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- (71) Applicant (*for all designated States except US*): **EX-ELIXIS, INC.** [US/US]; P.O. Box 511, 170 Harbor Way, South San Francisco, CA 94083-0511 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): **BELVIN, Marcia** [US/US]; 921 Santa Fe Avenue, Albany, CA 94706 (US). **FRANCIS-LANG, Helen** [GB/US]; 1782 Pacific Avenue, Apt. 2, San Francisco, CA 94109 (US). **PLOWMAN, Gregory, D.** [US/US]; 35 Winding Way, San Carlos, CA 94070 (US). **FUNKE, Roel, P.** [NL/US]; 343 California Avenue, South San Francisco, CA 94080 (US). **LI, Danxi** [US/US]; 90 Behr Avenue, #302, San Francisco, CA 94141 (US). **FRIEDMAN, Lori** [US/US]; 113 Arundel Road, San Carlos, CA 94070 (US).
- (74) Agents: **SHAYESTEH, Laleh** et al.; Exelixis, Inc., P.O. Box 511, 170 Harbor Way, South San Francisco, CA 94083-0511 (US).
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(54) Title: MODIFIER OF THE P53 PATHWAY AND METHODS OF USE

(57) Abstract: Human HM genes are identified as modulators of the p53 pathway, and thus are therapeutic targets for disorders associated with defective p53 function. Methods for identifying modulators of p53, comprising screening for agent that modulate the activity of HM are provided.



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MODIFIERS OF THE p53 PATHWAY AND METHODS OF USE

REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. provisional patent applications 60/338,733
5 filed 10/22/2001 and 60/357,600 filed 2/15/2002. The contents of the prior applications
are hereby incorporated in their entirety.

BACKGROUND OF THE INVENTION

The p53 gene is mutated in over 50 different types of human cancers, including
10 familial and spontaneous cancers, and is believed to be the most commonly mutated gene
in human cancer (Zambetti and Levine, FASEB (1993) 7:855-865; Hollstein, *et al.*,
Nucleic Acids Res. (1994) 22:3551-3555). Greater than 90% of mutations in the p53 gene
are missense mutations that alter a single amino acid that inactivates p53 function.
Aberrant forms of human p53 are associated with poor prognosis, more aggressive tumors,
15 metastasis, and short survival rates (Mitsudomi *et al.*, Clin Cancer Res 2000 Oct;
6(10):4055-63; Koshland, Science (1993) 262:1953).

The human p53 protein normally functions as a central integrator of signals including
DNA damage, hypoxia, nucleotide deprivation, and oncogene activation (Prives, Cell
(1998) 95:5-8). In response to these signals, p53 protein levels are greatly increased with
20 the result that the accumulated p53 activates cell cycle arrest or apoptosis depending on
the nature and strength of these signals. Indeed, multiple lines of experimental evidence
have pointed to a key role for p53 as a tumor suppressor (Levine, Cell (1997) 88:323-331).
For example, homozygous p53 "knockout" mice are developmentally normal but exhibit
nearly 100% incidence of neoplasia in the first year of life (Donehower *et al.*, Nature
25 (1992) 356:215-221).

The biochemical mechanisms and pathways through which p53 functions in normal
and cancerous cells are not fully understood, but one clearly important aspect of p53
function is its activity as a gene-specific transcriptional activator. Among the genes with
known p53-response elements are several with well-characterized roles in either regulation
30 of the cell cycle or apoptosis, including GADD45, p21/Waf1/Cip1, cyclin G, Bax, IGF-
BP3, and MDM2 (Levine, Cell (1997) 88:323-331).

Leucine-rich repeats (LRRs) are short motifs of 22-28 residues in length and are found
in various cytoplasmic, membrane, and extracellular proteins (Rothberg, J. *et al.* (1990)
Genes Dev (12A): 2169-87). These proteins play diverse roles, with protein-protein

interactions being the most common property. In vitro studies of a synthetic LRR from *Drosophila* Toll protein have implied that the peptides form gels by adopting beta-sheet structures that form extended filaments. These results support the idea that LRRs mediate protein-protein interactions and cellular adhesion (Gay, N. (1991) FEBS Lett; 291(1): 87-91). Other functions of LRR-containing proteins include the binding of enzymes (Tan, F. et al. (1990) J Biol Chem; 265(1): 13-9) and vascular repair (Hickey, M. (1989) Proc Natl Acad Sci U S A; 86(17): 6773-7). The 3-D structure of ribonuclease inhibitor, a protein containing 15 LRRs, has been determined (Kobe, B. and Deisenhofer, J. (1993) Nature; 366(6457): 751-6) demonstrating LRRs to be a new class of alpha/beta fold. LRRs form elongated non-globular structures and are often flanked by cysteine rich domains.

LRRN1 is a protein containing nine LRRs, leucine rich repeat C-terminal and N-terminal cysteine rich domains, and an immunoglobulin (Ig) domain. It shares sequence similarity with D2S448, a melanoma associated gene (Nagase, T. et al. (2000) DNA Res; 7(2): 143-50).

In vivo, insulin-like growth factors I (IGF1) and II (IGF2) are always complexed to one of a family of 6 IGF-binding proteins, IGFBP1, IGFBP2, IGFBP3, IGFBP4, IGFBP5, and IGFBP6. Until birth, binary IGFBP/IGF complexes predominate in serum. In juvenile and adult mammals, however, 80% to 85% of serum IGFs are found in a ternary complex composed of 1 molecule each of IGF, IGFBP3, and a protein that is found only in serum, the acid-labile subunit (ALS). ALS retains the IGFBP3/IGF complexes in the vascular compartment and extends the half life of IGFs in the circulation. Synthesis of ALS occurs mainly in liver after birth and is stimulated by growth hormone. Insulin-like growth factor binding protein acid-labile subunit (IGFALS) mediates the formation of IGF1 and IGFBP3 complex (Leong, S. R., et al (1992) Mol Endocrinol 6:870-6). IGFALS is required for postnatal accumulation of IGF1 and IGFBP3 but, consistent with findings supporting a predominant role for locally produced IGF1, is not critical for growth. IGFALS is necessary for blood sugar regulation, and shows deficiency in non islet cell tumor hypoglycemia syndrome and in liver cirrhosis (Ottesen, L. H., et al (2001) Liver 21: 350-6; Baxter, R. C. (1996) Horm Res 46, 195-201).

The DNA ligase activity in most proliferating mammalian cells is due to the high molecular weight enzyme designated DNA ligase I (LIG1). It acts as a DNA replication and repair enzyme (Lindahl, T.; Barnes, D. (1992) Annu. Rev. Biochem 61: 251-281). Mutations in genes in this location are known to cause a Bloom syndrome-like phenotype with immunodeficiency, growth retardation and predisposition to cancer (Barnes, D. et

al. (1992) Genomics 12: 164-166). LIG1 is thought to mediate increased expression in quiescent cells in response to growth factors.

5 NAG14 is a protein containing eight LRRs, leucine rich repeat C-terminal and N-terminal cysteine rich domains, and an immunoglobulin (Ig) domain. It is similar to chondroadherin (Shen, Z. et al. (1998) Biochem J; 330 (Pt 1):549-57).

KIAA1580 is a protein containing an immunoglobulin (Ig) domain, a leucine rich repeat N-terminal and C-terminal cysteine rich domain and nine LRRs. It is similar to glycoprotein V (Kitaguchi, T. et al. (1997) Thromb Res; 87(2):235-44).

10 DKFZp76 is a protein containing three LRRs, a leucine rich repeat C-terminal cysteine rich domain and an immunoglobulin (Ig) domain. It has a region of low homology to a region of melanoma associated gene D2S448.

The FLRT family of proteins structurally resembles small leucine-rich proteoglycans found in the extracellular matrix (ECM). The ECM is composed of collagens, proteoglycans, and noncollagenous glycoproteins, which provide cells and tissues with a mechanical scaffold for adhesion, migration, and signal transduction. These functions are varied and complex and depend on interactions between ECM components and cellular receptors, such as integrins and proteoglycans, which are located at the cell surface (Lacy, S. et al. (1999) Genomics 62: 417-426).

20 Fibronectin leucine rich transmembrane protein 1 (FLRT1) is a member of the FLRT family, which has a putative type I membrane protein with ten LRRs flanked by cysteine-rich regions (Lacy, S. et al. (1999) *supra*). FLRT1 is expressed in adult and fetal brain and kidney, and portions of the brain. FLRT1 functions in cell adhesion and/or receptor signaling (Lacy, S. et al. (1999) *supra*).

25 FLRT2 (KIAA0405) is a protein with eighteen LRRs, two leucine rich repeat C-terminal and two leucine rich repeat N-terminal cysteine rich domains and two fibronectin type III domains. It is similar to mouse fibromodulin (Ishikawa et al. (1997) DNA Res. 4: 307-313). FLRT2 is expressed in pancreas, skeletal muscle, brain, and heart. FLRT2 is also thought to be involved in cell adhesion and/or receptor signaling (Lacy, S. et al. (1999) *supra*).

30 Fibronectin leucine rich transmembrane protein 3 (FLRT3) is also a member of the FLRT family, which has a putative type I membrane protein with ten LRRs flanked by cysteine-rich regions. It may function as a receptor involved in cell-cell contact and cell adhesion (Lacy, S. et al. (1999) *supra*). FLRT3 is expressed in kidney, skeletal muscle, lung, and brain, and at lower levels in pancreas, liver, placenta, and heart.

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 BM et al., 1985 EMBO J 4:1551-1557; Gateff E. 1982 Adv. Cancer Res. 37: 33-74; Watson KL., et al., 1994 J Cell Sci. 18: 19-33; Miklos GL, and Rubin GM. 1996 Cell 86:521-529; Wassarman DA, et al., 1995 Curr Opin Gen Dev 5: 44-50; and Booth DR. 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 p53, modifier genes can be identified that may be attractive candidate targets for novel therapeutics.

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

We have discovered genes that modify the p53 pathway in *Drosophila*, and identified their human orthologs, hereinafter referred to as HM. The invention provides methods for utilizing these p53 modifier genes and polypeptides to identify HM-modulating agents that are candidate therapeutic agents that can be used in the treatment of disorders associated with defective or impaired p53 function and/or HM function. Preferred HM-modulating agents specifically bind to HM polypeptides and restore p53 function. Other preferred HM-modulating agents are nucleic acid modulators such as antisense oligomers and RNAi that repress HM gene expression or product activity by, for example, binding to and inhibiting the respective nucleic acid (i.e. DNA or mRNA).

HM modulating agents may be evaluated by any convenient *in vitro* or *in vivo* assay for molecular interaction with an HM polypeptide or nucleic acid. In one embodiment, candidate HM modulating agents are tested with an assay system comprising an HM polypeptide or nucleic acid. Agents that produce a change in the activity of the assay system relative to controls are identified as candidate p53 modulating agents. The assay system may be cell-based or cell-free. HM-modulating agents include HM related proteins (e.g. dominant negative mutants, and biotherapeutics); HM-specific antibodies; HM-specific antisense oligomers and other nucleic acid modulators; and chemical agents that specifically bind to or interact with HM or compete with HM binding partner (e.g. by binding to an HM binding partner). In one specific embodiment, a small molecule modulator is identified using a binding assay. In specific embodiments, the screening assay system is selected from an apoptosis assay, a cell proliferation assay, an angiogenesis assay, and a hypoxic induction assay.

In another embodiment, candidate p53 pathway modulating agents are further tested using a second assay system that detects changes in the p53 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 p53 pathway, such as an angiogenic, apoptotic, or cell proliferation disorder (e.g. cancer).

The invention further provides methods for modulating the HM function and/or the p53 pathway in a mammalian cell by contacting the mammalian cell with an agent that specifically binds an HM 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 the p53 pathway.

DETAILED DESCRIPTION OF THE INVENTION

Genetic screens were designed to identify modifiers of the p53 pathway in *Drosophila*, where a genetic modifier screen was carried out in which p53 was overexpressed in the wing (Ollmann M, et al., Cell 2000 101: 91-101). Modifiers of the p53 pathway were identified. Accordingly, vertebrate orthologs of these modifiers, and preferably the human orthologs, Human modifiers (HM) genes (i.e., nucleic acids and polypeptides) are attractive drug targets for the treatment of pathologies associated with a defective p53

signaling pathway, such as cancer. Table 1 lists the modifiers and their orthologs (see example II).

In vitro and in vivo methods of assessing HM function are provided herein. Modulation of the HM or their respective binding partners is useful for understanding the association of the p53 pathway and its members in normal and disease conditions and for developing diagnostics and therapeutic modalities for p53 related pathologies. HM-modulating agents that act by inhibiting or enhancing HM expression, directly or indirectly, for example, by affecting an HM function such as binding activity, can be identified using methods provided herein. HM modulating agents are useful in diagnosis, therapy and pharmaceutical development.

Nucleic acids and polypeptides of the invention

Sequences related to HM nucleic acids and polypeptides that can be used in the invention are disclosed in Genbank (referenced by Genbank identifier (GI) or RefSeq number), and shown in Table 1. SEQ ID NOs for each disclosed sequence is also indicated in Table 1.

The term "HM polypeptide" refers to a full-length HM protein or a functionally active fragment or derivative thereof. A "functionally active" HM fragment or derivative exhibits one or more functional activities associated with a full-length, wild-type HM protein, such as antigenic or immunogenic activity, ability to bind natural cellular substrates, etc. The functional activity of HM 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, New Jersey) and as further discussed below. In one embodiment, a functionally active HM polypeptide is an HM derivative capable of rescuing defective endogenous HM 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 HM, such as a binding domain. Protein domains can be identified using the PFAM program (Bateman A., et al., Nucleic Acids Res, 1999, 27:260-2). Methods for obtaining HM 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

any one of SEQ ID NOs:15-28 (an HM). In further preferred embodiments, the fragment comprises the entire functionally active domain.

The term "HM nucleic acid" refers to a DNA or RNA molecule that encodes an HM polypeptide. Preferably, the HM 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 HM. Methods of identifying orthologs 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 MA and Bork P, Proc Natl Acad Sci (1998) 95:5849-5856; Huynen MA *et al.*, Genome Research (2000) 10:1204-1210).

Programs for multiple sequence alignment, such as CLUSTAL (Thompson JD 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
5 conservative amino acid substitutions in addition to identical amino acids in the computation.

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
10 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.

