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(54) FIBROBLAST GROWTH FACTOR

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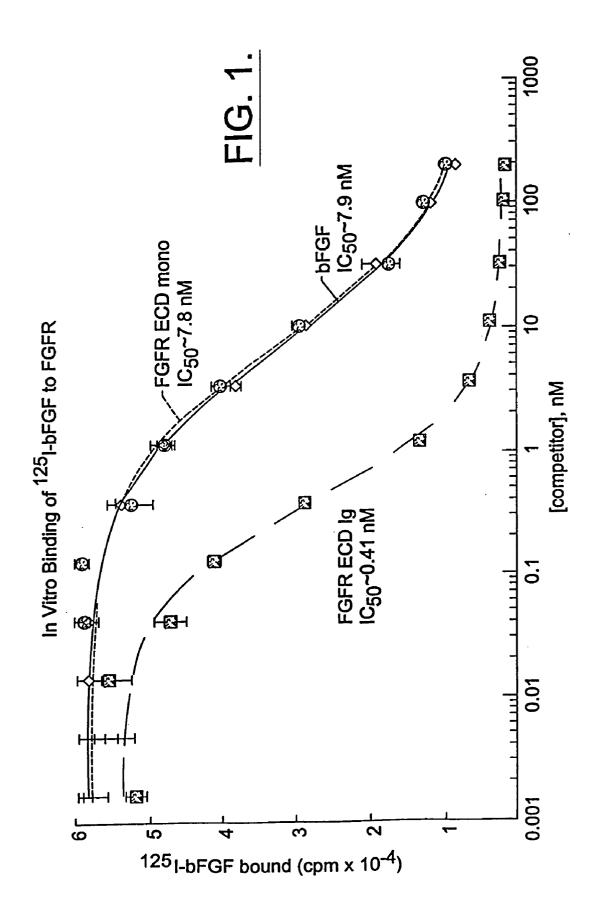
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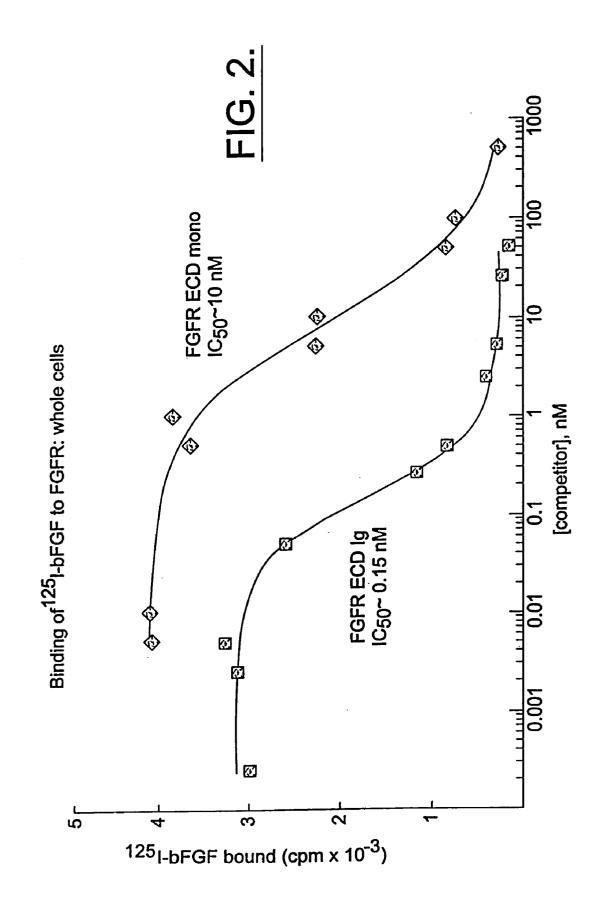
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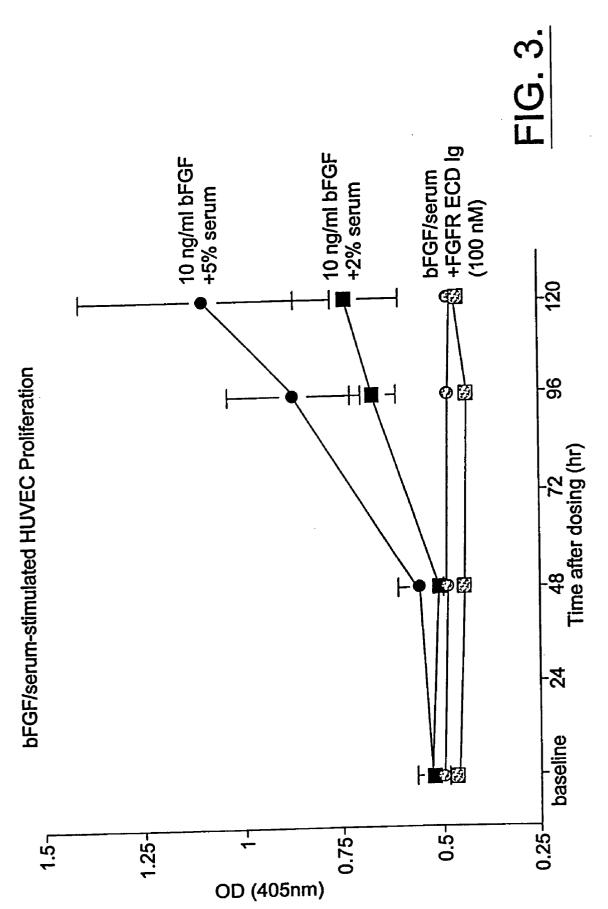
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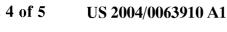
ABSTRACT (57)

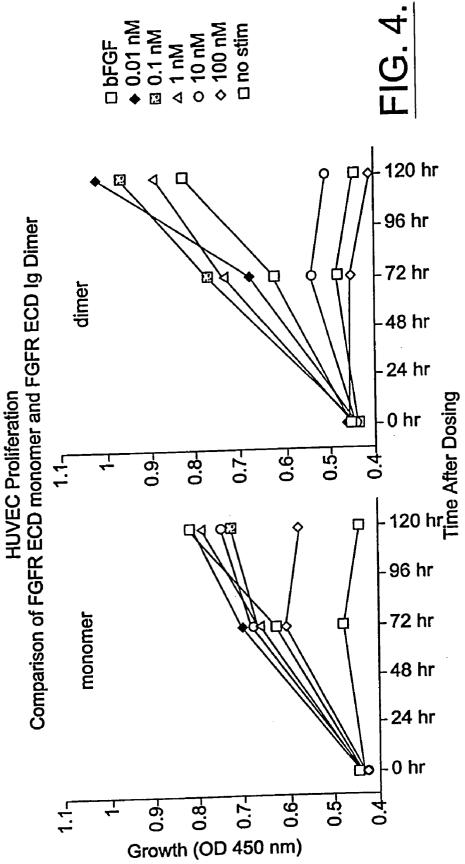
The invention relates to compositions and methods for inhibiting cell proliferation, especially angiogenesis. The invention specifically relates to fusions of the extracellular domain of a fibroblast growth factor receptor (FGFR) with a heterologous oligomerization domain, such as that contained in an immunoglobulin, to provide potent FGFR antagonists.

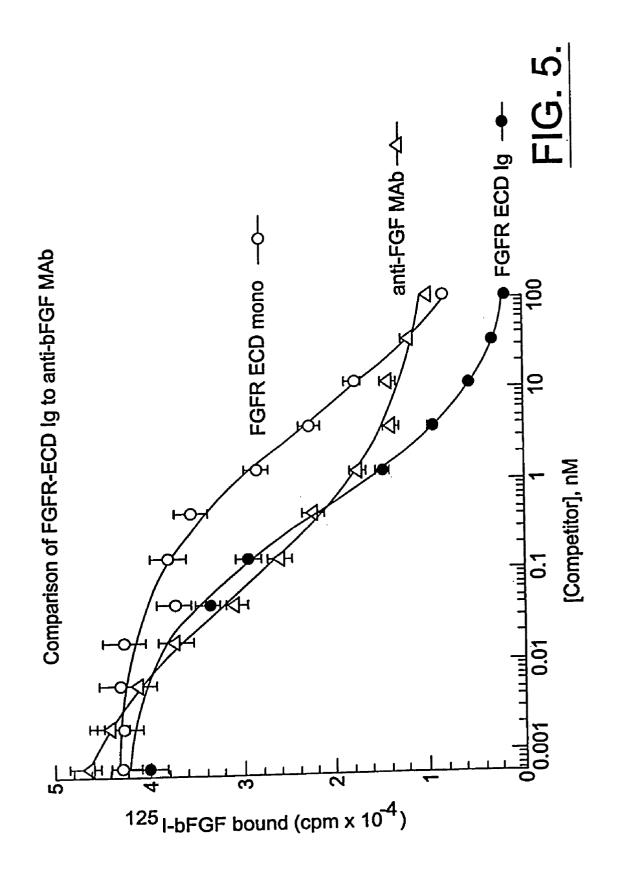












FIBROBLAST GROWTH FACTOR

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application is a divisional of U.S. application Ser. No. 09/499,846, filed Feb. 7, 2000, which claims the benefit of U.S. Provisional Application No. 60/119,002, filed Feb. 8, 1999; which are hereby incorporated herein in their entirety by reference.

FIELD OF THE INVENTION

[0002] The invention relates to compositions and methods for inhibiting cell proliferation, especially angiogenesis. The invention specifically relates to fusions of the extracellular domain of a fibroblast growth factor receptor (FGFR) with an immunoglobulin (Ig), especially an Ig Fc region, as an FGFR antagonist. The invention also relates to novel FGFR-Ig fusion proteins.

BACKGROUND OF THE INVENTION

[0003] Angiogenesis, or development of new blood vessels, is implicated in a host of diseases including tumorigenesis, metastasis and tumor growth, retinopathies, neovascular ocular disorders, and postangioplasty or postatherectomy restenosis (Bicknell et al. (1996) *Curr. Opin. Oncol.* 8:60-65; Gariano et al. (1996) *Survey Ophthalmol.* 40:481-490; and Wilcox (1993) *Am. J. Cardiol.* 72:88E-95E).

[0004] Expression and secretion of angiogenic factors by tumors has been investigated. It has been suggested that because tumors express multiple angiogenic factors, broad spectrum antagonists of angiogenesis can provide effective means of tumor stabilization. Anti-angiogenic approaches to tumor therapy have been defined to involve interference with growth, migration, and differentiation of blood vessels associated with tumor growth. Anti-angiogenic agents have been categorized to include protease inhibitors, modulators of cytokines, heparin-like molecules, and antagonists of vascular growth factors. Growth factor antagonists have been categorized to include heparin-like molecules, angiogenin antagonists, antisense fibroblast growth factor, DS 4152, suramin analogs, and protein-bound saccharide-K (Bicknell et al. (1996) *Curr. Opin. Oncol.* 8:60-65).

[0005] Various growth factors and growth factor receptors are known to be associated with particular types of tumors. At the molecular level, growth factors and growth factor receptors belong to multi-member families categorized based on structural and functional characteristics. Fibroblast growth factor (FGF) is involved in growth and differentiation of a number of cell types, and can contribute significantly to tumorigenicity. The FGF family includes FGF-1 or acidic FGF (aFGF), FGF-2 or basic FGF (bFGF), FGF-7 or KGF, oncogene products FGF-3 or int-2, hsp/Kaposi-FGF (K-FGF or FGF-4), FGF-5, and FGF-6. These members of the FGF family bind heparin, may exhibit mitogenic activity toward various cells, and may be potent mediators of angiogenesis (Pontaliano et al. (1994) *Biochemistry* 33:10229-10248; Kiefer et al. (1991) *Growth Factors* 5:115-127).

[0006] FGF receptor (FGFR) includes FGFR1 or flg, FGFR2 or bek, FGFR3 or cek2, and FGFR4 (Kiefer et al. (1991) *Growth Factors* 5:115-127). FGFR belongs to the

tyrosine kinase family of receptors and to the immunoglobulin (Ig) supergene family. mRNA splicing variants of FGFR exist that produce secreted and transmembrane forms of the receptors with various ligand binding affinities and specificities. In transmembrane forms of the receptor, the tyrosine kinase domain is intracellular and the (Ig)-like domains are extracellular. Both transmembrane and secreted forms bind FGF. Heparin and related compounds promote the interaction between FGF and FGFR by acting as cofactors in dimerization or higher oligomerization of FGFR. The dimerization process is thought to be necessary for activation of FGFR.

[0007] FGFR fusion proteins present the possibility of constructing preoligomerized, particularly predimerized forms of FGFR. Such preoligomerized forms would be useful as potent and therapeutically effective inhibitors of FGF-mediated cell proliferation. FGFR antagonists would be especially useful to treat diseases involving angiogenesis.

[0008] Monomeric forms of the FGFR extracellular domain have been used to inhibit FGF-mediated events (Kiefer et al. (1991) *Growth Factors* 5:115-127). However, preoligomerized forms of the FGFR extracellular domain have not been used as FGFR antagonists. Thus, there is a need for providing preoligomerized forms of FGFR extracellular domain as antagonists of FGFR. Given the implicated role of this ligand/receptor system in angiogenesis, and the breadth of involvement of angiogenesis in several malignancies and other disorders, the approach promises a useful tool in providing an effective therapy for such disorders.

