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(54) Title: COMPOSITIONS AND METHODS RELATED TO OBSTRUCTIVE SLEEP APNEA

(57) Abstract: The technology concerns methods and compositions for diagnosing and treating obstructive sleep apnea, a common condition observed in children. In certain embodiments, there are methods and compositions relating to inhibitors of phosphoserine phosphatase. In particular embodiments, an inhibitor of phosphoserine phosphatase is an siRNA against the phosphoserine phosphatase transcript.



WO 2010/051532 A1

DESCRIPTION

COMPOSITIONS AND METHODS RELATED TO OBSTRUCTIVE SLEEP APNEA

BACKGROUND OF THE INVENTION

[0001] This application claims priority to United States Provisional Patent Application Serial No. 61/110,113, filed on October 31, 2008, which is hereby incorporated by reference in its entirety.

[0002] This invention was made with U.S. Government support under grant number K08HL74223 awarded by the National Institutes of Health. The U.S. Government has certain rights in this invention.

I. FIELD OF THE INVENTION

[0003] The present invention relates generally to the field of obstructive sleep apnea. More particularly, it concerns the methods and compositions for diagnosing and treating obstructive sleep apnea, including the use of inhibitors of phosphatase. In certain embodiments, the phosphatase is a phosphoserine phosphatase.

II. BACKGROUND

[0004] Obstructive sleep apnea (OSA) is a prevalent disorder affecting up to 2-3% of children. It imposes substantial neurocognitive, behavioral, metabolic, and cardiovascular morbidities (Lumeng and Chervin, 2008; Capdevila *et al.*, 2008). This condition is characterized by repeated events of partial or complete obstruction of the upper airways during sleep, leading to recurring episodes of hypercapnia, hypoxemia, and arousal throughout the night (Muzumdar and Arens, 2008). Pediatric sleep apnea is a common disorder primarily caused by enlarged tonsils and adenoids impinging upon the patency of the upper airway during sleep. Mechanisms leading to the proliferation and enlargement of the tonsils and adenoids in children who subsequently develop obstructive sleep apnea remain unknown. Adenotonsillar hypertrophy is the major pathophysiological contributor to OSA in children (Arens *et al.*, 2003; Katz and D'Ambrosio, 2008). However, the mechanisms underlying the regulation of benign follicular lymphoid proliferation, hypertrophy, and hyperplasia are poorly understood, severely limiting the prediction of children who are at risk for developing adenotonsillar enlargement and OSA. Several epidemiological studies

have demonstrated that factors such as environmental smoking, allergies, and intercurrent respiratory infections are associated with either transient or persistent hypertrophy of lymphadenoid tissue in the upper airways of snoring children (Kaditis *et al.*, 2004; Teculescu *et al.*, 1992; Ersu *et al.*, 2004). Interestingly, all of these risk factors involve the generation of an inflammatory response, suggesting that the latter may promote the onset and maintenance of proliferative signals to lymphadenoid tissues.

[0005] Furthermore, the usual treatment for pediatric sleep apnea, *i.e.*, tonsillectomy and adenoidectomy, is costly, and fraught with measurable adverse consequences ranging from mild events such as pain to serious complications such as hemorrhage, infections, acute respiratory insufficiency, and potentially death (Ramsden, National Prospective Tonsillectomy Audit, 2005). In the vast majority of children diagnosed with OSA, the initial treatment approach is the surgical removal of enlarged tonsils and adenoids. Although 50-115 of every 10,000 children undergo tonsillectomy and adenoidectomy (Van Den Akker, 2004), the overall efficacy of this procedure has not been firmly established, with recent data suggesting that the success rate is significantly less than previously reported (Tauman *et al.*, 2006). Furthermore, this procedure is associated with pain, other morbidities, and substantial healthcare costs. Therefore, novel, nonsurgical therapeutic strategies are needed.

SUMMARY OF THE INVENTION

[0006] Information is provided that shows altered expression of certain genes related to proliferation of tonsillar tissue in the upper airway of children with obstructive sleep apnea (OSA). Embodiments concern compositions and methods that provide therapeutic and diagnostic applications for addressing OSA.

[0007] In some embodiments, there are methods for inhibiting adenoid or tonsillar cells comprising contacting the cells with an effective amount of a composition comprising an inhibitor of adenoid and/or tonsillar cells. In certain embodiments, adenoid or tonsillar cells are inhibited by inhibiting their proliferation, altering their phenotype or activity, and/or inducing their destruction. The term “adenotonsillar cells” refers to a mixture of cells comprising adenoid and tonsillar cells. It is contemplated that any embodiment described herein may be applied specifically with respect to only adenoid cells, only tonsillar cells, or a combination of

both cells. In some embodiments, the proliferation of adenoid and/or tonsillar cells is inhibited. In some cases, proliferation is inhibited by inducing apoptosis in these cells. Embodiments also include methods for treating obstructive sleep apnea in a patient. In some embodiments, methods involve administering to the patient an effective amount of a composition containing an inhibitor of adenoid and/or tonsillar cells. In certain embodiments, the inhibitor is a phosphatase inhibitor. In further embodiments, the inhibitor is a phosphoserine phosphatase inhibitor. It is contemplated that the patient may be suspected of having obstructive sleep apnea or that the patient has been diagnosed as having obstructive sleep apnea. The diagnosis may be based on results from one or more tests, reported symptoms or other observations from or about the patient, or from a patient history. In certain embodiments, one or more tests may be ordered to provide information about whether the patient has obstructive sleep apnea. In some cases, a test may provide information about whether the patient has apnea or whether adenotonsillar cells are hypertrophic. Based on this information, a diagnosis of obstructive sleep apnea may or may not be made. If a patient is diagnosed with obstructive sleep apnea or is suspected of having sleep apnea, they may be treated for the condition/disease.

[0008] In further embodiments, the adenoid or tonsillar cells are hypertrophic, meaning the cells are larger in size or greater in number than is seen with normal cells. Hypertrophic adenotonsillar cells are observed in patients with obstructive sleep apnea (OSA), particularly in pediatric patients (*i.e.*, patients who are under the age of 18). In certain embodiments, methods are applied specifically to pediatric patients. It is contemplated that subjects or patients may be mammals, including specifically humans.

[0009] Embodiments of the invention involve an inhibitor of adenoid or tonsillar cells, such as an inhibitor that induces apoptosis. The inhibitor may be a small molecule, a nucleic acid, a peptide or polypeptide, or a combination thereof. In certain cases, the inhibitor is a phosphatase inhibitor. In further embodiments, the phosphatase inhibitor is a phosphoserine phosphatase inhibitor.

[0010] In certain embodiments, the inhibitor is a nucleic acid. In specific cases, the nucleic acid is an siRNA. It is contemplated that compositions may contain multiple and different siRNAs against a single target transcript or multiple targets. In some embodiments, the siRNA is directed against a phosphatase; in some

embodiments, the phosphatase is a phosphoserine phosphatase. Methods and compositions may involve a composition containing a plurality of different siRNAs against one or more phosphatases. In some embodiments, an siRNA or plurality of siRNAs is directed against a phosphoserine phosphatase. It is contemplated that the inhibitors may be against only a phosphoserine phosphatase, such as encoded by PSPH, in some embodiments. In human patients or subjects, the gene that is targeted is a human sequence for that target. If compositions or methods are applied in a different animal subject, it is contemplated that the corresponding gene in that subject is targeted.

