(54) Title: MAT2As AS MODIFIERS OF THE p53 PATHWAY AND METHODS OF USE

(57) Abstract: Human MAT2A 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 agents that modulate the activity of MAT2A are provided.
MAT2As AS MODIFIERS OF THE p53 PATHWAY AND METHODS OF USE

REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. provisional patent application 60/437,162 filed 12/30/2002. The contents of the prior application are hereby incorporated in their entirety.

BACKGROUND OF THE INVENTION


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 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 (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 of the cell cycle or apoptosis, including GADD45, p21/Waf1/Cip1, cyclin G, Bax, IGF-BP3, and MDM2 (Levine, Cell (1997) 88:323-331).

Methionine adenosyltransferase catalyzes the biosynthesis of S-adenosylmethionine (AdoMet) from methionine and ATP. AdoMet is the major methyl donor for many of the transmethylation reactions in the body. AdoMet also participates in
the transsulfuration pathway and, after decarboxylation, serves as a propylamine group donor in the biosynthesis of polyamines. Three forms of MAT have been identified in mammalian tissues.

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 methionine adenosyltransferase II alpha (MAT2A). The invention provides methods for utilizing these p53 modifier genes and polypeptides to identify MAT2A-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 MAT2A function. Preferred MAT2A-modulating agents specifically
bind to MAT2A polypeptides and restore p53 function. Other preferred MAT2A-
modulating agents are nucleic acid modulators such as antisense oligomers and RNAi that
repress MAT2A gene expression or product activity by, for example, binding to and
inhibiting the respective nucleic acid (i.e. DNA or mRNA).

MAT2A modulating agents may be evaluated by any convenient in vitro or in vivo
assay for molecular interaction with a MAT2A polypeptide or nucleic acid. In one
embodiment, candidate MAT2A modulating agents are tested with an assay system
comprising a MAT2A 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. MAT2A-modulating agents
include MAT2A related proteins (e.g. dominant negative mutants, and biotherapeutics);
MAT2A -specific antibodies; MAT2A -specific antisense oligomers and other nucleic acid
modulators; and chemical agents that specifically bind to or interact with MAT2A or
compete with MAT2A binding partner (e.g. by binding to a MAT2A 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 MAT2A function
and/or the p53 pathway in a mammalian cell by contacting the mammalian cell with an
agent that specifically binds a MAT2A polypeptide or nucleic acid. The agent may be a
small molecule modulator, a nucleic acid modulator, or an antibody and may be
administered to a mammalian animal predetermined to have a pathology associated with
the 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). The CG2674 gene was identified as a modifier of the p53 pathway. Accordingly, vertebrate orthologs of the modifier, and preferably the human orthologs, MAT2A 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.

In vitro and in vivo methods of assessing MAT2A function are provided herein. Modulation of the MAT2A 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. MAT2A-modulating agents that act by inhibiting or enhancing MAT2A expression, directly or indirectly, for example, by affecting a MAT2A function such as enzymatic (e.g., catalytic) or binding activity, can be identified using methods provided herein. MAT2A modulating agents are useful in diagnosis, therapy and pharmaceutical development.

**Nucleic acids and polypeptides of the invention**

Sequences related to MAT2A nucleic acids and polypeptides that can be used in the invention are disclosed in Genbank (referenced by Genbank identifier (GI) number) as GI# 19923346 (SEQ ID NO:1) for nucleic acid, and GI# 5174529 (SEQ ID NO:2) for polypeptide.

The term “MAT2A polypeptide” refers to a full-length MAT2A protein or a functionally active fragment or derivative thereof. A “functionally active” MAT2A fragment or derivative exhibits one or more functional activities associated with a full-length, wild-type MAT2A protein, such as antigenic or immunogenic activity, enzymatic activity, ability to bind natural cellular substrates, etc. The functional activity of MAT2A 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 MAT2A polypeptide is a MAT2A derivative capable of rescuing defective endogenous MAT2A 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 a MAT2A, 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 MAT2A 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 a MAT2A. In further preferred embodiments, the fragment comprises the entire functionally active domain.

