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(54) Title: GROWTH DIFFERENTIATION FACTOR-11

(57) Abstract

Growth differentiation factor-11 (GDF-11) is disclosed along with its polynucleotide sequence and amino acid sequence. Also disclosed are diagnostic and therapeutic methods of using the GDF-11 polypeptide and polynucleotide sequences.

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# GROWTH DIFFERENTIATION FACTOR-11

## BACKGROUND OF THE INVENTION

### 1. *Field of the Invention*

The invention relates generally to growth factors and specifically to a new member of the transforming growth factor beta (TGF-β) superfamily, which is denoted, growth differentiation factor-11 (GDF-11).

### 2. *Description of Related Art*

The transforming growth factor β (TGF-β) superfamily encompasses a group of structurally-related proteins which affect a wide range of differentiation processes during embryonic development. The family includes, Mullerian inhibiting substance (MIS), which is required for normal male sex development (Behringer, *et al.*, *Nature*, 345:167, 1990), *Drosophila* decapentaplegic (DPP) gene product, which is required for dorsal-ventral axis formation and morphogenesis of the imaginal disks (Padgett, *et al., Nature*, 325:81-84, 1987), the *Xenopus* Vg-1 gene product, which localizes to the vegetal pole of eggs ((Weeks, *et al., Cell*, 51:861-867, 1987), the activins (Mason, *et al., Biochem, Biophys. Res. Commun.*, 135:957-964, 1986), which can induce the formation of mesoderm and anterior structures in *Xenopus* embryos (Thomsen, *et al., Cell*, 63:485, 1990), and the bone morphogenetic proteins (BMPs, osteogenin, OP-1) which can induce *de novo* cartilage and bone formation (Sampath, *et al., J. Biol. Chem.*, 265:13198, 1990). The TGF-βs can influence a variety of differentiation processes, including adipogenesis, myogenesis, chondrogenesis, hematopoiesis, and epithelial cell differentiation (for review, see Massague, *Cell* 49:437, 1987).

The proteins of the TGF-β family are initially synthesized as a large precursor protein which subsequently undergoes proteolytic cleavage at a cluster of basic residues

approximately 110-140 amino acids from the C-terminus. The C-terminal regions, or mature regions, of the proteins are all structurally related and the different family members can be classified into distinct subgroups based on the extent of their homology. Although the homologies within particular subgroups range from 70% to 90% amino acid sequence identity, the homologies between subgroups are significantly lower, generally ranging from only 20% to 50%. In each case, the active species appears to be a disulfide-linked dimer of C-terminal fragments. Studies have shown that when the pro-region of a member of the TGF-β family is coexpressed with a mature region of another member of the TGF-β family, intracellular dimerization and secretion of biologically active homodimers occur (Gray, A., and Maston, A., *Science*, 247:1328, 1990). Additional studies by Hammonds, *et al.*, (*Molec. Endocrin.* 5:149, 1991) showed that the use of the BMP-2 pro-region combined with the BMP-4 mature region led to dramatically improved expression of mature BMP-4. For most of the family members that have been studied, the homodimeric species has been found to be biologically active, but for other family members, like the inhibins (Ling, *et al., Nature*, 321:779, 1986) and the TGF-βs (Cheifetz, *et al., Cell*, 48:409, 1987), heterodimers have also been detected, and these appear to have different biological properties than the respective homodimers.

Identification of new factors that are tissue-specific in their expression pattern will provide a greater understanding of that tissue's development and function.

## SUMMARY OF THE INVENTION

The present invention provides a cell growth and differentiation factor, GDF-11, a polynucleotide sequence which encodes the factor, and antibodies which are bind to the factor. This factor appears to relate to various cell proliferative disorders, especially those involving muscle, neural, and uterine cells, as well as disorders related to the function of the immune system.

Thus, in one embodiment, the invention provides a method for detecting a cell proliferative disorder of muscle, neural, uterine, spleen, or thymus origin and which is associated with GDF-11. In another embodiment, the invention provides a method for treating a cell proliferative or immunologic disorder by suppressing or enhancing GDF-11 activity.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows the nucleotide and predicted amino acid sequences of murine (FIGURE 1a) and human (FIGURE 1b) GDF-11. The putative proteolytic processing sites are shown by the shaded boxes. In the human sequence, the potential N-linked glycosylation signal is shown by the open box, and the consensus polyadenylation signal is underlined; the poly A tail is not shown.

FIGURE 2 shows Northern blots of RNA prepared from adult (FIGURE 2a) or fetal and neonatal (FIGURE 2b) tissues probed with a murine GDF-11 probe.

FIGURE 3 shows amino acid homologies among different members of the TGF-β superfamily. Numbers represent percent amino acid identities between each pair calculated from the first conserved cysteine to the C-terminus. Boxes represent homologies among highly-related members within particular subgroups.

FIGURE 4 shows an alignment of the predicted amino acid sequences of human GDF-11 (top lines) with human GDF-8 (bottom lines). Vertical lines indicate identities. Dots represent gaps introduced in order to maximize the alignment. Numbers represent amino acid positions relative to the N-terminus. The putative proteolytic processing sites are shown by the open box. The conserved cysteine residues on the C-terminal region are shown by the shaded boxes.

FIGURE 5 shows the expression of GDF-11 in mammalian cells. Conditioned medium prepared from Chinese hamster ovary cells transfected with a hybrid GDF-8/GDF-11 gene (see text) cloned into the MSXND expression vector in either the antisense (lane 1) or sense (lane 2) orientation was dialyzed, lyophilized, and subjected to Western analysis using antibodies directed against the C-terminal portion of GDF-8 protein. Arrows at right indicate the putative unprocessed (pro-GDF-8/GDF-11) or processed GDF-11 proteins. Numbers at left indicate mobilities of molecular weight standards.

FIGURE 6 shows the chromosomal mapping of human GDF-11. DNA samples prepared from human/rodent somatic cell lines were subjected to PCR, electrophoresed on agarose gels, blotted, and probed. The human chromosome contained in each of the hybrid cell lines is identified at the top of each of the first 24 lanes (1-22, X, and Y). In the lanes designated CHO, M, and H, the starting DNA template was total genomic DNA from hamster, mouse, and human sources, respectively. In the lane marked B1, no template DNA was used. Numbers at left indicate the mobilities of DNA standards.

FIGURE 7 shows the FISH localization of GDF-11. Metaphase chromosomes derived from peripheral blood lymphocytes were hybridized with digoxigenin-labelled human GDF-11 probe (a) or a mixture of human GDF-11 genomic and chromosome 12-specific centromere probes (b) and analyzed as described in the text. A schematic showing the location of GDF-11 at position 12q13 is shown in panel (c).

FIGURE 8 shows the nucleotide and deduced amino acid sequence of murine GDF-8.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a growth and differentiation factor, GDF-11, and a polynucleotide sequence encoding GDF-11. GDF-11 is expressed at highest levels in muscle, brain, uterus, spleen, and thymus and at lower levels in other tissues. In one embodiment, the invention provides a method for detection of a cell proliferative or immunologic disorder of muscle, neural, uterine, spleen, or thymus origin which is associated with GDF-11 expression or function. In another embodiment, the invention provides a method for treating a cell proliferative or immunologic disorder by using an agent which suppresses or enhances GDF-11 activity.

The TGF-β superfamily consists of multifunctional polypeptides that control proliferation, differentiation, and other functions in many cell types. Many of the peptides have regulatory, both positive and negative, effects on other peptide growth factors. The structural homology between the GDF-11 protein of this invention and the members of the TGF-β family, indicates that GDF-11 is a new member of the family of growth and differentiation factors. Based on the known activities of many of the other members, it can be expected that GDF-11 will also possess biological activities that will make it useful as a diagnostic and therapeutic reagent.

Certain members of this superfamily have expression patterns or possess activities that relate to the function of the nervous system. For example, one family member, 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 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 family member, namely GDF-1, is nervous system-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 system. The expression of GDF-11 in brain and muscle suggests that GDF-11 may also possess activities that relate to the function of the nervous system. In particular, it is known, for example, that skeletal muscle produces a factor or factors that promote the survival of motor neurons (Brown, *Trends Neurosci.,* 7:10, 1984). The known neurotrophic activities of other members of this family and the expression of GDF-11 in muscle suggest that one activity of GDF-11 may be as a trophic factor for motor neurons; indeed, GDF-11 is highly related to GDF-8, which is virtually muscle-specific in its expression pattern. Alternatively, GDF-11 may have neurotrophic activities for other neuronal populations. Hence, GDF-11 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.

GDF-11 may also have applications in treating disease processes involving muscle, such as in musculodegenerative diseases or in tissue repair due to trauma. In this regard, many other members of the TGF-β family are also important mediators of tissue repair. TGF-β has been shown to have marked effects on the formation of collagen and to cause a striking angiogenic response in the newborn mouse (Roberts, et al., Proc. Natl. Acad. Sci., USA 83:4167, 1986). TGF-β has 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 GDF-11 could be exploited for maintaining cells prior to transplantation or for enhancing the efficiency of the fusion process.

GDF-11 may also have applications in the treatment of immunologic disorders. In particular, TGF-β has been shown to have a wide range of immunoregulatory activities,

including potent suppressive effects on B and T cell proliferation and function (for review, see Palladino, *et al., Ann. N.Y. Acad. Sci.,* 593:181, 1990). The expression of GDF-11 in spleen and thymus suggests that GDF-11 may possess similar activities and therefore, may be used as an anti-inflammatory agent or as a treatment for disorders related to abnormal proliferation or function of lymphocytes.

The term "substantially pure" as used herein refers to GDF-11 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 GDF-11 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 GDF-11 polypeptide can also be determined by amino-terminal amino acid sequence analysis. GDF-11 polypeptide includes functional fragments of the polypeptide, as long as the activity of GDF-11 remains. Smaller peptides containing the biological activity of GDF-11 are included in the invention.

The invention provides polynucleotides encoding the GDF-11 protein. These polynucleotides include DNA, cDNA and RNA sequences which encode GDF-11. It is understood that all polynucleotides encoding all or a portion of GDF-11 are also included herein, as long as they encode a polypeptide with GDF-11 activity. Such polynucleotides include naturally occurring, synthetic, and intentionally manipulated polynucleotides. For example, GDF-11 polynucleotide may be subjected to site-directed mutagenesis. The polynucleotide sequence for GDF-11 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 GDF-11 polypeptide encoded by the nucleotide sequence is functionally unchanged.

Specifically disclosed herein is a DNA sequence containing the human GDF-11 gene. The sequence contains an open reading frame encoding a polypeptide 407 amino acids in length. The sequence contains a putative RXXR proteolytic cleavage site at amino acids 295-298. Cleavage of the precursor at this site would generate an active C-terminal fragment 109 amino acids in length with a predicted molecular weight of approximately 12,500 kD. Also disclosed herein is a partial murine genomic sequence. Preferably, the human GDF-11 nucleotide sequence is SEQ ID NO:1 and the mouse nucleotide sequence is SEQ ID NO:3.

The polynucleotide encoding GDF-11 includes SEQ ID NO:1 and 3, as well as nucleic acid sequences complementary to SEQ ID NO's:1 and 3. 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 and 3 are 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 or 4 under physiological conditions.

The C-terminal region of GDF-11 following the putative proteolytic processing site shows significant homology to the known members of the TGF-β superfamily. The GDF-11 sequence contains most of the residues that are highly conserved in other family members (see FIGURE 1). Like the TGF-βs and inhibin βs, GDF-11 contains an extra pair of cysteine residues in addition to the 7 cysteines found in virtually all other family members. Among the known family members, GDF-11 is most homologous to GDF-8 (92% sequence identity) (see FIGURE 3).

Minor modifications of the recombinant GDF-11 primary amino acid sequence may result in proteins which have substantially equivalent activity as compared to the GDF-11 polypeptide described herein. 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 GDF-11 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 GDF-11 biological activity.

The nucleotide sequence encoding the GDF-11 polypeptide of the invention includes the disclosed sequence 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 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.

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 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.

Preferably the GDF-11 polynucleotide of the invention is derived from a mammalian organism, and most preferably from a mouse, rat, or 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 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-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 GDF-11 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).

A cDNA expression library, such as lambda gt11, can be screened indirectly for GDF-11 peptides having at least one epitope, using antibodies specific for GDF-11. Such antibodies can be either polyclonally or monoclonally derived and used to detect expression product indicative of the presence of GDF-11 cDNA.

DNA sequences encoding GDF-11 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.

