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(21) International Application Number: PCT/US96/06665 (22) International Filing Date: 10 May 1996 (10.05.96) (30) Priority Data: 08/438,439 12 May 1995 (12.05.95) US (71) Applicant: THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE [US/US]; 720 Rutland Avenue, Baltimore, MD 21205 (US). (72) Inventors: NATHANS, Jeremy; 5510 South Bend Road, Baltimore, MD 21209 (US). SMALLWOOD, Philip, M.; 5022 Woodbine Road, Woodbine, MD 21797 (US). MACKKE, Jennifer, P.; 7180 Talisman Road, Columbia, MD 21045 (US). (74) Agent: HAILE, Lisa, A.; Fish & Richardson P.C., Suite 1400, 4225 Executive Square, La Jolla, CA 92037 (US).		(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: FIBROBLAST GROWTH FACTOR HOMOLOGOUS FACTOR-2 AND METHODS OF USE		
(57) Abstract A novel growth factor, fibroblast growth factor homologous factor-2 (FHF-2) polypeptide, the polynucleotide sequence encoding FHF-2 and the deduced amino acid sequence are disclosed. Also disclosed are diagnostic and therapeutic methods of using the FHF-2 polypeptide and polynucleotide sequences and antibodies which specifically bind to FHF-2.		

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FIBROBLAST GROWTH FACTOR HOMOLOGOUS FACTOR-2 AND METHODS OF USE

5 BACKGROUND OF THE INVENTION

1. FIELD OF THE INVENTION

10 The invention relates generally to growth factors and specifically to a novel member of the fibroblast growth factor family, denoted fibroblast growth factor homologous factor-2 (FHF-2) and the polynucleotide encoding FHF-2.

2. DESCRIPTION OF RELATED ART

15 The fibroblast growth factor family encompasses a group of structurally related proteins with a wide range of growth promoting, survival, and/or differentiation activities in vivo and in vitro (reviewed in Baird, A., and Gospodarowicz, D. *Ann N.Y. Acad. Sci.* 638: 1, 1991; Eckenstein, F.P., *J. Neurobiology* 25: 1467, 1994; Mason, I.J. *Cell* 78: 547, 1994). As of December 1994, nine members of this family had
20 been characterized by molecular cloning. The first two members of the family to be characterized, acidic fibroblast growth factor (aFGF/FGF-1) and basic fibroblast growth factor (bFGF/FGF-2), have been found in numerous tissues, including for example brain, eye, kidney, placenta, and adrenal (Jaye *et al.*, *Science* 233: 541, 1986; Abraham *et al.*, *Science* 233: 545, 1986). These factors have been shown to
25 be potent mitogens and survival factors for a variety of mesoderm and neurectoderm-derived tissues, including fibroblasts, endothelial cells, hippocampal and cerebral cortical neurons, and astroglia (Burgess, W. H. and Maciag, T. *Ann. Rev. Biochemistry* 58: 575, 1989). Additional members of the FGF family include: int-2/FGF-3, identified as one of the frequent sites of integration of the mouse
30 mammary tumor virus, and therefore a presumptive oncogenic factor (Smith *et al.*, *EMBO J.* 7: 1013, 1988); FGF-4 (Delli-Bovi *et al.*, *Cell* 50: 729, 1987) and FGF-5 (Zhan *et al.*, *Mol. Cell Biol.* 8, 3487, 1988) as transforming genes in the NIH 3T3 transfection assay; FGF-6, isolated by molecular cloning based on its homology to FGF-4 (Marics *et al.*, *Oncogene* 4: 335 (1989); keratinocyte growth factor/ FGF-7, identified as a mitogen for keratinocytes (Finch *et al.*, *Science* 245: 752, 1989);
35 FGF-8 as an androgen-induced mitogen for mammary carcinoma cells (Tanaka *et*

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al., *Proc. Natl. Acad. Sci. USA* 89: 8928, 1992); and FGF-9 as a mitogen for primary astrocytes (Miyamoto *et al.*, *Mol. Cell Biol.* 13: 4251, 1993). Several of the FGFs, including aFGF and bFGF, lack a classical signal sequence; the mechanism by which they are secreted is not known.

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All members of the FGF family share approximately 25% or more amino acid sequence identity, a degree of homology indicating that they are likely to share nearly identical three-dimensional structures. Support for this inference comes from a comparison of the three-dimensional structures of bFGF and interleukin 1-beta determined by x-ray diffraction (Eriksson *et al.*, *Proc. Natl. Acad. Sci USA* 88: 3441, 1991; Zhang *et al.*, *Proc. Natl. Acad. Sci USA* 88: 3446, 1991; Ago *et al.*, *J. Biochem.* 110: 360, 1991). Although these proteins share only 10% amino acid identity, the alpha carbon backbones of the two crystal structures can be superimposed with a root-mean square deviation of less than 2 angstroms (Zhang *et al.*, *Proc. Natl. Acad. Sci USA* 88: 3446, 1991). Both proteins consist almost entirely of beta-sheets, which form a barrel composed of three copies of a four-stranded beta-meander motif. The likely heparin- and receptor-binding regions are located on nearby regions on one face of the protein.

aFGF, bFGF, and FGF-7/KGF have been shown to exert some or all of their biological activity through high affinity binding to cell surface tyrosine kinase receptors (*e.g.*, Lee, P. L. *et al.*, *Science* 245: 57, 1989; reviewed in Johnson, D.E. and Williams, L.T., *Adv. Cancer Res.* 60: 1, 1993). Many members of the FGF family also bind tightly to heparin, and a ternary complex of heparin, FGF, and transmembrane receptor may be the biologically relevant signalling species. Thus far four different genes have been identified that encode receptors for FGF family members. Recent work has shown that receptor diversity is increased by differential mRNA splicing within the extracellular ligand binding domain, with the result that multiple receptor isoforms with different ligand binding properties can be encoded by the same gene (Johnson, D.E. and Williams, L.T., *Adv. Cancer Res.* 60: 1, 1993). In tissue culture systems, the binding of aFGF or bFGF to its cell surface receptor activates phospholipase C-gamma (Burgess, W. H. *et al.*, *Mol. Cell Biol.* 10: 4770, 1990), a pathway known to integrate a variety of mitogenic signals.

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Identification and characterization of new members of the FGF family will provide insights into the mechanisms by which cells and organs control their growth, survival, senescence, differentiation, and recovery from injury.

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SUMMARY OF THE INVENTION

The present invention provides a cell growth, survival and differentiation factor, FHF-2, and a polynucleotide sequence which encodes the factor. This factor is involved in the growth, survival, and or differentiation of cells within the central nervous system (CNS) and in the heart.

The invention provides a method for detecting alterations in FHF-2 gene expression which are diagnostic of neurodegenerative, neoplastic, or cardiac disorders. In another embodiment, the invention provides a method for treating a neurodegenerative, neoplastic or cardiac disorder by enhancing or suppressing the expression or activity of FHF-2.

BRIEF DESCRIPTION OF THE DRAWINGS

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Figure 1 shows the nucleotide and predicted amino acid sequence of human FHF-2.

Figure 2 shows an alignment of the amino acid sequence of human FHF-2 and each of the published nine members of the FGF family. Conserved residues are highlighted. The FGF family members are: aFGF/FGF-1 (Jaye et al., Science 233: 541, 1986), bFGF/FGF-2 (Abraham et al., Science 233: 545, 1986), int-2/FGF-3 (Smith et al., EMBO J. 7: 1013, 1988), FGF-4 (Delli-Bovi et al., Cell 50: 729, 1987), FGF-5 (Zhan et al., Mol. Cell Biol. 8, 3487, 1988), FGF-6 (Marics et al., Oncogene 4: 335, 1989); keratinocyte growth factor/ FGF-7 (Finch et al., Science 245: 752, 1989), FGF-8 (Tanaka et al., Proc. Natl. Acad. Sci. USA 89: 8928, 1992), and FGF-9 (Miyamoto et al., Mol. Cell Biol. 13: 4251, 1993).

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Figure 3 shows a dendrogram in which the length of each path connecting any pair of FGF family members is proportional to the degree of amino acid sequence divergence of that pair.

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Figure 4 shows the location of the gene encoding FHF-2 on the human X-chromosome. A Southern blot was prepared from DNA derived from mouse-human or hamster-human hybrid cell lines, each of which contains a single human chromosome, indicated above each lane. The human specific hybridization is found

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Figure 5 shows the production of FHF-2 in transfected human embryonic kidney cells. Proteins were labeled biosynthetically with ³⁵S-methionine and resolved by SDS-polyacrylamide gel electrophoresis. Lanes 1, 3, 5, and 7: total cell protein; lanes 2, 4, 6, and 8: protein present in the medium (secreted protein). Lanes 1 and 2, mock transfected cells; lanes 3 and 4, transfection with cDNA encoding FHF-1, a closely related member of the FGF family; lanes 5 and 6, transfection with cDNA encoding FHF-2; lanes 7 and 8, transfection with cDNA encoding human growth hormone. Arrows indicate the FHF-1 and FHF-2 protein bands. Protein standards are shown to the left; from top to bottom their molecular masses are 220, 97, 66, 46, 30, 21.5, and 14.3 kD.

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Figure 6 shows the tissue specificity of FHF-2 expression. Ten micrograms of total RNA from the indicated mouse tissues was prepared (Chomczynski & Sacchi. Anal. Biochem. 162: 156, 1987) and used for RNase protection (Ausabel et al., Current Protocols in Molecular Biology; New York: Wiley Interscience, 1987) with a mouse FHF-2 antisense probe that spanned 197 bases of the most 3' coding region exon and the adjacent upstream 335 bases of intron sequence. RNase protection at the size expected for the 197 base exon region of the probe (arrowheads) was observed with RNA from brain, eye, and heart. A longer exposure reveals barely visible bands in all of the other tissues but not in the tRNA control sample.

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DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a growth factor, FHF-2, and a polynucleotide sequence encoding FHF-2. FHF-2 is expressed at high levels in brain and heart tissues. In one embodiment, the invention provides a method for detection of a cell proliferative or immunologic disorder of central nervous system or cardiac tissue origin which is associated with FHF-2 expression or function. In another embodiment, the invention provides a method for treating a cell proliferative or

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immunologic disorder by using an agent which suppresses or enhances FHF-2 expression or activity.

5 The structural homology between the FHF-2 protein of this invention and the members of the FGF family, indicates that FHF-2 is a new member of the family of growth factors. Based on the known activities of many of the other members, it can be expected that FHF-2 will also possess biological activities that will make it useful as a diagnostic and therapeutic reagent.