The term "MAT2A nucleic acid" refers to a DNA or RNA molecule that encodes a MAT2A polypeptide. Preferably, the MAT2A 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 MAT2A. Methods of identifying orthlogs are known in the art. Normally, orthlogs in different species retain the same function, due to presence of one or more protein motifs and/or 3-dimensional structures. Orthlogs are generally identified by sequence homology analysis, such as BLAST analysis, usually using protein bait sequences. Sequences are assigned as a potential orthlog 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 orthlogs. In evolution, when a gene duplication event follows speciation, a single gene in one species, such as Drosophila, may correspond to multiple genes (paralogs) in another, such as human. As used herein, the term "orthologs" encompasses paralogs. As used herein, "percent (%) sequence identity" with respect to a subject sequence, or a specified portion of a subject sequence, is defined as the percentage of nucleotides or amino acids in the
candidate derivative sequence identical with the nucleotides or amino acids in the subject sequence (or specified portion thereof), after aligning the sequences and introducing gaps, if necessary to achieve the maximum percent sequence identity, as generated by the program WU-BLAST-2.0a19 (Altschul et al., J. Mol. Biol. (1997) 215:403-410) with all the search parameters set to default values. The HSP S and HSP S2 parameters are dynamic values and are established by the program itself depending upon the composition of the particular sequence and composition of the particular database against which the sequence of interest is being searched. A % identity value is determined by the number of matching identical nucleotides or amino acids divided by the sequence length for which the percent identity is being reported. “Percent (%) amino acid sequence similarity” is determined by doing the same calculation as for determining % amino acid sequence identity, but including conservative amino acid substitutions in addition to identical amino acids in the computation.

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

Alternatively, an alignment for nucleic acid sequences is provided by the local homology algorithm of Smith and Waterman (Smith and Waterman, 1981, Advances in Applied Mathematics 2:482-489; database: European Bioinformatics Institute; Smith and Waterman, 1981, J. of Molec.Biol., 147:195-197; Nicholas et al., 1998, "A Tutorial on Searching Sequence Databases and Sequence Scoring Methods" (www.psc.edu) and references cited therein.; W.R. Pearson, 1991, Genomics 11:635-650). This algorithm can be applied to amino acid sequences by using the scoring matrix developed by Dayhoff (Dayhoff: Atlas of Protein Sequences and Structure, M. O. Dayhoff ed., 5 suppl. 3:353-358, National Biomedical Research Foundation, Washington, D.C., USA), and normalized by Gribskov (Gribskov 1986 Nucl. Acids Res. 14(6):6745-6763). The Smith-Waterman algorithm may be employed where default parameters are used for scoring (for example, gap open penalty of 12, gap extension penalty of two). From the data generated, the "Match" value reflects "sequence identity."
Derivative nucleic acid molecules of the subject nucleic acid molecules include sequences that hybridize to the nucleic acid sequence of a MAT2A. The stringency of hybridization can be controlled by temperature, ionic strength, pH, and the presence of denaturing agents such as formamide during hybridization and washing. Conditions routinely used are set out in readily available procedure texts (e.g., Current Protocol in Molecular Biology, Vol. 1, Chap. 2.10, John Wiley & Sons, Publishers (1994); Sambrook et al., Molecular Cloning, Cold Spring Harbor (1989)). In some embodiments, a nucleic acid molecule of the invention is capable of hybridizing to a nucleic acid molecule containing the nucleotide sequence of a MAT2A 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).

In other embodiments, moderately stringent hybridization conditions are used that are: pretreatment of filters containing nucleic acid for 6 h at 40°C in a solution containing 35% formamide, 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 are: 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 MAT2A Nucleic Acids and Polypeptides**

MAT2A nucleic acids and polypeptides are useful for identifying and testing agents that modulate MAT2A function and for other applications related to the
involvement of MAT2A in the p53 pathway. MAT2A 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 a MAT2A protein for assays used to assess MAT2A 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 MAT2A 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 a MAT2A polypeptide can be inserted into any appropriate expression vector. The necessary transcriptional and translational signals, including promoter/enhancer element, can derive from the native MAT2A 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 MAT2A gene product, the expression vector can comprise a promoter operably linked to a MAT2A 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 MAT2A gene product based on the physical or functional properties of the MAT2A protein in *in vitro* assay systems (e.g. immunoassays).