Alternatively, an alignment for nucleic acid sequences is provided by the local
15 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
20 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
25 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."

Derivative nucleic acid molecules of the subject nucleic acid molecules include sequences that hybridize to the nucleic acid sequence of any of SEQ ID NOs:1-14. The
30 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 any one of SEQ ID NOs:1-14 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 6X single strength citrate (SSC) (1X SSC is 0.15 M NaCl, 0.015 M Na citrate; pH 7.0), 5X 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 6X SSC, 1X Denhardt's solution, 100 µg/ml yeast tRNA and 0.05% sodium pyrophosphate; and washing of filters at 65° C for 1h in a solution containing 0.1X SSC and 0.1% SDS (sodium dodecyl sulfate).

10 In other embodiments, moderately stringent hybridization conditions are used that comprise: pretreatment of filters containing nucleic acid for 6 h at 40° C in a solution containing 35% formamide, 5X 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 18-20h at 40° C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH7.5), 5mM 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 2X SSC and 0.1% SDS.

Alternatively, low stringency conditions can be used that comprise: incubation for 8 hours to overnight at 37° C in a solution comprising 20% formamide, 5 x SSC, 50 mM sodium phosphate (pH 7.6), 5X 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 x SSC at about 37° C for 1 hour.

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

25 HM nucleic acids and polypeptides, useful for identifying and testing agents that modulate HM function and for other applications related to the involvement of HM in the p53 pathway. HM nucleic acids and derivatives 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 HM protein for assays used to assess HM 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 SJ and Hames BD (eds.) *Protein Expression: A Practical Approach*, Oxford University Press Inc., New York 1999; Stanbury PF 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 JE et al, *Current Protocols in Protein Science* (eds.), 1999, John Wiley & Sons, New York). In particular embodiments, recombinant HM is expressed in a cell line known to have defective p53 function (*e.g.* SAOS-2 osteoblasts, H1299 lung cancer cells, C33A and HT3 cervical cancer cells, HT-29 and DLD-1 colon cancer cells, among others, available from American Type Culture Collection (ATCC), Manassas, VA). The recombinant cells are used in cell-based screening assay systems of the invention, as described further below.

The nucleotide sequence encoding an HM polypeptide can be inserted into any appropriate expression vector. The necessary transcriptional and translational signals, including promoter/enhancer element, can derive from the native HM 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.

To detect expression of the HM gene product, the expression vector can comprise a promoter operably linked to an HM 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 HM gene product based on the physical or functional properties of the HM protein in *in vitro* assay systems (*e.g.* immunoassays).

The HM 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).

Once a recombinant cell that expresses the HM 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 HM 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.

The methods of this invention may also use cells that have been engineered for altered expression (mis-expression) of HM or other genes associated with the p53 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

Animal models that have been genetically modified to alter HM expression may be used in *in vivo* assays to test for activity of a candidate p53 modulating agent, or to further assess the role of HM in a p53 pathway process such as apoptosis or cell proliferation. Preferably, the altered HM expression results in a detectable phenotype, such as decreased or increased levels of cell proliferation, angiogenesis, or apoptosis compared to control animals having normal HM expression. The genetically modified animal may additionally have altered p53 expression (*e.g.* p53 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.

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. 5 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 10 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 15 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 20 International Publication Nos. WO 97/07668 and WO 97/07669).

In one embodiment, the transgenic animal is a "knock-out" animal having a heterozygous or homozygous alteration in the sequence of an endogenous HM gene that results in a decrease of HM function, preferably such that HM expression is undetectable or insignificant. Knock-out animals are typically generated by homologous recombination 25 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 HM gene is used to construct a 30 homologous recombination vector suitable for altering an endogenous HM 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 MH *et al.*, (1994) Scan J Immunol 40:257-264; Declerck PJ *et al.*, (1995) J Biol Chem. 270:8397-400).

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 HM gene, e.g., by introduction of additional copies of HM, or by operatively inserting a regulatory sequence that provides for altered expression of an endogenous copy of the HM gene. Such regulatory sequences include inducible, tissue-specific, and constitutive promoters and enhancer elements. The knock-in can be homozygous or heterozygous.

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 recombinase 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).

The genetically modified animals can be used in genetic studies to further elucidate the p53 pathway, as animal models of disease and disorders implicating defective p53 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 HM function and phenotypic changes are compared with appropriate control animals such as genetically modified animals that receive placebo treatment, and/or animals with unaltered HM expression that receive candidate therapeutic agent.

In addition to the above-described genetically modified animals having altered HM function, animal models having defective p53 function (and otherwise normal HM function), can be used in the methods of the present invention. For example, a p53 knockout mouse can be used to assess, *in vivo*, the activity of a candidate p53 modulating agent identified in one of the *in vitro* assays described below. p53 knockout mice are described in the literature (Jacks et al., Nature 2001;410:1111-1116, 1043-1044; Donehower *et al.*, supra). Preferably, the candidate p53 modulating agent when administered to a model system with cells defective in p53 function, produces a detectable phenotypic change in the model system indicating that the p53 function is restored, i.e., the cells exhibit normal cell cycle progression.

Modulating Agents

The invention provides methods to identify agents that interact with and/or modulate the function of HM and/or the p53 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 p53 pathway, as well as in further analysis of the HM protein and its contribution to the p53 pathway. Accordingly, the invention also provides methods for modulating the p53 pathway comprising the step of specifically modulating HM activity by administering an HM-interacting or -modulating agent.

As used herein, an "HM-modulating agent" is any agent that modulates HM function, for example, an agent that interacts with HM to inhibit or enhance HM activity or otherwise affect normal HM function. HM function can be affected at any level, including transcription, protein expression, protein localization, and cellular or extra-cellular activity. In a preferred embodiment, the HM - modulating agent specifically modulates the function of the HM. The phrases "specific modulating agent", "specifically modulates", etc., are used herein to refer to modulating agents that directly bind to the HM polypeptide or nucleic acid, and preferably inhibit, enhance, or otherwise alter, the function of the HM. These phrases also encompasses modulating agents that alter the interaction of the HM with a binding partner, substrate, or cofactor (e.g. by binding to a binding partner of an HM, or to a protein/binding partner complex, and altering HM function). In a further preferred embodiment, the HM- modulating agent is a modulator of the p53 pathway (e.g. it restores and/or upregulates p53 function) and thus is also a p53-modulating agent.

Preferred HM-modulating agents include small molecule compounds; HM-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.

Small molecule modulators

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 less than 10,000, preferably less than 5,000, more preferably less than 1,000, and most preferably less than 500. 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 HM 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 HM-modulating activity. Methods for generating and obtaining compounds are well known in the art (Schreiber SL, Science (2000) 151: 1964-1969; Radmann J and Gunther J, Science (2000) 151:1947-1948).

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 p53 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

Specific HM-interacting proteins are useful in a variety of diagnostic and therapeutic applications related to the p53 pathway and related disorders, as well as in validation assays for other HM-modulating agents. In a preferred embodiment, HM-interacting proteins affect normal HM function, including transcription, protein expression, protein localization, and cellular or extra-cellular activity. In another embodiment, HM-interacting proteins are useful in detecting and providing information about the function of HM proteins, as is relevant to p53 related disorders, such as cancer (e.g., for diagnostic means).

10 An HM-interacting protein may be endogenous, i.e. one that naturally interacts genetically or biochemically with an HM, such as a member of the HM pathway that modulates HM expression, localization, and/or activity. HM-modulators include dominant negative forms of HM-interacting proteins and of HM proteins themselves. Yeast two-hybrid and variant screens offer preferred methods for identifying endogenous HM-interacting proteins (Finley, R. L. et al. (1996) in DNA Cloning-Expression Systems: A
15 Practical Approach, eds. Glover D. & Hames B. D (Oxford University Press, Oxford, England), pp. 169-203; Fashema SF et al., Gene (2000) 250:1-14; Drees BL 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
20 the elucidation of protein complexes (reviewed in, e.g., Pandley A and Mann M, Nature (2000) 405:837-846; Yates JR 3rd, Trends Genet (2000) 16:5-8).

An HM-interacting protein may be an exogenous protein, such as an HM-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
25 antibodies: a laboratory manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press). HM antibodies are further discussed below.

In preferred embodiments, an HM-interacting protein specifically binds an HM protein. In alternative preferred embodiments, an HM-modulating agent binds an HM substrate, binding partner, or cofactor.

30

Antibodies

In another embodiment, the protein modulator is an HM specific antibody agonist or antagonist. The antibodies have therapeutic and diagnostic utilities, and can be used in screening assays to identify HM modulators. The antibodies can also be used in dissecting

the portions of the HM pathway responsible for various cellular responses and in the general processing and maturation of the HM.

Antibodies that specifically bind HM polypeptides can be generated using known methods. Preferably the antibody is specific to a mammalian ortholog of HM polypeptide, and more preferably, to human HM. Antibodies may be polyclonal, monoclonal (mAbs),
5 and more preferably, to human HM. Antibodies may be polyclonal, monoclonal (mAbs), humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab')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 HM which are particularly antigenic can be selected, for example, by routine screening of HM
10 polypeptides for antigenicity or by applying a theoretical method for selecting antigenic regions of a protein (Hopp and Wood (1981), Proc. Natl. 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 shown in any of SEQ ID NOs:15-28. Monoclonal antibodies with affinities of 10^8 M^{-1} preferably 10^9 M^{-1} to 10^{10} M^{-1} , or stronger can be
15 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 HM or substantially purified fragments thereof. If HM fragments are used, they preferably comprise at least 10, and more preferably, at least 20
20 contiguous amino acids of an HM protein. In a particular embodiment, HM-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
25 laboratory rabbit or mouse is immunized according to conventional protocols.

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

30 Chimeric antibodies specific to HM 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 LM, 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 SL. 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).

HM-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).

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*).

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).

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 (US Pat. No. 5,859,206; WO0073469).

Specific biotherapeutics

In a preferred embodiment, an HM-interacting protein may have biotherapeutic applications. Biotherapeutic agents formulated in pharmaceutically acceptable carriers and dosages may be used to activate or inhibit signal transduction pathways. This modulation may be accomplished by binding a ligand, thus inhibiting the activity of the pathway; or by binding a receptor, either to inhibit activation of, or to activate, the receptor. Alternatively, the biotherapeutic may itself be a ligand capable of activating or inhibiting a receptor. Biotherapeutic agents and methods of producing them are described in detail in U.S. Pat. No. 6,146,628.

When the HM is a ligand, it may be used as a biotherapeutic agent to activate or inhibit its natural receptor. Alternatively, antibodies against HM, as described in the previous section, may be used as biotherapeutic agents.

When the HM is a receptor, its ligand(s), antibodies to the ligand(s) or the HM itself may be used as biotherapeutics to modulate the activity of HM in the p53 pathway.

Nucleic Acid Modulators

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

In one embodiment, the antisense oligomer is an oligonucleotide that is sufficiently complementary to an HM mRNA to bind to and prevent translation, preferably by binding to the 5' untranslated region. HM-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.

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 JC, 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; US Pat. No. 5,235,033; and US Pat No. 5,378,841).

Alternative preferred HM 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 SM, et al., 2001 *Nature* 411:494-498).

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 JF, *et al*, *Current Concepts in Antisense Drug Design*, *J Med Chem.* (1993) 36:1923-1937; Tonkinson JL *et al.*, *Antisense Oligodeoxynucleotides as Clinical Therapeutic Agents*, *Cancer Invest.* (1996) 14:54-65). Accordingly, in one aspect of the invention, an HM-specific nucleic acid modulator is used in an assay to further elucidate the role of the HM in the p53 pathway, and/or its relationship to other members of the pathway. In another aspect of the invention, an HM-specific antisense oligomer is used as a therapeutic agent for treatment of p53-related disease states.

Assay Systems

The invention provides assay systems and screening methods for identifying specific modulators of HM 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 HM nucleic acid or protein. In general, secondary assays further assess the activity of an HM modulating agent identified by a primary assay and may confirm that the modulating agent affects HM in a manner relevant to the p53 pathway. In some cases, HM modulators will be directly tested in a secondary assay.

In a preferred embodiment, the screening method comprises contacting a suitable assay system comprising an HM 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. binding 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 HM activity, and hence the p53 pathway. The HM polypeptide or nucleic acid used in the assay may comprise any of the nucleic acids or polypeptides described above.

Primary Assays

The type of modulator tested generally determines the type of primary assay.

Primary assays for small molecule modulators

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 GS *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.

Cell-based screening assays usually require systems for recombinant expression of HM 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 HM-interacting proteins are used in

screens to identify small molecule modulators, the binding specificity of the interacting protein to the HM protein may be assayed by various known methods such as substrate processing (e.g. ability of the candidate HM-specific binding agents to function as negative effectors in HM-expressing cells), binding equilibrium constants (usually at least
5 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 HM 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.

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

Suitable assay formats that may be adapted to screen for HM modulators are known in
20 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 PB, Curr Opin Chem Biol (1998) 2:597-603; Sundberg SA, Curr Opin Biotechnol 2000, 11:47-53). In one preferred embodiment, screening assays uses fluorescence technologies, including fluorescence polarization, time-resolved
25 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 PR, Nat Struct Biol (2000) 7:730-4; Fernandes PB, *supra*; Hertzberg RP and Pope AJ, Curr Opin Chem Biol (2000) 4:445-451).

30 A variety of suitable assay systems may be used to identify candidate HM and p53 pathway modulators (e.g. U.S. Pat. Nos. 5,550,019 and 6,133,437 (apoptosis assays); U.S. Pat. No. 6,020,135 (p53 modulation), U.S. Pat. No. 6,114,132 (phosphatase and protease assays), 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.

Protein phosphatase assays. Protein phosphatases catalyze the removal of a gamma phosphate from a serine, threonine or tyrosine residue in a protein substrate. Since phosphatases act in opposition to kinases, appropriate assays measure the same parameters as kinase assays. In one example, the dephosphorylation of a fluorescently labeled peptide substrate allows trypsin cleavage of the substrate, which in turn renders the cleaved substrate significantly more fluorescent (Nishikata M *et al.*, Biochem J (1999) 343:35-391). In another example, fluorescence polarization (FP), a solution-based, homogeneous technique requiring no immobilization or separation of reaction components, is used to develop high throughput screening (HTS) assays for protein phosphatases. This assay uses direct binding of the phosphatase with the target, and increasing concentrations of target- phosphatase increase the rate of dephosphorylation, leading to a change in polarization (Parker GJ *et al.*, (2000) J Biomol Screen 5:77-88).