[0009] Receptor-immunoglobulin (Ig) fusion proteins have been used in the art. For example, an Ig fusion protein with a human tumor necrosis factor receptor has been applied to treatment of rheumatoid arthritis and septic shock (Moreland et al. (1997) New Engl. J. Med. 337:141-147; Fisher et al. (1996) New Engl. J. Med. 334:1697-1702). An Ig fusion protein with urokinase plasminogen activator (uPA) has been used as a uPA receptor antagonist to inhibit angiogenesis and tumor growth (Min et al. (1996) Cancer Res. 56:2428-2433). WO 95/21258 describes using FGFR-Ig fusion proteins in a method of identifying agonists and antagonists of FGFR. However, construction and use of the specific FGFR-Ig fusion proteins as antagonists of FGFR has not been suggested. Other examples of receptor-Ig fusion proteins include those described in U.S. Pat. Nos. 5,726,044; 5,707,632; and 5,750,375.

SUMMARY OF THE INVENTION

[0010] The invention is directed at providing oligomerized forms of FGFR as FGFR antagonists, constructed by fusing extracellular domains of FGFR with heterologous oligomerization domains. Compositions comprising polypeptides and polynucleotides encoding the fusion polypeptides are provided, as well as methods of using the compositions for treating disorders mediated by FGF, FGFR or angiogenesis, such as cancer and other hyperproliferative diseases.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] FIG. 1 depicts in vitro binding of ¹²⁵I-bFGF to FGFR. FGFR extracellular domain monomer, FGFR extracellular domain-Ig Fc fusion dimer, and bFGF are compared as competitors of ¹²⁵I-bFGF binding to immobilized FGFR

receptors. Bound ¹²⁵I-bFGF is plotted against a range of concentrations of each competitor.

[0012] FIG. 2 depicts binding of ¹²⁵I-bFGF to FGFR: whole cells. FGFR extracellular domain monomer and FGFR extracellular domain-Ig Fc fusion dimer are compared as competitors of ¹²⁵I-bFGF for binding to stable HEK293 cell lines overexpressing FGFR1. Bound ¹²⁵I-bFGF is plotted against a range of concentrations of each competitor.

[0013] FIG. 3 depicts bFGF/serum stimulated HUVEC proliferation. FGFR extracellular domain-Ig Fc fusion dimer is tested for its ability to inhibit proliferation of human umbilical vein endothelial cells (HUVEC) in serum- and bFGF-containing media. Optical density (OD) indicating growth is plotted against time after dosing.

[0014] FIG. 4 depicts a comparison of FGFR extracellular domain monomer and FGFR extracellular domain-Ig Fc dimmer on HUVEC proliferation. FGFR extracellular domain monomer and FGFR extracellular domain-Ig Fc fusion dimer are compared for their ability to inhibit HUVEC proliferation at indicated doses.

[0015] FIG. 5 depicts a comparison of FGFR extracellular domain-Ig Fc to anti-bFGF Mab. FGFR extracellular domain-Ig Fc fusion protein and an anti-bFGF monoclonal antibody (Mab) are compared as competitors of ¹²⁵I-bFGF binding to immobilized FGF receptors. Bound ¹²⁵I-bFGF is plotted against a range of concentrations of each competitor.

DETAILED DESCRIPTION OF THE INVENTION

[0016] Compositions

[0017] The invention is based on means to provide potent FGF receptor (FGFR) antagonists. Dimeric or higher-order oligomeric forms of FGF are required for activation of FGFR, which proceeds through receptor dimerization (Moy et al. (1997) Biochemistry 36:4782-4791; Pontaliano et al. (1994) Biochemistry 33:10229-10248). Accordingly, the invention provides polypeptides capable of forming oligomers of FGFR, particularly dimers. The polypeptides comprise FGFR extracellular domains fused to heterologous oligomerization domains. Such oligomerized FGF receptors are provided as FGFR antagonists with higher potency relative to the monomeric FGFR antagonist. More particularly, the invention provides polypeptides comprising specific FGFR extracellular domains fused to regions of immunoglobulin (Ig) molecules which are known to be capable of forming oligomers with other Ig regions.

[0018] "FGFR extracellular domain" as used herein includes that portion of FGFR that is extracellular in native transmembrane forms of the receptor, or is of such extracellular origin, or consists of all or part of the naturally secreted forms of the receptor. It is understood, however, that the extracellular domain could contain other regions of the FGFR receptor (i.e., non-extracellular portions) as long as these portions do not interfere with or-significantly alter the function of the extracellular domain that is relevant to the methods described herein.

[0019] The FGFR extracellular domain shares homology with the immunoglobulin supergene family. The FGFR extracellular domain contains Ig-like segments. Further-

more, extracellular domains of different members of the FGFR family contain different numbers of Ig-like segments. The Ig-like segments are classified by their position relative to the amino-terminus of the FGF receptor, and by sequence homology to known Ig-like domains. Such classifications are known by a person of ordinary skill in the art. See, for example, Pontaliano et al. (1993) *Biochemistry* 33:10229-10248; Note 1, 10229.

[0020] The Ig-like segments are generally designated by numbering according to the relative positions of the segments from the amino terminus of FGFR with three Ig-like domains. Such designations are known by a person of ordinary skill in the art and are used for the purposes of this application, unless indicated otherwise. For example, see Pontaliano et al. (1994) *Biochemistry* 33:10229-10248. Accordingly, in forms of FGFR having three Ig-like segments, the Ig I segment is classified as the first Ig-like segment of an FGFR extracellular domain from the amino terminus of the molecule, the Ig II segment as the second and the Ig III segment as the third.

[0021] In naturally or artificially truncated forms of FGFR which have less than three Ig-like segments by virtue of the truncation of the extracellular domain, the numbering of the Ig-like segments are retained as they were prior to the truncation. The numbers designating the Ig-like segments are not reassigned according to relative positions of the segments from the amino-terminus subsequent to the truncation. Such designations are known in the art and are used for the purposes of this application, unless indicated otherwise. For example, a truncated form of FGFR containing two Ig-like domains due to deletion of a region encompassing the Ig I segment, is referred to as containing the Ig II and Ig III segments, although in this truncated form, the Ig II segment is the first Ig-like segment from the amino-terminus and the Ig III segment is the second.

[0022] Extracellular domains of FGFR containing different numbers and types of such Ig-like segments are capable of binding various forms of FGF. The affinity and specificity of this binding is at least partially attributable to the type of Ig-like segment contained within the extracellular domain.

[0023] Thus, the invention relates to providing variants of the extracellular domain of FGFR such that FGFR antagonists can be created based on FGF binding capability, affinity, and specificity.

[0024] By "variants" is intended substantially similar sequences. Thus, for nucleotide sequences, variants include those sequences that encode corresponding parts of the fusion polypeptides of the invention, but that differ conservatively because of the degeneracy of the genetic code. These naturally occurring allelic variants can be identified with the use of well-known molecular biology techniques, such as polymerase chain reaction (PCR) and hybridization techniques as outlined below.

[0025] Variant nucleotide sequences also include synthetically derived nucleotide sequences that have been generated, for example, by using site-directed mutagenesis but which still encode corresponding parts of the fusion polypeptide sequences provided in the present invention as discussed below. Generally, nucleotide sequence variants of the invention will have at least 70%, preferably at least 80%, more preferably about 90 to 95% or more, and most preferably about 98% or more sequence identity to the provided nucleotide sequence.

[0026] A variant polypeptide can differ in amino acid sequence by one or more substitutions, deletions, insertions, inversions, fusions, and truncations, or a combination thereof. With respect to the amino acid sequences for various domains of the fusion polypeptides, variants include those domains that are derived from corresponding native domains by deletion (so-called truncation) or addition of one or more amino acids to the N-terminal and/or C-terminal end of the polypeptide; deletion or addition of one or more amino acids at one or more sites in the polypeptide; or substitution of one or more amino acids at one or more sites in the polypeptide. Such variants may result from, for example, genetic polymorphism or from human manipulation. Methods for such manipulations are generally known in the art.

[0027] Variants of the FGFR extracellular domain include deletion variants. In one embodiment, amino acid segments that do not contribute to the desired capability, affinity, and specificity of binding of FGF are deleted, while those segments that contribute favorably to these functional properties are retained.

[0028] Deletion variants also include those in which deletion of particular amino acid segments positively affects the desired affinity and binding of FGF.

[0029] Deletion could comprise any segment of the extracellular domain including but not limited to the Ig I segment. In this aspect, the invention provides FGFR extracellular domain fusion polypeptides which lack the Ig I segment. Natural variants of FGFR containing the Ig I segment and those which lack the Ig I segment are capable of binding acidic FGF (aFGF) and basic FGF (bFGF) (Kiefer et al. (1991) *Growth Factors* 5:115-127; Johnson and Williams (1993) *Adv. Cancer. Res.* 60:1-41). Thus, the Ig I segment is not necessary for binding of aFGF and bFGF. The Ig I deletion further increases the affinity for aFGF and heparin, protects the core of the molecule from proteolysis, and abrogates the heparin requirement for aFGF binding.

[0030] Deletions can range from portions of a segment to deletion of an entire segment. Further, deletions can include one or more deletions in one or more of the Ig-like segments of the FGFR extracellular domain.

[0031] The invention further provides fusion polypeptides in which the FGFR extracellular domain lacks the acid box segment. The acid box segment is a known common feature of the FGFR extracellular domain and is characterized by multiple acidic amino acid motifs. For example, see Kiefer et al. (1991) *Growth Factors* 5:115-127.

[0032] The Ig II segment is typically defined as the second Ig-like segment from the amino-terminus of an FGFR with three Ig-like segments. The invention encompasses oligomerized FGFR extracellular domains comprising polypeptides which are variants with respect to the Ig II segment. For example, it is recognized that fusion polypeptides lacking both Ig I and Ig II extracellular segments may have favorable FGF binding characteristics with respect to affinity and specificity of binding and be useful as an FGFR antagonist.

[0033] The Ig III segment is typically defined as the third Ig-like segment from the amino-terminus of an FGFR with three Ig-like segments. Sequence variants of the C-terminal half of the Ig III segment are associated with differential FGF binding affinities and specificities. The IIIc variant of the Ig III segment binds aFGF and bFGF with an equal

affinity, higher than that for FGF-7. The IIIb variant binds aFGF and FGF-7 with an equal affinity, higher than that for bFGF. The IIIa variant binds bFGF with a higher affinity than that for aFGF. The IIIc variant is the most widely expressed natural variant of the Ig III segment, and its deletion decreases the affinity for all ligands of the receptor. Further descriptions of the Ig IIIa, Ig IIIb, and Ig IIIc sequence variants are provided in Werner et al. (1992) *Mol. and Cell Biol.* 12:82-88, herein incorporated by reference.

[0034] The invention provides fusion polypeptides comprising sequence variants of the Ig III segment. The selection of the particular Ig III variants is based on the desired FGF binding affinities and specificities.

[0035] More preferred is a fusion polypeptide in which the FGFR extracellular domain comprises the IIIc sequence variant of the Ig III segment. The invention encompasses fusion polypeptides in which the FGFR extracellular domain comprises the IIIa or the IIIb sequence variants of the Ig III segment.

[0036] The invention further relates to providing a fusion polypeptide in which the FGFR extracellular domain comprises combinations of the above-described variants, including those with deletions of the Ig I segment, those with deletions of the acid box segment, and those comprising sequence variants of the Ig III segment.

[0037] More specifically, the invention provides human FGFR I extracellular domain fusion polypeptides having the amino acid sequences set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, and SEQ ID NO:12. The polypeptides having the sequences set forth in SEQ ID NO:4, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, and SEQ ID NO:12 comprise FGFR extracellular domain deletion variants of human FGFR1; lacking one or more segments as described above. Polynucleotide sequences encoding the above polypeptides are also provided and set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, and SEQ ID NO:11.

[0038] The FGF receptors of the invention also encompass polypeptides and polypeptide fragments that comprise FGFR extracellular domains of other members of the FGFR family, including but not limited to FGFR2, FGFR3, and FGFR4 (Kiefer et al. (1991) *Growth Factors* 5:115-127).

[0039] The invention also encompasses FGFR fusion polypeptides comprising other FGFR variants. These variants include substantially homologous FGFR proteins encoded by the same genetic locus, i.e., an allelic variant. The variants also include splicing variants of FGFR. The variants also encompass proteins derived from other genetic loci, but having substantial homology to the provided FGFR. The variants also include proteins substantially homologous to the provided FGFR but derived from another organism (i.e., non-human), i.e., an ortholog. The variants also include proteins substantially homologous that are produced by chemical synthesis. The variants also include proteins substantially homologous that are produced by recombinant methods.

[0040] "Homology" refers to the degree of similarity between x and y. The correspondence between the sequence from one form to another can be determined by techniques known in the art. For example, they can be determined by a direct comparison of the sequence information of the polynucleotide. Typically, two sequences, either polynucleotide or polypeptide, are homologous if the sequences exhibit at least 45% sequence identity; more typically, 50% sequence identity; more typically, 55% sequence identity; more typically, 60% sequence identity; more typically, 65% sequence identity; even more typically, 70% sequence identity. Usually, two sequences are homologous if the sequences exhibit at least 75% sequence identity; more usually, 80% sequence identity; even more usually, 85% sequence identity; even more usually, 90% sequence identity; and even more usually, 95% sequence identity.