The MAT2A 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 MAT2A 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 MAT2A 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 MAT2A 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 MAT2A 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 MAT2A in a p53 pathway process such as apoptosis or cell proliferation. Preferably, the altered MAT2A expression results in a detectable phenotype,
such as decreased or increased levels of cell proliferation, angiogenesis, or apoptosis compared to control animals having normal MAT2A 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.


In one embodiment, the transgenic animal is a “knock-out” animal having a heterozygous or homozygous alteration in the sequence of an endogenous MAT2A gene that results in a decrease of MAT2A function, preferably such that MAT2A expression is undetectable or insignificant. Knock-out animals are typically generated by homologous
recombination with a vector comprising a transgene having at least a portion of the gene to be knocked out. Typically a deletion, addition or substitution has been introduced into the transgene to functionally disrupt it. The transgene can be a human gene (e.g., from a human genomic clone) but more preferably is an ortholog of the human gene derived from the transgenic host species. For example, a mouse MAT2A gene is used to construct a homologous recombination vector suitable for altering an endogenous MAT2A 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-rat 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 MAT2A gene, e.g., by introduction of additional copies of MAT2A, or by operatively inserting a regulatory sequence that provides for altered expression of an endogenous copy of the MAT2A 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

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 MAT2A function and phenotypic changes are compared with appropriate control animals such as genetically modified animals that receive placebo treatment, and/or animals with unaltered MAT2A expression that receive candidate therapeutic agent.

In addition to the above-described genetically modified animals having altered MAT2A function, animal models having defective p53 function (and otherwise normal MAT2A 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 MAT2A 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 MAT2A 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 MAT2A activity by administering a MAT2A-interacting or -modulating agent.

As used herein, a "MAT2A-modulating agent" is any agent that modulates MAT2A function, for example, an agent that interacts with MAT2A to inhibit or enhance MAT2A activity or otherwise affect normal MAT2A function. MAT2A function can be affected at any level, including transcription, protein expression, protein localization, and
cellular or extra-cellular activity. In a preferred embodiment, the MAT2A-modulating agent specifically modulates the function of the MAT2A. The phrases “specific modulating agent”, “specifically modulates”, etc., are used herein to refer to modulating agents that directly bind to the MAT2A polypeptide or nucleic acid, and preferably inhibit, enhance, or otherwise alter, the function of the MAT2A. These phrases also encompass modulating agents that alter the interaction of the MAT2A with a binding partner, substrate, or cofactor (e.g. by binding to a binding partner of a MAT2A, or to a protein/binding partner complex, and altering MAT2A function). In a further preferred embodiment, the MAT2A-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 MAT2A-modulating agents include small molecule compounds; MAT2A-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 up to 10,000, preferably up to 5,000, more preferably up to 1,000, and most preferably up to 500 daltons. This class of modulators includes chemically synthesized molecules, for instance, compounds from combinatorial chemical libraries. Synthetic compounds may be rationally designed or identified based on known or inferred properties of the MAT2A 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 MAT2A-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 MAT2A-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 MAT2A-modulating agents. In a preferred embodiment, MAT2A-interacting proteins affect normal MAT2A function, including transcription, protein expression, protein localization, and cellular or extra-cellular activity. In another embodiment, MAT2A-interacting proteins are useful in detecting and providing information about the function of MAT2A proteins, as is relevant to p53 related disorders, such as cancer (e.g., for diagnostic means).


An MAT2A-interacting protein may be an exogenous protein, such as a MAT2A-specific antibody or a T-cell antigen receptor (see, e.g., Harlow and Lane (1988))

In preferred embodiments, a MAT2A-interacting protein specifically binds a MAT2A protein. In alternative preferred embodiments, a MAT2A-modulating agent binds a MAT2A substrate, binding partner, or cofactor.

**Antibodies**

In another embodiment, the protein modulator is a MAT2A specific antibody agonist or antagonist. The antibodies have therapeutic and diagnostic utilities, and can be used in screening assays to identify MAT2A modulators. The antibodies can also be used in dissecting the portions of the MAT2A pathway responsible for various cellular responses and in the general processing and maturation of the MAT2A.

