine and citrulline; or non-natural amino acids, such as amino hexanoic acid; or a combination of any of the foregoing amino acids).

[0067] The chain of atoms of the R_3 region of formula I is covalently attached to R_1 or R_2. Similarly the chain of atoms of the J_3 region of formula II is covalently attached to J_1 or J_2. The covalent bonds can be, for example, peptide, amide or ester bonds. Preferably, the R_3 and J_3 regions include a chain of a minimum of about six to nine atoms. More preferably, the R_3 and J_3 regions include a chain of a minimum of about twelve atoms. Most preferably, the R_3 and J_3 regions include a chain of a minimum of about fifteen atoms. For example, the R_3 and J_3 regions may be formed from a chain of at least two, at least three, at least four, at least five or at least six amino acids. Alternatively, the R_3 and J_3 regions may be formed from a chain of at least one, at least two, or at least three aminohexanoic acid residues.

[0068] Preferably, the R_3 and J_3 regions include a chain of a maximum of about fifty atoms. More preferably, the R_3 and J_3 regions include a chain of a maximum of about forty-five atoms. Most preferably, the R_3 and J_3 regions include a chain of a maximum of about thirty-five atoms. For example, the R_3 and J_3 regions may be formed from a chain of up to about twelve, up to about fifteen, or up to about seventeen amino acids.

[0069] The amino acid sequence of the R_3 and J_3 regions of formula I or II is preferably an artificial sequence, i.e. it does not include any amino acid sequence of four or more amino acid residues found in a natural ligand of a HBGF.

[0070] In a particular embodiment, the R_3 and J_3 regions include a hydrophobic amino acid residue, or a chain of hydrophobic amino acid residues. The R_3 and J_3 regions can, for example, include one or more aminohexanoic acid residues, such as one, two, three or more aminohexanoic acid residues. In another alternative embodiment, the R_3 and J_3 regions can include a combination of amino acid hydrophobic residues.

[0071] In another particular embodiment, the R_3 and J_3 regions of the molecule of formula I or II can include a branched or unbranched, saturated or unsaturated alkyl chain of between one and about twenty carbon atoms. In a further embodiment, the R_3 and J_3 regions can include a chain of

hydrophilic residues, such as for instance, ethylene glycol residues. For instance, the R_3 and J_3 regions can include at least about three, or at least about four, or at least about five ethylene glycol residues. Alternatively, the R_3 and J_3 regions can include up to about twelve, up to about fifteen, or up to about seventeen ethylene glycol residues. In another alternative embodiment, the R_3 and J_3 regions can include a combination of amino acid hydrophilic residues.

[0072] As used here and elsewhere, the following terms have the meanings given.

[0073] The term "alkene" includes unsaturated hydrocarbons that contain one or more double carbon-carbon bonds. Examples of such alkene groups include ethylene, propene, and the like.

[0074] The term "alkenyl" includes a linear monovalent hydrocarbon radical of two to six carbon atoms or a branched monovalent hydrocarbon radical of three to six carbon atoms containing at least one double bond; examples thereof include ethenyl, 2-propenyl, and the like.

[0075] The "alkyl" groups specified herein include those alkyl radicals of the designated length in either a straight or branched configuration. Examples of such alkyl radicals include methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, tertiary butyl, pentyl, isopentyl, hexyl, isohexyl, and the like.

[0076] The term "aryl" includes a monovalent or bicyclic aromatic hydrocarbon radical of 6 to 12 ring atoms, and optionally substituted independently with one or more substituents selected from alkyl, haloalkyl, cycloalkyl, alkoxy, alkylhio, halo, nitro, acyl, cyano, amino, monosubstituted amino, disubstituted amino, hydroxy, carboxy, or alkoxycarbonyl. Examples of an aryl group include phenyl, biphenyl, naphthyl, 1-naphthyl, and 2-naphthyl, derivatives thereof, and the like.

[0077] The term "aralkyl" includes a radical —R^aR^b where R^a is an alkylene (a bivalent alkyl) group and R^b is an aryl group as defined above. Examples of aralkyl groups include benzyl, phenylethyl, 3-(3-chlorophenyl)-2-methylpentyl, and the like. The term "aliphatic" includes compounds with hydrocarbon chains, such as for example alkanes, alkenes, alkynes, and derivatives thereof.

[0078] The term "acyl" includes a group RCO—, where R is an organic group. An example is the acetyl group CH_3CO—.

[0079] A peptide or aliphatic moiety is "acylated" when an alkyl or substituted alkyl group as defined above is bonded through one or more carbonyl {—(C═O)—} groups. A peptide is most usually acylated at the N-terminus.

[0080] An "amide" includes compounds that have a trivalent nitrogen attached to a carbonyl group (—$CO.NH_2$).

[0081] An "amine" includes compounds that contain an amino group (—NH_2).

[0082] FGF Synthetic Analogs

[0083] In another particular aspect, the invention provides a synthetic FGF peptide analog. The synthetic FGF analogs represented by any of formulas I and II above, wherein X is an FGF analog, is an analog of an FGF which can be any FGF, such as any of the known FGFs, including all 23 FGFs from FGF-1 to FGF-23.

[0084] The X region of the molecule of formulas I and II can include an amino acid sequence found in an FGF, such as for instance FGF-2 or FGF-7. Alternatively, the X region can include a sequence not found in the natural ligand of the FGFR bound by the molecule.

[0085] The R_3 and J_3 regions of the synthetic FGF peptide analogs of any of formulas I and II are not necessarily hydrophobic, and thus, if present, can be polar, basic, acidic, hydrophilic or hydrophobic. Thus, the amino acid residues of the R_3 and J_3 regions of synthetic FGF peptide analogs can include any amino acid, or polar, ionic, hydrophobic or hydrophilic group.

[0086] The X region of synthetic FGF peptide analogs can include an amino acid sequence that is 100% identical to the amino acid sequence found in a fibroblast growth factor or an amino acid sequence homologous to the amino acid sequence of a fibroblast growth factor. For instance, the X region can include an amino acid sequence that is at least about 50%, at least about 75%, or at least about 90% homologous to an amino acid sequence from a fibroblast growth factor. The fibroblast growth factor can be any fibroblast growth factor, including any of the known or yet to be identified fibroblast growth factors.

[0087] In a particular embodiment, the synthetic FGF analog of the invention is an agonist of the HBGFR. When bound to the HBGFR, the synthetic HBGF analog initiates a signal by the HBGFR.

[0088] In a further particular embodiment, the synthetic FGF analog of the invention is an antagonist of the HBGFR. When bound to the HBGFR, the synthetic HBGF analog blocks signaling by the HBGFR.

[0089] In another particular embodiment of the present invention, the synthetic FGF analog is an analog of FGF-2 (also known as basic FGF, or bFGF). In another particular embodiment of the present invention, the binding of the synthetic FGF analog to an FGF receptor initiates a signal by the FGF receptor. In a further particular embodiment, the binding of the synthetic FGF analog to the FGF receptor blocks signaling by the FGF receptor.

[0090] In a yet further particular embodiment, the present invention provides a synthetic FGF analog of FGF-2. In another particular embodiment, the present invention provides a synthetic FGF analog of FGF-2, wherein the amino acid sequence of the X region is YRSRKYTSWYVALKR (SEQ ID NO:1) from FGF-2. In yet another particular embodiment, the present invention provides a synthetic FGF analog wherein the amino acid sequence of the X region is NRFHSWDCIKTWASDTFVLVCYDDGSEA (SEQ ID NO:2). In yet another particular embodiment, the present invention provides a synthetic FGF-2 analog wherein the amino acid sequence of the X region is HIKLQLQAEERGVVS (SEQ ID NO:43).