In the present invention, the GDF-11 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 GDF-11 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).

Polynucleotide sequences encoding GDF-11 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. Preferably, the mature C-terminal region of GDF-11 is expressed from a DNA clone containing the entire coding sequence of GDF-11. Alternatively, the C-terminal portion of GDF-11 can be expressed as a fusion protein with the pro- region of another member of the TGF-β family or co-expressed with another pro- region (see for example, Hammonds, *et al., Molec. Endocrin.* 5:149, 1991; Gray, A., and Mason, A., *Science*, 247:1328, 1990).

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 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 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 GDF-11 of the invention, and a second foreign DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase 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).

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.

The GDF-11 polypeptides of the invention can also be used to produce antibodies which are immunoreactive or bind to epitopes of the GDF-11 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 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 epitopic

determinant. These antibody fragments retain some ability to selectively bind with its antigen or receptor and are defined as follows:

(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;

(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;

(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;

(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

(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).

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.

Antibodies which bind to the GDF-11 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 or chemical synthesis which can be conjugated to a carrier protein, if desired. Such commonly 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).

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, 1991, incorporated by reference).

It is also possible to use the anti-idiotype 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.

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 GDF-11 polynucleotide that is an antisense molecule is useful in treating malignancies of the various organ systems, particularly, for example, cells in muscle, uterus, spleen, thymus, or neural tissue. Essentially, any disorder which is etiologically linked to altered expression of GDF-11 could be considered susceptible to treatment with a GDF-11 suppressing reagent. One such disorder is a malignant cell proliferative disorder, for example.

The invention provides a method for detecting a cell proliferative disorder of muscle, uterine or neural tissue, for example, which comprises contacting an anti-GDF-11 antibody with a cell suspected of having a GDF-11 associated disorder and detecting binding to the antibody. The antibody reactive with GDF-11 is labeled with a compound which allows detection of binding to GDF-11. For purposes of the invention, an antibody specific for GDF-11 polypeptide may be used to detect the level of GDF-11 in biological fluids and tissues. Any specimen containing a detectable amount of antigen can be used. A preferred sample of this invention is muscle, uterus, spleen, thymus, or neural tissue. The level of GDF-11 in the suspect cell can be compared with the level in a normal cell to determine whether the subject has a GDF-11-associated cell proliferative disorder. Preferably the subject is human.

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.

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 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.

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 diethylenetriaminepentacetic 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.

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 $^{56}Fe$.

The monoclonal antibodies of the invention can be used *in vitro* and *in vivo* to monitor the course of amelioration of a GDF-11-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 GDF-11-associated disease is effective. The term "ameliorate" denotes a lessening of the detrimental effect of the GDF-11-associated disease in the subject receiving therapy.

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. Thus, where a cell-proliferative disorder is associated with the expression of GDF-11, nucleic acid sequences that interfere with GDF-11 expression at the translational level can be used. This approach utilizes, for example, antisense nucleic acid and ribozymes to block translation of a specific GDF-11 mRNA, either by masking that mRNA with an antisense nucleic acid or by cleaving it with a ribozyme. Such disorders include neurodegenerative diseases, for example.

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 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

GDF-11-producing cell. The use of antisense methods to inhibit the *in vitro* translation of genes is well known in the art (Marcus-Sakura, *Anal.Biochem.*, 172:289, 1988).

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

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 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.

The present invention also provides gene therapy for the treatment of cell proliferative or immunologic disorders which are mediated by GDF-11 protein. Such therapy would achieve its therapeutic effect by introduction of the GDF-11 antisense polynucleotide into cells having the proliferative disorder. Delivery of antisense GDF-11 polynucleotide can be achieved using a recombinant expression vector such as a 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.

Preferably, the retroviral vector is a derivative of a murine or avian retrovirus. Examples of retroviral vectors in which a single foreign gene can be inserted include, but are not limited to: Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV). A number of additional retroviral vectors can incorporate multiple genes. All of these vectors can transfer or incorporate a gene for a selectable marker so that transduced cells can be identified and generated. By inserting a GDF-11 sequence of interest into the viral vector, along with another gene which encodes the ligand for a receptor on a specific target cell, 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 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 GDF-11 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 Ψ2, 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 GDF-11 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 $\mu$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 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, cerebrosides, 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, dipalmitoylphosphatidylcholine 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 association with the liposomal bilayer. Various linking groups can be used for joining the lipid chains to the targeting ligand.

Due to the expression of GDF-11 in muscle, spleen, uterus, thymus, and 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, GDF-11 may be useful in various gene therapy procedures.

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.

## EXAMPLE 1

## IDENTIFICATION AND ISOLATION OF A NOVEL TGF-β FAMILY MEMBER

To identify novel members of the TGF-β superfamily, a murine genomic library was screened at reduced stringency using a murine GDF-8 probe (FIGURE 8; nucleotides 865-1234) spanning the region encoding the C-terminal portion of the GDF-8 precursor protein. Hybridization was carried out as described (Lee, *Mol. Endocrinol.*, 4:1034, 1990) at 65°C, and the final wash was carried out at the same temperature in a buffer containing 0.5 M NaCl. Among the hybridizing phage was one that could be distinguished from GDF-8-containing phage on the basis of its reduced hybridization intensity to the GDF-8 probe. Partial nucleotide sequence analysis of the genomic insert present in this weakly hybridizing phage showed that this clone contained a sequence highly related to but distinct from murine GDF-8.

A partial nucleotide sequence of the genomic insert present in this phage is shown in FIGURE 1a. The sequence contained an open reading frame extending from nucleotides 198 to 575 that showed significant homology to the known members of the TGF-β superfamily (see below). Preceding this sequence was a 3' splice consensus sequence at precisely the same position as in the GDF-8 gene. This new TGF-β family member was given the designation GDF-11 (growth/differentiation factor-11).

## EXAMPLE 2

## EXPRESSION OF GDF-11

To determine the expression pattern of GDF-11, RNA samples prepared from a variety of tissues were screened by Northern analysis. RNA isolation and Northern analysis were carried out as described previously (Lee, *Mol. Endocrinol.*, 4:1034, 1990) except that the hybridization was carried out in 5X SSPE, 10% dextran sulfate, 50% formamide, 1% SDS, 200$\mu$g/ml salmon DNA, and 0.1% each of bovine serum albumin, ficoll, and polyvinylpyrrolidone. Five micrograms of twice poly A-selected RNA prepared from each tissue (except for 2 day neonatal brain, for which only 3.3 $\mu$g RNA were used) were electrophoresed on formaldehyde gels, blotted, and probed with GDF-11. As shown in FIGURE 2, the GDF-11 probe detected two RNA species, approximately 4.2 and 3.2 kb in length, in adult thymus, brain, spleen, uterus, and muscle as well as in whole embryos isolated at day 12.5 or 18.5 and in brain samples taken at various stages of development. On longer exposures of these blots, lower levels of GDF-11 RNA could also be detected in a number of other tissues.

## EXAMPLE 3

## ISOLATION OF cDNA CLONES ENCODING GDF-11

In order to isolate cDNA clones encoding GDF-11, a cDNA library was prepared in the lambda ZAP II vector (Stratagene) using RNA prepared from human adult spleen. From 5 $\mu$g of twice poly A-selected RNA prepared from human spleen, a cDNA library consisting of 21 million recombinant phage was constructed according to the instructions provided by Stratagene. The library was screened without amplification. Library screening and characterization of cDNA inserts were carried out as described previously (Lee, *Mol. Endocrinol.*, 4:1034, 1990). From this library, 23 hybridizing phage were obtained.

The entire nucleotide sequence of the clone extending furthest toward the 5' end of the gene was determined. The 1258 base pair sequence contained a single long open reading frame beginning from the 5' end of the clone and extending to a TAA stop codon. Because the open reading frame and the homology with GDF-8 (see below) extended to the very 5' end of the clone, it seemed likely that this clone was missing the coding sequence corresponding to the N-terminal portion of the GDF-11 precursor protein. In order to obtain the remaining portion of the GDF-11 sequence, several genomic clones were isolated by screening a human genomic library with the human GDF-11 cDNA probe. Partial sequence analysis of one of these genomic clones showed that this clone contained the GDF-11 gene. From this clone, the remaining GDF-11 coding sequence was obtained. FIGURE 1b shows the predicted sequence of GDF-11 assembled from the genomic and cDNA sequences. Nucleotides 136 to 1393 represent the extent of the sequence obtained from a cDNA clone. Nucleotides 1 to 135 were obtained from a genomic clone. The sequence has been arbitrarily numbered beginning with a *Sac* II site present in the genomic clone, but the location of the mRNA start site is not known. The sequence contains a putative initiating methionine at nucleotide 54. Whether the sequence upstream of this methionine codon is all present in the mRNA is not known. Beginning with this methionine codon, the open reading frame extends for 407 amino acids. The sequence contains one potential N-linked glycosylation site at asparagine 94. The sequence contains a predicted RXXR proteolytic cleavage site at amino acids 295 to 298, and cleavage of the precursor at this site would generate an active C-terminal fragment 109 amino acids in length with a predicted molecular weight of approximately 12,500 kD. In this region, the predicted murine and human GDF-11 amino acid sequences are 100% identical. The high degree of sequence conservation across species suggests that GDF-11 plays an important role *in vivo*.

The C-terminal region following the predicted cleavage site contains all the hallmarks present in other TGF-β family members. GDF-11 contains most of the residues that are highly conserved in other family members, including the seven cysteine residues with their characteristic spacing. Like the TGF-β's, the inhibin β's, and GDF-8, GDF-11 also

contains two additional cysteine residues. In the case of TGF-β2, these additional cysteine residues are known to form an intramolecular disulfide bond (Daopin, *et al., Science,* 257:369, 1992; Schlunegger and Grutter, *Nature,* 358:430, 1992). A tabulation of the amino acid sequence homologies between GDF-11 and the other TGF-β family members is shown in FIGURE 3. Numbers represent percent amino acid identities between each pair calculated from the first conserved cysteine to the C-terminus. Boxes represent homologies among highly-related members within particular subgroups. In this region, GDF-11 is most highly related to GDF-8 (92% sequence identity).

An alignment of GDF-8 (SEQ ID NO:5) and GDF-11 (SEQ ID NO:6) amino acid sequences is shown in FIGURE 4. The two sequences contain potential N-linked glycosylation signals (NIS) and putative proteolytic processing sites (RSRR) at analogous positions. The two sequences are related not only in the C-terminal region following the putative cleavage site (90% amino acid sequence identity), but also in the pro-region of the molecules (45% amino acid sequence identity).

## EXAMPLE 4

## CONSTRUCTION OF A HYBRID GDF-8/GDF11 GENE

In order to express GDF-11 protein, a hybrid gene was constructed in which the N-terminal region of GDF-11 was replaced by the analogous region of GDF-8. Such hybrid constructs have been used to produce biologically-active BMP-4 (Hammonds, *et al., Mol. Endocrinol.,* 5:149, 1991) and Vg-1 (Thomsen and Melton, *Cell,* 74:433, 1993). In order to ensure that the GDF-11 protein produced from the hybrid construct would represent authentic GDF-11, the hybrid gene was constructed in such a manner that the fusion of the two gene fragments would occur precisely at the predicted cleavage sites. In particular, an *Ava*II restriction site is present in both sequences at the location corresponding to the predicted proteolytic cleavage site. The N-terminal pro-region of GDF-8 up to this *Ava*II site was obtained by partial digestion of the clone with *Ava*II and

fused to the C-terminal region of GDF-11 beginning at this *Ava*II site. The resulting hybrid construct was then inserted into the pMSXND mammalian expression vector (Lee and Nathans, *J. Biol. Chem.*, 263:3521) and transfected into Chinese hamster ovary cells. As shown in FIGURE 5, Western analysis of conditioned medium from G418-resistant cells using antibodies raised against the C-terminal portion of GDF-8 showed that these cells secreted GDF-11 protein into the medium and that at least some of the hybrid protein was proteolytically processed. Furthermore, these studies demonstrate that the antibodies directed against the C-terminal portion of GDF-8 will also react with GDF-11 protein.