10 Many growth factors have expression patterns or possess activities that relate to the function of the nervous system. For example, one growth factor in the TGF family, namely GDNF, has been shown to be a potent neurotrophic factor that can promote the survival of dopaminergic neurons (Lin, *et al.*, *Science*, 260:1130). Another family member, namely dorsalin-1, is capable of promoting the differentiation of neural crest
15 cells (Basler, *et al.*, *Cell*, 73:687, 1993). The inhibins and activins have been shown to be expressed in the brain (Meunier, *et al.*, *Proc. Nat'l. Acad. Sci., USA*, 85:247, 1988; Sawchenko, *et al.*, *Nature*, 334:615, 1988), and activin has been shown to be capable of functioning as a nerve cell survival molecule (Schubert, *et al.*, *Nature*, 344:868, 1990). Another TGF family member, namely GDF-1, is nervous system-
20 specific in its expression pattern (Lee, *Proc. Nat'l. Acad. Sci., USA*, 88:4250, 1991), and certain other family members, such as Vgr-1 (Lyons, *et al.*, *Proc. Nat'l. Acad. Sci., USA*, 86:4554, 1989; Jones, *et al.*, *Development*, 111:581, 1991), OP-1 (Ozkaynak, *et al.*, *J. Biol. Chem.*, 267:25220, 1992), and BMP-4 (Jones, *et al.*, *Development*, 111:531, 1991), are also known to be expressed in the nervous
25 system.

The expression of FHF-2 in brain and eye suggests that FHF-2 may also possess activities that relate to the function of the nervous system. The known neurotrophic activities of other members of this family and the expression of FHF-2 in muscle
30 suggest that one activity of FHF-2 may be as a trophic factor for motor neurons. Alternatively, FHF-2 may have neurotrophic activities for other neuronal populations. Hence, FHF-2 may have *in vitro* and *in vivo* applications in the treatment of neurodegenerative diseases, such as amyotrophic lateral sclerosis, or in maintaining cells or tissues in culture prior to transplantation.

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Growth factors have also been shown to inhibit the differentiation of myoblasts in culture (Massague, *et al.*, *Proc. Natl. Acad. Sci., USA* 83:8206, 1986). Moreover, because myoblast cells may be used as a vehicle for delivering genes to muscle for gene therapy, the properties of FHF-2, namely the elevated expression in heart tissue (*i.e.*, muscle), could be exploited for maintaining cells prior to transplantation or for enhancing the efficiency of the fusion process.

In a first embodiment, the invention provides substantially pure fibroblast growth factor homologous factor-2 (FHF-2) characterized by having a molecular weight of about 30kD as determined by reducing SDS-PAGE and having essentially the amino acid sequence of SEQ ID NO:2. The term "substantially pure" as used herein refers to FHF-2 which is substantially free of other proteins, lipids, carbohydrates or other materials with which it is naturally associated. One skilled in the art can purify FHF-2 using standard techniques for protein purification. The substantially pure polypeptide will yield a single major band on a non-reducing polyacrylamide gel. The purity of the FHF-2 polypeptide can also be determined by amino-terminal amino acid sequence analysis. FHF-2 polypeptide includes functional fragments of the polypeptide, as long as the activity of FHF-2 remains. Smaller peptides containing the biological activity of FHF-2 are included in the invention.

The invention provides polynucleotides encoding the FHF-2 protein. These polynucleotides include DNA, cDNA and RNA sequences which encode FHF-2. It is understood that all polynucleotides encoding all or a portion of FHF-2 are also included herein, as long as they encode a polypeptide with FHF-2 activity. Such polynucleotides include naturally occurring, synthetic, and intentionally manipulated polynucleotides. For example, FHF-2 polynucleotide may be subjected to site-directed mutagenesis. The polynucleotide sequence for FHF-2 also includes antisense sequences. The polynucleotides of the invention include sequences that are degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are included in the invention as long as the amino acid sequence of FHF-2 polypeptide encoded by the nucleotide sequence is functionally unchanged.

Specifically disclosed herein is a DNA sequence encoding the human FHF-2 gene. The sequence contains an open reading frame encoding a polypeptide 245 amino

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acids in length. The human FHF-2 initiator methionine codon shown in FIGURE 1 at position 352-354 corresponds to the location of the initiator methionine codon of another FGF family member, FHF-1, when the two sequences are aligned; a good consensus ribosome binding site (TGGCCATGG; Kozak, *Nucleic Acids Res.*, 15: 8125, 1987) is found at this position. The next methionine codon within the open reading frame is encountered 124 codons 3' of the putative initiator methionine codon. As observed for aFGF and bFGF, the amino-terminus of the primary translation product of FHF-2 does not conform to the consensus sequence for a signal peptide to direct cotranslational insertion across the endoplasmic reticulum membrane. The FHF-2 sequence has one potential asn-X-ser/thr site for asparagine-linked glycosylation four amino acids from the carboxy-terminus. Preferably, the human FHF-2 nucleotide sequence is SEQ ID NO:1 and the deduced amino acid sequence is probably SEQ ID NO:2.

The polynucleotide encoding FHF-2 includes SEQ ID NO:1 as well as nucleic acid sequences complementary to SEQ ID NO:1. A complementary sequence may include an antisense nucleotide. When the sequence is RNA, the deoxynucleotides A, G, C, and T of SEQ ID NO:1 is replaced by ribonucleotides A, G, C, and U, respectively. Also included in the invention are fragments of the above-described nucleic acid sequences that are at least 15 bases in length, which is sufficient to permit the fragment to selectively hybridize to DNA that encodes the protein of SEQ ID NO:2 under physiological conditions. Specifically, the fragments should hybridize to DNA encoding FHF-2 protein under stringent conditions.

The most homologous FGF family member is FGF-9, which shares 28% amino acid identity with FHF-2, when aligned with 6 gaps. Minor modifications of the FHF-2 primary amino acid sequence may result in proteins which have substantially equivalent activity as compared to the FHF-2 polypeptide described herein. Such proteins include those as defined by the term "having essentially the amino acid sequence of SEQ ID NO:2". Such modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous. All of the polypeptides produced by these modifications are included herein as long as the biological activity of FHF-2 still exists. Further, deletion of one or more amino acids can also result in a modification of the structure of the resultant molecule without significantly altering its biological activity. This can lead to the development of a smaller active molecule which would

have broader utility. For example, one can remove amino or carboxy terminal amino acids which are not required for FHF-2 biological activity.

5 The nucleotide sequence encoding the FHF-2 polypeptide of the invention includes the disclosed sequence (SEQ ID NO:2), and conservative variations thereof. The term "conservative variation" as used herein denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for
10 another, such as the substitution of arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine, and the like. The term "conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted polypeptide also immunoreact with the unsubstituted polypeptide.

15 DNA sequences of the invention can be obtained by several methods. For example, the DNA can be isolated using hybridization techniques which are well known in the art. These include, but are not limited to: 1) hybridization of genomic or cDNA libraries with probes to detect homologous nucleotide sequences, 2) polymerase
20 chain reaction (PCR) on genomic DNA or cDNA using primers capable of annealing to the DNA sequence of interest, and 3) antibody screening of expression libraries to detect cloned DNA fragments with shared structural features.

25 Preferably the FHF-2 polynucleotide of the invention is derived from a mammalian organism, and most preferably from human. Screening procedures which rely on nucleic acid hybridization make it possible to isolate any gene sequence from any organism, provided the appropriate probe is available. Oligonucleotide probes, which correspond to a part of the sequence encoding the protein in question, can be synthesized chemically. This requires that short, oligopeptide stretches of amino
30 acid sequence must be known. The DNA sequence encoding the protein can be deduced from the genetic code, however, the degeneracy of the code must be taken into account. It is possible to perform a mixed addition reaction when the sequence is degenerate. This includes a heterogeneous mixture of denatured double-stranded DNA. For such screening, hybridization is preferably performed on either single-
35 stranded DNA or denatured double-stranded DNA. Hybridization is particularly useful in the detection of cDNA clones derived from sources where an extremely low

amount of mRNA sequences relating to the polypeptide of interest are present. In other words, by using stringent hybridization conditions directed to avoid non-specific binding, it is possible, for example, to allow the autoradiographic visualization of a specific cDNA clone by the hybridization of the target DNA to that single probe in the mixture which is its complete complement (Wallace, *et al.*, *Nucl. Acid Res.*, 9:879, 1981; Maniatis, *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, N.Y. 1989).

The development of specific DNA sequences encoding FHF-2 can also be obtained by: 1) isolation of double-stranded DNA sequences from the genomic DNA; 2) chemical manufacture of a DNA sequence to provide the necessary codons for the polypeptide of interest; and 3) *in vitro* synthesis of a double-stranded DNA sequence by reverse transcription of mRNA isolated from a eukaryotic donor cell. In the latter case, a double-stranded DNA complement of mRNA is eventually formed which is generally referred to as cDNA.

Of the three above-noted methods for developing specific DNA sequences for use in recombinant procedures, the isolation of genomic DNA isolates is the least common. This is especially true when it is desirable to obtain the microbial expression of mammalian polypeptides due to the presence of introns.

The synthesis of DNA sequences is frequently the method of choice when the entire sequence of amino acid residues of the desired polypeptide product is known. When the entire sequence of amino acid residues of the desired polypeptide is not known, the direct synthesis of DNA sequences is not possible and the method of choice is the synthesis of cDNA sequences. Among the standard procedures for isolating cDNA sequences of interest is the formation of plasmid- or phage-carrying cDNA libraries which are derived from reverse transcription of mRNA which is abundant in donor cells that have a high level of genetic expression. When used in combination with polymerase chain reaction technology, even rare expression products can be cloned. In those cases where significant portions of the amino acid sequence of the polypeptide are known, the production of labeled single or double-stranded DNA or RNA probe sequences duplicating a sequence putatively present in the target cDNA may be employed in DNA/DNA hybridization procedures which are carried out on cloned copies of the cDNA which have been denatured into a single-stranded form (Jay, *et al.*, *Nucl. Acid Res.*, 11:2325, 1983).

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A cDNA expression library, such as lambda gt11, can be screened indirectly for FHF-2 peptides having at least one epitope, using antibodies specific for FHF-2. Such antibodies can be either polyclonally or monoclonally derived and used to detect expression product indicative of the presence of FHF-2 cDNA.

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DNA sequences encoding FHF-2 can be expressed *in vitro* by DNA transfer into a suitable host cell. "Host cells" are cells in which a vector can be propagated and its DNA expressed. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. However, such progeny are included when the term "host cell" is used. Methods of stable transfer, meaning that the foreign DNA is continuously maintained in the host, are known in the art.

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In the present invention, the FHF-2 polynucleotide sequences may be inserted into a recombinant expression vector. The term "recombinant expression vector" refers to a plasmid, virus or other vehicle known in the art that has been manipulated by insertion or incorporation of the FHF-2 genetic sequences. Such expression vectors contain a promoter sequence which facilitates the efficient transcription of the inserted genetic sequence of the host. The expression vector typically contains an origin of replication, a promoter, as well as specific genes which allow phenotypic selection of the transformed cells. Vectors suitable for use in the present invention include, but are not limited to the T7-based expression vector for expression in bacteria (Rosenberg, *et al.*, *Gene*, 56:125, 1987), the pMSXND expression vector for expression in mammalian cells (Lee and Nathans, *J. Biol. Chem.*, 263:3521, 1988) and baculovirus-derived vectors for expression in insect cells. The DNA segment can be present in the vector operably linked to regulatory elements, for example, a promoter (e.g., T7, metallothionein I, or polyhedrin promoters).