[0041] Thus, the invention encompasses polynucleotides having 75%, preferably 80%, more preferably 85%, even more preferably 90%, and most preferably 95% or greater sequence identity to the polynucleotide sequences set forth in SEQ ID NOS:1, 3, 5, 7, 9, and 11. The invention further encompasses polypeptides having 75%, preferably 80%, more preferably 85%, even more preferably 90%, and most preferably 95% or greater sequence identity to the polypeptide sequences set forth in SEQ ID NOS:2, 4, 6, 8, 10, and 12).

[0042] Alternatively, homology can be determined by hybridization of the polynucleotides under conditions which form stable duplexes between homologous regions. Stable duplexes are those, for example, which would withstand digestion with a single-stranded specific nuclease(s), such as S_1 . Such duplexes can be analyzed by various methods, such as size determination of digested fragments.

[0043] "Hybridization" refers to the association of two nucleic acid sequences to one another by hydrogen bonding. Typically, one sequence will be fixed to a solid support and the other will be free in solution. Then, the two sequences will be placed in contact with one another under conditions that favor hydrogen bonding. Factors that affect this bonding include: the type and volume of solvent; reaction temperature; time of hybridization; agitation; agents to block the non-specific attachment of the liquid phase sequence to the solid support (Denhardt's reagent or BLOTTO); concentration of the sequences; use of compounds to increase the rate of association of sequences (dextran sulfate or polyethylene glycol); and the stringency of the washing conditions following hybridization. See Sambrook et al. (1989) Molecular Cloning: A Laboratory Manual, 2nd ed., Volume 2, chapter 9, pages 9.47 to 9.57.

[0044] "Stringency" refers to conditions in a hybridization reaction that favor association of very similar sequences over sequences that differ. For example, the combination of temperature and salt concentration should be chosen that is approximately 12° C. to 20° C. below the calculated T_m of the hybrid under study. The temperature and salt conditions can often be determined empirically in preliminary experiments in which samples of genomic DNA immobilized on filters are hybridized to the sequence of interest and then washed under conditions of different stringencies. See Sambrook et al., above, at page 9.50.

[0045] Variables to consider when performing, for example, a Southern blot are (1) the complexity of the DNA being blotted and (2) the homology between the probe and the sequences being detected. The total amount of the fragment(s) to be studied can vary a magnitude of 10, from 0.1 to 1 μ g for a plasmid or phage digest to 10⁻⁹ to 10⁻⁸ μ g for a single copy gene in a highly complex eukaryotic

genome. For lower complexity polynucleotides, substantially shorter blotting, hybridization, and exposure times, a smaller amount of starting polynucleotides, and lower specific activity of probes can be used. For example, a singlecopy yeast gene can be detected with an exposure time of only 1 hour starting with 1 μ g of yeast DNA, blotting for two hours, and hybridizing for 4-8 hours with a probe of 10⁸ cpm/ μ g. For a single-copy mammalian gene a conservative approach would start with 10 μ g of DNA, blot overnight, and hybridize overnight in the presence of 10% dextran sulfate using a probe of greater than 10⁸ cpm/ μ g, resulting in an exposure time of ~24 hours.

[0046] Several factors can affect the melting temperature (T_m) of a DNA-DNA hybrid between the probe and the fragment of interest, and consequently, the appropriate conditions for hybridization and washing. In many cases the probe is not 100% homologous to the fragment. Other commonly encountered variables include the length and total G+C content of the hybridizing sequences and the ionic strength and formamide content of the hybridization buffer. The effects of all of these factors can be approximated by a single equation:

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T_{m}=81+16.6(log 10C_{i})+0.4[% G+C)]-0.6(% forma-
mide)-600/n-1.5 (% mismatch)
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[0047] where C_i is the salt concentration (monovalent ions) and n is the length of the hybrid in base pairs (slightly modified from Meinkoth and Wahl (1984) *Anal. Biochem.* 138:267-284).

[0048] In designing a hybridization experiment, some factors affecting nucleic acid hybridization can be conveniently altered. The temperature of the hybridization and washes and the salt concentration during the washes are the simplest to adjust. As the temperature of the hybridization increases (i.e., stringency), it becomes less likely for hybridization to occur between strands that are nonhomologous, and as a result, background decreases. If the radiolabeled probe is not completely homologous with the immobilized fragment (as is frequently the case in gene family and interspecies hybridization experiments), the hybridization temperature must be reduced, and background will increase. The temperature of the washes affects the intensity of the hybridizing band and the degree of background in a similar manner. The stringency of the washes is also increased with decreasing salt concentrations.

[0049] In general, convenient hybridization temperatures in the presence of 50% formamide are 42° C. for a probe which is 95% to 100% homologous to the target fragment, 37° C. for 90% to 95% homology, and 32° C. for 85% to 90% homology. For lower homologies, formamide content should be lowered and temperature adjusted accordingly, using the equation above. If the homology and between the probe and the target fragment are not known, the simplest approach is to start with both hybridization and wash conditions which are nonstringent. If nonspecific bands or high background are observed after autoradiography, the filter can be washed at high stringency and reexposed. If the time required for exposure makes this approach impractical, several hybridization and/or washing stringencies should be tested in parallel.

[0050] To determine the percent homology of two amino acid sequences, or of two nucleic acids, the sequences are aligned for optimal comparison purposes (e.g., gaps can be

introduced in the sequence of one protein or nucleic acid for optimal alignment with the other protein or nucleic acid). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in one sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the other sequence, then the molecules are homologous at that position. As used herein, amino acid or nucleic acid "homology" is equivalent to amino acid or nucleic acid "identity." The percent homology between the two sequences is a function of the number of identical positions shared by the sequences (i.e., percent homology equals the number of identical positions/total number of positions times 100).

[0051] The invention also encompasses fusion polypeptides having a lower degree of identity than those described above, but having sufficient similarity so as to perform one or more of the same functions performed by the fusion polypeptides of the invention. Similarity is determined by conserved amino acid substitution. Such substitutions are those that substitute a given amino acid in a polypeptide by another amino acid of like characteristics. Conservative substitutions are likely to be phenotypically silent. Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu, and Ile; interchange of the hydroxyl residues Ser and Thr; exchange of the acidic residues Asp and Glu; substitution between the amide residues Asn and Gln; exchange of the basic residues Lys and Arg; and replacements among the aromatic residues Phe, Tyr. Guidance concerning which amino acid changes are likely to be phenotypically silent are found in Bowie et al. (1990) Science 247:1306-1310.

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Conservative Amin	no Acid Substitutions.
Aromatic	Phenylalanine Tryptophan
Hydrophobic	Tyrosine Leucine Isoleucine Valine
Polar	Glutamine
Basic	Asparagine Arginine Lysine
Acidic	Histidine Aspartic Acid Glutamic Acid
Small	Alanine Serine Threonine Methionine Glycine

[0052] Both identity and similarity can be readily calculated (*Computational Molecular Biology*, ed. Lesk (Oxford University Press, New York, 1988); *Biocomputing: Informatics and Genome Projects*, ed. Smith (Academic Press, New York, 1993); *Computer Analysis of Sequence Data, Part* 1, ed. Griffin and Griffin (Humana Press, New Jersey, 1994); von Heinje (1987) *Sequence Analysis in Molecular Biology* (Academic Press); and *Sequence Analysis Primer*, ed. Gribskov and Devereux (M. Stockton Press, New York, 1991). **[0053]** Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCG program package (Devereux et al. (1984) *Nucleic Acids Res.* 12(1):387), BLASTP, BLASTN, FASTA (Atschul et al. (1990) *J. Molec. Biol.* 215:403).

[0054] A variant can differ in amino acid sequence by one or more substitutions, deletions, insertions, inversions, fusions, and truncations, or a combination of any of these.

[0055] Variant polypeptides can be fully functional or can lack function in one or more activities. Thus, in the present case, functions affected by variations include FGF binding capability, FGF binding affinity, FGF binding specificity, heparin binding, inhibition of FGF-stimulated cell proliferation, inhibition of FGF-mediated disorders, inhibition of FGFR-mediated disorders, inhibition of angiogenesis-mediated disorders, and inhibition of cancer and other hyperproliferative disorders.

[0056] Fully functional variants typically contain only conservative variation or variation in non-critical residues or in non-critical regions. Functional variants can also contain substitution of similar amino acids which result in no change or an insignificant change in function. Alternatively, such substitutions may positively or negatively effect function to some degree.

[0057] Non-functional variants typically contain one or more non-conservative amino acid substitutions, deletions, insertions, inversions, or truncation or a substitution, insertion, inversion, or deletion in a critical residue or critical region.

[0058] As indicated, variants can be naturally occurring or can be made by recombinant means or chemical synthesis to provide useful and novel characteristics for the receptor polypeptide. This includes preventing immunogenicity from pharmaceutical formulations by preventing protein aggregation.

[0059] Useful variations include alteration of ligand binding characteristics. For example, one specific embodiment encompasses fusion polypeptide dimers that bind aFGF and bFGF with equal affinity. A further embodiment encompasses fusion polypeptide dimers that bind aFGF and bFGF with equal affinity, but with higher than that for FGF-7. Another embodiment encompasses fusion polypeptide dimers that bind aFGF and bFGF with high affinity.

[0060] Another useful variation is one that provides for a protease cleavage site between the extracellular domain of FGFR and the Ig portion of the fusion polypeptide. While constructs containing the cleavage sites are not suited for in vivo use due to the presence of the cleavage site, they are useful as experimental controls. One resulting product from utilizing this cleavage site is FGFR extracellular domain monomer, which is useful as a control in assessing the functional characteristics of the corresponding FGFR extracellular domain-Ig dimer without the cleavage site.

[0061] The invention provides polypeptides comprising FGFR extracellular domains fused to heterologous oligomerization domains. By "heterologous oligomerization domain" is intended that domain of a polypeptide of the invention which is not naturally associated with the extracellular domain of the polypeptide, and is capable of forming oligomers, which are at least dimers, with other polypep-

tides. Specific examples of such heterologous oligomerization domains include, but are not limited to, the Fc region of an immunoglobulin molecule; the hinge region of an immunoglobulin molecule; the CH1 region of an immunoglobulin molecule; the CH2 region of an immunoglobulin molecule; the CH3 region of an immunoglobulin molecule; the CH3 region of an immunoglobulin molecule; the CH4 region of an immunoglobulin molecule; the light chain of an immunoglobulin molecule; and a peptide comprising a leucine zipper motif.

[0062] Leucine zipper motifs are known in the art, and are typically found in some of the DNA-binding proteins. Leucine zippers are formed from a region of α -helix that contain at least four leucines, each leucine separated by six amino acids from one another (i.e., Leu-X₆-Leu-X₆-Leu-X₆-Leu-X₆-Leu, where X is any common amino acid). See Devlin (1997) *Textbook of Biochemistry with Clinical Correlations* (4th ed., John Wiley and Sons, Inc.), pp. 110-111.

[0063] Examples of utilizing various immunoglobulin domains for constructing oligomeric fusion proteins are known in the art. See, for example, EP-A 0464 533 and U.S. Pat. No. 5,726,044, which describe fusion proteins comprising immunoglobulin Fc regions. See also U.S. Pat. No. 5,750,375, which describes fusion proteins comprising various heavy chain domains, as well as light chain kappa.

[0064] The heavy chain Fc region of an immunoglobulin molecule which contains the hinge region, but not the CH1 region of the heavy chain, is useful in therapy and diagnosis; and its incorporation into a protein results, for example, in improved pharmacokinetic properties of the protein. For example, see EP-A 0232 262. In drug discovery applications, human proteins have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists (Bennett et al. (1995) Journal of Molecular Recognition 8:52-58 and Johanson et al. (1995) The Journal of Biological Chemistry 270, 16:9459-9471). This invention encompasses soluble fusion proteins containing a receptor polypeptide and various portions of the constant regions of heavy or light chains of immunoglobulins of various subclass (IgG, IgD, IgM, IgA, IgE). Preferred as immunoglobulin is the constant part of the heavy chain of human IgG, particularly IgG1, where dimerization between two heavy chains takes place at the hinge region. It is recognized that inclusion of the CH2 and CH3 domains of the Fc region as part of the fusion polypeptide increases the in vivo circulation half-life of the polypeptide comprising the Fc region, and that of the oligomer or dimer comprising the polypeptide

[0065] Furthermore, the proteins of the invention are generally designed to eliminate or at least minimize the contribution of the Ig Fc fusion protein to immunogenic responses. To this end, native or mutated Ig Fc portions are preferred which have low or diminished affinity for Fc receptors, and have diminished capacities for interaction with complement; Duncan and Winter (1988) *Nature* 332:738-740; Xu et al. (1994) *J. Biol. Chem.* 269:3469-3474. For example, mutations of amino acids corresponding to Leu 235 and Pro 331 of human IgG1 to Glu and Ser respectively, are provided. More specifically, these mutations are provided as set forth in SEQ ID NOS:7 and 8 (Construct #4) and SEQ ID NOS:9 and 10 (Construct #5), and described in more detail in Example 6 below.

[0066] In order to express any of the fusion proteins of the invention in a secreted form, a signal peptide is typically

contained at the N-terminus of the fusion protein. Generally, the signal peptide that is native to the FGFR extracellular domain is comprised by the fusion proteins of the invention. Alternatively, signal peptides that are heterologous with respect to the extracellular domain may be used.