[0011] In some embodiments, an inhibitor is a small molecule. It is contemplated that a composition may contain different inhibitors. In some embodiments, the small molecule is okadaic acid, PPI2, and/or calyculin A.

[0012] It is also contemplated that in some embodiments, a modified inhibitor is employed. In certain embodiments, there is a modified nucleic acid. Inhibitors, such as nucleic acids, may be modified to alter stability, efficacy, availability, shelf-life, solubility, and ability to be formulated for a particular composition and/or route of administration.

[0013] In therapeutic methods, the composition may be administered one time or multiple times. It is contemplated that it may be formulated accordingly. In some embodiments, the composition is administered daily (in a single dose or multiple doses). In some embodiments, the composition is formulated for oral, topical or mucosal administration. In certain embodiments, the composition is a liquid, strip, lozenge, or lollipop. It may also be a mouthwash or spray.

[0014] Other embodiments include methods of diagnosing obstructive sleep apnea, or a risk thereof, in a subject. In some embodiments, methods involve diagnosing a subject with obstructive sleep apnea based on the results of a test indicating a measurable difference in the expression level of at least one biomarker in a biological sample from the subject, wherein the at least one biomarker is a product of a gene selected from Table 1. In further embodiments, at least one biomarker is an mRNA. In other embodiments, at least one biomarker is a polypeptide of interest. It is contemplated that multiple biomarkers may be evaluated, analyzed, measured, and/or determined. It is contemplated that in some embodiments a biological sample includes

lymphadenoid tissue and/or adenoid or tonsillar cells. In some circumstances, lymphadenoid tissue is selected from the group consisting of tonsillar tissue and adenoid tissue.

[0015] In some embodiments, method may involve ordering a test that provides information about a subject's having sleep apnea prior to diagnosing the subject. In specific embodiments, the test is a method involving biomarkers for sleep apnea.

[0016] Other embodiments concern methods of screening a candidate therapeutic for obstructive sleep apnea comprising: a) contacting hypertrophic adenoid and/or tonsillar cells with a candidate compound, wherein the candidate compound is an inhibitor of biomarker that is a product of a gene selected from Table 1, and b) determining a change on the phenotype or activity of the cells based on the contact with the candidate compound, wherein the change identifies the candidate compound as a potential therapeutic for obstructive sleep apnea. A potential therapeutic can be subsequently tested further using adenotonsillar cells, particularly hypertrophic cells. A potential therapeutic that reduces hypertrophy or the number of hypertrophic cells may be further tested in an *in vivo* system or animal.

[0017] The embodiments in the Example section are understood to be embodiments of the invention that are applicable to all aspects of the invention, including compositions and methods.

[0018] The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or." It is also contemplated that anything listed using the term "or" may also be specifically excluded.

[0019] Throughout this application, the term "about" is used to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value.

[0020] Following long-standing patent law, the words "a" and "an," when used in conjunction with the word "comprising" in the claims or specification, denotes one or more, unless specifically noted.

[0021] As used in this specification and claim(s), the words “comprising” (and any form of comprising, such as “comprise” and “comprises”), “having” (and any form of having, such as “have” and “has”), “including” (and any form of including, such as “includes” and “include”) or “containing” (and any form of containing, such as “contains” and “contain”) are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

[0022] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

DESCRIPTION OF THE DRAWINGS

[0023] So that the matter in which the above-recited features, advantages and objects of the invention as well as others which will become clear are attained and can be understood in detail, more particular descriptions and certain embodiments of the invention briefly summarized above are illustrated in the appended drawings. These drawings form a part of the specification. It is to be noted, however, that the appended drawings illustrate certain embodiments of the invention and therefore are not to be considered limiting in their scope.

[0024] **FIG. 1.** Proliferative rates in tonsil cell cultures from children with OSA following administration of okadaic acid, calyculin A, and PPI2 at increasing concentrations. In RI-derived tonsil cell cultures, no significant differences emerged for any of the compounds.

[0025] **FIG. 2.** Representative example of BrDU-based proliferation assay in tonsil cell cultures from a 6-year-old child with OSA treated with vehicle (left panel) or calyculin (right panel). Marked reductions in global cell proliferation, and in T-cell (CD+3 cells) and B-cell (CD19+ cells) proliferation are apparent.

[0026] **FIG. 3.** Representative example of Annexin V-based apoptosis assay in a 4-year old child with OSA treated with vehicle (left panel) or calyculin (right panel). Marked increases in global cell, T-cell (CD+3 cells), and B-cell (CD19+ cells) apoptosis are apparent.

[0027] **FIG. 4.** Histogram of significance scores for the OSA vs., IR samples. The relative frequency distribution and the score distribution are marked by the 95% cutoff of the score.

[0028] **FIG. 5.** Dose-dependent transfection efficiency assessed by GFP reporter in mixed cell cultures of tonsils. The negative GFP (-) scrambled control shRNA was used.

[0029] **FIG. 6.** SiRNA or shRNA transfection markedly reduced proliferative rates of tonsil mixed cell cultures (left panel), and increased apoptosis (right panel). Mean $\pm$ SD shown for n=7 experiments (4 with siRNA and 3 with shRNA)

DETAILED DESCRIPTION OF THE INVENTION

I. OBSTRUCTIVE SLEEP APNEA

[0030] A person with obstructive sleep apnea (OSA) will stop breathing periodically for a short time (typically less than 60 seconds) while sleeping; it is associated with an airway that may be blocked, which prevents air from reaching the lungs. While there are a number of factors that increase the risk of this having this condition, in children having large tonsils and adenoids is a factor. The diagnosis of this condition currently involves a physical exam and a survey about the patient's sleepiness, quality of sleep and bedtime habits. If a child is involved, questions will be posed to a parent or caregiver. A sleep study may be requested and performed to further evaluate for the presence of the condition. Other tests that may be performed include evaluation of arterial blood gases, electrocardiogram (ECG), echocardiogram, and/or thyroid function studies.

[0031] The inventors have previously demonstrated the effectiveness of nonsurgical approaches for treatment of mild sleep disordered breathing in children, including the use of anti-inflammatory agents such as oral leukotriene modifiers (Goldbart *et al.*, 2005) and intranasal steroids (Kheirandish *et al.*, 2006). Furthermore, we recently utilized adenotonsillar cell culture assays to confirm that these pharmacologic interventions promote an anti-proliferative phenotype (Kheirandish-Gozal *et al.*, 2009; Dayyat *et al.*, 2009).

[0032] As described herein, a genome-wide expression analysis was integrated with novel bioinformatic methods to identify putative functional networks involved in the proliferation of tonsillar tissue in the upper airway of children with

OSA. These analyses were conducted on tonsils from children with polysomnographically demonstrated OSA and from age-, gender-, and ethnicity-matched children without OSA who underwent tonsillectomy for recurrent tonsillar infections. A computational framework was developed to systematically narrow down differentially expressed genes to a restricted set derived from a network of proliferative pathways. To further validate this approach, the effect of antagonists directed against one of the network-associated candidate genes, phosphoserine phosphatase (PSPH), was assessed in a mixed tonsil cell culture system. This confirmed the pro-proliferative role played by PSPH in tonsillar tissue from children with OSA, but not in those derived from children with recurrent infections.