[0091] In a yet further particular embodiment, the invention provides a synthetic FGF analog of FGF-1, wherein the X region is YISKKHAEKNWFVGLKK (SEQ ID NO:3). This sequence is derived from amino acids bridging the beta 9 and beta 10 loop of FGF-1. In yet another particular embodiment, an FGF-1 analog is provided wherein the X region is HIQLQLSAESVGEVY (SEQ ID NO:4), corresponding to amino acids derived from the β-4 and β-5 region of FGF-1.

[0092] In a yet further particular embodiment, the invention provides a synthetic FGF analog of FGF-7, wherein the X region is YASAKWTHNGGEMFVALNQK (SEQ ID NO:5). In yet another embodiment of a synthetic FGF analog of FGF-7, the X regions is the amino acid sequence YNIMEIRTVAVGIVA (SEQ ID NO:6).

[0093] Other FGF receptor binding domains, derived largely from targeting sequences in the C-terminus of human FGF, include the following sequences shown in Table 1:

TABLE 1

CYTOKINE	PREFERRED X RECEPTOR BINDING DOMAIN	
FGF-10	YASFNWQHNGRQMYVALNQK	(SEQ ID NO: 44)
FGF-22	YASQRWRRRGQPNLALDRR	(SEQ ID NO: 45)
FGF-9	YSSNLYKHVDTGRRYYVALNK	(SEQ ID NO: 46)
FGF-16	YASTLYKHSDSERQYVALNK	(SEQ ID NO: 47)
FGF-20	YSSNIYKHGDTGRRFVALNK	(SEQ ID NO: 48)
FGF-4	YESYKYPGMFIALSKN	(SEQ ID NO: 49)
FGF-6	YESDLYQGTYILSKYGR	(SEQ ID NO: 50)
FGF-12	YSSTLYRQQESGRAWFLGNK	(SEQ ID NO: 51
FGF-14	YSSMLYRQQESGRAWFLGLNK	(SEQ ID NO: 52)
FGF-13	YSSMIYRQQQSGRGWYLGLNK	(SEQ ID NO: 53)
FGF-11	YASALYRQRRSGRAWYLDK	(SEQ ID NO: 54)

[0094] VEGF Synthetic Analogs

[0095] In another particular aspect, the invention provides a synthetic VEGF peptide analog. The synthetic VEGF analogs represented include, in one embodiment, a VEGF analog wherein the amino acid sequence of the X region is APMAE-GGGQNHHEWKFMDV (SEQ ID NO:7). In another embodiment, there is provided a synthetic VEGF peptide analog wherein the amino acid sequence of the X region is GATWLPPNPTK (SEQ ID NO:8). In yet another embodiment, there is provided a synthetic VEGF peptide analog wherein the amino acid sequence of the X region is NFLLSWVHWSLALLLYLHHA (SEQ ID NO:9).

[0096] BMP Synthetic Analogs

[0097] In another particular aspect, the invention provides a synthetic BMP peptide analog. The synthetic bone morphogenic protein analogs include embodiments wherein the X region is the amino acid sequence LYVDFSDVGWNDW (SEQ ID NO:10), AISMLYLDENEKWL (SEQ ID NO:11), ISIVILYLDENEKVVLKIIY (SEQ ID NO:12), EKWLKNYQDMWEG (SEQ ID NO:13), LVVKENED-LYLMSIAC (SEQ ID NO:14), AFYCHGECPFPLADHL (SEQ ID NO:15), or PFPLADHLNSTNHAIVQTLVNSV (SEQ ID NO:16).

[0098] Alternatively, in another particular aspect the invention provides synthetic BMP, TGF or GDF (growth differentiation factor) peptide analogs as shown in Table 2 wherein the transforming growth factor family member peptides are particularly useful in augmenting the activity of endogenous or artificial BMP peptides or TGF peptides, wherein is shown (under the heading "preferred X receptor binding domain") the sequence forming all or part of the X region of constructs of either formula I or II.

TABLE 2

CYTOKINE	PREFERRED X RECEPTOR BINDING DOMAIN	
TGF-β1	IVYYVGRKPKVEQLSNMIVRS	(SEQ ID NO: 17)
TGF-β2	TILYYIGKTPKIEQLSNMIVKS	(SEQ ID NO: 18)
TGF-β3	LTILYYVGRTPKVEQLSNMVV	(SEQ ID NO: 19)

TABLE 2-continued

CYTOKINE	PREFERRED X RECEPTOR BINDING DOMAIN	
BMP-2	AISMLYLDENEKVVLKNYQDMVV	(SEQ ID NO: 20)
BMP-3	SSLSILFFDENKNVVLKVYPNMTV	(SEQ ID NO: 21)
BMP-3β	NSLGVLFLDENRNVVLKVYPNMSV	(SEQ ID NO: 22)
BMP-4	AISMLYLDEYDKVVLKNYQEMVV	(SEQ ID NO: 23)
BMP-5	AISVLYFDDSSNVILKKYRNMVV	(SEQ ID NO: 24)
BMP-6	AISVLYFDDNSNVILKKYRNMVV	(SEQ ID NO: 25)
BMP-7	AISVLYFDDSSNVILKKYRNMVV	(SEQ ID NO: 26)
BMP-8	ATSVLYYDSSNNVILRKARNMVV	(SEQ ID NO: 27)
BMP-9	ISVLYKDDMGVPTLKYHYEGMSV	(SEQ ID NO: 28)
BMP-10	ISILYLDKGVVTYKFKYEGMAV	(SEQ ID NO: 29)
BMP-11	INMLYFNDKQQIIYGKIPGMVV	(SEQ ID NO: 30)
BMP-12	ISILYIDAANNVVYKQYEDMVV	(SEQ ID NO: 31)
BMP-13	ISILYIDAGNNVVYKQYEDMVV	(SEQ ID NO: 32)
BMP-14	ISILFIDSANNVVYKQYEDMVV	(SEQ ID NO: 33)
BMP-15	ISVLMIEANGSILYKEYEGMIA	(SEQ ID NO: 34)
GDF-1	ISVLFFDNSDNVVLRQYEDMVV	(SEQ ID NO: 35)
GDF-3	ISMLYQDNNDNVILRHYEDMVV	(SEQ ID NO: 36)
GDF-8	INMYLFNGKEQIIYGKIPAMVV	(SEQ ID NO: 37)
GDF-9	LSVLTIEPDGSIAYKEYEDMIA	(SEQ ID NO: 38)

[0099] Methods of Synthesizing the Heparin-Binding Growth Factor Analogs

[0100] The synthesis of the analogs of the invention can be achieved by any of a variety of chemical methods well known in the art. Such methods include bench scale solid phase synthesis and automated peptide synthesis in any one of the many commercially available peptide synthesizers. Preferably, the synthesizer has a per cycle coupling efficiency of greater than 99 percent.