Antibodies that specifically bind MAT2A polypeptides can be generated using known methods. Preferably the antibody is specific to a mammalian ortholog of MAT2A polypeptide, and more preferably, to human MAT2A. Antibodies may be polyclonal, monoclonal (mAbs), humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab').sub.2 fragments, fragments produced by a FAb expression library, idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above.


Monoclonal antibodies with affinities of $10^8$ M$^{-1}$ preferably $10^9$ M$^{-1}$ to $10^{10}$ M$^{-1}$, or stronger can be made by standard procedures as described (Harlow and Lane, supra; Goding (1986) Monoclonal Antibodies: Principles and Practice (2d ed) Academic Press, New York; and U.S. Pat. Nos. 4,381,292; 4,451,570; and 4,618,577). Antibodies may be generated against crude cell extracts of MAT2A or substantially purified fragments thereof. If MAT2A fragments are used, they preferably comprise at least 10, and more preferably, at least 20 contiguous amino acids of a MAT2A protein. In a particular embodiment, MAT2A-specific antigens and/or immunogens are coupled to carrier proteins that stimulate the immune response. For example, the subject polypeptides are covalently coupled to the keyhole limpet hemocyanin (KLH) carrier, and the conjugate is
emulsified in Freund's complete adjuvant, which enhances the immune response. An appropriate immune system such as a laboratory rabbit or mouse is immunized according to conventional protocols.

The presence of MAT2A-specific antibodies is assayed by an appropriate assay such as a solid phase enzyme-linked immunosorbant assay (ELISA) using immobilized corresponding MAT2A polypeptides. Other assays, such as radioimmunoassays or fluorescent assays might also be used.


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).

**Nucleic Acid Modulators**

Other preferred MAT2A-modulating agents comprise nucleic acid molecules, such as antisense oligomers or double stranded RNA (dsRNA), which generally inhibit MAT2A activity. Preferred nucleic acid modulators interfere with the function of the MAT2A nucleic acid such as DNA replication, transcription, translocation of the MAT2A
RNA to the site of protein translation, translation of protein from the MAT2A RNA, splicing of the MAT2A RNA to yield one or more mRNA species, or catalytic activity which may be engaged in or facilitated by the MAT2A RNA.

In one embodiment, the antisense oligomer is an oligonucleotide that is sufficiently complementary to a MAT2A mRNA to bind to and prevent translation, preferably by binding to the 5' untranslated region. MAT2A-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 phosphodiimidate 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).


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, a MAT2A-specific nucleic acid modulator is used in an assay to further elucidate the role of the MAT2A in the p53 pathway, and/or its relationship to other members of the pathway. In another aspect of the invention, a MAT2A-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 MAT2A 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 MAT2A nucleic acid or protein. In general, secondary assays further assess the activity of a MAT2A modulating agent identified by a primary assay and may confirm that the modulating agent affects MAT2A in a manner relevant to the p53 pathway. In some cases, MAT2A modulators will be directly tested in a secondary assay.

In a preferred embodiment, the screening method comprises contacting a suitable assay system comprising a MAT2A 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
MAT2A activity, and hence the p53 pathway. The MAT2A 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 MAT2A 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 MAT2A-interacting proteins are used in screens to identify small molecule modulators, the binding specificity of the interacting protein to the MAT2A protein may be assayed by various known methods such as substrate processing (*e.g.* ability of the candidate MAT2A-specific binding agents to function as negative effectors in MAT2A-expressing cells), binding
equilibrium constants (usually at least about $10^7 \text{ M}^{-1}$, preferably at least about $10^8 \text{ M}^{-1}$, more preferably at least about $10^9 \text{ M}^{-1}$), and immunogenicity (e.g. ability to elicit MAT2A 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 modulate activity of a MAT2A polypeptide, a fusion protein thereof, or to cells or membranes bearing the polypeptide or fusion protein. The MAT2A polypeptide can be full length or a fragment thereof that retains functional MAT2A activity. The MAT2A polypeptide may be fused to another polypeptide, such as a peptide tag for detection or anchoring, or to another tag. The MAT2A polypeptide is preferably human MAT2A, or is an ortholog or derivative thereof as described above. In a preferred embodiment, the screening assay detects candidate agent-based modulation of MAT2A interaction with a binding target, such as an endogenous or exogenous protein or other substrate that has MAT2A-specific binding activity, and can be used to assess normal MAT2A gene function.