## EXAMPLE 5

## CHROMOSOMAL LOCALIZATION OF GDF-11

In order to map the chromosomal location of GDF-11, DNA samples from human/rodent somatic cell hybrids (Drwinga, *et al., Genomics,* 16:311-313, 1993; Dubois and Naylor, *Genomics,* 16:315-319, 1993) were analyzed by polymerase chain reaction followed by Southern blotting. Polymerase chain reaction was carried out using primer #101, 5'-GAGTCCCGCTGCTGCCGATATCC-3', (SEQ ID NO:7) and primer #102, 5'-TAGAGCATGTTGATTGGGGACAT-3', (SEQ ID NO:8) for 35 cycles at 94°C for 2 minutes, 58°C for 1 minutes, and 72°C for 1 minute. These primers correspond to nucleotides 981 to 1003 and the reverse complement of nucleotides 1182 to 1204, respectively, in the human GDF-11 sequence. PCR products were electrophoresed on agarose gels, blotted, and probed with oligonucleotide #104, 5'-AAATATCCGCATACCCATTT-3', (SEQ ID NO:9) which corresponds to a sequence internal to the region flanked by primer #101 and #102. Filters were hybridized in 6 X SSC, 1 X Denhardt's solution, 100 $\mu$g/ml yeast transfer RNA, and 0.05% sodium pyrophosphate at 50°C.

As shown in FIGURE 6, the human-specific probe detected a band of the predicted size (approximately 224 base pairs) in the positive control sample (total human genomic DNA) and in a single DNA sample from the human/rodent hybrid panel. This positive signal corresponds to human chromosome 12. The human chromosome contained in each of the hybrid cell lines is identified at the top of each of the first 24 lanes (1-22, X, and Y). In the lanes designated CHO, M, and H, the starting DNA template was total genomic DNA from hamster, mouse, and human sources, respectively. In the lane marked B1, no template DNA was used. Numbers at left indicate the mobilities of DNA standards. These data show that the human GDF-11 gene is located on chromosome 12.

In order to determine the more precise location of GDF-11 on chromosome 12, the GDF-11 gene was localized by florescence *in situ* hybridization (FISH). These FISH localization studies were carried out by contract to BIOS laboratories (New Haven, Connecticut). Purified DNA from a human GDF-11 genomic clone was labelled with digoxigenin dUTP by nick translation. Labelled probe was combined with sheared human DNA and hybridized to normal metaphase chromosomes derived from PHA stimulated peripheral blood lymphocytes in a solution containing 50% formamide, 10% dextran sulfate and 2xSSC. Specific hybridization signals were detected by incubating the hybridized slides in fluorescein-conjugated sheep antidigoxigenin antibodies. Slides were then counterstained with propidium iodide and analyzed. As shown in FIGURE 7a, this experiment resulted in the specific labelling of the proximal long arm of a group C chromosome, the size and morphology of which were consistent with chromosome 12. In order to confirm the identity of the specifically labelled chromosome, a second experiment was conducted in which a chromosome 12- specific centromere probe was cohybridized with GDF-11. As shown in FIGURE 7b, this experiment clearly demonstrated that GDF-11 is located at a position which is 23% of the distance from the centromere to the telomere of the long arm of chromosome 12, an area which corresponds to band 12q13 (FIGURE 7c). A total of 85 metaphase cells were analyzed and 80 exhibited specific labelling.

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

**SEQUENCE LISTING**

(1) GENERAL INFORMATION:

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

(ii) TITLE OF INVENTION: GROWTH DIFFERENTIATION FACTOR-11

(iii) NUMBER OF SEQUENCES: 9

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Fish & Richardson P.C.
(B) STREET: 4225 Executive Square, Suite 1400
(C) CITY: La Jolla
(D) STATE: California
(E) COUNTRY: US
(F) ZIP: 92037

(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 #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/US95/
(B) FILING DATE: 07-JUL-1995
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: HAILE, PH.D., LISA A.
(B) REGISTRATION NUMBER: 38,347
(C) REFERENCE/DOCKET NUMBER: 07265/036WO1

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 619/678-5070
(B) TELEFAX: 619/678-5099

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1393 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: HUMAN GDF-11

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 54..1274

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

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CCGCGGGACT CCGGCGTCCC CGCCCCCCAG TCCTCCCTCC CCTCCCCTCC AGC ATG      56
                                                            Met
                                                             1

GTG CTC GCG GCC CCG CTG CTG CTG GGC TTC CTG CTC CTC GCC CTG GAG    104
Val Leu Ala Ala Pro Leu Leu Leu Gly Phe Leu Leu Leu Ala Leu Glu
              5                  10                  15

CTG CGG CCC CGG GGG GAG GCG GCC GAG GGC CCC GCG GCG GCG GCG GCG    152
Leu Arg Pro Arg Gly Glu Ala Ala Glu Gly Pro Ala Ala Ala Ala Ala
         20                  25                  30

GCG GCG GCG GCG GCG GCA GCG GCG GGG GTC GGG GGG GAG CGC TCC AGC    200
Ala Ala Ala Ala Ala Ala Ala Ala Gly Val Gly Gly Glu Arg Ser Ser
     35                  40                  45

CGG CCA GCC CCG TCC GTG GCG CCC GAG CCG GAC GGC TGC CCC GTG TGC    248
Arg Pro Ala Pro Ser Val Ala Pro Glu Pro Asp Gly Cys Pro Val Cys
 50                  55                  60                  65

GTT TGG CGG CAG CAC AGC CGC GAG CTG CGC CTA GAG AGC ATC AAG TCG    296
Val Trp Arg Gln His Ser Arg Glu Leu Arg Leu Glu Ser Ile Lys Ser
                 70                  75                  80
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CAG ATC TTG AGC AAA CTG CGG CTC AAG GAG GCG CCC AAC ATC AGC CGC      344
Gln Ile Leu Ser Lys Leu Arg Leu Lys Glu Ala Pro Asn Ile Ser Arg
            85                  90                  95

GAG GTG GTG AAG CAG CTG CTG CCC AAG GCG CCG CCG CTG CAG CAG ATC      392
Glu Val Val Lys Gln Leu Leu Pro Lys Ala Pro Pro Leu Gln Gln Ile
        100                 105                 110

CTG GAC CTA CAC GAC TTC CAG GGC GAC GCG CTG CAG CCC GAG GAC TTC      440
Leu Asp Leu His Asp Phe Gln Gly Asp Ala Leu Gln Pro Glu Asp Phe
    115                 120                 125

CTG GAG GAG GAC GAG TAC CAC GCC ACC ACC GAG ACC GTC ATT AGC ATG      488
Leu Glu Glu Asp Glu Tyr His Ala Thr Thr Glu Thr Val Ile Ser Met
130                 135                 140                 145

GCC CAG GAG ACG GAC CCA GCA GTA CAG ACA GAT GGC AGC CCT CTC TGC      536
Ala Gln Glu Thr Asp Pro Ala Val Gln Thr Asp Gly Ser Pro Leu Cys
                150                 155                 160

TGC CAT TTT CAC TTC AGC CCC AAG GTG ATG TTC ACA AAG GTA CTG AAG      584
Cys His Phe His Phe Ser Pro Lys Val Met Phe Thr Lys Val Leu Lys
            165                 170                 175

GCC CAG CTG TGG GTG TAC CTA CGG CCT GTA CCC CGC CCA GCC ACA GTC      632
Ala Gln Leu Trp Val Tyr Leu Arg Pro Val Pro Arg Pro Ala Thr Val
        180                 185                 190

TAC CTG CAG ATC TTG CGA CTA AAA CCC CTA ACT GGG GAA GGG ACC GCA      680
Tyr Leu Gln Ile Leu Arg Leu Lys Pro Leu Thr Gly Glu Gly Thr Ala
    195                 200                 205

GGG GGA GGG GGC GGA GGC CGG CGT CAC ATC CGT ATC CGC TCA CTG AAG      728
Gly Gly Gly Gly Gly Gly Arg Arg His Ile Arg Ile Arg Ser Leu Lys
210                 215                 220                 225

ATT GAG CTG CAC TCA CGC TCA GGC CAT TGG CAG AGC ATC GAC TTC AAG      776
Ile Glu Leu His Ser Arg Ser Gly His Trp Gln Ser Ile Asp Phe Lys
                230                 235                 240

CAA GTG CTA CAC AGC TGG TTC CGC CAG CCA CAG AGC AAC TGG GGC ATC      824
Gln Val Leu His Ser Trp Phe Arg Gln Pro Gln Ser Asn Trp Gly Ile
            245                 250                 255
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GAG ATC AAC GCC TTT GAT CCC AGT GGC ACA GAC CTG GCT GTC ACC TCC      872
Glu Ile Asn Ala Phe Asp Pro Ser Gly Thr Asp Leu Ala Val Thr Ser
        260                 265                 270

CTG GGG CCG GGA GCC GAG GGG CTG CAT CCA TTC ATG GAG CTT CGA GTC      920
Leu Gly Pro Gly Ala Glu Gly Leu His Pro Phe Met Glu Leu Arg Val
    275                 280                 285

CTA GAG AAC ACA AAA CGT TCC CGG CGG AAC CTG GGT CTG GAC TGC GAC      968
Leu Glu Asn Thr Lys Arg Ser Arg Arg Asn Leu Gly Leu Asp Cys Asp
290                 295                 300                 305

GAG CAC TCA AGC GAG TCC CGC TGC TGC CGA TAT CCC CTC ACA GTG GAC      1016
Glu His Ser Ser Glu Ser Arg Cys Cys Arg Tyr Pro Leu Thr Val Asp
                310                 315                 320

TTT GAG GCT TTC GGC TGG GAC TGG ATC ATC GCA CCT AAG CGC TAC AAG      1064
Phe Glu Ala Phe Gly Trp Asp Trp Ile Ile Ala Pro Lys Arg Tyr Lys
            325                 330                 335

GCC AAC TAC TGC TCC GGC CAG TGC GAG TAC ATG TTC ATG CAA AAA TAT      1112
Ala Asn Tyr Cys Ser Gly Gln Cys Glu Tyr Met Phe Met Gln Lys Tyr
        340                 345                 350

CCG CAT ACC CAT TTG GTG CAG CAG GCC AAT CCA AGA GGC TCT GCT GGG      1160
Pro His Thr His Leu Val Gln Gln Ala Asn Pro Arg Gly Ser Ala Gly
    355                 360                 365

CCC TGT TGT ACC CCC ACC AAG ATG TCC CCA ATC AAC ATG CTC TAC TTC      1208
Pro Cys Cys Thr Pro Thr Lys Met Ser Pro Ile Asn Met Leu Tyr Phe
370                 375                 380                 385

AAT GAC AAG CAG CAG ATT ATC TAC GGC AAG ATC CCT GGC ATG GTG GTG      1256
Asn Asp Lys Gln Gln Ile Ile Tyr Gly Lys Ile Pro Gly Met Val Val
                390                 395                 400

GAT CGC TGT GGC TGC TCT TAAGTGGGTC ACTACAAGCT GCTGGAGCAA             1304
Asp Arg Cys Gly Cys Ser
            405

AGACTTGGTG GGTGGGTAAC TTAACCTCTT CACAGAGGAT AAAAAATGCT TGTGAGTATG    1364

ACAGAAGGGA ATAAACAGGC TTAAAGGGT                                      1393
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 407 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

```
Met Val Leu Ala Ala Pro Leu Leu Leu Gly Phe Leu Leu Leu Ala Leu
 1               5                  10                  15

Glu Leu Arg Pro Arg Gly Glu Ala Ala Glu Gly Pro Ala Ala Ala Ala
            20                  25                  30

Ala Ala Ala Ala Ala Ala Ala Ala Ala Gly Val Gly Gly Glu Arg Ser
        35                  40                  45

Ser Arg Pro Ala Pro Ser Val Ala Pro Glu Pro Asp Gly Cys Pro Val
    50                  55                  60

Cys Val Trp Arg Gln His Ser Arg Glu Leu Arg Leu Glu Ser Ile Lys
65                  70                  75                  80

Ser Gln Ile Leu Ser Lys Leu Arg Leu Lys Glu Ala Pro Asn Ile Ser
                85                  90                  95

Arg Glu Val Val Lys Gln Leu Leu Pro Lys Ala Pro Pro Leu Gln Gln
            100                 105                 110

Ile Leu Asp Leu His Asp Phe Gln Gly Asp Ala Leu Gln Pro Glu Asp
        115                 120                 125

Phe Leu Glu Glu Asp Glu Tyr His Ala Thr Thr Glu Thr Val Ile Ser
    130                 135                 140

Met Ala Gln Glu Thr Asp Pro Ala Val Gln Thr Asp Gly Ser Pro Leu
145                 150                 155                 160

Cys Cys His Phe His Phe Ser Pro Lys Val Met Phe Thr Lys Val Leu
                165                 170                 175

Lys Ala Gln Leu Trp Val Tyr Leu Arg Pro Val Pro Arg Pro Ala Thr
```