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Polynucleotide sequences encoding FHF-2 can be expressed in either prokaryotes or eukaryotes. Hosts can include microbial, yeast, insect and mammalian organisms. Methods of expressing DNA sequences having eukaryotic or viral sequences in prokaryotes are well known in the art. Biologically functional viral and plasmid DNA vectors capable of expression and replication in a host are known in the art. Such vectors are used to incorporate DNA sequences of the invention.

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Transformation of a host cell with recombinant DNA may be carried out by conventional techniques as are well known to those skilled in the art. Where the host is prokaryotic, such as *E. coli*, competent cells which are capable of DNA uptake can be prepared from cells harvested after exponential growth phase and subsequently
5 treated by the CaCl_2 method using procedures well known in the art. Alternatively, MgCl_2 or RbCl can be used. Transformation can also be performed after forming a protoplast of the host cell if desired.

When the host is a eukaryote, such methods of transfection of DNA as calcium phosphate co-precipitates, conventional mechanical procedures such as
10 microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors may be used. Eukaryotic cells can also be cotransformed with DNA sequences encoding the FHF-2 of the invention, and a second foreign DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase
15 gene. Another method is to use a eukaryotic viral vector, such as simian virus 40 (SV40) or bovine papilloma virus, to transiently infect or transform eukaryotic cells and express the protein. (see for example, *Eukaryotic Viral Vectors*, Cold Spring Harbor Laboratory, Gluzman ed., 1982).

20 Isolation and purification of microbial expressed polypeptide, or fragments thereof, provided by the invention, may be carried out by conventional means including preparative chromatography and immunological separations involving monoclonal or polyclonal antibodies.

25 The FHF-2 polypeptides of the invention can also be used to produce antibodies which are immunoreactive or bind to epitopes of the FHF-2 polypeptides. Antibody which consists essentially of pooled monoclonal antibodies with different epitopic specificities, as well as distinct monoclonal antibody preparations are provided. Monoclonal antibodies are made from antigen containing fragments of the protein by
30 methods well known in the art (Kohler, *et al.*, *Nature*, 256:495, 1975; *Current Protocols in Molecular Biology*, Ausubel, *et al.*, ed., 1989).

The term "antibody" as used in this invention includes intact molecules as well as fragments thereof, such as Fab, F(ab')_2 , and Fv which are capable of binding the
35 epitopic determinant. These antibody fragments retain some ability to selectively bind with its antigen or receptor and are defined as follows:

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(1) Fab, the fragment which contains a monovalent antigen-binding fragment of an antibody molecule can be produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and a portion of one heavy chain;

5 (2) Fab', the fragment of an antibody molecule can be obtained by treating whole antibody with pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain; two Fab' fragments are obtained per antibody molecule;

10 (3) (Fab')₂, the fragment of the antibody that can be obtained by treating whole antibody with the enzyme pepsin without subsequent reduction; F(ab')₂ is a dimer of two Fab' fragments held together by two disulfide bonds;

15 (4) Fv, defined as a genetically engineered fragment containing the variable region of the light chain and the variable region of the heavy chain expressed as two chains; and

20 (5) Single chain antibody ("SCA"), defined as a genetically engineered molecule containing the variable region of the light chain, the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule.

Methods of making these fragments are known in the art. (See for example, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York (1988), incorporated herein by reference).

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As used in this invention, the term "epitope" means any antigenic determinant on an antigen to which the paratope of an antibody binds. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics.

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Antibodies which bind to the FHF-2 polypeptide of the invention can be prepared using an intact polypeptide or fragments containing small peptides of interest as the immunizing antigen. The polypeptide or a peptide used to immunize an animal can be derived from translated cDNA (see for example, EXAMPLE 4) or chemical synthesis which can be conjugated to a carrier protein, if desired. Such commonly

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used carriers which are chemically coupled to the peptide include keyhole limpet hemocyanin (KLH), thyroglobulin, bovine serum albumin (BSA), and tetanus toxoid. The coupled peptide is then used to immunize the animal (e.g., a mouse, a rat, or a rabbit).

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If desired, polyclonal or monoclonal antibodies can be further purified, for example, by binding to and elution from a matrix to which the polypeptide or a peptide to which the antibodies were raised is bound. Those of skill in the art will know of various techniques common in the immunology arts for purification and/or concentration of polyclonal antibodies, as well as monoclonal antibodies (See for example, Coligan, *et al.*, Unit 9, *Current Protocols in Immunology*, Wiley Interscience, 1994, incorporated by reference).

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It is also possible to use the anti-idiotypic technology to produce monoclonal antibodies which mimic an epitope. For example, an anti-idiotypic monoclonal antibody made to a first monoclonal antibody will have a binding domain in the hypervariable region which is the "image" of the epitope bound by the first monoclonal antibody.

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The term "cell-proliferative disorder" denotes malignant as well as non-malignant cell populations which often appear to differ from the surrounding tissue both morphologically and genotypically. Malignant cells (*i.e.* cancer) develop as a result of a multistep process. The FHF-2 polynucleotide that is an antisense molecule is useful in treating malignancies of the various organ systems, particularly, for example, cells in the central nervous system, including neural tissue, heart, and cells of the eye. Essentially, any disorder which is etiologically linked to altered expression of FHF-2 could be considered susceptible to treatment with a FHF-2 suppressing reagent. One such disorder is a malignant cell proliferative disorder, for example.

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For purposes of the invention, an antibody or nucleic acid probe specific for FHF-2 may be used to detect FHF-2 polypeptide (using antibody) or polynucleotide (using nucleic acid probe) in biological tissues or fluids. The invention provides a method for detecting a cell proliferative disorder of cardiac tissue or neural tissue, for example, which comprises contacting an anti-FHF-2 antibody or nucleic acid probe with a cell suspected of having a FHF-2 associated disorder and detecting binding

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of FHF-2 antigen or mRNA to the antibody or nucleic acid probe, respectively. The antibody or nucleic acid probe reactive with FHF-2 is preferably labeled with a compound which allows detection of binding to FHF-2. Any specimen containing a detectable amount of antigen can be used. A preferred sample of this invention is neural tissue or heart tissue. The level of FHF-2 in the suspect cell can be compared with the level in a normal cell to determine whether the subject has a FHF-2-associated cell proliferative disorder. Preferably the subject is human.

When the cell component is nucleic acid, it may be necessary to amplify the nucleic acid prior to binding with an FHF-2 specific probe. Preferably, polymerase chain reaction (PCR) is used, however, other nucleic acid amplification procedures such as ligase chain reaction (LCR), ligated activated transcription (LAT) and nucleic acid sequence-based amplification (NASBA) may be used.

The antibodies of the invention can be used in any subject in which it is desirable to administer *in vitro* or *in vivo* immunodiagnosis or immunotherapy. The antibodies of the invention are suited for use, for example, in immunoassays in which they can be utilized in liquid phase or bound to a solid phase carrier. In addition, the antibodies in these immunoassays can be detectably labeled in various ways. Examples of types of immunoassays which can utilize antibodies of the invention are competitive and non-competitive immunoassays in either a direct or indirect format. Examples of such immunoassays are the radioimmunoassay (RIA) and the sandwich (immunometric) assay. Detection of the antigens using the antibodies of the invention can be done utilizing immunoassays which are run in either the forward, reverse, or simultaneous modes, including immunohistochemical assays on physiological samples. Those of skill in the art will know, or can readily discern, other immunoassay formats without undue experimentation.

The antibodies of the invention can be bound to many different carriers and used to detect the presence of an antigen comprising the polypeptide of the invention. Examples of well-known carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses and magnetite. The nature of the carrier can be either soluble or insoluble for purposes of the invention. Those skilled in the art will know of other suitable carriers for binding antibodies, or will be able to ascertain such, using routine experimentation.

- 15 -

There are many different labels and methods of labeling known to those of ordinary skill in the art. Examples of the types of labels which can be used in the present invention include enzymes, radioisotopes, fluorescent compounds, colloidal metals, chemiluminescent compounds, phosphorescent compounds, and bioluminescent compounds. Those of ordinary skill in the art will know of other suitable labels for binding to the antibody, or will be able to ascertain such, using routine experimentation.

Another technique which may also result in greater sensitivity consists of coupling the antibodies to low molecular weight haptens. These haptens can then be specifically detected by means of a second reaction. For example, it is common to use such haptens as biotin, which reacts with avidin, or dinitrophenyl, puridoxal, and fluorescein, which can react with specific antihapten antibodies.

In using the monoclonal antibodies of the invention for the *in vivo* detection of antigen, the detectably labeled antibody is given a dose which is diagnostically effective. The term "diagnostically effective" means that the amount of detectably labeled monoclonal antibody is administered in sufficient quantity to enable detection of the site having the antigen comprising a polypeptide of the invention for which the monoclonal antibodies are specific.

The concentration of detectably labeled monoclonal antibody which is administered should be sufficient such that the binding to those cells having the polypeptide is detectable compared to the background. Further, it is desirable that the detectably labeled monoclonal antibody be rapidly cleared from the circulatory system in order to give the best target-to-background signal ratio.

As a rule, the dosage of detectably labeled monoclonal antibody for *in vivo* diagnosis will vary depending on such factors as age, sex, and extent of disease of the individual. Such dosages may vary, for example, depending on whether multiple injections are given, antigenic burden, and other factors known to those of skill in the art.

For *in vivo* diagnostic imaging, the type of detection instrument available is a major factor in selecting a given radioisotope. The radioisotope chosen must have a type of decay which is detectable for a given type of instrument. Still another important

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factor in selecting a radioisotope for *in vivo* diagnosis is that deleterious radiation with respect to the host is minimized. Ideally, a radioisotope used for *in vivo* imaging will lack a particle emission, but produce a large number of photons in the 140-250 keV range, which may readily be detected by conventional gamma cameras.

5

For *in vivo* diagnosis radioisotopes may be bound to immunoglobulin either directly or indirectly by using an intermediate functional group. Intermediate functional groups which often are used to bind radioisotopes which exist as metallic ions to immunoglobulins are the bifunctional chelating agents such as diethylenetriaminepentaacetic acid (DTPA) and ethylenediaminetetraacetic acid (EDTA) and similar molecules. Typical examples of metallic ions which can be bound to the monoclonal antibodies of the invention are ^{111}In , ^{97}Ru , ^{67}Ga , ^{68}Ga , ^{72}As , ^{89}Zr , and ^{201}Tl .