Apoptosis assays. 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). An apoptosis assay system may comprise a cell that expresses an HM, and that optionally has defective p53 function (e.g. p53 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 p53 modulating agents. In some embodiments of the invention, an apoptosis assay may be used as a secondary assay to test a candidate p53 modulating agents that is initially identified using a cell-free assay system. An apoptosis assay may also be used to test whether HM function plays a direct role in apoptosis. For example, an apoptosis assay may be performed on cells that over- or under-express HM relative to wild type cells. Differences in apoptotic response compared to wild type cells suggests that the HM plays a direct role in the apoptotic response. Apoptosis assays are described further in US Pat. No. 6,133,437.

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.

5 Cell Proliferation may also be examined using [³H]-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 [³H]-thymidine into newly synthesized DNA. Incorporation can then be measured by standard techniques such as by counting of
10 radioisotope in a scintillation counter (e.g., Beckman LS 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 SL *et al.*, 1998, *In Vitro Cell Dev Biol Anim* 34:239-46).

15 Cell proliferation may also be assayed by colony formation in soft agar (Sambrook *et al.*, *Molecular Cloning*, Cold Spring Harbor (1989)). For example, cells transformed with HM are seeded in soft agar plates, and colonies are measured and counted after two weeks incubation.

 Involvement of a gene in the cell cycle may be assayed by flow cytometry (Gray JW *et al.* (1986) *Int J Radiat Biol Relat Stud Phys Chem Med* 49:237-55). Cells transfected with
20 an HM 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.

 Accordingly, a cell proliferation or cell cycle assay system may comprise a cell that
25 expresses an HM, and that optionally has defective p53 function (e.g. p53 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 p53 modulating agents. In some embodiments of the invention, the cell proliferation or cell cycle assay may be used as a secondary assay to
30 test a candidate p53 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 HM 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

HM relative to wild type cells. Differences in proliferation or cell cycle compared to wild type cells suggests that the HM plays a direct role in cell proliferation or cell cycle.

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 HM, and that optionally has defective p53 function (e.g. p53 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 p53 modulating agents. In some embodiments of the invention, the angiogenesis assay may be used as a secondary assay to test a candidate p53 modulating agents that is initially identified using another assay system. An angiogenesis assay may also be used to test whether HM function plays a *direct role in cell proliferation*. For example, an angiogenesis assay may be performed on cells that over- or under-express HM relative to wild type cells. Differences in angiogenesis compared to wild type cells suggests that the HM plays a direct role in angiogenesis. U.S. Pat. Nos. 5,976,782, 6,225,118 and 6,444,434, among others.

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 glycolytic enzymes and VEGF. Induction of such genes by hypoxic conditions may be assayed by growing cells transfected with HM 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 HM, and that optionally has a mutated p53 (e.g. p53 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 p53 modulating agents. In some embodiments of the invention, the hypoxic induction assay may be used as a secondary assay to test a candidate p53 modulating agents that is initially identified using another assay system. A hypoxic induction assay may also be used to test whether HM 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 HM relative to wild type cells. Differences in hypoxic response compared to wild type cells suggests that the HM plays a direct role in hypoxic induction.

10 **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 2.5g/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.

20 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.

30 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 JR et al., Bioconjug Chem. 2001 May-Jun;12(3):346-53).

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 JH 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, MA), 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, CA), 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 HM's response to a variety of pro-angiogenic factors, including tumor angiogenic and inflammatory angiogenic agents, and culturing the cells in serum free medium.

Primary assays for antibody modulators

For antibody modulators, appropriate primary assays test is a binding assay that tests the antibody's affinity to and specificity for the HM 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 HM-specific antibodies; others include FACS assays, radioimmunoassays, and fluorescent assays.

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

Primary assays for nucleic acid modulators

For nucleic acid modulators, primary assays may test the ability of the nucleic acid modulator to inhibit or enhance HM gene expression, preferably mRNA expression. In general, expression analysis comprises comparing HM expression in like populations of cells (*e.g.*, two pools of cells that endogenously or recombinantly express HM) 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 HM mRNA expression is reduced in cells treated with the nucleic acid modulator (*e.g.*, Current Protocols in Molecular Biology (1994) Ausubel FM *et al.*, eds., John Wiley & Sons, Inc., chapter 4; Freeman WM *et al.*, Biotechniques (1999) 26:112-125; Kallioniemi OP, Ann Med 2001, 33:142-147; Blohm DH 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 HM 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*).

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

Secondary Assays

Secondary assays may be used to further assess the activity of HM-modulating agent identified by any of the above methods to confirm that the modulating agent affects HM in a manner relevant to the p53 pathway. As used herein, HM-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 HM.

Secondary assays generally compare like populations of cells or animals (*e.g.*, two pools of cells or animals that endogenously or recombinantly express HM) in the presence and absence of the candidate modulator. In general, such assays test whether treatment of cells or animals with a candidate HM-modulating agent results in changes in the p53 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 p53 or interacting pathways.

Cell-based assays

5 Cell based assays may use a variety of mammalian cell lines known to have defective p53 function (e.g. SAOS-2 osteoblasts, H1299 lung cancer cells, C33A and HT3 cervical cancer cells, HT-29 and DLD-1 colon cancer cells, among others, available from American Type Culture Collection (ATCC), Manassas, VA). Cell based assays may detect endogenous p53 pathway activity or may rely on recombinant expression of p53
10 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

15 A variety of non-human animal models of normal or defective p53 pathway may be used to test candidate HM modulators. Models for defective p53 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 p53 pathway. Assays generally require systemic delivery of the candidate modulators, such as by oral administration, injection,
20 etc.

In a preferred embodiment, p53 pathway activity is assessed by monitoring neovascularization and angiogenesis. Animal models with defective and normal p53 are used to test the candidate modulator’s affect on HM in Matrigel® assays. Matrigel® is an extract of basement membrane proteins, and is composed primarily of laminin, collagen
25 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 HM. The mixture is then injected subcutaneously(SC) into female athymic nude mice (Taconic, Germantown, NY) to support an intense vascular response. Mice with Matrigel® pellets
30 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.

In another preferred embodiment, the effect of the candidate modulator on HM is assessed via tumorigenicity assays. In one example, a xenograft comprising human cells from a pre-existing tumor or a tumor cell line is used. 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 HM endogenously are injected in the flank, 1×10^5 to 1×10^7 cells per mouse in a volume of 100 μ L 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.

In another preferred embodiment, tumorigenicity is monitored using a hollow fiber assay, which is described in U.S. Pat No. US 5,698,413. Briefly, the method comprises implanting into a laboratory animal a biocompatible, semi-permeable 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. Tumorigenicity 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.

In another preferred embodiment, a tumorigenicity 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). Tumorigenicity 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.

15 **Diagnostic and therapeutic uses**

Specific HM-modulating agents are useful in a variety of diagnostic and therapeutic applications where disease or disease prognosis is related to defects in the p53 pathway, such as angiogenic, apoptotic, or cell proliferation disorders. Accordingly, the invention also provides methods for modulating the p53 pathway in a cell, preferably a cell pre-determined to have defective or impaired p53 function (e.g. due to overexpression, underexpression, or misexpression of p53, or due to gene mutations), comprising the step of administering an agent to the cell that specifically modulates HM activity. Preferably, the modulating agent produces a detectable phenotypic change in the cell indicating that the p53 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 p53 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 p53 function by administering a therapeutically effective amount of an HM -modulating agent that modulates the p53 pathway. The invention further provides methods for modulating HM function in a cell, preferably a cell pre-determined to have defective or impaired HM function, by administering an HM -modulating agent. Additionally, the invention provides a method for treating disorders or

disease associated with impaired HM function by administering a therapeutically effective amount of an HM -modulating agent.

The discovery that HM is implicated in p53 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 p53 pathway and for the identification of subjects having a predisposition to such diseases and disorders.

Various expression analysis methods can be used to diagnose whether HM 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 FM *et al.*, eds., John Wiley & Sons, Inc., chapter 4; Freeman WM *et al.*, Biotechniques (1999) 26:112-125; Kallioniemi OP, 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 p53 signaling that express an HM, are identified as amenable to treatment with an HM modulating agent. In a preferred application, the p53 defective tissue overexpresses an HM 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 HM cDNA sequences as probes, can determine whether particular tumors express or overexpress HM. Alternatively, the TaqMan® is used for quantitative RT-PCR analysis of HM expression in cell lines, normal tissues and tumor samples (PE Applied Biosystems).

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

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 HM expression, the method comprising: a) obtaining a biological sample from the patient; b) contacting the sample with a probe for HM 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 2. The probe may be either DNA or protein, including an antibody.

EXAMPLES

5 The following experimental section and examples are offered by way of illustration and not by way of limitation.

I. Drosophila p53 screen

10 The *Drosophila* p53 gene was overexpressed specifically in the wing using the vestigial margin quadrant enhancer. Increasing quantities of *Drosophila* p53 (titrated using different strength transgenic inserts in 1 or 2 copies) caused deterioration of normal wing morphology from mild to strong, with phenotypes including disruption of pattern and polarity of wing hairs, shortening and thickening of wing veins, progressive crumpling of the wing and appearance of dark "death" inclusions in wing blade. In a screen designed to
15 identify enhancers and suppressors of *Drosophila* p53, homozygous females carrying two copies of p53 were crossed to 5663 males carrying random insertions of a piggyBac transposon (Fraser M *et al.*, Virology (1985) 145:356-361). Progeny containing insertions were compared to non-insertion-bearing sibling progeny for enhancement or suppression of the p53 phenotypes. Sequence information surrounding the piggyBac insertion site was
20 used to identify the modifier genes. Modifiers of the wing phenotype were identified as members of the p53 pathway. Human orthologs of the modifiers are referred to herein as HM.

II. Analysis of Table 1

25 BLAST analysis (Altschul et al., *supra*) was employed to identify Targets from *Drosophila* modifiers. The column "HM name" provides a symbol or the known name abbreviations for the Targets, where available, from Genbank. "HM RefSeq_NA or GI_NA", "HM GI_AA", and "HM Description" provide the reference DNA sequences for the HMs as available from National Center for Biology Information (NCBI), HM protein
30 Genbank identifier number (GI#), and HM description, all available from Genbank, respectively. The respective SEQ ID NO for each nucleic acid and polypeptide sequence is indicated next to the sequence. The length of each amino acid is in the "HM Protein Length" column.

Names and Protein sequences of *Drosophila* modifiers of p53 from screen (Example I), are represented in the "Modifier Name" and "Modifier GI_AA" column by GI#, respectively.

5 Table 1

	HM_Na me	HM RefS eq_ NA or GI_ NA	A SE Q ID N O	HM_GI_ AA	AA SEQ ID NO	HM_Description	HM prot ein lengt h	Modif ier Name	Modifier GI_AA
1	LRR N1	1449 5560	1	gi 795925 5 dbj BAA 96021.1	15	KIAA1497 protein [Homo sapiens]; NP_032542 leucine rich repeat protein 1, neuronal [Mus musculus]	730	caps	gi 3885974 gb A AC78144.1
2	LOC 9246 8	1475 1033	2	gi 147510 34 ref XP_ 045260.1	16	similar to neuronal leucine-rich repeat protein-3 ; WUGSC:H_RG118D07.1, Homo sapiens BAC clone CTB-118D7 from 7q31; similar to murine leucine-rich repeat protein; possible role in neural development by protein-protein interactions; 93% similarity to D49802 (PID:g1369906)	708	caps	gi 3885974 gb A AC78144.1
3	bA4 38B 23.1	1004 5383	3	gi 123096 30 emb C AC22713. 1	17	bA438B23.1 (neuronal leucine-rich repeat protein) [Homo sapiens]	606	caps	gi 3885974 gb A AC78144.1
4	XP_ 0531 44	1530 1269	4	gi 153012 70 ref XP_ 053144.1	18	hypothetical protein XP_053144 [Homo sapiens]	614	caps	gi 3885974 gb A AC78144.1

5	IGF ALS	4826 771	5	gi 482677 2 ref NP_0 04961.1	19	insulin-like growth factor binding protein, acid labile subunit; INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN COMPLEX ACID LABILE CHAIN PRECURSOR [Homo sapiens]	605	caps	gi 3885974 gb A AC78144.1
6	LIG 1	1442 3348	6	gi 144233 49 gb AA K62357.1 AF381545 _1	20	Homo sapiens membrane glycoprotein LIG-1 mRNA,	###	caps	gi 3885974 gb A AC78144.1
7	NA G14	1449 5560	7	gi 144955 61 gb AA G28019.2 AF196976 _1	21	Homo sapiens brain tumor associated protein NAG14 (NAG14)	653	caps	gi 3885974 gb A AC78144.1
8	KIA A15 80	1004 7234	8	gi 100472 35 dbj BA B13406.1	22	KIAA1580 protein	640	caps	gi 3885974 gb A AC78144.1
9	DKF Zp76 1A1 79	6808 025	9	gi 680802 6 emb CA B70743.1	23	Homo sapiens Mrna; cDNA DKFZp761A179	422	caps	gi 3885974 gb A AC78144.1
10	KIA A06 44	7662 219	10	gi 766222 0 ref NP_0 55632.1	24	KIAA0644 gene product [Homo sapiens]	811	caps	gi 3885974 gb A AC78144.1
11	FLR T1	8051 591	11	gi 701937 9 ref NP_0 37412.1	25	fibronectin leucine rich transmembrane protein 1 [Homo sapiens]	674	caps	gi 3885974 gb A AC78144.1
12	FLR T2	6808 604	12	gi 680860 5 gb AAF 28460.1 A F169676_ 1	26	leucine-rich repeat transmembrane protein FLRT2	660	caps	gi 3885974 gb A AC78144.1
13	FLR T3	6808 606	13	gi 680860 7 gb AAF 28461.1 A F169677_ 1	27	Homo sapiens leucine-rich repeat transmembrane protein FLRT3	649	caps	gi 3885974 gb A AC78144.1

1	BG7	1400	14	gi 128526	28		279	tyrosin	gi 7301043 gb A
4	2419	3385		96 dbj BA				e	AF56179.1
	8			B29504.1				phosp	
				MOUSE				hatase	
				261aa ;				CG10	
				gi 128495				371	
				78 dbj BA					
				B28400.1					
				mOUSE					
				279aa;					
				ref NT_00					
				8978.5 Hs					
				11_9135 =					
				human					
				genomic					

III. High-Throughput In Vitro Fluorescence Polarization Assay

Fluorescently-labeled HM 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 HM activity.