180 185 190

Val Tyr Leu Gln Ile Leu Arg Leu Lys Pro Leu Thr Gly Glu Gly Thr
195 200 205

Ala Gly Gly Gly Gly Gly Gly Arg Arg His Ile Arg Ile Arg Ser Leu
210 215 220

Lys Ile Glu Leu His Ser Arg Ser Gly His Trp Gln Ser Ile Asp Phe
225 230 235 240

Lys Gln Val Leu His Ser Trp Phe Arg Gln Pro Gln Ser Asn Trp Gly
245 250 255

Ile Glu Ile Asn Ala Phe Asp Pro Ser Gly Thr Asp Leu Ala Val Thr
260 265 270

Ser Leu Gly Pro Gly Ala Glu Gly Leu His Pro Phe Met Glu Leu Arg
275 280 285

Val Leu Glu Asn Thr Lys Arg Ser Arg Arg Asn Leu Gly Leu Asp Cys
290 295 300

Asp Glu His Ser Ser Glu Ser Arg Cys Cys Arg Tyr Pro Leu Thr Val
305 310 315 320

Asp Phe Glu Ala Phe Gly Trp Asp Trp Ile Ile Ala Pro Lys Arg Tyr
325 330 335

Lys Ala Asn Tyr Cys Ser Gly Gln Cys Glu Tyr Met Phe Met Gln Lys
340 345 350

Tyr Pro His Thr His Leu Val Gln Gln Ala Asn Pro Arg Gly Ser Ala
355 360 365

Gly Pro Cys Cys Thr Pro Thr Lys Met Ser Pro Ile Asn Met Leu Tyr
370 375 380

Phe Asn Asp Lys Gln Gln Ile Ile Tyr Gly Lys Ile Pro Gly Met Val
385 390 395 400

Val Asp Arg Cys Gly Cys Ser
405

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 630 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: MOUSE GDF-11

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 198..575

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
TCTAGATGTC AAGAGAAGTG GTCACAATGT CTGGGTGGGA GCCGTAAACA AGCCAAGAGG      60

TTATGGTTTC TGGTCTGATG CTCCTGTTGA GATCAGGAAA TGTTCAGGAA ATCCCCTGTT     120

GAGATGTAGG AAAGTAAGAG GTAAGAGACA TTGTTGAGGG TCATGTCACA TCTCTTTCCC     180

CTCTCCCTGA CCCTCAG CAT CCT TTC ATG GAG CTT CGA GTC CTA GAG AAC         230
                  His Pro Phe Met Glu Leu Arg Val Leu Glu Asn
                    1               5                  10

ACG AAA AGG TCC CGG CGG AAC CTA GGC CTG GAC TGC GAT GAA CAC TCG       278
Thr Lys Arg Ser Arg Arg Asn Leu Gly Leu Asp Cys Asp Glu His Ser
            15                  20                  25

AGT GAG TCC CGC TGC TGC CGA TAT CCT CTC ACA GTG GAC TTT GAG GCT       326
Ser Glu Ser Arg Cys Cys Arg Tyr Pro Leu Thr Val Asp Phe Glu Ala
        30                  35                  40

TTT GGC TGG GAC TGG ATC ATC GCA CCT AAG CGC TAC AAG GCC AAC TAC       374
Phe Gly Trp Asp Trp Ile Ile Ala Pro Lys Arg Tyr Lys Ala Asn Tyr
    45                  50                  55

TGC TCC GGC CAG TGC GAA TAC ATG TTC ATG CAA AAG TAT CCA CAC ACC       422
Cys Ser Gly Gln Cys Glu Tyr Met Phe Met Gln Lys Tyr Pro His Thr
```

```
60                  65                  70                  75

CAC TTG GTG CAA CAG GCC AAC CCA AGA GGC TCT GCT GGG CCC TGC TGC      470
His Leu Val Gln Gln Ala Asn Pro Arg Gly Ser Ala Gly Pro Cys Cys
                80                  85                  90

ACC CCT ACC AAG ATG TCC CCA ATC AAC ATG CTC TAC TTC AAT GAC AAG      518
Thr Pro Thr Lys Met Ser Pro Ile Asn Met Leu Tyr Phe Asn Asp Lys
            95                  100                 105

CAG CAG ATT ATC TAC GGC AAG ATC CCT GGC ATG GTG GTG GAT CGA TGT      566
Gln Gln Ile Ile Tyr Gly Lys Ile Pro Gly Met Val Val Asp Arg Cys
        110                 115                 120

GGC TGC TCC TAAGTTGTGG GCTACAGTGG ATGCCTCCCT CAGACCCTAC              615
Gly Cys Ser
    125

CCCAAGAACC CCAGC                                                    630
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 126 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
His Pro Phe Met Glu Leu Arg Val Leu Glu Asn Thr Lys Arg Ser Arg
 1               5                  10                  15

Arg Asn Leu Gly Leu Asp Cys Asp Glu His Ser Ser Glu Ser Arg Cys
            20                  25                  30

Cys Arg Tyr Pro Leu Thr Val Asp Phe Glu Ala Phe Gly Trp Asp Trp
        35                  40                  45

Ile Ile Ala Pro Lys Arg Tyr Lys Ala Asn Tyr Cys Ser Gly Gln Cys
    50                  55                  60
```

Glu Tyr Met Phe Met Gln Lys Tyr Pro His Thr His Leu Val Gln Gln
65 70 75 80

Ala Asn Pro Arg Gly Ser Ala Gly Pro Cys Cys Thr Pro Thr Lys Met
85 90 95

Ser Pro Ile Asn Met Leu Tyr Phe Asn Asp Lys Gln Gln Ile Ile Tyr
100 105 110

Gly Lys Ile Pro Gly Met Val Val Asp Arg Cys Gly Cys Ser
115 120 125

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 375 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: GDF-8

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..375

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

Met Gln Lys Leu Gln Leu Cys Val Tyr Ile Tyr Leu Phe Met Leu Ile
1 5 10 15

Val Ala Gly Pro Val Asp Leu Asn Glu Asn Ser Glu Gln Lys Glu Asn
20 25 30

Val Glu Lys Glu Gly Leu Cys Asn Ala Cys Thr Trp Arg Gln Asn Thr
35 40 45

Lys Ser Ser Arg Ile Glu Ala Ile Lys Ile Gln Ile Leu Ser Lys Leu
50 55 60

Arg Leu Glu Thr Ala Pro Asn Ile Ser Lys Asp Val Ile Arg Gln Leu
65 70 75 80

Leu Pro Lys Ala Pro Pro Leu Arg Glu Leu Ile Asp Gln Tyr Asp Val
85 90 95

Gln Arg Asp Asp Ser Ser Asp Gly Ser Leu Glu Asp Asp Asp Tyr His
100 105 110

Ala Thr Thr Glu Thr Ile Ile Thr Met Pro Thr Glu Ser Asp Phe Leu
115 120 125

Met Gln Val Asp Gly Lys Pro Lys Cys Cys Phe Phe Lys Phe Ser Ser
130 135 140

Lys Ile Gln Tyr Asn Lys Val Val Lys Ala Gln Leu Trp Ile Tyr Leu
145 150 155 160

Arg Pro Val Glu Thr Pro Thr Thr Val Phe Val Gln Ile Leu Arg Leu
165 170 175

Ile Lys Pro Met Lys Asp Gly Thr Arg Tyr Thr Gly Ile Arg Ser Leu
180 185 190

Lys Leu Asp Met Asn Pro Gly Thr Gly Ile Trp Gln Ser Ile Asp Val
195 200 205

Lys Thr Val Leu Gln Asn Trp Leu Lys Gln Pro Glu Ser Asn Leu Gly
210 215 220

Ile Glu Ile Lys Ala Leu Asp Glu Asn Gly His Asp Leu Ala Val Thr
225 230 235 240

Phe Pro Gly Pro Gly Glu Asp Gly Leu Asn Pro Phe Leu Glu Val Lys
245 250 255

Val Thr Asp Thr Pro Lys Arg Ser Arg Arg Asp Phe Gly Leu Asp Cys
260 265 270

Asp Glu His Ser Thr Glu Ser Arg Cys Cys Arg Tyr Pro Leu Thr Val
275 280 285

Asp Phe Glu Ala Phe Gly Trp Asp Trp Ile Ile Ala Pro Lys Arg Tyr
290 295 300

```
Lys Ala Asn Tyr Cys Ser Gly Glu Cys Glu Phe Val Phe Leu Gln Lys
305                 310                 315                 320

Tyr Pro His Thr His Leu Val His Gln Ala Asn Pro Arg Gly Ser Ala
                325                 330                 335

Gly Pro Cys Cys Thr Pro Thr Lys Met Ser Pro Ile Asn Met Leu Tyr
            340                 345                 350

Phe Asn Gly Lys Glu Gln Ile Ile Tyr Gly Lys Ile Pro Ala Met Val
        355                 360                 365

Val Asp Arg Cys Gly Cys Ser
    370                 375
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 407 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: GDF-11

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..407

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Val Leu Ala Ala Pro Leu Leu Leu Gly Phe Leu Leu Leu Ala Leu
1               5                   10                  15

Glu Leu Arg Pro Arg Gly Glu Ala Ala Glu Gly Pro Ala Ala Ala Ala
            20                  25                  30