10

The monoclonal antibodies of the invention can also be labeled with a paramagnetic isotope for purposes of *in vivo* diagnosis, as in magnetic resonance imaging (MRI) or electron spin resonance (ESR). In general, any conventional method for visualizing diagnostic imaging can be utilized. Usually gamma and positron emitting radioisotopes are used for camera imaging and paramagnetic isotopes for MRI. Elements which are particularly useful in such techniques include ^{157}Gd , ^{55}Mn , ^{162}Dy , ^{52}Cr , and ^{56}Fe .

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The monoclonal antibodies or polynucleotides of the invention can be used *in vitro* and *in vivo* to monitor the course of amelioration of a FHF-2-associated disease in a subject. Thus, for example, by measuring the increase or decrease in the number of cells expressing antigen comprising a polypeptide of the invention or changes in the concentration of such antigen present in various body fluids, it would be possible to determine whether a particular therapeutic regimen aimed at ameliorating the FHF-2-associated disease is effective. The term "ameliorate" denotes a lessening of the detrimental effect of the FHF-2-associated disease in the subject receiving therapy.

25

30

The present invention identifies a nucleotide sequence that can be expressed in an altered manner as compared to expression in a normal cell, therefore it is possible to design appropriate therapeutic or diagnostic techniques directed to this sequence. Detection of elevated levels of FHF-2 expression is accomplished by hybridization of nucleic acids isolated from a cell suspected of having an FHF-2 associated

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proliferative disorder with an FHF-2 polynucleotide of the invention. Analyses, such as Northern Blot analysis, are utilized to quantitate expression of FHF-2. Other standard nucleic acid detection techniques will be known to those of skill in the art.

5 Treatment of an FHF-2 associated cell proliferative disorder include modulation of FHF-2 gene expression and FHF-2 activity. The term "modulate" envisions the suppression of expression of FHF-2 when it is over-expressed, or augmentation of FHF-2 expression when it is under-expressed. Where a cell-proliferative disorder is associated with the expression of FHF-2, nucleic acid sequences that interfere with
10 FHF-2 expression at the translational level can be used. This approach utilizes, for example, antisense nucleic acid, ribozymes, or triplex agents to block transcription or translation of a specific FHF-2 mRNA, either by masking that mRNA with an antisense nucleic acid or triplex agent, or by cleaving it with a ribozyme. Such disorders include neurodegenerative diseases, for example.

15 Antisense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific mRNA molecule (Weintraub, *Scientific American*, 262:40, 1990). In the cell, the antisense nucleic acids hybridize to the corresponding mRNA, forming a double-stranded molecule. The antisense nucleic acids interfere with the
20 translation of the mRNA, since the cell will not translate a mRNA that is double-stranded. Antisense oligomers of about 15 nucleotides are preferred, since they are easily synthesized and are less likely to cause problems than larger molecules when introduced into the target FHF-2-producing cell. The use of antisense methods to inhibit the *in vitro* translation of genes is well known in the art (Marcus-Sakura,
25 *Anal.Biochem.*, 172:289, 1988).

Use of an oligonucleotide to stall transcription is known as the triplex strategy since the oligomer winds around double-helical DNA, forming a three-strand helix. Therefore, these triplex compounds can be designed to recognize a unique site on
30 a chosen gene (Maher, *et al.*, *Antisense Res. and Dev.*, 1(3):227, 1991; Helene, C., *Anticancer Drug Design*, 6(6):569, 1991).

Ribozymes are RNA molecules possessing the ability to specifically cleave other single-stranded RNA in a manner analogous to DNA restriction endonucleases.
35 Through the modification of nucleotide sequences which encode these RNAs, it is possible to engineer molecules that recognize specific nucleotide sequences in an

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RNA molecule and cleave it (Cech, *J.Amer.Med. Assn.*, 260:3030, 1988). A major advantage of this approach is that, because they are sequence-specific, only mRNAs with particular sequences are inactivated.

5 There are two basic types of ribozymes namely, *tetrahymena*-type (Hasselhoff, *Nature*, 334:585, 1988) and "hammerhead"-type. *Tetrahymena*-type ribozymes recognize sequences which are four bases in length, while "hammerhead"-type ribozymes recognize base sequences 11-18 bases in length. The longer the recognition sequence, the greater the likelihood that the sequence will occur exclusively in
10 the target mRNA species. Consequently, hammerhead-type ribozymes are preferable to *tetrahymena*-type ribozymes for inactivating a specific mRNA species and 18-based recognition sequences are preferable to shorter recognition sequences.

15 The present invention also provides gene therapy for the treatment of cell proliferative or immunologic disorders which are mediated by FHF-2 protein. Such therapy would achieve its therapeutic effect by introduction of the FHF-2 antisense polynucleotide into cells having the proliferative disorder. Delivery of antisense FHF-2 polynucleotide can be achieved using a recombinant expression vector such as a
20 chimeric virus or a colloidal dispersion system. Especially preferred for therapeutic delivery of antisense sequences is the use of targeted liposomes.

Various viral vectors which can be utilized for gene therapy as taught herein include adenovirus, herpes virus, vaccinia, or, preferably, an RNA virus such as a retrovirus.
25 Preferably, the retroviral vector is a derivative of a murine or avian retrovirus. Examples of retroviral vectors in which a single foreign gene can be inserted include, but are not limited to: Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV). Preferably, when the subject is a human, a vector such as
30 the gibbon ape leukemia virus (GaLV) is utilized. A number of additional retroviral vectors can incorporate multiple genes. All of these vectors can transfer or incorporate a gene for a selectable marker so that transduced cells can be identified and generated. By inserting a FHF-2 sequence of interest into the viral vector, along with another gene which encodes the ligand for a receptor on a specific target cell,
35 for example, the vector is now target specific. Retroviral vectors can be made target specific by attaching, for example, a sugar, a glycolipid, or a protein. Preferred

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targeting is accomplished by using an antibody to target the retroviral vector. Those of skill in the art will know of, or can readily ascertain without undue experimentation, specific polynucleotide sequences which can be inserted into the retroviral genome or attached to a viral envelope to allow target specific delivery of the retroviral vector containing the FHF-2 antisense polynucleotide.

Since recombinant retroviruses are defective, they require assistance in order to produce infectious vector particles. This assistance can be provided, for example, by using helper cell lines that contain plasmids encoding all of the structural genes of the retrovirus under the control of regulatory sequences within the LTR. These plasmids are missing a nucleotide sequence which enables the packaging mechanism to recognize an RNA transcript for encapsidation. Helper cell lines which have deletions of the packaging signal include, but are not limited to Ψ 2, PA317 and PA12, for example. These cell lines produce empty virions, since no genome is packaged. If a retroviral vector is introduced into such cells in which the packaging signal is intact, but the structural genes are replaced by other genes of interest, the vector can be packaged and vector virion produced.

Alternatively, NIH 3T3 or other tissue culture cells can be directly transfected with plasmids encoding the retroviral structural genes *gag*, *pol* and *env*, by conventional calcium phosphate transfection. These cells are then transfected with the vector plasmid containing the genes of interest. The resulting cells release the retroviral vector into the culture medium.

Another targeted delivery system for FHF-2 antisense polynucleotides is a colloidal dispersion system. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. The preferred colloidal system of this invention is a liposome. Liposomes are artificial membrane vesicles which are useful as delivery vehicles *in vitro* and *in vivo*. It has been shown that large unilamellar vesicles (LUV), which range in size from 0.2-4.0 μ m can encapsulate a substantial percentage of an aqueous buffer containing large macromolecules. RNA, DNA and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (Fraley, *et al.*, *Trends Biochem. Sci.*, 6:77, 1981). In addition to mammalian cells, liposomes have been used for delivery of polynucleotides in plant, yeast and bacterial cells. In order for a liposome to be

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an efficient gene transfer vehicle, the following characteristics should be present: (1) encapsulation of the genes of interest at high efficiency while not compromising their biological activity; (2) preferential and substantial binding to a target cell in comparison to non-target cells; (3) delivery of the aqueous contents of the vesicle to the target cell cytoplasm at high efficiency; and (4) accurate and effective expression of genetic information (Mannino, *et al.*, *Biotechniques*, 6:682, 1988).

The composition of the liposome is usually a combination of phospholipids, particularly high-phase-transition-temperature phospholipids, usually in combination with steroids, especially cholesterol. Other phospholipids or other lipids may also be used. The physical characteristics of liposomes depend on pH, ionic strength, and the presence of divalent cations.

Examples of lipids useful in liposome production include phosphatidyl compounds, such as phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids, cerebroside, and gangliosides. Particularly useful are diacylphosphatidylglycerols, where the lipid moiety contains from 14-18 carbon atoms, particularly from 16-18 carbon atoms, and is saturated. Illustrative phospholipids include egg phosphatidylcholine, d-
ipalmitoylphosphatidylcholine and distearoylphosphatidylcholine.

The targeting of liposomes can be classified based on anatomical and mechanistic factors. Anatomical classification is based on the level of selectivity, for example, organ-specific, cell-specific, and organelle-specific. Mechanistic targeting can be distinguished based upon whether it is passive or active. Passive targeting utilizes the natural tendency of liposomes to distribute to cells of the reticulo-endothelial system (RES) in organs which contain sinusoidal capillaries. Active targeting, on the other hand, involves alteration of the liposome by coupling the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein, or by changing the composition or size of the liposome in order to achieve targeting to organs and cell types other than the naturally occurring sites of localization.

The surface of the targeted delivery system may be modified in a variety of ways. In the case of a liposomal targeted delivery system, lipid groups can be incorporated into the lipid bilayer of the liposome in order to maintain the targeting ligand in stable

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association with the liposomal bilayer. Various linking groups can be used for joining the lipid chains to the targeting ligand.

5 Due to the expression of FHF-2 in heart, eye, and brain, or neural tissue, there are a variety of applications using the polypeptide, polynucleotide, and antibodies of the invention, related to these tissues. Such applications include treatment of cell proliferative and immunologic disorders involving these and other tissues. In addition, FHF-2 may be useful in various gene therapy procedures.

10 The identification of a novel member of the FGF family provides a useful tool for diagnosis, prognosis and therapeutic strategies associated with FHF-2 mediated disorders. Measurement of FHF-2 levels using anti-FHF-2 antibodies is useful diagnostic for following the progression or recovery from diseases of the nervous system, including: cancer, stroke, neurodegenerative diseases such as Parkinson's
15 disease or Alzheimer's disease, retinal diseases such as retinitis pigmentosa, or viral encephalitis. The presence of high levels of FHF-2 in the central nervous system suggests that the observed low level of FHF-2 in a number of peripheral tissues could reflect FHF-2 in peripheral nerve, and therefore measurement of FHF-2 levels using anti-FHF-2 antibodies could be diagnostic for peripheral neuropathy. The
20 presence of high levels of FHF-2 in the heart suggests that measurement of FHF-2 levels using anti-FHF-2 antibodies is useful as a diagnostic for myocardial infarction, viral endocarditis, or other cardiac disorders.