IV. High-Throughput In Vitro Binding Assay.

³³P-labeled HM peptide is added in an assay buffer (100 mM KCl, 20 mM HEPES pH 7.6, 1 mM MgCl₂, 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 p53 modulating agents.

V. Immunoprecipitations and Immunoblotting

For coprecipitation of transfected proteins, 3×10^6 appropriate recombinant cells containing the HM 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 $15,000 \times g$ for 15 min. The cell lysate is incubated
5 with 25 μ l of M2 beads (Sigma) for 2 h at 4 °C with gentle rocking.

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
10 appropriate secondary antibodies and the enhanced chemiluminescence (ECL) Western blotting detection system (Amersham Pharmacia Biotech).

VI. Expression analysis

All cell lines used in the following experiments are NCI (National Cancer Institute)
15 lines, and are available from ATCC (American Type Culture Collection, Manassas, VA 20110-2209). Normal and tumor tissues were obtained from Impath, UC Davis, Clontech, Stratagene, and Ambion.

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

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

25 Primers for expression analysis using TaqMan assay (Applied Biosystems, Foster City, CA) 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.

Taqman reactions were carried out following manufacturer's protocols, in 25 μ l total
30 volume for 96-well plates and 10 μ l total volume for 384-well plates, using 300nM primer and 250 nM probe, and approximately 25ng 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 18S rRNA (universally expressed in all tissues and cells).

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., $\text{Tumor} - \text{average}(\text{all normal samples}) > 2 \times \text{STDEV}(\text{all normal samples})$).

Results are shown in Table 2. 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. "ND" means not done. 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 2

SEQ ID NO	Breas t	# of Pairs	Colo n	# of Pai rs	Kidney	# of Pai rs	Lung	# of Pai rs	Ovary	# of Pai rs	Uterus	# of Pai rs	Prostat e	# of Pai rs	Skin	# of Pai rs
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12	0.0%	12	33.3 %	30	ND	0	21.4 %	14	0.0%	7	ND	0	ND	0	ND	0
13	41.7%	12	26.7 %	30	ND	0	14.3 %	14	28.6%	7	ND	0	ND	0	ND	0
10	33.3%	12	30.0 %	30	ND	0	14.3 %	14	28.6%	7	ND	0	ND	0	ND	0
8	8.3%	12	30.0 %	30	ND	0	14.3 %	14	14.3%	7	ND	0	ND	0	ND	0
6	25.0%	12	10.0 %	30	ND	0	14.3 %	14	42.9%	7	ND	0	ND	0	ND	0
7	0.0%	12	16.7 %	30	ND	0	14.3 %	14	28.6%	7	ND	0	ND	0	ND	0
3	8.3%	12	39.3 %	28	ND	0	14.3 %	14	28.6%	7	ND	0	ND	0	ND	0

WHAT IS CLAIMED IS:

1. A method of identifying a candidate p53 pathway modulating agent, said method comprising the steps of:
 - 5 a. providing an assay system comprising a purified HM polypeptide or nucleic acid or a functionally active fragment or derivative thereof;
 - 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
 - 10 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 p53 pathway modulating agent.
- 15 2. The method of Claim 1 wherein the assay system comprises cultured cells that express the HM polypeptide.
3. The method of Claim 2 wherein the cultured cells additionally have defective p53 function.
- 20 4. The method of Claim 1 wherein the assay system includes a screening assay comprising an HM polypeptide, and the candidate test agent is a small molecule modulator.
5. The method of Claim 4 wherein the assay is a binding assay.
- 25 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.
- 30 7. The method of Claim 1 wherein the assay system includes a binding assay comprising an HM 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 HM 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.
- 5 11. The method of Claim 1 additionally comprising:
- d. administering the candidate p53 pathway modulating agent identified in (c) to a model system comprising cells defective in p53 function and, detecting a phenotypic change in the model system that indicates that the p53 function is restored.
- 10 12. The method of Claim 11 wherein the model system is a mouse model with defective p53 function.
- 15 13. A method for modulating a p53 pathway of a cell comprising contacting a cell defective in p53 function with a candidate modulator that specifically binds to an HM polypeptide, whereby p53 function is restored.
- 20 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 p53 function.
15. The method of Claim 13 wherein the candidate modulator is selected from the group consisting of an antibody and a small molecule.
- 25 16. The method of Claim 1, comprising the additional steps of:
- a. providing a secondary assay system comprising cultured cells or a non-human animal expressing HM ,
- b. 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
- 30 c. detecting an agent-biased activity of the second assay system,

17. 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 p53 pathway modulating agent,
18. and wherein the second assay detects an agent-biased change in the p53 pathway.
- 5
19. The method of Claim 16 wherein the secondary assay system comprises cultured cells.
20. The method of Claim 16 wherein the secondary assay system comprises a non-
- 10 human animal.
21. The method of Claim 18 wherein the non-human animal mis-expresses a p53 pathway gene.
- 15
22. A method of modulating p53 pathway in a mammalian cell comprising contacting the cell with an agent that specifically binds an HM polypeptide or nucleic acid.
23. The method of Claim 20 wherein the agent is administered to a mammalian animal predetermined to have a pathology associated with the p53 pathway.
- 20
24. The method of Claim 20 wherein the agent is a small molecule modulator, a nucleic acid modulator, or an antibody.
25. A method for diagnosing a disease in a patient comprising:
- 25
- a. obtaining a biological sample from the patient;
- b. contacting the sample with a probe for HM expression;
- c. comparing results from step (b) with a control;
- d. determining whether step (c) indicates a likelihood of disease.
- 30
26. The method of claim 23 wherein said disease is cancer.
27. The method according to claim 24, wherein said cancer is a cancer as shown in Table 2 as having >25% expression level.

SEQUENCE LISTING

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<120> MODIFIERS OF THE p53 PATHWAY AND METHODS OF USE

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<212> DNA

<213> Homo sapiens

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35 40 45

Leu Cys Val Cys Glu Ile Arg Pro Trp Phe Thr Pro Gln Ser Thr Tyr
50 55 60

Arg Glu Ala Thr Thr Val Asp Cys Asn Asp Leu Arg Leu Thr Arg Ile
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Pro Ser Asn Leu Ser Ser Asp Thr Gln Val Leu Leu Leu Gln Ser Asn
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115 120 125

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Ile Thr Glu Met Thr Asp Tyr Cys Leu Gln Asp Leu Ser Asn Leu Gln

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Ile Asp Asn Pro His Ile Thr Tyr Thr Ala Arg Val Pro Val Asp Val						
	580			585		590
His Glu Tyr Asn Leu Thr His Leu Gln Pro Ser Thr Asp Tyr Glu Val						
	595			600		605
Cys Leu Thr Val Ser Asn Ile His Gln Gln Thr Gln Lys Ser Cys Val						
	610			615		620
Asn Val Thr Thr Lys Asn Ala Ala Phe Ala Val Asp Ile Ser Asp Gln						

<400> 16

Lys Lys Met Pro Gln Leu Leu Ser Val Tyr Leu Glu Glu Asn Lys Leu
 115 120 125

Thr Glu Leu Pro Glu Lys Cys Leu Ser Glu Leu Ser Asn Leu Gln Glu
 130 135 140

Leu Tyr Ile Asn His Asn Leu Leu Ser Thr Ile Ser Pro Gly Ala Phe
 145 150 155 160

Ile Gly Leu His Asn Leu Leu Arg Leu His Leu Asn Ser Asn Arg Leu
 165 170 175

Gln Met Ile Asn Ser Lys Trp Phe Asp Ala Leu Pro Asn Leu Glu Ile
 180 185 190

Leu Met Ile Gly Glu Asn Pro Ile Ile Arg Ile Lys Asp Met Asn Phe
 195 200 205

Lys Pro Leu Ile Asn Leu Arg Ser Leu Val Ile Ala Gly Ile Asn Leu
 210 215 220

Thr Glu Ile Pro Asp Asn Ala Leu Val Gly Leu Glu Asn Leu Glu Ser
 225 230 235 240

Ile Ser Phe Tyr Asp Asn Arg Leu Ile Lys Val Pro His Val Ala Leu
 245 250 255

Gln Lys Val Val Asn Leu Lys Phe Leu Asp Leu Asn Lys Asn Pro Ile
 260 265 270

Asn Arg Ile Arg Arg Gly Asp Phe Ser Asn Met Leu His Leu Lys Glu
 275 280 285

Leu Gly Ile Asn Asn Met Pro Glu Leu Ile Ser Ile Asp Ser Leu Ala
 290 295 300

Val Asp Asn Leu Pro Asp Leu Arg Lys Ile Glu Ala Thr Asn Asn Pro
 305 310 315 320

Arg Leu Ser Tyr Ile His Pro Asn Ala Phe Phe Arg Leu Pro Lys Leu
 325 330 335

Glu Ser Leu Met Leu Asn Ser Asn Ala Leu Ser Ala Leu Tyr His Gly
 340 345 350

Thr Ile Glu Ser Leu Pro Asn Leu Lys Glu Ile Ser Ile His Ser Asn
 355 360 365
 Pro Ile Arg Cys Asp Cys Val Ile Arg Trp Met Asn Met Asn Lys Thr
 370 375 380
 Asn Ile Arg Phe Met Glu Pro Asp Ser Leu Phe Cys Val Asp Pro Pro
 385 390 395 400
 Glu Phe Gln Gly Gln Asn Val Arg Gln Val His Phe Arg Asp Met Met
 405 410 415
 Glu Ile Cys Leu Pro Leu Ile Ala Pro Glu Ser Phe Pro Ser Asn Leu
 420 425 430
 Asn Val Glu Ala Gly Ser Tyr Val Ser Phe His Cys Arg Ala Thr Ala
 435 440 445
 Glu Pro Gln Pro Glu Ile Tyr Trp Ile Thr Pro Ser Gly Gln Lys Leu
 450 455 460
 Leu Pro Asn Thr Leu Thr Asp Lys Phe Tyr Val His Ser Glu Gly Thr
 465 470 475 480
 Leu Asp Ile Asn Gly Val Thr Pro Lys Glu Gly Gly Leu Tyr Thr Cys
 485 490 495
 Ile Ala Thr Asn Leu Val Gly Ala Asp Leu Lys Ser Val Met Ile Lys
 500 505 510
 Val Asp Gly Ser Phe Pro Gln Asp Asn Asn Gly Ser Leu Asn Ile Lys
 515 520 525
 Ile Arg Asp Ile Gln Ala Asn Ser Val Leu Val Ser Trp Lys Ala Ser
 530 535 540
 Ser Lys Ile Leu Lys Ser Ser Val Lys Trp Thr Ala Phe Val Lys Thr
 545 550 555 560
 Glu Asn Ser His Ala Ala Gln Ser Ala Arg Ile Pro Ser Asp Val Lys
 565 570 575
 Val Tyr Asn Leu Thr His Leu Asn Pro Ser Thr Glu Tyr Lys Ile Cys
 580 585 590

