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(54) **ITPKS AS MODIFIERS OF THE IGFR PATHWAY AND METHODS OF USE**

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(57) **ABSTRACT**

Human MIGFR genes are identified as modulators of the IGFR pathway, and thus are therapeutic targets for disorders associated with defective IGFR function. Methods for identifying modulators of IGFR, comprising screening for agents that modulate the activity of MIGFR are provided.

# ITPKS AS MODIFIERS OF THE IGFR PATHWAY AND METHODS OF USE

## REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. provisional patent application 60/539,837 filed Jan. 28, 2004. The contents of the prior applications are hereby incorporated in their entirety.

## BACKGROUND OF THE INVENTION

[0002] Somatic mutations in the PTEN (Phosphatase and Tensin homolog deleted on chromosome 10) gene are known to cause tumors in a variety of human tissues. In addition, germline mutations in PTEN are the cause of human diseases (Cowden disease and Bannayan-Zonana syndrome) associated with increased risk of breast and thyroid cancer (Nelen M R et al. (1997) Hum Mol Genet, 8:1383-1387; Liaw D et al. (1997) Nat Genet, 1:64-67; Marsh D J et al. (1998) Hum Mol Genet, 3:507-515). PTEN is thought to act as a tumor suppressor by regulating several signaling pathways through the second messenger phosphatidylinositol 3,4,5 triphosphate (PIP3). PTEN dephosphorylates the D3 position of PIP3 and downregulates signaling events dependent on PIP3 levels (Maehama T and Dixon J E (1998) J Biol Chem, 22, 13375-8). In particular, pro-survival pathways downstream of the insulin-like growth factor (IGF) pathway are regulated by PTEN activity. Stimulation of the IGF pathway, or loss of PTEN function, elevates PIP3 levels and activates pro-survival pathways associated with tumorigenesis (Stambolic V et al. (1998) Cell, 95:29-39). Consistent with this model, elevated levels of insulin-like growth factors I and II correlate with increased risk of cancer (Yu H et al (1999) J Natl Cancer Inst 91:151-156) and poor prognosis (Takanami I et al, 1996, J Surg Oncol 61(3):205-8). In addition, increased levels or activity of positive effectors of the IGF pathway, such as Akt and PI(3) kinase, have been implicated in several types of human cancer (Nicholson K M and Anderson N G (2002) Cellular Signalling, 14:381-395).

[0003] In *Drosophila melanogaster*, as in vertebrates, the Insulin Growth Factor Receptor (IGFR) pathway includes the positive effectors PI(3) kinase, Akt, and PDK and the inhibitor, PTEN. These proteins have been implicated in multiple processes, including the regulation of cell growth and size as well as cell division and survival (Oldham S and Hafen E. (2003) Trends Cell Biol. 13:79-85; Garafolo R S. (2002) Trends Endocr. Metab. 13:156-162; Backman S A et al. (2002) Curr. Op. Neurobio. 12:1-7; Tapon N et al. (2001) Curr Op. Cell Biol. 13:731-737). Activation of the pathway in Drosophila can result in increases in cell size, cell number and organ size (Oldham S et al. (2002) Dev. 129:4103-4109; Prober D A and Edgar B A. (2002) Genes & Dev. 16:2286-2299; Potter C J et al. (2001) Cell 105:357-368; Verdu J et al. (1999) Cell Biol. 1:500-506).

[0004] Inositol 1,4,5-trisphosphate 3-kinase (ITPK) catalyzes the phosphorylation of Ins(1,4,5)P3 to Ins(1,3,4,5)P4, both of which are modulators of calcium homeostasis. ITPK isoforms include ITPKA, ITPKB, and ITPKC, all of which contain a conserved catalytic unit in their C termini, but have unique N-terminal sequences and tissue distributions. ITPKA regulates inositol phosphate metabolism by phosphorylation of second messenger inositol 1,4,5-trisphosphate to Ins(1,3,4,5)P4. The activity of the inositol 1,4,5-trisphosphate 3-kinase is responsible for regulating the levels of a large number of inositol polyphosphates that are important in cellular signaling. Both calcium/calmodulin and protein phosphorylation mechanisms control its activity. It is also a substrate for the cyclic AMP-dependent protein kinase, calcium/calmodulin-dependent protein kinase II, and protein kinase C in vitro. ITPKB regulates inositol phosphate metabolism by phosphorylation of second messenger inositol 1,4,5-trisphosphate to Ins(1,3,4,5)P4. The activity of ITPKB is responsible for regulating the levels of a large number of inositol polyphosphates that are important in cellular signaling. Both calcium/calmodulin and protein phosphorylation mechanisms control ITPKB activity. ITPKC has no calmodulin kinase II phosphorylation site. Functional analysis indicated that ITPKC is relatively weakly activated by the calcium-calmodulin complex.

[0005] The ability to manipulate the genomes of model organisms such as *Drosophila* provides a powerful means to analyze biochemical processes that, due to significant evolutionary conservation, have direct relevance to more complex vertebrate organisms. Due to a high level of gene and pathway conservation, the strong similarity of cellular processes, and the functional conservation of genes between these model organisms and mammals, identification of the involvement of novel genes in particular pathways and their functions in such model organisms can directly contribute to the understanding of the correlative pathways and methods of modulating them in mammals (see, for example, Mechler B M et al., 1985 EMBO J 4:1551-1557; Gateff E. 1982 Adv. Cancer Res. 37: 33-74; Watson K L., et al., 1994 J Cell Sci. 18: 19-33; Miklos G L, and Rubin G M. 1996 Cell 86:521-529; Wassarman D A, et al., 1995 Curr Opin Gen Dev 5: 44-50; and Booth D R. 1999 Cancer Metastasis Rev. 18: 261-284). For example, a genetic screen can be carried out in an invertebrate model organism having underexpression (e.g. knockout) or overexpression of a gene (referred to as a "genetic entry point") that yields a visible phenotype. Additional genes are mutated in a random or targeted manner. When a gene mutation changes the original phenotype caused by the mutation in the genetic entry point, the gene is identified as a "modifier" involved in the same or overlapping pathway as the genetic entry point. When the genetic entry point is an ortholog of a human gene implicated in a disease pathway, such as IGFR, modifier genes can be identified that may be attractive candidate targets for novel therapeutics.

[0006] All references cited herein, including patents, patent applications, publications, and sequence information in referenced Genbank identifier numbers, are incorporated herein in their entireties.

## SUMMARY OF THE INVENTION

[0007] We have discovered genes that modify the IGFR pathway in *Drosophila*, and identified their human orthologs, hereinafter referred to as Inositol 1,4,5-trisphosphate 3-kinase (ITPK). The invention provides methods for utilizing these IGFR modifier genes and polypeptides to identify ITPK-modulating agents that are candidate therapeutic agents that can be used in the treatment of disorders associated with defective or impaired IGFR function and/or ITPK function. Preferred ITPK-modulating agents specifically bind to ITPK polypeptides and restore IGFR function.

Other preferred ITPK-modulating agents are nucleic acid modulators such as antisense oligomers and RNAi that repress ITPK gene expression or product activity by, for example, binding to and inhibiting the respective nucleic acid (i.e. DNA or mRNA).

[0008] ITPK modulating agents may be evaluated by any convenient in vitro or in vivo assay for molecular interaction with an ITPK polypeptide or nucleic acid. In one embodiment, candidate ITPK modulating agents are tested with an assay system comprising an ITPK polypeptide or nucleic acid. Agents that produce a change in the activity of the assay system relative to controls are identified as candidate IGFR modulating agents. The assay system may be cell-based or cell-free. ITPK-modulating agents include ITPK related proteins (e.g. dominant negative mutants, and biotherapeutics); ITPK-specific antibodies; ITPK-specific antisense oligomers and other nucleic acid modulators; and chemical agents that specifically bind to or interact with ITPK or compete with ITPK binding partner (e.g. by binding to an ITPK binding partner). In one specific embodiment, a small molecule modulator is identified using a kinase assay. In specific embodiments, the screening assay system is selected from a binding assay, an apoptosis assay, a cell proliferation assay, an angiogenesis assay, and a hypoxic induction assay.

[0009] In another embodiment, candidate IGFR pathway modulating agents are further tested using a second assay system that detects changes in the IGFR pathway, such as angiogenic, apoptotic, or cell proliferation changes produced by the originally identified candidate agent or an agent derived from the original agent. The second assay system may use cultured cells or non-human animals. In specific embodiments, the secondary assay system uses non-human animals, including animals predetermined to have a disease or disorder implicating the IGFR pathway, such as an angiogenic, apoptotic, or cell proliferation disorder (e.g. cancer).

[0010] The invention further provides methods for modulating the ITPK function and/or the IGFR pathway in a mammalian cell by contacting the mammalian cell with an agent that specifically binds an ITPK polypeptide or nucleic acid. The agent may be a small molecule modulator, a nucleic acid modulator, or an antibody and may be administered to a mammalian animal predetermined to have a pathology associated with the IGFR pathway.

## DETAILED DESCRIPTION OF THE INVENTION

[0011] A dominant loss of function screen was carried out in *Drosophila* to identify genes that interact with or modulate the IGFR signaling pathway. Modifiers of the IGFR pathway and their orthologs were identified. The CG4026 (IP3K1) gene was identified as a modifier of the IGFR pathway. Accordingly, vertebrate orthologs of these modifiers, and preferably the human orthologs, ITPK genes (i.e., nucleic acids and polypeptides) are attractive drug targets for the treatment of pathologies associated with a defective IGFR signaling pathway, such as cancer.

[0012] In vitro and in vivo methods of assessing ITPK function are provided herein. Modulation of the ITPK or their respective binding partners is useful for understanding the association of the IGFR pathway and its members in normal and disease conditions and for developing diagnostics and therapeutic modalities for IGFR related pathologies. ITPK-modulating agents that act by inhibiting or enhancing ITPK expression, directly or indirectly, for example, by affecting an ITPK function such as enzymatic (e.g., catalytic) or binding activity, can be identified using methods provided herein. ITPK modulating agents are useful in diagnosis, therapy and pharmaceutical development.

### Nucleic Acids and Polypeptides of the Invention

[0013] Sequences related to ITPK nucleic acids and polypeptides that can be used in the invention are disclosed in Genbank (referenced by Genbank identifier (GI) number) as GI#s 4504788 (SEQ ID NO:1), 20072242 (SEQ ID NO:2), 38569399 (SEQ ID NO:3), 4504790 (SEQ ID NO:4), 15929097 (SEQ ID NO:5), 40254974 (SEQ ID NO:6), and 18643382 (SEQ ID NO:7) for nucleic acid, and GI#s 4504789 (SEQ ID NO:8), 38569400 (SEQ ID NO:9), and 18643383 (SEQ ID NO:10) for polypeptide sequences.

[0014] The term "ITPK polypeptide" refers to a full-length ITPK protein or a functionally active fragment or derivative thereof. A "functionally active" ITPK fragment or derivative exhibits one or more functional activities associated with a full-length, wild-type ITPK protein, such as antigenic or immunogenic activity, enzymatic activity, ability to bind natural cellular substrates, etc. The functional activity of ITPK proteins, derivatives and fragments can be assayed by various methods known to one skilled in the art (Current Protocols in Protein Science (1998) Coligan et al., eds., John Wiley & Sons, Inc., Somerset, N.J.) and as further discussed below. In one embodiment, a functionally active ITPK polypeptide is an ITPK derivative capable of rescuing defective endogenous ITPK activity, such as in cell based or animal assays; the rescuing derivative may be from the same or a different species. For purposes herein, functionally active fragments also include those fragments that comprise one or more structural domains of an ITPK, such as a kinase domain or a binding domain. Protein domains can be identified using the PFAM program (Bateman A., et al., Nucleic Acids Res, 1999, 27:260-2). For example, the Inositol polyphosphate kinase domain (PFAM 03770) of ITPK from each of GI#s 4504789, 38569400, and 18643383 (SEQ ID NOs:8, 9, and 10, respectively) is located respectively at approximately amino acid residues 171 to 456, 652 to 937, and 393 to 678. Methods for obtaining ITPK polypeptides are also further described below. In some embodiments, preferred fragments are functionally active, domain-containing fragments comprising at least 25 contiguous amino acids, preferably at least 50, more preferably 75, and most preferably at least 100 contiguous amino acids of an ITPK. In further preferred embodiments, the fragment comprises the entire functionally active domain.

[0015] The term "ITPK nucleic acid" refers to a DNA or RNA molecule that encodes an ITPK polypeptide. Preferably, the ITPK polypeptide or nucleic acid or fragment thereof is from a human, but can also be an ortholog, or derivative thereof with at least 70% sequence identity, preferably at least 80%, more preferably 85%, still more preferably 90%, and most preferably at least 95% sequence identity with human ITPK. Methods of identifying orthlogs are known in the art. Normally, orthologs in different species retain the same function, due to presence of one or more protein motifs and/or 3-dimensional structures. Orthologs

are generally identified by sequence homology analysis, such as BLAST analysis, usually using protein bait sequences. Sequences are assigned as a potential ortholog if the best hit sequence from the forward BLAST result retrieves the original query sequence in the reverse BLAST (Huynen M A and Bork P, Proc Natl Acad Sci (1998) 95:5849-5856; Huynen M A et al., Genome Research (2000) 10:1204-1210). Programs for multiple sequence alignment, such as CLUSTAL (Thompson J D et al, 1994, Nucleic Acids Res 22:4673-4680) may be used to highlight conserved regions and/or residues of orthologous proteins and to generate phylogenetic trees. In a phylogenetic tree representing multiple homologous sequences from diverse species (e.g., retrieved through BLAST analysis), orthologous sequences from two species generally appear closest on the tree with respect to all other sequences from these two species. Structural threading or other analysis of protein folding (e.g., using software by ProCeryon, Biosciences, Salzburg, Austria) may also identify potential orthologs. In evolution, when a gene duplication event follows speciation, a single gene in one species, such as *Drosophila*, may correspond to multiple genes (paralogs) in another, such as human. As used herein, the term "orthologs" encompasses paralogs. As used herein, "percent (%) sequence identity" with respect to a subject sequence, or a specified portion of a subject sequence, is defined as the percentage of nucleotides or amino acids in the candidate derivative sequence identical with the nucleotides or amino acids in the subject sequence (or specified portion thereof), after aligning the sequences and introducing gaps, if necessary to achieve the maximum percent sequence identity, as generated by the program WU-BLAST-2.0a19 (Altschul et al., J. Mol. Biol. (1997) 215:403-410) with all the search parameters set to default values. The HSP S and HSP S2 parameters are dynamic values and are established by the program itself depending upon the composition of the particular sequence and composition of the particular database against which the sequence of interest is being searched. A % identity value is determined by the number of matching identical nucleotides or amino acids divided by the sequence length for which the percent identity is being reported. "Percent (%) amino acid sequence similarity" is determined by doing the same calculation as for determining % amino acid sequence identity, but including conservative amino acid substitutions in addition to identical amino acids in the computation.

**[0016]** A conservative amino acid substitution is one in which an amino acid is substituted for another amino acid having similar properties such that the folding or activity of the protein is not significantly affected. Aromatic amino acids that can be substituted for each other are phenylalanine, tryptophan, and tyrosine; interchangeable hydrophobic amino acids are leucine, isoleucine, methionine, and valine; interchangeable polar amino acids are glutamine and asparagine; interchangeable basic amino acids are arginine, lysine and histidine; interchangeable acidic amino acids are aspartic acid and glutamic acid; and interchangeable small amino acids are alanine, serine, threonine, cysteine and glycine.

**[0017]** Alternatively, an alignment for nucleic acid sequences is provided by the local homology algorithm of Smith and Waterman (Smith and Waterman, 1981, Advances in Applied Mathematics 2:482-489; database: European Bioinformatics Institute; Smith and Waterman, 1981, J. of Molec. Biol., 147:195-197; Nicholas et al., 1998, "A Tutorial on Searching Sequence Databases and Sequence Scoring Methods" (www.psc.edu) and references cited therein.; W. R. Pearson, 1991, Genomics 11:635-650). This algorithm can be applied to amino acid sequences by using the scoring matrix developed by Dayhoff (Dayhoff: Atlas of Protein Sequences and Structure, M. O. Dayhoff ed., 5 suppl. 3:353-358, National Biomedical Research Foundation, Washington, D.C., USA), and normalized by Gribskov (Gribskov 1986 Nucl. Acids Res. 14(6):6745-6763). The Smith-Waterman algorithm may be employed where default parameters are used for scoring (for example, gap open penalty of 12, gap extension penalty of two). From the data generated, the "Match" value reflects "sequence identity."

**[0018]** Derivative nucleic acid molecules of the subject nucleic acid molecules include sequences that hybridize to the nucleic acid sequence of an ITPK. The stringency of hybridization can be controlled by temperature, ionic strength, pH, and the presence of denaturing agents such as formamide during hybridization and washing. Conditions routinely used are set out in readily available procedure texts (e.g., Current Protocol in Molecular Biology, Vol. 1, Chap. 2.10, John Wiley & Sons, Publishers (1994); Sambrook et al., Molecular Cloning, Cold Spring Harbor (1989)). In some embodiments, a nucleic acid molecule of the invention is capable of hybridizing to a nucleic acid molecule containing the nucleotide sequence of an ITPK under high stringency hybridization conditions that are: prehybridization of filters containing nucleic acid for 8 hours to overnight at 65° C. in a solution comprising 6×single strength citrate (SSC) (1×SSC is 0.15 M NaCl, 0.015 M Na citrate; pH 7.0), 5×Denhardt's solution, 0.05% sodium pyrophosphate and 100 μg/ml herring sperm DNA; hybridization for 18-20 hours at 65° C. in a solution containing 6×SSC, 1×Denhardt's solution, 100 μg/ml yeast tRNA and 0.05% sodium pyrophosphate; and washing of filters at 65° C. for 1 h in a solution containing 0.1×SSC and 0.1% SDS (sodium dodecyl sulfate).

**[0019]** In other embodiments, moderately stringent hybridization conditions are used that are: pretreatment of filters containing nucleic acid for 6 h at 40° C. in a solution containing 35% formamide, 5×SSC, 50 mM Tris-HCl (pH7.5), 5mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, and 500 μg/ml denatured salmon sperm DNA; hybridization for 18-20 h at 40° C. in a solution containing 35% formamide, 5×SSC, 50 mM Tris-HCl (pH7.5), 5 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 μg/ml salmon sperm DNA, and 10% (wt/vol) dextran sulfate; followed by washing twice for 1 hour at 55° C. in a solution containing 2×SSC and 0.1% SDS.

**[0020]** Alternatively, low stringency conditions can be used that are: incubation for 8 hours to overnight at 37° C. in a solution comprising 20% formamide, 5×SSC, 50 mM sodium phosphate (pH 7.6), 5×Denhardt's solution, 10% dextran sulfate, and 20 μg/ml denatured sheared salmon sperm DNA; hybridization in the same buffer for 18 to 20 hours; and washing of filters in 1×SSC at about 37° C. for 1 hour.

Isolation, Production, Expression, and Mis-expression of ITPK Nucleic Acids and Polypeptides

**[0021]** ITPK nucleic acids and polypeptides are useful for identifying and testing agents that modulate ITPK function and for other applications related to the involvement of ITPK in the IGFR pathway. ITPK nucleic acids and deriva-

tives and orthologs thereof may be obtained using any available method. For instance, techniques for isolating cDNA or genomic DNA sequences of interest by screening DNA libraries or by using polymerase chain reaction (PCR) are well known in the art. In general, the particular use for the protein will dictate the particulars of expression, production, and purification methods. For instance, production of proteins for use in screening for modulating agents may require methods that preserve specific biological activities of these proteins, whereas production of proteins for antibody generation may require structural integrity of particular epitopes. Expression of proteins to be purified for screening or antibody production may require the addition of specific tags (e.g., generation of fusion proteins). Overexpression of an ITPK protein for assays used to assess ITPK function, such as involvement in cell cycle regulation or hypoxic response, may require expression in eukaryotic cell lines capable of these cellular activities. Techniques for the expression, production, and purification of proteins are well known in the art; any suitable means therefore may be used (e.g., Higgins S J and Hames B D (eds.) Protein Expression: A Practical Approach, Oxford University Press Inc., New York 1999; Stanbury P F et al., Principles of Fermentation Technology, 2nd edition, Elsevier Science, New York, 1995; Doonan S (ed.) Protein Purification Protocols, Humana Press, New Jersey, 1996; Coligan J E et al, Current Protocols in Protein Science (eds.), 1999, John Wiley & Sons, New York). In particular embodiments, recombinant ITPK is expressed in a cell line known to have defective IGFR function. The recombinant cells are used in cell-based screening assay systems of the invention, as described further below.

[0022] The nucleotide sequence encoding an ITPK polypeptide can be inserted into any appropriate expression vector. The necessary transcriptional and translational signals, including promoter/enhancer element, can derive from the native ITPK gene and/or its flanking regions or can be heterologous. A variety of host-vector expression systems may be utilized, such as mammalian cell systems infected with virus (e.g. vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g. baculovirus); microorganisms such as yeast containing yeast vectors, or bacteria transformed with bacteriophage, plasmid, or cosmid DNA. An isolated host cell strain that modulates the expression of, modifies, and/or specifically processes the gene product may be used.

[0023] To detect expression of the ITPK gene product, the expression vector can comprise a promoter operably linked to an ITPK gene nucleic acid, one or more origins of replication, and, one or more selectable markers (e.g. thymidine kinase activity, resistance to antibiotics, etc.). Alternatively, recombinant expression vectors can be identified by assaying for the expression of the ITPK gene product based on the physical or functional properties of the ITPK protein in in vitro assay systems (e.g. immunoassays).

[0024] The ITPK protein, fragment, or derivative may be optionally expressed as a fusion, or chimeric protein product (i.e. it is joined via a peptide bond to a heterologous protein sequence of a different protein), for example to facilitate purification or detection. A chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other using standard methods and expressing the chimeric product. A chimeric product may also be made by protein synthetic techniques, e.g. by use of a peptide synthesizer (Hunkapiller et al., Nature (1984) 310:105-111).