[0101] The analogs of the present invention can be produced by stepwise synthesis or by synthesis of a series of fragments that can be coupled by similar well known techniques. See, for instance, Nyfeler, Peptide synthesis via fragment condensation. Methods Mol Biol 35:303-16 (1994); and Merrifield, Concept and early development of solid-phase peptide synthesis. Methods in Enzymol 289:3-13 (1997). These methods are routinely used for the preparation of individual peptides. It is possible to assemble the analogs of the present invention in component parts, such as peptides constituting the X, R_3 and J_3 region components thereof, and to thereafter couple such component parts to assemble the analog. See, for instance, Dawson and Kent, Synthesis of native proteins by chemical ligation. Annu. Rev. Biochem. 69:923-960 (2000); and Eom et al., Tandem ligation of multipartite peptides with cell-permeable activity. J. Am. Chem. Soc. 125:73-82 2003).

[0102] Advantageously, given that the analogs of formulas I and II of the invention include two identical X region amino

acid sequences, the synthesis of these identical X region peptides may be performed in parallel. By this method each cycle of addition adds an amino acid to both of the X region peptides, greatly facilitating the synthesis of these branched molecules.

[0103] Peptide libraries that can be used to screen for a desired property, such as binding to an HBGFR can be prepared by adaptations of these methods. See for instance, Fox, Multiple peptide synthesis, Mol. Biotechnol. 3:249-58 (1995); and Wade and Tregear, Solid phase peptide synthesis: recent advances and applications. Austral. Biotechnol. 3:332-6 (1993).

[0104] In a particular embodiment, the synthetic HBGF analog of the invention is an agonist of the HBGFR. When bound to the HBGFR, the synthetic HBGF analog initiates a signal by the HBGFR.

[0105] In another particular embodiment, the synthetic HBGF analog of the invention is an antagonist of the HBGFR. When bound to the HBGFR, the synthetic HBGF analog blocks signaling by the HBGFR.

[0106] In a particular aspect, the invention provides a method for stimulating growth factor receptor signaling in a cell by contacting the cell with an effective amount of a synthetic HBGF analog according to formulas I and II. The effective amount can be readily determined by one of skill in the art. The signaling can result in cytokine release from the cell, stimulation or inhibition of proliferation or differentiation of the cell, chemotaxis of the cell, stimulation or inhibition of the immune system of the mammal.

[0107] Methods of Use of the Hbgfs of the Invention

[0108] The HBGF analogs of the invention provide a cost effective and potentially unlimited source of biologically active molecules that are useful in a number of ways, including as soluble prophylactic or therapeutic pharmaceutical agents, such as for instance for administration as a soluble drug for prevention or treatment of various diseases, including for example, uses in cancer therapy and radioprotection.

[0109] The synthetic HBGF analogs of present invention are also useful as biologically active agents for coating of medical devices, such as for instance, sutures, implants and medical instruments to promote biological responses, for instance, to stimulate growth and proliferation of cells, or healing of wounds.

[0110] In one aspect, the present invention provides a method for treating a mammal that has been exposed to a harmful dose of radiation. The method includes administering an effective dose of a synthetic HBGF analog of the invention which is an FGF analog to the mammal. The treatment is particularly useful in the prevention or treatment of mucositis, gastrointestinal syndrome (G.I. syndrome), or radionecrosis such as can result from exposure to radiation. The HBGF analog can be administered parenterally, orally, or topically. Alternatively, the HBGF analog can be delivered loco-regionally, e.g. on an analog coated medical device. In a related embodiment, the present invention provides a method for treating a mammal that has been administered a dose of a chemotherapeutic agent, to ameliorate the toxicity of the chemotherapeutic agent to the mammal. In a particular embodiment of the above-described methods, the mammal is a human. In another particular embodiment of the method, the HBGF analog is an FGF-2 analog or an FGF-7 analog.

[0111] The term "medical device" as used herein means a device that has one or more surfaces in contact with an organ, tissue, blood or other bodily fluid in an organism, preferably a mammal, particularly, a human. Medical devices include, for example, extracorporeal devices for use in surgery such as blood oxygenators, blood pumps, blood sensors, tubing used to carry blood, and the like which contact blood that is returned to the patient. The term can also include endoprostheses implanted in blood contact in a human or animal body, such as vascular grafts, stents, pacemaker leads, heart valves, and the like that are implanted in blood vessels or in the heart. The term can further include devices for temporary intravascular use such as catheters, guide wires, and the like that are placed in blood vessels or the heart for purposes of monitoring or repair. The term can further include nerve electrodes, muscle electrodes, implantable pulse generators, implantable drug pumps, and defibrillators. Moreover, the term medical device can include sutures, graft materials, wound coverings, nerve guides, bone wax, aneurysm coils, embolization particles, microbeads, dental implants, bone prostheses, tissue scaffolds, artificial joints or a controlled release drug delivery devices.

[0112] The surface of the medical device can be formed from any of the commonly used materials suitable for use in medical devices, such as for instance, stainless steel, titanium, platinum, tungsten, ceramics, polyurethane, polytetrafluoroethylene, extended polytetrafluoroethylene, polycarbonate, polyester, polypropylene, polyethylene, polystyrene, polyvinyl chloride, polyamide, polyacrylate, polyurethane, polyvinyl alcohol, polycaprolactone, polylactide, polyglycolide, polysiloxanes (such as 2,4,6,8-tetramethylcyclotetrasiloxane), natural rubbers, or artificial rubbers, or block polymers or copolymers thereof.

[0113] Methods for coating biological molecules onto the surfaces of medical devices are known. See for instance U.S. Pat. No. 5,866,113 to Hendriks et al., the specification of which is hereby incorporated by reference. Tsang et al. in U.S. Pat. No. 5,955,588 teach a non-thrombogenic coating composition and methods for using the same on medical devices, and is incorporated herein by reference. Zamora et al. in U.S. Pat. No. 6,342,591 teach an amphipathic coating for medical devices for modulating cellular adhesion composition, and is incorporated herein by reference.

[0114] In one embodiment the invention provides a method for delivering an active peptide to a mammal, the method includes (i) providing a medical device coated on its surface with a synthetic HBGF analog of formulas I and II, the synthetic HBGF analog being bound to the surface of the medical device by non-covalent bonds; and (ii) placing the medical device onto a surface of, or implanting the medical device into, the mammal.

[0115] In a particular embodiment of the above method, the non-covalent bonds are associations between the heparin binding domain of the synthetic HBGF analog and a heparin-containing compound bound to the surface of the medical device. The heparin-containing compound bound to the surface of the medical device can be any heparin-containing compound, such as for instance, benzyl-bis(dimethylsilylmethyl)oxy carbamoyl-heparin.

[0116] In another particular embodiment of the above method, the medical device is not pre-coated with a heparin-containing compound before being coated with the synthetic HBGF analog of formulas I and II.

[0117] Heparin-Binding Growth Factor Analog Pharmaceutical Applications

[0118] The HBGF analogs of this invention can be used for as an active ingredient in pharmaceutical compositions for

both medical applications and animal husbandry or veterinary applications. Typically, the HBGF analog or pharmaceutical composition is used in humans, but may also be used in other mammals. The term "patient" is intended to denote a mammalian individual, and is so used throughout the specification and in the claims. The primary applications of this invention involve human patients, but this invention may be applied to laboratory, farm, zoo, wildlife, pet, sport or other animals.

[0119] The HBGF analogs of this invention may be in the form of any pharmaceutically acceptable salt. The term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic bases or acids including inorganic or organic bases and inorganic or organic acids. Salts derived from inorganic bases include aluminum, ammonium, calcium, copper, ferric, ferrous, lithium, magnesium, manganic salts, manganous, potassium, sodium, zinc, and the like. Particularly preferred are the ammonium, calcium, lithium, magnesium, potassium, and sodium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines, and basic ion exchange resins, such as arginine, betaine, caffeine, choline, N,N'-dibenzylethylenediamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethylmorpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, tromethamine, and the like.