II. DIAGNOSTIC AND THERAPEUTIC METHODS

[0033] In some embodiments there are therapeutic methods, and in other embodiments there are diagnostic methods related to obstructive sleep apnea.

[0034] Diagnostic methods are based on the identification of biomarkers in a sample from a subject. A “biomarker” is a molecule useful as an indicator of a biologic state in a subject. With reference to the present subject matter, the OSA biomarkers disclosed herein can be polypeptides that exhibit a change in expression or state, which can be correlated with the risk of developing, the presence of, or the progression of OSA in a subject. The OSA biomarkers are contemplated to constitute the markers identified on Table 1. In certain embodiments, specific biomarkers on Table 1 are contemplated. In certain embodiments, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 of the biomarkers on Table 1, or a range derivable therein, may be employed in embodiments described herein. In addition, the biomarkers disclosed herein can include messenger RNAs (mRNAs) encoding the biomarker polypeptides, as measurement of a change in expression of an mRNA can be correlated with changes in expression of the polypeptide encoded by the mRNA. Changes in expression may be an increase (up-regulation) in expression in OSA cells or a decrease (down-regulation) in expression in OSA cells compared to the control cells. Whether a particular biomarker is increased or decreased is shown in Table 1. In some embodiments, that change of expression, within a range of As such, determining an expression level of a gene of interest in a biological sample is inclusive of determining an amount of a polypeptide biomarker and/or an amount of an mRNA

encoding the polypeptide biomarker either by direct or indirect (*e.g.*, by measure of a complementary DNA (cDNA) synthesized from the mRNA) measure of the mRNA.

[0035] Therapeutic methods concern the use of an inhibitor against a biomarker that is upregulated in OSA compared to non-OSA patients. In certain embodiments, the inhibitor reduces cell growth, proliferation, size, or number of adenoid and/or tonsillar cells. In certain embodiments, the inhibitor targets a phosphatase, which means it directly or indirectly inhibits the function, activity, expression level (transcript or protein) of phosphatase in adenoid and/or tonsillar cells. A direct inhibitor is one that acts on a phosphatase gene or its gene products to inhibit its activity or function. An indirect inhibitor is one that acts on a different entity but that has an inhibitory effect on the activity or function of the phosphatase gene or its gene products (transcript or protein product). In some embodiments, the phosphatase is a phosphoserine phosphatase.

[0036] Phosphoserine phosphatase belongs to a subfamily of phosphotransferases and catalyzes the rate-limiting step in serine biosynthesis by converting L-phosphoserine to L-serine (Cohen, 1989). The only reported case of PSPH deficiency in a child described pre- and postnatal growth retardation, moderate psychomotor retardation, facial abnormalities and reduced PSPH activity in lymphoblasts and fibroblasts (Jaeken *et al.*, 1997). Treatment with oral serine led to normalization of serine levels and some improvement in head growth. In mice, PSPH is abundantly expressed in proliferating embryonic and hematopoietic stem cells, and in neural progenitors in the developing brain (Geschwind *et al.*, 2001; Nakano *et al.*, 2007). Furthermore, siRNA knockdown of PSPH expression inhibited neural stem cell proliferation (Nakano *et al.*, 2007) suggesting that this enzyme can be selectively targeted to affect cellular proliferation. To our knowledge, a role for PSPH in promoting tonsillar tissue hypertrophy has not been previously reported. The inventors used several strategies to target PSPH expression and activity in adenotonsillar primary cell cultures, including pharmacological and siRNA inhibition, resulting in significant anti-proliferative effects specifically in cell cultures derived from children with OSA. Furthermore, inhibition of PSPH appears to promote programmed cell death in tonsillar cell cultures. Together, these observations suggest that PSPH is a logical therapeutic target in reversing the adenotonsillar enlargement of pediatric OSA.

[0037] Dual specificity phosphatases have also been extensively investigated as critical modulators of lymphocyte function, proliferation, and apoptosis. Indeed, immune cell functions are intimately associated with the enzymatically catalyzed addition of phosphate to key tyrosine, threonine and serine residues in proteins (Tanzola and Kersh, 2006; Liu, 2009; Won and Lee, 2008). While the specific role of DUSP1 in tonsillar proliferation remains to be established in the context of pediatric OSA, the preliminary experiments using siRNA approaches would also support a regulatory role of this group of phosphatases in the regulation of proliferation and apoptosis among critical lymphocyte populations within tonsillar tissues.

A. Nucleic Acids

[0038] Embodiments concern polynucleotides or nucleic acid molecules relating to an OSA biomarker nucleic acid sequence in diagnostic, therapeutic, and preventative applications. Certain embodiments specifically concern a nucleic acid that is targeted for inhibition for the prevention or treatment of OSA. In other embodiments, the present invention concerns a nucleic acid that can be used to diagnose OSA based on the detection of an OSA biomarker. Nucleic acids or polynucleotides may be DNA or RNA, and they may be oligonucleotides (100 residues or fewer) in certain embodiments. Moreover, they may be recombinantly produced or synthetically produced.

[0039] Other embodiments concern the use of primers or hybridizable segments that may be used to identify and/or quantify OSA biomarkers, particularly in diagnostic methods. It is contemplated that the discussion below is relevant to embodiments concerning such methods and compositions related to diagnostic applications in the context of the OSA biomarkers.

[0040] These polynucleotides or nucleic acid molecules may be isolatable and purifiable from cells or they may be synthetically produced. In some embodiments, a nucleic acid targets or identifies an OSA biomarker. In other embodiments, a nucleic acid is an inhibitor, such as a ribozyme, siRNA, or shRNA that reduces the level of phosphatase expression.

[0041] As used in this application, the term “polynucleotide” refers to a nucleic acid molecule, RNA or DNA, that has been isolated free of total genomic nucleic acid. Therefore, a “polynucleotide encoding an OSA biomarker” refers to a

nucleic acid sequence (RNA or DNA) that contains an OSA biomarker coding sequences, yet may be isolated away from, or purified and free of, total genomic DNA and proteins. An OSA biomarker inhibitor refers to an inhibitor of an OSA biomarker.

[0042] The term “cDNA” is intended to refer to DNA prepared using RNA as a template. The advantage of using a cDNA, as opposed to genomic DNA or an RNA transcript is stability and the ability to manipulate the sequence using recombinant DNA technology (*See* Sambrook, 2001; Ausubel, 1996). There may be times when the full or partial genomic sequence is some. Alternatively, cDNAs may be advantageous because it represents coding regions of a polypeptide and eliminates introns and other regulatory regions. In certain embodiments, nucleic acids are complementary or identical to all or part of cDNA encoding sequences, such as a phosphoserine phosphatase sequence.