[0025] Once a recombinant cell that expresses the ITPK gene sequence is identified, the gene product can be isolated and purified using standard methods (e.g. ion exchange, affinity, and gel exclusion chromatography; centrifugation; differential solubility; electrophoresis). Alternatively, native ITPK proteins can be purified from natural sources, by standard methods (e.g. immunoaffinity purification). Once a protein is obtained, it may be quantified and its activity measured by appropriate methods, such as immunoassay, bioassay, or other measurements of physical properties, such as crystallography.

[0026] The methods of this invention may also use cells that have been engineered for altered expression (mis-expression) of ITPK or other genes associated with the IGFR pathway. As used herein, mis-expression encompasses ectopic expression, over-expression, under-expression, and non-expression (e.g. by gene knock-out or blocking expression that would otherwise normally occur).

Genetically Modified Animals

[0027] Animal models that have been genetically modified to alter ITPK expression may be used in in vivo assays to test for activity of a candidate IGFR modulating agent, or to further assess the role of ITPK in a IGFR pathway process such as apoptosis or cell proliferation. Preferably, the altered ITPK expression results in a detectable phenotype, such as decreased or increased levels of cell proliferation, angiogenesis, or apoptosis compared to control animals having normal ITPK expression. The genetically modified animal may additionally have altered IGFR expression (e.g. IGFR knockout). Preferred genetically modified animals are mammals such as primates, rodents (preferably mice or rats), among others. Preferred non-mammalian species include zebrafish, *C. elegans,* and *Drosophila*. Preferred genetically modified animals are transgenic animals having a heterologous nucleic acid sequence present as an extrachromosomal element in a portion of its cells, i.e. mosaic animals (see, for example, techniques described by Jakobovits, 1994, Curr. Biol. 4:761-763.) or stably integrated into its germ line DNA (i.e., in the genomic sequence of most or all of its cells). Heterologous nucleic acid is introduced into the germ line of such transgenic animals by genetic manipulation of, for example, embryos or embryonic stem cells of the host animal.

[0028] Methods of making transgenic animals are well-known in the art (for transgenic mice see Brinster et al., Proc. Nat. Acad. Sci. USA 82: 4438-4442 (1985), U.S. Pat. Nos. 4,736,866 and 4,870,009, both by Leder et al., U.S. Pat. No. 4,873,191 by Wagner et al., and Hogan, B., Manipulating the Mouse Embryo, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1986); for particle bombardment see U.S. Pat. No., 4,945,050, by Sandford et al.; for transgenic *Drosophila* see Rubin and Spradling, Science (1982) 218:348-53 and U.S. Pat. No. 4,670,388; for transgenic insects see Berghammer A. J. et al., A Universal Marker for Transgenic Insects (1999) Nature 402:370-371; for transgenic Zebrafish see Lin S., Transgenic Zebrafish, Methods Mol Biol. (2000);136:375-3830); for microinjection procedures for fish, amphibian eggs and birds see Houdebine and Chourrout, Experientia (1991) 47:897-905;

for transgenic rats see Hammer et al., Cell (1990) 63:1099-1112; and for culturing of embryonic stem (ES) cells and the subsequent production of transgenic animals by the introduction of DNA into ES cells using methods such as electroporation, calcium phosphate/DNA precipitation and direct injection see, e.g., Teratocarcinomas and Embryonic Stem Cells, A Practical Approach, E. J. Robertson, ed., IRL Press (1987)). Clones of the nonhuman transgenic animals can be produced according to available methods (see Wilmut, I. et al. (1997) Nature 385:810-813; and PCT International Publication Nos. WO 97/07668 and WO 97/07669).

[0029] In one embodiment, the transgenic animal is a "knock-out" animal having a heterozygous or homozygous alteration in the sequence of an endogenous ITPK gene that results in a decrease of ITPK function, preferably such that ITPK expression is undetectable or insignificant. Knock-out animals are typically generated by homologous recombination with a vector comprising a transgene having at least a portion of the gene to be knocked out. Typically a deletion, addition or substitution has been introduced into the transgene to functionally disrupt it. The transgene can be a human gene (e.g., from a human genomic clone) but more preferably is an ortholog of the human gene derived from the transgenic host species. For example, a mouse ITPK gene is used to construct a homologous recombination vector suitable for altering an endogenous ITPK gene in the mouse genome. Detailed methodologies for homologous recombination in mice are available (see Capecchi, Science (1989) 244:1288-1292; Joyner et al., Nature (1989) 338:153-156). Procedures for the production of non-rodent transgenic mammals and other animals are also available (Houdebine and Chourrout, supra; Pursel et al., Science (1989) 244:1281-1288; Simms et al., Bio/Technology (1988) 6:179-183). In a preferred embodiment, knock-out animals, such as mice harboring a knockout of a specific gene, may be used to produce antibodies against the human counterpart of the gene that has been knocked out (Claesson M H et al., (1994) Scan J Immunol 40:257-264; Declerck P J et al., (1995) J Biol Chem. 270:8397-400).

[0030] In another embodiment, the transgenic animal is a "knock-in" animal having an alteration in its genome that results in altered expression (e.g., increased (including ectopic) or decreased expression) of the ITPK gene, e.g., by introduction of additional copies of ITPK, or by operatively inserting a regulatory sequence that provides for altered expression of an endogenous copy of the ITPK gene. Such regulatory sequences include inducible, tissue-specific, and constitutive promoters and enhancer elements. The knock-in can be homozygous or heterozygous.

[0031] Transgenic nonhuman animals can also be produced that contain selected systems allowing for regulated expression of the transgene. One example of such a system that may be produced is the cre/loxP recoinbinase system of bacteriophage P1 (Lakso et al., PNAS (1992) 89:6232-6236; U.S. Pat. No. 4,959,317). If a cre/loxP recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the Cre recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, e.g., by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase. Another example of a recombinase system is the FLP recombinase system of Saccharomyces cerevisiae (O'Gorman et al. (1991) Science 251:1351-1355; U.S. Pat. No. 5,654,182). In a preferred embodiment, both Cre-LoxP and Flp-Frt are used in the same system to regulate expression of the transgene, and for sequential deletion of vector sequences in the same cell (Sun X et al (2000) Nat Genet 25:83-6).

[0032] The genetically modified animals can be used in genetic studies to further elucidate the IGFR pathway, as animal models of disease and disorders implicating defective IGFR function, and for in vivo testing of candidate therapeutic agents, such as those identified in screens described below. The candidate therapeutic agents are administered to a genetically modified animal having altered ITPK function and phenotypic changes are compared with appropriate control animals such as genetically modified animals that receive placebo treatment, and/or animals with unaltered ITPK expression that receive candidate therapeutic agent.

[0033] In addition to the above-described genetically modified animals having altered ITPK function, animal models having defective IGFR function (and otherwise normal ITPK function), can be used in the methods of the present invention. For example, a IGFR knockout mouse can be used to assess, in vivo, the activity of a candidate IGFR modulating agent identified in one of the in vitro assays described below. Preferably, the candidate IGFR modulating agent when administered to a model system with cells defective in IGFR function, produces a detectable phenotypic change in the model system indicating that the IGFR function is restored, i.e., the cells exhibit normal cell cycle progression.

Modulating Agents

[0034] The invention provides methods to identify agents that interact with and/or modulate the function of ITPK and/or the IGFR pathway. Modulating agents identified by the methods are also part of the invention. Such agents are useful in a variety of diagnostic and therapeutic applications associated with the IGFR pathway, as well as in further analysis of the ITPK protein and its contribution to the IGFR pathway. Accordingly, the invention also provides methods for modulating the IGFR pathway comprising the step of specifically modulating ITPK activity by administering an ITPK-interacting or -modulating agent.

[0035] As used herein, an "ITPK-modulating agent" is any agent that modulates ITPK function, for example, an agent that interacts with ITPK to inhibit or enhance ITPK activity or otherwise affect normal ITPK function. ITPK function can be affected at any level, including transcription, protein expression, protein localization, and cellular or extra-cellular activity. In a preferred embodiment, the ITPK-modulating agent specifically modulates the function of the ITPK. The phrases "specific modulating agent", "specifically modulates", etc., are used herein to refer to modulating agents that directly bind to the ITPK polypeptide or nucleic acid, and preferably inhibit, enhance, or otherwise alter, the function of the ITPK. These phrases also encompass modulating agents that alter the interaction of the ITPK with a binding partner, substrate, or cofactor (e.g. by binding to a binding partner of an ITPK, or to a protein/binding partner complex, and altering ITPK function). In a further preferred embodiment, the ITPK-modulating agent is a modulator of the IGFR pathway (e.g. it restores and/or upregulates IGFR function) and thus is also an IGFR-modulating agent.

[0036] Preferred ITPK-modulating agents include small molecule compounds; ITPK-interacting proteins, including antibodies and other biotherapeutics; and nucleic acid modulators such as antisense and RNA inhibitors. The modulating agents may be formulated in pharmaceutical compositions, for example, as compositions that may comprise other active ingredients, as in combination therapy, and/or suitable carriers or excipients. Techniques for formulation and administration of the compounds may be found in "Remington's Pharmaceutical Sciences" Mack Publishing Co., Easton, Pa., 19th edition.

[0037] Small Molecule Modulators

[0038] Small molecules are often preferred to modulate function of proteins with enzymatic function, and/or containing protein interaction domains. Chemical agents, referred to in the art as "small molecule" compounds are typically organic, non-peptide molecules, having a molecular weight up to 10,000, preferably up to 5,000, more preferably up to 1,000, and most preferably up to 500 daltons. This class of modulators includes chemically synthesized molecules, for instance, compounds from combinatorial chemical libraries. Synthetic compounds may be rationally designed or identified based on known or inferred properties of the ITPK protein or may be identified by screening compound libraries. Alternative appropriate modulators of this class are natural products, particularly secondary metabolites from organisms such as plants or fungi, which can also be identified by screening compound libraries for ITPK-modulating activity. Methods for generating and obtaining compounds are well known in the art (Schreiber S L, Science (2000) 151: 1964-1969; Radmann J and Gunther J, Science (2000) 151:1947-1948).

[0039] Small molecule modulators identified from screening assays, as described below, can be used as lead compounds from which candidate clinical compounds may be designed, optimized, and synthesized. Such clinical compounds may have utility in treating pathologies associated with the IGFR pathway. The activity of candidate small molecule modulating agents may be improved several-fold through iterative secondary functional validation, as further described below, structure determination, and candidate modulator modification and testing. Additionally, candidate clinical compounds are generated with specific regard to clinical and pharmacological properties. For example, the reagents may be derivatized and re-screened using in vitro and in vivo assays to optimize activity and minimize toxicity for pharmaceutical development.

Protein Modulators

[0040] Specific ITPK-interacting proteins are useful in a variety of diagnostic and therapeutic applications related to the IGFR pathway and related disorders, as well as in validation assays for other ITPK-modulating agents. In a preferred embodiment, ITPK-interacting proteins affect normal ITPK function, including transcription, protein expression, protein localization, and cellular or extra-cellular activity. In another embodiment, ITPK-interacting proteins are useful in detecting and providing information about the function of ITPK proteins, as is relevant to IGFR related disorders, such as cancer (e.g., for diagnostic means).

[0041] An ITPK-interacting protein may be endogenous, i.e. one that naturally interacts genetically or biochemically with an ITPK, such as a member of the ITPK pathway that modulates ITPK expression, localization, and/or activity. ITPK-modulators include dominant negative forms of ITPK-interacting proteins and of ITPK proteins themselves. Yeast two-hybrid and variant screens offer preferred methods for identifying endogenous ITPK-interacting proteins (Finley, R. L. et al. (1996) in DNA Cloning-Expression Systems: A Practical Approach, eds. Glover D. & Hames B. D (Oxford University Press, Oxford, England), pp. 169-203; Fashema S F et al., Gene (2000) 250:1-14; Drees B L Curr Opin Chem Biol (1999) 3:64-70; Vidal M and Legrain P Nucleic Acids Res (1999) 27:919-29; and U.S. Pat. No. 5,928,868). Mass spectrometry is an alternative preferred method for the elucidation of protein complexes (reviewed in, e.g., Pandley A and Mann M, Nature (2000) 405:837-846; Yates J R 3rd, Trends Genet (2000) 16:5-8).

[0042] An ITPK-interacting protein may be an exogenous protein, such as an ITPK-specific antibody or a T-cell antigen receptor (see, e.g., Harlow and Lane (1988) Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory; Harlow and Lane (1999) Using antibodies: a laboratory manual. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press). ITPK antibodies are further discussed below.

[0043] In preferred embodiments, an ITPK-interacting protein specifically binds an ITPK protein. In alternative preferred embodiments, an ITPK-modulating agent binds an ITPK substrate, binding partner, or cofactor.

Antibodies

[0044] In another embodiment, the protein modulator is an ITPK specific antibody agonist or antagonist. The antibodies have therapeutic and diagnostic utilities, and can be used in screening assays to identify ITPK modulators. The antibodies can also be used in dissecting the portions of the ITPK pathway responsible for various cellular responses and in the general processing and maturation of the ITPK.

[0045] Antibodies that specifically bind ITPK polypeptides can be generated using known methods. Preferably the antibody is specific to a mammalian ortholog of ITPK polypeptide, and more preferably, to human ITPK. Antibodies may be polyclonal, monoclonal (mAbs), humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab').sub.2 fragments, fragments produced by a FAb expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. Epitopes of ITPK which are particularly antigenic can be selected, for example, by routine screening of ITPK polypeptides for antigenicity or by applying a theoretical method for selecting antigenic regions of a protein (Hopp and Wood (1981), Proc. Nati. Acad. Sci. U.S.A. 78:3824-28; Hopp and Wood, (1983) Mol. Immunol. 20:483-89; Sutcliffe et al., (1983) Science 219:660-66) to the amino acid sequence of an ITPK. Monoclonal antibodies with affinities of $10^8$ $M^{-1}$ preferably $10^9$ $M^{-1}$ to $10^{10}$ $M^{-1}$, or stronger can be made by standard procedures as described (Harlow and Lane, supra; Goding (1986) Monoclonal Antibodies: Principles and Practice (2d ed) Academic Press, New York; and U.S. Pat. Nos. 4,381, 292; 4,451,570; and 4,618,577). Antibodies may be generated against crude cell extracts of ITPK or substantially purified fragments thereof. If ITPK fragments are used, they preferably comprise at least 10, and more preferably, at least 20 contiguous amino acids of an ITPK protein. In a particu-

lar embodiment, ITPK-specific antigens and/or immunogens are coupled to carrier proteins that stimulate the immune response. For example, the subject polypeptides are covalently coupled to the keyhole limpet hemocyanin (KLH) carrier, and the conjugate is emulsified in Freund's complete adjuvant, which enhances the immune response. An appropriate immune system such as a laboratory rabbit or mouse is immunized according to conventional protocols.

[0046] The presence of ITPK-specific antibodies is assayed by an appropriate assay such as a solid phase enzyme-linked immunosorbant assay (ELISA) using immobilized corresponding ITPK polypeptides. Other assays, such as radioimmunoassays or fluorescent assays might also be used.

[0047] Chimeric antibodies specific to ITPK polypeptides can be made that contain different portions from different animal species. For instance, a human immunoglobulin constant region may be linked to a variable region of a murine mAb, such that the antibody derives its biological activity from the human antibody, and its binding specificity from the murine fragment. Chimeric antibodies are produced by splicing together genes that encode the appropriate regions from each species (Morrison et al., Proc. Natl. Acad. Sci. (1984) 81:6851-6855; Neuberger et al., Nature (1984) 312:604-608; Takeda et al., Nature (1985) 31:452-454). Humanized antibodies, which are a form of chimeric antibodies, can be generated by grafting complementary-determining regions (CDRs) (Carlos, T. M., J. M. Harlan. 1994. Blood 84:2068-2101) of mouse antibodies into a background of human framework regions and constant regions by recombinant DNA technology (Riechmann L M, et al., 1988 Nature 323: 323-327). Humanized antibodies contain ~10% murine sequences and ~90% human sequences, and thus further reduce or eliminate immunogenicity, while retaining the antibody specificities (Co MS, and Queen C. 1991 Nature 351: 501-501; Morrison S L. 1992 Ann. Rev. Immun. 10:239-265). Humanized antibodies and methods of their production are well-known in the art (U.S. Pat. Nos. 5,530,101, 5,585,089, 5,693,762, and 6,180,370).

[0048] ITPK-specific single chain antibodies which are recombinant, single chain polypeptides formed by linking the heavy and light chain fragments of the Fv regions via an amino acid bridge, can be produced by methods known in the art (U.S. Pat. No. 4,946,778; Bird, Science (1988) 242:423-426; Huston et al., Proc. Natl. Acad. Sci. USA (1988) 85:5879-5883; and Ward et al., Nature (1989) 334:544-546).

[0049] Other suitable techniques for antibody production involve in vitro exposure of lymphocytes to the antigenic polypeptides or alternatively to selection of libraries of antibodies in phage or similar vectors (Huse et al., Science (1989) 246:1275-1281). As used herein, T-cell antigen receptors are included within the scope of antibody modulators (Harlow and Lane, 1988, supra).

[0050] The polypeptides and antibodies of the present invention may be used with or without modification. Frequently, antibodies will be labeled by joining, either covalently or non-covalently, a substance that provides for a detectable signal, or that is toxic to cells that express the targeted protein (Menard S, et al., Int J. Biol Markers (1989) 4:131-134). A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent moieties, fluorescent emitting lanthanide metals, chemiluminescent moieties, bioluminescent moieties, magnetic particles, and the like (U.S. Pat. Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241). Also, recombinant immunoglobulins may be produced (U.S. Pat. No. 4,816,567). Antibodies to cytoplasmic polypeptides may be delivered and reach their targets by conjugation with membrane-penetrating toxin proteins (U.S. Pat. No. 6,086,900).

[0051] When used therapeutically in a patient, the antibodies of the subject invention are typically administered parenterally, when possible at the target site, or intravenously. The therapeutically effective dose and dosage regimen is determined by clinical studies. Typically, the amount of antibody administered is in the range of about 0.1 mg/kg-to about 10 mg/kg of patient weight. For parenteral administration, the antibodies are formulated in a unit dosage injectable form (e.g., solution, suspension, emulsion) in association with a pharmaceutically acceptable vehicle. Such vehicles are inherently nontoxic and non-therapeutic. Examples are water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Nonaqueous vehicles such as fixed oils, ethyl oleate, or liposome carriers may also be used. The vehicle may contain minor amounts of additives, such as buffers and preservatives, which enhance isotonicity and chemical stability or otherwise enhance therapeutic potential. The antibodies' concentrations in such vehicles are typically in the range of about 1 mg/ml to about 10 mg/ml. Immunotherapeutic methods are further described in the literature (U.S. Pat. No. 5,859,206; WO0073469).

Nucleic Acid Modulators

[0052] Other preferred ITPK-modulating agents comprise nucleic acid molecules, such as antisense oligomers or double stranded RNA (dsRNA), which generally inhibit ITPK activity. Preferred nucleic acid modulators interfere with the function of the ITPK nucleic acid such as DNA replication, transcription, translocation of the ITPK RNA to the site of protein translation, translation of protein from the ITPK RNA, splicing of the ITPK RNA to yield one or more mRNA species, or catalytic activity which may be engaged in or facilitated by the ITPK RNA.

[0053] In one embodiment, the antisense oligomer is an oligonucleotide that is sufficiently complementary to an ITPK mRNA to bind to and prevent translation, preferably by binding to the 5' untranslated region. ITPK-specific antisense oligonucleotides, preferably range from at least 6 to about 200 nucleotides. In some embodiments the oligonucleotide is preferably at least 10, 15, or 20 nucleotides in length. In other embodiments, the oligonucleotide is preferably less than 50, 40, or 30 nucleotides in length. The oligonucleotide can be DNA or RNA or a chimeric mixture or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone. The oligonucleotide may include other appending groups such as peptides, agents that facilitate transport across the cell membrane, hybridization-triggered cleavage agents, and intercalating agents.

[0054] In another embodiment, the antisense oligomer is a phosphothioate morpholino oligomer (PMO). PMOs are

assembled from four different morpholino subunits, each of which contain one of four genetic bases (A, C, G, or T) linked to a six-membered morpholine ring. Polymers of these subunits are joined by non-ionic phosphodiamidate intersubunit linkages. Details of how to make and use PMOs and other antisense oligomers are well known in the art (e.g. see WO99/18193; Probst J C, Antisense Oligodeoxynucleotide and Ribozyme Design, Methods. (2000) 22(3):271-281; Summerton J, and Weller D. 1997 Antisense Nucleic Acid Drug Dev.: 7:187-95; U.S. Pat. No. 5,235,033; and U.S. Pat. No. 5,378,841).

[0055] Alternative preferred ITPK nucleic acid modulators are double-stranded RNA species mediating RNA interference (RNAi). RNAi is the process of sequence-specific, post-transcriptional gene silencing in animals and plants, initiated by double-stranded RNA (dsRNA) that is homologous in sequence to the silenced gene. Methods relating to the use of RNAi to silence genes in *C. elegans, Drosophila*, plants, and humans are known in the art (Fire A, et al., 1998 Nature 391:806-811; Fire, A. Trends Genet. 15, 358-363 (1999); Sharp, P. A. RNA interference 2001. Genes Dev. 15, 485-490 (2001); Hammond, S. M., et al., Nature Rev. Genet. 2, 110-1119 (2001); Tuschl, T. Chem. Biochem. 2, 239-245 (2001); Hamilton, A. et al., Science 286, 950-952 (1999); Hammond, S. M., et al., Nature 404, 293-296 (2000); Zamore, P. D., et al., Cell 101, 25-33 (2000); Bernstein, E., et al., Nature 409, 363-366 (2001); Elbashir, S. M., et al., Genes Dev. 15, 188-200 (2001); WO0129058; WO9932619; Elbashir S M, et al., 2001 Nature 411:494-498; Novina C D and Sharp P. 2004 Nature 430:161-164; Soutschek J et al 2004 Nature 432:173-178).