[0067] Heparin is known to be required for optimal FGF binding to FGFR. Thus, in constructing the fusion proteins, the heparin binding site is generally retained as part of the FGFR extracellular domain.

[0068] Methods for testing the function of the fusion proteins of the invention include, but are not limited to, the following methods, herein incorporated by reference: in vitro and in situ growth factor binding assays (Pontaliano et al. (1994) *Biochemistry* 33:10229-10248; Kiefer et al. (1991) *Growth Factors* 5:115-127; U.S. Pat. No. 5,229,501; cell proliferation assays (U.S. Pat. No. 5,229,501; WST cell proliferation assay, Boehringer Mannheim); in vivo and ex vivo assessments of angiogenesis (Min et al. (1996) *Cancer Res.* 56:2428-2433; Bickness et al. (1996) *Curr. Opin. Oncol.* 8:60-65); and assessments of tumor growth (Kim et al. (1993) *Nature* 362:841-844; Millauer et al. (1993) Nature 367:576-579).

[0069] It is understood that other methodologies associated with particular pathologies, biological conditions, or processes may be employed when applicable to testing the fusion proteins of the invention. Examples of such methodologies include molecular biological, immunochemical, histochemical, and morphological assessments relevant to cell proliferation in restenosis (Wilcox (1993) Am. J. Cardiol. 72:88E-95E) and ocular diseases (Gariano et al. (1996) Survey Ophthalm. 40:481-490). Additional methodologies for assessing vascular density and tumor growth; biochemical assays utilizing markers of angiogenesis; and in vivo methods of assessing therapeutic effects of antiangiogenic agents disclosed by Bickness et al. (1996) Curr. Opin. Oncol. 8:60-65, may also be employed in testing the fusion proteins of the invention, and are herein incorporated by reference.

[0070] Polynucleotide Constructs and Host Cells

[0071] DNA constructs encoding the FGFR fusion polypeptides of the invention, DNA constructs capable of expressing the FGFR fusion polypeptides, and host cells containing or capable of expressing such constructs are also provided by the invention.

[0072] Routine techniques for the construction of the vectors comprising the fusion proteins of the present invention are well-known to those of ordinary skill in the art and can be found in such references as Sambrook et al. (1989) Molecular Cloning: A Laboratory Manual (2nd ed., Cold Spring Harbor, N.Y.). A variety of strategies are available for ligating fragments of DNA, the choice of which depends on the nature of the termini of the DNA fragments and which choices can be readily made by those of skill in the art. Generally, and unless otherwise specified, the 3' end of the DNA segment encoding the desired FGFR extracellular domains will be ligated in frame to the 5' end of a DNA segment encoding the desired oligomerization domain, such that a contiguous fusion protein is produced upon expression of the ligated DNA. These strategies may encompass PCR techniques in obtaining or modifying pertinent DNA segments. Also available to one of ordinary skill in the art is a variety of host cells for containing and expressing the desired constructs.

[0073] Constructs include native sequences and variants, as well as sequences that hybridize under stringent conditions.

[0074] Methods of Using FGFR-IG Fusions

[0075] Working examples of the invention provide methods of inhibiting FGF-stimulated cell proliferation by administering an FGFR antagonist comprising a fusion of the extracellular domain of FGFR with the Fc region of an Ig molecule as the heterologous oligomerization domain in an amount effective to inhibit the proliferation. The invention also provides methods of inhibiting angiogenesis by administering to cells capable of undergoing angiogenesis the fusion protein in an amount effective to inhibit angiogenesis. The inhibition could be in vitro or in vivo.

[0076] "Amount effective to inhibit" is intended to mean that amount of the fusion protein which prevents or induces a measurable inhibition of FGF-stimulated cell proliferation. Methods of measuring such inhibition are known to those skilled in the art and include available commercial kits which are based on measuring numbers of viable cells.

[0077] Where the inhibition is in vivo, the amount effective to inhibit can induce a concentration of the fusion protein in the target organ or tissue needed to inhibit cell proliferation in the cells of the target organ or tissue.

[0078] The invention provides methods of treating disorders which are FGF-mediated, FGFR-mediated, or angiogenesis-mediated. The methods encompass administering therapeutically effective amounts of the polypeptides of the invention or vectors comprising polynucleotides encoding the polypeptides of the invention to a mammal. The administration can be alone or in conjunction with other agents, including other inhibitors of angiogenesis, tumorigenesis, anticancer agents, and the like.

[0079] Where the treating of a disorder involves administering polynucleotides comprising coding regions comprising the polypeptides of the invention, the polynucleotides are provided in expression vectors capable of expressing the polynucleotides in a particular organism, organ, tissue, or cell type; such that the coding region is operably linked to the promoter of the expression vector.

[0080] The therapeutic polynucleotides and polypeptides of the present invention may be utilized in gene delivery vehicles. The gene delivery vehicle may be of viral or non-viral origin (see generally, Jolly (1994) *Cancer Gene Therapy* 1:51-64; Kimura (1994) *Human Gene Therapy* 1:185-193; and Kaplitt (1994) *Nature Genetics* 6:148-153). Gene therapy vehicles for delivery of constructs including a coding sequence of a therapeutic of the invention can be administered either locally or systemically. These constructs can utilize viral or non-viral vector approaches. Expression of such coding sequences can be induced using endogenous mammalian or heterologous promoters. Expression of the coding sequence can be either constitutive or regulated.

[0081] The present invention can employ recombinant retroviruses which are constructed to carry or express a selected nucleic acid molecule of interest. Retrovirus vectors that can be employed include those described in EP 0 415 731; WO 90/07936; WO 94/03622; WO 93/25698; WO 93/25234; U.S. Pat. No. 5,219,740; WO 93/11230; WO

93/10218; Vile and Hart (1993) *Cancer Res.* 53:3860-3864; Vile and Hart (1993) *Cancer Res.* 53:962-967; Ram et al. (1993) *Cancer Res.* 53:83-88; Takamiya et al. (1993) *J. Neurosci. Res.* 33:493-503; Baba et al. (1993) *J. Neuroscurg.* 79:729-735; U.S. Pat. No. 4,777,127; GB Patent No. 2,200, 651; and EP 0 345 242. Preferred recombinant retroviruses include those described in WO 91/02805.

[0082] Packaging cell lines suitable for use with the above-described retroviral vector constructs may be readily prepared (see PCT publications WO 95/30763 and WO 92/05266), and used to create producer cell lines (also termed vector cell lines) for the production of recombinant vector particles. Within particularly preferred embodiments of the invention, packaging cell lines are made from human (such as HT1080 cells) or mink parent cell lines, thereby allowing production of recombinant retroviruses that can survive inactivation in human serum.

[0083] The present invention also employs alphavirusbased vectors that can function as gene delivery vehicles. Such vectors can be constructed from a wide variety of alphaviruses, including, for example, Sindbis virus vectors, Semliki forest virus (ATCC VR-67; ATCC VR-1247), Ross River virus (ATCC VR-373; ATCC VR-1246) and Venezuelan equine encephalitis virus (ATCC VR-923; ATCC VR-1250; ATCC VR 1249; ATCC VR-532). Representative examples of such vector systems include those described in U.S. Pat. Nos. 5,091,309; 5,217,879; and 5,185,440; and PCT Nos. WO 92/10578; WO 94/21792; WO 95/27069; WO 95/27044; and WO 95/07994.

[0084] Gene delivery vehicles of the present invention can also employ parvovirus such as adeno-associated virus (AAV) vectors. Representative examples include the AAV vectors disclosed by Srivastava in WO 93/09239, Samulski et al. (1989) *J. Vir.* 63:3822-3828; Mendelson et al. (1988) *Virol.* 166:154-165; and Flotte et al. (1993) *PNAS* 90:10613-10617.

[0085] Representative examples of adenoviral vectors include those described by Berkner (1988) Biotechniques 6:616-627; Rosenfeld et al. (1991) Science 252:431-434; WO 93/19191; Kolls et al. (1994) PNAS 91:215-219; Kass-Eisler et al. (1993) PNAS 90:11498-11502; Guzman et al. (1993) Circulation 88:2838-2848; Guzman et al. (1993) Cir. Res. 73:1202-1207; Zabner et al. (1993) Cell 75:207-216; Li et al. (1993) Hum. Gene Ther. 4:403-409; Cailaud et al. (1993) Eur. J. Neurosci. 5:1287-1291; Vincent et al. (1993) Nat. Genet. 5:130-134; Jaffe et al. (1992) Nat. Genet. 1:372-378; and Levrero et al. (1991) Gene 101:195-202. Exemplary adenoviral gene therapy vectors employable in this invention also include those described in WO 94/12649; WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984; and WO 95/00655. Administration of DNA linked to killed adenovirus as described in Curiel (1992) Human Gene Therapy 3:147-154 maybe employed.

[0086] Other gene delivery vehicles and methods may be employed, including polycationic condensed DNA linked or unlinked to killed adenovirus alone, for example Curiel (1992) *Human Gene Therapy* 3:147-154; ligand linked DNA, for example see Wu (1989) *Biol. Chem.* 264:16985-16987; eukaryotic cell delivery vehicles cells, for example see U.S. Ser. No. 08/240,030, filed May 9, 1994, and U.S. Ser. No. 08/404,796; deposition of photopolymerized hydrogel materials; hand-held gene transfer particle gun, as

described in U.S. Pat. No. 5,149,655; ionizing radiation as described in U.S. Pat. No. 5,206,152 and in WO 92/11033; nucleic charge neutralization or fusion with cell membranes. Additional approaches are described in Philip (1994) *Mol. Cell Biol.* 14:2411-2418, and in Woffendin (1994) *Proc. Natl. Acad. Sci. USA* 91:1581-1585.

[0087] Naked DNA may also be employed. Exemplary naked DNA introduction methods are described in WO 90/11092 and U.S. Pat. No. 5,580,859. Uptake efficiency may be improved using biodegradable latex beads. DNA coated latex beads are efficiently transported into cells after endocytosis initiation by the beads. The method may be improved further by treatment of the beads to increase hydrophobicity and thereby facilitate disruption of the endosome and release of the DNA into the cytoplasm. Liposomes that can act as gene delivery vehicles are described in U.S. Pat. No. 5,422,120; PCT Nos. WO 95/13796, WO 94/23697, and WO 91/14445; and EP No. 0 524 968.

[0088] Further non-viral delivery suitable for use includes mechanical delivery systems such as the approach described in Woffendin et al. (1994) *Proc. Natl. Acad. Sci. USA* 91(24):11581-11585. Moreover, the coding sequence and the product of expression of such can be delivered through deposition of photopolymerized hydrogel materials. Other conventional methods for gene delivery that can be used for delivery of the coding sequence include, for example, use of hand-held gene transfer particle gun, as described in U.S. Pat. No. 5,149,655; use of ionizing radiation for activating transferred gene, as described in U.S. Pat. No. 5,206,152 and PCT No. WO 92/11033.

[0089] The invention also provides a method of diagnosing disorders involving FGF-stimulated cell proliferation, angiogenesis and tumorigenesis.

[0090] In one embodiment, the method encompasses administering to test cells an effective amount of the fusion proteins of the invention and assessing inhibition compared to a control.

[0091] In another embodiment of the invention, the abovedescribed methods of diagnosis encompass in vitro administration of any of the described fusion protein antagonists to cells in culture.

[0092] In another embodiment of the invention, the abovedescribed methods of diagnosis involve administering any of the described fusion proteins in vivo.

[0093] In one embodiment, therapeutically effective amounts of pharmacological compositions containing fusion protein antagonists as described herein are administered to a patient or an animal model in need of such administration. The methods of treatment or prevention encompass administering effective amounts of a pharmacological composition containing a fusion protein of the invention as described herein.

[0094] Methods of constructing therapeutically effective fusion protein antagonists are provided. In one embodiment of the invention, binding affinities and specificities of the fusion proteins are first characterized in vitro. Next, proteins with desired affinities and specificities against various forms of FGF are selected and further assessed for inhibition of FGF-stimulated cell proliferation. Next, new fusion proteins are constructed by deletion of segments determined not to be

necessary for desired affinities and specificities of binding to FGF or high potency of inhibition of FGF-stimulated cell proliferation. The above-described assessments and selections are repeated with the smaller deletion constructs; until a minimal protein structure having the desired affinities, specificities and potencies is constructed. The therapeutic effectiveness of selected minimal constructs are then assessed in vivo.

[0095] The administration includes, but is not limited to, administration to animal models and patients manifesting the following disorders: restenosis after angioplasty or atherectomy (Wilcox (1993) *Am. J. Cardiol.* 72:88E-95E), ophthalmological disorders involving excessive vasoproliferation (Gariano et al. (1996) *Survey Ophthalm.* 40:481-490), various tumors and cancers (Kim et al. (1993) *Nature* 334:841-844; Kim et al. (1993) *Nature* 362:841-844; Min et al. (1996) *Cancer Res.* 56:2428-2433) including AIDS-related Kaposi sarcoma (Pontaliano et al. (1994) *Biochemistry* 33:10229-10248).

[0096] Pharmacological Compositions

[0097] The invention provides pharmacological compositions comprising the fusion polypeptides of the invention or polynucleotides encoding the polypeptides described herein. The pharmacological compositions may also contain any of the described variants of FGFR.

[0098] Compounds useful for formulating polypeptides and/or protein pharmaceutical compositions can be used with fusion proteins.