Ala Ala Ala Ala Ala Ala Ala Ala Ala Gly Val Gly Gly Glu Arg Ser
        35                  40                  45
```

Ser Arg Pro Ala Pro Ser Val Ala Pro Glu Pro Asp Gly Cys Pro Val
50 55 60

Cys Val Trp Arg Gln His Ser Arg Glu Leu Arg Leu Glu Ser Ile Lys
65 70 75 80

Ser Gln Ile Leu Ser Lys Leu Arg Leu Lys Glu Ala Pro Asn Ile Ser
85 90 95

Arg Glu Val Val Lys Gln Leu Leu Pro Lys Ala Pro Pro Leu Gln Gln
100 105 110

Ile Leu Asp Leu His Asp Phe Gln Gly Asp Ala Leu Gln Pro Glu Asp
115 120 125

Phe Leu Glu Glu Asp Glu Tyr His Ala Thr Thr Glu Thr Val Ile Ser
130 135 140

Met Ala Gln Glu Thr Asp Pro Ala Val Gln Thr Asp Gly Ser Pro Leu
145 150 155 160

Cys Cys His Phe His Phe Ser Pro Lys Val Met Phe Thr Lys Val Leu
165 170 175

Lys Ala Gln Leu Trp Val Tyr Leu Arg Pro Val Pro Arg Pro Ala Thr
180 185 190

Val Tyr Leu Gln Ile Leu Arg Leu Lys Pro Leu Thr Gly Glu Gly Thr
195 200 205

Ala Gly Gly Gly Gly Gly Gly Arg Arg His Ile Arg Ile Arg Ser Leu
210 215 220

Lys Ile Glu Leu His Ser Arg Ser Gly His Trp Gln Ser Ile Asp Phe
225 230 235 240

Lys Gln Val Leu His Ser Trp Phe Arg Gln Pro Gln Ser Asn Trp Gly
245 250 255

Ile Glu Ile Asn Ala Phe Asp Pro Ser Gly Thr Asp Leu Ala Val Thr
260 265 270

Ser Leu Gly Pro Gly Ala Glu Gly Leu His Pro Phe Met Glu Leu Arg
275 280 285

Val Leu Glu Asn Thr Lys Arg Ser Arg Arg Asn Leu Gly Leu Asp Cys
290 295 300

Asp Glu His Ser Ser Glu Ser Arg Cys Cys Arg Tyr Pro Leu Thr Val
305 310 315 320

Asp Phe Glu Ala Phe Gly Trp Asp Trp Ile Ile Ala Pro Lys Arg Tyr
325 330 335

Lys Ala Asn Tyr Cys Ser Gly Gln Cys Glu Tyr Met Phe Met Gln Lys
340 345 350

Tyr Pro His Thr His Leu Val Gln Gln Ala Asn Pro Arg Gly Ser Ala
355 360 365

Gly Pro Cys Cys Thr Pro Thr Lys Met Ser Pro Ile Asn Met Leu Tyr
370 375 380

Phe Asn Asp Lys Gln Gln Ile Ile Tyr Gly Lys Ile Pro Gly Met Val
385 390 395 400

Val Asp Arg Cys Gly Cys Ser
405

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..23

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GAGTCCCGCT GCTGCCGATA TCC 23

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..23

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TAGAGCATGT TGATTGGGGA CAT 23

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AAATATCCGC ATACCCATTT 20

CLAIMS

1. Substantially pure growth differentiation factor-11 (GDF-11).

2. An isolated polynucleotide sequence encoding the GDF-11 polypeptide of claim 1.

3. The polynucleotide of claim 2, wherein the GDF-11 nucleotide sequence is selected from the group consisting of:

a. SEQ ID NO:1, wherein T can also be U;

b. SEQ ID NO:3, wherein T can also be U;

c. nucleic acid sequences complementary to SEQ ID NO:1;

d. nucleic acid sequences complementary to SEQ ID NO:3;

e. fragments of a. or c. that are at least 15 bases in length and that will selectively hybridize to DNA which encodes the GDF-11 protein of SEQ ID NO:2; and

f. fragments of b. or d. that are at least 15 bases in length and that will selectively hybridize to DNA which encodes the GDF-11 protein of SEQ ID NO:4.

4. The polynucleotide sequence of claim 2, wherein the polynucleotide is isolated from a mammalian cell.

5. The polynucleotide of claim 4, wherein the mammalian cell is selected from the group consisting of mouse, rat, and human cell.

6. An expression vector including the polynucleotide of claim 2.

7. The vector of claim 6, wherein the vector is a plasmid.

8. The vector of claim 6, wherein the vector is a virus.

9. A host cell stably transformed with the vector of claim 6.

10. The host cell of claim 9, wherein the cell is prokaryotic.

11. The host cell of claim 9, wherein the cell is eukaryotic.

12. Antibodies that bind to the polypeptide of claim 1 or fragments thereof.

13. The antibodies of claim 12, wherein the antibodies are polyclonal.

14. The antibodies of claim 12, wherein the antibodies are monoclonal.

15. A method of detecting a cell proliferative disorder comprising contacting the antibody of claim 12 with a specimen of a subject suspected of having a GDF-11 associated disorder and detecting binding of the antibody.

16. The method of claim 15, wherein the cell is a muscle cell.

17. The method of claim 15, wherein the detecting is *in vivo.*

18. The method of claim 17, wherein the antibody is detectably labeled.

19. The method of claim 18, wherein the detectable label is selected from the group consisting of a radioisotope, a fluorescent compound, a bioluminescent compound and a chemiluminescent compound.

20. The method of claim 15, wherein the detection is *in vitro.*

21. The method of claim 20, wherein the antibody is detectably labeled.

22. The method of claim 21, wherein the label is selected from the group consisting of a radioisotope, a fluorescent compound, a bioluminescent compound, a chemoluminescent compound and an enzyme.

23. A method of treating a cell proliferative disorder associated with expression of GDF-11, comprising contacting the cells with a reagent which suppresses the GDF-11 activity.

24. The method of claim 23, wherein the reagent is an anti-GDF-11 antibody.

25. The method of claim 23, wherein the reagent is a GDF-11 antisense sequence.

26. The method of claim 23, wherein the cell is a muscle cell.

27. The method of claim 23, wherein the reagent which suppresses GDF-11 activity is introduced to a cell using a vector.

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

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

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

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

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

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

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

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

37. The method of claim 36, wherein the vector is a virus.

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

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

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

41. The method of claim 40, wherein a moiety for target specificity is encoded by a polynucleotide inserted into the retroviral genome.

42. The method of claim 40, wherein a moiety for target specificity is selected from the group consisting of a sugar, a glycolipid, and a protein.

43. The method of claim 42, wherein the protein is an antibody.

```
  1  TCTAGATGTCAAGAGAAGTGGTCACAATGTCTGGGTGGGAGCCGTAAACAAGCCAAGAGG  60
 61  TTATGGTTTCTGGTCTGATGCTCCTGTTGAGATCAGGAAATGTTCAGGAAATCCCCTGTT 120
121  GAGATGTAGGAAAGTAAGAGGTAAGAGACATTGTTGAGGGTCATGTCACATCTCTTTCCC 180
181  CTCTCCCTGACCCTCAGCATCCTTTCATGGAGCTTCGAGTCCTAGAGAACACGAAAAGGT 240
                     H  P  F  M  E  L  R  V  L  E  N  T  K  R  S
241  CCCGGCGGAACCTAGGCCTGGACTGCGATGAACACTCGAGTGAGTCCCGCTGCTGCCGAT 300
       R  R  N  L  G  L  D  C  D  E  H  S  S  E  S  R  C  C  R  Y
301  ATCCTCTCACAGTGGACTTTGAGGCTTTTGGCTGGGACTGGATCATCGCACCTAAGCGCT 360
       P  L  T  V  D  F  E  A  F  G  W  D  W  I  I  A  P  K  R  Y
361  ACAAGGCCAACTACTGCTCCGGCCAGTGCGAATACATGTTCATGCAAAAGTATCCACACA 420
       K  A  N  Y  C  S  G  Q  C  E  Y  M  F  M  Q  K  Y  P  H  T
421  CCCACTTGGTGCAACAGGCCAACCCAAGAGGCTCTGCTGGGCCCTGCTGCACCCCTACCA 480
       H  L  V  Q  Q  A  N  P  R  G  S  A  G  P  C  C  T  P  T  K
481  AGATGTCCCCAATCAACATGCTCTACTTCAATGACAAGCAGCAGATTATCTACGGCAAGA 540
       M  S  P  I  N  M  L  Y  F  N  D  K  Q  Q  I  I  Y  G  K  I
541  TCCCTGGCATGGTGGTGGATCGATGTGGCTGCTCCTAAGTTGTGGGCTACAGTGGATGCC 600
       P  G  M  V  V  D  R  C  G  C  S  *
601  TCCCTCAGACCCTACCCCAAGAACCCCAGC  630
```

FIG. 1a