25 Like other members of the FGF family, FHF-2 likely has mitogenic and/or cell survival activity, therefore FHF-2 or an analogue that mimics FHF-2 action could be used to promote tissue repair or replacement. The presence of FHF-2 in the CNS suggests such a therapeutic role in diseases of the nervous system, including: stroke, neurodegenerative diseases such as Parkinson's disease or Alzheimer's disease, or in retinal degenerative diseases such as retinitis pigmentosa or macular degeneration,
30 or in peripheral neuropathies. The presence of high levels of FHF-2 in the heart suggests that FHF-2 or an analogue of FHF-2 could be used to accelerate recovery from myocardial infarction or could promote increased cardiac output by increasing heart muscle. Conversely, blocking FHF-2 action either with anti-FHF-2 antibodies or with an FHF-2 antagonist might slow or ameliorate diseases in which excess cell
35 growth is pathological, most obviously cancer.

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The following examples are intended to illustrate but not limit the invention. While they are typical of those that might be used, other procedures known to those skilled in the art may alternatively be used.

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EXAMPLE 1

ISOLATION OF FHF-2, A NOVEL MEMBER OF THE FGF FAMILY

10 To identify novel gene products expressed in the human retina, random segments of human retina cDNA clones were partially sequenced, and the resulting partial sequences compared to the sequences available in the public databases.

15 In detail, an adult human retina cDNA library constructed in lambda gt10 (Nathans, *et al.*, *Science*, 232: 193, 1986) was amplified, and the cDNA inserts were excised en mass by cleavage with EcoR I and purified free of the vector by agarose gel electrophoresis. Following heat denaturation of the purified cDNA inserts, a synthetic oligonucleotide containing an EcoR I site at its 5' end and six random nucleotides at its 3' end (5' GACGAGATATTAGAATTCTACTCGNNNNNN) (SEQ ID NO:3) was used to prime two sequential rounds of DNA synthesis in the presence of the Klenow
20 fragment of E. coli DNA polymerase. The resulting duplex molecules were amplified using the polymerase chain reaction (PCR) with a primer corresponding to the unique 5' flanking sequence (5' CCCCCCCCCGACGAGATATTAGAATTCTACTCG) (SEQ ID NO:4). These PCR products, representing a random sampling of the original cDNA inserts, were cleaved with EcoR I, size fractionated by preparative agarose gel
25 electrophoresis to include only segments of approximately 500 bp in length, and cloned into lambda gt10. Three thousand single plaques from this derivative library were arrayed in 96-well trays and from these clones the inserts were amplified by PCR using flanking vector primers and then sequenced using the dideoxy method and automated fluorescent detection (Applied Biosystems). A single sequencing run
30 from one end of each insert was conceptually translated on both strands in all three reading frames and the six resulting amino acid sequences were used to search for homology in the GenBank nonredundant protein database using the BLASTX searching algorithm.

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One partial cDNA sequence was found that showed statistically significant homology to previously described members of the FGF family. Using this partial cDNA as a probe, multiple independent cDNA clones were isolated from the human retina cDNA library, including two that encompass the entire open reading frame and from which complete nucleotide sequences were determined. This sequence was named FHF-1 and is the subject of a pending patent application. A search of partial cDNA sequences ('expressed sequence tags', ESTs) in the public databases revealed a human fetal brain cDNA fragment (NCBI ID 28057, EST ID EST06895, Genbank ID T09003; Adams, *et al.*, *Nature Genetics*, 4: 373, 1993) with strong homology to FHF-1, but only weak homology to other members of the FGF family. The homology between this EST and other members of the FGF family is sufficiently low that no indication of that homology was noted in the description associated with the clone in Genbank or in the publication describing the EST sequence (Adams, *et al.*, *supra*, 1993). Based on the EST sequence, this fragment was amplified by PCR from a human retina cDNA library and used as a probe to isolate multiple independent cDNA clones from that library, including two that encompass the entire open reading frame and from which complete nucleotide sequences were determined. This sequence was named FHF-2.

EXAMPLE 2

DEDUCED PRIMARY STRUCTURE OF FHF-2

FIGURE 1 shows the sequence of human FHF-2 deduced from the nucleotide sequences of two independent human retina cDNA clones. The primary translation product of human FHF-2 is predicted to be 245 amino acids in length. The human FHF-2 initiator methionine codon shown in Figure 1 at position 352-354 corresponds to the location of the initiator methionine codon of FHF-1 when the two sequences are aligned; a good consensus ribosome binding site (TGGCCATGG; Kozak, *Nucleic Acids Res.*, 15: 8125, 1987) (SEQ ID NO:5) is found at this position. The next methionine codon within the open reading frame is encountered 124 codons 3' of the putative initiator methionine codon. As observed for aFGF and bFGF, the amino-terminus of the primary translation product of FHF-2 does not conform to the consensus sequence for a signal peptide to direct cotranslational insertion across the endoplasmic reticulum membrane. The FHF-2 sequence has one potential asn-X-ser/thr site for asparagine-linked glycosylation four amino acids from the carboxy-terminus.

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Alignment of FHF-2 with the other known members of the FGF family is shown in FIGURE 2 and a dendrogram showing the degree of amino acid similarity is shown in FIGURE 3. The most homologous FGF family member is FGF-9 which shows 28% amino acid identity with FHF-2 when aligned with 6 gaps. Note that in the central region of each polypeptide, all FGF family members, including FHF-2, share 11 invariant amino acids.

EXAMPLE 3

CHROMOSOMAL LOCALIZATION OF FHF-2

The chromosomal location of FHF-2 was determined by probing a Southern blot containing restriction enzyme digested DNA derived from a panel of 24 human-mouse and human-hamster cell lines, each containing a different human chromosome (Oncor, Gaithersburg, MD). As seen in FIGURE 4, hybridization of the human FHF-2 probe to human, mouse, and hamster genomic DNA produces distinct hybridizing fragment sizes. The human-specific hybridization pattern is seen only in the lane corresponding to the hybrid cell line carrying the human X-chromosome.

EXAMPLE 4

PRODUCTION OF FHF-2 IN TRANSFECTED HUMAN CELLS

To express FHF-2 in human cells, the complete open reading frame was inserted into the eukaryotic expression vector pCIS (Gorman, *et al.*, *DNA Protein Eng. Tech.*, 2: 3, 1990). To increase the efficiency of translation, the region immediately 5' of the initiator methionine coding was converted to an optimal ribosome binding site (CCACCATGG) (SEQ ID NO:5) by cutting the FHF-2 coding region at the initiator methionine with Nco I (which recognizes CCATGG) and ligating to the expression vector. Following transient transfection of human embryonic kidney cells with the expression construct and a plasmid expressing the simian virus 40 (SV40) large T-antigen (pRSV-TAg; Gorman *et al.*, *supra*), cells were metabolically labeled with ³⁵S methionine for 6 hours in the absence of serum. As shown in FIGURE 5, cells transfected with FHF-2 synthesize a single polypeptide with an apparent molecular mass of 30 kD that is not produced by untransfected cells or by cells transfected with an unrelated construct. This polypeptide corresponds closely to the predicted molecular mass of the primary

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translation product, 27.6 kD. Figure 5 also shows that cells transfected with a human growth hormone (hGH) expression plasmid efficiently secrete hGH, whereas FHF-2 accumulates within the transfected cells and fails to be secreted in detectable quantities.

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EXAMPLE 5

TISSUE DISTRIBUTION OF FHF-2 mRNA

To determine the tissue distribution of FHF-2 mRNA, RNase protection analysis
10 was performed on total RNA from mouse brain, eye, heart, kidney, liver, lung, spleen, and testis, as well as a yeast tRNA negative control. The probe used was derived from a segment of the mouse FHF-2 gene isolated by hybridization with the full-length human FHF-2 cDNA. As seen in Figure 6, the highest levels of FHF-2 expression are in the brain, eye, and heart. Very low levels of FHF-2
15 expression were detected in all of the other tissues on a longer exposure of the autoradiogram.

Although the invention has been described with reference to the presently preferred embodiment, it should be understood that various modifications can be
20 made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

5

(i) APPLICANT: The Johns Hopkins University School of Medicine

10

(ii) TITLE OF INVENTION: FIBROBLAST GROWTH FACTOR HOMOLOGOUS FACTOR-2
AND METHODS OF USE

(iii) NUMBER OF SEQUENCES: 5

15

(iv) CORRESPONDENCE ADDRESS:

- (A) ADDRESSEE: Fish & Richardson P.C.
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- (D) STATE: California
- (E) COUNTRY: USA
- (F) ZIP: 92037

20

(v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30

25

(vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER: US
- (B) FILING DATE: 10-MAY-1996
- (C) CLASSIFICATION:

30

(viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: Haile, Ph.D., Lisa A.,
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35

(ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: (619) 678-5070
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40

(2) INFORMATION FOR SEQ ID NO:1:

45

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1150 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

50

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:

- (B) CLONE: FHF-2

55

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 353..1087

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AATTCCGCTT GCACAGTGTC CGCCGGGCGC AGGGGCCGAC CGCACGCAGT CGCGCAGTTC

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	TGCCTCCGCC TGCCAGTCTC GCCCGCGATC CCGGCCCGGG GCTGTGGCGT CCACTCCGAC	120
	CCAGGCAGCC AGCAGCCCGC GCGGGAGCCG GACCGCCGCC GGAGGAGCTC GGACGGCATG	180
5	CTGAGCCCCC TCCTTGCTG AAGCCCGAGT GCGGAGAAGC CCGGGCAAAC GCAGGCTAAG	240
	GAGACCAAAG CGGCGAAGTC GCGAGACAGC GGACAAGCAG CGGAGGAGAA GGAGGAGGAG	300
10	GCGAACCCAG AGAGGGGCGAG CAAAAGAAGC GGTGGTGGTG GCGTCGTGG CC ATG Met 1	355
	GCG GCG GCT ATC GCC AGC TCG CTC ATC CGT CAG AAG AGG CAA GCC CGC	403
15	Ala Ala Ala Ile Ala Ser Ser Leu Ile Arg Gln Lys Arg Gln Ala Arg 5 10 15	
	GAG CGC GAG AAA TCC AAC GCC TGC AAG TGT GTC AGC AGC CCC AGC AAA	451
20	Glu Arg Glu Lys Ser Asn Ala Cys Lys Cys Val Ser Ser Pro Ser Lys 20 25 30	
	GGC AAG ACC AGC TGC GAC AAA AAC AAG TTA AAT GTC TTT TCC CGG GTC	499
	Gly Lys Thr Ser Cys Asp Lys Asn Lys Leu Asn Val Phe Ser Arg Val 35 40 45	
25	AAA CTC TTC GGC TCC AAG AAG AGG CGC AGA AGA AGA CCA GAG CCT CAG	547
	Lys Leu Phe Gly Ser Lys Lys Arg Arg Arg Arg Arg Pro Glu Pro Gln 50 55 60 65	
30	CTT AAG GGT ATA GTT ACC AAG CTA TAC AGC CGA CAA GGC TAC CAC TTG	595
	Leu Lys Gly Ile Val Thr Lys Leu Tyr Ser Arg Gln Gly Tyr His Leu 70 75 80	
	CAG CTG CAG GCG GAT GGA ACC ATT GAT GGC ACC AAA GAT GAG GAC AGC	643
35	Gln Leu Gln Ala Asp Gly Thr Ile Asp Gly Thr Lys Asp Glu Asp Ser 85 90 95	
	ACT TAC ACT CTG TTT AAC CTC ATC CCT GTG GGT CTG CGA GTG GTG GCT	691
40	Thr Tyr Thr Leu Phe Asn Leu Ile Pro Val Gly Leu Arg Val Val Ala 100 105 110	
	ATC CAA GGA GTT CAA ACC AAG CTG TAC TTG GCA ATG AAC AGT GAG GGA	739
45	Ile Gln Gly Val Gln Thr Lys Leu Tyr Leu Ala Met Asn Ser Glu Gly 115 120 125	
	TAC TTG TAC ACC TCG GAA CTT TTC ACA CCT GAG TGC AAA TTC AAA GAA	787
	Tyr Leu Tyr Thr Ser Glu Leu Phe Thr Pro Glu Cys Lys Phe Lys Glu 130 135 140 145	
50	TCA GTG TTT GAA AAT TAT TAT GTG ACA TAT TCA TCA ATG ATA TAC CGT	835
	Ser Val Phe Glu Asn Tyr Tyr Val Thr Tyr Ser Ser Met Ile Tyr Arg 150 155 160	
55	CAG CAG CAG TCA GGC CGA GGG TGG TAT CTG GGT CTG AAC AAA GAA GGA	883
	Gln Gln Gln Ser Gly Arg Gly Trp Tyr Leu Gly Leu Asn Lys Glu Gly 165 170 175	
	GAG ATC ATG AAA GGC AAC CAT GTG AAG AAG AAC AAG CCT GCA GCT CAT	931
60	Glu Ile Met Lys Gly Asn His Val Lys Lys Asn Lys Pro Ala Ala His 180 185 190	
	TTT CTG CCT AAA CCA CTG AAA GTG GCC ATG TAC AAG GAG CCA TCA CTG	979
	Phe Leu Pro Lys Pro Leu Lys Val Ala Met Tyr Lys Glu Pro Ser Leu 195 200 205	

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CAC GAT CTC ACG GAG TTC TCC CGA TCT GGA AGC GGG ACC CCA ACC AAG 1027
 His Asp Leu Thr Glu Phe Ser Arg Ser Gly Ser Gly Thr Pro Thr Lys
 210 215 220 225

5 AGC AGA AGT GTC TCT GGC GTG CTG AAC GGA GGC AAA TCC ATG AGC CAC 1075
 Ser Arg Ser Val Ser Gly Val Leu Asn Gly Gly Lys Ser Met Ser His
 230 235 240

10 AAT GAA TCA ACG TAGCCAGTGA GGGCAAAGA AGGGCTCTGT AACAGAACCT 1127
 Asn Glu Ser Thr
 245

TACCTCCAGG TGCTGTTGAA TTC 1150

15 (2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 245 amino acids
 20 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala Ala Ala Ile Ala Ser Ser Leu Ile Arg Gln Lys Arg Gln Ala
 1 5 10 15

30 Arg Glu Arg Glu Lys Ser Asn Ala Cys Lys Cys Val Ser Ser Pro Ser
 20 25 30

35 Lys Gly Lys Thr Ser Cys Asp Lys Asn Lys Leu Asn Val Phe Ser Arg
 35 40 45

Val Lys Leu Phe Gly Ser Lys Lys Arg Arg Arg Arg Arg Pro Glu Pro
 50 55 60

40 Gln Leu Lys Gly Ile Val Thr Lys Leu Tyr Ser Arg Gln Gly Tyr His
 65 70 75 80

45 Leu Gln Leu Gln Ala Asp Gly Thr Ile Asp Gly Thr Lys Asp Glu Asp
 85 90 95

Ser Thr Tyr Thr Leu Phe Asn Leu Ile Pro Val Gly Leu Arg Val Val
 100 105 110

50 Ala Ile Gln Gly Val Gln Thr Lys Leu Tyr Leu Ala Met Asn Ser Glu
 115 120 125

Gly Tyr Leu Tyr Thr Ser Glu Leu Phe Thr Pro Glu Cys Lys Phe Lys
 130 135 140

55 Glu Ser Val Phe Glu Asn Tyr Tyr Val Thr Tyr Ser Ser Met Ile Tyr
 145 150 155 160

60 Arg Gln Gln Gln Ser Gly Arg Gly Trp Tyr Leu Gly Leu Asn Lys Glu
 165 170 175

Gly Glu Ile Met Lys Gly Asn His Val Lys Lys Asn Lys Pro Ala Ala
 180 185 190

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His Phe Leu Pro Lys Pro Leu Lys Val Ala Met Tyr Lys Glu Pro Ser
 195 200 205
 5 Leu His Asp Leu Thr Glu Phe Ser Arg Ser Gly Ser Gly Thr Pro Thr
 210 215 220
 Lys Ser Arg Ser Val Ser Gly Val Leu Asn Gly Gly Lys Ser Met Ser
 225 230 235 240
 10 His Asn Glu Ser Thr
 245

- (2) INFORMATION FOR SEQ ID NO:3:
- 15 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- 20 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- 25 (ix) FEATURE:
- (A) NAME/KEY: CDS
- (B) LOCATION: 1..30
- 30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GACGAGATAT TAGAATTCTA CTCGNNNNNN

30

- (2) INFORMATION FOR SEQ ID NO:4:
- 35 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- 40 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- 45 (ix) FEATURE:
- (A) NAME/KEY: CDS
- (B) LOCATION: 1..33
- 50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CCCCCCCCCG ACGAGATATT AGAATTCTAC TCG

33

- (2) INFORMATION FOR SEQ ID NO:5:
- 55 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 9 base pairs
- (B) TYPE: nucleic acid
- 60 (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)

-30-

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..9

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TGGCCATGG

9

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. Substantially pure fibroblast growth factor homologous factor-2 (FHF-2) characterized by:

- 5 (a) having a molecular weight of about 30kD as determined by reducing SDS-PAGE; and
(b) having essentially the amino acid sequence of SEQ ID NO:2.

10 2. An isolated polynucleotide sequence encoding the FHF-2 polypeptide of claim 1.

3. An isolated polynucleotide encoding the FHF-2 polypeptide or a fragment thereof, wherein the nucleotide sequence is selected from the group consisting of:

- 15 (a) SEQ ID NO:1, wherein T can also be U;
(b) nucleic acid sequence complementary to SEQ ID NO:1;
(c) fragments of (a) or (b) that are at least 15 bases in length and that will selectively hybridize to DNA which encodes the FHF-2 protein of SEQ ID NO:2, under stringent conditions.

20 4. A polynucleotide sequence according to claim 2 or claim 3, wherein the polynucleotide is isolated from a mammalian cell.

25 5. A polynucleotide according to claim 4, wherein the mammalian cell is a human cell.

6. An expression vector including a polynucleotide according to claim 2 or claim 3.

30 7. A vector according to claim 6, wherein the vector is a plasmid.

8. A vector according to claim 6, wherein the vector is a virus.

9. A host cell stably transformed with a vector according to claim 6.

35

10. A host cell according to claim 9, wherein the cell is prokaryotic.



11. A host cell according to claim 9, wherein the cell is eukaryotic.
12. An antibody that binds to FHF-2 polypeptide or immunoreactive
5 fragments thereof.
13. An antibody according to claim 12, wherein the antibody is polyclonal.
14. An antibody according to claim 12, wherein the antibody is monoclonal.
10
15. A method of detecting a cell proliferative disorder including contacting a specimen of a subject suspected of having a FHF-2 associated cell proliferative disorder with a reagent that binds to FHF-2 and detecting binding of the reagent to FHF-2.
15
16. A method according to claim 15, wherein the cell is selected from the group consisting of brain, heart and eye cell.
17. A method according to claim 15 or claim 16, wherein the reagent is an antibody which binds to FHF-2.
20
18. A method according to claim 15 or claim 16, wherein the reagent is a polynucleotide which encodes FHF-2 polypeptide, or fragments thereof.
19. A method according to any one of claims 15 to 18, wherein the detecting is *in vivo*.
25
20. A method according to any one of claims 15 to 18, wherein the detection is *in vitro*.
30
21. A method according to claim 17 or 18, wherein the reagent is detectably labeled.
22. A method of claim 21, wherein the detectable label is selected from the group consisting of a radioisotope, a fluorescent compound, a bioluminescent compound and a chemiluminescent compound.
35



23. A method of treating a cell proliferative disorder associated with expression of FHF-2, including contacting a cell having or suspected of having the disorder with a reagent which suppresses the FHF-2 activity.

5

24. A method according to claim 23, wherein the reagent is an anti-FHF-2 antibody.

10

25. A method according to claim 23, wherein the reagent is a FHF-2 antisense sequence.

26. A method according to any one of claims 23 to 25, wherein the cell is a heart, brain or eye cell.

15

27. A method according to any one of claims 23 to 26, wherein the reagent which suppresses FHF-2 activity is introduced to a cell using a vector.

28. A method of claim 27, wherein the vector is a colloidal dispersion system.

20

29. A method of claim 28, wherein the colloidal dispersion system is a liposome.

30. A method of claim 29, wherein the liposome is essentially target specific.

25

31. A method of claim 30, wherein the liposome is anatomically targeted.

32. A method of claim 31, wherein the liposome is mechanistically targeted.

30

33. A method of claim 32, wherein the mechanistic targeting is passive.

34. A method of claim 32, wherein the mechanistic targeting is active.

35

35. A method of claim 34, wherein the liposome is actively targeted by coupling with a moiety selected from the group consisting of a sugar, a glycolipid, and a protein.



36. A method of claim 35, wherein the protein moiety is an antibody.

37. A method of claim 27, wherein the vector is a virus.

5 38. A method of claim 37, wherein the virus is an RNA virus.

39. A method of claim 38, wherein the RNA virus is a retrovirus.

10 40. A method of claim 39, wherein the retrovirus is essentially target specific.

41. A substantially pure fibroblast growth factor according to claim 1 substantially as herein described with reference to any one of the Examples or Figures.

15

Dated this eighth day of January 1999.