[0099] The pharmaceutical compositions will comprise a therapeutically effective amount of any of the proteins of the claimed invention.

[0100] The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent to treat or prevent a disorder sufficient to exhibit a detectable preventive, ameliorative, curative or other therapeutic effect. The effect may include, for example, treatment, amelioration, or prevention of any physical or biochemical condition, for example, including but not limited to hyperproliferative growth, angiogenesis and cancer.

[0101] The effect can be detected by, for example, biochemical or histological means of assessing angiogenesis. Therapeutic effects also include reduction in physical symptoms, such as decreased tumor size. The precise effective amount for a subject will depend upon the subject's size and health, the nature and extent of the condition, and the therapeutics or combination of therapeutics selected for administration. Thus, it is not useful to specify an exact effective amount in advance. However, the effective amount for a given situation can be determined by routine experimentation.

[0102] In this aspect of the invention, by "FGF-mediated disorder," "FGFR-mediated disorder," or "angiogenesis-mediated disorder" is intended an adverse biological or biochemical condition that is exacerbated by FGF, FGFR, or angiogenesis. Examples of such disorders include, but are not limited to, tumorigenesis, neovascularization, hyperproliferation of vascular smooth muscle cells, and the like.

[0103] Tumors include, but are not limited to, bladder, breast, node-negative breast, lung, rectal, colorectal, testis,

and cervical tumors; glioblastoma; childhood brain tumors; squamous cancer of the tongue; etc.

[0104] Disorders involving neovascularization include, but are not limited to, diabetic retinopathy, retinopathy of prematurity (ROP), choroidal neovascularization, neovascular glaucoma, wound healing after surgery and injury, corneal scarring, ocular neoplasia, and breakdown of blood-retina barrier.

[0105] Disorders involving hyperproliferation of vascular smooth muscle cells include, but are not limited to, postangioplasty and postatherectomy restenosis.

[0106] It is recognized that depending on the type and stage of a particular disorder, the disorder may be mediated by FGF, FGFR, angiogenesis, or combinations thereof.

[0107] For purposes of the present invention, an effective dose will be from about 1 pg/kg to 10 mg/kg of the fusion protein in the individual to which it is administered.

[0108] A pharmaceutical composition can also contain a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent, such as antibodies or a polypeptide, genes, and other therapeutic agents. The term refers to any pharmaceutical carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition, and which may be administered without undue toxicity. Suitable carriers may be large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid coknown to those of ordinary skill in the art.

[0109] Pharmaceutically acceptable salts can be used therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of pharmaceutically acceptable excipients are available in *Remington's Pharmaceutical Sciences* (Mack Pub. Co., NJ, 1991).

[0110] Pharmaceutically acceptable carriers in therapeutic compositions may contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles. Typically, the therapeutic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. Liposomes are included within the definition of a pharmaceutically acceptable carrier.

[0111] Administration Methods

[0112] Once formulated, the fusion protein compositions of the invention can be (1) administered directly to the subject; or delivered ex vivo, to cells derived from the subject.

[0113] Direct delivery of the compositions will generally be accomplished by injection, either subcutaneously, intraperitoneally, intravenously, or intramuscularly, or delivered to the interstitial space of a tissue. The compositions can also be administered into a tumor or lesion. Other modes of

administration include oral and pulmonary administration, suppositories, and transdermal applications, needles, and gene guns, or hyposprays. Dosage treatment may be a single dose schedule or a multiple dose schedule.

[0114] Additional pharmaceutical methods may be employed to control the duration of action. Controlled release preparations may be achieved by the use of polymers to complex or absorb the compositions. The controlled delivery may be exercised by selecting appropriate macromolecules (for example, polyesters, polyamino acids, polyvinyl pyrrolidone, ethylene-vinylacetate, methylcellulose, carboxymethylcellulose, or protamine sulfate). The rate of drug release may also be controlled by altering the concentration of such macromolecules.

[0115] Another possible method for controlling the duration of action comprises incorporating the therapeutic agents into particles of a polymeric substance such as polyesters, polyamino acids, hydrogels, poly(lactic acid), or ethylene vinylacetate copolymers. Alternatively, it is possible to entrap the therapeutic agents in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, by the use of hydroxymethyl cellulose or gelatin-microcapsules or poly(methylmethacrylate) microcapsules, respectively, or in a colloid drug delivery system, for example, liposomes, albumin, microspheres, microemulsions, nanoparticles, nanocapsules, or in macroemulsions. Such teachings are disclosed in *Remington's Pharmaceutical Sciences* (1980).

[0116] A preferred controlled release composition for delivery of such compositions will encompass liposome encapsulation as described in, but not limited to U.S. Pat. No. 4,522,803; EP 0 280 503 B1; and WO 95/13796.

[0117] Detecting Cell Proliferation

[0118] The invention provides methods of diagnosing FGF-stimulated cell proliferation and angiogenesis by administering to test cells an effective amount of any of the fusion proteins and assessing inhibition against a control. The cells can be in vivo or in vitro. In this aspect, the administration could be directed at animal models or patients, including those described above, or directed at primary cultures or cell lines derived from tissues affected by disease states potentially involving FGF-stimulation. The method of diagnosis is, thus, useful for determining the propensity of an animal to respond to treatment with fusion protein antagonists as provided by the invention.

[0119] In this manner, the invention relates to providing receptor oligomer fusion constructs which can effect clinically determinable inhibition of angiogenesis or diseases involving angiogenesis when administered in effective amounts to patients or animal models in need of such administration.

EXPERIMENTAL

[0120] FGFR-Ig Fc fusion protein dimer (FGFR ECD Ig), according to Construct #1 (Example 6) was characterized with respect to bFGF binding in vitro (**FIG. 1**) and in whole cells (**FIG. 2**). Determined IC_{50} values indicate that the dimerized receptor is an effective antagonist of FGF binding at subnanomolar concentrations, and is 20-50 fold more potent than the monomeric form (FGFR ECD mono) in competing for bFGF binding.

Example 1

In Vitro Binding of ¹²⁵I-bFGF to FGFR (FIG. 1)

[0121] FGFR monomer (U.S. Pat. No. 5,229,501) and FGFR-Ig Fc fusion dimer according to Construct #1 (Example 6) were tested for their ability to compete with ¹²⁵I-bFGF for binding to immobilized FGF receptors. Immulon 2 microtiter plates were coated with 10 nM FGFR-IgFc fusion protein (Construct #1) in 50 mM Na₂CO₃, pH 9.6 overnight at 4° C. The plates were then blocked with 1% gelatin in Dulbecco's PBS (DPBS) for 1 hr at room temperature, and washed with DPBS plus 0.05% Tween 20. Serial dilutions in duplicate of FGFR-Ig fusion dimer (Construct #1, Example 6), FGFR monomer, or bFGF were added, followed by a subsaturating amount (0.5 nM final) ¹²⁵I-bFGF in DPBS plus 0.1% gelatin, 0.1% triton X-100, and 10 µM Heparin, pH 7.75. After 2 hr at room temperature, wells were washed and the bound radioactivity determined by gamma counting. Displacement curves were analyzed with a four parameter fit to obtain IC_{50} values. The results indicate that the FGFR-Ig Fc fusion dimer is active as a FGF antagonist at subnanomolar concentrations, and is approximately 20-fold more potent than the FGFR monomer protein as a competitor of bFGF binding to immobilized FGF receptors.

Example 2

Binding of ¹²⁵I-bFGF to FGFR: Whole Cells (FIG. 2)

[0122] FGFR monomer (U.S. Pat. No. 5,229,501) and FGFR-Ig fusion dimer according to Construct #1 (Example 6) were tested for their ability to compete with 125 I-bFGF for binding to stable HEK293 cell lines overexpressing FGFR1. FGFR was overexpressed (0.3×10⁶ receptors/cell) in HEK293 cells by transfection of the FGFR1 cDNA (U.S. Pat. No. 5,229,501) in the high copy number plasmid pcDNA3 and selecting clones resistant to G418. 1.5×10⁵ cells were plated in 24 well plates in DMEM plus 10% serum. Following overnight incubation, the cells were washed twice with 1 ml DMEM plus 0.2% gelatin and 15 units/ml heparin. Serial dilutions of FGFR extracellular domain-Fg Fc fusion dimer, FGFR monomer, or bFGF were pre-mixed with 0.1 nM 125 I-bFGF (1138 Ci/mmol) and 250 μ l of this mixture was added to each well, and incubated at 37° C. for 30 minutes. The media was removed, and the cells washed three times with 1 ml DMEM containing 150 mM NaCl, 0.2% gelatin, and 15 units/ml heparin. The cells were lysed in 250 μ l 0.1% SDS and lysates counted in a gamma counter. Displacement curves were analyzed with a four parameter fit to obtain IC₅₀ values. In this assay, the FGFR-Ig fusion dimer is approximately 50-fold more potent than the FGFR monomer protein as a competitor of bFGF binding to cells.

Example 3

bFGF/Serum Stimulated HUVEC Proliferation (FIG. 3)

[0123] FGFR-Ig fusion dimer (FGFR ECD Ig), according to Construct #1 (Example 6) was tested for its ability to inhibit proliferation of human umbilical vein endothelial cells (HUVEC) in serum- and bFGF-containing media.

HUVEC cells were plated in gelatin coated 96-well plates at a density of 2000 cells/well in 50 μ l of EBM (endothelial basal media-Clonetics). The cells were incubated overnight (37° C. 5% CO₂), the media was removed and 200 μ l of media was added containing 10 ng/ml bFGF+90 μ g/ml heparin+either 2% or 5% FBS and +/-0.1 μ M FGFR fusion (Construct #1). The FGFR fusion was preincubated with bFGF and heparin for at least 30 minutes prior to addition to cell wells. Proliferation indexes were determined on days 0, 1, 2, 3, 4 and 5 using the WST-1 cell proliferation assay (Boehringer Mannheim), which measures number of viable cells. 100 nM FGFR-Ig fusion dimer completely inhibits bFGF-stimulated HUVEC proliferation, even in the presence of 5% serum.

Example 4

HUVEC (Proliferation: Comparison of FGFR Monomer and FGFR-Ig Dimer FIG. 4)

[0124] FGFR monomer (U.S. Pat. No. 5,229,501) and FGFR-Ig fusion dimer (FGFR ECD Ig), according to Construct #1 (Example 6) were compared for their ability to inhibit HUVEC proliferation at different doses in the presence of 10 ng/ml bFGF+90 μ g/ml heparin+5% FBS as described in Example 3 above. The FGFR-Ig fusion dimer is more than 10-fold more potent than the FGFR monomer as an inhibit of HUVEC proliferation in the presence of serum and bFGF, can inhibit proliferation completely, and can inhibit proliferation even below the level seen in the absence of FGF. These results are consistent with the data from in vitro and whole-cell binding assays of FIGS. 1-3.

[0125] FIGS. 3 and 4 indicate that not only FGFR-Ig is a more potent inhibitor of this proliferation than monomeric extracellular domains of FGFR, the fusion protein is capable of 100% inhibition of the proliferation.

Example 5

Comparison of FGFR-Ig to Anti-bFGF Mab (FIG. 5)

[0126] The ability of the FGFR-Ig fusion dimer (FGFR ECD Ig), according to Construct #1 (Example 6) to compete with ¹²⁵I-bFGF for binding to immobilized receptors was compared to the highest affinity neutralizing mouse monoclonal antibody to bFGF which is available (Upstate Biotechnology Inc). The assay was performed as in (A). The overall IC₅₀s for these two protein is similar, but the antibody displays some non-competitive binding behavior, and is unable to completely inhibit ¹²⁵I-bFGF binding, even at high concentrations. In contrast, the FGFR-Ig fusion protein completely inhibits binding.

Example 6

FGFR Extracellular Domain-Ig Fc Fusion Constructs

[0127] SEQ ID NOS:1-12 set forth nucleotide and amino acid sequences for fusion protein Constructs #1-6 comprising segments of an FGFR extracellular domain fused to the Fc region of an immunoglobulin molecule.

[0128] Construct #1

[0129] The polynucleotide and amino acid sequences of Construct #1 are set forth in SEQ ID NOS:1 and 2, respectively.

[0130] Construct #1 contains, in order from the 5'/NH2terminus to the 3'/COOH-terminus: human FGFR1 signal peptide (comprised by amino acids 1-21); human FGFR1 extracellular domain (nucleotides 64-1123, amino acids 22-374) which contains the Ig I segment (comprised by nucleotides 163-303, amino acids 55-101), the acid box segment (nucleotides 376-399, amino acids 126-133), the Ig II segment (nucleotides 526-684, amino acids 176-228), and the IIIc variant of Ig III segment (nucleotides 823-1017, amino acids 275-339); a linker sequence with a thrombin cleavage site (nucleotides 1123-1170, amino acids 375-390); and the Fc portion of human IgG1 which includes the hinge region, and domains CH2 and CH3 (nucleotides 1171-1869, amino acids 391-622).

[0131] Construct #2

[0132] The polynucleotide and amino acid sequences of Construct #2 are set forth in SEQ ID NOS:3 and 4, respectively.

[0133] Construct #2 comprises deleting from Construct #1 the Ig I segment plus additional flanking sequences (nucleotides 91-357, amino acids 31-119, as numbered in Construct #1), and part of the linker encompassing the thrombin cleavage site (nucleotides 1123-1146, amino acids 375-382, as numbered in Construct #1).