[0043] The term “gene” is used for simplicity to refer to a functional protein, polypeptide, or peptide-encoding nucleic acid unit. As will be understood by those in the art, this functional term includes genomic sequences, cDNA sequences, and smaller engineered gene segments that express, or may be adapted to express, proteins, polypeptides, domains, peptides, fusion proteins, and mutants. The nucleic acid molecule hybridizing to all or part of a phosphatase nucleic acid sequence may comprise a contiguous nucleic acid sequence of the following lengths or at least the following lengths: 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 441, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830,

840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1010, 1020, 1030, 1040, 1050, 1060, 1070, 1080, 1090, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 4000, 4100, 4200, 4300, 4400, 4500, 4600, 4700, 4800, 4900, 5000, 5100, 5200, 5300, 5400, 5500, 5600, 5700, 5800, 5900, 6000, 6100, 6200, 6300, 6400, 6500, 6600, 6700, 6800, 6900, 7000, 7100, 7200, 7300, 7400, 7500, 7600, 7700, 7800, 7900, 8000, 8100, 8200, 8300, 8400, 8500, 8600, 8700, 8800, 8900, 9000, 9100, 9200, 9300, 9400, 9500, 9600, 9700, 9800, 9900, 10000, 10100, 10200, 10300, 10400, 10500, 10600, 10700, 10800, 10900, 11000, 11100, 11200, 11300, 11400, 11500, 11600, 11700, 11800, 11900, 12000 or more (or any range derivable therein) nucleotides, nucleosides, or base pairs of a phosphatase sequence. In specific embodiments, the sequence concerns a phosphoserine phosphatase. The GenBank Accession number for the human phosphoserine phosphatase (PSPH) sequence (nucleic acid and protein) is NM_004577, which is hereby incorporated by reference. The transcript sequence is provided in SEQ ID NO:1 and the protein sequence is provided in SEQ ID NO:2. Nucleic acid molecules used in embodiments described herein may be identical or complementary to SEQ ID NO:1, as set forth in the previous paragraph.

[0044] Accordingly, sequences that have or have at least or at most 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, and any range derivable therein, of nucleic acids that are identical or complementary to a nucleic acid sequence of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190,

191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 441, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1010, 1020, 1030, 1040, 1050, 1060, 1070, 1080, 1090, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 4000, 4100, 4200, 4300, 4400, 4500, 4600, 4700, 4800, 4900, or 5000 contiguous bases (or any range derivable therein) of SEQ ID NO:1 or SEQ ID NOs:3-9 are contemplated as part of the invention. They may be used as PBEF inhibitors or as detection probes or primers for methods of the invention.

[0045] “Isolated substantially away from other coding sequences” means that the gene of interest forms part of the coding region of the nucleic acid segment, and that the segment does not contain large portions of naturally-occurring coding nucleic acid, such as large chromosomal fragments or other functional genes or cDNA coding regions. Of course, this refers to the nucleic acid segment as originally isolated, and does not exclude genes or coding regions later added to the segment by human manipulation.

1. Antisense Sequences, Including siRNAs and shRNAs

[0046] Particular embodiments concern isolated nucleic acid segments and recombinant vectors incorporating DNA sequences that encode phosphoserine phosphatase inhibitors, such as phosphoserine phosphatase siRNAs, ribozymes and phosphoserine phosphatase antibodies and other phosphoserine phosphatase binding proteins or proteins that inhibit expression of phosphoserine phosphatase transcripts.

[0047] In some embodiments, a nucleic acid may encode an antisense construct. Antisense methodology takes advantage of the fact that nucleic acids tend to pair with “complementary sequences.” By complementary, it is meant that polynucleotides are those which are capable of base-pairing according to the standard Watson-Crick complementarity rules. Inclusion of less common bases such as inosine, 5-methylcytosine, 6-methyladenine, hypoxanthine and others in hybridizing sequences does not interfere with pairing.

[0048] Antisense polynucleotides, when introduced into a target cell, specifically bind to their target polynucleotide and interfere with transcription, RNA processing, transport, translation and/or stability. Antisense RNA constructs, or DNA encoding such antisense RNA's, may be employed to inhibit gene transcription or translation or both within a host cell, either *in vitro* or *in vivo*, such as within a host animal, including a human subject.

[0049] Antisense constructs may be designed to bind to the promoter and other control regions, exons, introns or even exon-intron boundaries of a gene. It is contemplated that the most effective antisense constructs will include regions complementary to intron/exon splice junctions. Thus, it is proposed that a preferred embodiment includes an antisense construct with complementarity to regions within 50-200 bases of an intron-exon splice junction. It has been observed that some exon sequences can be included in the construct without seriously affecting the target selectivity thereof. The amount of exonic material included will vary depending on the particular exon and intron sequences used. One can readily test whether too much exon DNA is included simply by testing the constructs *in vitro* to determine whether normal cellular function is affected or whether the expression of related genes having complementary sequences is affected.

[0050] As stated above, "complementary" or "antisense" means polynucleotide sequences that are substantially complementary over their entire length and have very few base mismatches. For example, sequences of fifteen bases in length may be termed complementary when they have complementary nucleotides at thirteen or fourteen positions. Naturally, sequences which are completely complementary will be sequences which are entirely complementary throughout their entire length and have no base mismatches. Other sequences with lower degrees of homology also are contemplated. For example, an antisense construct which has limited regions of high homology, but also contains a non-homologous region (*e.g.*, ribozyme; see below) could be designed. These molecules, though having less than 50% homology, would bind to target sequences under appropriate conditions.

[0051] It may be advantageous to combine portions of genomic DNA with cDNA or synthetic sequences to generate specific constructs. For example, where an intron is desired in the ultimate construct, a genomic clone will need to be used. The cDNA or a synthesized polynucleotide may provide more convenient restriction sites

for the remaining portion of the construct and, therefore, would be used for the rest of the sequence.

[0052] In certain embodiments, the nucleic acid encodes an interfering RNA or siRNA. RNA interference (also referred to as "RNA-mediated interference" or RNAi) is a mechanism by which gene expression can be reduced or eliminated. Double-stranded RNA (dsRNA) has been observed to mediate the reduction, which is a multi-step process. dsRNA activates post-transcriptional gene expression surveillance mechanisms that appear to function to defend cells from virus infection and transposon activity (Fire *et al.*, 1998; Grishok *et al.*, 2000; Ketting *et al.*, 1999; Lin and Avery, 1999; Montgomery *et al.*, 1998; Sharp and Zamore, 2000; Tabara *et al.*, 1999). Activation of these mechanisms targets mature, dsRNA-complementary mRNA for destruction. Advantages of RNAi include a very high specificity, ease of movement across cell membranes, and prolonged down-regulation of the targeted gene (Fire *et al.*, 1998; Grishok *et al.*, 2000; Ketting *et al.*, 1999; Lin and Avery *et al.*, 1999; Montgomery *et al.*, 1998; Sharp *et al.*, 1999; Sharp and Zamore, 2000; Tabara *et al.*, 1999). Moreover, dsRNA has been shown to silence genes in a wide range of systems, including plants, protozoans, fungi, *C. elegans*, *Trypanosoma*, *Drosophila*, and mammals (Grishok *et al.*, 2000; Sharp *et al.*, 1999; Sharp and Zamore, 2000; Elbashir *et al.*, 2001). It is generally accepted that RNAi acts post-transcriptionally, targeting RNA transcripts for degradation. It appears that both nuclear and cytoplasmic RNA can be targeted (Bosher and Labouesse, 2000).

[0053] siRNAs are designed so that they are specific and effective in suppressing the expression of the genes of interest. Methods of selecting the target sequences, *i.e.*, those sequences present in the gene or genes of interest to which the siRNAs will guide the degradative machinery, are directed to avoiding sequences that may interfere with the siRNA's guide function while including sequences that are specific to the gene or genes. Typically, siRNA target sequences of about 21 to 23 nucleotides in length are most effective. This length reflects the lengths of digestion products resulting from the processing of much longer RNAs as described above (Montgomery *et al.*, 1998).