[0120] When the HBGF analog of the present invention is basic, acid addition salts may be prepared from pharmaceutically acceptable non-toxic acids, including inorganic and organic acids. Such acids include acetic, benzenesulfonic, benzoic, camphorsulfonic, carboxylic, citric, ethanesulfonic, formic, fumaric, gluconic, glutamic, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, mandelic, methanesulfonic, malonic, mucic, nitric, pamoic, pantothenic, phosphoric, propionic, succinic, sulfuric, tartaric, p-toluenesulfonic acid, trifluoroacetic acid, and the like. Acid addition salts of the HBGF analogs of this invention are prepared in a suitable solvent for the HBGF analog and an excess of an acid, such as hydrochloric, hydrobromic, sulfuric, phosphoric, acetic, trifluoroacetic, citric, tartaric, maleic, succinic or methanesulfonic acid. The acetate salt form is especially useful. Where the HBGF analogs of this invention include an acidic moiety, suitable pharmaceutically acceptable salts may include alkali metal salts, such as sodium or potassium salts, or alkaline earth metal salts, such as calcium or magnesium salts.

[0121] The invention provides a pharmaceutical composition that includes a HBGF analog of this invention and a pharmaceutically acceptable carrier. The carrier may be a liquid formulation, and in one embodiment a buffered, isotonic, aqueous solution. Pharmaceutically acceptable carriers also include excipients, such as diluents, carriers and the like, and additives, such as stabilizing agents, preservatives, solubilizing agents, buffers and the like, as hereafter described.

[0122] Thus the HBGF analog compositions of this invention may be formulated or compounded into pharmaceutical compositions that include at least one HBGF analog of this invention together with one or more pharmaceutically acceptable carriers, including excipients, such as diluents, carriers and the like, and additives, such as stabilizing agents, preservatives, solubilizing agents, buffers and the like, as may be desired. Formulation excipients may include polyvinylpyrrolidone, gelatin, hydroxy cellulose, acacia, polyethylene glycol, manniton, sodium chloride and sodium citrate. For injection or other liquid administration formulations, water containing at least one or more buffering constituents is preferred, and stabilizing agents, preservatives and solubilizing agents may also be employed. For solid administration formulations, any of a variety of thickening, filler, bulking and carrier additives may be employed, such as starches, sugars, fatty acids and the like. For topical administration formulations, any of a variety of creams, ointments, gels, lotions and the like may be employed. For most pharmaceutical formulations, non-active ingredients will constitute the greater part, by weight or volume, of the preparation. For pharmaceutical formulations, it is also contemplated that any of a variety of measured-release, slow-release or time-release formulations and additives may be employed, so that the dosage may be formulated so as to effect delivery of a HBGF analog of this invention over a period of time.

[0123] In practical use, the HBGF analogs of the invention can be combined as the active ingredient in an admixture with a pharmaceutical carrier according to conventional pharmaceutical compounding techniques. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, for example, oral, parenteral (including intravenous), urethral, vaginal, nasal, buccal, sublingual, or the like. In preparing the compositions for oral dosage form, any of the usual pharmaceutical media may be employed, such as, for example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents and the like in the case of oral liquid preparations, such as, for example, suspensions, elixirs and solutions; or carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents and the like in the case of oral solid preparations such as, for example, powders, hard and soft capsules and tablets.

[0124] The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that it may be administered by syringe. The form must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, a polyol, for example glycerol, propylene glycol or liquid polyethylene glycol, suitable mixtures thereof, and vegetable oils.

[0125] If the HBGF analog pharmaceutical composition is administered by injection, the injection may be intravenous, subcutaneous, intramuscular, intraperitoneal or other means known in the art. The HBGF analogs of this invention may alternatively be formulated by any means known in the art, including but not limited to formulation as tablets, capsules, caplets, suspensions, powders, lyophilized preparations, suppositories, ocular drops, skin patches, oral soluble formulations, sprays, aerosols and the like, and may be mixed and formulated with buffers, binders, excipients, stabilizers, antioxidants and other agents known in the art. In general, any route of administration by which the HBGF analogs of invention are introduced across an epidermal layer of cells may be employed. Administration means may thus include adminis-

tration through mucous membranes, buccal administration, oral administration, dermal administration, inhalation administration, nasal administration, urethral administration, vaginal administration, and the like.

[0126] In general, the actual quantity of HBGF analog of this invention administered to a patient will vary between fairly wide ranges depending upon the mode of administration, the formulation used, and the response desired. The dosage for treatment is administration, by any of the foregoing means or any other means known in the art, of an amount sufficient to bring about the desired therapeutic effect.

[0127] Heparin-Binding Growth Factors

[0128] The fibroblast growth factors, FGFs constitute a family of related proteins controlling normal growth and differentiation of mesenchymal, epithelial, and neuroectodermal cell types. Homologs have been found in a wide variety of species. FGFs show a very high affinity to heparin and are therefore also referred to as heparin-binding growth factors (HBGFs). As used herein, the term HBGFs includes all FGFs.

[0129] Two main types of FGF are known. The first type of FGF was isolated initially from brain tissue. It was identified by its proliferation-enhancing activities for murine fibroblasts, such as 3T3 cells. Due to its basic pl the factor was named basic FGF (bFGF, or HBGF-2, heparin-binding growth factor-2) and is now generally referred to as FGF-2. This is the prototype of the FGF family.

[0130] Another type of FGF, also initially isolated from brain tissues, is acidic FGF (aFGF, also known as HBGF-1, heparin-binding growth factor-1 or HBGF-α, heparin-binding growth factor-α), now generally referred to as FGF-1. It was identified by its proliferation-enhancing activity for myoblasts.

[0131] Other fibroblast growth factors belonging to the same family include FGF-3 (or HBGF-3, heparin-binding growth factor-3, originally called int-2; see Fekete, Trends in Neurosci. 23:332 (2000)), FGF-4 (HBGF-4, heparin-binding growth factor-4, initially recognized as the product of the oncogene hst; see Sakamoto et al., Proc. Natl. Acad. Sci. USA 91:12368-72), and FGF-5 (originally called HBGF-5, see Bates et al. Biosynthesis of human fibroblast growth factor 5. Mol. Cell. Biol. 11:1840-1845 (1991)); Burgess and Maciag, The heparin-binding (fibroblast) growth factor family of proteins. Ann. Rev. Biochem. 58: 575-606 (1989); and Zhan et al. The human FGF-5 oncogene encodes a novel protein related to fibroblast growth factors. Mol. Cell. Biol. 8:3487-3495 (1988)).

[0132] FGF-6 is also known as HBGF-6, and sometimes called hst-2 or oncogene hst-1 related growth factor, see lida et al. Human hst-2 (FGF-6) oncogene: cDNA cloning and characterization. Oncogene 7:303-9 (1992); and Marics et al. Characterization of the HST-related FGF-6 gene, a new member of the fibroblast growth factor gene family. Oncogene 4:335-40 (1989).

[0133] FGF-7 or K-FGF is also known as KGF or keratinocyte growth factor (See Aaronson et al. Keratinocyte growth factor. A fibroblast growth factor family member with unusual target cell specificity. Annals NY Acad. Sci. 638:62-77 (1991)); Finch et al. Human KGF is FGF-related with properties of a paracrine effector of epithelial cell growth. Science 245:752-5 (1989); Marchese et al. Human keratinocyte growth factor activity on proliferation and differentiation of human keratinocytes: differentiation response distinguishes KGF from EGF family. J. Cellular Physiol. 144: 326-32 (1990)).