[0056] Nucleic acid modulators are commonly used as research reagents, diagnostics, and therapeutics. For example, antisense oligonucleotides, which are able to inhibit gene expression with exquisite specificity, are often used to elucidate the function of particular genes (see, for example, U.S. Pat. No. 6,165,790). Nucleic acid modulators are also used, for example, to distinguish between functions of various members of a biological pathway. For example, antisense oligomers have been employed as therapeutic moieties in the treatment of disease states in animals and man and have been demonstrated in numerous clinical trials to be safe and effective (Milligan J F, et al, Current Concepts in Antisense Drug Design, J Med Chem. (1993) 36:1923-1937; Tonkinson J L et al., Antisense Oligodeoxynucleotides as Clinical Therapeutic Agents, Cancer Invest. (1996) 14:54-65). Accordingly, in one aspect of the invention, an ITPK-specific nucleic acid modulator is used in an assay to further elucidate the role of the ITPK in the IGFR pathway, and/or its relationship to other members of the pathway. In another aspect of the invention, an ITPK-specific antisense oligomer is used as a therapeutic agent for treatment of IGFR-related disease states.

Assay Systems

[0057] The invention provides assay systems and screening methods for identifying specific modulators of ITPK activity. As used herein, an "assay system" encompasses all the components required for performing and analyzing results of an assay that detects and/or measures a particular event. In general, primary assays are used to identify or confirm a modulator's specific biochemical or molecular effect with respect to the ITPK nucleic acid or protein. In general, secondary assays further assess the activity of an ITPK modulating agent identified by a primary assay and may confirm that the modulating agent affects ITPK in a manner relevant to the IGFR pathway. In some cases, ITPK modulators will be directly tested in a secondary assay.

[0058] In a preferred embodiment, the screening method comprises contacting a suitable assay system comprising an ITPK polypeptide or nucleic acid with a candidate agent under conditions whereby, but for the presence of the agent, the system provides a reference activity (e.g. kinase activity), which is based on the particular molecular event the screening method detects. A statistically significant difference between the agent-biased activity and the reference activity indicates that the candidate agent modulates ITPK activity, and hence the IGFR pathway. The ITPK polypeptide or nucleic acid used in the assay may comprise any of the nucleic acids or polypeptides described above.

Primary Assays

[0059] The type of modulator tested generally determines the type of primary assay.

Primary Assays for Small Molecule Modulators

[0060] For small molecule modulators, screening assays are used to identify candidate modulators. Screening assays may be cell-based or may use a cell-free system that recreates or retains the relevant biochemical reaction of the target protein (reviewed in Sittampalam G S et al., Curr Opin Chem Biol (1997) 1:384-91 and accompanying references). As used herein the term "cell-based" refers to assays using live cells, dead cells, or a particular cellular fraction, such as a membrane, endoplasmic reticulum, or mitochondrial fraction. The term "cell free" encompasses assays using substantially purified protein (either endogenous or recombinantly produced), partially purified or crude cellular extracts. Screening assays may detect a variety of molecular events, including protein-DNA interactions, protein-protein interactions (e.g., receptor-ligand binding), transcriptional activity (e.g., using a reporter gene), enzymatic activity (e.g., via a property of the substrate), activity of second messengers, immunogenicity and changes in cellular morphology or other cellular characteristics. Appropriate screening assays may use a wide range of detection methods including fluorescent, radioactive, colorimetric, spectrophotometric, and amperometric methods, to provide a read-out for the particular molecular event detected.

[0061] Cell-based screening assays usually require systems for recombinant expression of ITPK and any auxiliary proteins demanded by the particular assay. Appropriate methods for generating recombinant proteins produce sufficient quantities of proteins that retain their relevant biological activities and are of sufficient purity to optimize activity and assure assay reproducibility. Yeast two-hybrid and variant screens, and mass spectrometry provide preferred methods for determining protein-protein interactions and elucidation of protein complexes. In certain applications, when ITPK-interacting proteins are used in screens to identify small molecule modulators, the binding specificity of the interacting protein to the ITPK protein may be assayed by various known methods such as substrate processing (e.g. ability of the candidate ITPK-specific binding agents to function as negative effectors in ITPK-expressing cells), binding equilibrium constants (usually at least about $10^7$

$M^{-1}$, preferably at least about $10^8$ $M^{-1}$, more preferably at least about $10^9$ $M^{-1}$), and immunogenicity (e.g. ability to elicit ITPK specific antibody in a heterologous host such as a mouse, rat, goat or rabbit). For enzymes and receptors, binding may be assayed by, respectively, substrate and ligand processing.

[0062] The screening assay may measure a candidate agent's ability to specifically bind to or modulate activity of an ITPK polypeptide, a fusion protein thereof, or to cells or membranes bearing the polypeptide or fusion protein. The ITPK polypeptide can be full length or a fragment thereof that retains functional ITPK activity. The ITPK polypeptide may be fused to another polypeptide, such as a peptide tag for detection or anchoring, or to another tag. The ITPK polypeptide is preferably human ITPK, or is an ortholog or derivative thereof as described above. In a preferred embodiment, the screening assay detects candidate agent-based modulation of ITPK interaction with a binding target, such as an endogenous or exogenous protein or other substrate that has ITPK-specific binding activity, and can be used to assess normal ITPK gene function.

[0063] Suitable assay formats that may be adapted to screen for ITPK modulators are known in the art. Preferred screening assays are high throughput or ultra high throughput and thus provide automated, cost-effective means of screening compound libraries for lead compounds (Fernandes P B, Curr Opin Chem Biol (1998) 2:597-603; Sundberg S A, Curr Opin Biotechnol 2000, 11:47-53). In one preferred embodiment, screening assays uses fluorescence technologies, including fluorescence polarization, time-resolved fluorescence, and fluorescence resonance energy transfer. These systems offer means to monitor protein-protein or DNA-protein interactions in which the intensity of the signal emitted from dye-labeled molecules depends upon their interactions with partner molecules (e.g., Selvin P R, Nat Struct Biol (2000) 7:730-4; Fernandes P B, supra; Hertzberg R P and Pope A J, Curr Opin Chem Biol (2000) 4:445-451).

[0064] A variety of suitable assay systems may be used to identify candidate ITPK and IGFR pathway modulators (e.g. U.S. Pat. No. 6,165,992 and U.S. Pat. No. 6,720,162 (kinase assays); U.S. Pat. Nos. 5,550,019 and 6,133,437 (apoptosis assays); and U.S. Pat. Nos. 5,976,782, 6,225,118 and 6,444,434 (angiogenesis assays), among others). Specific preferred assays are described in more detail below.

[0065] Kinase assays. In some preferred embodiments the screening assay detects the ability of the test agent to modulate the kinase activity of an ITPK polypeptide. In further embodiments, a cell-free kinase assay system is used to identify a candidate IGFR modulating agent, and a secondary, cell-based assay, such as an apoptosis or hypoxic induction assay (described below), may be used to further characterize the candidate IGFR modulating agent. Many different assays for kinases have been reported in the literature and are well known to those skilled in the art (e.g. U.S. Pat. No. 6,165,992; Zhu et al., Nature Genetics (2000) 26:283-289; and WO0073469). Radioassays, which monitor the transfer of a gamma phosphate are frequently used. For instance, a scintillation assay for p56 (lck) kinase activity monitors the transfer of the gamma phosphate from gamma -$^{33}$P ATP to a biotinylated peptide substrate; the substrate is captured on a streptavidin coated bead that transmits the signal (Beveridge M et al., J Biomol Screen (2000) 5:205-212). This assay uses the scintillation proximity assay (SPA), in which only radio-ligand bound to receptors tethered to the surface of an SPA bead are detected by the scintillant immobilized within it, allowing binding to be measured without separation of bound from free ligand.

[0066] Other assays for protein kinase activity may use antibodies that specifically recognize phosphorylated substrates. For instance, the kinase receptor activation (KIRA) assay measures receptor tyrosine kinase activity by ligand stimulating the intact receptor in cultured cells, then capturing solubilized receptor with specific antibodies and quantifying phosphorylation via phosphotyrosine ELISA (Sadick M D, Dev Biol Stand (1999) 97:121-133).

[0067] Another example of antibody based assays for protein kinase activity is TRF (time-resolved fluorometry). This method utilizes europium chelate-labeled anti-phosphotyrosine antibodies to detect phosphate transfer to a polymeric substrate coated onto microtiter plate wells. The amount of phosphorylation is then detected using time-resolved, dissociation-enhanced fluorescence (Braunwalder A F, et al., Anal Biochem Jul. 1, 1996;238(2):159-64).

[0068] Yet other assays for kinases involve uncoupled, pH sensitive assays that can be used for high-throughput screening of potential inhibitors or for determining substrate specificity. Since kinases catalyze the transfer of a gamma-phosphoryl group from ATP to an appropriate hydroxyl acceptor with the release of a proton, a pH sensitive assay is based on the detection of this proton using an appropriately matched buffer/indicator system (Chapman E and Wong C H (2002) Bioorg Med Chem. 10:551-5).

[0069] Apoptosis assays. Apoptosis or programmed cell death is a suicide program is activated within the cell, leading to fragmentation of DNA, shrinkage of the cytoplasm, membrane changes and cell death. Apoptosis is mediated by proteolytic enzymes of the caspase family. Many of the altering parameters of a cell are measurable during apoptosis. Assays for apoptosis may be performed by terminal deoxynucleotidyl transferase-mediated digoxigenin-11-dUTP nick end labeling (TUNEL) assay. The TUNEL assay is used to measure nuclear DNA fragmentation characteristic of apoptosis (Lazebnik et al., 1994, Nature 371, 346), by following the incorporation of fluorescein-dUTP (Yonehara et al., 1989, J. Exp. Med. 169, 1747). Apoptosis may further be assayed by acridine orange staining of tissue culture cells (Lucas, R., et al., 1998, Blood 15:4730-41). Other cell-based apoptosis assays include the caspase-3/7 assay and the cell death nucleosome ELISA assay. The caspase 3/7 assay is based on the activation of the caspase cleavage activity as part of a cascade of events that occur during programmed cell death in many apoptotic pathways. In the caspase 3/7 assay (commercially available Apo-ONE™ Homogeneous Caspase-3/7 assay from Promega, cat# 67790), lysis buffer and caspase substrate are mixed and added to cells. The caspase substrate becomes fluorescent when cleaved by active caspase 3/7. The nucleosome ELISA assay is a general cell death assay known to those skilled in the art, and available commercially (Roche, Cat# 1774425). This assay is a quantitative sandwich-enzyme-immunoassay which uses monoclonal antibodies directed against DNA and histones respectively, thus specifically determining amount of mono- and oligonucleo-

somes in the cytoplasmic fraction of cell lysates. Mono and oligonucleosomes are enriched in the cytoplasm during apoptosis due to the fact that DNA fragmentation occurs several hours before the plasma membrane breaks down, allowing for accumulation in the cytoplasm. Nucleosomes are not present in the cytoplasmic fraction of cells that are not undergoing apoptosis. The Phospho-histone H2B assay is another apoptosis assay, based on phosphorylation of histone H2B as a result of apoptosis. Fluorescent dyes that are associated with phosphohistone H2B may be used to measure the increase of phosphohistone H2B as a result of apoptosis. Apoptosis assays that simultaneously measure multiple parameters associated with apoptosis have also been developed. In such assays, various cellular parameters that can be associated with antibodies or fluorescent dyes, and that mark various stages of apoptosis are labeled, and the results are measured using instruments such as Cellomics™ ArrayScan® HCS System. The measurable parameters and their markers include anti-active caspase-3 antibody which marks intermediate stage apoptosis, anti-PARP-p85 antibody (cleaved PARP) which marks late stage apoptosis, Hoechst labels which label the nucleus and are used to measure nuclear swelling as a measure of early apoptosis and nuclear condensation as a measure of late apoptosis, TOTO-3 fluorescent dye which labels DNA of dead cells with high cell membrane permeability, and anti-alpha-tubulin or F-actin labels, which assess cytoskeletal changes in cells and correlate well with TOTO-3 label.

[0070] An apoptosis assay system may comprise a cell that expresses an ITPK, and that optionally has defective IGFR function (e.g. IGFR is over-expressed or under-expressed relative to wild-type cells). A test agent can be added to the apoptosis assay system and changes in induction of apoptosis relative to controls where no test agent is added, identify candidate IGFR modulating agents. In some embodiments of the invention, an apoptosis assay may be used as a secondary assay to test a candidate IGFR modulating agents that is initially identified using a cell-free assay system. An apoptosis assay may also be used to test whether ITPK function plays a direct role in apoptosis. For example, an apoptosis assay may be performed on cells that over- or under-express ITPK relative to wild type cells. Differences in apoptotic response compared to wild type cells suggests that the ITPK plays a direct role in the apoptotic response. Apoptosis assays are described further in U.S. Pat. No. 6,133,437.

[0071] Cell proliferation and cell cycle assays. Cell proliferation may be assayed via bromodeoxyuridine (BRDU) incorporation. This assay identifies a cell population undergoing DNA synthesis by incorporation of BRDU into newly-synthesized DNA. Newly-synthesized DNA may then be detected using an anti-BRDU antibody (Hoshino et al., 1986, Int. J. Cancer 38, 369; Campana et al., 1988, J. Immunol. Meth. 107, 79), or by other means.

[0072] Cell proliferation is also assayed via phosphohistone H3 staining, which identifies a cell population undergoing mitosis by phosphorylation of histone H3. Phosphorylation of histone H3 at serine 10 is detected using an antibody specific to the phosphorylated form of the serine 10 residue of histone H3. (Chadlee, D. N. 1995, J. Biol. Chem 270:20098-105). Cell Proliferation may also be examined using [$^3$H]-thymidine incorporation (Chen, J., 1996, Oncogene 13:1395-403; Jeoung, J., 1995, J. Biol. Chem. 270:18367-73). This assay allows for quantitative characterization of S-phase DNA syntheses. In this assay, cells synthesizing DNA will incorporate [$^3$H]-thymidine into newly synthesized DNA. Incorporation can then be measured by standard techniques such as by counting of radioisotope in a scintillation counter (e.g., Beckman L S 3800 Liquid Scintillation Counter). Another proliferation assay uses the dye Alamar Blue (available from Biosource International), which fluoresces when reduced in living cells and provides an indirect measurement of cell number (Voytik-Harbin S L et al., 1998, In Vitro Cell Dev Biol Anim 34:239-46). Yet another proliferation assay, the MTS assay, is based on in vitro cytotoxicity assessment of industrial chemicals, and uses the soluble tetrazolium salt, MTS. MTS assays are commercially available, for example, the Promega CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay (Cat.# G5421).

[0073] Cell proliferation may also be assayed by colony formation in soft agar, or clonogenic survival assay (Sambrook et al., Molecular Cloning, Cold Spring Harbor (1989)). For example, cells transformed with ITPK are seeded in soft agar plates, and colonies are measured and counted after two weeks incubation.

[0074] Cell proliferation may also be assayed by measuring ATP levels as indicator of metabolically active cells. Such assays are commercially available, for example Cell Titer-Glo™, which is a luminescent homogeneous assay available from Promega.

[0075] Involvement of a gene in the cell cycle may be assayed by flow cytometry (Gray J W et al. (1986) Int J Radiat Biol Relat Stud Phys Chem Med 49:237-55). Cells transfected with an ITPK may be stained with propidium iodide and evaluated in a flow cytometer (available from Becton Dickinson), which indicates accumulation of cells in different stages of the cell cycle.

[0076] Involvement of a gene in cell cycle may also be assayed by FOXO nuclear translocation assays. The FOXO family of transcription factors are mediators of various cellular functions including cell cycle progression and cell death, and are negatively regulated by activation of the PI3 kinase pathway. Akt phosphorylation of FOXO family members leads to FOXO sequestration in the cytoplasm and transcriptional inactivation (Medema, R. H et al (2000) Nature 404: 782-787). PTEN is a negative regulator of P13 kinase pathway. Activation of PTEN, or loss of P13 kinase or AKT, prevents phosphorylation of FOXO, leading to accumulation of FOXO in the nucleus, transcriptional activation of FOXO regulated genes, and apoptosis. Alternatively, loss of PTEN leads to pathway activation and cell survival (Nakamura, N. et al (2000) Mol Cell Biol 20: 8969-8982). FOXO translocation into the cytoplasm is used in assays and screens to identify members and/or modulators of the PTEN pathway. FOXO translocation assays using GFP or luciferase as detection reagents are known in the art (e.g., Zhang X et al (2002) J Biol Chem 277:45276-45284; and Li et al (2003) Mol Cell Biol 23:104-118).

[0077] Accordingly, a cell proliferation or cell cycle assay system may comprise a cell that expresses an ITPK, and that optionally has defective IGFR function (e.g. IGFR is over-expressed or under-expressed relative to wild-type cells). A test agent can be added to the assay system and changes in cell proliferation or cell cycle relative to controls where no

test agent is added, identify candidate IGFR modulating agents. In some embodiments of the invention, the cell proliferation or cell cycle assay may be used as a secondary assay to test a candidate IGFR modulating agents that is initially identified using another assay system such as a cell-free assay system. A cell proliferation assay may also be used to test whether ITPK function plays a direct role in cell proliferation or cell cycle. For example, a cell proliferation or cell cycle assay may be performed on cells that over- or under-express ITPK relative to wild type cells. Differences in proliferation or cell cycle compared to wild type cells suggests that the ITPK plays a direct role in cell proliferation or cell cycle.

[0078] Angiogenesis. Angiogenesis may be assayed using various human endothelial cell systems, such as umbilical vein, coronary artery, or dermal cells. Suitable assays include Alamar Blue based assays (available from Biosource International) to measure proliferation; migration assays using fluorescent molecules, such as the use of Becton Dickinson Falcon HTS FluoroBlock cell culture inserts to measure migration of cells through membranes in presence or absence of angiogenesis enhancer or suppressors; and tubule formation assays based on the formation of tubular structures by endothelial cells on Matrigel® (Becton Dickinson). Accordingly, an angiogenesis assay system may comprise a cell that expresses an ITPK, and that optionally has defective IGFR function (e.g. IGFR is over-expressed or under-expressed relative to wild-type cells). A test agent can be added to the angiogenesis assay system and changes in angiogenesis relative to controls where no test agent is added, identify candidate IGFR modulating agents. In some embodiments of the invention, the angiogenesis assay may be used as a secondary assay to test a candidate IGFR modulating agents that is initially identified using another assay system. An angiogenesis assay may also be used to test whether ITPK function plays a direct role in cell proliferation. For example, an angiogenesis assay may be performed on cells that over- or under-express ITPK relative to wild type cells. Differences in angiogenesis compared to wild type cells suggests that the ITPK plays a direct role in angiogenesis. U.S. Pat. Nos. 5,976,782, 6,225,118 and 6,444,434, among others, describe various angiogenesis assays.

[0079] Hypoxic induction. The alpha subunit of the transcription factor, hypoxia inducible factor-1 (HIF-1), is upregulated in tumor cells following exposure to hypoxia in vitro. Under hypoxic conditions, HIF-1 stimulates the expression of genes known to be important in tumour cell survival, such as those encoding glyolytic enzymes and VEGF. Induction of such genes by hypoxic conditions may be assayed by growing cells transfected with ITPK in hypoxic conditions (such as with 0.1% O2, 5% CO2, and balance N2, generated in a Napco 7001 incubator (Precision Scientific)) and normoxic conditions, followed by assessment of gene activity or expression by Taqman®. For example, a hypoxic induction assay system may comprise a cell that expresses an ITPK, and that optionally has defective IGFR function (e.g. IGFR is over-expressed or under-expressed relative to wild-type cells). A test agent can be added to the hypoxic induction assay system and changes in hypoxic response relative to controls where no test agent is added, identify candidate IGFR modulating agents. In some embodiments of the invention, the hypoxic induction assay may be used as a secondary assay to test a candidate IGFR modulating agents that is initially identified using another assay system. A hypoxic induction assay may also be used to test whether ITPK function plays a direct role in the hypoxic response. For example, a hypoxic induction assay may be performed on cells that over- or under-express ITPK relative to wild type cells. Differences in hypoxic response compared to wild type cells suggests that the ITPK plays a direct role in hypoxic induction.

[0080] Cell adhesion. Cell adhesion assays measure adhesion of cells to purified adhesion proteins, or adhesion of cells to each other, in presence or absence of candidate modulating agents. Cell-protein adhesion assays measure the ability of agents to modulate the adhesion of cells to purified proteins. For example, recombinant proteins are produced, diluted to 2.5 g/mL in PBS, and used to coat the wells of a microtiter plate. The wells used for negative control are not coated. Coated wells are then washed, blocked with 1% BSA, and washed again. Compounds are diluted to 2×final test concentration and added to the blocked, coated wells. Cells are then added to the wells, and the unbound cells are washed off. Retained cells are labeled directly on the plate by adding a membrane-permeable fluorescent dye, such as calcein-AM, and the signal is quantified in a fluorescent microplate reader.

[0081] Cell-cell adhesion assays measure the ability of agents to modulate binding of cell adhesion proteins with their native ligands. These assays use cells that naturally or recombinantly express the adhesion protein of choice. In an exemplary assay, cells expressing the cell adhesion protein are plated in wells of a multiwell plate. Cells expressing the ligand are labeled with a membrane-permeable fluorescent dye, such as BCECF, and allowed to adhere to the monolayers in the presence of candidate agents. Unbound cells are washed off, and bound cells are detected using a fluorescence plate reader.

[0082] High-throughput cell adhesion assays have also been described. In one such assay, small molecule ligands and peptides are bound to the surface of microscope slides using a microarray spotter, intact cells are then contacted with the slides, and unbound cells are washed off. In this assay, not only the binding specificity of the peptides and modulators against cell lines are determined, but also the functional cell signaling of attached cells using immunofluorescence techniques in situ on the microchip is measured (Falsey J R et al., Bioconjug Chem. May-June 2001;12(3):346-53).