Suitable assay formats that may be adapted to screen for MAT2A modulators are known in the art. Preferred screening assays are high throughput or ultra high throughput and thus provide automated, cost-effective means of screening compound libraries for lead compounds (Fernandes 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 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).

**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). Other cell-based apoptosis assays include the caspase-3/7 assay and the cell
death nucleosome ELISA assay. The caspase 3/7 assay is based on the activation of the caspase cleavage activity as part of a cascade of events that occur during programmed cell death in many apoptotic pathways. In the caspase 3/7 assay (commercially available Apo-ONE\textsuperscript{TM} Homogeneous Caspase-3/7 assay from Promega, cat\# 67790), lysis buffer and caspase substrate are mixed and added to cells. The caspase substrate becomes fluorescent when cleaved by active caspase 3/7. The nucleosome ELISA assay is a general cell death assay known to those skilled in the art, and available commercially (Roche, Cat# 1774425). This assay is a quantitative sandwich-enzyme-immunoassay which uses monoclonal antibodies directed against DNA and histones respectively, thus specifically determining amount of mono- and oligonucleosomes in the cytoplasmic fraction of cell lysates. Mono and oligonucleosomes are enriched in the cytoplasm during apoptosis due to the fact that DNA fragmentation occurs several hours before the plasma membrane breaks down, allowing for accumulation in the cytoplasm. Nucleosomes are not present in the cytoplasmic fraction of cells that are not undergoing apoptosis. An apoptosis assay system may comprise a cell that expresses a MAT2A, 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 MAT2A function plays a direct role in apoptosis. For example, an apoptosis assay may be performed on cells that over- or under-express MAT2A relative to wild type cells. Differences in apoptotic response compared to wild type cells suggests that the MAT2A 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.
Cell proliferation is also assayed via phospho-histone H3 staining, which identifies a cell population undergoing mitosis by phosphorylation of histone H3. Phosphorylation of histone H3 at serine 10 is detected using an antibody specific to the phosphorylated form of the serine 10 residue of histone H3. (Chadlee, D.N. 1995, J. Biol. Chem 270:20098-105). Cell Proliferation may also be examined using [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 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 (Voytk-Harbin SL et al., 1998, In Vitro Cell Dev Biol Anim 34:239-46). Yet another proliferation assay, the MTS assay, is based on in vitro cytotoxicity assessment of industrial chemicals, and uses the soluble tetrazolium salt, MTS. MTS assays are commercially available, for example, the Promega Cell Titer 96® AQueous Non-Radioactive Cell Proliferation Assay (Cat.# G5421).

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 MAT2A are seeded in soft agar plates, and colonies are measured and counted after two weeks incubation.

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

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 a MAT2A 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 expresses a MAT2A, 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 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 MAT2A 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 MAT2A relative to wild type cells. Differences in proliferation or cell cycle compared to wild type cells suggests that the MAT2A 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 a MAT2A, 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 MAT2A function plays a direct role in cell proliferation. For example, an angiogenesis assay may be performed on cells that over- or under-express MAT2A relative to wild type cells. Differences in angiogenesis compared to wild type cells suggests that the MAT2A plays a direct role in angiogenesis. U.S. Pat. Nos. 5,976,782, 6,225,118 and 6,444,434, among others, describe various angiogenesis assays.

**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 MAT2A 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 a MAT2A, 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 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 MAT2A 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 MAT2A relative to wild type cells. Differences in hypoxic response compared to wild type cells suggests that the MAT2A plays a direct role in hypoxic induction.

**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 2x 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.

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.

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).