[0134] FGF-8 was found to be identical to androgen-induced growth factor, AIGF and has been well studied (See Blunt et al. Overlapping expression and redundant activation of mesenchymal fibroblast growth factor (FGF) receptors by alternatively spliced FGF-8 ligands. J. Biol. Chem. 272:3733-8 (1997)); Dubrulle et al. FGF signaling controls somite boundary position and regulates segmentation clock control of spatiotemporal Hox gene activation. Cell 106:219-232 (2001); Gemel et al. Structure and sequence of human FGF8. Genomics 35:253-257 (1996); Tanaka et al. A novel isoform of human fibroblast growth factor 8 is induced by androgens and associated with progression of esophageal carcinoma. Dig. Dis. Sci. 46:1016-21 (2001)).

[0135] FGF-9 was originally called glia activating factor, or HBGF-9. See Miyamoto et al. Molecular cloning of a novel cytokine cDNA encoding the ninth member of the fibroblast growth factor family, which has a unique secretion pattern. Mol. Cell. Biol. 13:4251-9 (1993); and Naruo et al. Novel secretory heparin-binding factors from human glioma cells (glia-activating factors) involved in glial cell growth. J. Biol. Chem. 268: 2857-64 (1993).

[0136] FGF-10 is also called KGF-2, keratinocyte growth factor-2 (see Kok et al. Cloning and characterization of a cDNA encoding a novel fibroblast growth factor preferentially expressed in human heart. Biochem. Biophys. Res. Comm. 255:717-721, (1999)).

[0137] Several FGF-related factors have been described as fibroblast growth factor homologous factors (FHFs) and are also referred to as FGF-11 (FHF-3), FGF-12 (FHF-1), FGF-13 (FHF-2, see Greene et al. Identification and characterization of a novel member of the fibroblast growth factor family. Eur. J. Neurosci. 10:1911-1925 (1998)), and FGF-14 (FHF-4).

[0138] FGF-15 is expressed in the developing nervous system and was identified as a gene regulated by transcription factor E2A-Pbx1. McWhirter et al. A novel fibroblast growth factor gene expressed in the developing nervous system is a downstream target of the chimeric homeodomain oncoprotein E2A-Pbx1. Development 124:3221-3232 (1997).

[0139] FGF-16 was isolated as a cDNA clone from rat heart by homology-based polymerase chain reaction expressing an FGF of 207 amino acids. FGF-16 is 73% identical to FGF-9. Miyake et al. Structure and expression of a novel member, FGF-16, of the fibroblast growth factor family. Biochem. Biophys. Res. Commun. 243:148-152 (1998).

[0140] The cDNA encoding FGF-17 was isolated from rat embryos and encodes a protein of 216 amino acids. When expressed in 3T3 fibroblasts, mouse FGF-17 is transforming. During embryogenesis, FGF-17 is expressed at specific sites in forebrain, the midbrain-hindbrain junction, the developing skeleton and in developing arteries. See Hoshikawa et al. Structure and expression of a novel fibroblast growth factor, FGF-17, preferentially expressed in the embryonic brain. Biochem. Biophys. Res. Commun. 244:187-191 (1998); and Xu et al. Genomic structure, mapping, activity and expression of fibroblast growth factor 17. Mechanisms of Development 83:165-178 (1999).

[0141] The cDNA encoding FGF-18 was isolated from rat embryos encoding a protein of 207 amino acids. FGF-18 is a glycosylated protein and is most similar to FGF-8 and FGF-17. Injection of recombinant murine FGF-18 has been shown to induce proliferation in tissues of both epithelial and mesenchymal origin, particularly in liver and small intestine. Recombinant rat FGF-18 induces neurite outgrowth in PC12

cells. Recombinant murine FGF-18 protein stimulates proliferation in NIH 3T3 fibroblasts in vitro in a heparan sulfate-dependent manner. For general information see Hu et al. FGF-18, a novel member of the fibroblast growth factor family, stimulates hepatic and intestinal proliferation. Mol. Cell. Biol. 18:6063-6074 (1998); and Ohbayashi et al. Structure and expression of the mRNA encoding a novel fibroblast growth factor, FGF-18. J. Biol. Chem. 273:18161-18164 (1998).

[0142] FGF-19 is related distantly to other members of the FGF family. FGF-19 mRNA is expressed in several tissues including fetal cartilage, skin, and retina, as well as adult gall bladder. It is overexpressed in a colon adenocarcinoma cell line. FGF-19 is a high affinity, heparin-dependent ligand for the FGF-4 receptor. See Xie et al. FGF-19, a novel fibroblast growth factor with unique specificity for FGFR4 Cytokine 11:729-735 (1999).

[0143] FGF-20 is expressed in normal brain, particularly the cerebellum, and in some cancer cell lines. FGF-20 mRNA is expressed preferentially in the substantia nigra pars compacta. Recombinant FGF-20 protein induces DNA synthesis in a variety of cell types and is recognized by multiple FGF receptors. FGF-20 functions like an oncogene, causing a transformed phenotype when expressed in the 3T3 fibroblast cell line. These transformed cells are tumorigenic in nude mice. See Jeffers et al. Identification of a novel human fibroblast growth factor and characterization of its role in oncogenesis. Cancer Res. 61:3131-8 (2001); and Ohmachi et al. FGF-20, a novel neurotrophic factor, preferentially expressed in the substantia nigra pars compacta of rat brain. Biochem. Biophys. Res. Commun. 277:355-60 (2000).

[0144] FGF-21 was isolated from mouse embryos. FGF-21 mRNA is most abundant in the liver with lower levels in the thymus. FGF-21 is most similar to human FGF-19. See Nishimura et al. Identification of a novel FGF, FGF-21, preferentially expressed in the liver. Biochim. Biophys. Acta 1492: 203-6 (2000).

[0145] The cDNA encoding FGF-22 (170 amino acids) was isolated from human placenta. FGF-22 is most similar to FGF-10 and FGF-7. Murine FGF-22 mRNA is expressed preferentially in the skin. FGF-22 mRNA in the skin is found preferentially in the inner root sheath of the hair follicle. See Nakatake et al. Identification of a novel fibroblast growth factor, FGF-22, preferentially expressed in the inner root sheath of the hair follicle. Biochim. Biophys. Acta 1517: 460-3 (2001).

[0146] FGF-23 is most similar to FGF-21 and FGF-19. The human FGF-23 gene maps to chromosome 12p13 linked to human FGF-6 gene. FGF-23 mRNA is expressed mainly in the brain (preferentially in the ventrolateral thalamic nucleus) and thymus at low levels. Missense mutations in the FGF-23 gene have been found in patients with autosomal dominant hypophosphataemic rickets. Overproduction of FGF23 causes tumor-induced osteomalacia, a paraneoplastic disease characterized by hypophosphatemia caused by renal phosphate wasting. See Yamashita et al. Identification of a novel fibroblast growth factor, FGF-23, preferentially expressed in the ventrolateral thalamic nucleus of the brain. Biochem. Biophys. Res. Commun. 277:494-8 (2000); and Shimada et al. Cloning and characterization of FGF23 as a causative factor of tumor-induced osteomalacia. Proc. Natl. Acad. Sci. (USA) 98:6500-5 (2001).