**THE JOHNS HOPKINS UNIVERSITY
SCHOOL OF MEDICINE**

Patent Attorneys for the Applicant:

F B RICE & CO



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GAATTCCGCT TGCACAGTGT CCGCCGGGCG CAGGGGCCGA CCGCAGCGAG TCGCGCAGTT 60
 CTGCCTCCGC CTGCCAGTCT CGCCCGCGAT CCCGGCCCCG GGCTGTGGCG TCGACTCCGA 120
 CCCAGGCAGC CAGCAGCCCC CGCGGGAGCC GGACCGCCGC CGGAGAGCTC GGACGGCATG 180
 CTGAGCCCCC TCCTTGGCTG AAGCCCGAGT GCGGAGAAGC CCGGGCAAAC GCAGGCTAAG 240
 GAGACCAAAG CGGCGAAGTC GCGAGACAGC GGACAAGCAG CGGAGGAGAA GGAGGAGGAG 300
 GCGAACCCAG AGAGGGGCAG CAAAAGAAGC GGTGGTGGTG GCGTCTGTGG CCATGGCGGC 360
 M A A
 GGCTATCGCC AGCTCGCTCA TCCGTCAGAA GAGGCAAGCC CGCGAGCGCG AGAAATCCAA 420
 A I A S S L I R Q K R Q A R E R E K S N
 CGCCTGCAAG TGTGTCAGCA GCCCCAGCAA AGGCAAGACC AGCTGCGACA AAAACAAGTT 480
 A C K C V S S P S K G K T S C D K N K L
 AAATGTCTTT TCCCGGGTCA AACTCTTCGG CTCCAAGAAG AGGCGCAGAA GAAGACCAGA 540
 N V F S R V K L F G S K K R R R R R P E
 GCCTCAGCTT AAGGGTATAG TTACCAAGCT ATACAGCCGA CAAGGCTACC ACTTGCAGCT 600
 P Q L K G I V T K L Y S R Q G Y H L Q L
 GCAGGCGGAT GGAACCATTG ATGGCACCAA AGATGAGGAC AGCACTTACA CTCTGTTTAA 660
 Q A D G T I D G T K D E D S T Y T L F N
 CCTCATCCCT GTGGGTCTGC GAGTGGTGGC TATCCAAGGA GTTCAAACCA AGCTGTACTT 720
 L I P V G L R V V A I Q G V Q T K L Y L
 GGCAATGAAC AGTGAGGGAT ACTTGTAACAC CTCGGAAGTT TTCACACCTG AGTGCAAATT 780
 A M N S E G Y L Y T S E L F T P E C K F
 CAAAGAATCA GTGTTTGAAA ATTATTATGT GACATATTCA TCAATGATAT ACCGTCAGCA 840
 K E S V F E N Y Y V T Y S S M I Y R Q Q
 GCAGTCAGGC CGAGGGTGGT ATCTGGGTCT GAACAAAGAA GGAGAGATCA TGAAAGGCAA 900
 Q S G R G W Y L G L N K E G E I M K G N
 CCATGTGAAG AAGAACAAGC CTGCAGCTCA TTTTCTGCCT AAACCACTGA AAGTGGCCAT 960
 H V K K N K P A A H F L P K P L K V A M
 GTACAAGGAG CCATCACTGC ACGATCTCAC GGAGTTCTCC CGATCTGGAA GCGGGACCCC 1020
 Y K E P S L H D L T E F S R S G S G T P
 AACCAAGAGC AGAAGTGTCT CTGGCGTGCT GAACGGAGGC AAATCCATGA GCCACAATGA 1080
 T K S R S V S G V L N G G K S M S H N E
 ATCAACGTAG CCAAGTGGGG CAAAAGAAGG GCTCTGTAAC AGAACCTTAC CTCCAGGTGC 1140
 S T

TGTTGAATTC

FIG. 1

SUBSTITUTE SHEET (RULE 26)

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FGF-8	MSPRSALS-	--CLLLH---	-----LLVLC	LQAQVTVQSS	PNFTQHVVREQ	39
FGF-3	MGLIWLLLL-	--SLLEPSW-	-----PT-	-TGPGTRLR-	-----RDA-	29
FGF-5	MSLSFLLLLF	FSHLILSAWA	HGEKRLAPKG	QPGPAATDRN	PIGSSSRQSS	50
FGF-4	MSGPGTAAV-	--ALLPAVLL	ALLAPWAGRG	GAAAPTAPNG	TLEAELERRW	47
FGF-6	MSRGAGRLQ-	--GTLWA--L	VFLGILVGMV	VPSPAGTRAN	NTLLD-SRGW	44
FHF-2	MAAAIASSL-	--IRQKRQAR	EREKSNACKC	VSSPSKGKTS	CDKNK-LNVF	46
FGF-9	MAPLGEVGN-	--YFGVQDAV	PFGNV-PVLP	VDSPVLLSDH	LGQSE-AGGL	45
FGF-1	MAEGEITTF-	-----	-----	---TALTE--	-----KFNL	18
FGF-2	MAAGSITTL-	-----	-----	---PALPE--	---DGGSGAF	21
FGF-7	MHKWILTWI-	----LPT--L	LYRSCFHIIC	LVGTISLACN	DMTPEQMATN	43

FGF-8	S-----	-----LV	TDQLSR----	-RLIRTY--Q	LYSRTS-GKH	64
FGF-3	-----	-----GGR	GGVYEHLG--	-GAPRRR--K	LYC--ATKYH	55
FGF-5	SSAMSSSSAS	SSPAASLGSO	GSGLEQSSFO	WSPSGRRTGS	LYCRVGIGFH	100
FGF-4	ESLVALSLAR	LPVAAQPKEA	AVQSGAGDYL	LGIKRLR--R	LYCNVGIGFH	95
FGF-6	GTL--LSRSR	AGLAGE--IA	GVNWESG-YL	VGIKRQR--R	LYCNVGIGFH	87
FHF-2	SRVKLFGSKK	-----RRRRR	PEPQLK----	-GIVT-K---	LYSRQ--GYH	80
FGF-9	PR---GPA-	-----VT	DLDHLK----	-GILR-RRQ-	LYCRT--GFH	73
FGF-1	-----	-----P	PGNYKK----	--PKL-----	LYCSNG-GHF	37
FGF-2	-----	-----P	PGHFKD----	--PKR-----	LYCKNG-GFF	40
FGF-7	VNC--SSPER	-----HTR	SYDYMEG---	-GDIRVR--R	LYCRT--QWY	76

FGF-8	VQVLANKRIN	AMAEDGDPFA	KLIVETDTFG	SVRVVGAET	GLYICMKNKG	114
FGF-3	LQLHPSGRVN	GSLENS-AYS	ILEITAVEVG	V-VAIKGLFS	GRYLAMNKEG	103
FGF-5	LQIYPDGKVN	GSHEAN-MLS	VLEIFAVSQG	I-VGIRGVFS	NKFLAMSKKG	148
FGF-4	LQALPDGRIG	GAHADT-RDS	LLELSPVERG	V-VSIFGVAS	RFVAMSSKG	143
FGF-6	LQVLPDGRIS	GTHEEN-PYS	LLEISTVERG	V-VSLFGVRS	ALFVAMNSKG	135
FHF-2	LQLQADGTID	GTKDEDSTYT	LBNLIPVGLR	V-VAIQGVOT	KLYLAMNSEG	129
FGF-9	LEIFPNGTIQ	GTRKDHSRFG	ILEFISIAVG	L-VSIRGVDS	GLYLGMNEKG	122
FGF-1	LRILPDGTVD	GTRDRSDQHI	QLQLSAESVG	E-VYIKSTET	GOYLAMDTDG	86
FGF-2	LRIHPDGRVD	GVREKSDPHI	KLQLQAEERG	V-VSIKGVCA	NRYLAMKEDG	89
FGF-7	LRIDKRGKVK	GTOEMKNNYN	IMEIRTVAVG	I-VAIKGVES	EFYLAMNKEG	125

FGF-8	KLIAKSNGKG	KLOVEIEIVL	ENNYTALQNA	KYE-----	-----G	148
FGF-3	RLYASDHYN	-ECFEEVERIH	ELGYNTYASR	LYRTGSSGPG	AQRQPGAQRP	152
FGF-5	KLIHASAKFTD	-DCKFERFERFQ	ENSNTYASA	IHRTEKTG--	-----RE	187
FGF-4	KLYGSPFFTD	-ECIFKEILL	PNNYNAYESY	KYPM-----	-----	177
FGF-6	RLYATPSFQE	-ECKFERETLL	PNNYNAYESD	LYQGT-----	-----	169
FHF-2	YLYTSELFTEP	-ECKFERESVF	ENYNTYSSM	IYRQQQSG--	-----RG	168
FGF-9	ELYGSEKLTQ	-ECMFREQFE	ENWNTYSSN	LYKHVDTG--	-----RR	161
FGF-1	ILYGSQTPNE	-ECLELERLE	ENHYNTYISK	KHA-----	-----EKN	121
FGF-2	RLASKCVTD	-ECFFERLE	SNNYNTYRSR	KYT-----	-----S	122
FGF-7	KLIYAKKECNE	-DCKELIL	ENHYNTYASA	KWTHNG----	-----GE	162

FIG. 2
SUBSTITUTE SHEET (RULE 26)

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FGF-8	WYMAFTRKGR	PRKGSKTR--	-----QHQ	RE--VHFMKR	LPRGHHTTEQ	187
FGF-3	WYVSVNGKGR	PRRGEKTRRT	QKSSLFLPRV	LGHKDHMVR	LLQSSQPRAP	202
FGF-5	WYVALNKRKGR	AKRGCSPR--	VK-----PQH	IS--THFLPR	FKQSEQPELS	228
FGF-4	-FIALSKNGK	TKKGNRVS--	-----PTM	KV--THFLPR	L-----	206
FGF-6	-YIALSKYGR	VKRGSKVS--	-----PIM	TV--THFLPR	I-----	198
FHF-2	WYLGLNKEGE	IMKGNHVKKK	KP-----A	----AHFLPK	PLKVAMYKEP	207
FGF-9	YYVALNKDGT	PREGTRTKRH	QK-----F	----THFLPR	PVDPD--KVP	198
FGF-1	WEVGLKKNGS	CKRGPRTH--	-----YGQ	KA--ILFLPL	PVSSD-----	155
FGF-2	WYVALKRTGO	YKLGSKTG--	-----PGQ	KA--ILFLPM	SAKS-----	155
FGF-7	MEVALNQKGI	PVRGKKTG--	-----KEQ	KT--AHFLPM	AIT-----	194
FGF-8	---SLR--FE	FLNYPPFTRS	LRGSQRTWAP	-----EPR		215
FGF-3	GEGSQPRQRR	QKKQSPGDHG	KMETLSTRAT	PSTQLHTGGL	AVA	245
FGF-5	FTVTVP---E	KKNPPSPIKS	KIPLSAPRKN	TNSVKYRLKF	RFG	268
FGF-4	-----	-----	-----	-----	---	206
FGF-6	-----	-----	-----	-----	---	198
FHF-2	---SLHDLTE	FSRSGSGTPT	KSRVSGVLN	GGKSMSHNE-	STT	246
FGF-9	---EL-----	Y-----	-----KDIL-	-----SQS		208
FGF-1	-----	-----	-----	-----	---	155
FGF-2	-----	-----	-----	-----	---	155
FGF-7	-----	-----	-----	-----	---	194