```
   1  CCGCGGGACTCCGGCGTCCCCGCCCCCCAGTCCTCCCTCCCCTCCCCTCCAGCATGGTGC   60
                                                          M  V  L
  61  TCGCGGCCCCGCTGCTGCTGGGCTTCCTGCTCCTCGCCCTGGAGCTGCGGCCCCGGGGGG  120
       A  A  P  L  L  L  G  F  L  L  L  A  L  E  L  R  P  R  G  E
 121  AGGCGGCCGAGGGCCCCGCGGCGGCGGCGGCGGCGGCGGCGGCGGCAGCGGCGGGGG  180
       A  A  E  G  P  A  A  A  A  A  A  A  A  A  A  A  A  A  G  V
 181  TCGGGGGGGAGCGCTCCAGCCGGCCAGCCCCGTCCGTGGCGCCCGAGCCGGACGGCTGCC  240
       G  G  E  R  S  S  R  P  A  P  S  V  A  P  E  P  D  G  C  P
 241  CCGTGTGCGTTTGGCGGCAGCACAGCCGCGAGCTGCGCCTAGAGAGCATCAAGTCGCAGA  300
       V  C  V  W  R  Q  H  S  R  E  L  R  L  E  S  I  K  S  Q  I
 301  TCTTGAGCAAACTGCGGCTCAAGGAGGCGCCCAACATCAGCCGCGAGGTGGTGAAGCAGC  360
       L  S  K  L  R  L  K  E  A  P  N  I  S  R  E  V  V  K  Q  L
 361  TGCTGCCCAAGGCGCCGCCGCTGCAGCAGATCCTGGACCTACACGACTTCCAGGGCGACG  420
       L  P  K  A  P  P  L  Q  Q  I  L  D  L  H  D  F  Q  G  D  A
 421  CGCTGCAGCCCGAGGACTTCCTGGAGGAGGACGAGTACCACGCCACCACCGAGACCGTCA  480
       L  Q  P  E  D  F  L  E  E  D  E  Y  H  A  T  T  E  T  V  I
 481  TTAGCATGGCCCAGGAGACGGACCCAGCAGTACAGACAGATGGCAGCCCTCTCTGCTGCC  540
       S  M  A  Q  E  T  D  P  A  V  Q  T  D  G  S  P  L  C  C  H
 541  ATTTTCACTTCAGCCCCAAGGTGATGTTCACAAAGGTACTGAAGGCCCAGCTGTGGGTGT  600
       F  H  F  S  P  K  V  M  F  T  K  V  L  K  A  Q  L  W  V  Y
 601  ACCTACGGCCTGTACCCCGCCCAGCCACAGTCTACCTGCAGATCTTGCGACTAAAACCCC  660
       L  R  P  V  P  R  P  A  T  V  Y  L  Q  I  L  R  L  K  P  L
 661  TAACTGGGGAAGGGACCGCAGGGGGAGGGGGCGGAGGCCGGCGTCACATCCGTATCCGCT  720
       T  G  E  G  T  A  G  G  G  G  G  R  R  H  I  R  I  R  S
 721  CACTGAAGATTGAGCTGCACTCACGCTCAGGCCATTGGCAGAGCATCGACTTCAAGCAAG  780
       L  K  I  E  L  H  S  R  S  G  H  W  Q  S  I  D  F  K  Q  V
 781  TGCTACACAGCTGGTTCCGCCAGCCACAGAGCAACTGGGGCATCGAGATCAACGCCTTTG  840
       L  H  S  W  F  R  Q  P  Q  S  N  W  G  I  E  I  N  A  F  D
 841  ATCCCAGTGGCACAGACCTGGCTGTCACCTCCCTGGGGCCGGGAGCCGAGGGGCTGCATC  900
       P  S  G  T  D  L  A  V  T  S  L  G  P  G  A  E  G  L  H  P
 901  CATTCATGGAGCTTCGAGTCCTAGAGAACACAAAACGTTCCCGGCGGAACCTGGGTCTGG  960
       F  M  E  L  R  V  L  E  N  T  K  R  S  R  R  N  L  G  L  D
 961  ACTGCGACGAGCACTCAAGCGAGTCCCGCTGCTGCCGATATCCCCTCACAGTGGACTTTG 1020
       C  D  E  H  S  S  E  S  R  C  C  R  Y  P  L  T  V  D  F  E
1021  AGGCTTTCGGCTGGGACTGGATCATCGCACCTAAGCGCTACAAGGCCAACTACTGCTCCG 1080
        A  F  G  W  D  W  I  I  A  P  K  R  Y  K  A  N  Y  C  S  G
1081  GCCAGTGCGAGTACATGTTCATGCAAAAATATCCGCATACCCATTTGGTGCAGCAGGCCA 1140