[0134] Accordingly, Construct #2 comprises from the 5'/NH2-terminus to the 3'/COOH-terminus: human FGFR1 signal peptide, human FGFR1 extracellular domain which contains the acid box segment, the Ig II segment and the IIIc variant of Ig III segment; and the Fc portion of human IgG1 which includes the hinge region, and domains CH2 and CH3.

[0135] Construct #3

[0136] The polynucleotide and amino acid sequences of Construct #3 are set forth in SEQ ID NOS:5 and 6, respectively.

[0137] Construct #3 comprises deleting from Construct #1 the Ig I segment as well as the acid box and flanking sequences (nucleotides 91-441, amino acids 31-147, as numbered in Construct #1), and part of the linker encompassing the thrombin cleavage site (nucleotides 1123-1146, amino acids 375-382, as numbered in Construct #1).

[0138] Accordingly, Construct #3 comprises from the 5'/NH2-terminus to the 3'/COOH-terminus: human FGFR1 signal peptide, human FGFR1 extracellular domain which contains the Ig II segment and the IIIc variant of the Ig III segment; and the Fc portion of human IgG1 which includes the hinge region, and domains CH2 and CH3.

[0139] Construct #4

[0140] The polynucleotide and amino acid sequences of Construct #4 are set forth in SEQ ID NOS:7 and 8, respectively.

[0141] Construct #4 is the same as Construct #2 with two additional changes:

[0142] a) Nucleotides 937 to 938 were changed from "CT" to "GA", which changes amino acid 313 from LEU to GLU (as numbered in Constructs #2 and 4).

This mutation decreases the affinity of the Fc portion for Fc receptors.

[0143] b) Nucleotide 1225 was changed from "C" to "T", which changes amino acid 409 from PRO to SER (as numbered in sequences #2 and 4). This mutation decreases the affinity of the Fc portion for complement.

[0144] The positions correspond to amino acids 235 (LEU to GLU) and 331 (Pro to Ser) of human IgG1.

[0145] Construct #5

[0146] The polynucleotide and amino acid sequences of Construct #5 are set forth in SEQ ID NOS:9 and 10, respectively.

[0147] Construct #5 is the same as Construct #3 with two additional changes:

- **[0148]** a) Nucleotides 853 to 854 have been changed from "CT" to "GA", which changes amino acid 285 from LEU to GLU (as numbered in sequences #3 and 5).
- [0149] b) Nucleotide 1141 has been changed from "C" to "T", which changes amino acid 385 from PRO to SER.

[0150] The positions changed correspond to amino acids 235 (LEU to GLU) and 331 (Pro to Ser) of human IgG1, and result in decreased affinities of the Fc portion for Fc receptors and complement respectively.

[0151] Construct #6

[0152] The polynucleotide and amino acid sequences of Construct #6 are set forth in SEQ ID NOS:11 and 12, respectively.

[0153] Construct #6 is the same as Construct #5 with one change: Nucleotides 772-798 (amino acids 258-266), as numbered in Construct #5, were deleted. Thus, this construct lacks all the residues from the linker sequence encompassing the thrombin cleavage site as described in Construct #1, and is potentially the least immunogenic construct.

[0154] The purpose of this construct was to eliminate all residual amino acids left over from the original linker segment in Construct #1. Construct #6 has the same activity as the other constructs and is the preferred construct for administration as a therapeutic, because it is potentially the least immunogenic construct.

[0155] Other modifications and embodiments of the invention will come to mind in one skilled in the art to which this invention pertains having the benefit of the teachings presented herein. Therefore, it is to be understood that the invention is not to be limited to the specific embodiments disclosed. Although specific terms are employed, they are used in generic and descriptive sense only and not for purposes of limitation, and that modifications and embodiments are intended to be included within the scope of the appended claims.

SEQUENCE LISTING

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Pro Glu 465	Asn	Asn	Tyr	L y s 470	Thr	Thr	Pro	Pro	Val 475	Leu	Asp	Ser	Asp	Gl y 480	
Ser Phe	Phe	Leu	Tyr 485	Ser	Lys	Leu	Thr	Val 490	Asp	Lys	Ser	Arg	Trp 495	Gln	
Gln Gly	Asn	Val 500	Phe	Ser	Сув	Ser	Val 505	Met	His	Glu	Ala	Leu 510	His	Asn	
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gtg ccg Val Pro 50															192
cca aac Pro Asn 65			-	-		-					-				240
gac cac Asp His															288
ata atg Ile Met															336
gtg gag Val Glu															384
gtg gag Val Glu 130															432
aac aaa Asn Lys 145						-									480
tac agt Tyr Ser															528
aat ggg Asn Gl y															576
aag act Lys Thr															624

									tgc Cys		672
									acc Thr		720
									ctg Leu		768
									tgt Cys 270		816
									GJ À ddd		864
									atg Met		912
									cac His		960
									gtg Val		1008
									tac Tyr 350		1056
-	-	-		-	-	-	-	 -	ggc Gly	-	 1104
									atc Ile		1152
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Ala Pr	o Tyr 35	Trp	Thr	Ser	Pro	Glu 40	Lys	Met	Glu	Lys	Lys 45	Leu	His	Ala
Val Pr 50	o Ala	Ala	Lys	Thr	Val 55	Lys	Phe	Lys	Суз	Pro 60	Ser	Ser	Gly	Thr
Pro As 65	n Pro	Thr	Leu	Arg 70	Trp	Leu	Lys	Asn	Gly 75	Lys	Glu	Phe	Lys	Pro 80
Asp Hi	s Arg	Ile	Gly 85	Gly	Tyr	Lys	Val	Arg 90	Tyr	Ala	Thr	Trp	Ser 95	Ile
Ile Me	t Asp	Ser 100	Val	Val	Pro	Ser	A sp 105	Lys	Gly	Asn	Tyr	Thr 110	Cys	Ile
Val Gl	u Asn 115		Tyr	Gly	Ser	Ile 120	Asn	His	Thr	Tyr	Gln 125	Leu	Asp	Val
Val Gl 13		Ser	Pro	His	Arg 135	Pro	Ile	Leu	Gln	Ala 140	Gly	Leu	Pro	Ala
Asn Ly 145	s Thr	Val	Ala	Leu 150	Gly	Ser	Asn	Val	Glu 155	Phe	Met	Сув	Lys	Val 160
Tyr Se	r Asp	Pro	Gln 165	Pro	His	Ile	Gln	Trp 170	Leu	Lys	His	Ile	Glu 175	Val
Asn Gl	y Ser	Lys 180	Ile	Gly	Pro	Asp	As n 185	Leu	Pro	Tyr	Val	Gln 190	Ile	Leu
Lys Th	r Ala 195	Gly	Val	Asn	Thr	Thr 200	Asp	Lys	Glu	Met	Glu 205	Val	Leu	His
Leu Ar 21		Val	Ser	Phe	Glu 215	Asp	Ala	Gly	Glu	Ty r 220	Thr	Суз	Leu	Ala
Gly As 225	n Ser	Ile	Gly	Leu 230	Ser	His	His	Ser	Ala 235	Trp	Leu	Thr	Val	Leu 240
Glu Al	a Leu	Glu	Glu 245	Arg	Pro	Ala	Val	Met 250	Thr	Ser	Pro	Leu	T y r 255	Leu
Glu Gl	y Ser	Gly 260	Ser	Pro	Gly	Leu	Gln 265	Glu	Pro	Lys	Ser	C y s 270	Asp	Lys
Thr Hi	s Thr 275	Сув	Pro	Pro	Сув	Pro 280	Ala	Pro	Glu	Leu	Leu 285	Gly	Gly	Pro
Ser Va 29		Leu	Phe	Pro	Pro 295	Lys	Pro	Lys	Asp	Thr 300	Leu	Met	Ile	Ser
Arg Th 305	r Pro	Glu	Val	Thr 310	Сув	Val	Val	Val	Asp 315	Val	Ser	His	Glu	Asp 320
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Ala Ly	s Thr	Lys 340	Pro	Arg	Glu	Glu	Gln 345	Tyr	Asn	Ser	Thr	Ty r 350	Arg	Val
Val Se	r Val 355	Leu	Thr	Val	Leu	His 360	Gln	Asp	Trp	Leu	Asn 365	Gly	Lys	Glu

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rc His Arg Pro IL Leu Gln Ála GÍY Leu Pro Ála Asn Lys Thr Ýal 165 176 170 177 170 175 175 175 176 175 186 187 180 187 180 189 180 180 187 180 190 180 180 187 190 190 190 190 189 190 190 189 191 190 189 191 191 191 191 191 192 193 181 190 194 189 190 197 189 189 197 181 610 191 192 193 191 195 197 189 189 197 194 191 194 193 184 619 210 210 210 215 220 111 197 189 189 197 181 610 191 192 193 177 Ala Gly 210 210 213 220 111 184 180 197 177 181 610 192 213 220 111 181 1820 Arg Asn Leu Pro Tyr Val Gln 118 Leu Lys Thr Ala Gly 210 210 213 220 111 184 189 184 610 192 123 220 111 181 1820 Arg Asn Val 220 20 111 184 189 184 610 192 123 220 121 122 220 111 184 184 184 192 235 220 121 122 235 220 121 122 124 220 121 122 124 124 192 117 Thr Cys Leu Ala Gly Asn Ser Ile 245 255 246 127 210 225 257 24 184 187 192 197 Thr Cys Leu Ala Gly Asn Ser Ile 245 250 245 250 270 210 266 270 210 225 270 210 225 270 210 225 270 210 225 270 210 210 226 270 210 226 270 210 225 270 210 210 210 210 210 210 210 210 210 21	145					150					155					160	
la Leú GÍy Sér Asn Val Glú Phe Met Cys Lys Val Tyr Sér Asp Pró180185ag coc cac dto cag tyg ta at gog ago cag gt gg ta at gog ago cag cac cac dto cag tyg ta ta go cac at cag ag at gag act got gg ta ta go cag act act cag ag at gag ag ag ag ag ta cac ta cac aga as ag ag ta gag tg tg tt cac tta aga at gtc195195196197197Val Ghn He Leu Lys Thr Ala Gly2102152102152102152102152102152102152102152112202122152122152132302142302252402524025240252402524025240252402524025240265255262552726027260260270261261270261281282282270283284290285290295291291292295292295293294293295294295295294295295296295297295298295298295299295299295 <t< td=""><td></td><td></td><td></td><td></td><td>Ile</td><td></td><td></td><td></td><td></td><td>Leu</td><td></td><td></td><td></td><td></td><td>Thr</td><td></td><td>528</td></t<>					Ile					Leu					Thr		528
In Pro His Ile Gln Trp Leu Lys His Ile Glu Val Asn Gly Ser Lys 200(72) 200tt ggc Cca gac aac ctg cct tat gtc cag atc ttg aag act gct gg 210(72) 210tt aat acc acc gac aaa gag atg gag gtg ctt cac tta aga aat gtc 230(72) 220tt aat acc acc gac agg gag atg acg gg gt gt ta cac tta aga aat gtc 230(72) 220cc tt gag gac gca ggg gag tat acg tgc ttg gcg ggt aac tca tac 245(76) 250cc tt gag gac gca ggg gag tat acg tgc ttg gcg ggt aac tca tac 245(76) 250cc tt gag gac gca ggg gag tat acg tgc ttg gcg ggt aac tca tac 245(76) 250ga ct ctc cat cac tt gca tgg ttg acc gtt ctg gaa gcc tg ga 260(76) 250ga gag cg ga gtg atg acg ctg tg tag acc tg tac gg gg 260(76) 270ad agg ccg ga gtg atg acc tg cc ctg tac ctg gag gcg ggt 275(864) 280u Arg Pro Ala Val Met Thr Ser Pro Leu Tyr Leu Glu Gly Ser Gly 290(864) 290u Arg Pro Ala Val Met Thr Ser Pro Leu Tyr Leu Glu Gly Ser Gly 290(10) 295cc ccc gg gt gc acg cc tag acc gag ccc aaa tct ga ga gcc gca dc tc ctc 310(10) 310cc cc ca aaa ccc aag gac acc ct atg atc tcc gg acc cct gag 310(10) 310tc ccc cca aaa cca aag gac acc ct at ga tc tcc ccg acc ct gag 310(10) 320tc ccc ca aaa cca aag gac acc ct at ga cc ga gac acc gad gac acc aag 310(10) 320tc ccc ca aaa cca aag gac acc ct at ga cc ga gac cc gag gac acg 310(10) 320tc ccc ca aaa cca aag gac acc ct at ga cc ga gac cc gag gac acc 310(10) 320tc ccc ca aaa cca aag gcg gtg gag gag gag ac acc gag gac acc gag gac gac				Ser					Met					Ser			576
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al Asn Thr Thr Ásp Lys Ĝlu Met Ĝlu Val 230 230 230 230 230 230 230 230 230 230		Gly		-		-	Pro		-	-		Leu	-		-		672
er Phe Glu Asp Ala Glý Glu Tyr Thr Cys Leu Ala Gly Asn Ser Ile 245 250 250 255 255 255 255 255 255 255 25	-				-	Lys		-			Leu			-		Val	720
ly Leu Ser His His Ser Àla Trỳ Leu Try Val Leu Glu Àla Leu Glu 265Xan Leu Glu Ala Leu Glu 270Xan Leu Glu 270Xan Leu Glu 270Xan Leu Glu 270Xan Leu Glu 270Xan Leu Glu 					Ala					Cys					Ser		768
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er Pro Gly Leu Gly Gly Pro Lys Ser Cys Asp Lys Thr His Thr Cys 290 295 300 300 300 300 300 300 ca ccg tcg cca gca cct gaa ctc gag ggg ggg cca tc ctc ctc ctc ctc ccd ada ccc ada ccd ada ccd ada ccd gac ccd gac<			Pro					Ser					Glu				864
ro Pró Cýs Pro Åla Pro Ĝlu Leu Ĝlu Ĝlý Ĝiy Pro Ser Val Phe Leu 310 315 315 320 320 310 320 310 315 310 320 310 315 310 320 320 320 320 320 320 320 320 320 32		Pro		-	-		Pro			-	-	Lys				-	912
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he Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys 355 360 360 365 1152 cg cgg gag gag cag tac ac gt c ct Arg Glu Glu Glu Glu Glu Tyr Asn 375 Ser Thr Tyr Arg Val Val Ser Val Leu 380 1152 cc gtc ctg cac cag gac tgg ctg at ggc aag gag tac aag tgc aag 380 1200 380 1200 cc gtc ctg cac cag gac tgg ctg ct ct c ca ag gac tgg ctg at ggc aag gag tac aag tgc aag 400 1200 1200 hr Val Leu His Gln Asp Trp Leu Asn Gly Lys 390 390 390 1200 tc tcc aac aaa gcc ctc cca gcc tcc atc gag aaa acc atc tcc aaa 1248 al Ser Asn Lys Ala Leu Pro Ala Ser Ile Glu Lys Thr Ile Ser Lys 405 1152 cc aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc 1296 1296 la Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser 420 430 gg gat gag ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa 430 1344 rg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys 445 1392 gc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc aat ggg cag 1392 1392				Val					Ser					Glu			1056
ro Arg Glu Glu Glu Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu 370 375 375 380 380 1200 380 1200 380 1200 380 1200 380 1200 380 1200 1200 1200 1200 1200 1200 1200 12			Trp					Val					Ala				1104
hr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys 85 390 395 400 tc tcc aac aaa gcc ctc cca gcc tcc atc gag aaa acc atc tcc aaa 1248 al Ser Asn Lys Ala Leu Pro Ala Ser Ile Glu Lys Thr Ile Ser Lys 405 410 415 cc aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc 1296 1296 1297 420 425 430 gg gat gag ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa rg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys 435 440 445 gc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc aat ggg cag 1392		Arg					Asn					Val					1152
al Ser Asn Lys Ala Leu Pro Ala Ser Ile Glu Lys Thr Ile Ser Lys 405 410 415 415 415 1296 cc aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc 1296 la Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser 420 425 430 1344 gg gat gag ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa 1344 rg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys 435 440 445 1392		-	_		_	Åsp					Lys			-	_	Lys	1200
la Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser 420 425 430 gg gat gag ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa 1344 rg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys 435 440 445 gc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc aat ggg cag 1392					Ala					Ile					Ser		1248
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			Glu					Gln					Cys				1344
																	1392