Ile Asp Ile Pro Thr Ile Tyr Gln Lys Asn Arg Lys Lys Cys Val Asn
595 600 605

Val Thr Thr Lys Gly Leu His Pro Asp Gln Lys Glu Tyr Glu Lys Asn
610 615 620

Asn Thr Thr Thr Leu Met Ala Cys Leu Gly Gly Leu Leu Gly Ile Ile
625 630 635 640

Gly Val Ile Cys Leu Ile Ser Cys Leu Ser Pro Glu Met Asn Cys Asp
645 650 655

Gly Gly His Ser Tyr Val Arg Asn Tyr Leu Gln Lys Pro Thr Phe Ala
660 665 670

Leu Gly Glu Leu Tyr Pro Pro Leu Ile Asn Leu Trp Glu Ala Gly Lys
675 680 685

Glu Lys Ser Thr Ser Leu Lys Val Lys Ala Thr Val Ile Gly Leu Pro
690 695 700

Thr Asn Met Ser
705

<210> 17
<211> 606
<212> PRT
<213> Homo sapiens

<400> 17

Met Leu His Thr Ala Ile Ser Cys Trp Gln Pro Phe Leu Gly Leu Ala
1 5 10 15

Val Val Leu Ile Phe Met Gly Ser Thr Ile Gly Cys Pro Ala Arg Cys
20 25 30

Glu Cys Ser Ala Gln Asn Lys Ser Val Ser Cys His Arg Arg Arg Leu
35 40 45

Ile Ala Ile Pro Glu Gly Ile Pro Ile Glu Thr Lys Ile Leu Asp Leu
50 55 60

Ser Lys Asn Arg Leu Lys Ser Val Asn Pro Glu Glu Phe Ile Ser Tyr
65 70 75 80

Pro Leu Leu Glu Glu Ile Asp Leu Ser Asp Asn Ile Ile Ala Asn Val
85 90 95

Glu Pro Gly Ala Phe Asn Asn Leu Phe Asn Leu Arg Ser Leu Arg Leu
100 105 110

Lys Gly Asn Arg Leu Lys Leu Val Pro Leu Gly Val Phe Thr Gly Leu
115 120 125

Ser Asn Leu Thr Lys Leu Asp Ile Ser Glu Asn Lys Ile Val Ile Leu
130 135 140

Leu Asp Tyr Met Phe Gln Asp Leu His Asn Leu Lys Ser Leu Glu Val
145 150 155 160

Gly Asp Asn Asp Leu Val Tyr Ile Ser His Arg Ala Phe Ser Gly Leu
165 170 175

Leu Ser Leu Glu Gln Leu Thr Leu Glu Lys Cys Asn Leu Thr Ala Val
180 185 190

Pro Thr Glu Ala Leu Ser His Leu Arg Ser Leu Ile Ser Leu His Leu
195 200 205

Lys His Leu Asn Ile Asn Asn Met Pro Val Tyr Ala Phe Lys Arg Leu
210 215 220

Phe His Leu Lys His Leu Glu Ile Asp Tyr Trp Pro Leu Leu Asp Met
225 230 235 240

Met Pro Ala Asn Ser Leu Tyr Gly Leu Asn Leu Thr Ser Leu Ser Val
245 250 255

Thr Asn Thr Asn Leu Ser Thr Val Pro Phe Leu Ala Phe Lys His Leu
260 265 270

Val Tyr Leu Thr His Leu Asn Leu Ser Tyr Asn Pro Ile Ser Thr Ile
275 280 285

Glu Ala Gly Met Phe Ser Asp Leu Ile Arg Leu Gln Glu Leu His Ile
290 295 300

Val Gly Ala Gln Leu Arg Thr Ile Glu Pro His Ser Phe Gln Gly Leu
305 310 315 320

Arg Phe Leu Arg Val Leu Asn Val Ser Gln Asn Leu Leu Glu Thr Leu
 325 330 335

Glu Glu Asn Val Phe Ser Ser Pro Arg Ala Leu Glu Val Leu Ser Ile
 340 345 350

Asn Asn Asn Pro Leu Ala Cys Asp Cys Arg Leu Leu Trp Ile Leu Gln
 355 360 365

Arg Gln Pro Thr Leu Gln Phe Gly Gly Gln Gln Pro Met Cys Ala Gly
 370 375 380

Pro Asp Thr Ile Arg Glu Arg Ser Phe Lys Asp Phe His Ser Thr Ala
 385 390 395 400

Leu Ser Phe Tyr Phe Thr Cys Lys Lys Pro Lys Ile Arg Glu Lys Lys
 405 410 415

Leu Gln His Leu Leu Val Asp Glu Gly Gln Thr Val Gln Leu Glu Cys
 420 425 430

Ser Ala Asp Gly Asp Pro Gln Pro Val Ile Ser Trp Val Thr Pro Arg
 435 440 445

Arg Arg Phe Ile Thr Thr Lys Ser Asn Gly Arg Ala Thr Val Leu Gly
 450 455 460

Asp Gly Thr Leu Glu Ile Arg Phe Ala Gln Asp Gln Asp Ser Gly Met
 465 470 475 480

Tyr Val Cys Ile Ala Ser Asn Ala Ala Gly Asn Asp Thr Phe Thr Ala
 485 490 495

Ser Leu Thr Val Lys Gly Phe Ala Ser Asp Arg Phe Leu Tyr Ala Asn
 500 505 510

Arg Thr Pro Met Tyr Met Thr Asp Ser Asn Asp Thr Ile Ser Asn Gly
 515 520 525

Thr Asn Ala Asn Thr Phe Ser Leu Asp Leu Lys Thr Ile Leu Val Ser
 530 535 540

Thr Ala Met Gly Cys Phe Thr Phe Leu Gly Val Val Leu Phe Cys Phe
 545 550 555 560

Leu Leu Leu Phe Val Trp Ser Arg Gly Lys Gly Lys His Lys Asn Ser
 565 570 575

Ile Asp Leu Glu Tyr Val Pro Arg Lys Asn Asn Gly Ala Val Val Glu
 580 585 590

Gly Glu Val Ala Gly Pro Arg Arg Phe Asn Met Lys Met Ile
 595 600 605

<210> 18
 <211> 614
 <212> PRT
 <213> Homo sapiens

<400> 18

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

Trp Gln Pro Ile Leu Leu Leu Val Leu Gly Ser Val Leu Ser Gly Ser
 20 25 30

Ala Thr Gly Cys Pro Pro Arg Cys Glu Cys Ser Ala Gln Asp Arg Ala
 35 40 45

Val Leu Cys His Arg Lys Arg Phe Val Ala Val Pro Glu Gly Ile Pro
 50 55 60

Thr Glu Thr Arg Leu Leu Asp Leu Gly Lys Asn Arg Ile Lys Thr Leu
 65 70 75 80

Asn Gln Asp Glu Phe Ala Ser Phe Pro His Leu Glu Glu Leu Glu Leu
 85 90 95

Asn Glu Asn Ile Val Ser Ala Val Glu Pro Gly Ala Phe Asn Asn Leu
 100 105 110

Phe Asn Leu Arg Thr Leu Gly Leu Arg Ser Asn Arg Leu Lys Leu Ile
 115 120 125

Pro Leu Gly Val Phe Thr Gly Leu Ser Asn Leu Thr Lys Leu Asp Ile
 130 135 140

Ser Glu Asn Lys Ile Val Ile Leu Leu Asp Tyr Met Phe Gln Asp Leu
 145 150 155 160

Tyr Asn Leu Lys Ser Leu Glu Val Gly Asp Asn Asp Leu Val Tyr Ile
 165 170 175

Ser His Arg Ala Phe Ser Gly Leu Asn Ser Leu Glu Gln Leu Thr Leu
 180 185 190

Glu Lys Cys Asn Leu Thr Ser Ile Pro Thr Glu Ala Leu Ser His Leu
 195 200 205

His Gly Leu Ile Val Leu Arg Leu Arg His Leu Asn Ile Asn Ala Ile
 210 215 220

Arg Asp Tyr Ser Phe Lys Arg Leu Tyr Arg Leu Lys Val Leu Glu Ile
 225 230 235 240

Ser His Trp Pro Tyr Leu Asp Thr Met Thr Pro Asn Cys Leu Tyr Gly
 245 250 255

Leu Asn Leu Thr Ser Leu Ser Ile Thr His Cys Asn Leu Thr Ala Val
 260 265 270

Pro Tyr Leu Ala Val Arg His Leu Val Tyr Leu Arg Phe Leu Asn Leu
 275 280 285

Ser Tyr Asn Pro Ile Ser Thr Ile Glu Gly Ser Met Leu His Glu Leu
 290 295 300

Leu Arg Leu Gln Glu Ile Gln Leu Val Gly Gly Gln Leu Ala Val Val
 305 310 315 320

Glu Pro Tyr Ala Phe Arg Gly Leu Asn Tyr Leu Arg Val Leu Asn Val
 325 330 335

Ser Gly Asn Gln Leu Thr Thr Leu Glu Glu Ser Val Phe His Ser Val
 340 345 350

Gly Asn Leu Glu Thr Leu Ile Leu Asp Ser Asn Pro Leu Ala Cys Asp
 355 360 365

Cys Arg Leu Leu Trp Val Phe Arg Arg Arg Trp Arg Leu Asn Phe Asn
 370 375 380

Arg Gln Gln Pro Thr Cys Ala Thr Pro Glu Phe Val Gln Gly Lys Glu
 385 390 395 400

Phe Lys Asp Phe Pro Asp Val Leu Leu Pro Asn Tyr Phe Thr Cys Arg
 405 410 415

Arg Ala Arg Ile Arg Asp Arg Lys Ala Gln Gln Val Phe Val Asp Glu
 420 425 430

Gly His Thr Val Gln Phe Val Cys Arg Ala Asp Gly Asp Pro Pro Pro
 435 440 445

Ala Ile Leu Trp Leu Ser Pro Arg Lys His Leu Val Ser Ala Lys Ser
 450 455 460

Asn Gly Arg Leu Thr Val Phe Pro Asp Gly Thr Leu Glu Val Arg Tyr
 465 470 475 480

Ala Gln Val Gln Asp Asn Gly Thr Tyr Leu Cys Ile Ala Ala Asn Ala
 485 490 495

Gly Gly Asn Asp Ser Met Pro Ala His Leu His Val Arg Ser Tyr Ser
 500 505 510

Pro Asp Trp Pro His Gln Pro Asn Lys Thr Phe Ala Phe Ile Ser Asn
 515 520 525

Gln Pro Gly Glu Gly Glu Ala Asn Ser Thr Arg Ala Thr Val Pro Phe
 530 535 540

Pro Phe Asp Ile Lys Thr Leu Ile Ile Ala Thr Thr Met Gly Phe Ile
 545 550 555 560

Ser Phe Leu Gly Val Val Leu Phe Cys Leu Val Leu Leu Phe Leu Trp
 565 570 575

Ser Arg Gly Lys Gly Asn Thr Lys His Asn Ile Glu Ile Glu Tyr Val
 580 585 590

Pro Arg Lys Ser Asp Ala Gly Ile Ser Ser Ala Asp Ala Pro Arg Lys
 595 600 605

Phe Asn Met Lys Met Ile
 610

<210> 19

<211> 605

<212> PRT

<213> Homo sapiens

<400> 19

Met Ala Leu Arg Lys Gly Gly Leu Ala Leu Ala Leu Leu Leu Ser
 1 5 10 15

Trp Val Ala Leu Gly Pro Arg Ser Leu Glu Gly Ala Asp Pro Gly Thr
 20 25 30

Pro Gly Glu Ala Glu Gly Pro Ala Cys Pro Ala Ala Cys Val Cys Ser
 35 40 45

Tyr Asp Asp Asp Ala Asp Glu Leu Ser Val Phe Cys Ser Ser Arg Asn
 50 55 60

Leu Thr Arg Leu Pro Asp Gly Val Pro Gly Gly Thr Gln Ala Leu Trp
 65 70 75 80

Leu Asp Gly Asn Asn Leu Ser Ser Val Pro Pro Ala Ala Phe Gln Asn
 85 90 95

Leu Ser Ser Leu Gly Phe Leu Asn Leu Gln Gly Gly Gln Leu Gly Ser
 100 105 110

Leu Glu Pro Gln Ala Leu Leu Gly Leu Glu Asn Leu Cys His Leu His
 115 120 125

Leu Glu Arg Asn Gln Leu Arg Ser Leu Ala Leu Gly Thr Phe Ala His
 130 135 140

Thr Pro Ala Leu Ala Ser Leu Gly Leu Ser Asn Asn Arg Leu Ser Arg
 145 150 155 160

Leu Glu Asp Gly Leu Phe Glu Gly Leu Gly Ser Leu Trp Asp Leu Asn
 165 170 175

Leu Gly Trp Asn Ser Leu Ala Val Leu Pro Asp Ala Ala Phe Arg Gly
 180 185 190

Leu Gly Ser Leu Arg Glu Leu Val Leu Ala Gly Asn Arg Leu Ala Tyr
 195 200 205

Leu Gln Pro Ala Leu Phe Ser Gly Leu Ala Glu Leu Arg Glu Leu Asp
 210 215 220

Leu Ser Arg Asn Ala Leu Arg Ala Ile Lys Ala Asn Val Phe Val Gln

225		230		235		240
Leu Pro Arg	Leu Gln Lys	Leu Tyr Leu	Asp Arg Asn	Leu Ile Ala	Ala	
	245		250		255	
Val Ala Pro	Gly Ala Phe	Leu Gly Leu	Lys Ala Leu	Arg Trp Leu	Asp	
	260		265		270	
Leu Ser His	Asn Arg Val	Ala Gly Leu	Leu Glu Asp	Thr Phe Pro	Gly	
	275		280		285	
Leu Leu Gly	Leu Arg Val	Leu Arg Leu	Ser His Asn	Ala Ile Ala	Ser	
	290		295		300	
Leu Arg Pro	Arg Thr Phe	Lys Asp Leu	His Phe Leu	Glu Glu Leu	Gln	
	305		310		315	320
Leu Gly His	Asn Arg Ile	Arg Gln Leu	Ala Glu Arg	Ser Phe Glu	Gly	
	325		330		335	
Leu Gly Gln	Leu Glu Val	Leu Thr Leu	Asp His Asn	Gln Leu Gln	Glu	
	340		345		350	
Val Lys Ala	Gly Ala Phe	Leu Gly Leu	Thr Asn Val	Ala Val Met	Asn	
	355		360		365	
Leu Ser Gly	Asn Cys Leu	Arg Asn Leu	Pro Glu Gln	Val Phe Arg	Gly	
	370		375		380	
Leu Gly Lys	Leu His Ser	Leu His Leu	Glu Gly Ser	Cys Leu Gly	Arg	
	385		390		395	400
Ile Arg Pro	His Thr Phe	Thr Gly Leu	Ser Gly Leu	Arg Arg Leu	Phe	
	405		410		415	
Leu Lys Asp	Asn Gly Leu	Val Gly Ile	Glu Glu Gln	Ser Leu Trp	Gly	
	420		425		430	
Leu Ala Glu	Leu Leu Glu	Leu Asp Leu	Thr Ser Asn	Gln Leu Thr	His	
	435		440		445	
Leu Pro His	Arg Leu Phe	Gln Gly Leu	Gly Lys Leu	Glu Tyr Leu	Leu	
	450		455		460	
Leu Ser Arg	Asn Arg Leu	Ala Glu Leu	Pro Ala Asp	Ala Leu Gly	Pro	

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Ser Glu Ile Asp Pro Ala Gly Phe Glu Asp Leu Pro Asn Leu Gln Glu
 85 90 95
 Val Tyr Leu Asn Asn Asn Glu Leu Thr Ala Val Pro Ser Leu Gly Ala
 100 105 110
 Ala Ser Ser His Val Val Ser Leu Phe Leu Gln His Asn Lys Ile Arg
 115 120 125
 Ser Val Glu Gly Ser Gln Leu Lys Ala Tyr Leu Ser Leu Glu Val Leu
 130 135 140
 Asp Leu Ser Leu Asn Asn Ile Thr Glu Val Arg Asn Thr Cys Phe Pro
 145 150 155 160
 His Gly Pro Pro Ile Lys Glu Leu Asn Leu Ala Gly Asn Arg Ile Gly
 165 170 175
 Thr Leu Glu Leu Gly Ala Phe Asp Gly Leu Ser Arg Ser Leu Leu Thr
 180 185 190
 Leu Arg Leu Ser Lys Asn Arg Ile Thr Gln Leu Pro Val Arg Ala Phe
 195 200 205
 Lys Leu Pro Arg Leu Thr Gln Leu Asp Leu Asn Arg Asn Arg Ile Arg
 210 215 220
 Leu Ile Glu Gly Leu Thr Phe Gln Gly Leu Asn Ser Leu Glu Val Leu
 225 230 235 240
 Lys Leu Gln Arg Asn Asn Ile Ser Lys Leu Thr Asp Gly Ala Phe Trp
 245 250 255
 Gly Leu Ser Lys Met His Val Leu His Leu Glu Tyr Asn Ser Leu Val
 260 265 270
 Glu Val Asn Ser Gly Ser Leu Tyr Gly Leu Thr Ala Leu His Gln Leu
 275 280 285
 His Leu Ser Asn Asn Ser Ile Ala Arg Ile His Arg Lys Gly Trp Ser
 290 295 300
 Phe Cys Gln Lys Leu His Glu Leu Val Leu Ser Phe Asn Asn Leu Thr
 305 310 315 320