       Q  C  E  Y  M  F  M  Q  K  Y  P  H  T  H  L  V  Q  Q  A  N
1141  ATCCAAGAGGCTCTGCTGGGCCCTGTTGTACCCCCACCAAGATGTCCCCAATCAACATGC 1200
       P  R  G  S  A  G  P  C  C  T  P  T  K  M  S  P  I  N  M  L
1201  TCTACTTCAATGACAAGCAGCAGATTATCTACGGCAAGATCCCTGGCATGGTGGTGGATC 1260
       Y  F  N  D  K  Q  Q  I  I  Y  G  K  I  P  G  M  V  V  D  R
1261  GCTGTGGCTGCTCTTAAGTGGGTCACTACAAGCTGCTGGAGCAAAGACTTGGTGGGTGGG 1320
       C  G  C  S  *
1321  TAACTTAACCTCTTCACAGAGGATAAAAAATGCTTGTGAGTATGACAGAAGGGAATAAAC 1380
1381  AGGCTTAAAGGGT 1393
```

FIG. 1b

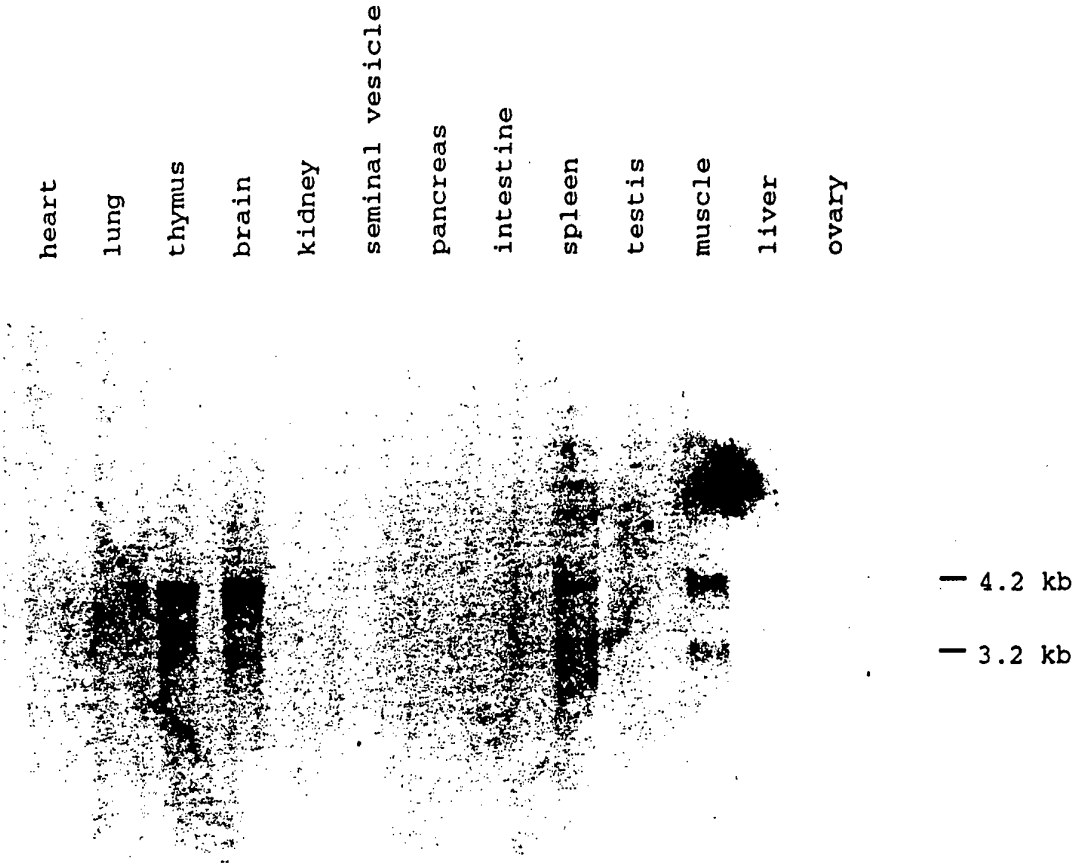

heart
lung
thymus
brain
kidney
seminal vesicle
pancreas
intestine
spleen
testis
muscle
liver
ovary
4.2 kb
3.2 kb

FIG. 2a

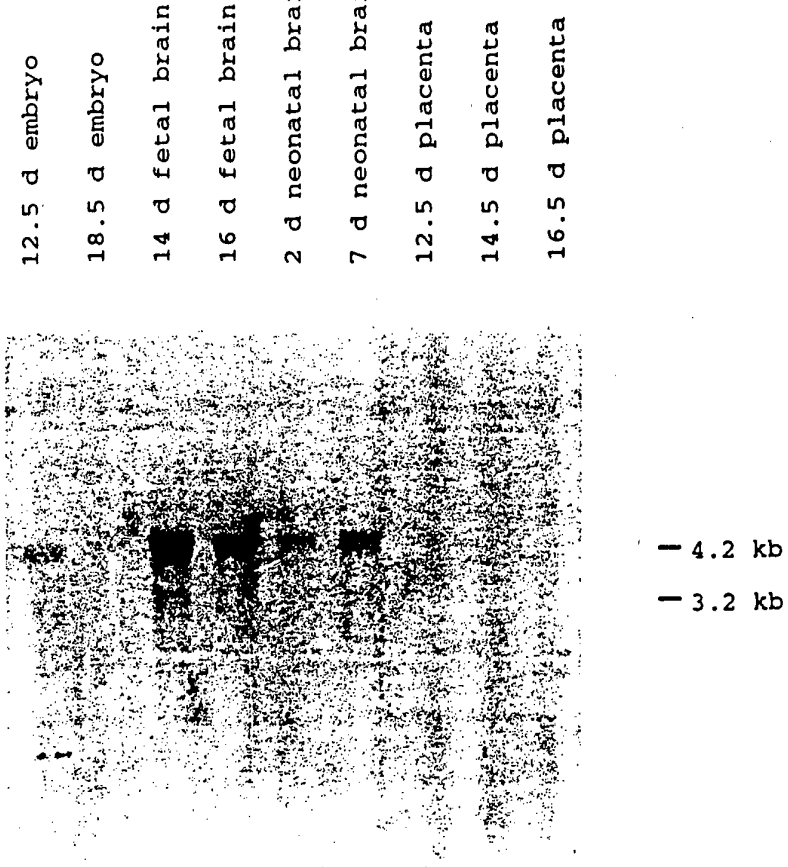

12.5 d embryo
18.5 d embryo
14 d fetal brain
16 d fetal brain
2 d neonatal brain
7 d neonatal brain
12.5 d placenta
14.5 d placenta
16.5 d placenta
4.2 kb
3.2 kb

FIG. 2b

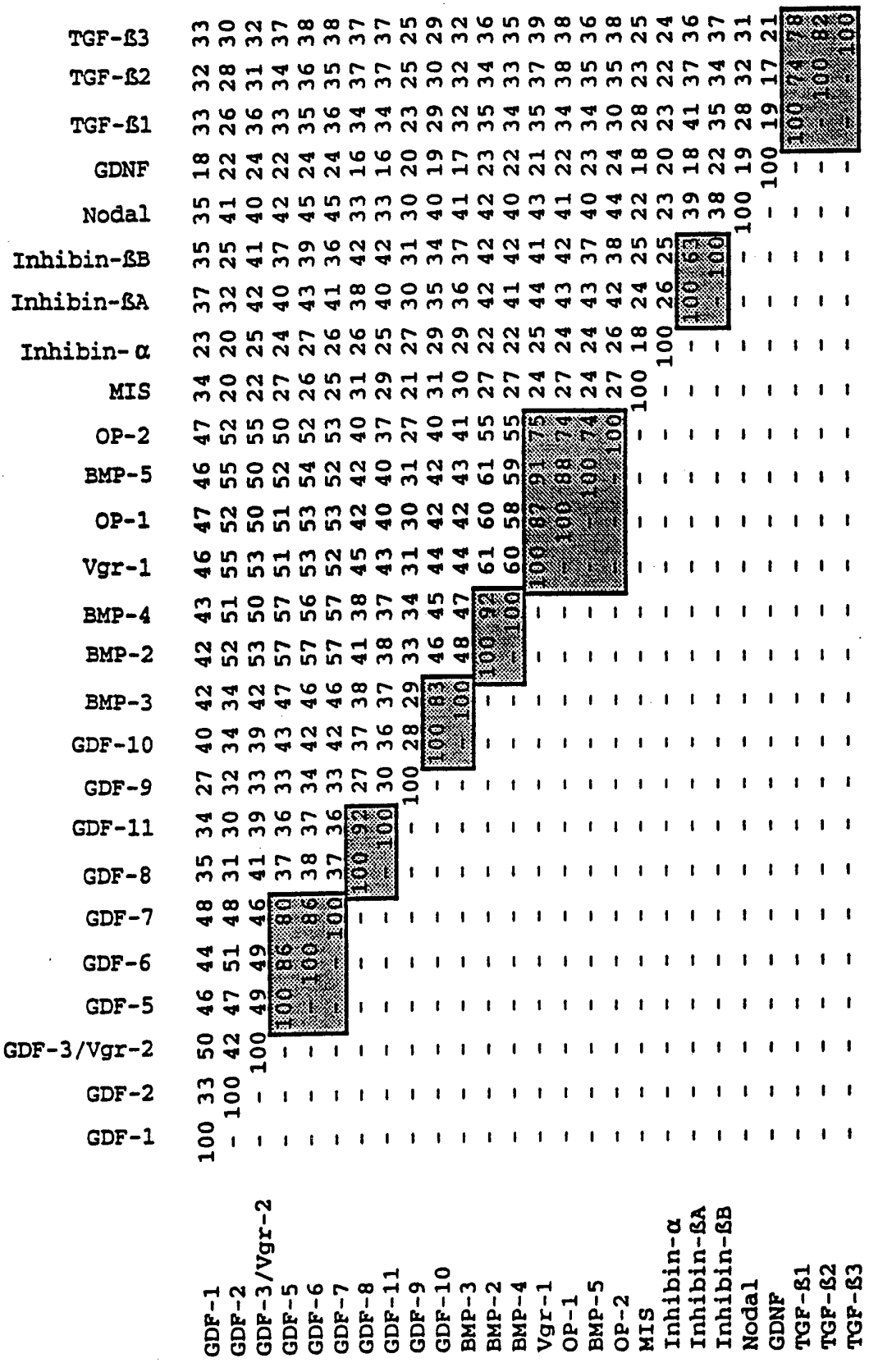

GDF-1
GDF-2
GDF-3/Vgr-2
GDF-5
GDF-6
GDF-7
GDF-8
GDF-11
GDF-9
GDF-10
BMP-3
BMP-2
BMP-4
Vgr-1
OP-1
BMP-5
OP-2
MIS
Inhibin-α
Inhibin-ßA
Inhibin-ßB
Nodal
GDNF
TGF-ß1
TGF-ß2
TGF-ß3

FIG. 3

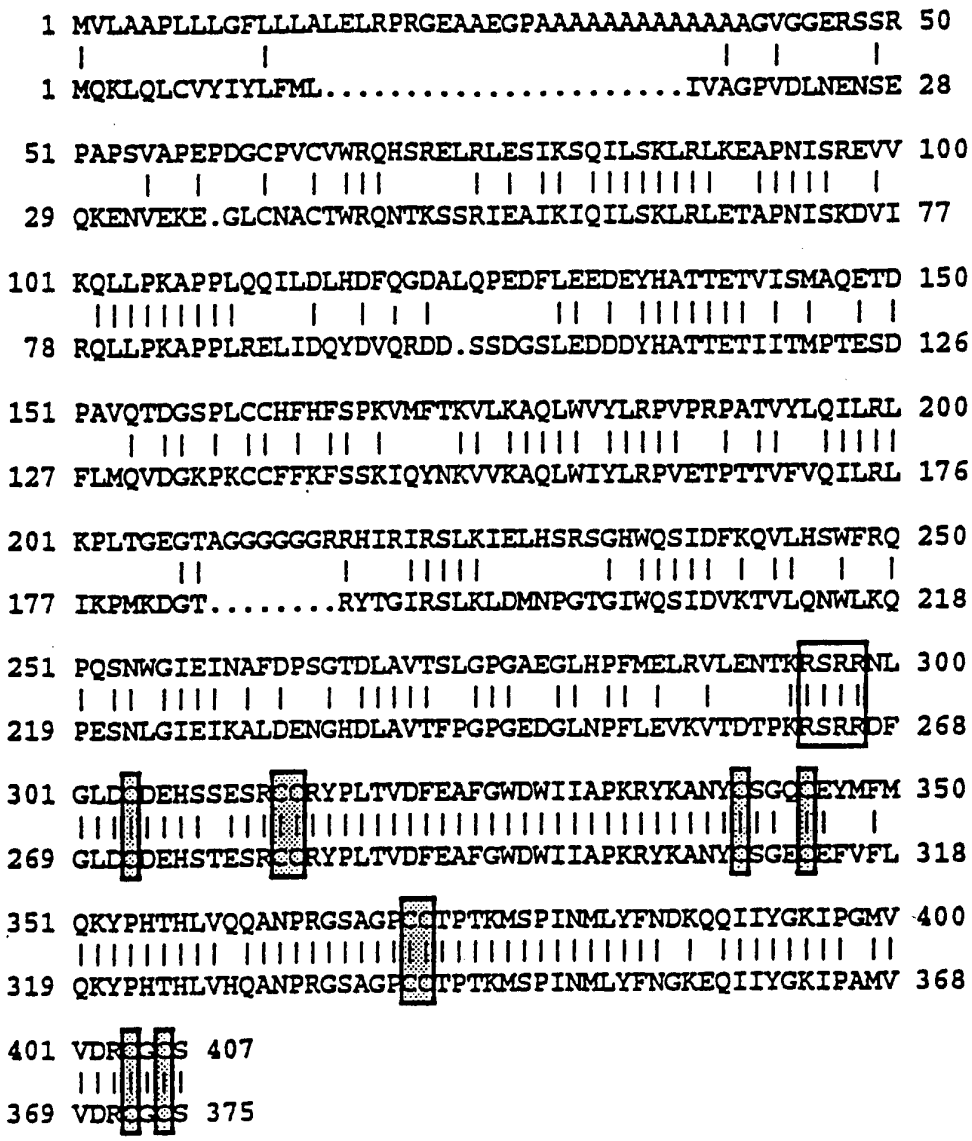

1 MVLAAPLLLGFLLLALELRPRGEAAEGPAAAAAAAAAAAAAAGVGGERSSR 50
1 MQKLQLCVYIYLFML.....IVAGPVDLNENSE 28
51 PAPSVAPEPDGCPVCVWRQHSRELRLESIKSQILSKLRLKEAPNISREVV 100
29 QKENVEKE.GLCNACTWRQNTKSSRIEAIKIQILSKLRLETAPNISKDVI 77
101 KQLLPKAPPLQQILDLHDFQGDALQPEDFLEEDEYHATTETVISMAQETD 150
78 RQLLPKAPPLRELIDQYDVQRDD.SSDGSLEDDDYHATTETIITMPTESD 126
151 PAVQTDGSPLCCHFHFSPKVMFTKVLKAQLWVYLRPVPRPATVYLQILRL 200
127 FLMQVDGKPKCCFFKFSSKIQYNKVVKAQLWIYLRPVETPTTVFVQILRL 176
201 KPLTGEGTAGGGGGRRHIRIRSLKIELHSRSGHWQSIDFKQVLHSWFRQ 250
177 IKPMKDGT.....RYTGIRSLKLDMNPGTGIWQSIDVKTVLQNWLKQ 218
251 PQSNWGIEINAFDPSGTDLAVTSLGPGAEGLHPFMELRVLENTKRSRRNL 300
219 PESNLGIEIKALDENGHDLAVTFPGPGEDGLNPFLEVKVTDTPKRSRRDF 268
301 GLDCDEHSSESRCCRYPLTVDFEAFGWDWIIAPKRYKANYCSGQCEYMFM 350
269 GLDCDEHSTESRCCRYPLTVDFEAFGWDWIIAPKRYKANYCSGECEFVFL 318
351 QKYPHTHLVQQANPRGSAGPCCTPTKMSPINMLYFNDKQQIIYGKIPGMV 400
319 QKYPHTHLVHQANPRGSAGPCCTPTKMSPINMLYFNGKEQIIYGKIPAMV 368
401 VDRCCCS 407
369 VDRCCCS 375

FIG. 4

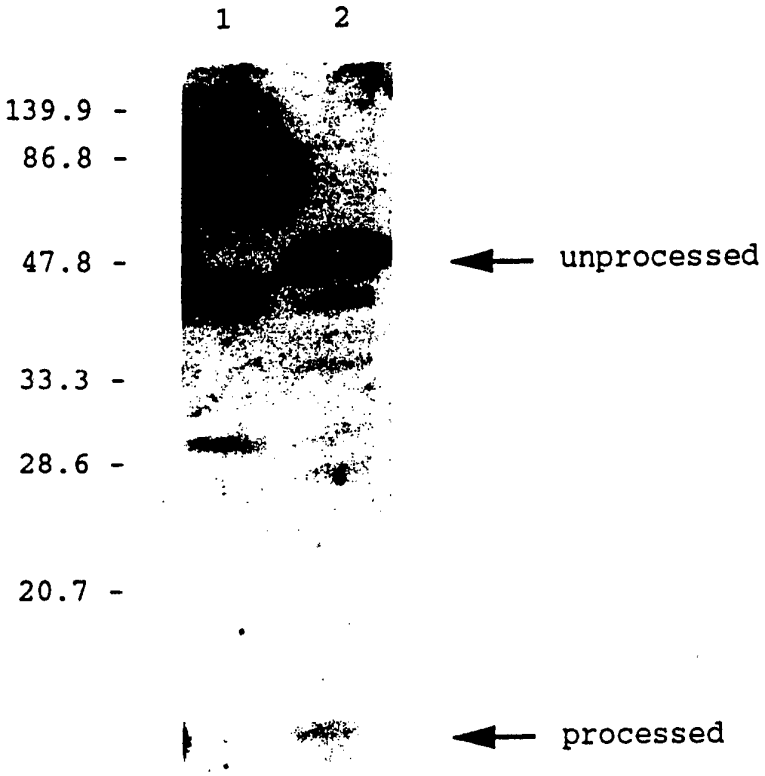



FIG. 5

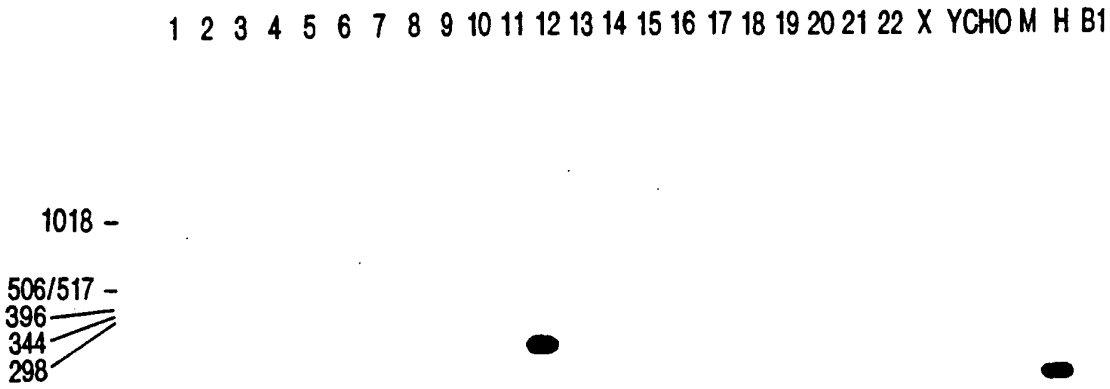

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X YCHO M H B1
1018 –
506/517 –
396
344
298

FIG. 6

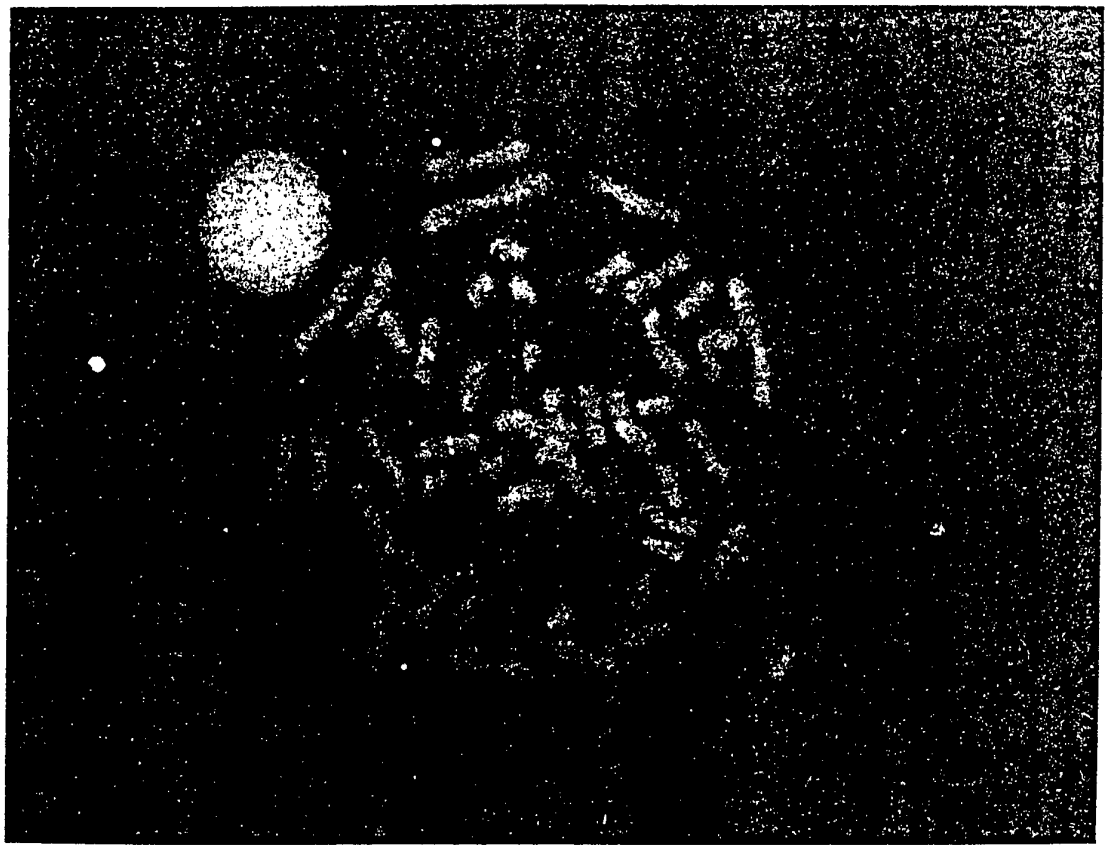


FIG. 7a

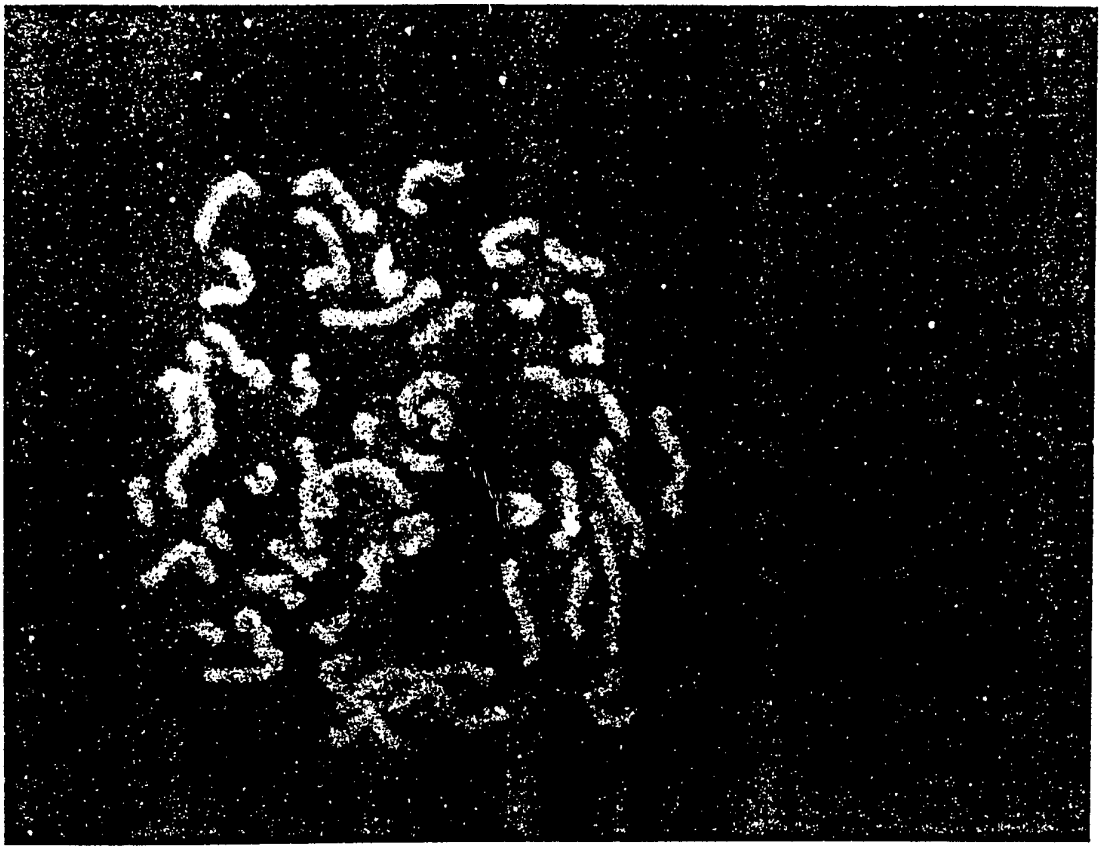
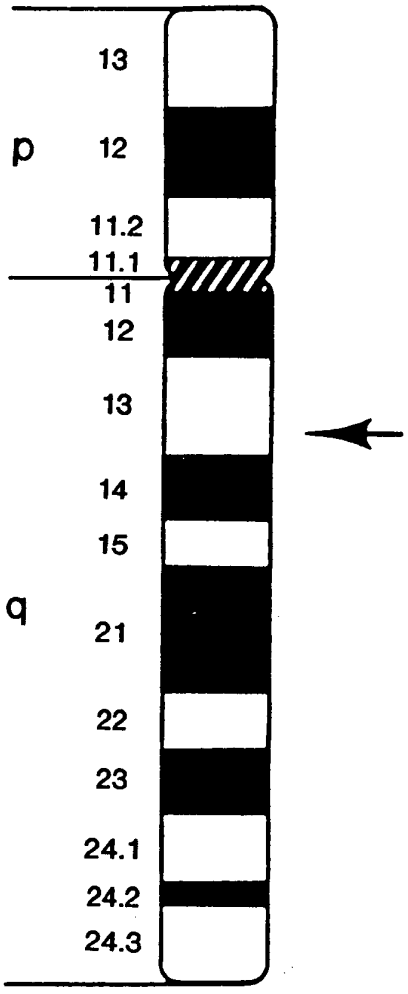


FIG. 7b




12

FIG. 7c