[0147] HBBM (Heparin-binding brain mitogen) was isolated initially as a heparin binding protein from brain tissues of several species and is identical to heparin-binding neurite promoting factor. See Huber et al. Amino-terminal sequences of a novel heparin-binding protein with mitogenic activity for endothelial cells from human bovine, rat, and chick brain: high interspecies homology. Neurochem. Res. 15:435-439 (1990).

[0148] HB-GAF (heparin-binding growth associated factor) is a neurotrophic and mitogenic factor identical to HBNF (heparin-binding neurite-promoting factor). See Kuo et al. Characterization of heparin-binding growth-associated factor receptor in NIH 3T3 cells. Biochem. Biophys. Res. Commun. 182:188-194 (1992).

[0149] HB-EGF (heparin-binding EGF-like factor) is found in conditioned media of cell line U937 and is also synthesized by macrophages and human vascular smooth muscle cells. HB-EGF is a monomeric heparin-binding O-glycosylated protein of 86 amino acids and is processed from a precursor of 208 amino acids. Several truncated forms of HB-EGF have been described. HB-EGF is a potent mitogen for NIH 3T3 cells, keratinocytes and smooth muscle cells, but not for endothelial cells. The mitogenic activity on smooth muscle cells is much stronger than for EGF and appears to involve interactions with cell surface heparan sulfate proteoglycans. HB-EGF is a major growth factor component of wound fluid and may play an important role in wound healing. See Abraham et al. Heparin-binding EGF-like growth factor: characterization of rat and mouse cDNA clones, protein domain conservation across species, and transcript expression in tissues. Biochem. Biophys. Res. Commun. 190:125-133 (1993); Higashiyama et al. A heparin-binding growth factor secreted by macrophage like cells that is related to EGF. Science 251:936-9 (1991); and Marikovsky et al. Appearance of heparin-binding EGF-like growth factor in wound fluid as a response to injury. Proc. Natl. Acad. Sci. (USA) 90:3889-93.

[0150] HB-GAM (heparin-binding growth associated molecule) also referred to as HBNF (heparin-binding neurite promoting factor) is a protein of 15.3 kDa isolated as a heparin binding protein from brain tissues of several species. HB-GAM promotes growth of SW-13 cells in soft agar. Courty et al. Mitogenic properties of a new endothelial cell growth factor related to pleiotrophin. Biochem. Biophys. Res. Commun. 180: 145-151 (1991); and Hampton et al. Structural and functional characterization of full-length heparin-binding growth associated molecule. Mol. Biol. Cell. 3:85-93 (1992).

[0151] TGF-beta (TGF-β) exists in at least five isoforms, known TGF-β1, TGF-β2, TGF-β3, TGF-β4 and TGF-β5, that are not related to TGF-α. Their amino acid sequences display homologies on the order of 70-80 percent. TGF-β1 is the prevalent form and is found almost ubiquitously while the other isoforms are expressed in a more limited spectrum of cells and tissues.

[0152] TGF-beta is the prototype of a family of proteins known as the TGF-beta superfamily. This family includes inhibins, Activin A, MIS (Mullerian activating substance) and BMPs (Bone morphogenic proteins). Burt, Evolutionary grouping of the transforming growth factor-beta superfamily. Biochem. Biophys. Res. Commun. 184:590-5 (1992).

EXAMPLES

Example 1

[0153] The synthetic HBGF analog, PBA1-1, was synthesized by standard solid phase peptide synthesis methods.

PBA1-1 has a structure according to formula II, in which the amino acid sequences of the X region is WVLFNANTRDIL-RRSI (SEQ ID NO:39) (considering the sequence in the conventional N C orientation, notwithstanding that it is branched sequence). Each of the two X region peptides of SEQ ID NO:39 are covalently linked by amide bonds to a lysine residue, the lysine residues corresponding to J_1 and J_2. The J_2 Lys is bound by means of a covalent peptide bond to one terminus of a tripeptide formed from two glycine amino acid residues and corresponding to J3.

[0154] The peptides were assembled stepwise by solid-phase synthesis on a substituted benzhydrylamine resin, using Fmoc chemistry for temporary protection of amino groups in the repetitive cycles. Branching of the chain was accomplished by stepwise growth of identical chains from the side-chain amino groups of consecutive lysyl residues. The completed peptide chains were cleaved from the resin as C-terminal amides by acidolysis, which also removed the acid-labile side-chain protecting groups.

[0155] Peptide fractions were concentrated by loading onto Sep-Pak® C18 cartridges (Waters, Milford, Mass., USA), washed with 10 column volumes of water, and then eluted with 80% acetonitrile. Eluted fractions were lyophilized, redissolved in water, and the concentration was determined by BCA® Protein Assay Kit (Pierce Endogen, Rockford, Ill., USA) using bovine serum albumin as a reference.

[0156] The resulting analog had the following structure:

NH_2-K-K-G-G-amide

I I

S S

R R

R R

L L

I I

D D

R R

T T

N N

A A

N N

F F

L L

V V

W W

Example 2

[0157] A synthetic HBGF analog, PBA2-1, was synthesized by standard solid phase peptide synthesis methods. The amino acid sequence KKRVIEIKRV (SEQ ID NO:40) (again considering the sequence in the conventional N→C orientation, notwithstanding that it is branched sequence) corresponds to the two X region peptides.

[0158] The crude preparation was purified as described above in Example 1. The resulting analog had the following structure:

NH_2-K-K-G-G-amide

V V

R R

K K

I I

E E

I I

V V

R R

K K

K K

Example 3

[0159] A synthetic HBGF analog is synthesized by standard solid phase peptide synthesis methods. The amino acid sequence ISRRLIDRTNANFLVW (SEQ ID NO:41) (again considering the sequence in the conventional N→C orientation, notwithstanding that it is branched sequence) corresponds to the two X region peptides, and is a portion of the known platelet-derived growth factor beta isoform 2 sequence.

[0160] The crude preparation is purified as described above in Example 1. The resulting analog has the following structure:

NH_2-K-K-G-G-amide

W W

V V

L L

F F

N N

A A

N N

T T

R R

D D

I I

L L

R R

R R

S S

I I

Example 4

[0161] A synthetic HBGF analog is synthesized by standard solid phase peptide synthesis methods. The amino acid sequence VRKIEIVRKK (SEQ ID NO:42) (again considering the sequence in the conventional N→C orientation, notwithstanding that it is branched sequence) corresponds to the two X region peptides, and is a portion of the known platelet-derived growth factor beta isoform 2 sequence.

[0162] The crude preparation is purified as described above in Example 1. The resulting analog has the following structure:

NH_2-K-K-G-G-amide

K K

K K

R R

V V

I I

E E

I I

K K

R R

V V

Example 5

[0163] A synthetic HBGF analog is synthesized by standard solid phase peptide synthesis methods. The amino acid sequence VRKIEIVRKK (SEQ ID NO:42) (again considering the sequence in the conventional N→C orientation, notwithstanding that it is branched sequence) here too corresponds to the two X region peptides, where the analog is of formula I wherein R_1 is not a trifunctional amino acid residue, but is rather glycine, and analog accordingly is single branched.