[0083] Tubulogenesis. Tubulogenesis assays monitor the ability of cultured cells, generally endothelial cells, to form tubular structures on a matrix substrate, which generally simulates the environment of the extracellular matrix. Exemplary substrates include Matrigel™ (Becton Dickinson), an extract of basement membrane proteins containing laminin, collagen IV, and heparin sulfate proteoglycan, which is liquid at 4° C. and forms a solid gel at 37° C. Other suitable matrices comprise extracellular components such as collagen, fibronectin, and/or fibrin. Cells are stimulated with a pro-angiogenic stimulant, and their ability to form tubules is detected by imaging. Tubules can generally be detected after an overnight incubation with stimuli, but longer or shorter time frames may also be used. Tube formation assays are well known in the art (e.g., Jones M K et al., 1999, Nature Medicine 5:1418-1423). These assays have tradition-

ally involved stimulation with serum or with the growth factors FGF or VEGF. Serum represents an undefined source of growth factors. In a preferred embodiment, the assay is performed with cells cultured in serum free medium, in order to control which process or pathway a candidate agent modulates. Moreover, we have found that different target genes respond differently to stimulation with different pro-angiogenic agents, including inflammatory angiogenic factors such as TNF-alpa. Thus, in a further preferred embodiment, a tubulogenesis assay system comprises testing an ITPK's response to a variety of factors, such as FGF, VEGF, phorbol myristate acetate (PMA), TNF-alpha, ephrin, etc.

[0084] Cell Migration. An invasion/migration assay (also called a migration assay) tests the ability of cells to overcome a physical barrier and to migrate towards pro-angiogenic signals. Migration assays are known in the art (e.g., Paik J H et al., 2001, J Biol Chem 276:11830-11837). In a typical experimental set-up, cultured endothelial cells are seeded onto a matrix-coated porous lamina, with pore sizes generally smaller than typical cell size. The matrix generally simulates the environment of the extracellular matrix, as described above. The lamina is typically a membrane, such as the transwell polycarbonate membrane (Corning Costar Corporation, Cambridge, Mass.), and is generally part of an upper chamber that is in fluid contact with a lower chamber containing pro-angiogenic stimuli. Migration is generally assayed after an overnight incubation with stimuli, but longer or shorter time frames may also be used. Migration is assessed as the number of cells that crossed the lamina, and may be detected by staining cells with hemotoxylin solution (VWR Scientific, South San Francisco, Calif.), or by any other method for determining cell number. In another exemplary set up, cells are fluorescently labeled and migration is detected using fluorescent readings, for instance using the Falcon HTS FluoroBlok (Becton Dickinson). While some migration is observed in the absence of stimulus, migration is greatly increased in response to pro-angiogenic factors. As described above, a preferred assay system for migration/invasion assays comprises testing an ITPK's response to a variety of pro-angiogenic factors, including tumor angiogenic and inflammatory angiogenic agents, and culturing the cells in serum free medium.

[0085] Sprouting assay. A sprouting assay is a three-dimensional in vitro angiogenesis assay that uses a cell-number defined spheroid aggregation of endothelial cells ("spheroid"), embedded in a collagen gel-based matrix. The spheroid can serve as a starting point for the sprouting of capillary-like structures by invasion into the extracellular matrix (termed "cell sprouting") and the subsequent formation of complex anastomosing networks (Korff and Augustin, 1999, J Cell Sci 112:3249-58). In an exemplary experimental set-up, spheroids are prepared by pipetting 400 human umbilical vein endothelial cells into individual wells of a nonadhesive 96-well plates to allow overnight spheroidal aggregation (Korff and Augustin: J Cell Biol 143: 1341-52, 1998). Spheroids are harvested and seeded in 900 μl of methocel-collagen solution and pipetted into individual wells of a 24 well plate to allow collagen gel polymerization. Test agents are added after 30 min by pipetting 100 μl of 10-fold concentrated working dilution of the test substances on top of the gel. Plates are incubated at 37° C. for 24 h. Dishes are fixed at the end of the experimental incubation period by addition of paraformaldehyde. Sprouting intensity of endothelial cells can be quantitated by an automated image analysis system to determine the cumulative sprout length per spheroid.

Primary Assays for Antibody Modulators

[0086] For antibody modulators, appropriate primary assays test is a binding assay that tests the antibody's affinity to and specificity for the ITPK protein. Methods for testing antibody affinity and specificity are well known in the art (Harlow and Lane, 1988, 1999, supra). The enzyme-linked immunosorbant assay (ELISA) is a preferred method for detecting ITPK-specific antibodies; others include FACS assays, radioimmunoassays, and fluorescent assays.

[0087] In some cases, screening assays described for small molecule modulators may also be used to test antibody modulators.

Primary assays for Nucleic Acid Modulators

[0088] For nucleic acid modulators, primary assays may test the ability of the nucleic acid modulator to inhibit or enhance ITPK gene expression, preferably mRNA expression. In general, expression analysis comprises comparing ITPK expression in like populations of cells (e.g., two pools of cells that endogenously or recombinantly express ITPK) in the presence and absence of the nucleic acid modulator. Methods for analyzing mRNA and protein expression are well known in the art. For instance, Northern blotting, slot blotting, ribonuclease protection, quantitative RT-PCR (e.g., using the TaqMan®, PE Applied Biosystems), or microarray analysis may be used to confirm that ITPK mRNA expression is reduced in cells treated with the nucleic acid modulator (e.g., Current Protocols in Molecular Biology (1994) Ausubel F M et al., eds., John Wiley & Sons, Inc., chapter 4; Freeman W M et al., Biotechniques (1999) 26:112-125; Kallioniemi O P, Ann Med 2001, 33:142-147; Blohm D H and Guiseppi-Elie, A Curr Opin Biotechnol 2001, 12:41-47). Protein expression may also be monitored. Proteins are most commonly detected with specific antibodies or antisera directed against either the ITPK protein or specific peptides. A variety of means including Western blotting, ELISA, or in situ detection, are available (Harlow E and Lane D, 1988 and 1999, supra).

[0089] In some cases, screening assays described for small molecule modulators, particularly in assay systems that involve ITPK mRNA expression, may also be used to test nucleic acid modulators.

Secondary Assays

[0090] Secondary assays may be used to further assess the activity of ITPK-modulating agent identified by any of the above methods to confirm that the modulating agent affects ITPK in a manner relevant to the IGFR pathway. As used herein, ITPK-modulating agents encompass candidate clinical compounds or other agents derived from previously identified modulating agent. Secondary assays can also be used to test the activity of a modulating agent on a particular genetic or biochemical pathway or to test the specificity of the modulating agent's interaction with ITPK.

[0091] Secondary assays generally compare like populations of cells or animals (e.g., two pools of cells or animals that endogenously or recombinantly express ITPK) in the presence and absence of the candidate modulator. In general, such assays test whether treatment of cells or animals with

a candidate ITPK-modulating agent results in changes in the IGFR pathway in comparison to untreated (or mock- or placebo-treated) cells or animals. Certain assays use "sensitized genetic backgrounds", which, as used herein, describe cells or animals engineered for altered expression of genes in the IGFR or interacting pathways.

Cell-Based Assays

[0092] Cell based assays may detect endogenous IGFR pathway activity or may rely on recombinant expression of IGFR pathway components. Any of the aforementioned assays may be used in this cell-based format. Candidate modulators are typically added to the cell media but may also be injected into cells or delivered by any other efficacious means.

Animal Assays

[0093] A variety of non-human animal models of normal or defective IGFR pathway may be used to test candidate ITPK modulators. Models for defective IGFR pathway typically use genetically modified animals that have been engineered to mis-express (e.g., over-express or lack expression in) genes involved in the IGFR pathway. Assays generally require systemic delivery of the candidate modulators, such as by oral administration, injection, etc.

[0094] In a preferred embodiment, IGFR pathway activity is assessed by monitoring neovascularization and angiogenesis. Animal models with defective and normal IGFR are used to test the candidate modulator's affect on ITPK in Matrigel® assays. Matrigel® is an extract of basement membrane proteins, and is composed primarily of laminin, collagen IV, and heparin sulfate proteoglycan. It is provided as a sterile liquid at 4° C., but rapidly forms a solid gel at 37° C. Liquid Matrigel® is mixed with various angiogenic agents, such as bFGF and VEGF, or with human tumor cells which over-express the ITPK. The mixture is then injected subcutaneously(SC) into female athymic nude mice (Taconic, Germantown, N.Y.) to support an intense vascular response. Mice with Matrigel® pellets may be dosed via oral (PO), intraperitoneal (IP), or intravenous (IV) routes with the candidate modulator. Mice are euthanized 5 -12 days post-injection, and the Matrigel® pellet is harvested for hemoglobin analysis (Sigma plasma hemoglobin kit). Hemoglobin content of the gel is found to correlate the degree of neovascularization in the gel.

[0095] In another preferred embodiment, the effect of the candidate modulator on ITPK is assessed via tumorigenicity assays. Tumor xenograft assays are known in the art (see, e.g., Ogawa K et al., 2000, Oncogene 19:6043-6052). Xenografts are typically implanted SC into female athymic mice, 6-7 week old, as single cell suspensions either from a pre-existing tumor or from in vitro culture. The tumors which express the ITPK endogenously are injected in the flank, $1\times10^5$ to $1\times10^7$ cells per mouse in a volume of 100 μL using a 27 gauge needle. Mice are then ear tagged and tumors are measured twice weekly. Candidate modulator treatment is initiated on the day the mean tumor weight reaches 100 mg. Candidate modulator is delivered IV, SC, IP, or PO by bolus administration. Depending upon the pharmacokinetics of each unique candidate modulator, dosing can be performed multiple times per day. The tumor weight is assessed by measuring perpendicular diameters with a caliper and calculated by multiplying the measurements of diameters in two dimensions. At the end of the experiment, the excised tumors maybe utilized for biomarker identification or further analyses. For immunohistochemistry staining, xenograft tumors are fixed in 4% paraformaldehyde, 0.1M phosphate, pH 7.2, for 6 hours at 4° C., immersed in 30% sucrose in PBS, and rapidly frozen in isopentane cooled with liquid nitrogen.

[0096] In another preferred embodiment, tumorogenicity is monitored using a hollow fiber assay, which is described in U.S. Pat No. 5,698,413. Briefly, the method comprises implanting into a laboratory animal a biocompatible, semipermeable encapsulation device containing target cells, treating the laboratory animal with a candidate modulating agent, and evaluating the target cells for reaction to the candidate modulator. Implanted cells are generally human cells from a pre-existing tumor or a tumor cell line. After an appropriate period of time, generally around six days, the implanted samples are harvested for evaluation of the candidate modulator. Tumorogenicity and modulator efficacy may be evaluated by assaying the quantity of viable cells present in the macrocapsule, which can be determined by tests known in the art, for example, MTT dye conversion assay, neutral red dye uptake, trypan blue staining, viable cell counts, the number of colonies formed in soft agar, the capacity of the cells to recover and replicate in vitro, etc.

[0097] In another preferred embodiment, a tumorogenicity assay use a transgenic animal, usually a mouse, carrying a dominant oncogene or tumor suppressor gene knockout under the control of tissue specific regulatory sequences; these assays are generally referred to as transgenic tumor assays. In a preferred application, tumor development in the transgenic model is well characterized or is controlled. In an exemplary model, the "RIP1-Tag2" transgene, comprising the SV40 large T-antigen oncogene under control of the insulin gene regulatory regions is expressed in pancreatic beta cells and results in islet cell carcinomas (Hanahan D, 1985, Nature 315:115-122; Parangi S et al, 1996, Proc Natl Acad Sci USA 93: 2002-2007; Bergers G et al, 1999, Science 284:808-812). An "angiogenic switch," occurs at approximately five weeks, as normally quiescent capillaries in a subset of hyperproliferative islets become angiogenic. The RIP1-TAG2 mice die by age 14 weeks. Candidate modulators may be administered at a variety of stages, including just prior to the angiogenic switch (e.g., for a model of tumor prevention), during the growth of small tumors (e.g., for a model of intervention), or during the growth of large and/or invasive tumors (e.g., for a model of regression). Tumorogenicity and modulator efficacy can be evaluating life-span extension and/or tumor characteristics, including number of tumors, tumor size, tumor morphology, vessel density, apoptotic index, etc.

Diagnostic and Therapeutic Uses

[0098] Specific ITPK-modulating agents are useful in a variety of diagnostic and therapeutic applications where disease or disease prognosis is related to defects in the IGFR pathway, such as angiogenic, apoptotic, or cell proliferation disorders. Accordingly, the invention also provides methods for modulating the IGFR pathway in a cell, preferably a cell pre-determined to have defective or impaired IGFR function (e.g. due to overexpression, underexpression, or misexpression of IGFR, or due to gene mutations), comprising the step of administering an agent to the cell that specifically modu-

lates ITPK activity. Preferably, the modulating agent produces a detectable phenotypic change in the cell indicating that the IGFR function is restored. The phrase "function is restored", and equivalents, as used herein, means that the desired phenotype is achieved, or is brought closer to normal compared to untreated cells. For example, with restored IGFR function, cell proliferation and/or progression through cell cycle may normalize, or be brought closer to normal relative to untreated cells. The invention also provides methods for treating disorders or disease associated with impaired IGFR function by administering a therapeutically effective amount of an ITPK -modulating agent that modulates the IGFR pathway. The invention further provides methods for modulating ITPK function in a cell, preferably a cell pre-determined to have defective or impaired ITPK function, by administering an ITPK -modulating agent. Additionally, the invention provides a method for treating disorders or disease associated with impaired ITPK function by administering a therapeutically effective amount of an ITPK -modulating agent.

[0099] The discovery that ITPK is implicated in IGFR pathway provides for a variety of methods that can be employed for the diagnostic and prognostic evaluation of diseases and disorders involving defects in the IGFR pathway and for the identification of subjects having a predisposition to such diseases and disorders.

[0100] Various expression analysis methods can be used to diagnose whether ITPK expression occurs in a particular sample, including Northern blotting, slot blotting, ribonuclease protection, quantitative RT-PCR, and microarray analysis. (e.g., Current Protocols in Molecular Biology (1994) Ausubel F M et al., eds., John Wiley & Sons, Inc., chapter 4; Freeman W M et al., Biotechniques (1999) 26:112-125; Kallioniemi O P, Ann Med 2001, 33:142-147; Blohm and Guiseppi-Elie, Curr Opin Biotechnol 2001, 12:41-47). Tissues having a disease or disorder implicating defective IGFR signaling that express an ITPK, are identified as amenable to treatment with an ITPK modulating agent. In a preferred application, the IGFR defective tissue overexpresses an ITPK relative to normal tissue. For example, a Northern blot analysis of mRNA from tumor and normal cell lines, or from tumor and matching normal tissue samples from the same patient, using full or partial ITPK cDNA sequences as probes, can determine whether particular tumors express or overexpress ITPK. Alternatively, the TaqMan® is used for quantitative RT-PCR analysis of ITPK expression in cell lines, normal tissues and tumor samples (PE Applied Biosystems).

[0101] Various other diagnostic methods may be performed, for example, utilizing reagents such as the ITPK oligonucleotides, and antibodies directed against an ITPK, as described above for: (1) the detection of the presence of ITPK gene mutations, or the detection of either over- or under-expression of ITPK mRNA relative to the non-disorder state; (2) the detection of either an over- or an under-abundance of ITPK gene product relative to the non-disorder state; and (3) the detection of perturbations or abnormalities in the signal transduction pathway mediated by ITPK.

[0102] Kits for detecting expression of ITPK in various samples, comprising at least one antibody specific to ITPK, all reagents and/or devices suitable for the detection of antibodies, the immobilization of antibodies, and the like, and instructions for using such kits in diagnosis or therapy are also provided.

[0103] Thus, in a specific embodiment, the invention is drawn to a method for diagnosing a disease or disorder in a patient that is associated with alterations in ITPK expression, the method comprising: a) obtaining a biological sample from the patient; b) contacting the sample with a probe for ITPK expression; c) comparing results from step (b) with a control; and d) determining whether step (c) indicates a likelihood of the disease or disorder. Preferably, the disease is cancer, most preferably a cancer as shown in TABLE 1. The probe may be either DNA or protein, including an antibody.

## EXAMPLES

[0104] The following experimental section and examples are offered by way of illustration and not by way of limitation.

### I. IGFR Overexpression Screen

[0105] A dominant loss of function screen was carried out in *Drosophila* to identify genes that interact with or modulate the IGFR signaling pathway. Activation of the pathway by overexpression of IGFR at early stages in the developing *Drosophila* eye leads to an increase in cell number which results in a larger and rougher adult eye (Potter C J et al. (2001) Cell 105:357-368; Huang et al., 1999. Dev. 126:5365-5372). We generated a fly stock with an enlarged eye due to overexpression of IGFR and identified modifiers of this phenotype. We then identified human orthologues of these modifiers.

[0106] The screening stock carried two transgenes. The genotype is as follows:

[0107] +; +; P{DmIGFR-pExp-UAS)} P{Gal4-pExp-1Xey}/TM6B

[0108] Screening stock females of the above genotype were crossed to males from a collection of 3 classes of piggyBac-based transposons. The resulting progeny, which contain both the transgenes and the transposon, were scored for the effect of the transposon on the eye overgrowth phenotype (either enhancement, suppression or no effect). All data was recorded and all modifiers were retested with a repeat of the original cross. CG4026 (IP3K1) was a suppressor of the eye phenotype. Orthologs of the modifiers are referred to herein as ITPK.

[0109] BLAST analysis (Altschul et al., supra) was employed to identify orthologs of *Drosophila* modifiers. For example, representative sequences from ITPK, GI#s 4504789 (SEQ ID NO:8), 38569400 (SEQ ID NO:9) and GI# 18643383 (SEQ ID NO:10) share 45%, 38%, and 40% amino acid identity, respectively, with the *Drosopila* CG4026 (IP3K1).].

[0110] Various domains, signals, and functional subunits in proteins were analyzed using the PSORT (Nakai K., and Horton P., Trends Biochem Sci, 1999, 24:34-6; Kenta Nakai, Protein sorting signals and prediction of subcellular localization, Adv. Protein Chem. 54, 277-344 (2000)), PFAM (Bateman A., et al., Nucleic Acids Res, 1999, 27:260-2), SMART (Ponting C P, et al., SMART: identification and annotation of domains from signaling and extracellular

protein sequences. Nucleic Acids Res. Jan. 1, 1999;27(1):229-32), TM-HMM (Erik L. L. Sonnhammer, Gunnar von Heijne, and Anders Krogh: A hidden Markov model for predicting transmembrane helices in protein sequences. In Proc. of Sixth Int. Conf. on Intelligent Systems for Molecular Biology, p 175-182 Ed J. Glasgow, T. Littlejohn, F. Major, R. Lathrop, D. Sankoff, and C. Sensen Menlo Park, Calif.: AAAI Press, 1998), and clust (Remm M, and Sonnhammer E. Classification of transmembrane protein families in the Caenorhabditis elegans genome and identification of human orthologs. Genome Res. November 2000;10(11):1679-89) programs. For example, the Inositol polyphosphate kinase domain (PFAM 03770) of ITPK from each of GI#s 4504789, 38569400, and 18643383 (SEQ ID NOs:8, 9, and 10, respectively) is located respectively at approximately amino acid residues 171 to 456, 652 to 937, and 393 to 678.

II. High-Throughput In Vitro Fluorescence Polarization Assay

[0111] Fluorescently-labeled ITPK peptide/substrate are added to each well of a 96-well microtiter plate, along with a test agent in a test buffer (10 mM HEPES, 10 mM NaCl, 6 mM magnesium chloride, pH 7.6). Changes in fluorescence polarization, determined by using a Fluorolite FPM-2 Fluorescence Polarization Microtiter System (Dynatech Laboratories, Inc), relative to control values indicates the test compound is a candidate modifier of ITPK activity.

III. High-Throughput In Vitro Binding Assay.

[0112] $^{33}$P-labeled ITPK peptide is added in an assay buffer (100 mM KCl, 20 mM HEPES pH 7.6, 1 mM $MgCl_2$, 1% glycerol, 0.5% NP-40, 50 mM beta-mercaptoethanol, 1 mg/ml BSA, cocktail of protease inhibitors) along with a test agent to the wells of a Neutralite-avidin coated assay plate and incubated at 25° C. for 1 hour. Biotinylated substrate is then added to each well and incubated for 1 hour. Reactions are stopped by washing with PBS, and counted in a scintillation counter. Test agents that cause a difference in activity relative to control without test agent are identified as candidate IGFR modulating agents.

IV. Immunoprecipitations and Immunoblotting

[0113] For coprecipitation of transfected proteins, $3\times10^6$ appropriate recombinant cells containing the ITPK proteins are plated on 10-cm dishes and transfected on the following day with expression constructs. The total amount of DNA is kept constant in each transfection by adding empty vector. After 24 h, cells are collected, washed once with phosphate-buffered saline and lysed for 20 min on ice in 1 ml of lysis buffer containing 50 mM Hepes, pH 7.9, 250 mM NaCl, 20 mM-glycerophosphate, 1 mM sodium orthovanadate, 5 mM p-nitrophenyl phosphate, 2 mM dithiothreitol, protease inhibitors (complete, Roche Molecular Biochemicals), and 1% Nonidet P-40. Cellular debris is removed by centrifugation twice at 15,000×g for 15 min. The cell lysate is incubated with 25 μl of M2 beads (Sigma) for 2 h at 4° C. with gentle rocking.

[0114] After extensive washing with lysis buffer, proteins bound to the beads are solubilized by boiling in SDS sample buffer, fractionated by SDS-polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membrane and blotted with the indicated antibodies. The reactive bands are visualized with horseradish peroxidase coupled to the appropriate secondary antibodies and the enhanced chemiluminescence (ECL) Western blotting detection system (Amersham Pharmacia Biotech).