```
   1  GTCTCTCGGACGGTACATGCACTAATATTTCACTTGGCATTACTCAAAAGCAAAAAGAAG   60
  61  AAATAAGAACAAGGGAAAAAAAAAGATTGTGCTGATTTTTAAAATGATGCAAAAACTGCA  120
                                                  M  M  Q  K  L  Q
 121  AATGTATGTTTATATTTACCTGTTCATGCTGATTGCTGCTGGCCCAGTGGATCTAAATGA  180
       M  Y  V  Y  I  Y  L  F  M  L  I  A  A  G  P  V  D  L  N  E
 181  GGGCAGTGAGAGAGAAGAAAATGTGGAAAAAGAGGGGCTGTGTAATGCATGTGCGTGGAG  240
       G  S  E  R  E  E  N  V  E  K  E  G  L  C  N  A  C  A  W  R
 241  ACAAAACACGAGGTACTCCAGAATAGAAGCCATAAAAATTCAAATCCTCAGTAAGCTGCG  300
       Q  N  T  R  Y  S  R  I  E  A  I  K  I  Q  I  L  S  K  L  R
 301  CCTGGAAACAGCTCCTAACATCAGCAAAGATGCTATAAGACAACTTCTGCCAAGAGCGCC  360
       L  E  T  A  P  N  I  S  K  D  A  I  R  Q  L  L  P  R  A  P
 361  TCCACTCCGGGAACTGATCGATCAGTACGACGTCCAGAGGGATGACAGCAGTGATGGCTC  420
       P  L  R  E  L  I  D  Q  Y  D  V  Q  R  D  D  S  S  D  G  S
 421  TTTGGAAGATGACGATTATCACGCTACCACGGAAACAATCATTACCATGCCTACAGAGTC  480
       L  E  D  D  D  Y  H  A  T  T  E  T  I  I  T  M  P  T  E  S
 481  TGACTTTCTAATGCAAGCGGATGGCAAGCCCAAATGTTGCTTTTTTAAATTTAGCTCTAA  540
       D  F  L  M  Q  A  D  G  K  P  K  C  C  F  F  K  F  S  S  K
 541  AATACAGTACAACAAAGTAGTAAAAGCCCAACTGTGGATATATCTCAGACCCGTCAAGAC  600
       I  Q  Y  N  K  V  V  K  A  Q  L  W  I  Y  L  R  P  V  K  T
 601  TCCTACAACAGTGTTTGTGCAAATCCTGAGACTCATCAAACCCATGAAAGACGGTACAAG  660
       P  T  T  V  F  V  Q  I  L  R  L  I  K  P  M  K  D  G  T  R
 661  GTATACTGGAATCCGATCTCTGAAACTTGACATGAGCCCAGGCACTGGTATTTGGCAGAG  720
       Y  T  G  I  R  S  L  K  L  D  M  S  P  G  T  G  I  W  Q  S
 721  TATTGATGTGAAGACAGTGTTGCAAAATTGGCTCAAACAGCCTGAATCCAACTTAGGCAT  780
       I  D  V  K  T  V  L  Q  N  W  L  K  Q  P  E  S  N  L  G  I
 781  TGAAATCAAAGCTTTGGATGAGAATGGCCATGATCTTGCTGTAACCTTCCCAGGACCAGG  840
       E  I  K  A  L  D  E  N  G  H  D  L  A  V  T  F  P  G  P  G
 841  AGAAGATGGGCTGAATCCCTTTTTAGAAGTCAAGGTGACAGACACACCCAAGAGGTCCCG  900
       E  D  G  L  N  P  F  L  E  V  K  V  T  D  T  P  K  R  S  R
 901  GAGAGACTTTGGGCTTGACTGCGATGAGCACTCCACGGAATCCCGGTGCTGCCGCTACCC  960
       R  D  F  G  L  D  C  D  E  H  S  T  E  S  R  C  C  R  Y  P
 961  CCTCACGGTCGATTTTGAAGCCTTTGGATGGGACTGGATTATCGCACCCAAAAGATATAA 1020
       L  T  V  D  F  E  A  F  G  W  D  W  I  I  A  P  K  R  Y  K
1021  GGCCAATTACTGCTCAGGAGAGTGTGAATTTGTGTTTTTACAAAAATATCCGCATACTCA 1080
       A  N  Y  C  S  G  E  C  E  F  V  F  L  Q  K  Y  P  H  T  H
1081  TCTTGTGCACCAAGCAAACCCCAGAGGCTCAGCAGGCCCTTGCTGCACTCCGACAAAAAT 1140
       L  V  H  Q  A  N  P  R  G  S  A  G  P  C  C  T  P  T  K  M
1141  GTCTCCCATTAATATGCTATATTTTAATGGCAAAGAACAAATAATATATGGGAAAATTCC 1200
       S  P  I  N  M  L  Y  F  N  G  K  E  Q  I  I  Y  G  K  I  P
1201  AGCCATGGTAGTAGACCGCTGTGGGTGCTCATGAGCTTTGCATTAGGTTAGAAACTTCCC 1260
       A  M  V  V  D  R  C  G  C  S  *
```

FIG. 8a

1261 AAGTCATGGAAGGTCTTCCCCTCAATTTCGAAACTGTGAATTCAAGCACCACAGGCTGTA 1320
1321 GGCCTTGAGTATGCTCTAGTAACGTAAGCACAAGCTACAGTGTATGAACTAAAAGAGAGA 1380
1381 ATAGATGCAATGGTTGGCATTCAACCACCAAAATAAACCATACTATAGGATGTTGTATGA 1440
1441 TTTCCAGAGTTTTTGAAATAGATGGAGATCAAATTACATTTATGTCCATATATGTATATT 1500
1501 ACAACTACAATCTAGGCAAGGAAGTGAGAGCACATCTTGTGGTCTGCTGAGTTAGGAGGG 1560
1561 TATGATTAAAAGGTAAAGTCTTATTTCCTAACAGTTTCACTTAATATTTACAGAAGAATC 1620
1621 TATATGTAGCCTTTGTAAAGTGTAGGATTGTTATCATTTAAAAACATCATGTACACTTAT 1680
1681 ATTTGTATTGTATACTTGGTAAGATAAAATTCCACAAAGTAGGAATGGGGCCTCACATAC 1740
1741 ACATTGCCATTCCTATTATAATTGGACAATCCACCACGGTGCTAATGCAGTGCTGAATGG 1800
1801 CTCCTACTGGACCTCTCGATAGAACACTCTACAAAGTACGAGTCTCTCTCTCCCTTCCAG 1860
1861 GTGCATCTCCACACACACAGCACTAAGTGTTCAATGCATTTTCTTTAAGGAAAGAAGAAT 1920
1921 CTTTTTTTCTAGAGGTCAACTTTCAGTCAACTCTAGCACAGCGGGAGTGACTGCTGCATC 1980
1981 TTAAAAGGCAGCCAAACAGTATTCATTTTTTAATCTAAATTTCAAAATCACTGTCTGCCT 2040
2041 TTATCACATGGCAATTTTGTGGTAAAATAATGGAAATGACTGGTTCTATCAATATTGTAT 2100
2101 AAAAGACTCTGAAACAATTACATTTATATAATATGTATACAATATTGTTTTGTAAATAAG 2160
2161 TGTCTCCTTTTATATTTACTTTGGTATATTTTTACACTAATGAAATTTCAAATCATTAAA 2220
2221 GTACAAAGACATGTCATGTATCACAAAAAAGGTGACTGCTTCTATTTCAGAGTGAATTAG 2280
2281 CAGATTCAATAGTGGTCTTAAAACTCTGTATGTTAAGATTAGAAGGTTATATTACAATCA 2340
2341 ATTTATGTATTTTTTACATTATCAACTTATGGTTTCATGGTGGCTGTATCTATGAATGTG 2400
2401 GCTCCCAGTCAAATTTCAATGCCCCACCATTTTAAAAATTACAAGCATTACTAAACATAC 2460
2461 CAACATGTATCTAAAGAAATACAAATATGGTATCTCAATAACAGCTACTTTTTTATTTTA 2520
2521 TAATTTGACAATGAATACATTTCTTTTATTTACTTCAGTTTTATAAATTGGAACTTTGTT 2580
2581 TATCAAATGTATTGTACTCATAGCTAAATGAAATTATTTCTTACATAAAAATGTGTAGAA 2640
2641 ACTATAAATTAAAGTGTTTTCACATTTTTGAAAGGC 2676

FIG. 8b

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/08543

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07K 14/52, 14/495; C12N 15/19, 15/63, 5/10, 1/21, 1/15
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350, 351, 395; 536/23.5, 24.31; 435/240.2, 252.3, 254.11, 320.1, 69.1, 69.5

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

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

Please See Extra Sheet.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | *Proceedings of the National Academy of Sciences USA*, Volume 90, issued July 1993, T. K. Sampath et al., "*Drosophila* transforming growth factor $\beta$ superfamily proteins induce endochondral bone formation in mammals", pages 6004-6008. | 1-11 |
| Y,P | WO, A, 94/21681 (LEE ET AL.) 29 September 1994, especially Figs. 5a and 5b and Claim 3. | 3 |
| --- | | ------- |
| A,P | | 1, 2, 4-11 |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

| | | | |
|---|---|---|---|
| * | Special categories of cited documents: | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" | document defining the general state of the art which is not considered to be of particular relevance | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "E" | earlier document published on or after the international filing date | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "L" | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "&" | document member of the same patent family |
| "O" | document referring to an oral disclosure, use, exhibition or other means | | |
| "P" | document published prior to the international filing date but later than the priority date claimed | | |

| | |
|---|---|
| Date of the actual completion of the international search<br>17 AUGUST 1995 | Date of mailing of the international search report<br>12 OCT 1995 |
| Name and mailing address of the ISA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231<br>Facsimile No. (703) 305-3230 | Authorized officer<br>MP Woodward for<br>DAVID L. FITZGERALD<br>Telephone No. (703) 308-0196 |

Form PCT/ISA/210 (second sheet)(July 1992)*

# INTERNATIONAL SEARCH REPORT

Int...national application No.
PCT/US95/08543

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

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

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

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

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

Please See Extra Sheet.

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

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

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

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-11

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/08543

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

530/350, 351, 395; 536/23.5, 24.31; 435/240.2, 252.3, 254.11, 320.1, 69.1, 69.5

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

Keyword databases: Medline, Biosis, SciSearch, Derwent WPI, USPTO-APS
search terms: growth differentiation factor; TGF-$\beta$ [super]family
Sequence databases: GenBank/EMBL/DDBJ, GeneSeq, SwissProt, PIR

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

I. Claims 1-11, directed to a GDF-11 protein, a nucleic acid encoding it, and corresponding vectors and transformed cells.

II. Claims 12-22, directed to an antibody which binds to a GDF-11 polypeptide and an immunoassay using the same.

III. Claims 23-43, directed to therapeutic methods involving the suppression of GDF-11 activity.

The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons.

The special technical feature of Group I which defines an advance over the art is the novel protein, GDF-11. Neither Group II nor Group III shares this special technical feature because each relates to product(s) which are materially unlike the products of Group I, and the inventions of these groups are not required to make or use the invention of that group. Since the GDF-11 peptide is closely related to GDF-8 and other members of the TGF-$\beta$ family, the antibodies of Group II may be alternatively made using, e.g., GDF-8 as an antigen. The methods of Group III relate to the suppression of GDF-11 activity; they relate to methods and reagents which are wholly independent of the GDF-11 protein itself. Each of Groups II and III thus requires an advance over the art which is not dependent on the special technical feature embodied in the GDF-11 protein of Group I.

Groups II and III do not share a special technical feature. The special technical feature of Group II involves antibodies characterized by their ability to bind to GDF-11 and the exploitation of such binding in an analytical context. Group III does not share this special technical feature because it relates to the suppression of GDF-11 activity rather than the detection of the protein.

For the above reasons, this Authority considers that the inventions are not so linked by any special technical feature so as to form a single inventive concept within the meaning of PCT Rule 13.2.