	450					455					460					
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	ttc Phe															1488
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Leu	Pro	Ser 35	Ser	Glu	Asp	Asp	Asp 40	Asp	Asp	Asp	Asp	Ser 45	Ser	Ser	Glu	
Glu	Lys 50	Glu	Thr	Asp	Asn	Thr 55	Lys	Pro	Asn	Pro	Val 60	Ala	Pro	Tyr	Trp	
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Lys	Thr	Val	Lys	Phe 85	Lys	Cys	Pro	Ser	Ser 90	Gly	Thr	Pro	Asn	Pro 95	Thr	
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Gly	Gly	Tyr 115	Lys	Val	Arg	Tyr	Ala 120	Thr	Trp	Ser	Ile	Ile 125	Met	Asp	Ser	
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Ser	Phe	Glu	Asp	Ala 245	Gly	Glu	Tyr	Thr	Cys 250	Leu	Ala	Gly	Asn	Ser 255	Ile	
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cos acc coc cac etg up tug tug tug aca at gop and gas tic and cot 65 240 gac cac up att grt grt da aca up to opt tat goo acc tug age att has his hard to the diy Giy Tyr by Val Arp Tyr har hir try ber lie 100 280 gac cac up att grt got cac age goo acc tag age att has atg got cot grt got cac age goo acc tag age att 100 336 gat got got att grt got cac to got age goo acc tag age att 100 336 git gong and got age got att acc cac cac to att 110 334 git gong ogg to cot cac acc got got att the acc cac tyg att 110 334 git gong ogg to cot cac acc got cot to tag as age got tyg cor got 110 312 git gong ogg to cot cac acc got cot the acc age got cat tag and 110 313 git gong ogg to cot cac acc got cot the acc age tug the cor got 110 313 git gong got cot cac acc got cot the acc age cac tag tug cor got 110 312 git gong got to cot cac acc got got got tat tug cas att got 110 315 tas att got cit got age cac acc dot cat tug and got cit got 110 314 314 tas att got cit got age acc acc dot cag acc as tug got 110 315 316 tas att got cit got acc age acc dot cat tug tug tug cac acc age acc act acc age acc age acc acc age acc age acc acc age acc age acc age acc age acc acc age acc age acc age acc acc acc acc age acc age acc acc age acc age acc acc acc acc age acc age acc age acc acc acc acc acc														CTII	ueu			
App His Arg 1le Giy Giy Tyr Lys Val Arg Tyr Ala Thr Trp Ser Ile 95ata atg gac tct gtg gtg ccc tct gac aag ggc aac tac acc tgc att 100336Tie Met App Cr tal Yal Pro Ser App Tyr Cyr Oly Ann Tyr Trp Cyr 1le 100334gtg gag aat gag tac ggc agr atc anc cac aca tac cac ctg gtg gtg cc 115384gtg Gag Cyd tcc att ccc gg ccc atc ctg cas gcg gtg tc 120384gtg Gag Cyd tcc att ccc gg ccc atc ctg cas gcg gtg tc 130 kg Ger Pro His Cyg ccc atc ctg cas gcg gtg ttg ccc gtg 130 kg Ger Pro His Cyg ccc atc ctg cas gcg gtg ttg ccc 140432acc aaa aca gtg gc cc atc ctg cas acc gtg gtg tta tag gtg Ann Lye Thr Val Ala Lee Giy Ser Ann Val Clu Phe Met Cys Lye Val 155480tas dyt gac ccg aca gtg gtg ct atg gtg ct atg tag gtg 165100tas dyt gac ccg aca gtg gtg ct atg gtg gtg ct tag 165100tas dyt gac acg gtg gtg gtg ct atg gtg gtg gtg gtg 165100tas dyt gac acg gtg gtg gtg gtg gtg gtg gtg gtg gtg 160101tas dyt gac acg gtg gtg gtg gtg gtg gtg gtg gtg gtg 160101tas dyt gac acg gtg gtg gtg gtg gtg gtg gtg gtg gtg 160101tas dyt gac ga atg gtg gac gca gyg gtg gtg gtg gtg gtg gtg 180101tas aga aat gtc co ct tt gat gac gca gtg gtg gtg gtg gtg gtg 230101tas aga at gtc to ct tt gat gag gac gca gtg gtg gtg gtg gtg gtg 180101tas aga at gtc to ct tt gat gac gca gtg gtg gtg gtg gtg gtg 230101tas aga at gtc to ct tt gat gag gac gca gtg gtg gtg gtg gtg gtg 230101tas aga at gtc to ct tt gat gac gca gtg gtg gtg gtg gtg gtg 230102tas aga at gtc to ct gat gtg gtg gtg gtg gt	Pro				-	Arg		-			Gly		-			Pro	240	
ILE Het Åp Ser Vol Val Pro Ser Åpp Lyg Gly Am Tyr Thr Cys ILE 110 110 110 110 110 110 110 110 110 11					Gly					Arg					Ser		288	
<pre>val fui Aen du Tyr Gly Ser IIe Aen Nie Thr Tyr Cli Ler Aep Val 115 115 116 116 117 118 118 118 119 119 119 119 119 119 119</pre>				Ser					Asp					Thr			336	
Val dit årg ser Pro Hi Årg Pro Ile Lee un Ala dit Lee Pro Åla130aac aaa acc gtg goo otg ggt agc aac gtg gag tot atg ttg taag gtg Am Lyg Thr Val Åla Lee dit Ser Aan Val Gilu Phe Net Cys Lys Val 155480145145tar agt gac oog oog oo ag ac at cog tgg cta ag ooc at c gag gtg 166528tar agt gac oog oog oo ag ac at cog tgg cta ag ooc at c gag gtg 167528aat ggg ago ag att ggo oo ag ac ac otg oot tat gto oog at ttg 160576aat ggg ago ag att ggo oo ag ac ac ofg oot tat gto oog at ttg 180576aat ggg ago ag att ggo oot gac ac oo gac aac ofg oot tat gto oog at ttg 180576aat ggg ago ag att ggo oot gac oog ag ag ag ag oog ggg gt to too too 190624Lys Thr Ale ool y val Aan Thr Thr App Lys oll Met Oll val Lee His 210672ggt aac tot atc ggg ac to co cat cac ct tge at gg ttg ct ct ct 210720gga go ofg ga to to co dt cac tt gea tgg ttg ac gt ttg gog 110720gaa goo ofg ga ag ag go g go g gt gt gt co oot gt ac ottg 210768gaa goo ofg ga ag ag go g go go go at to try the col Lee Thr Val Lee 220768gaa goo gt too coo gge ttg od gac oo ac at to the ga ga to too coo ttg ac ottg 200768gaa goo cot g ga ag ag go go go coc ag ac coo the gac of the ga gg ga coc 200768gaa goo cot ga ag ag ag oo go coc ag ac coc the ga ac to ta go for clu Lee Oll Val Lee 200864210245700gaa goo cot ge coc of go coc go coc cot ga coc the ga gg gg ag coc 200864211215216gaa goo coc go coc gt coc coc ga coc coc ga ga coc aaa toc			Asn					Ile					Gln				384	
Am Lys Thr Vai Ai a Lei Gily Ser Am Vai Gili Phe Met Cys Lys Vai145145146145146146147148149141141149141142142143144144144144144144144144144144144144144		Glu					Arg					Ala					432	
Tyr Ser Asp Pro Gin Prö His Ile Gin Trp Leu Lys His Ile Gin Val 165 at ggg dc aag at ggc cca gac ac ctg cct tat gtc cag atc ttg 180 180 185 185 185 186 185 185 185 195 195 195 195 195 195 195 19	Asn				-	Leu		-			Glu		-	-	-	Val	480	
Asn ốlý ser Lyš 1le ốly Pro Âsp Asn Leú Pro Tyr Val Cln Il LeúIn Il Leú180185190ang act gyt gq 4gt at act acc gac Gac aca gag agt g agt gt gt tt cac624Lys Thr Ala Gly Val Asn Thr Thr Asp Lys Glu Met Glu Val Leu His672tta aga aat gtc tc tt tt gag gac gca ggg gag tat acg tgc ttg gcg672ggt aac tct atc gga ctc tcc cat cac tct gca tgg tg acc gt ctg720210215215221220ggt aac tct atc gga cg cg agt g atg acc tgc cc tg tac720225230230ga agc ctg gaa gag agg cg gca gtg atg acc tgc cc tg tac ctg768Glu Ala Leu Glu Glu Arg Pro Ala Val Met Thr Ser Pro Leu Tyr Leu255gag ggc agc ggt tcc ccc ggg ttg cag gag cac tc tg ga ggg gga ccg864Glu Gly Ser Gly Ser Pro Gly Leu Glu Glu Pro Lys Ser Cys Asp Lys864260265270act cac aca tgc cca cac agt gc ca gac acc cag ac acc tc atg at ct ccc912ser val Phe Leu Phe Pro Pro Cys Pro Ala Pro Glu Leu Glu Glu Gly Gly Pro285tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc912255260285cc ag tc ttc ct dt tc ccc caa acc agg gac acg ggt agg acc ag aga cac gas agg960305310310310315320320310315320320cc aga cc tg ag gtc aca tac aga ac aga ac aga agg agg cc gg gag agg ag					Gln					Trp					Glu		528	
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Gíy Asn Ser Ile Gíy Leu Ser His His Ser Ála Trp Leu Thr Val Leu 230Leu Trp Leu 240Tr Val Leu 240gaa goc etg gaa gag agg ceg gca gtg atg acc teg coc etg tac etg Glu Ala Leu Glu Glu Arg Pro Ala Val Met Thr Ser Pro Leu Tyr Leu 250768gag ggc agc ggt tec coc ggg ttg cag gag coc aaa tet tgt gac aaa Glu Gly Ser Gly Ser Pro Gly Leu Gln Glu Pro Lys Ser Cys Asp Lys 260816act cac aca tgc cca ccg tgc cca gca cet gaa ctc gag ggg gga ccg 270864Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Glu Gly Gly Gly Pro 270912act cac aca tgc cca ccg tgc cca aaa coc aag gac acc et atg atc tcc 270912Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser 290912cg acc cot gag gtc aca tgg gtg gtg gtg gac gtg agc cag gaa gac acc cot gag gtc aaa teg gtg agg tac at the Ser His Glu Nap 310960Arg Thr Pro Glu Val Thr Cys Val Val Val Nap Val Ser His Glu Nap 3101008cct gag gtc aag tte aac tgg tac gtg gac ggc ggc gtg gag gtg cat aat 3301008cct gaa gac aag ccg cgg gg gga cag tac aac acg acg tac cgt gtg gtg 3301008gac caag aca aag ccg cgg gg gga cgg tac aac aca acg acg tac cgt gtg 3401056at Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val 340104		Arg					Glu					Tyr					672	
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Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp 290Thr Leu Met Ile Ser 300960cgg acc cct gag gtc aca tgc gtg gtg gtg gtg gac gtg agc cac gaa gac Arg Thr Pro Glu Val Thr Cys Val Val Val Asp 310960cct gag gtc aag ttc aac tgg tac gtg gg			Thr					Pro					Glu				864	
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Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn 325330335gcc aag aca aag ccg cgg gag gag cag tac aac agc acg tac cgt gtg Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val 3401056gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag gag Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu1104	Arg					Thr					Åsp					Åsp	960	
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Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu				Lys					Gln					Tyr			1056	
			Val					His					Asn				1104	