Arg Leu Asp Glu Glu Ser Leu Ala Glu Leu Ser Ser Leu Ser Val Leu
 325 330 335

Arg Leu Ser His Asn Ser Ile Ser His Ile Ala Glu Gly Ala Phe Lys
 340 345 350

Gly Leu Arg Ser Leu Arg Val Leu Asp Leu Asp His Asn Glu Ile Ser
 355 360 365

Gly Thr Ile Glu Asp Thr Ser Gly Ala Phe Ser Gly Leu Asp Ser Leu
 370 375 380

Ser Lys Leu Thr Leu Phe Gly Asn Lys Ile Lys Ser Val Ala Lys Arg
 385 390 395 400

Ala Phe Ser Gly Leu Glu Gly Leu Glu His Leu Asn Leu Gly Gly Asn
 405 410 415

Ala Ile Arg Ser Val Gln Phe Asp Ala Phe Val Lys Met Lys Asn Leu
 420 425 430

Lys Glu Leu His Ile Ser Ser Asp Ser Phe Leu Cys Asp Cys Gln Leu
 435 440 445

Lys Trp Leu Pro Pro Trp Leu Ile Gly Arg Met Leu Gln Ala Phe Val
 450 455 460

Thr Ala Thr Cys Ala His Pro Glu Ser Leu Lys Gly Gln Ser Ile Phe
 465 470 475 480

Ser Val Pro Pro Glu Ser Phe Val Cys Asp Asp Phe Leu Lys Pro Gln
 485 490 495

Ile Ile Thr Gln Pro Glu Thr Thr Met Ala Met Val Gly Lys Asp Ile
 500 505 510

Arg Phe Thr Cys Ser Ala Ala Ser Ser Ser Ser Ser Pro Met Thr Phe
 515 520 525

Ala Trp Lys Lys Asp Asn Glu Val Leu Thr Asn Ala Asp Met Glu Asn
 530 535 540

Phe Val His Val His Ala Gln Asp Gly Glu Val Met Glu Tyr Thr Thr
 545 550 555 560

Ile Leu His Leu Arg Gln Val Thr Phe Gly His Glu Gly Arg Tyr Gln
 565 570 575

Cys Val Ile Thr Asn His Phe Gly Ser Thr Tyr Ser His Lys Ala Arg
 580 585 590

Leu Thr Val Asn Val Leu Pro Ser Phe Thr Lys Thr Pro His Asp Ile
 595 600 605

Thr Ile Arg Thr Thr Thr Val Ala Arg Leu Glu Cys Ala Ala Thr Gly
 610 615 620

His Pro Asn Pro Gln Ile Ala Trp Gln Lys Asp Gly Gly Thr Asp Phe
 625 630 635 640

Pro Ala Ala Arg Glu Arg Arg Met His Val Met Pro Asp Asp Asp Val
 645 650 655

Phe Phe Ile Thr Asp Val Lys Ile Asp Asp Ala Gly Val Tyr Ser Cys
 660 665 670

Thr Ala Gln Asn Ser Ala Gly Ser Ile Ser Ala Asn Ala Thr Leu Thr
 675 680 685

Val Leu Glu Thr Pro Ser Leu Val Val Pro Leu Glu Asp Arg Val Val
 690 695 700

Ser Val Gly Glu Thr Val Ala Leu Gln Cys Lys Ala Thr Gly Asn Pro
 705 710 715 720

Pro Pro Arg Ile Thr Trp Phe Lys Gly Asp Arg Pro Leu Ser Leu Thr
 725 730 735

Glu Arg His His Leu Thr Pro Asp Asn Gln Leu Leu Val Val Gln Asn
 740 745 750

Val Val Ala Glu Asp Ala Gly Arg Tyr Thr Cys Glu Met Ser Asn Thr
 755 760 765

Leu Gly Thr Glu Arg Ala His Ser Gln Leu Ser Val Leu Pro Ala Ala
 770 775 780

Gly Cys Arg Lys Asp Gly Thr Thr Val Gly Ile Phe Thr Ile Ala Val
 785 790 795 800

Val Ser Ser Ile Val Leu Thr Ser Leu Val Trp Val Cys Ile Ile Tyr
 805 810 815

Gln Thr Arg Lys Lys Ser Glu Glu Tyr Ser Val Thr Asn Thr Asp Glu
 820 825 830

Thr Val Val Pro Pro Asp Val Pro Ser Tyr Leu Ser Ser Gln Gly Thr
 835 840 845

Leu Ser Asp Arg Gln Glu Thr Val Val Arg Thr Glu Gly Gly Pro Gln
 850 855 860

Ala Asn Gly His Ile Glu Ser Asn Gly Val Cys Pro Arg Asp Ala Ser
 865 870 875 880

His Phe Pro Glu Pro Asp Thr His Ser Val Ala Cys Arg Gln Pro Lys
 885 890 895

Leu Cys Ala Gly Ser Ala Tyr His Lys Glu Pro Trp Lys Ala Met Glu
 900 905 910

Lys Ala Glu Gly Thr Pro Gly Pro His Lys Met Glu His Gly Gly Arg
 915 920 925

Val Val Cys Ser Asp Cys Asn Thr Glu Val Asp Cys Tyr Ser Arg Gly
 930 935 940

Gln Ala Phe His Pro Gln Pro Val Ser Arg Asp Ser Ala Gln Pro Ser
 945 950 955 960

Ala Pro Asn Gly Pro Glu Pro Gly Gly Ser Asp Gln Glu His Ser Pro
 965 970 975

His His Gln Cys Ser Arg Thr Ala Ala Gly Ser Cys Pro Glu Cys Gln
 980 985 990

Gly Ser Leu Tyr Pro Ser Asn His Asp Arg Met Leu Thr Ala Val Lys
 995 1000 1005

Lys Lys Pro Met Ala Ser Leu Asp Gly Lys Gly Asp Ser Ser Trp
 1010 1015 1020

Thr Leu Ala Arg Leu Tyr His Pro Asp Ser Thr Glu Leu Gln Pro
 1025 1030 1035

Ala Ser Ser Leu Thr Ser Gly Ser Pro Glu Arg Ala Glu Ala Gln
1040 1045 1050

Tyr Leu Leu Val Ser Asn Gly His Leu Pro Lys Ala Cys Asp Ala
1055 1060 1065

Ser Pro Glu Ser Thr Pro Leu Thr Gly Gln Leu Pro Gly Lys Gln
1070 1075 1080

Arg Val Pro Leu Leu Leu Ala Pro Lys Ser
1085 1090

<210> 21
<211> 653
<212> PRT
<213> Homo sapiens

<400> 21

Met Lys Leu Leu Trp Gln Val Thr Val His His His Thr Trp Asn Ala
1 5 10 15

Ile Leu Leu Pro Phe Val Tyr Leu Thr Ala Gln Val Trp Ile Leu Cys
20 25 30

Ala Ala Ile Ala Ala Ala Ala Ser Ala Gly Pro Gln Asn Cys Pro Ser
35 40 45

Val Cys Ser Cys Ser Asn Gln Phe Ser Lys Val Val Cys Thr Arg Arg
50 55 60

Gly Leu Ser Glu Val Pro Gln Gly Ile Pro Ser Asn Thr Arg Tyr Leu
65 70 75 80

Asn Leu Met Glu Asn Asn Ile Gln Met Ile Gln Ala Asp Thr Phe Arg
85 90 95

His Leu His His Leu Glu Val Leu Gln Leu Gly Arg Asn Ser Ile Arg
100 105 110

Gln Ile Glu Val Gly Ala Phe Asn Gly Leu Ala Ser Leu Asn Thr Leu
115 120 125

Glu Leu Phe Asp Asn Trp Leu Thr Val Ile Pro Ser Gly Ala Phe Glu
130 135 140

Tyr Leu Ser Lys Leu Arg Glu Leu Trp Leu Arg Asn Asn Pro Ile Glu
 145 150 155 160

Ser Ile Pro Ser Tyr Ala Phe Asn Arg Val Pro Ser Leu Met Arg Leu
 165 170 175

Asp Leu Gly Glu Leu Lys Lys Leu Glu Tyr Ile Ser Glu Gly Ala Phe
 180 185 190

Glu Gly Leu Phe Asn Leu Lys Tyr Leu Asn Leu Gly Met Cys Asn Ile
 195 200 205

Lys Asp Met Pro Asn Leu Thr Pro Leu Val Gly Leu Glu Glu Leu Glu
 210 215 220

Met Ser Gly Asn His Phe Pro Glu Ile Arg Pro Gly Ser Phe His Gly
 225 230 235 240

Leu Ser Ser Leu Lys Lys Leu Trp Val Met Asn Ser Gln Val Ser Leu
 245 250 255

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

Leu Ala His Asn Asn Leu Ser Ser Leu Pro His Asp Leu Phe Thr Pro
 275 280 285

Leu Arg Tyr Leu Val Glu Leu His Leu His His Asn Pro Trp Asn Cys
 290 295 300

Asp Cys Asp Ile Leu Trp Leu Ala Trp Trp Leu Arg Glu Tyr Ile Pro
 305 310 315 320

Thr Asn Ser Thr Cys Cys Gly Arg Cys His Ala Pro Met His Met Arg
 325 330 335

Gly Arg Tyr Leu Val Glu Val Asp Gln Ala Ser Phe Gln Cys Ser Ala
 340 345 350

Pro Phe Ile Met Asp Ala Pro Arg Asp Leu Asn Ile Ser Glu Gly Arg
 355 360 365

Met Ala Glu Leu Lys Cys Arg Thr Pro Pro Met Ser Ser Val Lys Trp
 370 375 380

Leu Leu Pro Asn Gly Thr Val Leu Ser His Ala Ser Arg His Pro Arg
 385 390 395 400

Ile Ser Val Leu Asn Asp Gly Thr Leu Asn Phe Ser His Val Leu Leu
 405 410 415

Ser Asp Thr Gly Val Tyr Thr Cys Met Val Thr Asn Val Ala Gly Asn
 420 425 430

Ser Asn Ala Ser Ala Tyr Leu Asn Val Ser Thr Ala Glu Leu Asn Thr
 435 440 445

Ser Asn Tyr Ser Phe Phe Thr Thr Val Thr Val Glu Thr Thr Glu Ile
 450 455 460

Ser Pro Glu Asp Thr Thr Arg Lys Tyr Lys Pro Val Pro Thr Thr Ser
 465 470 475 480

Thr Gly Tyr Gln Pro Ala Tyr Thr Thr Ser Thr Thr Val Leu Ile Gln
 485 490 495

Thr Thr Arg Val Pro Lys Gln Val Ala Val Pro Ala Thr Asp Thr Thr
 500 505 510

Asp Lys Met Gln Thr Ser Leu Asp Glu Val Met Lys Thr Thr Lys Ile
 515 520 525

Ile Ile Gly Cys Phe Val Ala Val Thr Leu Leu Ala Ala Ala Met Leu
 530 535 540

Ile Val Phe Tyr Lys Leu Arg Lys Arg His Gln Gln Arg Ser Thr Val
 545 550 555 560

Thr Ala Ala Arg Thr Val Glu Ile Ile Gln Val Asp Glu Asp Ile Pro
 565 570 575

Ala Ala Thr Ser Ala Ala Ala Thr Ala Ala Pro Ser Gly Val Ser Gly
 580 585 590

Glu Gly Ala Val Val Leu Pro Thr Ile His Asp His Ile Asn Tyr Asn
 595 600 605

Thr Tyr Lys Pro Ala His Gly Ala His Trp Thr Glu Asn Ser Leu Gly
 610 615 620

Asn Ser Leu His Pro Thr Val Thr Thr Ile Ser Glu Pro Tyr Ile Ile
625 630 635 640

Gln Thr His Thr Lys Asp Lys Val Gln Glu Thr Gln Ile
645 650

<210> 22
<211> 640
<212> PRT
<213> Homo sapiens

<400> 22

Met Leu Asn Lys Met Thr Leu His Pro Gln Gln Ile Met Ile Gly Pro
1 5 10 15

Arg Phe Asn Arg Ala Leu Phe Asp Pro Leu Leu Val Val Leu Leu Ala
20 25 30

Leu Gln Leu Leu Val Val Ala Gly Leu Val Arg Ala Gln Thr Cys Pro
35 40 45

Ser Val Cys Ser Cys Ser Asn Gln Phe Ser Lys Val Ile Cys Val Arg
50 55 60

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

Leu Asn Leu His Glu Asn Gln Ile Gln Ile Ile Lys Val Asn Ser Phe
85 90 95

Lys His Leu Arg His Leu Glu Ile Leu Gln Leu Ser Arg Asn His Ile
100 105 110

Arg Thr Ile Glu Ile Gly Ala Phe Asn Gly Leu Ala Asn Leu Asn Thr
115 120 125

Leu Glu Leu Phe Asp Asn Arg Leu Thr Thr Ile Pro Asn Gly Ala Phe
130 135 140

Val Tyr Leu Ser Lys Leu Lys Glu Leu Trp Leu Arg Asn Asn Pro Ile
145 150 155 160

Glu Ser Ile Pro Ser Tyr Ala Phe Asn Arg Ile Pro Ser Leu Arg Arg
165 170 175

Leu Asp Leu Gly Glu Leu Lys Arg Leu Ser Tyr Ile Ser Glu Gly Ala
 180 185 190

Phe Glu Gly Leu Ser Asn Leu Arg Tyr Leu Asn Leu Ala Met Cys Asn
 195 200 205

Leu Arg Glu Ile Pro Asn Leu Thr Pro Leu Ile Lys Leu Asp Glu Leu
 210 215 220

Asp Leu Ser Gly Asn His Leu Ser Ala Ile Arg Pro Gly Ser Phe Gln
 225 230 235 240

Gly Leu Met His Leu Gln Lys Leu Trp Met Ile Gln Ser Gln Ile Gln
 245 250 255

Val Ile Glu Arg Asn Ala Phe Asp Asn Leu Gln Ser Leu Val Glu Ile
 260 265 270

Asn Leu Ala His Asn Asn Leu Thr Leu Leu Pro His Asp Leu Phe Thr
 275 280 285

Pro Leu His His Leu Glu Arg Ile His Leu His His Asn Pro Trp Asn
 290 295 300

Cys Asn Cys Asp Ile Leu Trp Leu Ser Trp Trp Ile Lys Asp Met Ala
 305 310 315 320

Pro Ser Asn Thr Ala Cys Cys Ala Arg Cys Asn Thr Pro Pro Asn Leu
 325 330 335

Lys Gly Arg Tyr Ile Gly Glu Leu Asp Gln Asn Tyr Phe Thr Cys Tyr
 340 345 350

Ala Pro Val Ile Val Glu Pro Pro Ala Asp Leu Asn Val Thr Glu Gly
 355 360 365

Met Ala Ala Glu Leu Lys Cys Arg Ala Ser Thr Ser Leu Thr Ser Val
 370 375 380

Ser Trp Ile Thr Pro Asn Gly Thr Val Met Thr His Gly Ala Tyr Lys
 385 390 395 400

Val Arg Ile Ala Val Leu Ser Asp Gly Thr Leu Asn Phe Thr Asn Val
 405 410 415

Thr Val Gln Asp Thr Gly Met Tyr Thr Cys Met Val Ser Asn Ser Val
 420 425 430

Gly Asn Thr Thr Ala Ser Ala Thr Leu Asn Val Thr Ala Ala Thr Thr
 435 440 445

Thr Pro Phe Ser Tyr Phe Ser Thr Val Thr Val Glu Thr Met Glu Pro
 450 455 460

Ser Gln Asp Glu Ala Arg Thr Thr Asp Asn Asn Val Gly Pro Thr Pro
 465 470 475 480

Val Val Asp Trp Glu Thr Thr Asn Val Thr Thr Ser Leu Thr Pro Gln
 485 490 495

Ser Thr Arg Ser Thr Glu Lys Thr Phe Thr Ile Pro Val Thr Asp Ile
 500 505 510

Asn Ser Gly Ile Pro Gly Ile Asp Glu Val Met Lys Thr Thr Lys Ile
 515 520 525

Ile Ile Gly Cys Phe Val Ala Ile Thr Leu Met Ala Ala Val Met Leu
 530 535 540

Val Ile Phe Tyr Lys Met Arg Lys Gln His His Arg Gln Asn His His
 545 550 555 560

Ala Pro Thr Arg Thr Val Glu Ile Ile Asn Val Asp Asp Glu Ile Thr
 565 570 575

Gly Asp Thr Pro Met Glu Ser His Leu Pro Met Pro Ala Ile Glu His
 580 585 590

Glu His Leu Asn His Tyr Asn Ser Tyr Lys Ser Pro Phe Asn His Thr
 595 600 605

Thr Thr Val Asn Thr Ile Asn Ser Ile His Ser Ser Val His Glu Pro
 610 615 620

Leu Leu Ile Arg Met Asn Ser Lys Asp Asn Val Gln Glu Thr Gln Ile
 625 630 635 640

<210> 23

<211> 422

<212> PRT

<213> Homo sapiens

<400> 23

Met Cys Asn Leu Lys Asp Ile Pro Asn Leu Thr Ala Leu Val Arg Leu
 1 5 10 15

Glu Glu Leu Glu Leu Ser Gly Asn Arg Leu Asp Leu Ile Arg Pro Gly
 20 25 30

Ser Phe Gln Gly Leu Thr Ser Leu Arg Lys Leu Trp Leu Met His Ala
 35 40 45

Gln Val Ala Thr Ile Glu Arg Asn Ala Phe Asp Asp Leu Lys Ser Leu
 50 55 60

Glu Glu Leu Asn Leu Ser His Asn Asn Leu Met Ser Leu Pro His Asp
 65 70 75 80

Leu Phe Thr Pro Leu His Arg Leu Glu Arg Val His Leu Asn His Asn
 85 90 95

Pro Trp His Cys Asn Cys Asp Val Leu Trp Leu Ser Trp Trp Leu Lys
 100 105 110

Glu Thr Val Pro Ser Asn Thr Thr Cys Cys Ala Arg Cys His Ala Pro
 115 120 125

Ala Gly Leu Lys Gly Arg Tyr Ile Gly Glu Leu Asp Gln Ser His Phe
 130 135 140

Thr Cys Tyr Ala Pro Val Ile Val Glu Pro Pro Thr Asp Leu Asn Val
 145 150 155 160

Thr Glu Gly Met Ala Ala Glu Leu Lys Cys Arg Thr Gly Thr Ser Met
 165 170 175

Thr Ser Val Asn Trp Leu Thr Pro Asn Gly Thr Leu Met Thr His Gly
 180 185 190

Ser Tyr Arg Val Arg Ile Ser Val Leu His Asp Gly Thr Leu Asn Phe
 195 200 205

Thr Asn Val Thr Val Gln Asp Thr Gly Gln Tyr Thr Cys Met Val Thr
 210 215 220

Asn Ser Ala Gly Asn Thr Thr Ala Ser Ala Thr Leu Asn Val Ser Ala

<400> 24

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Leu Ala Leu Pro Pro Leu Ala Glu Pro Val Cys Pro Glu Arg Cys Asp
 20 25 30