[0054] The making of siRNAs has been mainly through direct chemical synthesis; or through an *in vitro* system derived from S2 cells. Chemical synthesis proceeds by making two single stranded RNA-oligomers followed by the annealing of

the two single stranded oligomers into a double-stranded RNA. Methods of chemical synthesis are diverse. Non-limiting examples are provided in U.S. Patents 5,889,136, 4,415,723, and 4,458,066, expressly incorporated herein by reference, and in Wincott *et al.* (1995).

[0055] Several further modifications to siRNA sequences have been suggested in order to alter their stability or improve their effectiveness. It is suggested that synthetic complementary 21-mer RNAs having di-nucleotide overhangs (*i.e.*, 19 complementary nucleotides + 3' non-complementary dimers) may provide the greatest level of suppression. These protocols primarily use a sequence of two (2'-deoxy) thymidine nucleotides as the di-nucleotide overhangs. These dinucleotide overhangs are often written as dTdT to distinguish them from the typical nucleotides incorporated into RNA. The literature has indicated that the use of dT overhangs is primarily motivated by the need to reduce the cost of the chemically synthesized RNAs. It is also suggested that the dTdT overhangs might be more stable than UU overhangs, though the data available shows only a slight (< 20%) improvement of the dTdT overhang compared to an siRNA with a UU overhang.

[0056] In some embodiments, the invention concerns an siRNA that is capable of triggering RNA interference, a process by which a particular RNA sequence is destroyed. siRNA are dsRNA molecules that are 100 bases or fewer in length (or have 100 basepairs or fewer in its complementarity region). In some cases, it has a 2 nucleotide 3' overhang and a 5' phosphate. The particular RNA sequence is targeted as a result of the complementarity between the dsRNA and the particular RNA sequence. It will be understood that dsRNA or siRNA of the invention can effect at least a 20, 30, 40, 50, 60, 70, 80, 90 percent or more reduction of expression of a targeted RNA in a cell. dsRNA of the invention (the term "dsRNA" will be understood to include "siRNA") is distinct and distinguishable from antisense and ribozyme molecules by virtue of the ability to trigger RNAi. Structurally, dsRNA molecules for RNAi differ from antisense and ribozyme molecules in that dsRNA has at least one region of complementarity within the RNA molecule. The complementary (also referred to as "complementarity") region comprises at least or at most 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73,

74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 441, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, or 1000 contiguous bases, or any range derivable therein, to sequences (or their complements) disclosed herein. In some embodiments, the sequence is SEQ ID NO:1. In other embodiments, the sequence is identical or complementary to SEQ ID NO:3-SEQ ID NO:234 (or any specific SEQ ID NO identified between the numbers of 3 and 234, inclusive). In some embodiments a phosphoserine phosphatase inhibitor is specifically modified or designed based on the sequence. Such rational design methods are described in US Patent Publication 20090182134, which is hereby incorporated by reference.

[0057] In some embodiments, long dsRNA are employed in which “long” refers to dsRNA that are 1000 bases or longer (or 1000 basepairs or longer in complementarity region). The term “dsRNA” includes “long dsRNA” and “intermediate dsRNA” unless otherwise indicated. In some embodiments of the invention, dsRNA can exclude the use of siRNA, long dsRNA, and/or “intermediate” dsRNA (lengths of 100 to 1000 bases or basepairs in complementarity region). It is specifically contemplated that a dsRNA may be a molecule comprising two separate RNA strands in which one strand has at least one region complementary to a region on the other strand. Alternatively, a dsRNA includes a molecule that is single stranded yet has at least one complementarity region as described above (see Sui *et al.*, 2002 and Brummelkamp *et al.*, 2002 in which a single strand with a hairpin loop is used as a dsRNA for RNAi). For convenience, lengths of dsRNA may be referred to in terms of bases, which simply refers to the length of a single strand or in terms of basepairs, which refers to the length of the complementarity region. It is specifically contemplated that embodiments discussed herein with respect to a dsRNA comprised of two strands are contemplated for use with respect to a dsRNA comprising a single strand, and vice versa. In a two-stranded dsRNA molecule, the strand that has a sequence that is complementary to the targeted mRNA is referred to as the “antisense strand” and the strand with a sequence identical to the targeted mRNA is referred to

as the “sense strand.” Similarly, with a dsRNA comprising only a single strand, it is contemplated that the “antisense region” has the sequence complementary to the targeted mRNA, while the “sense region” has the sequence identical to the targeted mRNA. Furthermore, it will be understood that sense and antisense region, like sense and antisense strands, are complementary (*i.e.*, can specifically hybridize) to each other.

[0058] The single RNA strand or two complementary double strands of a dsRNA molecule may be of at least or at most the following lengths: 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 441, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 31, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 4000, 4100, 4200, 4300, 4400, 4500, 4600, 4700, 4800, 4900, 5000, 6000, 7000, 8000, 9000, 10000 or more (including the full-length of a particular’s gene’s mRNA without the poly-A tail) bases or basepairs. If the dsRNA is composed of two separate strands, the two strands may be the same length or different lengths. If the dsRNA is a single strand, in addition to the complementarity region, the strand may have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 or more bases on either or both ends (5’ and/or 3’) or as forming a hairpin loop between the complementarity regions.

[0059] In some embodiments, there is a cocktail of siRNA molecules, which means there is a plurality of siRNAs having different sequences, which may target the

same transcript (at different positions) and/or different transcripts. It is contemplated that a cocktail may have, have at least, or have at most 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more (or any range derivable therein) different siRNA molecules.

[0060] In certain embodiments, siRNAs or shRNAs targeting phosphoserine phosphatase are part of methods or compositions. In these embodiments, one or more siRNA molecules are included. The siRNA molecules may be single or double stranded. As a single strand, the siRNA may include just the antisense sequence that targets the mRNA, or it may include an overlapping sense sequence that hybridizes with the siRNA antisense sequence (in a hairpin or hairpin-like conformation). The tables below provides sequence information regarding siRNAs that may be used. It is contemplated that an siRNA molecule may have, have at least, or have at most 14, 15, 16, 17, 18, 19, or 20 nucleotides (or any range derivable therein) from any of the antisense strand sequences identified below. Alternatively, an siRNA molecule may be at least 85%, 90%, 95%, or 100% identical to any antisense sequence (excluding the "tt" on the end) below.