V. Kinase Assay

[0115] A purified or partially purified ITPK is diluted in a suitable reaction buffer, e.g., 50 mM Hepes, pH 7.5, containing magnesium chloride or manganese chloride (1-20 mM) and a peptide or polypeptide substrate, such as myelin basic protein or casein (1-10 μg/ml). The final concentration of the kinase is 1-20 mM. The enzyme reaction is conducted in microtiter plates to facilitate optimization of reaction conditions by increasing assay throughput. A 96-well microtiter plate is employed using a final volume 30-100 μl. The reaction is initiated by the addition of $^{33}$P-gamma-ATP (0.5 μCi/ml) and incubated for 0.5 to 3 hours at room temperature. Negative controls are provided by the addition of EDTA, which chelates the divalent cation ($Mg2^+$ or $Mn^{2+}$) required for enzymatic activity. Following the incubation, the enzyme reaction is quenched using EDTA. Samples of the reaction are transferred to a 96-well glass fiber filter plate (MultiScreen, Millipore). The filters are subsequently washed with phosphate-buffered saline, dilute phosphoric acid (0.5%) or other suitable medium to remove excess radiolabeled ATP. Scintillation cocktail is added to the filter plate and the incorporated radioactivity is quantitated by scintillation counting (Wallac/Perkin Elmer). Activity is defined by the amount of radioactivity detected following subtraction of the negative control reaction value (EDTA quench).

VI. Expression analysis

[0116] All cell lines used in the following experiments are NCI (National Cancer Institute) lines, and are available from ATCC (American Type Culture Collection, Manassas, Va. 20110-2209). Normal and tumor tissues were obtained from Impath, U C Davis, Clontech, Stratagene, Ardais, Genome Collaborative, and Ambion.

[0117] TaqMan ® analysis was used to assess expression levels of the disclosed genes in various samples.

[0118] RNA was extracted from each tissue sample using Qiagen (Valencia, Calif.) RNeasy kits, following manufacturer's protocols, to a final concentration of 50 ng/μl. Single stranded cDNA was then synthesized by reverse transcribing the RNA samples using random hexamers and 500 ng of total RNA per reaction, following protocol 4304965 of Applied Biosystems (Foster City, Calif.).

[0119] Primers for expression analysis using TaqMan® assay (Applied Biosystems, Foster City, Calif.) were prepared according to the TaqMan® protocols, and the following criteria: a) primer pairs were designed to span introns to eliminate genomic contamination, and b) each primer pair produced only one product. Expression analysis was performed using a 7900 HT instrument.

[0120] TaqMan® reactions were carried out following manufacturer's protocols, in 25 μl total volume for 96-well plates and 10 μl total volume for 384-well plates, using 300 nM primer and 250 nM probe, and approximately 25 ng of cDNA. The standard curve for result analysis was prepared using a universal pool of human cDNA samples, which is a mixture of cDNAs from a wide variety of tissues so that the chance that a target will be present in appreciable amounts

is good. The raw data were normalized using 18 S rRNA (universally expressed in all tissues and cells).

[0121] For each expression analysis, tumor tissue samples were compared with matched normal tissues from the same patient. A gene was considered overexpressed in a tumor when the level of expression of the gene was 2 fold or higher in the tumor compared with its matched normal sample. In cases where normal tissue was not available, a universal pool of cDNA samples was used instead. In these cases, a gene was considered overexpressed in a tumor sample when the difference of expression levels between a tumor sample and the average of all normal samples from the same tissue type was greater than 2 times the standard deviation of all normal samples (i.e., Tumor−average(all normal samples)>2×STDEV(all normal samples)).

[0122] Results are shown in Table 1. Number of pairs of tumor samples and matched normal tissue from the same patient are shown for each tumor type. Percentage of the samples with at least two-fold overexpression for each tumor type is provided. A modulator identified by an assay described herein can be further validated for therapeutic effect by administration to a tumor in which the gene is overexpressed. A decrease in tumor growth confirms therapeutic utility of the modulator. Prior to treating a patient with the modulator, the likelihood that the patient will respond to treatment can be diagnosed by obtaining a tumor sample from the patient, and assaying for expression of the gene targeted by the modulator. The expression data for the gene(s) can also be used as a diagnostic marker for disease progression. The assay can be performed by expression analysis as described above, by antibody directed to the gene target, or by any other available detection method.

TABLE 1

| | ITPKA (SEQ ID NO: 1) | ITPKB (SEQ ID NO: 3) | ITPKC (SEQ ID NO: 7) |
|---|---|---|---|
| Breast | 55% | 6% | 6% |
| # of Pairs | 33 | 36 | 36 |
| Colon | 12% | 8% | 10% |
| # of Pairs | 40 | 40 | 40 |
| Head And Neck | 85% | 23% | 15% |
| # of Pairs | 13 | 13 | 13 |
| Liver | 78% | 33% | 0% |
| # of Pairs | 9 | 9 | 9 |
| Lung | 64% | 5% | 8% |
| # of Pairs | 39 | 40 | 40 |
| Lymphoma | 0% | 25% | 0% |
| # of Pairs | 4 | 4 | 4 |
| Ovary | 42% | 21% | 11% |
| # of Pairs | 19 | 19 | 18 |
| Pancreas | 50% | 50% | 67% |
| # of Pairs | 12 | 12 | 12 |
| Prostate | 8% | 8% | 12% |
| # of Pairs | 24 | 24 | 24 |
| Skin | 57% | 43% | 0% |
| # of Pairs | 7 | 7 | 7 |
| Stomach | 18% | 27% | 27% |
| # of Pairs | 11 | 11 | 11 |
| Testis | 12% | 12% | 0% |
| # of Pairs | 8 | 8 | 8 |
| Thyroid Gland | 43% | 0% | 8% |
| # of Pairs | 14 | 14 | 13 |
| Uterus | 39% | 0% | 9% |
| # of Pairs | 23 | 23 | 23 |

VII. ITPK Functional Assays

[0123] RNAi experiments were carried out to knock down expression of ITPKA, ITPKB, and ITPKC (SEQ ID NOs:1, 4, and 7, respectively) in various cell lines using small interfering RNAs (siRNA, Elbashir et al, supra).

[0124] Effect of ITPK RNAi on cell proliferation and growth. BrdU and Cell Titer-Glo™ assays, as described above, were employed to study the effects of decreased ITPK expression on cell proliferation. The results of these experiments indicated that RNAi of ITPKA and IPTKC (SEQ ID Nos: 1 and 7) decreased proliferation in 231T breast cancer cells, A549 lung cancer cells, PC3 prostate cancer cells, and U87MG glioblastoma cells. RNAi of ITPKB (SEQ ID NO:4) decreased proliferation in 231T breast cancer cells, A549 lung cancer cells, and U87MG glioblastoma cells.

[0125] Standard colony growth assays, as described above, were employed to study the effects of decreased ITPK expression on cell growth and cell count. Results indicated that RNAi of SEQ ID NO:1 decreased cell count in 231T cells, A549 cells, PC3 cells, and A2780 ovarian cancer cells, and also decreased cell growth in A549 and A2780 cells. RNAi of SEQ ID NO:4 decreased cell count and cell growth in A549 cells. RNAi of SEQ ID NO:7 decreased cell count in 231T and A549 cells, and decreased cell growth in A549 cells.

[0126] [$^3$H]-thymidine incorporation assay, as described above, was also employed to study the effects of decreased ITPK expression on cell proliferation. The results of this experiment indicated that RNAi of ITPKA and ITPKB (SEQ ID Nos:1 and 4, respectively), decreased proliferation in A549 cells and RD1 rhabdomyosarcome cells, and RNAi of ITPKC (SEQ ID NO:7) decreased proliferation in A549, RD1 and A2780 cells.

[0127] Effect of ITPK RNAi on apoptosis. The Phosphohistone H2B assay, as described above, was employed to study the effects of decreased ITPK expression on apoptosis. The results of this experiment indicated that RNAi of ITPKA (SEQ ID NO:1) increased apoptosis in PC3 cells; RNAi of ITPKB (SEQ ID NO:4) increased apoptosis in U87MG cells; and RNAi of ITPKC (SEQ ID NO:7) increased apoptosis in 231T, A549, PC3, and U87MG cells.

[0128] Multiple paramater apoptosis assay, as described above, was also used to study the effects of decreased ITPK expression on apoptosis. The results of this experiment indicated that RNAi of each ITPKA, ITPKB, and ITPKC (SEQ ID Nos:1, 4, and 7, respectively) increased apoptosis in A549 and A2780 cells.

[0129] Involvement of ITPK in IGF pathway: ITPK FOXO nuclear translocation assays. FOXO nuclear translocation assays, as described above, were employed to assess involvement of ITPK in the PTEN/IGF pathway. In one set of experiments, cells with reduced expression of ITPK by RNAi were transiently transfected with a plasmid expressing GFP-tagged FOXO. Automated imaging of cellular compo-

nents, such as nucleus and cytoplasm were then carried out to assess translocation of FOXO. Results indicated that reduced expression of ITPKA and IPTKC (SEQ ID Nos:1 and 7) led to translocation of FOXO to the cytoplasm, similar to loss of PTEN in U20S osteosarcoma cells. In another set of experiments, cells were co-transfected with siRNA directed to ITPK along with a plasmid containing FOXO, and a cassette containing a promoter, a FOXO response element, and luciferase. Cells were then analyzed for luciferase activity and compared with cells with no siRNA. Results indicated that reduced expression of ITPKB (SEQ ID NO:4) led to translocation of FOXO to the cytoplasm in PC3 and A2780 cells, and reduced expression of ITPKC (SEQ ID NO:7) led to translocation of FOXO to the cytoplasm in A2780 cells. These results suggest involvement of ITPK in the PTEN/IGF pathway.

**[0130]** Pan-AKT assays. This assay was developed to detect involvement of ITPK in the PTEN/IGF pathway. The assay detects changes in phosphorylation for several substrates of AKT, such as PRAS40, BAD, 4EBP1, and RPS6. For this experiment, antibodies were raised against phosporylated AKT substrates, including the consensus phosphorylated AKT substrate sequence RxRxxS/T. Expression levels of phosphorylated substrates were then quantitated at normal levels, in presence of a negative control, a positive control (AKT), and then withreduced expression of ITPK. For example, when AKT levels were reduced, expression of all its substrates was also reduced. RNAi of ITPKa and ITPKB (SEQ ID NOs:1 and 4) decreased the level of phosphorylated AKT substrates in 231T and A549 cells. RNAi of ITPKC (SEQ ID NO:7) decreased the level of phosphorylated AKT substrates in 231T, PC3, and A549 cells.

**[0131]** We then used RPS6 assay for a subset of experiments. RPS6 is an IGF dependent substrate of AKT. IGF1 treatment increases cytoplasmic RPS6 levels. Alternatively, Lily compound LY294002, a PI3K inhibitor, reduces AKT and cytoplasmic RPS6 levels. Cells were plated in 96 well plates, transfected with RNAi for ITPK, fixed, treated with RPS6 antibody, and stained. Measurements were based on percentage of population of cells with increased or decreased staining compared with negative or positive control cells. Results of this experiment showed that RNAi of ITPKA (SEQ ID NO: 1) caused a reduction in the level of phosphorylated RPS6 in PC3 cells, and RNAi of ITPKC (SEQ ID NO:7) caused a reduction in the level of phosphorylated RPS6 in A549 cells.

**[0132]** We also used 4EBP1 as another specific substrate for another subset of experiments. For this substrate, AKT pathway inhibition causes decreased cytoplasmic staining and increased nuclear staining. Cells were plated in 96 well plates, transfected with RNAi for ITPK, fixed, treated with 4EBP1 antibody, and stained. Measurements were based on percentage of population of cells with increased or decreased nuclear/cytoplasmic staining ratio compared with negative or positive control cells. Results of this experiment showed that RNAi of ITPKA (SEQ ID NO:1) decreased the level of phopshorylated 4EBP1 in PC3 and A549 cells, while RNAi of ITPKB (SEQ ID NO:4) decreased the level of phopshorylated 4EBP1 in 231T and A549 cells, and RNAi of ITPKC (SEQ ID NO:7) decreased the level of phopshorylated 4EBP1 in A549 cells.

**[0133]** For the last subset of these experiments, we used PRAS40 as the substrate. For this substrate, pathway inhibition causes decreased cytoplasmic staining and increased nuclear and perinuclear staining. Cells were plated in 96 well plates, transfected with RNAi for ITPK, fixed, treated with PRAS40 antibody, and stained. Measurements were based on percentage of population of cells with increased or decreased nuclear/cytoplasmic staining ratio compared with negative or positive control cells. Results of this experiment showed that RNAi of ITPKA (SEQ ID NO:1) decreased the level of phosphorylated PRAS40 in PC3 cells, and RNAi of ITPKC (SEQ ID NO:7) decreased the level of phosphorylated PRAS40 in A549 and PC3 cells. Taken together, the results of these experiments suggest involvement of IPTK in the IGFR pathway.

**[0134]** High Throughput PTEN/IGF Transcriptional readout assay. This assay is an expanded TaqMan® transcriptional readout assay monitoring changes in the mRNA levels of endogenous PTEN/IGF regulated genes. This assay measures changes in expression of PTEN/IGF regulated cellular genes as a readout for pathway signaling activity.

**[0135]** We identified a panel of genes that were transcriptionally regulated by PTEN/IGF signaling, then designed and tested TaqMan® primer/probes sets. We reduced expression of PTEN/IGF by RNAi, and tested its affect on the expression of the transcriptionally regulated genes in multiple cell types. The panel readout was then narrowed to the ten most robust probes.

**[0136]** We then treated cancer cells with siRNAs of the target genes of interest, such as ITPK, and tested how the reduced levels of the target genes affected the expression levels of the PTEN/IGF regulated gene panel.

**[0137]** Genes that when knocked out via at least 2 different RNAi oligos, demonstrated the same pattern of activity on at least one third of the panel genes as a PTEN/IGF knockout, were identified as involved in the PTEN/IGF pathway.

**[0138]** TaqMan® assays were performed on the RNAs in a 384 well format.

**[0139]** RNAi of ITPKA (SEQ ID NO:1) and ITPKC (SEQ ID NO:7) showed the same pattern of activity as PTEN/IGF RNAi for at least 2 RNAi oligos on at least one third of the transcriptionally regulated genes in 231T cells.

**[0140]** In a last set of experiments we showed that RNAi of AKT1, AKT2, and CyclinD1 increases mRNA level of ITPKA (SEQ ID NO:1) in 231T, A549, and PC3 cells, again suggesting a direct involvement of ITPK in the IGFR pathway.

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gttctggagg agaggacccc ccccgcctgc cgagaggctc tcttcctgtt ctgcacccct 5760

ctccccatgg gaccttggag aaaactgaac tgttacaaac ccctgcacag tgcctgtcaa 5820

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<210> SEQ ID NO 4
<211> LENGTH: 4505
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

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gttgggacgc tgcgaaagat ctgaaagaac ctcagtgccc tcctggggac agggtgggtg 180

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cgcgtggcac aggggtgcaa tcagagggga cttgggaaag ccagcggcag gacagtgatg 300

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ccttggagga gacgcaggga agccctcggg gcaacctgcc cctgaggaaa ctgtcctctt 480

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<210> SEQ ID NO 5
<211> LENGTH: 3010
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 5

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tctgagaaac tgcagccgca cggttgttta tggagctttg ggcgggggct gagcccgcgg 180

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aggggaccgg cagcaggtgg tggcagccgg taccctctcc ccgccagggc cggaggaggc 720

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<210> SEQ ID NO 6
<211> LENGTH: 3398
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 6

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Gly Ala Ala Cys Cys Gly Ala Ala Gly Gly Ala Gly Cys Gly Gly Gly
20 25 30

Cys Ala Thr Gly Ala Gly Gly Cys Gly Cys Thr Gly Cys Cys Cys Gly
35 40 45

Thr Gly Cys Cys Gly Thr Gly Gly Gly Ala Gly Cys Cys Thr Gly Ala
50 55 60

Ala Cys Gly Ala Gly Gly Cys Gly Gly Ala Gly Gly Cys Cys Gly Gly
65 70 75 80

Gly Gly Cys Gly Cys Thr Gly Cys Cys Cys Gly Cys Gly Gly Cys Gly
85 90 95

Gly Cys Cys Cys Gly Cys Ala Thr Gly Gly Gly Ala Cys Thr Gly Gly
100 105 110

Ala Gly Gly Cys Gly Cys Cys Gly Cys Gly Ala Gly Gly Ala Gly Gly
115 120 125

Gly Cys Gly Gly Cys Gly Gly Cys Gly Gly Cys Ala Gly Cys Cys Gly
130 135 140

Gly Gly Ala Cys Ala Gly Cys Ala Gly Cys Gly Ala Cys Cys Thr Gly
145 150 155 160

Gly Gly Cys Cys Cys Gly Gly Cys Gly Cys Ala Gly Gly Gly Gly Cys
165 170 175

Cys Cys Cys Gly Gly Cys Gly Gly Gly Gly Cys Gly Gly Cys Cys Gly
180 185 190

Gly Ala Gly Gly Gly Gly Gly Gly Cys Gly Gly Gly Cys Cys Cys Thr
195 200 205

Gly Gly Gly Cys Cys Cys Gly Gly Ala Cys Ala Gly Ala Gly Gly Gly
210 215 220

Gly Thr Cys Cys Ala Gly Cys Cys Thr Cys Cys Ala Cys Ala Gly Cys
225 230 235 240

Gly Ala Gly Cys Cys Thr Gly Ala Gly Ala Gly Gly Gly Cys Cys Gly
245 250 255

Gly Cys Cys Thr Cys Gly Gly Gly Cys Cys Thr Gly Cys Gly Cys Cys
260 265 270

Gly Gly Gly Gly Ala Cys Ala Gly Ala Gly Ala Gly Thr Cys Cys Gly
275 280 285

Cys Ala Gly Gly Cys Ala Gly Ala Ala Thr Thr Cys Thr Gly Gly Ala
290 295 300

Cys Ala Gly Ala Cys Gly Gly Ala Cys Ala Gly Ala Cys Thr Gly Ala
305 310 315 320

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Gly Cys Cys Cys Gly Cys Gly Gly Cys Ala Gly Cys Thr Gly Gly Cys
                325                 330                 335

Cys Thr Thr Gly Gly Ala Gly Thr Ala Gly Ala Gly Ala Cys Cys Gly
            340                 345                 350

Ala Gly Ala Gly Gly Cys Cys Cys Ala Ala Gly Cys Ala Ala Ala Ala
        355                 360                 365

Gly Ala Cys Gly Gly Ala Gly Cys Cys Ala Gly Ala Cys Ala Gly Gly
    370                 375                 380

Thr Cys Cys Ala Gly Cys Cys Thr Cys Cys Gly Gly Ala Cys Gly Cys
385                 390                 395                 400

Ala Thr Cys Thr Ala Gly Ala Ala Thr Gly Gly Ala Gly Cys Thr Gly
                405                 410                 415

Gly Thr Cys Ala Gly Ala Gly Cys Thr Gly Gly Ala Gly Ala Cys Gly
            420                 425                 430

Ala Cys Thr Thr Gly Thr Cys Thr Thr Thr Gly Gly Ala Cys Gly Gly
        435                 440                 445

Ala Gly Ala Cys Cys Gly Gly Gly Ala Cys Ala Gly Ala Thr Gly Gly
    450                 455                 460

Cys Cys Thr Thr Thr Gly Gly Ala Cys Thr Gly Ala Thr Cys Cys Gly
465                 470                 475                 480

Cys Ala Cys Ala Gly Gly Thr Cys Cys Gly Ala Cys Cys Thr Cys Cys
                485                 490                 495

Ala Gly Thr Thr Thr Cys Ala Gly Cys Cys Cys Gly Ala Gly Gly Ala
            500                 505                 510

Gly Gly Cys Cys Ala Gly Cys Cys Cys Cys Thr Gly Gly Ala Cys Ala
        515                 520                 525

Cys Ala Gly Cys Cys Ala Gly Gly Gly Gly Thr Thr Cys Ala Thr Gly
    530                 535                 540

Gly Gly Cys Cys Cys Thr Gly Gly Ala Cys Ala Gly Ala Gly Cys Thr
545                 550                 555                 560

Gly Gly Ala Ala Ala Cys Gly Cys Ala Thr Gly Gly Gly Thr Cys Ala
                565                 570                 575

Cys Ala Gly Ala Cys Thr Cys Ala Gly Cys Cys Ala Gly Ala Gly Ala
            580                 585                 590

Gly Gly Gly Thr Cys Ala Ala Gly Thr Cys Cys Thr Gly Gly Gly Cys
        595                 600                 605

Thr Gly Ala Thr Ala Ala Cys Cys Thr Cys Thr Gly Gly Ala Cys Cys
    610                 615                 620

Cys Ala Cys Cys Ala Gly Ala Ala Cys Ala Gly Thr Thr Cys Cys Ala
625                 630                 635                 640

Gly Cys Cys Thr Cys Cys Ala Gly Ala Cys Thr Cys Ala Cys Cys Cys
                645                 650                 655

Ala Gly Ala Ala Gly Gly Ala Gly Cys Cys Thr Gly Thr Cys Cys Cys
            660                 665                 670

Thr Cys Ala Ala Ala Ala Gly Ala Gly Cys Cys Ala Ala Gly Thr Gly
        675                 680                 685

Cys Thr Gly Ala Thr Gly Gly Cys Thr Cys Cys Thr Gly Gly Ala Ala
    690                 695                 700

Ala Gly Ala Ala Thr Thr Gly Thr Ala Thr Ala Cys Thr Gly Ala Thr
705                 710                 715                 720

Gly Gly Cys Thr Cys Cys Ala Gly Gly Ala Cys Ala Cys Ala Ala Cys
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               725                 730                 735

Ala Gly Gly Ala Thr Ala Thr Thr Gly Ala Ala Gly Gly Thr Cys Cys
            740                 745                 750

Cys Thr Gly Gly Ala Cys Ala Gly Ala Gly Cys Cys Ala Thr Ala Thr
        755                 760                 765

Ala Cys Thr Gly Ala Thr Gly Gly Cys Thr Cys Cys Cys Ala Gly Ala
    770                 775                 780