**Tubulogenesis.** Tubulogenesis assays monitor the ability of cultured cells, generally endothelial cells, to form tubular structures on a matrix substrate, which generally simulates the environment of the extracellular matrix. Exemplary substrates include Matrigel™ (Becton Dickinson), an extract of basement membrane proteins containing laminin, collagen IV, and heparin sulfate proteoglycan, which is liquid at 4°C and forms a solid gel at 37°C. Other suitable matrices comprise extracellular components such as collagen, fibronectin, and/or fibrin. Cells are stimulated with a pro-angiogenic stimulant, and their ability to form tubules is detected by imaging. Tubules can generally be detected after an overnight incubation with stimuli, but longer or shorter time frames may also be used. Tube formation assays are well known in the art (e.g., Jones MK et al., 1999, Nature Medicine 5:1418-1423). These assays have traditionally involved stimulation with serum or with the growth factors FGF or VEGF. Serum represents an undefined source of growth factors. In a preferred embodiment, the assay is performed with cells cultured in serum free medium, in order to control which process or pathway a candidate agent modulates. Moreover, we have found that different target genes respond differently to stimulation with different pro-angiogenic agents, including inflammatory angiogenic factors such as TNF-alpha. Thus, in a further preferred embodiment, a tubulogenesis assay system comprises testing a MAT2A’s response to a variety of factors, such as FGF, VEGF, phorbol myristate acetate (PMA), TNF-alpha, ephrin, etc.

**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 a MAT2A's response to a variety of pro-angiogenic factors, including tumor angiogenic and inflammatory angiogenic agents, and culturing the cells in serum free medium.

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

For antibody modulators, appropriate primary assays test is a binding assay that tests the antibody's affinity to and specificity for the MAT2A 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 MAT2A-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 MAT2A gene expression, preferably mRNA expression. In general, expression analysis comprises comparing MAT2A expression in like populations of cells (e.g., two pools of cells that endogenously or recombinantly express MAT2A) 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 MAT2A 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; Kallioniem 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 antiserum directed against either the MAT2A 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 MAT2A mRNA expression, may also be used to test nucleic acid modulators.

Secondary Assays

Secondary assays may be used to further assess the activity of MAT2A-modulating agent identified by any of the above methods to confirm that the modulating agent affects
MAT2A in a manner relevant to the p53 pathway. As used herein, MAT2A-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 MAT2A.

Secondary assays generally compare like populations of cells or animals (e.g., two pools of cells or animals that endogenously or recombinantly express MAT2A) in the presence and absence of the candidate modulator. In general, such assays test whether treatment of cells or animals with a candidate MAT2A-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**

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 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**

A variety of non-human animal models of normal or defective p53 pathway may be used to test candidate MAT2A 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, 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 MAT2A in Matrigel® assays. Matrigel®
is an extract of basement membrane proteins, and is composed primarily of laminin, collagen IV, and heparin sulfate proteoglycan. It is provided as a sterile liquid at 4°C, but rapidly forms a solid gel at 37°C. Liquid Matrigel® is mixed with various angiogenic agents, such as bFGF and VEGF, or with human tumor cells which over-express the MAT2A. 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 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 MAT2A is assessed via tumorigenicity assays. Tumor xenograft assays are known in the art (see, e.g., Ogawa K et al., 2000, Oncogene 19:6043-6052). Xenografts are typically implanted SC into female athymic mice, 6-7 week old, as single cell suspensions either from a pre-existing tumor or from in vitro culture. The tumors which express the MAT2A endogenously are injected in the flank, 1 x 10^5 to 1 x 10^7 cells per mouse in a volume of 100 µL using a 27 gauge needle. Mice are then ear tagged and tumors are measured twice weekly. Candidate modulator treatment is initiated on the day the mean tumor weight reaches 100 mg. Candidate modulator is delivered IV, SC, IP, or PO by bolus administration. Depending upon the pharmacokinetics of each unique candidate modulator, dosing can be performed multiple times per day. The tumor weight is assessed by measuring perpendicular diameters with a caliper and calculated by multiplying the measurements of diameters in two dimensions. At the end of the experiment, the excised tumors maybe utilized for biomarker identification or further analyses. For immunohistochemistry staining, xenograft tumors are fixed in 4% paraformaldehyde, 0.1M phosphate, pH 7.2, for 6 hours at 4°C, immersed in 30% sucrose in PBS, and rapidly frozen in isopentane cooled with liquid nitrogen.