Position in gene sequence: 208

GC content: 61.9%

Sense strand siRNA: GCACCCUCCCCCGGAAUCAtt (SEQ ID NO:3)

Antisense strand siRNA: UGAUUCCGGGGGAGGGUGCtt (SEQ ID NO:4)

Position in gene sequence: 224

GC content: 57.1%

Sense strand siRNA: UCAUGC GGUGCUGUGAGGCtt (SEQ ID NO:5)

Antisense strand siRNA: GCCUCACAGCACCGCAUGAtt (SEQ ID NO:6)

Position in gene sequence: 254

GC content: 33.3%

Sense strand siRNA: GAUGAAGAUAGAAUGCAAGtt (SEQ ID NO:7)

Antisense strand siRNA: CUUGCAUUCUAUCUUCAUCtt (SEQ ID NO:8)

Position in gene sequence: 260

GC content: 33.3%

Sense strand siRNA: GAUAGAAUGCAAGGUAGAAtt (SEQ ID NO:9)

Antisense strand siRNA: UUCUACCUUGCAUUCUAUCtt (SEQ ID NO:10)

Position in gene sequence: 267

GC content: 42.9%

Sense strand siRNA: UGCAAGGUAGAAAGUGCUGtt (SEQ ID NO:11)

Antisense strand siRNA: CAGCACUUUCUACCUUGCAtt (SEQ ID NO:12)

Position in gene sequence: 272

GC content: 42.9%

Sense strand siRNA: GGUAGAAAGUGCUGGAUACtt (SEQ ID NO:13)

Antisense strand siRNA: GUAUCCAGCACUUUCUACCtt (SEQ ID NO:14)

Position in gene sequence: 279

GC content: 38.1%

Sense strand siRNA: AGUGCUGGAUACCUUUAGAtt (SEQ ID NO:15)

Antisense strand siRNA: UCUAAAGGUAUCCAGCACUtt (SEQ ID NO:16)

Position in gene sequence: 299

GC content: 47.6%

Sense strand siRNA: AGCUGCAGGACU301GGUGtt (SEQ ID NO:17)

Antisense strand siRNA: CACC103AGUCCUGCAGCUtt (SEQ ID NO:18)

Position in gene sequence: 338

GC content: 61.9%

Sense strand siRNA: GAACCUGCCCGUCCGUAGGtt (SEQ ID NO:19)

Antisense strand siRNA: CCUACGGACGGGCAGGUUCtt (SEQ ID NO:20)

Position in gene sequence: 341

GC content: 66.7%

Sense strand siRNA: CCUGCCCGUCCGUAGGGCUtt (SEQ ID NO:21)

Antisense strand siRNA: AGCCCUACGGACGGGCAGGtt (SEQ ID NO:22)

Position in gene sequence: 405

GC content: 38.1%

Sense strand siRNA: AAUUCACCCUUGUAGAGUCtt (SEQ ID NO:23)

Antisense strand siRNA: GACUCUACAAGGGUGAAUtt (SEQ ID NO:24)

Position in gene sequence: 407

GC content: 38.1%

Sense strand siRNA: UUCACCCUUGUAGAGUCAUtt (SEQ ID NO:25)

Antisense strand siRNA: AUGACUCUACAAGGGUGAAtt (SEQ ID NO:26)

Position in gene sequence: 457

GC content: 47.6%

Sense strand siRNA: GCAGAUCAAGAGAGCGUtt (SEQ ID NO:27)

Antisense strand siRNA: ACGCUCUCUUGAGAUCUGCtt (SEQ ID NO:28)

Position in gene sequence: 468

GC content: 61.9%

Sense strand siRNA: GAGAGCGUUCGGUGGAGGCtt (SEQ ID NO:29)

Antisense strand siRNA: GCCUCCACCGAACGCUCUCtt (SEQ ID NO:30)

Position in gene sequence: 521

GC content: 71.4%

Sense strand siRNA: CUGCUCGCCCAGCGUCGGtt (SEQ ID NO:31)

Antisense strand siRNA: CCGACGCUCGGGCGAGCAGtt (SEQ ID NO:32)

Position in gene sequence: 571

GC content: 66.7%

Sense strand siRNA: GGUUGCAGCCGAGGAGCCtt (SEQ ID NO:33)

Antisense strand siRNA: GGCUCCUGCGGCUGCAACCtt (SEQ ID NO:34)

Position in gene sequence: 631

GC content: 42.9%

Sense strand siRNA: UCCACAGGUCUUUCUUGAGtt (SEQ ID NO:35)

Antisense strand siRNA: CUCAAGAAAGACCUGUGGAt (SEQ ID NO:36)

Position in gene sequence: 653

GC content: 33.3%

Sense strand siRNA: AUCUGUAGACAGAACUUUGtt (SEQ ID NO:37)

Antisense strand siRNA: CAAAGUUCUGUCUACAGAUtt (SEQ ID NO:38)

Position in gene sequence: 667

GC content: 33.3%

Sense strand siRNA: CUUUGUGCUGCGUUUUUAUtt (SEQ ID NO:39)

Antisense strand siRNA: AUAAAAACGCAGCACAAAGtt (SEQ ID NO:40)

Position in gene sequence: 697

GC content: 47.6%

Sense strand siRNA: GGAACAGAAGAGUGUCGUCtt (SEQ ID NO:41)

Antisense strand siRNA: GACGACACUCUUCUGUUCtt (SEQ ID NO:42)

Position in gene sequence: 701

GC content: 47.6%

Sense strand siRNA: CAGAAGAGUGUCGUCUCCUtt (SEQ ID NO:43)

Antisense strand siRNA: AGGAGACGACACUCUUCUGtt (SEQ ID NO:44)

Position in gene sequence: 706

GC content: 42.9%

Sense strand siRNA: GAGUGUCGUCUCCUAGAAAt (SEQ ID NO:45)

Antisense strand siRNA: UUUCUAGGAGACGACACUCtt (SEQ ID NO:46)

Position in gene sequence: 724

GC content: 42.9%

Sense strand siRNA: AUCUAGCACUGGAGAAACGtt (SEQ ID NO:47)

Antisense strand siRNA: CGUUUCUCCAGUGCUAGAUtt (SEQ ID NO:48)

Position in gene sequence: 740

GC content: 28.6%

Sense strand siRNA: ACGAGGAAAAUU721CUUCtt (SEQ ID NO:49)

Antisense strand siRNA: GAAG127AAUUUCCUCGUtt (SEQ ID NO:50)

Position in gene sequence: 748

GC content: 33.3%

Sense strand siRNA: AAUU721CUUCCAGCGAUGtt (SEQ ID NO:51)

Antisense strand siRNA: CAUCGCUGGAAG127AAUtt (SEQ ID NO:52)

Position in gene sequence: 750

GC content: 38.1%

Sense strand siRNA: UU721CUUCCAGCGAUGGUtt (SEQ ID NO:53)

Antisense strand siRNA: ACCAUCGCUGGAAG127AAtt (SEQ ID NO:54)

Position in gene sequence: 790

GC content: 38.1%

Sense strand siRNA: GCUUUUCUACUCAGCAGAUtt (SEQ ID NO:55)

Antisense strand siRNA: AUCUGCUGAGUAGAAAAGCtt (SEQ ID NO:56)

Position in gene sequence: 851

GC content: 42.9%

Sense strand siRNA: GAAGGAAUCGAUGAGCUAGtt (SEQ ID NO:57)

Antisense strand siRNA: CUAGCUCAUCGAUCCUUCtt (SEQ ID NO:58)

Position in gene sequence: 854

GC content: 47.6%

Sense strand siRNA: GGAAUCGAUGAGCUAGCCAtt (SEQ ID NO:59)

Antisense strand siRNA: UGGCUAGCUCAUCGAUCCtt (SEQ ID NO:60)

Position in gene sequence: 858

GC content: 38.1%

Sense strand siRNA: UCGAUGAGCUAGCCAAAAUtt (SEQ ID NO:61)

Antisense strand siRNA: AUUUUGGCUAGCUCAUCGAtt (SEQ ID NO:62)

Position in gene sequence: 874

GC content: 38.1%

Sense strand siRNA: AAUC841UGUGGCGUUGAGtt (SEQ ID NO:63)