Ala Ala Ala Ala Ala Cys Ala Gly Gly Ala Thr Ala Cys Thr Gly Ala
785                 790                 795                 800

Ala Gly Cys Ala Gly Cys Cys Ala Gly Gly Ala Ala Ala Cys Ala Gly
               805                 810                 815

Cys Cys Thr Gly Gly Cys Ala Cys Thr Gly Gly Thr Gly Gly Thr Thr
            820                 825                 830

Thr Cys Cys Ala Ala Ala Thr Ala Cys Ala Ala Cys Ala Gly Gly Ala
        835                 840                 845

Thr Ala Cys Thr Gly Ala Thr Gly Gly Cys Thr Cys Cys Thr Gly Gly
    850                 855                 860

Ala Cys Ala Cys Ala Ala Cys Cys Thr Ala Gly Cys Ala Cys Thr Gly
865                 870                 875                 880

Ala Cys Gly Gly Thr Thr Cys Cys Cys Ala Gly Ala Cys Ala Gly Cys
               885                 890                 895

Ala Cys Cys Thr Gly Gly Gly Ala Cys Ala Gly Ala Cys Thr Gly Cys
            900                 905                 910

Cys Thr Cys Thr Thr Gly Gly Gly Ala Gly Ala Gly Cys Cys Thr Gly
        915                 920                 925

Ala Gly Gly Ala Thr Gly Gly Cys Cys Cys Ala Thr Thr Ala Gly Ala
    930                 935                 940

Gly Gly Ala Ala Cys Cys Ala Gly Ala Gly Cys Cys Thr Gly Gly Ala
945                 950                 955                 960

Gly Ala Ala Thr Thr Gly Cys Thr Gly Ala Cys Thr Cys Ala Cys Cys
               965                 970                 975

Thr Gly Thr Ala Cys Thr Cys Thr Cys Ala Cys Cys Thr Gly Ala Ala
            980                 985                 990

Gly Thr Gly Thr Ala Gly Cys Cys  Cys Cys Cys Thr Gly  Thr Gly Cys
        995                 1000                1005

Cys Cys  Thr Gly Thr Gly Cys  Cys Cys Cys Gly Cys  Cys Thr Cys
    1010                1015                1020

Ala Thr  Cys Ala Thr Thr Ala  Cys Cys Cys Cys Thr  Gly Ala Gly
    1025                1030                1035

Ala Cys  Cys Cys Cys Thr Gly  Ala Gly Cys Cys Thr  Gly Ala Gly
    1040                1045                1050

Gly Cys  Cys Cys Ala Gly Cys  Cys Ala Gly Thr Gly  Gly Gly Ala
    1055                1060                1065

Cys Cys  Cys Cys Cys Cys Thr  Cys Cys Cys Gly Gly  Gly Thr Thr
    1070                1075                1080

Gly Ala  Gly Gly Gly Gly Gly  Gly Cys Ala Gly Cys  Gly Gly Cys
    1085                1090                1095

Gly Gly  Cys Thr Thr Cys Thr  Cys Cys Thr Cys Thr  Gly Cys Cys
    1100                1105                1110

Thr Cys  Thr Thr Cys Thr Thr  Thr Cys Gly Ala Cys  Gly Ala Gly
    1115                1120                1125
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Thr Cys  Thr Gly Ala Gly Gly  Ala Thr Gly Ala Cys  Gly Thr Gly
    1130                 1135                 1140

Gly Thr  Gly Gly Cys Cys Gly  Gly Gly Gly Gly Cys  Gly Gly Ala
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Gly Gly  Thr Gly Cys Cys Ala  Gly Cys Gly Ala Thr  Cys Cys Cys
    1160                 1165                 1170

Gly Ala  Gly Gly Ala Cys Ala  Gly Gly Thr Cys Thr  Gly Gly Gly
    1175                 1180                 1185

Ala Gly  Cys Ala Ala Ala Cys  Cys Cys Thr Gly Gly  Ala Ala Gly
    1190                 1195                 1200

Ala Ala  Gly Cys Thr Gly Ala  Ala Gly Ala Cys Ala  Gly Thr Thr
    1205                 1210                 1215

Cys Thr  Gly Ala Ala Gly Thr  Ala Thr Thr Cys Ala  Cys Cys Cys
    1220                 1225                 1230

Thr Thr  Thr Gly Thr Gly Gly  Thr Cys Thr Cys Cys  Thr Thr Cys
    1235                 1240                 1245

Cys Gly  Ala Ala Ala Ala Cys  Ala Cys Thr Ala Cys  Cys Cys Thr
    1250                 1255                 1260

Thr Gly  Gly Gly Thr Cys Cys  Ala Gly Cys Thr Thr  Thr Cys Thr
    1265                 1270                 1275

Gly Gly  Ala Cys Ala Thr Gly  Cys Thr Gly Gly Gly  Ala Ala Cys
    1280                 1285                 1290

Thr Thr  Cys Cys Ala Gly Gly  Cys Ala Gly Gly Ala  Gly Ala Gly
    1295                 1300                 1305

Gly Ala  Thr Gly Gly Thr Cys  Gly Gly Ala Thr Thr  Cys Thr Gly
    1310                 1315                 1320

Ala Ala  Ala Cys Gly Thr Thr  Thr Cys Thr Gly Thr  Cys Ala Gly
    1325                 1330                 1335

Thr Gly  Thr Gly Ala Gly Cys  Ala Gly Cys Gly Cys  Ala Gly Cys
    1340                 1345                 1350

Cys Thr  Gly Gly Ala Gly Cys  Ala Gly Cys Thr Gly  Ala Thr Gly
    1355                 1360                 1365

Ala Ala  Ala Gly Ala Cys Cys  Cys Gly Cys Thr Gly  Cys Gly Ala
    1370                 1375                 1380

Cys Cys  Thr Thr Thr Cys Gly  Thr Gly Cys Cys Thr  Gly Cys Cys
    1385                 1390                 1395

Thr Ala  Cys Thr Ala Thr Gly  Gly Cys Ala Thr Gly  Gly Thr Gly
    1400                 1405                 1410

Cys Thr  Gly Cys Ala Gly Gly  Ala Thr Gly Gly Cys  Cys Ala Gly
    1415                 1420                 1425

Ala Cys  Cys Thr Thr Cys Ala  Ala Cys Cys Ala Gly  Ala Thr Gly
    1430                 1435                 1440

Gly Ala  Ala Gly Ala Cys Cys  Thr Cys Cys Thr Gly  Gly Cys Thr
    1445                 1450                 1455

Gly Ala  Cys Thr Thr Thr Gly  Ala Gly Gly Gly Cys  Cys Cys Cys
    1460                 1465                 1470

Thr Cys  Cys Ala Thr Thr Ala  Thr Gly Gly Ala Cys  Thr Gly Cys
    1475                 1480                 1485

Ala Ala  Gly Ala Thr Gly Gly  Gly Cys Ala Gly Cys  Ala Gly Gly
    1490                 1495                 1500
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    1520                 1525                 1530

Cys Gly  Gly Gly Ala Ala Cys  Gly Thr Cys Cys Cys  Cys Gly Thr
    1535                 1540                 1545

Cys Cys  Cys Cys Gly Gly Ala  Ala Gly Gly Ala Cys  Ala Thr Gly
    1550                 1555                 1560

Thr Ala  Thr Gly Ala Gly Ala  Ala Gly Ala Thr Gly  Gly Thr Gly
    1565                 1570                 1575

Gly Cys  Thr Gly Thr Gly Gly  Ala Cys Cys Cys Thr  Gly Gly Gly
    1580                 1585                 1590

Gly Cys  Cys Cys Cys Thr Ala  Cys Cys Cys Cys Thr  Gly Ala Gly
    1595                 1600                 1605

Gly Ala  Gly Cys Ala Thr Gly  Cys Cys Cys Ala Gly  Gly Gly Thr
    1610                 1615                 1620

Gly Cys  Ala Gly Thr Cys Ala  Cys Cys Ala Ala Gly  Cys Cys Cys
    1625                 1630                 1635

Cys Gly  Cys Thr Ala Cys Ala  Thr Gly Cys Ala Gly  Thr Gly Gly
    1640                 1645                 1650

Ala Gly  Gly Gly Ala Ala Ala  Cys Cys Ala Thr Gly  Ala Gly Cys
    1655                 1660                 1665

Thr Cys  Cys Ala Cys Cys Thr  Cys Thr Ala Cys Cys  Cys Thr Gly
    1670                 1675                 1680

Gly Gly  Cys Thr Thr Cys Cys  Gly Gly Ala Thr Cys  Gly Ala Gly
    1685                 1690                 1695

Gly Gly  Cys Ala Thr Cys Ala  Ala Gly Ala Ala Gly  Gly Cys Ala
    1700                 1705                 1710

Gly Ala  Thr Gly Gly Gly Ala  Cys Cys Thr Gly Thr  Ala Ala Cys
    1715                 1720                 1725

Ala Cys  Cys Ala Ala Cys Thr  Thr Cys Ala Ala Gly  Ala Ala Gly
    1730                 1735                 1740

Ala Cys  Gly Cys Ala Gly Gly  Cys Ala Cys Thr Gly  Gly Ala Gly
    1745                 1750                 1755

Cys Ala  Gly Gly Thr Gly Ala  Cys Ala Ala Ala Ala  Gly Thr Gly
    1760                 1765                 1770

Cys Thr  Gly Gly Ala Gly Gly  Ala Cys Thr Thr Cys  Gly Thr Gly
    1775                 1780                 1785

Gly Ala  Thr Gly Gly Ala Gly  Ala Cys Cys Ala Cys  Gly Thr Cys
    1790                 1795                 1800

Ala Thr  Cys Cys Thr Gly Cys  Ala Ala Ala Ala Gly  Thr Ala Cys
    1805                 1810                 1815

Gly Thr  Gly Gly Cys Ala Thr  Gly Cys Cys Thr Ala  Gly Ala Ala
    1820                 1825                 1830

Gly Ala  Ala Cys Thr Thr Cys  Gly Thr Gly Ala Ala  Gly Cys Thr
    1835                 1840                 1845

Cys Thr  Gly Gly Ala Gly Ala  Thr Cys Thr Cys Cys  Cys Cys Cys
    1850                 1855                 1860

Thr Thr  Cys Thr Thr Cys Ala  Ala Gly Ala Cys Cys  Cys Ala Cys
    1865                 1870                 1875

Gly Ala  Gly Gly Thr Gly Gly  Thr Ala Gly Gly Cys  Ala Gly Cys
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    1880                1885                1890

Thr Cys  Cys Cys Thr Cys Cys  Thr Cys Thr Thr Cys  Gly Thr Gly
    1895                1900                1905

Cys Ala  Cys Gly Ala Cys Cys  Ala Cys Ala Cys Cys  Gly Gly Cys
    1910                1915                1920

Cys Thr  Gly Gly Cys Cys Ala  Ala Gly Gly Thr Cys  Thr Gly Gly
    1925                1930                1935

Ala Thr  Gly Ala Thr Ala Gly  Ala Cys Thr Thr Cys  Gly Gly Cys
    1940                1945                1950

Ala Ala  Gly Ala Cys Gly Gly  Thr Gly Gly Cys Cys  Thr Thr Gly
    1955                1960                1965

Cys Cys  Cys Gly Ala Cys Cys  Ala Cys Cys Ala Gly  Ala Cys Gly
    1970                1975                1980

Cys Thr  Cys Ala Gly Cys Cys  Ala Cys Ala Gly Gly  Cys Thr Gly
    1985                1990                1995

Cys Cys  Cys Thr Gly Gly Gly  Cys Thr Gly Ala Gly  Gly Gly Cys
    2000                2005                2010

Ala Ala  Cys Cys Gly Thr Gly  Ala Gly Gly Ala Cys  Gly Gly Cys
    2015                2020                2025

Thr Ala  Cys Cys Thr Cys Thr  Gly Gly Gly Gly Cys  Cys Thr Gly
    2030                2035                2040

Gly Ala  Cys Ala Ala Cys Ala  Thr Gly Ala Thr Cys  Thr Gly Cys
    2045                2050                2055

Cys Thr  Cys Cys Thr Gly Cys  Ala Gly Gly Gly Gly  Cys Thr Gly
    2060                2065                2070

Gly Cys  Ala Cys Ala Gly Ala  Gly Cys Thr Gly Ala  Gly Cys Thr
    2075                2080                2085

Gly Cys  Thr Cys Ala Gly Cys  Cys Ala Cys Cys Ala  Thr Cys Ala
    2090                2095                2100

Gly Gly  Thr Thr Ala Ala Thr  Thr Gly Gly Ala Thr  Gly Gly Cys
    2105                2110                2115

Gly Cys  Cys Ala Gly Thr Cys  Thr Gly Gly Cys Thr  Gly Gly Ala
    2120                2125                2130

Gly Gly  Ala Gly Cys Cys Cys  Thr Gly Ala Gly Ala  Thr Gly Cys
    2135                2140                2145

Cys Ala  Thr Gly Gly Gly Ala  Gly Gly Cys Cys Thr  Gly Ala Gly
    2150                2155                2160

Gly Thr  Thr Gly Gly Cys Cys  Ala Cys Gly Gly Gly  Gly Gly Ala
    2165                2170                2175

Gly Cys  Thr Gly Gly Cys Cys  Thr Cys Cys Ala Gly  Gly Gly Ala
    2180                2185                2190

Cys Gly  Gly Gly Ala Gly Ala  Gly Ala Thr Thr Gly  Thr Gly Thr
    2195                2200                2205

Cys Ala  Thr Gly Thr Gly Cys  Cys Ala Cys Ala Cys  Gly Ala Gly
    2210                2215                2220

Ala Cys  Cys Ala Ala Cys Gly  Thr Gly Gly Ala Ala  Ala Ala Gly
    2225                2230                2235

Thr Cys  Thr Gly Ala Ala Gly  Gly Gly Cys Cys Thr  Thr Gly Gly
    2240                2245                2250

Gly Ala  Gly Ala Cys Cys Ala  Gly Gly Thr Ala Gly  Cys Ala Cys
    2255                2260                2265
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Cys Thr  Gly Gly Cys Cys Cys  Cys Ala Thr Cys Ala  Thr Gly Ala
    2270                 2275                 2280

Thr Gly  Cys Ala Gly Gly Gly  Gly Thr Thr Thr Thr  Gly Gly Gly
    2285                 2290                 2295

Gly Ala  Cys Cys Thr Gly Gly  Ala Ala Gly Gly Ala  Ala Gly Gly
    2300                 2305                 2310

Thr Gly  Ala Thr Gly Ala Gly  Gly Cys Ala Gly Thr  Gly Ala Gly
    2315                 2320                 2325

Thr Cys  Ala Gly Ala Ala Ala  Ala Ala Cys Cys Ala  Gly Ala Ala
    2330                 2335                 2340

Cys Gly  Gly Gly Gly Thr Cys  Cys Cys Cys Gly Gly  Ala Thr Cys
    2345                 2350                 2355

Thr Gly  Cys Cys Gly Gly Gly  Ala Ala Gly Gly Cys  Thr Thr Cys
    2360                 2365                 2370

Thr Gly  Ala Gly Gly Gly Gly  Cys Thr Gly Cys Cys  Cys Thr Gly
    2375                 2380                 2385

Ala Gly  Ala Gly Cys Ala Thr  Thr Cys Ala Gly Thr  Thr Cys Ala
    2390                 2395                 2400

Cys Ala  Thr Gly Thr Cys Ala  Cys Ala Gly Gly Gly  Thr Ala Thr
    2405                 2410                 2415

Gly Gly  Thr Gly Thr Gly Ala  Cys Ala Gly Gly Gly  Thr Gly Cys
    2420                 2425                 2430

Cys Thr  Gly Thr Gly Gly Ala  Cys Ala Cys Ala Thr  Gly Ala Ala
    2435                 2440                 2445

Thr Cys  Ala Cys Thr Thr Cys  Thr Ala Ala Cys Cys  Thr Gly Cys
    2450                 2455                 2460

Cys Thr  Cys Cys Cys Thr Gly  Thr Cys Ala Gly Cys  Cys Thr Cys
    2465                 2470                 2475

Cys Ala  Gly Gly Cys Thr Gly  Cys Cys Ala Gly Cys  Thr Gly Gly
    2480                 2485                 2490

Cys Thr  Gly Ala Gly Gly Cys  Cys Ala Gly Gly Gly  Ala Cys Thr
    2495                 2500                 2505

Gly Gly  Gly Thr Cys Ala Gly  Gly Cys Thr Cys Ala  Thr Cys Thr
    2510                 2515                 2520

Gly Thr  Gly Gly Cys Gly Cys  Cys Thr Cys Ala Gly  Ala Gly Gly
    2525                 2530                 2535

Gly Thr  Cys Ala Gly Cys Ala  Thr Cys Ala Thr Thr  Gly Gly Thr
    2540                 2545                 2550

Gly Ala  Ala Cys Ala Gly Ala  Thr Gly Cys Ala Gly  Gly Cys Gly
    2555                 2560                 2565

Cys Thr  Gly Cys Thr Gly Gly  Ala Cys Cys Ala Thr  Cys Thr Gly
    2570                 2575                 2580

Gly Gly  Gly Ala Gly Ala Gly  Thr Gly Ala Cys Ala  Gly Thr Cys
    2585                 2590                 2595

Cys Ala  Thr Gly Thr Cys Thr  Thr Cys Ala Cys Cys  Ala Gly Gly
    2600                 2605                 2610

Gly Ala  Gly Cys Cys Ala Thr  Thr Thr Gly Ala Gly  Thr Gly Cys
    2615                 2620                 2625

Thr Gly  Ala Gly Cys Gly Ala  Cys Ala Ala Gly Ala  Gly Gly Cys
    2630                 2635                 2640
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Thr Cys  Ala Gly Ala Gly Gly  Gly Cys Ala Thr Gly  Ala Cys Cys
    2645                 2650                 2655

Cys Cys  Ala Thr Gly Gly Gly  Ala Cys Thr Gly Gly  Ala Thr Gly
    2660                 2665                 2670

Cys Gly  Gly Cys Cys Thr Gly  Ala Gly Gly Gly Cys  Thr Gly Ala
    2675                 2680                 2685

Thr Ala  Cys Cys Gly Cys Thr  Gly Gly Gly Cys Gly  Gly Thr Ala
    2690                 2695                 2700

Thr Cys  Cys Thr Gly Cys Cys  Cys Thr Gly Cys Thr  Gly Thr Gly
    2705                 2710                 2715

Gly Cys  Cys Cys Thr Gly Thr  Gly Gly Gly Ala Thr  Cys Cys Thr
    2720                 2725                 2730

Cys Cys  Gly Thr Gly Thr Thr  Cys Cys Thr Cys Gly  Gly Cys Gly
    2735                 2740                 2745

Gly Ala  Cys Thr Cys Thr Gly  Cys Thr Gly Ala Cys  Cys Thr Cys
    2750                 2755                 2760

Cys Thr  Gly Cys Ala Gly Ala  Cys Cys Cys Ala Ala  Ala Cys Cys
    2765                 2770                 2775

Ala Cys  Ala Gly Cys Cys Ala  Cys Ala Thr Cys Cys  Cys Ala Gly
    2780                 2785                 2790

Cys Thr  Thr Cys Thr Gly Thr  Gly Cys Cys Ala Gly  Cys Ala Cys
    2795                 2800                 2805

Thr Gly  Thr Gly Ala Cys Ala  Gly Thr Ala Cys Cys  Thr Cys Gly
    2810                 2815                 2820

Cys Thr  Cys Cys Thr Cys Thr  Gly Thr Gly Cys Ala  Cys Cys Ala
    2825                 2830                 2835

Gly Ala  Thr Cys Cys Gly Gly  Cys Cys Thr Cys Ala  Gly Gly Ala
    2840                 2845                 2850

Cys Thr  Thr Ala Cys Ala Cys  Cys Thr Cys Cys Thr  Gly Cys Cys
    2855                 2860                 2865

Thr Gly  Ala Cys Cys Cys Cys  Cys Ala Gly Gly Cys  Thr Thr Cys
    2870                 2875                 2880

Thr Cys  Thr Cys Thr Cys Cys  Thr Thr Thr Cys Thr  Cys Cys Cys
    2885                 2890                 2895

Ala Gly  Cys Ala Ala Ala Cys  Thr Gly Cys Ala Gly  Thr Gly Gly
    2900                 2905                 2910

Cys Ala  Gly Ala Ala Ala Gly  Gly Ala Gly Gly Thr  Thr Cys Ala
    2915                 2920                 2925

Gly Ala  Gly Gly Cys Thr Gly  Gly Gly Ala Ala Ala  Gly Thr Gly
    2930                 2935                 2940

Gly Gly  Cys Cys Thr Cys Cys  Cys Cys Thr Thr Gly  Cys Ala Ala
    2945                 2950                 2955

Cys Thr  Cys Ala Gly Ala Gly  Cys Thr Gly Cys Thr  Gly Cys Ala
    2960                 2965                 2970

Cys Thr  Cys Ala Gly Gly Ala  Gly Gly Gly Cys Cys  Cys Cys Ala
    2975                 2980                 2985

Thr Cys  Cys Ala Ala Thr Cys  Cys Cys Gly Gly Gly  Cys Cys Cys
    2990                 2995                 3000

Cys Thr  Gly Cys Ala Gly Gly  Gly Ala Ala Ala Ala  Gly Cys Gly
    3005                 3010                 3015

Cys Thr  Gly Gly Gly Thr Gly  Thr Gly Thr Gly Thr  Cys Ala Gly
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    3020                3025                3030

Ala Gly  Gly Cys Gly Cys Ala  Gly Gly Gly Thr Gly  Gly Gly Thr
    3035                3040                3045

Gly Gly  Gly Gly Cys Thr Gly  Cys Cys Ala Gly Cys  Cys Ala Gly
    3050                3055                3060

Gly Ala  Cys Cys Cys Thr Gly  Gly Cys Cys Thr Gly  Cys Ala Gly
    3065                3070                3075