In another preferred embodiment, tumorogenticity 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. Tumorogenicity and modulator efficacy may be evaluated by assaying the quantity of viable cells present in the macrocapsule, which can be determined by tests known in the art, for example, MTT dye conversion assay, neutral red dye uptake, trypan blue staining, viable cell counts, the number of colonies formed in soft agar, the capacity of the cells to recover and replicate in vitro, etc.

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

**Diagnostic and therapeutic uses**

Specific MAT2A-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 MAT2A 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 a MAT2A-modulating agent that modulates the p53 pathway. The invention further provides methods for modulating MAT2A function in a cell, preferably a cell pre-determined to have defective or impaired MAT2A function, by administering a MAT2A-modulating agent. Additionally, the invention provides a method for treating disorders or disease associated with impaired MAT2A function by administering a therapeutically effective amount of a MAT2A-modulating agent.

The discovery that MAT2A 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 MAT2A 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 a MAT2A, are identified as amenable to treatment with a MAT2A modulating agent. In a preferred application, the p53 defective tissue overexpresses a MAT2A 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 MAT2A cDNA sequences as probes, can determine whether particular tumors express or overexpress MAT2A. Alternatively, the TaqMan® is used for quantitative RT-PCR analysis of MAT2A 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 MAT2A oligonucleotides, and antibodies directed against a MAT2A, as described above for: (1) the detection of the presence of MAT2A gene mutations, or the detection of either over- or under-expression of MAT2A mRNA relative to the non-
disorder state; (2) the detection of either an over- or an under-abundance of MAT2A gene product relative to the non-disorder state; and (3) the detection of perturbations or abnormalities in the signal transduction pathway mediated by MAT2A.

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 MAT2A expression, the method comprising: a) obtaining a biological sample from the patient; b) contacting the sample with a probe for MAT2A 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. The probe may be either DNA or protein, including an antibody.

EXAMPLES

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

15

I. Drosophila p53 screen

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 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 used to identify the modifier genes. Modifiers of the wing phenotype were identified as members of the p53 pathway. CG2674 was an enhancer of the wing phenotype.

Orthologs of the modifiers are referred to herein as MAT2A.

BLAST analysis (Altschul et al., supra) was employed to identify orthologs of Drosophila modifiers.

Various domains, signals, and functional subunits in proteins were analyzed using the PSORT (Nakai K., and Horton P., Trends Biochem Sci, 1999, 24:34-6; Kenta Nakai,

II. High-Throughput In Vitro Fluorescence Polarization Assay

Fluorescently-labeled MAT2A 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 MAT2A activity.

III. High-Throughput In Vitro Binding Assay

$^{33}$P-labeled MAT2A peptide is added in an assay buffer (100 mM KCl, 20 mM HEPES pH 7.6, 1 mM MgCl$_2$, 1% glycerol, 0.5% NP-40, 50 mM beta-mercaptoethanol, 1 mg/ml BSA, cocktail of protease inhibitors) along with a test agent to the wells of a Neutralite-avidin coated assay plate and incubated at 25°C for 1 hour. Biotinylated substrate is then added to each well and incubated for 1 hour. Reactions are stopped by washing with PBS, and counted in a scintillation counter. Test agents that cause a difference in activity relative to control without test agent are identified as candidate p53 modulating agents.

IV. Immunoprecipitations and Immunoblotting

For coprecipitation of transfected proteins, $3 \times 10^6$ appropriate recombinant cells containing the MAT2A proteins are plated on 10-cm dishes and transfected on the following day with expression constructs. The total amount of DNA is kept constant in
each transfection by adding empty vector. After 24 h, cells are collected, washed once
with phosphate-buffered saline and lysed for 20 min on ice in 1 ml of lysis buffer
containing 50 mM Hepes, pH 7.9, 250 mM NaCl, 20 mM -glycerophosphate, 1 mM
sodium orthovanadate, 5 mM p-nitrophenyl phosphate, 2 mM dithiothreitol, protease
inhibitors (complete, Roche Molecular Biochemicals), and 1% Nonidet P-40. Cellular
debris is removed by centrifugation twice at 15,000 × g for 15 min. The cell lysate is
incubated with 25 μl of M2 beads (Sigma) for 2 h at 4 °C with gentle rocking.