Cys Gln His Pro Gln His Leu Leu Cys Thr Asn Arg Gly Leu Arg Val
 35 40 45

Val Pro Lys Thr Ser Ser Leu Pro Ser Pro His Asp Val Leu Thr Tyr
 50 55 60

Ser Leu Gly Gly Asn Phe Ile Thr Asn Ile Thr Ala Phe Asp Phe His
 65 70 75 80

Arg Leu Gly Gln Leu Arg Arg Leu Asp Leu Gln Tyr Asn Gln Ile Arg
 85 90 95

Ser Leu His Pro Lys Thr Phe Glu Lys Leu Ser Arg Leu Glu Glu Leu
 100 105 110

Tyr Leu Gly Asn Asn Leu Leu Gln Ala Leu Ala Pro Gly Thr Leu Ala
 115 120 125

Pro Leu Arg Lys Leu Arg Ile Leu Tyr Ala Asn Gly Asn Glu Ile Ser
 130 135 140

Arg Leu Ser Arg Gly Ser Phe Glu Gly Leu Glu Ser Leu Val Lys Leu
 145 150 155 160

Arg Leu Asp Gly Asn Ala Leu Gly Ala Leu Pro Asp Ala Val Phe Ala
 165 170 175

Pro Leu Gly Asn Leu Leu Tyr Leu His Leu Glu Ser Asn Arg Ile Arg
 180 185 190

Phe Leu Gly Lys Asn Ala Phe Ala Gln Leu Gly Lys Leu Arg Phe Leu
 195 200 205

Asn Leu Ser Ala Asn Glu Leu Gln Pro Ser Leu Arg His Ala Ala Thr
 210 215 220

Phe Ala Pro Leu Arg Ser Leu Ser Ser Leu Ile Leu Ser Ala Asn Ser
 225 230 235 240

Leu Gln His Leu Gly Pro Arg Ile Phe Gln His Leu Pro Arg Leu Gly
 245 250 255

Leu Leu Ser Leu Arg Gly Asn Gln Leu Thr His Leu Ala Pro Glu Ala
 260 265 270

Phe Trp Gly Leu Glu Ala Leu Arg Glu Leu Arg Leu Glu Gly Asn Arg
 275 280 285

Leu Ser Gln Leu Pro Thr Ala Leu Leu Glu Pro Leu His Ser Leu Glu
 290 295 300

Ala Leu Asp Leu Ser Gly Asn Glu Leu Ser Ala Leu His Pro Ala Thr
 305 310 315 320

Phe Gly His Leu Gly Arg Leu Arg Glu Leu Ser Leu Arg Asn Asn Ala
 325 330 335

Leu Ser Ala Leu Ser Gly Asp Ile Phe Ala Ala Ser Pro Ala Leu Tyr
 340 345 350

Arg Leu Asp Leu Asp Gly Asn Gly Trp Thr Cys Asp Cys Arg Leu Arg
 355 360 365

Gly Leu Lys Arg Trp Met Gly Asp Trp His Ser Gln Gly Arg Leu Leu
 370 375 380

Thr Val Phe Val Gln Cys Arg His Pro Pro Ala Leu Arg Gly Lys Tyr
 385 390 395 400

Leu Asp Tyr Leu Asp Asp Gln Gln Leu Gln Asn Gly Ser Cys Ala Asp
 405 410 415

Pro Ser Pro Ser Ala Ser Leu Thr Ala Asp Arg Arg Arg Gln Pro Leu
 420 425 430

Pro Thr Ala Ala Gly Glu Glu Met Thr Pro Pro Ala Gly Leu Ala Glu
 435 440 445

Glu Leu Pro Pro Gln Pro Gln Leu Gln Gln Gln Gly Arg Phe Leu Ala
 450 455 460

Gly Val Ala Trp Asp Gly Ala Ala Arg Glu Leu Val Gly Asn Arg Ser
 465 470 475 480

Ala Leu Arg Leu Ser Arg Arg Gly Pro Gly Leu Gln Gln Pro Ser Pro
 485 490 495

Ser Val Ala Ala Ala Ala Gly Pro Ala Pro Gln Ser Leu Asp Leu His
 500 505 510

Lys Lys Pro Gln Arg Gly Arg Pro Thr Arg Ala Asp Pro Ala Leu Ala
 515 520 525

Glu Pro Thr Pro Thr Ala Ser Pro Gly Ser Ala Pro Ser Pro Ala Gly
 530 535 540

Asp Pro Trp Gln Arg Ala Thr Lys His Arg Leu Gly Thr Glu His Gln
 545 550 555 560

Glu Arg Ala Ala Gln Ser Asp Gly Gly Ala Gly Leu Pro Pro Leu Val
 565 570 575

Ser Asp Pro Cys Asp Phe Asn Lys Phe Ile Leu Cys Asn Leu Thr Val
 580 585 590

Glu Ala Val Gly Ala Asp Ser Ala Ser Val Arg Trp Ala Val Arg Glu
 595 600 605

His Arg Ser Pro Arg Pro Leu Gly Gly Ala Arg Phe Arg Leu Leu Phe
 610 615 620

Asp Arg Phe Gly Gln Gln Pro Lys Phe His Arg Phe Val Tyr Leu Pro
 625 630 635 640

Glu Ser Ser Asp Ser Ala Thr Leu Arg Glu Leu Arg Gly Asp Thr Pro
 645 650 655

Tyr Leu Val Cys Val Glu Gly Val Leu Gly Gly Arg Val Cys Pro Val
 660 665 670

Ala Pro Arg Asp His Cys Ala Gly Leu Val Thr Leu Pro Glu Ala Gly
 675 680 685

Ser Arg Gly Gly Val Asp Tyr Gln Leu Leu Thr Leu Ala Leu Leu Thr
 690 695 700

Val Asn Ala Leu Leu Val Leu Leu Ala Leu Ala Ala Trp Ala Ser Arg
 705 710 715 720

Trp Leu Arg Arg Lys Leu Arg Ala Arg Arg Lys Gly Gly Ala Pro Val
 725 730 735

His Val Arg His Met Tyr Ser Thr Arg Arg Pro Leu Arg Ser Met Gly
 740 745 750

Thr Gly Val Ser Ala Asp Phe Ser Gly Phe Gln Ser His Arg Pro Arg
 755 760 765

Thr Thr Val Cys Ala Leu Ser Glu Ala Asp Leu Ile Glu Phe Pro Cys
 770 775 780

Asp Arg Phe Met Asp Ser Ala Gly Gly Gly Ala Gly Gly Ser Leu Arg
 785 790 795 800

Arg Glu Asp Arg Leu Leu Gln Arg Phe Ala Asp
 805 810

<210> 25
 <211> 674
 <212> PRT
 <213> Homo sapiens

<400> 25

Met Val Val Ala His Pro Thr Ala Thr Ala Thr Thr Thr Pro Thr Ala
 1 5 10 15

Thr Val Thr Ala Thr Val Val Met Thr Thr Ala Thr Met Asp Leu Arg
 20 25 30

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

Ile Asp Ser Thr Thr Cys Pro Ser Val Cys Arg Cys Asp Asn Gly Phe
 50 55 60

Ile Tyr Cys Asn Asp Arg Gly Leu Thr Ser Ile Pro Ala Asp Ile Pro
 65 70 75 80

Asp Asp Ala Thr Thr Leu Tyr Leu Gln Asn Asn Gln Ile Asn Asn Ala
 85 90 95

Gly Ile Pro Gln Asp Leu Lys Thr Lys Val Asn Val Gln Val Ile Tyr
 100 105 110

Leu Tyr Glu Asn Asp Leu Asp Glu Phe Pro Ile Asn Leu Pro Arg Ser
 115 120 125

Leu Arg Glu Leu His Leu Gln Asp Asn Asn Val Arg Thr Ile Ala Arg
 130 135 140

Asp Ser Leu Ala Arg Ile Pro Leu Leu Glu Lys Leu His Leu Asp Asp
 145 150 155 160

Asn Ser Val Ser Thr Val Ser Ile Glu Glu Asp Ala Phe Ala Asp Ser
 165 170 175

Lys Gln Leu Lys Leu Leu Phe Leu Ser Arg Asn His Leu Ser Ser Ile
 180 185 190

Pro Ser Gly Leu Pro His Thr Leu Glu Glu Leu Arg Leu Asp Asp Asn
 195 200 205

Arg Ile Ser Thr Ile Pro Leu His Ala Phe Lys Gly Leu Asn Ser Leu
 210 215 220

Arg Arg Leu Val Leu Asp Gly Asn Leu Leu Ala Asn Gln Arg Ile Ala
 225 230 235 240

Asp Asp Thr Phe Ser Arg Leu Gln Asn Leu Thr Glu Leu Ser Leu Val
 245 250 255

Arg Asn Ser Leu Ala Ala Pro Pro Leu Asn Leu Pro Ser Ala His Leu
 260 265 270

Gln Lys Leu Tyr Leu Gln Asp Asn Ala Ile Ser His Ile Pro Tyr Asn
 275 280 285

Thr Leu Ala Lys Met Arg Glu Leu Glu Arg Leu Asp Leu Ser Asn Asn
 290 295 300

Asn Leu Thr Thr Leu Pro Arg Gly Leu Phe Asp Asp Leu Gly Asn Leu
 305 310 315 320

Ala Gln Leu Leu Leu Arg Asn Asn Pro Trp Phe Cys Gly Cys Asn Leu
 325 330 335

Met Trp Leu Arg Asp Trp Val Lys Ala Arg Ala Ala Val Val Asn Val
 340 345 350

Arg Gly Leu Met Cys Gln Gly Pro Glu Lys Val Arg Gly Met Ala Ile
 355 360 365

Lys Asp Ile Thr Ser Glu Met Asp Glu Cys Phe Glu Thr Gly Pro Gln
 370 375 380

Gly Gly Val Ala Asn Ala Ala Ala Lys Thr Thr Ala Ser Asn His Ala
 385 390 395 400

Ser Ala Thr Thr Pro Gln Gly Ser Leu Phe Thr Leu Lys Ala Lys Arg
 405 410 415

Pro Gly Leu Arg Leu Pro Asp Ser Asn Ile Asp Tyr Pro Met Ala Thr
 420 425 430

Gly Asp Gly Ala Lys Thr Leu Ala Ile His Val Lys Ala Leu Thr Ala
 435 440 445

Asp Ser Ile Arg Ile Thr Trp Lys Ala Thr Leu Pro Ala Ser Ser Phe
 450 455 460

Arg Leu Ser Trp Leu Arg Leu Gly His Ser Pro Ala Val Gly Ser Ile
 465 470 475 480

Thr Glu Thr Leu Val Gln Gly Asp Lys Thr Glu Tyr Leu Leu Thr Ala
 485 490 495

Leu Glu Pro Lys Ser Thr Tyr Ile Ile Cys Met Val Thr Met Glu Thr
 500 505 510

Ser Asn Ala Tyr Val Ala Asp Glu Thr Pro Val Cys Ala Lys Ala Glu
 515 520 525

Thr Ala Asp Ser Tyr Gly Pro Thr Thr Thr Leu Asn Gln Glu Gln Asn
 530 535 540

Ala Gly Pro Met Ala Ser Leu Pro Leu Ala Gly Ile Ile Gly Gly Ala
 545 550 555 560

Val Ala Leu Val Phe Leu Phe Leu Val Leu Gly Ala Ile Cys Trp Tyr
 565 570 575

Val His Gln Ala Gly Glu Leu Leu Thr Arg Glu Arg Ala Tyr Asn Arg
 580 585 590

Gly Ser Arg Glu Lys Asp Asp Tyr Met Glu Ser Gly Thr Lys Lys Asp
 595 600 605

Asn Ser Ile Leu Glu Ile Arg Gly Pro Gly Leu Gln Met Leu Pro Ile
 610 615 620

Asn Pro Tyr Arg Ala Lys Glu Glu Tyr Val Val His Thr Ile Phe Pro