Cys Cys  Thr Gly Ala Thr Cys  Cys Ala Ala Ala Cys  Cys Ala Ala
    3080                3085                3090

Ala Gly  Ala Cys Thr Gly Thr  Ala Gly Ala Ala Cys  Cys Cys Thr
    3095                3100                3105

Gly Gly  Gly Gly Thr Gly Thr  Gly Gly Cys Thr Ala  Ala Cys Gly
    3110                3115                3120

Gly Cys  Cys Cys Cys Thr Cys  Cys Ala Gly Cys Ala  Cys Cys Cys
    3125                3130                3135

Ala Thr  Ala Gly Cys Cys Ala  Gly Gly Thr Cys Thr  Thr Cys Cys
    3140                3145                3150

Thr Gly  Gly Cys Cys Cys Thr  Thr Gly Ala Gly Gly  Cys Thr Gly
    3155                3160                3165

Gly Gly  Cys Thr Gly Gly Cys  Gly Gly Ala Cys Ala  Gly Gly Cys
    3170                3175                3180

Ala Cys  Cys Thr Ala Cys Cys  Thr Cys Thr Thr Cys  Cys Thr Thr
    3185                3190                3195

Ala Ala  Gly Cys Thr Gly Ala  Ala Gly Cys Thr Cys  Cys Cys Ala
    3200                3205                3210

Cys Ala  Cys Thr Gly Thr Cys  Thr Thr Cys Cys Ala  Gly Gly Gly
    3215                3220                3225

Cys Thr  Gly Ala Gly Gly Ala  Gly Ala Thr Gly Cys  Thr Cys Thr
    3230                3235                3240

Cys Cys  Thr Thr Thr Thr Cys  Thr Ala Cys Thr Gly  Ala Cys Cys
    3245                3250                3255

Ala Thr  Cys Thr Thr Gly Ala  Thr Ala Cys Thr Thr  Ala Thr Thr
    3260                3265                3270

Thr Ala  Thr Ala Cys Gly Ala  Gly Ala Gly Gly Cys  Ala Gly Thr
    3275                3280                3285

Thr Gly  Cys Thr Gly Gly Ala  Cys Gly Gly Gly Gly  Thr Ala Gly
    3290                3295                3300

Thr Ala  Cys Thr Gly Gly Gly  Ala Ala Gly Cys Ala  Gly Gly Ala
    3305                3310                3315

Gly Gly  Cys Ala Gly Ala Ala  Thr Gly Gly Cys Thr  Cys Thr Gly
    3320                3325                3330

Cys Thr  Gly Ala Gly Cys Cys  Thr Cys Cys Thr Ala  Cys Cys Cys
    3335                3340                3345

Ala Thr  Gly Ala Cys Ala Ala  Cys Ala Cys Cys Cys  Cys Ala Ala
    3350                3355                3360

Thr Ala  Ala Ala Cys Ala Gly  Ala Ala Cys Ala Thr  Thr Cys Ala
    3365                3370                3375

Gly Ala  Gly Cys Cys Ala Ala  Ala Ala Ala Ala Ala  Ala Ala Ala
    3380                3385                3390

Ala Ala  Ala Ala Ala
    3395
```

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<210> SEQ ID NO 7
<211> LENGTH: 2052
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 7

```
Ala Thr Gly Ala Gly Gly Cys Gly Cys Thr Gly Cys Cys Cys Gly Thr
1               5                   10                  15
Gly Cys Cys Gly Thr Gly Gly Gly Ala Gly Cys Cys Thr Gly Ala Ala

            20                  25                  30
Cys Gly Ala Gly Gly Cys Gly Gly Ala Gly Gly Cys Cys Gly Gly Gly

        35                  40                  45
Gly Cys Gly Cys Thr Gly Cys Cys Cys Gly Cys Gly Gly Cys Gly Gly

    50                  55                  60
Cys Cys Cys Gly Cys Ala Thr Gly Gly Gly Ala Cys Thr Gly Gly Ala

65                  70                  75                  80
Gly Gly Cys Gly Cys Cys Gly Cys Gly Ala Gly Gly Ala Gly Gly Gly

                85                  90                  95
Cys Gly Gly Cys Gly Gly Cys Gly Gly Cys Ala Gly Cys Cys Gly Gly

            100                 105                 110
Gly Ala Cys Ala Gly Cys Ala Gly Cys Gly Ala Cys Cys Thr Gly Gly

        115                 120                 125
Gly Cys Cys Cys Gly Gly Cys Gly Cys Ala Gly Gly Gly Gly Cys Cys

    130                 135                 140
Cys Cys Gly Gly Cys Gly Gly Gly Gly Cys Gly Gly Cys Cys Gly Gly

145                 150                 155                 160
Ala Gly Gly Gly Gly Gly Gly Cys Gly Gly Gly Cys Cys Cys Thr Gly

                165                 170                 175
Gly Gly Cys Cys Cys Gly Gly Ala Cys Ala Gly Ala Gly Gly Gly Gly

            180                 185                 190
Thr Cys Cys Ala Gly Cys Cys Thr Cys Cys Ala Cys Ala Gly Cys Gly

        195                 200                 205
Ala Gly Cys Cys Thr Gly Ala Gly Ala Gly Gly Gly Cys Cys Gly Gly

    210                 215                 220
Cys Cys Thr Cys Gly Gly Gly Cys Cys Thr Gly Cys Gly Cys Cys Gly

225                 230                 235                 240
Gly Gly Gly Ala Cys Ala Gly Ala Gly Ala Gly Thr Cys Cys Gly Cys

                245                 250                 255
Ala Gly Gly Cys Ala Gly Ala Ala Thr Thr Cys Thr Gly Gly Ala Cys

            260                 265                 270
Ala Gly Ala Cys Gly Gly Ala Cys Ala Gly Ala Cys Thr Gly Ala Gly

        275                 280                 285
Cys Cys Cys Gly Cys Gly Gly Cys Ala Gly Cys Thr Gly Gly Cys Cys

    290                 295                 300
Thr Thr Gly Gly Ala Gly Thr Ala Gly Ala Gly Ala Cys Cys Gly Ala

305                 310                 315                 320
Gly Ala Gly Gly Cys Cys Cys Ala Ala Gly Cys Ala Ala Ala Ala Gly

                325                 330                 335
Ala Cys Gly Gly Ala Gly Cys Cys Ala Gly Ala Cys Ala Gly Gly Thr

            340                 345                 350
Cys Cys Ala Gly Cys Cys Thr Cys Cys Gly Gly Ala Cys Gly Cys Ala
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        355                 360                 365
Thr Cys Thr Ala Gly Ala Ala Thr Gly Gly Ala Gly Cys Thr Gly Gly

    370                 375                 380
Thr Cys Ala Gly Ala Gly Cys Thr Gly Gly Ala Gly Ala Cys Gly Ala

385                 390                 395                 400
Cys Thr Thr Gly Thr Cys Thr Thr Thr Gly Gly Ala Cys Gly Gly Ala

                405                 410                 415
Gly Ala Cys Cys Gly Gly Gly Ala Cys Ala Gly Ala Thr Gly Gly Cys

            420                 425                 430
Cys Thr Thr Thr Gly Gly Ala Cys Thr Gly Ala Thr Cys Cys Gly Cys

        435                 440                 445
Ala Cys Ala Gly Gly Thr Cys Cys Gly Ala Cys Cys Thr Cys Cys Ala

    450                 455                 460
Gly Thr Thr Thr Cys Ala Gly Cys Cys Cys Gly Ala Gly Gly Ala Gly

465                 470                 475                 480
Gly Cys Cys Ala Gly Cys Cys Cys Cys Thr Gly Gly Ala Cys Ala Cys

                485                 490                 495
Ala Gly Cys Cys Ala Gly Gly Gly Gly Thr Thr Cys Ala Thr Gly Gly

            500                 505                 510
Gly Cys Cys Cys Thr Gly Gly Ala Cys Ala Gly Ala Gly Cys Thr Gly

        515                 520                 525
Gly Ala Ala Ala Cys Gly Cys Ala Thr Gly Gly Gly Thr Cys Ala Cys

    530                 535                 540
Ala Gly Ala Cys Thr Cys Ala Gly Cys Cys Ala Gly Ala Gly Ala Gly

545                 550                 555                 560
Gly Gly Thr Cys Ala Ala Gly Thr Cys Cys Thr Gly Gly Gly Cys Thr

                565                 570                 575
Gly Ala Thr Ala Ala Cys Cys Thr Cys Thr Gly Gly Ala Cys Cys Cys

            580                 585                 590
Ala Cys Cys Ala Gly Ala Ala Cys Ala Gly Thr Thr Cys Cys Ala Gly

        595                 600                 605
Cys Cys Thr Cys Cys Ala Gly Ala Cys Thr Cys Ala Cys Cys Cys Ala

    610                 615                 620
Gly Ala Ala Gly Gly Ala Gly Cys Cys Thr Gly Thr Cys Cys Cys Thr

625                 630                 635                 640
Cys Ala Ala Ala Ala Gly Ala Gly Cys Cys Ala Ala Gly Thr Gly Cys

                645                 650                 655
Thr Gly Ala Thr Gly Gly Cys Thr Cys Cys Thr Gly Gly Ala Ala Ala

            660                 665                 670
Gly Ala Ala Thr Thr Gly Thr Ala Thr Ala Cys Thr Gly Ala Thr Gly

        675                 680                 685
Gly Cys Thr Cys Cys Ala Gly Gly Ala Cys Ala Cys Ala Ala Cys Ala

    690                 695                 700
Gly Gly Ala Thr Ala Thr Thr Gly Ala Ala Gly Gly Thr Cys Cys Cys

705                 710                 715                 720
Thr Gly Gly Ala Cys Ala Gly Ala Gly Cys Cys Ala Thr Ala Thr Ala

                725                 730                 735
Cys Thr Gly Ala Thr Gly Gly Cys Thr Cys Cys Cys Ala Gly Ala Ala

            740                 745                 750
Ala Ala Ala Ala Cys Ala Gly Gly Ala Thr Ala Cys Thr Gly Ala Ala

        755                 760                 765
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```
Gly Cys Ala Gly Cys Cys Ala Gly Gly Ala Ala Ala Cys Ala Gly Cys

    770                 775                 780
Cys Thr Gly Gly Cys Ala Cys Thr Gly Gly Thr Gly Gly Thr Thr Thr

785                 790                 795                 800
Cys Cys Ala Ala Ala Thr Ala Cys Ala Ala Cys Ala Gly Gly Ala Thr

                805                 810                 815
Ala Cys Thr Gly Ala Thr Gly Gly Cys Thr Cys Cys Thr Gly Gly Ala

            820                 825                 830
Cys Ala Cys Ala Ala Cys Cys Thr Ala Gly Cys Ala Cys Thr Gly Ala

        835                 840                 845
Cys Gly Gly Thr Thr Cys Cys Cys Ala Gly Ala Cys Ala Gly Cys Ala

    850                 855                 860
Cys Cys Thr Gly Gly Gly Ala Cys Ala Gly Ala Cys Thr Gly Cys Cys

865                 870                 875                 880
Thr Cys Thr Thr Gly Gly Gly Ala Gly Ala Gly Cys Cys Thr Gly Ala

                885                 890                 895
Gly Gly Ala Thr Gly Gly Cys Cys Cys Ala Thr Thr Ala Gly Ala Gly

            900                 905                 910
Gly Ala Ala Cys Cys Ala Gly Ala Gly Cys Cys Thr Gly Gly Ala Gly

        915                 920                 925
Ala Ala Thr Thr Gly Cys Thr Gly Ala Cys Thr Cys Ala Cys Cys Thr

    930                 935                 940
Gly Thr Ala Cys Thr Cys Thr Cys Ala Cys Cys Thr Gly Ala Ala Gly

945                 950                 955                 960
Thr Gly Thr Ala Gly Cys Cys Cys Cys Cys Thr Gly Thr Gly Cys Cys

                965                 970                 975
Cys Thr Gly Thr Gly Cys Cys Cys Cys Gly Cys Cys Thr Cys Ala Thr

            980                 985                 990
Cys Ala Thr Thr Ala Cys Cys Cys  Cys Thr Gly Ala Gly  Ala Cys Cys

        995                 1000                1005
Cys Cys  Thr Gly Ala Gly Cys  Cys Thr Gly Ala Gly  Gly Cys Cys

    1010                1015                1020
Cys Ala  Gly Cys Cys Ala Gly  Thr Gly Gly Gly Ala  Cys Cys Cys

    1025                1030                1035
Cys Cys  Cys Thr Cys Cys Cys  Gly Gly Gly Thr Thr  Gly Ala Gly

    1040                1045                1050
Gly Gly  Gly Gly Gly Cys Ala  Gly Cys Gly Gly Cys  Gly Gly Cys

    1055                1060                1065
Thr Thr  Cys Thr Cys Cys Thr  Cys Thr Gly Cys Cys  Thr Cys Thr

    1070                1075                1080
Thr Cys  Thr Thr Thr Cys Gly  Ala Cys Gly Ala Gly  Thr Cys Thr

    1085                1090                1095
Gly Ala  Gly Gly Ala Thr Gly  Ala Cys Gly Thr Gly  Gly Thr Gly

    1100                1105                1110
Gly Cys  Cys Gly Gly Gly Gly  Gly Cys Gly Gly Ala  Gly Gly Thr

    1115                1120                1125
Gly Cys  Cys Ala Gly Cys Gly  Ala Thr Cys Cys Cys  Gly Ala Gly

    1130                1135                1140
Gly Ala  Cys Ala Gly Gly Thr  Cys Thr Gly Gly Gly  Ala Gly Cys