FIG. 2

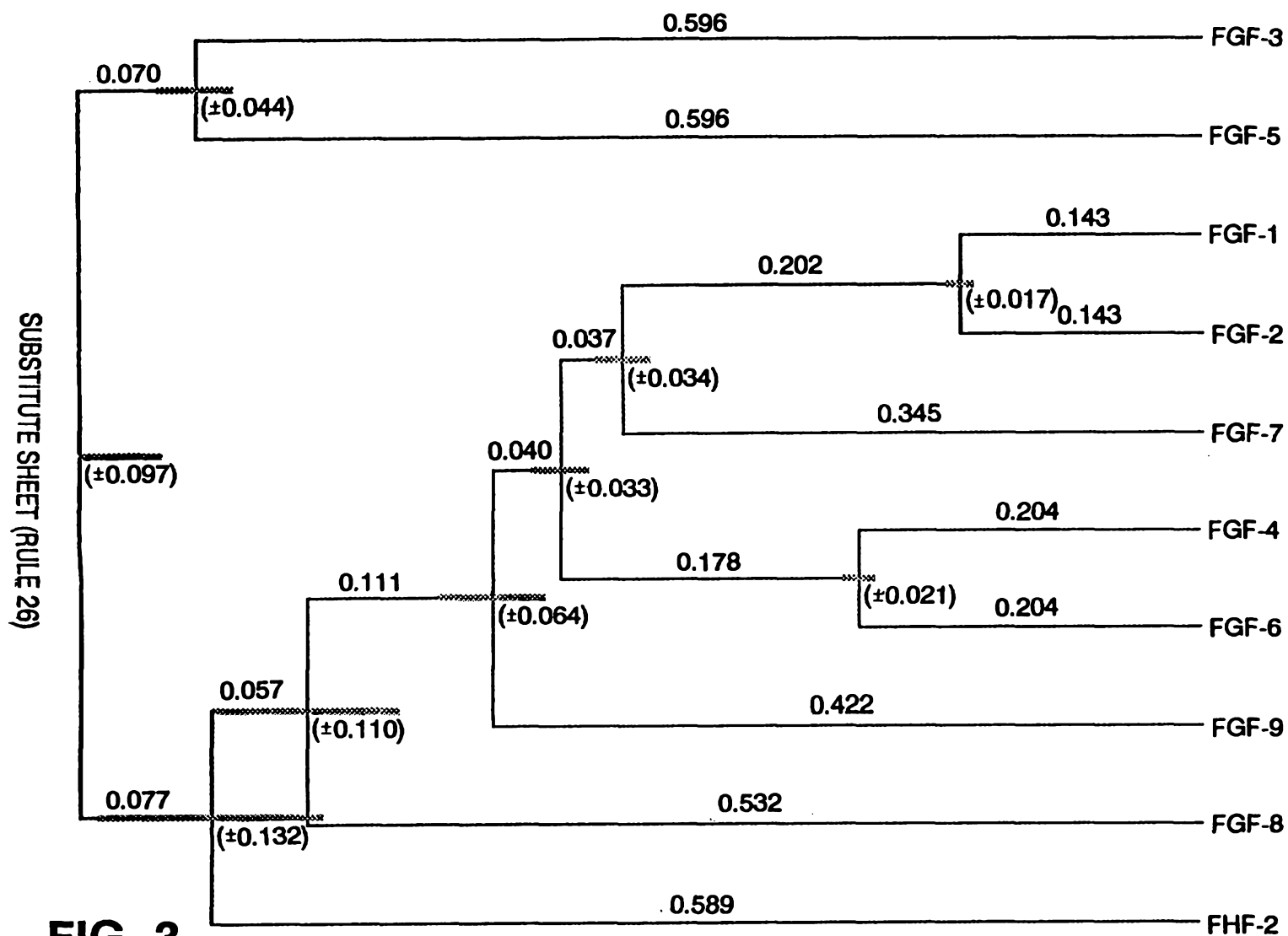


FIG. 3

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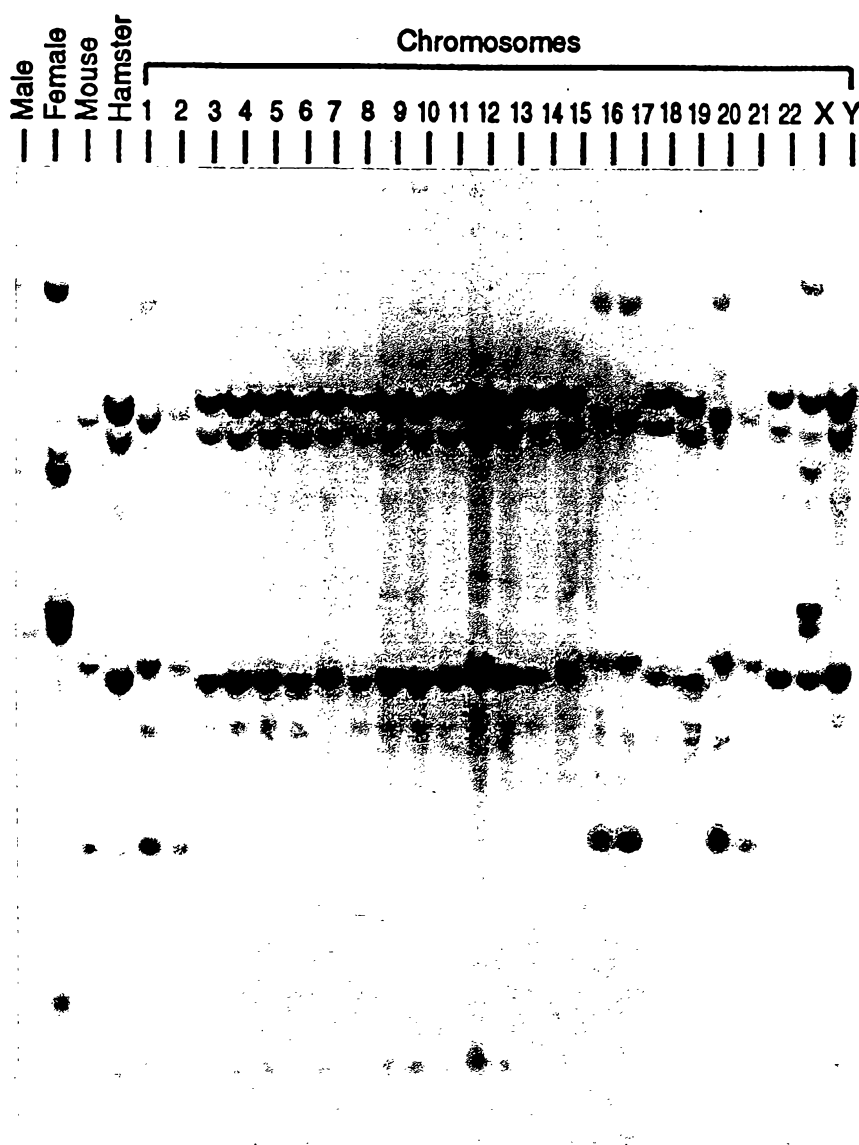
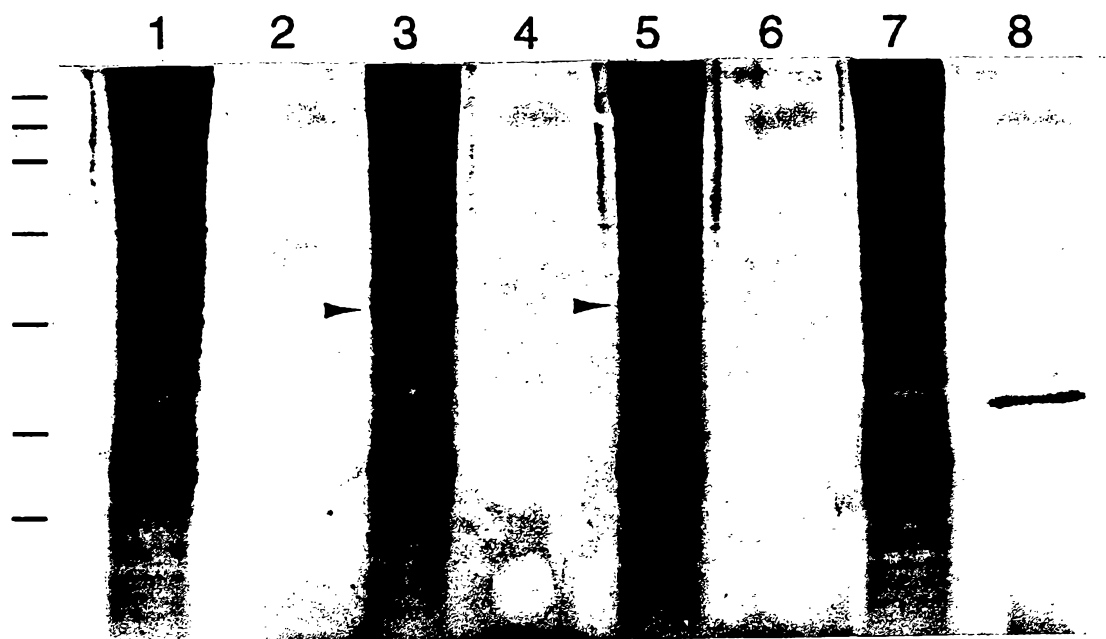
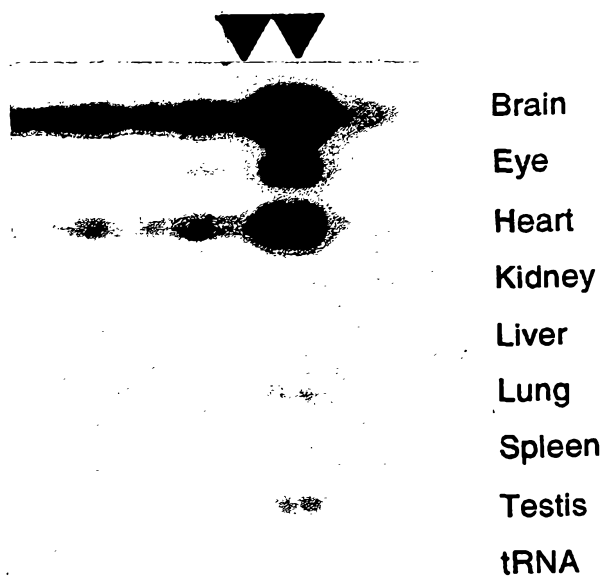


FIG. 4

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**FIG. 5****FIG. 6**