 625 630 635 640

Ser Asn Gly Ser Ser Leu Cys Lys Ala Thr His Thr Ile Gly Tyr Gly
 645 650 655

Thr Thr Arg Gly Tyr Arg Asp Gly Gly Ile Pro Asp Ile Asp Tyr Ser
 660 665 670

Tyr Thr

<210> 26
 <211> 660
 <212> PRT
 <213> Homo sapiens

<400> 26

Met Gly Leu Gln Thr Thr Lys Trp Pro Ser His Gly Ala Phe Phe Leu
 1 5 10 15

Lys Ser Trp Leu Ile Ile Ser Leu Gly Leu Tyr Ser Gln Val Ser Lys
 20 25 30

Leu Leu Ala Cys Pro Ser Val Cys Arg Cys Asp Arg Asn Phe Val Tyr
 35 40 45

Cys Asn Glu Arg Ser Leu Thr Ser Val Pro Leu Gly Ile Pro Glu Gly
 50 55 60

Val Thr Val Leu Tyr Leu His Asn Asn Gln Ile Asn Asn Ala Gly Phe
 65 70 75 80

Pro Ala Glu Leu His Asn Val Gln Ser Val His Thr Val Tyr Leu Tyr
 85 90 95

Gly Asn Gln Leu Asp Glu Phe Pro Met Asn Leu Pro Lys Asn Val Arg
 100 105 110

Val Leu His Leu Gln Glu Asn Asn Ile Gln Thr Ile Ser Arg Ala Ala
 115 120 125

Leu Ala Gln Leu Leu Lys Leu Glu Glu Leu His Leu Asp Asp Asn Ser
 130 135 140

Ile Ser Thr Val Gly Val Glu Asp Gly Ala Phe Arg Glu Ala Ile Ser
 145 150 155 160

Leu Lys Leu Leu Phe Leu Ser Lys Asn His Leu Ser Ser Val Pro Val
 165 170 175

Gly Leu Pro Val Asp Leu Gln Glu Leu Arg Val Asp Glu Asn Arg Ile
 180 185 190

Ala Val Ile Ser Asp Met Ala Phe Gln Asn Leu Thr Ser Leu Glu Arg
 195 200 205

Leu Ile Val Asp Gly Asn Leu Leu Thr Asn Lys Gly Ile Ala Glu Gly
 210 215 220

Thr Phe Ser His Leu Thr Lys Leu Lys Glu Phe Ser Ile Val Arg Asn
 225 230 235 240

Ser Leu Ser His Pro Pro Pro Asp Leu Pro Gly Thr His Leu Ile Arg
 245 250 255

Leu Tyr Leu Gln Asp Asn Gln Ile Asn His Ile Pro Leu Thr Ala Phe
 260 265 270

Ser Asn Leu Arg Lys Leu Glu Arg Leu Asp Ile Ser Asn Asn Gln Leu
 275 280 285

Arg Met Leu Thr Gln Gly Val Phe Asp Asn Leu Ser Asn Leu Lys Gln
 290 295 300

Leu Thr Ala Arg Asn Asn Pro Trp Phe Cys Asp Cys Ser Ile Lys Trp
 305 310 315 320

Val Thr Glu Trp Leu Lys Tyr Ile Pro Ser Ser Leu Asn Val Arg Gly
 325 330 335

Phe Met Cys Gln Gly Pro Glu Gln Val Arg Gly Met Ala Val Arg Glu
 340 345 350

Leu Asn Met Asn Leu Leu Ser Cys Pro Thr Thr Thr Pro Gly Leu Pro
 355 360 365

Leu Phe Thr Pro Ala Pro Ser Thr Ala Ser Pro Thr Thr Gln Pro Pro
 370 375 380
 Thr Leu Ser Ile Pro Asn Pro Ser Arg Ser Tyr Thr Pro Pro Thr Pro
 385 390 395 400
 Thr Thr Ser Lys Leu Pro Thr Ile Pro Asp Trp Asp Gly Arg Glu Arg
 405 410 415
 Val Thr Pro Pro Ile Ser Glu Arg Ile Gln Leu Ser Ile His Phe Val
 420 425 430
 Asn Asp Thr Ser Ile Gln Val Ser Trp Leu Ser Leu Phe Thr Val Met
 435 440 445
 Ala Tyr Lys Leu Thr Trp Val Lys Met Gly His Ser Leu Val Gly Gly
 450 455 460
 Ile Val Gln Glu Arg Ile Val Ser Gly Glu Lys Gln His Leu Ser Leu
 465 470 475 480
 Val Asn Leu Glu Pro Arg Ser Thr Tyr Arg Ile Cys Leu Val Pro Leu
 485 490 495
 Asp Ala Phe Asn Tyr Arg Ala Val Glu Asp Thr Ile Cys Ser Glu Ala
 500 505 510
 Thr Thr His Ala Ser Tyr Leu Asn Asn Gly Ser Asn Thr Ala Ser Ser
 515 520 525
 His Glu Gln Thr Thr Ser His Ser Met Gly Ser Pro Phe Leu Leu Ala
 530 535 540
 Gly Leu Ile Gly Gly Ala Val Ile Phe Val Leu Val Val Leu Leu Ser
 545 550 555 560
 Val Phe Cys Trp His Met His Lys Lys Gly Arg Tyr Thr Ser Gln Lys
 565 570 575
 Trp Lys Tyr Asn Arg Gly Arg Arg Lys Asp Asp Tyr Cys Glu Ala Gly
 580 585 590
 Thr Lys Lys Asp Asn Ser Ile Leu Glu Met Thr Glu Thr Ser Phe Gln
 595 600 605

Ile Val Ser Leu Asn Asn Asp Gln Leu Leu Lys Gly Asp Phe Arg Leu
 610 615 620

Gln Pro Ile Tyr Thr Pro Asn Gly Gly Ile Asn Tyr Thr Asp Cys His
 625 630 635 640

Ile Pro Asn Asn Met Arg Tyr Cys Asn Ser Ser Val Pro Asp Leu Glu
 645 650 655

His Cys His Thr
 660

<210> 27
 <211> 649
 <212> PRT
 <213> Homo sapiens

<400> 27

Met Ile Ser Ala Ala Trp Ser Ile Phe Leu Ile Gly Thr Lys Ile Gly
 1 5 10 15

Leu Phe Leu Gln Val Ala Pro Leu Ser Val Met Ala Lys Ser Cys Pro
 20 25 30

Ser Val Cys Arg Cys Asp Ala Gly Phe Ile Tyr Cys Asn Asp Arg Phe
 35 40 45

Leu Thr Ser Ile Pro Thr Gly Ile Pro Glu Asp Ala Thr Thr Leu Tyr
 50 55 60

Leu Gln Asn Asn Gln Ile Asn Asn Ala Gly Ile Pro Ser Asp Leu Lys
 65 70 75 80

Asn Leu Leu Lys Val Glu Arg Ile Tyr Leu Tyr His Asn Ser Leu Asp
 85 90 95

Glu Phe Pro Thr Asn Leu Pro Lys Tyr Val Lys Glu Leu His Leu Gln
 100 105 110

Glu Asn Asn Ile Arg Thr Ile Thr Tyr Asp Ser Leu Ser Lys Ile Pro
 115 120 125

Tyr Leu Glu Glu Leu His Leu Asp Asp Asn Ser Val Ser Ala Val Ser
 130 135 140

Ile Glu Glu Gly Ala Phe Arg Asp Ser Asn Tyr Leu Arg Leu Leu Phe

145		150		155		160
Leu Ser Arg Asn	His Leu Ser Thr	Ile Pro Trp Gly	Leu Pro Arg Thr			
	165	170	175			
Ile Glu Glu Leu	Arg Leu Asp Asp	Asn Arg Ile Ser	Thr Ile Ser Ser			
	180	185	190			
Pro Ser Leu Gln	Gly Leu Thr Ser	Leu Lys Arg Leu	Val Leu Asp Gly			
	195	200	205			
Asn Leu Leu Asn	Asn His Gly Leu	Gly Asp Lys Val	Phe Phe Asn Leu			
	210	215	220			
Val Asn Leu Thr	Glu Leu Ser Leu	Val Arg Asn Ser	Leu Thr Ala Ala			
	225	230	235			240
Pro Val Asn Leu	Pro Gly Thr Asn	Leu Arg Lys Leu	Tyr Leu Gln Asp			
	245	250	255			
Asn His Ile Asn	Arg Val Pro Pro	Asn Ala Phe Ser	Tyr Leu Arg Gln			
	260	265	270			
Leu Tyr Arg Leu	Asp Met Ser Asn	Asn Asn Leu Ser	Asn Leu Pro Gln			
	275	280	285			
Gly Ile Phe Asp	Asp Leu Asp Asn	Ile Thr Gln Leu	Ile Leu Arg Asn			
	290	295	300			
Asn Pro Trp Tyr	Cys Gly Cys Lys	Met Lys Trp Val	Arg Asp Trp Leu			
	305	310	315			320
Gln Ser Leu Pro	Val Lys Val Asn	Val Arg Gly Leu	Met Cys Gln Ala			
	325	330	335			
Pro Glu Lys Val	Arg Gly Met Ala	Ile Lys Asp Leu	Asn Ala Glu Leu			
	340	345	350			
Phe Asp Cys Lys	Asp Ser Gly Ile	Val Ser Thr Ile	Gln Ile Thr Thr			
	355	360	365			
Ala Ile Pro Asn	Thr Val Tyr Pro	Ala Gln Gly Gln	Trp Pro Ala Pro			
	370	375	380			
Val Thr Lys Gln	Pro Asp Ile Lys	Asn Pro Lys Leu	Thr Lys Asp His			

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625

630

635

640

Ile Pro Asp Ser Asp His Ser His Ser
645

<210> 28

<211> 261

<212> PRT

<213> Homo sapiens

<400> 28

Met Ala Pro Val Pro Gly Ser Leu Gly Gln Gly Arg Asp Ser Gly Asp
1 5 10 15

Ser Ala Ser Lys Ser Arg Glu Ala Ser Gly Gly Pro Gln Leu Ser Ser
20 25 30

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

Trp Pro Leu Arg Leu Ala Gly Trp Gly Ala Ser Pro Leu Arg Leu Ala
50 55 60

Gly Trp Gly Gly Met Ala Ala Ser Ala Trp Leu Glu Ala Gly Leu Ala
65 70 75 80

Arg Val Leu Phe Tyr Pro Thr Leu Leu Tyr Thr Val Phe Arg Gly Arg
85 90 95

Val Arg Gly Pro Ala His Arg Asp Trp Tyr His Arg Ile Asp His Thr
100 105 110

Val Leu Leu Gly Ala Leu Pro Leu Lys Asn Met Thr Arg Arg Leu Val
115 120 125

Leu Asp Glu Asn Val Arg Gly Val Ile Thr Met Asn Glu Glu Tyr Glu
130 135 140

Thr Arg Phe Leu Cys Asn Thr Ser Lys Glu Trp Lys Lys Ala Gly Val
145 150 155 160

Glu Gln Leu Arg Leu Ser Thr Val Asp Met Thr Gly Val Pro Thr Leu
165 170 175

Ala Asn Leu His Lys Gly Val Gln Phe Ala Leu Lys Tyr Gln Ala Leu
180 185 190

Gly Gln Cys Val Tyr Val His Cys Lys Ala Gly Arg Ser Arg Ser Ala
195 200 205

Thr Met Val Ala Ala Tyr Leu Ile Gln Val His Asn Trp Ser Pro Glu
210 215 220

Glu Ala Ile Glu Ala Ile Ala Lys Ile Arg Ser His Ile Ser Ile Arg
225 230 235 240

Pro Ser Gln Leu Glu Val Leu Lys Glu Phe His Lys Glu Ile Thr Ala
245 250 255

Arg Ala Ala Lys Asn
260