Antisense strand siRNA: CUCAACGCCACA148GAUUtt (SEQ ID NO:64)

Position in gene sequence: 876

GC content: 42.9%

Sense strand siRNA: UC841UGUGGCGUUGAGGAtt (SEQ ID NO:65)

Antisense strand siRNA: UCCUCAACGCCACA148GAtt (SEQ ID NO:66)

Position in gene sequence: 908

GC content: 57.1%

Sense strand siRNA: AUGACACGGCGAGCCAUGGtt (SEQ ID NO:67)

Antisense strand siRNA: CCAUGGCUCGCCGUGUCAUtt (SEQ ID NO:68)

Position in gene sequence: 949

GC content: 57.1%

Sense strand siRNA: AGCUGCUCUCACAGAGCGCtt (SEQ ID NO:69)

Antisense strand siRNA: GCGCUCUGUGAGAGCAGCUtt (SEQ ID NO:70)

Position in gene sequence: 1025

GC content: 71.4%

Sense strand siRNA: CCCCCACACCUGACCCCCGtt (SEQ ID NO:71)

Antisense strand siRNA: CGGGGGUCAGGUGUGGGGGtt (SEQ ID NO:72)

Position in gene sequence: 1050

GC content: 52.4%

Sense strand siRNA: GGGAGCUGGUAAGUCGC10tt (SEQ ID NO:73)

Antisense strand siRNA: 01GCGACUUACCAGCUCCCtt (SEQ ID NO:74)

Position in gene sequence: 1062

GC content: 47.6%

Sense strand siRNA: GUCGC1021CUACAGGAGCtt (SEQ ID NO:75)

Antisense strand siRNA: GCUCCUGUAG1201GCGACtt (SEQ ID NO:76)

Position in gene sequence: 1084

GC content: 28.6%

Sense strand siRNA: AUGUUCAGGUUUUCCUAAUtt (SEQ ID NO:77)

Antisense strand siRNA: AUUAGGAAAACCUGAACAUtt (SEQ ID NO:78)

Position in gene sequence: 1102

GC content: 42.9%

Sense strand siRNA: UAUCUGGUGGCUUUAGGAGtt (SEQ ID NO:79)

Antisense strand siRNA: CUCCUAAAGCCACCAGAUAtt (SEQ ID NO:80)

Position in gene sequence: 1148

GC content: 42.9%

Sense strand siRNA: AGCUCAAUAUCCCAGCAACtt (SEQ ID NO:81)

Antisense strand siRNA: GUUGCUGGGAUUUGAGCUtt (SEQ ID NO:82)

Position in gene sequence: 1155

GC content: 38.1%

Sense strand siRNA: UAUCCCAGCAACCAAUGUAAtt (SEQ ID NO:83)

Antisense strand siRNA: UACAUUGGUUGCUGGGUAAtt (SEQ ID NO:84)

Position in gene sequence: 1166

GC content: 33.3%

Sense strand siRNA: CCAAUGUAUUUGCCAAUAGtt (SEQ ID NO:85)

Antisense strand siRNA: CUAUUGGCAAUAACAUUGGtt (SEQ ID NO:86)

Position in gene sequence: 1170

GC content: 38.1%

Sense strand siRNA: UGUAAUUUGCCAAUAGGCUGtt (SEQ ID NO:87)

Antisense strand siRNA: CAGCCUAUUGGCAAUAACAtt (SEQ ID NO:88)

Position in gene sequence: 1182

GC content: 23.8%

Sense strand siRNA: UAGGCUGAAAUUC1141UAAtt (SEQ ID NO:89)

Antisense strand siRNA: UA1411GAAUUUCAGCCUAAtt (SEQ ID NO:90)

Position in gene sequence: 1191

GC content: 23.8%

Sense strand siRNA: AUUC1141UACUUUAACGGtt (SEQ ID NO:91)

Antisense strand siRNA: CCGUAAAAGUA1411GAAUtt (SEQ ID NO:92)

Position in gene sequence: 1207

GC content: 38.1%

Sense strand siRNA: CGGUGAAUAUGCAGGUUUUtt (SEQ ID NO:93)

Antisense strand siRNA: AAAACCUGCAUAUUCACCGtt (SEQ ID NO:94)

Position in gene sequence: 1214

GC content: 33.3%

Sense strand siRNA: UAUGCAGGUUUUGAUGAGAtt (SEQ ID NO:95)

Antisense strand siRNA: UCUCAUCAAACCUGCAUAtt (SEQ ID NO:96)

Position in gene sequence: 1242

GC content: 42.9%

Sense strand siRNA: CAGCUGAAUCUGGUGGA12tt (SEQ ID NO:97)

Antisense strand siRNA: 21UCCACCAGAUUCAGCUGtt (SEQ ID NO:98)

Position in gene sequence: 1250

GC content: 33.3%

Sense strand siRNA: UCUGGUGGA1201AAAGGAtt (SEQ ID NO:99)

Antisense strand siRNA: UCCUUU1021UCCACCAGAtt (SEQ ID NO:100)

Position in gene sequence: 1265

GC content: 23.8%

Sense strand siRNA: AGGAAAAGUGAUUAAACUUtt (SEQ ID NO:101)

Antisense strand siRNA: AAGUUUAAUCACUUUCCUtt (SEQ ID NO:102)

Position in gene sequence: 1270

GC content: 14.3%

Sense strand siRNA: AAGUGAUUAAACUUUUAAAAtt (SEQ ID NO:103)

Antisense strand siRNA: UUUAAAAGUUUAAUCACUUtt (SEQ ID NO:104)

Position in gene sequence: 1272

GC content: 23.8%

Sense strand siRNA: GUGAUUAAACUUUAAAAGGtt (SEQ ID NO:105)

Antisense strand siRNA: CCUUUAAAAGUUUAAUCACtt (SEQ ID NO:106)

Position in gene sequence: 1280

GC content: 14.3%

Sense strand siRNA: ACUUUUAAAGGAAAAAUUUtt (SEQ ID NO:107)

Antisense strand siRNA: AAAUUUUUCCUUUAAAAGUtt (SEQ ID NO:108)

Position in gene sequence: 1288

GC content: 14.3%

Sense strand siRNA: AGGAAAAAUUCAUUUUAAtt (SEQ ID NO:109)

Antisense strand siRNA: UUAAAAUGAAAUUUUUCCUtt (SEQ ID NO:110)

Position in gene sequence: 1293

GC content: 9.5%

Sense strand siRNA: AAAUUUCAUUUUAAGAAAAtt (SEQ ID NO:111)

Antisense strand siRNA: UUUUCUAAAAUGAAAUUUtt (SEQ ID NO:112)

Position in gene sequence: 1295

GC content: 9.5%

Sense strand siRNA: AUUUCAUUUUAAGAAAAUtt (SEQ ID NO:113)

Antisense strand siRNA: UAUUUUCUAAAAUGAAAUtt (SEQ ID NO:114)

Position in gene sequence: 1307

GC content: 14.3%

Sense strand siRNA: GAAAUAUCAUGAUU126tt (SEQ ID NO:115)

Antisense strand siRNA: 621AAUCAUGAUUAUUUUCtt (SEQ ID NO:116)