[0164] The crude preparation is purified as described above in Example 1. The resulting analog has the following structure:

VRKIEIVRKK-G-K-G-G-G-amide

K

K

R

V

I

E

I

K

-continued

R

V

Example 6

[0165] A synthetic BMP-2 HBGF analog is synthesized by standard solid phase peptide synthesis methods. The amino acid sequence AISIMLYLDENEKVVL (SEQ ID NO:11) (again considering the sequence in the conventional N→C orientation, notwithstanding that it is branched sequence) corresponds to the two X region peptides, but this analog is of formula I wherein J_3 is hydrophobic, consisting of the sequence Hex-Hex-Hex where Hex is 6-aminohexanoic acid.

[0166] The crude preparation is purified as described above in Example 1. The resulting analog has the following structure:

NH_2-K-K-Hex-Hex-Hex-amide

L L

V V

V V

K K

E E

N N

E E

D D

L L

Y Y

L L

M M

S S

I I

A A

[0167] The preceding examples can be repeated with similar success by substituting the generically or specifically described reactants and/or operating conditions of this invention for those used in the preceding examples.

[0168] Although the invention has been described in detail with particular reference to these preferred embodiments, other embodiments can achieve the same results. Variations and modifications of the present invention will be obvious to those skilled in the art and it is intended to cover in the appended claims all such modifications and equivalents. The entire disclosures of all references, applications, patents, and publications cited above are hereby incorporated by reference.

SEQUENCE LISTING

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Phe Val Leu Val Cys Tyr Asp Asp Gly Ser Glu Ala
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Lys

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Leu Asn Gln Lys
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Phe Met Asp Val
20

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Leu His His Ala
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1 5 10 15

Tyr

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Asn Met Ile Val Lys Ser
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1 5 10 15

Ser Asn Met Val Val
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Asn Tyr Gln Asp Met Val Val
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Lys Val Tyr Pro Asn Met Thr Val
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Lys Val Tyr Pro Asn Met Ser Val
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Asn Tyr Gln Glu Met Val Val
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Lys Tyr Arg Asn Met Val Val
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Lys Tyr Arg Asn Met Val Val
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Lys Tyr Arg Asn Met Val Val
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Lys Ala Arg Asn Met Val Val
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His Tyr Glu Gly Met Ser Val
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1 5 10 15

Tyr Glu Gly Met Ala Val
20

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20

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<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic BMP-12 analog

<400> SEQUENCE: 31

Ile Ser Ile Leu Tyr Ile Asp Ala Ala Asn Asn Val Val Tyr Lys Gln
1 5 10 15

Tyr Glu Asp Met Val Val
20

<210> SEQ ID NO 32
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic BMP-13 analog

<400> SEQUENCE: 32

Ile Ser Ile Leu Tyr Ile Asp Ala Gly Asn Asn Val Val Tyr Lys Gln
1 5 10 15

Tyr Glu Asp Met Val Val
20

<210> SEQ ID NO 33
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic BMP-14 analog

<400> SEQUENCE: 33

Ile Ser Ile Leu Phe Ile Asp Ser Ala Asn Asn Val Val Tyr Lys Gln
1 5 10 15

Tyr Glu Asp Met Val Val
20

<210> SEQ ID NO 34
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic BMP-15 analog

<400> SEQUENCE: 34

Ile Ser Val Leu Met Ile Glu Ala Asn Gly Ser Ile Leu Tyr Lys Glu
1 5 10 15

Tyr Glu Gly Met Ile Ala
20

<210> SEQ ID NO 35
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic GDF-1 analog

<400> SEQUENCE: 35

Ile Ser Val Leu Phe Phe Asp Asn Ser Asp Asn Val Val Leu Arg Gln
1 5 10 15

-continued

Tyr Glu Asp Met Val Val
20

<210> SEQ ID NO 36
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic GDF-3 analog

<400> SEQUENCE: 36

Ile Ser Met Leu Tyr Gln Asp Asn Asn Asp Asn Val Ile Leu Arg His
1 5 10 15

Tyr Glu Asp Met Val Val
20

<210> SEQ ID NO 37
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic GDF-8 analog

<400> SEQUENCE: 37

Ile Asn Met Tyr Leu Phe Asn Gly Lys Glu Gln Ile Ile Tyr Gly Lys
1 5 10 15

Ile Pro Ala Met Val Val
20

<210> SEQ ID NO 38
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic GDF-9 analog

<400> SEQUENCE: 38

Leu Ser Val Leu Thr Ile Glu Pro Asp Gly Ser Ile Ala Tyr Lys Glu
1 5 10 15

Tyr Glu Asp Met Ile Ala
20

<210> SEQ ID NO 39
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic HBGF analog

<400> SEQUENCE: 39

Trp Val Leu Phe Asn Ala Asn Thr Arg Asp Ile Leu Arg Arg Ser Ile
1 5 10 15

<210> SEQ ID NO 40
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic HBGF analog

<400> SEQUENCE: 40

Lys Lys Arg Val Ile Glu Ile Lys Arg Val
1 5 10

-continued

<210> SEQ ID NO 41
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic HBGF analog

<400> SEQUENCE: 41

Ile Ser Arg Arg Leu Ile Asp Arg Thr Asn Ala Asn Phe Leu Val Trp
1 5 10 15

<210> SEQ ID NO 42
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic HBGF analog

<400> SEQUENCE: 42

Val Arg Lys Ile Glu Ile Val Arg Lys Lys
1 5 10

<210> SEQ ID NO 43
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic FGF-2 analog

<400> SEQUENCE: 43

His Ile Lys Leu Gln Leu Gln Ala Glu Glu Arg Gly Val Val Ser
1 5 10 15

<210> SEQ ID NO 44
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic FGF-10 analog

<400> SEQUENCE: 44

Tyr Ala Ser Phe Asn Trp Gln His Asn Gly Arg Gln Met Tyr Val Ala
1 5 10 15

Leu Asn Gln Lys
20

<210> SEQ ID NO 45
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic FGF-22 analog

<400> SEQUENCE: 45

Tyr Ala Ser Gln Arg Trp Arg Arg Arg Gly Gln Pro Asn Leu Ala Leu
1 5 10 15

Asp Arg Arg

<210> SEQ ID NO 46
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Artificial

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<220> FEATURE:
<223> OTHER INFORMATION: synthetic FGF-9 analog

<400> SEQUENCE: 46

Tyr Ser Ser Asn Leu Tyr Lys His Val Asp Thr Gly Arg Arg Tyr Tyr
1 5 10 15

Val Ala Leu Asn Lys
20

<210> SEQ ID NO 47
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic FGF-16 analog

<400> SEQUENCE: 47

Tyr Ala Ser Thr Leu Tyr Lys His Ser Asp Ser Glu Arg Gln Tyr Val
1 5 10 15

Ala Leu Asn Lys
20

<210> SEQ ID NO 48
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic FGF-20 analog

<400> SEQUENCE: 48

Tyr Ser Ser Asn Ile Tyr Lys His Gly Asp Thr Gly Arg Arg Phe Val
1 5 10 15

Ala Leu Asn Lys
20

<210> SEQ ID NO 49
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic FGF-4 analog

<400> SEQUENCE: 49

Tyr Glu Ser Tyr Lys Tyr Pro Gly Met Phe Ile Ala Leu Ser Lys Asn
1 5 10 15

<210> SEQ ID NO 50
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic FGF-6 analog

<400> SEQUENCE: 50

Tyr Glu Ser Asp Leu Tyr Gln Gly Thr Tyr Ile Leu Ser Lys Tyr Gly
1 5 10 15

Arg

<210> SEQ ID NO 51
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial

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<220> FEATURE:
<223> OTHER INFORMATION: synthetic FGF-12 analog