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Ala Pro Tyr Trp 35	Thr Ser Pro	Glu Lys Met 40	Glu Lys Lys Leu 45	His Ala
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Pro Asn Pro Thr 65	Leu Arg Trp 70	-	Gly Lys Glu Phe 75	e Lys Pro 80
Asp His Arg Ile	Gly Gly Tyr 85	Lys Val Arg 90	Tyr Ala Thr Trp	95
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			Gln Ala Gly Leu 140	a Pro Ala
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Asn																			
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G1 y 225	Asn	Ser	IIe	GIY	Leu 230	Ser	H1S	H1S	Ser	A1a 235	Trp	Leu	Thr	Val	Leu 240				
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	-	-			cca Pro	-	-			-	-	-	57	6
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			-	-	aaa Lys			-		-	-	-	81	6
					ccg Pro								86	4
					tcc Ser 295								91	2
					gac Asp								96	0

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<pre>Ser Leu Ser Leu <210> SEQ ID NO <211> LENGTH: 4 <212> TYPE: PRT <213> ORGANISM: <400> SEQUENCE: Met Trp Ser Trp 1</pre>	Ser Pro 485 12 38 Homo sap 12 12 Lys Cys 5	Gly Lys piens Leu Leu	. Phe	10				15		1467
Ser Leu Ser Leu <210> SEQ ID NO <211> LENGTH: 4 <212> TYPE: PRT <213> ORGANISM: <400> SEQUENCE: Met Trp Ser Trp	Ser Pro 485 12 38 Homo sap 12 12 Lys Cys 5	Gly Lys piens Leu Leu	. Phe	10				15		1467
<pre>Ser Leu Ser Leu <210> SEQ ID NO <211> LENGTH: 4 <212> TYPE: PRT <213> ORGANISM: <400> SEQUENCE: Met Trp Ser Trp 1 Thr Leu Cys Thr</pre>	Ser Pro 485 12 38 Homo sap 12 Lys Cys 5 Ala Arg	Gly Lys piens Leu Leu Pro Ser	Phe Pro 25	10 Thr I	Leu Pi	ro Glu	Gln 30	15 Pro	Val	1467
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Val Glu Arg Ser Pro His Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala Asn Lys Thr Val Ala Leu Gly Ser Asn Val Glu Phe Met Cys Lys Val Tyr Ser Asp Pro Gln Pro His Ile Gln Trp Leu Lys His Ile Glu Val Asn Gly Ser Lys Ile Gly Pro Asp Asn Leu Pro Tyr Val Gln Ile Leu Lys Thr Ala Gly Val As
n Thr Thr Asp Lys Glu Met Glu Val Leu His Leu Arg Asn Val Ser Phe Glu Asp Ala Gly Glu Tyr Thr Cys Leu Ala Gly Asn Ser Ile Gly Leu Ser His His Ser Ala Trp Leu Thr Val Leu Glu Ala Leu Glu Glu Arg Pro Ala Val Met Thr Ser Pro Leu Tyr Leu Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Glu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys

1. A polypeptide comprising a fibroblast growth factor (FGF) receptor extracellular domain fused to a heterologous oligomerization domain wherein said extracellular domain lacks the Ig I segment, and wherein said heterologous oligomerization domain is selected from the group consisting of

a) the Fc region of an immunoglobulin molecule;

b) the hinge region of an immunoglobulin molecule;

c) the CH1 region of an immunoglobulin molecule;

d) the CH2 region of an immunoglobulin molecule;

e) the CH3 region of an immunoglobulin molecule;

f) the CH4 region of an immunoglobulin molecule;

g) the light chain of an immunoglobulin molecule; and

h) a peptide comprising a leucine zipper motif.

2. The polypeptide of claim 1, wherein said oligomerization domain is capable of forming an oligomer which is at least a dimer with at least one other polypeptide comprising said oligomerization domain of claim 1.

3. The polypeptide of claim 2, wherein said oligomer is a homodimer.

4. The polypeptide of claim 2, wherein said oligomer is a heterodimer.

5. The polypeptide of claim 1, wherein said polypeptide is capable of binding FGF.

6. The polypeptide of claim 1 wherein said immonoglobulin is selected from the group IgG, IgE, IgA, IgD, and IgM.

7. The polypeptide of claim 6, wherein said immunoglobulin is human immunoglobulin.

8. The polypeptide of claim 1, wherein said oligomerization domain is the Fc region of an immunoglobulin molecule, and

- wherein the hinge region contained in said Fc region is capable of self dimerization, and
- wherein the CH2 and CH3 regions contained in said Fc region are capable of increasing the circulating half-life of said polypeptide, and

wherein said polypeptide is capable of binding FGF.

9. The polypeptide of claim 1 lacking the acid box segment of said extracellular domain.

10. The polypeptide of claim 1 further comprising a variant of the Ig III segment of said extracellular domain.

11. The polypeptide of claim 1 lacking the acid box segment of said extracellular domain and further comprising a variant of the Ig III segment of said extracellular domain.

12. The polypeptide of claim 1, wherein said FGF receptor is selected from the group consisting of FGFR1, FGFR2, FGFR3 and FGFR4.

13. The polypeptide of claim 1, wherein said FGF receptor is human FGFR1.

14. A polypeptide having the amino acid sequence selected from the group consisting of:

- a) an amino acid sequence set forth in SEQ ID NO:4, 6, 8, 10, or 12;
- b) an amino acid sequence having at least 75% identity to the amino acid sequence set forth in SEQ ID NO:4, 6, 8, 10, or 12; and

c) an amino acid sequence having at least 95% identity to the amino acid sequence set forth in SEQ ID NO:4, 6, 8, 10, or 12.

15. A polynucleotide construct encoding said amino acid sequence of claim 14.

16. A polynucleotide construct having the sequence selected from the group consisting of:

- a) the nucleotide sequence set forth in SEQ ID NO:3, 5, 7, 9, or 11;
- b) a nucleotide sequence having at least 75% identity to the nucleotide sequence set forth in SEQ ID NO:3, 5, 7, 9, or 11; and
- c) a nucleotide sequence having at least 95% identity to the nucleotide sequence set forth in SEQ ID NO:3, 5, 7, 9, or 11.

17. A polynucleotide construct encoding the polypeptide of claim 1.

18. A viral vector comprising the polynucleotide construct of claim 17.

19. An isolated host cell containing or capable of expressing the polynucleotide construct of claim 17.

20. A pharmacological composition comprising the vector of claim 19.

21. A pharmacological composition comprising the polypeptide of claim 1.

22. A method for treating a fibroblast growth factor (FGF)-mediated disorder in a mammal, said method comprising administering to said mammal a therapeutically effective amount of a polypeptide comprising an FGF receptor extracellular domain fused to a heterologous oligomerization domain, wherein said heterologous oligomerization domain is selected from the group consisting of

a) the Fc region of an immunoglobulin molecule;

b) the hinge region of an immunoglobulin molecule;

c) the CH1 region of an immunoglobulin molecule;

d) the CH2 region of an immunoglobulin molecule;

e) the CH3 region of an immunoglobulin molecule;

- f) the CH4 region of an immunoglobulin molecule;
- g) the light chain of an immunoglobulin molecule;

h) a peptide comprising a leucine zipper motif; and

wherein said oligomerization domain is capable of forming an oligomer which is at least a dimer with at least one other polypeptide comprising said oligomerization domain.

23. The method of claim 22, wherein said mammal is human and wherein said FGF receptor extracellular domain and said oligomerization domain are human polypeptides.

24. The method of claim 22, wherein said polypeptide has the amino acid sequence selected from the group consisting of:

- a) the amino acid sequence set forth in SEQ ID NO:2, 4, 6, 8, 10, or 12;
- b) an amino acid sequence having at least 75% identity to the amino acid sequence set forth in SEQ ID NO:2, 4, 6, 8, 10, or 12;

c) an amino acid sequence having at least 95% identity to the amino acid sequence set forth in SEQ ID NO:2, 4, 6, 8, 10, or 12.

25. The method of claim 22, wherein said polypeptide has the amino acid sequence encoded by a polynucleotide having the sequence selected from the group consisting of:

- a) the nucleotide sequence set forth in SEQ ID NO:1, 3, 5, 7, 9, or 11;
- b) a nucleotide sequence having at least 75% identity to the nucleotide sequence set forth in SEQ ID NO:1, 3, 5, 7, 9, or 11; and
- c) a nucleotide sequence having at least 95% identity to the nucleotide sequence set forth in SEQ ID NO:1, 3, 5, 7, 9, or 11.

26. A method for treating a fibroblast growth factor (FGF)-mediated disorder in a mammal, said method comprising administering to said mammal a therapeutically effective amount of a polynucleotide comprising a coding region encoding a polypeptide comprising an FGF receptor extracellular domain fused to a heterologous oligomerization domain, wherein said heterologous oligomerization domain is selected from the group consisting of

a) the Fc region of an immunoglobulin molecule;

b) the hinge region of an immunoglobulin molecule;

c) the CH1 region of an immunoglobulin molecule;

d) the CH2 region of an immunoglobulin molecule;

e) the CH3 region of an immunoglobulin molecule;

f) the CH4 region of an immunoglobulin molecule;

g) the light chain of an immunoglobulin molecule;

h) a peptide comprising a leucine zipper motif; and

wherein said oligomerization domain is capable of forming an oligomer which is at least a dimer with at least one other polypeptide comprising said oligomerization domain.

27. The method of claim 26, wherein said polynucleotide has the sequence selected from the group consisting of:

- a) the nucleotide sequence set forth in SEQ ID NO:1, 3, 5, 7, 9, or 11;
- b) a nucleotide sequence having at least 75% identity to the nucleotide sequence set forth in SEQ ID NO:1, 3, 5, 7, 9, or 11; and
- c) a nucleotide sequence having at least 95% identity to the nucleotide sequence set forth in SEQ ID NO:1, 3, 5, 7, 9, or 11.

28. A method for treating an angiogenesis-mediated disorder in a mammal, said method comprising administering to said mammal a therapeutically effective amount of a polypeptide comprising a fibroblast growth factor (FGF) receptor extracellular domain fused to a heterologous oli-

gomerization domain, wherein said heterologous oligomerization domain is selected from the group consisting of:

- a) the Fc region of an immunoglobulin molecule;
- b) the hinge region of an immunoglobulin molecule;
- c) the CH1 region of an immunoglobulin molecule;
- d) the CH2 region of an immunoglobulin molecule;
- e) the CH3 region of an immunoglobulin molecule;
- f) the CH4 region of an immunoglobulin molecule;
- g) the light chain of an immunoglobulin molecule;
- h) a peptide comprising a leucine zipper motif; and
- wherein said oligomerization domain is capable of forming an oligomer which is at least a dimer with at least one other polypeptide comprising said oligomerization domain.

29. A method for treating an angiogenesis-mediated disorder in a mammal, said method comprising administering to said mammal a therapeutically effective amount of a polynucleotide comprising a coding region encoding a polypeptide comprising a fibroblast growth factor (FGF) receptor extracellular domain fused to a heterologous oligomerization domain, wherein said heterologous oligomerization domain is selected from the group consisting of

a) the Fc region of an immunoglobulin molecule;

- b) the hinge region of an immunoglobulin molecule;
- c) the CH1 region of an immunoglobulin molecule;
- d) the CH2 region of an immunoglobulin molecule;
- e) the CH3 region of an immunoglobulin molecule;
- f) the CH4 region of an immunoglobulin molecule;
- g) the light chain of an immunoglobulin molecule;
- h) a peptide comprising a leucine zipper motif; and
- wherein said oligomerization domain is capable of forming an oligomer which is at least a dimer with at least one other polypeptide comprising said oligomerization domain.

30. The method of claim 29, wherein said polynucleotide has the sequence selected from the group consisting of:

- a) the nucleotide sequence set forth in SEQ ID NO:1, 3, 5, 7, 9, or 11;
- b) a nucleotide sequence having at least 75% identity to the nucleotide sequence set forth in SEQ ID NO:1, 3, 5, 7, 9, or 11; and
- c) a nucleotide sequence having at least 95% identity to the nucleotide sequence set forth in SEQ ID NO:1, 3, 5, 7, 9, or 11.

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