    1145                1150                1155
Ala Ala  Ala Cys Cys Cys Thr  Gly Gly Ala Ala Gly  Ala Ala Gly
```

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```
    1160                1165                1170
Cys Thr  Gly Ala Ala Gly Ala  Cys Ala Gly Thr Thr  Cys Thr Gly

    1175                1180                1185
Ala Ala  Gly Thr Ala Thr Thr  Cys Ala Cys Cys Cys  Thr Thr Thr

    1190                1195                1200
Gly Thr  Gly Gly Thr Cys Thr  Cys Cys Thr Thr Cys  Cys Gly Ala

    1205                1210                1215
Ala Ala  Ala Cys Ala Cys Thr  Ala Cys Cys Cys Thr  Thr Gly Gly

    1220                1225                1230
Gly Thr  Cys Cys Ala Gly Cys  Thr Thr Thr Cys Thr  Gly Gly Ala

    1235                1240                1245
Cys Ala  Thr Gly Cys Thr Gly  Gly Gly Ala Ala Cys  Thr Thr Cys

    1250                1255                1260
Cys Ala  Gly Gly Cys Ala Gly  Gly Ala Gly Ala Gly  Gly Ala Thr

    1265                1270                1275
Gly Gly  Thr Cys Gly Gly Ala  Thr Thr Cys Thr Gly  Ala Ala Ala

    1280                1285                1290
Cys Gly  Thr Thr Thr Cys Thr  Gly Thr Cys Ala Gly  Thr Gly Thr

    1295                1300                1305
Gly Ala  Gly Cys Ala Gly Cys  Gly Cys Ala Gly Cys  Cys Thr Gly

    1310                1315                1320
Gly Ala  Gly Cys Ala Gly Cys  Thr Gly Ala Thr Gly  Ala Ala Ala

    1325                1330                1335
Gly Ala  Cys Cys Cys Gly Cys  Thr Gly Cys Gly Ala  Cys Cys Thr

    1340                1345                1350
Thr Thr  Cys Gly Thr Gly Cys  Cys Thr Gly Cys Cys  Thr Ala Cys

    1355                1360                1365
Thr Ala  Thr Gly Gly Cys Ala  Thr Gly Gly Thr Gly  Cys Thr Gly

    1370                1375                1380
Cys Ala  Gly Gly Ala Thr Gly  Gly Cys Cys Ala Gly  Ala Cys Cys

    1385                1390                1395
Thr Thr  Cys Ala Ala Cys Cys  Ala Gly Ala Thr Gly  Gly Ala Ala

    1400                1405                1410
Gly Ala  Cys Cys Thr Cys Cys  Thr Gly Gly Cys Thr  Gly Ala Cys

    1415                1420                1425
Thr Thr  Thr Gly Ala Gly Gly  Gly Cys Cys Cys Cys  Thr Cys Cys

    1430                1435                1440
Ala Thr  Thr Ala Thr Gly Gly  Ala Cys Thr Gly Cys  Ala Ala Gly

    1445                1450                1455
Ala Thr  Gly Gly Gly Cys Ala  Gly Cys Ala Gly Gly  Ala Cys Cys

    1460                1465                1470
Thr Ala  Thr Cys Thr Gly Gly  Ala Ala Gly Ala Gly  Gly Ala Gly

    1475                1480                1485
Cys Thr  Ala Gly Thr Gly Ala  Ala Gly Gly Cys Ala  Cys Gly Gly

    1490                1495                1500
Gly Ala  Ala Cys Gly Thr Cys  Cys Cys Cys Gly Thr  Cys Cys Cys

    1505                1510                1515
Cys Gly  Gly Ala Ala Gly Gly  Ala Cys Ala Thr Gly  Thr Ala Thr

    1520                1525                1530
Gly Ala  Gly Ala Ala Gly Ala  Thr Gly Gly Thr Gly  Gly Cys Thr
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```
    1535                1540                1545
Gly Thr  Gly Gly Ala Cys Cys  Cys Thr Gly Gly Gly  Gly Cys Cys

    1550                1555                1560
Cys Cys  Thr Ala Cys Cys Cys  Cys Thr Gly Ala Gly  Gly Ala Gly

    1565                1570                1575
Cys Ala  Thr Gly Cys Cys Cys  Ala Gly Gly Gly Thr  Gly Cys Ala

    1580                1585                1590
Gly Thr  Cys Ala Cys Cys Ala  Ala Gly Cys Cys Cys  Cys Gly Cys

    1595                1600                1605
Thr Ala  Cys Ala Thr Gly Cys  Ala Gly Thr Gly Gly  Ala Gly Gly

    1610                1615                1620
Gly Ala  Ala Ala Cys Cys Ala  Thr Gly Ala Gly Cys  Thr Cys Cys

    1625                1630                1635
Ala Cys  Cys Thr Cys Thr Ala  Cys Cys Cys Thr Gly  Gly Gly Cys

    1640                1645                1650
Thr Thr  Cys Cys Gly Gly Ala  Thr Cys Gly Ala Gly  Gly Gly Cys

    1655                1660                1665
Ala Thr  Cys Ala Ala Gly Ala  Ala Gly Gly Cys Ala  Gly Ala Thr

    1670                1675                1680
Gly Gly  Gly Ala Cys Cys Thr  Gly Thr Ala Ala Cys  Ala Cys Cys

    1685                1690                1695
Ala Ala  Cys Thr Thr Cys Ala  Ala Gly Ala Ala Gly  Ala Cys Gly

    1700                1705                1710
Cys Ala  Gly Gly Cys Ala Cys  Thr Gly Gly Ala Gly  Cys Ala Gly

    1715                1720                1725
Gly Thr  Gly Ala Cys Ala Ala  Ala Ala Gly Thr Gly  Cys Thr Gly

    1730                1735                1740
Gly Ala  Gly Gly Ala Cys Thr  Thr Cys Gly Thr Gly  Gly Ala Thr

    1745                1750                1755
Gly Gly  Ala Gly Ala Cys Cys  Ala Cys Gly Thr Cys  Ala Thr Cys

    1760                1765                1770
Cys Thr  Gly Cys Ala Ala Ala  Ala Gly Thr Ala Cys  Gly Thr Gly

    1775                1780                1785
Gly Cys  Ala Thr Gly Cys Cys  Thr Ala Gly Ala Ala  Gly Ala Ala

    1790                1795                1800
Cys Thr  Thr Cys Gly Thr Gly  Ala Ala Gly Cys Thr  Cys Thr Gly

    1805                1810                1815
Gly Ala  Gly Ala Thr Cys Thr  Cys Cys Cys Cys Cys  Thr Thr Cys

    1820                1825                1830
Thr Thr  Cys Ala Ala Gly Ala  Cys Cys Cys Ala Cys  Gly Ala Gly

    1835                1840                1845
Gly Thr  Gly Gly Thr Ala Gly  Gly Cys Ala Gly Cys  Thr Cys Cys

    1850                1855                1860
Cys Thr  Cys Cys Thr Cys Thr  Thr Cys Gly Thr Gly  Cys Ala Cys

    1865                1870                1875
Gly Ala  Cys Cys Ala Cys Ala  Cys Cys Gly Gly Cys  Cys Thr Gly

    1880                1885                1890
Gly Cys  Cys Ala Ala Gly Gly  Thr Cys Thr Gly Gly  Ala Thr Gly

    1895                1900                1905
Ala Thr  Ala Gly Ala Cys Thr  Thr Cys Gly Gly Cys  Ala Ala Gly

    1910                1915                1920
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```
Ala Cys  Gly Gly Thr Gly Gly  Cys Cys Thr Thr Gly  Cys Cys Cys

    1925                1930                1935
Gly Ala  Cys Cys Ala Cys Cys  Ala Gly Ala Cys Gly  Cys Thr Cys

    1940                1945                1950
Ala Gly  Cys Cys Ala Cys Ala  Gly Gly Cys Thr Gly  Cys Cys Cys

    1955                1960                1965
Thr Gly  Gly Gly Cys Thr Gly  Ala Gly Gly Gly Cys  Ala Ala Cys

    1970                1975                1980
Cys Gly  Thr Gly Ala Gly Gly  Ala Cys Gly Gly Cys  Thr Ala Cys

    1985                1990                1995
Cys Thr  Cys Thr Gly Gly Gly  Gly Cys Cys Thr Gly  Gly Ala Cys

    2000                2005                2010
Ala Ala  Cys Ala Thr Gly Ala  Thr Cys Thr Gly Cys  Cys Thr Cys

    2015                2020                2025
Cys Thr  Gly Cys Ala Gly Gly  Gly Gly Cys Thr Gly  Gly Cys Ala

    2030                2035                2040
Cys Ala  Gly Ala Gly Cys Thr  Gly Ala

    2045                2050


<210> SEQ ID NO 8
<211> LENGTH: 461
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 8

Met Thr Leu Pro Gly Gly Pro Thr Gly Met Ala Arg Pro Gly Gly Ala
1               5                   10                  15

Arg Pro Cys Ser Pro Gly Leu Glu Arg Ala Pro Arg Arg Ser Val Gly
            20                  25                  30

Glu Leu Arg Leu Leu Phe Glu Ala Arg Cys Ala Ala Val Ala Ala Ala
        35                  40                  45

Ala Ala Ala Gly Glu Pro Arg Ala Arg Gly Ala Lys Arg Arg Gly Gly
    50                  55                  60

Gln Val Pro Asn Gly Leu Pro Arg Ala Pro Pro Ala Pro Val Ile Pro
65                  70                  75                  80

Gln Leu Thr Val Thr Ala Glu Glu Pro Asp Val Pro Pro Thr Ser Pro
                85                  90                  95

Gly Pro Pro Glu Arg Glu Arg Asp Cys Leu Pro Ala Ala Gly Ser Ser
            100                 105                 110

His Leu Gln Gln Pro Arg Arg Leu Ser Thr Ser Ser Val Ser Ser Thr
        115                 120                 125

Gly Ser Ser Ser Leu Leu Glu Asp Ser Glu Asp Asp Leu Leu Ser Asp
    130                 135                 140

Ser Glu Ser Arg Ser Arg Gly Asn Val Gln Leu Glu Ala Gly Glu Asp
145                 150                 155                 160

Val Gly Gln Lys Asn His Trp Gln Lys Ile Arg Thr Met Val Asn Leu
                165                 170                 175

Pro Val Ile Ser Pro Phe Lys Lys Arg Tyr Ala Trp Val Gln Leu Ala
            180                 185                 190

Gly His Thr Gly Ser Phe Lys Ala Ala Gly Thr Ser Gly Leu Ile Leu
        195                 200                 205

Lys Arg Cys Ser Glu Pro Glu Arg Tyr Cys Leu Ala Arg Leu Met Ala
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    210                 215                 220

Asp Ala Leu Arg Gly Cys Val Pro Ala Phe His Gly Val Val Glu Arg
225                 230                 235                 240

Asp Gly Glu Ser Tyr Leu Gln Leu Gln Asp Leu Leu Asp Gly Phe Asp
                245                 250                 255

Gly Pro Cys Val Leu Asp Cys Lys Met Gly Val Arg Thr Tyr Leu Glu
            260                 265                 270

Glu Glu Leu Thr Lys Ala Arg Glu Arg Pro Lys Leu Arg Lys Asp Met
        275                 280                 285

Tyr Lys Lys Met Leu Ala Val Asp Pro Glu Ala Pro Thr Glu Glu Glu
    290                 295                 300

His Ala Gln Arg Ala Val Thr Lys Pro Arg Tyr Met Gln Trp Arg Glu
305                 310                 315                 320

Gly Ile Ser Ser Ser Thr Thr Leu Gly Phe Arg Ile Glu Gly Ile Lys
                325                 330                 335

Lys Ala Asp Gly Ser Cys Ser Thr Asp Phe Lys Thr Thr Arg Ser Arg
            340                 345                 350

Glu Gln Val Leu Arg Val Phe Glu Glu Phe Val Gln Gly Asp Glu Glu
        355                 360                 365

Val Leu Arg Arg Tyr Leu Asn Arg Leu Gln Gln Ile Arg Asp Thr Leu
    370                 375                 380

Glu Val Ser Glu Phe Phe Arg Arg His Glu Val Ile Gly Ser Ser Leu
385                 390                 395                 400

Leu Phe Val His Asp His Cys His Arg Ala Gly Val Trp Leu Ile Asp
                405                 410                 415

Phe Gly Lys Thr Thr Pro Leu Pro Asp Gly Gln Ile Leu Asp His Arg
            420                 425                 430

Arg Pro Trp Glu Glu Gly Asn Arg Glu Asp Gly Tyr Leu Leu Gly Leu
        435                 440                 445

Asp Asn Leu Ile Gly Ile Leu Ala Ser Leu Ala Glu Arg
    450                 455                 460


<210> SEQ ID NO 9
<211> LENGTH: 946
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 9

Met Ala Val Tyr Cys Tyr Ala Leu Asn Ser Leu Val Ile Met Asn Ser
1               5                   10                  15

Ala Asn Glu Met Lys Ser Gly Gly Gly Pro Gly Pro Ser Gly Ser Glu
            20                  25                  30

Thr Pro Pro Pro Pro Arg Arg Ala Val Leu Ser Pro Gly Ser Val Phe
        35                  40                  45

Ser Pro Gly Arg Gly Ala Ser Phe Leu Phe Pro Pro Ala Glu Ser Leu
    50                  55                  60

Ser Pro Glu Glu Pro Arg Ser Pro Gly Gly Trp Arg Ser Gly Arg Arg
65                  70                  75                  80

Arg Leu Asn Ser Ser Ser Gly Ser Gly Ser Gly Ser Ser Gly Ser Ser
                85                  90                  95

Val Ser Ser Pro Ser Trp Ala Gly Arg Leu Arg Gly Asp Arg Gln Gln
            100                 105                 110
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Val Val Ala Ala Gly Thr Leu Ser Pro Pro Gly Pro Glu Glu Ala Lys
        115                 120                 125

Arg Lys Leu Arg Ile Leu Gln Arg Glu Leu Gln Asn Val Gln Val Asn
    130                 135                 140

Gln Lys Val Gly Met Phe Glu Ala His Ile Gln Ala Gln Ser Ser Ala
145                 150                 155                 160

Ile Gln Ala Pro Arg Ser Pro Arg Leu Gly Arg Ala Arg Ser Pro Ser
                165                 170                 175

Pro Cys Pro Phe Arg Ser Ser Ser Gln Pro Pro Gly Arg Val Leu Val
            180                 185                 190

Gln Gly Ala Arg Ser Glu Glu Arg Arg Thr Lys Ser Trp Gly Glu Gln
        195                 200                 205

Cys Pro Glu Thr Ser Gly Thr Asp Ser Gly Arg Lys Gly Gly Pro Ser
    210                 215                 220

Leu Cys Ser Ser Gln Val Lys Lys Gly Met Pro Pro Leu Pro Gly Arg
225                 230                 235                 240

Ala Ala Pro Thr Gly Ser Glu Ala Gln Gly Pro Ser Ala Phe Val Arg
                245                 250                 255

Met Glu Lys Gly Ile Pro Ala Ser Pro Arg Cys Gly Ser Pro Thr Ala
            260                 265                 270

Met Glu Ile Asp Lys Arg Gly Ser Pro Thr Pro Gly Thr Arg Ser Cys
        275                 280                 285

Leu Ala Pro Ser Leu Gly Leu Phe Gly Ala Ser Leu Thr Met Ala Thr
    290                 295                 300

Glu Val Ala Ala Arg Val Thr Ser Thr Gly Pro His Arg Pro Gln Asp
305                 310                 315                 320

Leu Ala Leu Thr Glu Pro Ser Gly Arg Ala Arg Glu Leu Glu Asp Leu
                325                 330                 335

Gln Pro Pro Glu Ala Leu Val Glu Arg Gln Gly Gln Phe Leu Gly Ser
            340                 345                 350

Glu Thr Ser Pro Ala Pro Glu Arg Gly Gly Pro Arg Asp Gly Glu Pro
        355                 360                 365

Pro Gly Lys Met Gly Lys Gly Tyr Leu Pro Cys Gly Met Pro Gly Ser
    370                 375                 380

Gly Glu Pro Glu Val Gly Lys Arg Pro Glu Glu Thr Thr Val Ser Val
385                 390                 395                 400

Gln Ser Ala Glu Ser Ser Asp Ser Leu Ser Trp Ser Arg Leu Pro Arg
                405                 410                 415

Ala Leu Ala Ser Val Gly Pro Glu Glu Ala Arg Ser Gly Ala Pro Val
            420                 425                 430

Gly Gly Gly Arg Trp Gln Leu Ser Asp Arg Val Glu Gly Gly Ser Pro
        435                 440                 445

Thr Leu Gly Leu Leu Gly Gly Ser Pro Ser Ala Gln Pro Gly Thr Gly
    450                 455                 460

Asn Val Glu Ala Gly Ile Pro Ser Gly Arg Met Leu Glu Pro Leu Pro
465                 470                 475                 480

Cys Trp Asp Ala Ala Lys Asp Leu Lys Glu Pro Gln Cys Pro Pro Gly
                485                 490                 495

Asp Arg Val Gly Val Gln Pro Gly Asn Ser Arg Val Trp Gln Gly Thr
            500                 505                 510

Met Glu Lys Ala Gly Leu Ala Trp Thr Arg Gly Thr Gly Val Gln Ser
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        515                 520                 525

Glu Gly Thr Trp Glu Ser Gln Arg Gln Asp Ser Asp Ala Leu Pro Ser
    530                 535                 540

Pro Glu Leu Leu Pro Gln Asp Gln Asp Lys Pro Phe Leu Arg Lys Ala
545                 550                 555                 560

Cys Ser Pro Ser Asn Ile Pro Ala Val Ile Ile Thr Asp Met Gly Thr
                565                 570                 575

Gln Glu Asp Gly Ala Leu Glu Glu Thr Gln Gly Ser Pro Arg Gly Asn
            580                 585                 590

Leu Pro Leu Arg Lys Leu Ser Ser Ser Ser Ala Ser Ser Thr Gly Phe
        595                 600                 605

Ser Ser Ser Tyr Glu Asp Ser Glu Glu Asp Ile Ser Ser Asp Pro Glu
    610                 615                 620

Arg Thr Leu Asp Pro Asn Ser Ala Phe Leu His Thr Leu Asp Gln Gln
625                 630                 635                 640

Lys Pro Arg Val Ser Lys Ser Trp Arg Lys Ile Lys Asn Met Val His
                645                 650                 655

Trp Ser Pro Phe Val Met Ser Phe Lys Lys Lys Tyr Pro Trp Ile Gln
            660                 665                 670

Leu Ala Gly His Ala Gly Ser Phe Lys Ala Ala Ala Asn Gly Arg Ile
        675                 680                 685

Leu Lys Lys His Cys Glu Ser Glu Gln Arg Cys Leu Asp Arg Leu Met
    690                 695                 700

Val Asp Val Leu Arg Pro Phe Val Pro Ala Tyr His Gly Asp Val Val
705                 710                 715                 720

Lys Asp Gly Glu Arg Tyr Asn Gln Met Asp Asp Leu Leu Ala Asp Phe
                725                 730                 735

Asp Ser Pro Cys Val Met Asp Cys Lys Met Gly Ile Arg Thr Tyr Leu
            740                 745                 750

Glu Glu Glu Leu Thr Lys Ala Arg Lys Lys Pro Ser Leu Arg Lys Asp
        755                 760                 765

Met Tyr Gln Lys Met Ile Glu Val Asp Pro Glu Ala Pro Thr Glu Glu
    770                 775                 780

Glu Lys Ala Gln Arg Ala Val Thr Lys Pro Arg Tyr Met Gln Trp Arg
785                 790                 795                 800

Glu Thr Ile Ser Ser Thr Ala Thr Leu Gly Phe Arg Ile Glu Gly Ile
                805                 810                 815

Lys Lys Glu Asp Gly Thr Val Asn Arg Asp Phe Lys Lys Thr Lys Thr
            820                 825                 830

Arg Glu Gln Val Thr Glu Ala Phe Arg Glu Phe Thr Lys Gly Asn His
        835                 840                 845

Asn Ile Leu Ile Ala Tyr Arg Asp Arg Leu Lys Ala Ile Arg Thr Thr
    850                 855                 860

Leu Glu Val Ser Pro Phe Phe Lys Cys His Glu Val Ile Gly Ser Ser
865                 870                 875                 880

Leu Leu Phe Ile His Asp Lys Lys Glu Gln Ala Lys Val Trp Met Ile
                885                 890                 895

Asp Phe Gly Lys Thr Thr Pro Leu Pro Glu Gly Gln Thr Leu Gln His
            900                 905                 910

Asp Val Pro Trp Gln Glu Gly Asn Arg Glu Asp Gly Tyr Leu Ser Gly
        915                 920                 925
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Leu Asn Asn Leu Val Asp Ile Leu Thr Glu Met Ser Gln Asp Ala Pro
    930                 935                 940

Leu Ala
945
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<210> SEQ ID NO 10
<211> LENGTH: 683
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 10

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Met Arg Arg Cys Pro Cys Arg Gly Ser Leu Asn Glu Ala Glu Ala Gly
1               5                   10                  15

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

Arg Arg Arg Gln Pro Gly Gln Gln Arg Pro Gly Pro Gly Ala Gly Ala
        35                  40                  45

Pro Ala Gly Arg Pro Glu Gly Gly Gly Pro Trp Ala Arg Thr Glu Gly
    50                  55                  60

Ser Ser Leu His Ser Glu Pro Glu Arg Ala Gly Leu Gly Pro Ala Pro
65                  70                  75                  80

Gly Thr Glu Ser Pro Gln Ala Glu Phe Trp Thr Asp Gly Gln Thr Glu
                85                  90                  95

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

Thr Glu Pro Asp Arg Ser Ser Leu Arg Thr His Leu Glu Trp Ser Trp
        115                 120                 125

Ser Glu Leu Glu Thr Thr Cys Leu Trp Thr Glu Thr Gly Thr Asp Gly
    130                 135                 140

Leu Trp Thr Asp Pro His Arg Ser Asp Leu Gln Phe Gln Pro Glu Glu
145                 150                 155                 160

Ala Ser Pro Trp Thr Gln Pro Gly Val His Gly Pro Trp Thr Glu Leu
                165                 170                 175

Glu Thr His Gly Ser Gln Thr Gln Pro Glu Arg Val Lys Ser Trp Ala
            180                 185                 190

Asp Asn Leu Trp Thr His Gln Asn Ser Ser Ser Leu Gln Thr His Pro
        195                 200                 205

Glu Gly Ala Cys Pro Ser Lys Glu Pro Ser Ala Asp Gly Ser Trp Lys
    210                 215                 220

Glu Leu Tyr Thr Asp Gly Ser Arg Thr Gln Gln Asp Ile Glu Gly Pro
225                 230                 235                 240

Trp Thr Glu Pro Tyr Thr Asp Gly Ser Gln Lys Lys Gln Asp Thr Glu
                245                 250                 255

Ala Ala Arg Lys Gln Pro Gly Thr Gly Gly Phe Gln Ile Gln Gln Asp
            260                 265                 270

Thr Asp Gly Ser Trp Thr Gln Pro Ser Thr Asp Gly Ser Gln Thr Ala
        275                 280                 285

Pro Gly Thr Asp Cys Leu Leu Gly Glu Pro Glu Asp Gly Pro Leu Glu
    290                 295                 300

Glu Pro Glu Pro Gly Glu Leu Leu Thr His Leu Tyr Ser His Leu Lys
305                 310                 315                 320

Cys Ser Pro Leu Cys Pro Val Pro Arg Leu Ile Ile Thr Pro Glu Thr
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               325                 330                 335

Pro Glu Pro Glu Ala Gln Pro Val Gly Pro Pro Ser Arg Val Glu Gly
            340                 345                 350

Gly Ser Gly Gly Phe Ser Ser Ala Ser Ser Phe Asp Glu Ser Glu Asp
        355                 360                 365

Asp Val Val Ala Gly Gly Gly Gly Ala Ser Asp Pro Glu Asp Arg Ser
    370                 375                 380

Gly Ser Lys Pro Trp Lys Lys Leu Lys Thr Val Leu Lys Tyr Ser Pro
385                 390                 395                 400

Phe Val Val Ser Phe Arg Lys His Tyr Pro Trp Val Gln Leu Ser Gly
                405                 410                 415

His Ala Gly Asn Phe Gln Ala Gly Glu Asp Gly Arg Ile Leu Lys Arg
            420                 425                 430

Phe Cys Gln Cys Glu Gln Arg Ser Leu Glu Gln Leu Met Lys Asp Pro
        435                 440                 445

Leu Arg Pro Phe Val Pro Ala Tyr Tyr Gly Met Val Leu Gln Asp Gly
    450                 455                 460

Gln Thr Phe Asn Gln Met Glu Asp Leu Leu Ala Asp Phe Glu Gly Pro
465                 470                 475                 480

Ser Ile Met Asp Cys Lys Met Gly Ser Arg Thr Tyr Leu Glu Glu Glu
                485                 490                 495

Leu Val Lys Ala Arg Glu Arg Pro Arg Pro Arg Lys Asp Met Tyr Glu
            500                 505                 510

Lys Met Val Ala Val Asp Pro Gly Ala Pro Thr Pro Glu Glu His Ala
        515                 520                 525

Gln Gly Ala Val Thr Lys Pro Arg Tyr Met Gln Trp Arg Glu Thr Met
    530                 535                 540

Ser Ser Thr Ser Thr Leu Gly Phe Arg Ile Glu Gly Ile Lys Lys Ala
545                 550                 555                 560

Asp Gly Thr Cys Asn Thr Asn Phe Lys Lys Thr Gln Ala Leu Glu Gln
                565                 570                 575

Val Thr Lys Val Leu Glu Asp Phe Val Asp Gly Asp His Val Ile Leu
            580                 585                 590

Gln Lys Tyr Val Ala Cys Leu Glu Glu Leu Arg Glu Ala Leu Glu Ile
        595                 600                 605

Ser Pro Phe Phe Lys Thr His Glu Val Val Gly Ser Ser Leu Leu Phe
    610                 615                 620

Val His Asp His Thr Gly Leu Ala Lys Val Trp Met Ile Asp Phe Gly
625                 630                 635                 640

Lys Thr Val Ala Leu Pro Asp His Gln Thr Leu Ser His Arg Leu Pro
                645                 650                 655

Trp Ala Glu Gly Asn Arg Glu Asp Gly Tyr Leu Trp Gly Leu Asp Asn
            660                 665                 670

Met Ile Cys Leu Leu Gln Gly Leu Ala Gln Ser
        675                 680
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What is claimed is:

1. A method of identifying a candidate IGFR pathway modulating agent, said method comprising the steps of:

(a) providing an assay system comprising an ITPK polypeptide or nucleic acid;

(b) contacting the assay system with a test agent under conditions whereby, but for the presence of the test agent, the system provides a reference activity; and

(c) detecting a test agent-biased activity of the assay system, wherein a difference between the test agent-biased activity and the reference activity identifies the test agent as a candidate IGFR pathway modulating agent.

2. The method of claim 1 wherein the assay system comprises cultured cells that express the ITPK polypeptide.

3. The method of claim 2 wherein the cultured cells additionally have defective IGFR function.

4. The method of claim 1 wherein the assay system includes a screening assay comprising an ITPK polypeptide, and the candidate test agent is a small molecule modulator.

5. The method of claim 4 wherein the assay is a kinase assay.

6. The method of claim 1 wherein the assay system is selected from the group consisting of an apoptosis assay system, a cell proliferation assay system, an angiogenesis assay system, and a hypoxic induction assay system.

7. The method of claim 1 wherein the assay system includes a binding assay comprising an ITPK polypeptide and the candidate test agent is an antibody.

8. The method of claim 1 wherein the assay system includes an expression assay comprising an ITPK nucleic acid and the candidate test agent is a nucleic acid modulator.

9. The method of claim 8 wherein the nucleic acid modulator is an antisense oligomer.

10. The method of claim 8 wherein the nucleic acid modulator is a PMO.

11. The method of claim 1 additionally comprising:

(d) administering the candidate IGFR pathway modulating agent identified in (c) to a model system comprising cells defective in IGFR function and, detecting a phenotypic change in the model system that indicates that the IGFR function is restored.

12. The method of claim 11 wherein the model system is a mouse model with defective IGFR function.

13. A method for modulating a IGFR pathway of a cell comprising contacting a cell defective in IGFR function with a candidate modulator that specifically binds to an ITPK polypeptide, whereby IGFR function is restored.

14. The method of claim 13 wherein the candidate modulator is administered to a vertebrate animal predetermined to have a disease or disorder resulting from a defect in IGFR function.

15. The method of claim 13 wherein the candidate modulator is selected from the group consisting of an antibody and a small molecule.

16. The method of claim 1, comprising the additional steps of:

(d) providing a secondary assay system comprising cultured cells or a non-human animal expressing ITPK,

(e) contacting the secondary assay system with the test agent of (b) or an agent derived therefrom under conditions whereby, but for the presence of the test agent or agent derived therefrom, the system provides a reference activity; and

(f) detecting an agent-biased activity of the second assay system,

wherein a difference between the agent-biased activity and the reference activity of the second assay system confirms the test agent or agent derived therefrom as a candidate IGFR pathway modulating agent,

and wherein the second assay detects an agent-biased change in the IGFR pathway.

17. The method of claim 16 wherein the secondary assay system comprises cultured cells.

18. The method of claim 16 wherein the secondary assay system comprises a non-human animal.

19. The method of claim 18 wherein the non-human animal mis-expresses a IGFR pathway gene.

20. A method of modulating IGFR pathway in a mammalian cell comprising contacting the cell with an agent that specifically binds an ITPK polypeptide or nucleic acid.

21. The method of claim 20 wherein the agent is administered to a mammalian animal predetermined to have a pathology associated with the IGFR pathway.

22. The method of claim 20 wherein the agent is a small molecule modulator, a nucleic acid modulator, or an antibody.

23. A method for diagnosing a disease in a patient comprising:

obtaining a biological sample from the patient;

contacting the sample with a probe for ITPK expression;

comparing results from step (b) with a control;

determining whether step (c) indicates a likelihood of disease.

24. The method of claim 23 wherein said disease is cancer.

25. The method according to claim 24, wherein said cancer is a cancer as shown in Table 1 as having >25% expression level.

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