<400> SEQUENCE: 51

Tyr Ser Ser Thr Leu Tyr Arg Gln Gln Glu Ser Gly Arg Ala Trp Phe
1 5 10 15

Leu Gly Asn Lys
20

<210> SEQ ID NO 52
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic FGF-14 analog

<400> SEQUENCE: 52

Tyr Ser Ser Met Leu Tyr Arg Gln Gln Glu Ser Gly Arg Ala Trp Phe
1 5 10 15

Leu Gly Leu Asn Lys
20

<210> SEQ ID NO 53
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic FGF-13 analog

<400> SEQUENCE: 53

Tyr Ser Ser Met Ile Tyr Arg Gln Gln Gln Ser Gly Arg Gly Trp Tyr
1 5 10 15

Leu Gly Leu Asn Lys
20

<210> SEQ ID NO 54
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic FGF-11 analog

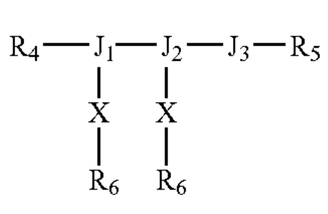
<400> SEQUENCE: 54

Tyr Ala Ser Ala Leu Tyr Arg Gln Arg Arg Ser Gly Arg Ala Trp Tyr
1 5 10 15

Leu Asp Lys

What is claimed is:

1. A heparin-binding growth factor (HBGF) analog of formula II:



II

wherein:

R_4 and R_6 are each independently NH_2, an acyl group with a linear or branched C_1 to C_{17} alkyl, aryl, heteroaryl, alkene, alkenyl or aralkyl chain including an N-terminus NH_2, NH_3^+, or NH group or a corresponding acylated derivative, or is amino acid, a dipeptide or a tripeptide with an N-terminus NH_2, NH_3^+, or NH group or a corresponding acylated derivative;

R_5 is —OH, NH_2, NH—R_6, or is an amino acid, a dipeptide or a tripeptide with a C-terminus —OH, NH_2, or NH—R_7;

R_7 is an aliphatic C_1 to C_{17} chain;

each X is a peptide chain that (i) has a minimum of three amino acid residues, (ii) has a maximum of about fifty amino acid residues, and (iii) binds a heparin-binding growth factor receptor (HBGFR);

J_1 and J_2 are each independently a trifunctional alpha amino acid residue, wherein each X is covalently bonded through a side chain of J_1 or J_2;

J_3 is a chain comprising a chain from 0 to about 50 atoms covalently bonded to J_2;

n is 0 or 1, wherein when n=1 the synthetic peptide chains X are identical.

2. The heparin-binding growth factor analog of claim **7** wherein X and J_3 are synthetic peptide chains.

3. The heparin-binding growth factor analog of claim **8** which is a synthetic heparin-binding growth factor analog.

4. The heparin-binding growth factor analog of claim **7** wherein J_3 comprises a chain of a minimum of about 9 and a maximum of about 50 atoms, and J_3 is not found in the natural ligand of the heparin-binding growth factor receptor (HB-GFR) which X binds.

5. The heparin-binding growth factor analog of claim **7** wherein binding of the heparin-binding growth factor analog to the heparin-binding growth factor receptor initiates a signal by the heparin-binding growth factor receptor.

6. The heparin-binding growth factor analog of claim **7** wherein binding of the heparin-binding growth factor analog to the heparin-binding growth factor receptor blocks signaling by the heparin-binding growth factor receptor.

7. The heparin-binding growth factor analog of claim **7** wherein J_1 and J_2 are diamine amino acid residues.

8. The heparin-binding growth factor analog of claim **13** wherein the diamine amino acid residue is a 2,3 diamino propionyl amino acid residue, a 2,4 diamino butylic amino acid residue, lysine or ornithine.

9. The heparin-binding growth factor analog of claim **7** wherein the covalent bond between X and J_1 and J_2 comprises an amide, disulfide, thioether, Schiff base, reduced Schiff base, imide, secondary amine, carbonyl, urea, hydrazone or oxime bond.

10. The heparin-binding growth factor analog of claim **7** wherein the side chains of J_1 and J_2 comprise reactive carboxyl groups.

11. The heparin-binding growth factor analog of claim **7** wherein X comprises an amino acid sequence found in any of FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, FGF-15, FGF-16, FGF-17, FGF-18, FGF-19, FGF-20, FGF-21, FGF-22, FGF-23, HBBM (heparin-binding brain mitogen), HB-GAF (heparin-binding growth associated factor), HB-EGF (heparin-binding EGF-like factor) HB-GAM (heparin-binding growth associated molecule, also known as pleiotrophin, PTN, HARP), TGF-α (transforming growth factor-α), TGF-βs (transforming growth factor-βs), VEGF (vascular endothelial growth factor), EGF (epidermal growth factor), IGF-1 (insulin-like growth factor-1), IGF-2 (insulin-like growth factor-2), PDGF (platelet derived growth factor), RANTES, SDF-1, secreted frizzled-related protein-1 (SFRP-1), small inducible cytokine A3 (SCYA3), inducible cytokine subfamily A member 20 (SCYA20), inducible cytokine subfamily B member 14 (SCYB14), inducible cytokine subfamily D member 1 (SCYD1), stromal cell-derived factor-1 (SDF-1), thrombospondins 1, 2, 3 and 4 (THBS1-4), platelet factor 4 (PF4), lens epithelium-derived growth factor (LEDGF), midikine (MK), macrophage inflammatory protein (MIP-1), moesin (MSN), hepatocyte growth factor (HGF, also called SF), placental growth factor, IL-1 (interleukin-1), IL-2 (interleukin-2), IL-3 (interleukin-3), IL-6 (interleukin-6), IL-7 (interleukin-7), IL-10 (interleukin-10), IL-12 (interleukin-12), IFN-α (interferon-α), IFN-γ (interferon-γ), TNF-α (tumor necrosis factor-α), SDGF (Schwannoma-derived growth factor), nerve growth factor, neurite growth-promoting factor 2 (NEGF2), neurotrophin, BMP-2 (bone morphogenic protein 2), OP-1 (osteogenic protein 1, also called BMP-7), keratinocyte growth factor (KGF), interferon-γ inducible protein-20, RANTES, and HIV-tat-transactivating factor, amphiregulin (AREG), angio-associated migratory cell protein (AAMP), angiostatin, betacellulin (BTC), connective tissue growth factor (CTGF), cysteine-rich angiogenic inducer 61 (CYCR61), endostatin, fractalkine/neuroactin, glial derived neurotrophic factor (GDNF), GRO2, hepatoma-derived growth factor (HDGF), and granulocyte-macrophage colony stimulating factor (GMCSF).

12. The heparin-binding growth factor analog of claim **7** wherein the peptide chains X are cross-linked or cyclized.

13. The heparin-binding growth factor analog of claim **7** wherein J_3 comprises between one and about thirty-three ethylene glycol units.

14. The heparin-binding growth factor analog of claim **7** wherein J_3 comprises a branched or unbranched, saturated or unsaturated alkyl chain of between one and about twenty carbon atoms.

15. The heparin-binding growth factor analog of claim **7** wherein J_3 comprises $[NH_2—(CH_2)_pCO]_q$ wherein p is from 1 to about 10 and q is from 1 to about 20.

16. The heparin-binding growth factor analog of claim **7** wherein J_3 comprises a peptide sequence comprising from one to about 16 Gly residues.

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