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

V. Expression analysis

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

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

RNA is extracted from each tissue sample using Qiagen (Valencia, CA) RNeasy
kits, following manufacturer’s protocols, to a final concentration of 50ng/μl. Single
stranded cDNA is 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).

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

TaqMan® reactions are carried out following manufacturer’s protocols, in 25 μl
total volume for 96-well plates and 10 μl total volume for 384-well plates, using 300nM
primer and 250 nM probe, and approximately 25ng of cDNA. The standard curve for
result analysis is 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 are normalized using 18S rRNA (universally expressed in all tissues and cells).

For each expression analysis, tumor tissue samples are compared with matched normal tissues from the same patient. A gene is considered overexpressed in a tumor when the level of expression of the gene is 2 fold or higher in the tumor compared with its matched normal sample. In cases where normal tissue is not available, a universal pool of cDNA samples is used instead. In these cases, a gene is 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 is greater than 2 times the standard deviation of all normal samples (i.e., Tumor – average(all normal samples) ≥ 2 x STDEV(all normal samples)).

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.
WHAT IS CLAIMED IS:

1. A method of identifying a candidate p53 pathway modulating agent, said method comprising the steps of:
   (a) providing an assay system comprising a MAT2A polypeptide or nucleic acid;
   (b) contacting the assay system with a test agent under conditions whereby, but for the presence of the test agent, the system provides a reference activity; and
   (c) detecting a test agent-biased activity of the assay system, wherein a difference between the test agent-biased activity and the reference activity identifies the test agent as a candidate p53 pathway modulating agent.

2. The method of Claim 1 wherein the assay system comprises cultured cells that express the MAT2A polypeptide.

3. The method of Claim 2 wherein the cultured cells additionally have defective p53 function.

4. The method of Claim 1 wherein the assay system includes a screening assay comprising a MAT2A polypeptide, and the candidate test agent is a small molecule modulator.

5. The method of Claim 4 wherein the assay is a binding assay.

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

7. The method of Claim 1 wherein the assay system includes a binding assay comprising a MAT2A polypeptide and the candidate test agent is an antibody.

8. The method of Claim 1 wherein the assay system includes an expression assay comprising a MAT2A nucleic acid and the candidate test agent is a nucleic acid modulator.
9. The method of claim 8 wherein the nucleic acid modulator is an antisense oligomer.

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

11. The method of Claim 1 additionally comprising:
   (d) administering the candidate 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.

12. The method of Claim 11 wherein the model system is a mouse model with defective
    p53 function.

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 a MAT2A
    polypeptide, whereby p53 function is restored.

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

16. The method of Claim 1, comprising the additional steps of:
   (e) providing a secondary assay system comprising cultured cells or a non-human
   animal expressing MAT2A,
   (f) 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
   (g) detecting an agent-biased activity of the second assay system,
   wherein a difference between the agent-biased activity and the reference activity of
   the second assay system confirms the test agent or agent derived therefrom as a candidate
   p53 pathway modulating agent,
   and wherein the second assay detects an agent-biased change in the p53 pathway.
17. The method of Claim 16 wherein the secondary assay system comprises cultured cells.

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


20. A method of modulating p53 pathway in a mammalian cell comprising contacting the cell with an agent that specifically binds a MAT2A polypeptide or nucleic acid.

21. The method of Claim 20 wherein the agent is administered to a mammalian animal predetermined to have a pathology associated with the p53 pathway.

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

23. A method for diagnosing a disease in a patient comprising:
   (a) obtaining a biological sample from the patient;
   (b) contacting the sample with a probe for MAT2A expression;
   (c) comparing results from step (b) with a control;
   (d) determining whether step (c) indicates a likelihood of disease.

24. The method of Claim 23 wherein said disease is cancer.
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Gly Trp Gly Ala His Gly Gly Gly Ala Phe Ser Gly Lys Asp Tyr Thr
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