Position in gene sequence: 1310

GC content: 19%

Sense strand siRNA: AAUAAUCAUGAUU1261GGtt (SEQ ID NO:117)

Antisense strand siRNA: CC1621AAUCAUGAUUAUUtt (SEQ ID NO:118)

Position in gene sequence: 1312

GC content: 23.8%

Sense strand siRNA: UAAUCAUGAUU1261GGAGtt (SEQ ID NO:119)

Antisense strand siRNA: CUCC1621AAUCAUGAUUAtt (SEQ ID NO:120)

Position in gene sequence: 1315

GC content: 28.6%

Sense strand siRNA: UCAUGAUU1261GGAGAUGtt (SEQ ID NO:121)

Antisense strand siRNA: CAUCUCC1621AAUCAUGAtt (SEQ ID NO:122)

Position in gene sequence: 1351

GC content: 57.1%

Sense strand siRNA: GCCUGUCCUCCUGCUGAUGtt (SEQ ID NO:123)

Antisense strand siRNA: CAUCAGCAGGAGGACAGGctt (SEQ ID NO:124)

Position in gene sequence: 1395

GC content: 38.1%

Sense strand siRNA: AUGUGAUCAGGCAACAAGUtt (SEQ ID NO:125)

Antisense strand siRNA: ACUUGUUGCCUGAUCACAUtt (SEQ ID NO:126)

Position in gene sequence: 1409

GC content: 42.9%

Sense strand siRNA: CAAGUCAAGGAUAACGCCAtt (SEQ ID NO:127)

Antisense strand siRNA: UGGCGUUAUCCUUGACUUGtt (SEQ ID NO:128)

Position in gene sequence: 1412

GC content: 38.1%

Sense strand siRNA: GUCAAGGAUAACGCCAAAUtt (SEQ ID NO:129)

Antisense strand siRNA: AUUUGGCGUUAUCCUUGACtt (SEQ ID NO:130)

Position in gene sequence: 1417

GC content: 38.1%

Sense strand siRNA: GGAUAACGCCAAAUGGUAUtt (SEQ ID NO:131)

Antisense strand siRNA: AUACCAUUUGGCGUUAUCCtt (SEQ ID NO:132)

Position in gene sequence: 1423

GC content: 38.1%

Sense strand siRNA: CGCCAAAUGGUUAUAUCACUtt (SEQ ID NO:133)

Antisense strand siRNA: AGUGAUUAUACCAUUUGGCGtt (SEQ ID NO:134)

Position in gene sequence: 1429

GC content: 23.8%

Sense strand siRNA: AUGGUUAUAUCACUGAUUUUtt (SEQ ID NO:135)

Antisense strand siRNA: AAAAUCAGUGAUUAUACCAUtt (SEQ ID NO:136)

Position in gene sequence: 1470

GC content: 33.3%

Sense strand siRNA: CUGGAAGAAUAACAUCCAUtt (SEQ ID NO:137)

Antisense strand siRNA: AUGGAUGUUAUUCUCCAGtt (SEQ ID NO:138)

Position in gene sequence: 1476

GC content: 33.3%

Sense strand siRNA: GAAUAACAUCCAUUGUCGUtt (SEQ ID NO:139)

Antisense strand siRNA: ACGACAAUGGAUGUUAUUCtt (SEQ ID NO:140)

Position in gene sequence: 1479

GC content: 33.3%

Sense strand siRNA: UAACAUCCAUUGUCGUACAtt (SEQ ID NO:141)

Antisense strand siRNA: UGUACGACAAUGGAUGUUAtt (SEQ ID NO:142)

Position in gene sequence: 1482

GC content: 42.9%

Sense strand siRNA: CAUCCAUUGUCGUACAGCUtt (SEQ ID NO:143)

Antisense strand siRNA: AGCUGUACGACAAUGGAUGtt (SEQ ID NO:144)

Position in gene sequence: 1505

GC content: 23.8%

Sense strand siRNA: ACAACUUCAG1441AUGAAtt (SEQ ID NO:145)

Antisense strand siRNA: UUCAU1441CUGAAGUUGUtt (SEQ ID NO:146)

Position in gene sequence: 1509

GC content: 19%

Sense strand siRNA: CUUCAG1441AUGAAUUUUtt (SEQ ID NO:147)

Antisense strand siRNA: AAAAUUCAU1441CUGAAGtt (SEQ ID NO:148)

Position in gene sequence: 1524

GC content: 19%

Sense strand siRNA: UUUUUACAAGUUAUACAGAtt (SEQ ID NO:149)

Antisense strand siRNA: UCUGUAUAACUUGUAAAAAtt (SEQ ID NO:150)

Position in gene sequence: 1533

GC content: 28.6%

Sense strand siRNA: GUUAUACAGAUUGAUACUGtt (SEQ ID NO:151)

Antisense strand siRNA: CAGUAUCAAUCUGUAUAACtt (SEQ ID NO:152)

Position in gene sequence: 1576

GC content: 23.8%

Sense strand siRNA: CUU1501GCUAUAGAAAGUtt (SEQ ID NO:153)

Antisense strand siRNA: ACUUUCUAUAGC1051AAGtt (SEQ ID NO:154)

Position in gene sequence: 1592

GC content: 33.3%

Sense strand siRNA: AGUUGGUACAAAUGAUCUGtt (SEQ ID NO:155)

Antisense strand siRNA: CAGAUCAUUUGUACCAACUtt (SEQ ID NO:156)

Position in gene sequence: 1603

GC content: 23.8%

Sense strand siRNA: AUGAUCUGUACUUUAAACUtt (SEQ ID NO:157)

Antisense strand siRNA: AGUUUAAAGUACAGAUCAUtt (SEQ ID NO:158)

Position in gene sequence: 1619

GC content: 33.3%

Sense strand siRNA: ACUACAGUUAGGAAUCCUAtt (SEQ ID NO:159)

Antisense strand siRNA: UAGGAUUCCUACUGUAGUtt (SEQ ID NO:160)

Position in gene sequence: 1633

GC content: 28.6%

Sense strand siRNA: UCCUAGAAGA1561UUGCUtt (SEQ ID NO:161)

Antisense strand siRNA: AGCAA1651UCUUCUAGGAt (SEQ ID NO:162)

Position in gene sequence: 1641

GC content: 14.3%

Sense strand siRNA: GA1561UUGCUUUUUUUUUtt (SEQ ID NO:163)

Antisense strand siRNA: AAAAAAAAAAGCAA1651UCtt (SEQ ID NO:164)

Position in gene sequence: 1668

GC content: 28.6%

Sense strand siRNA: CUGUAGUCCAGUAUUAUAAt (SEQ ID NO:165)

Antisense strand siRNA: UAUAAUACUGGAACUACAGtt (SEQ ID NO:166)

Position in gene sequence: 1740

GC content: 52.4%

Sense strand siRNA: UCUUGCUCUGUUGCCCAGGtt (SEQ ID NO:167)

Antisense strand siRNA: CCUGGGCAACAGAGCAAGAt (SEQ ID NO:168)

Position in gene sequence: 1798

GC content: 57.1%

Sense strand siRNA: GCUCUGCCUCCCAGGUUCAAt (SEQ ID NO:169)

Antisense strand siRNA: UGAACCUGGGAGGCAGAGCtt (SEQ ID NO:170)

Position in gene sequence: 1888

GC content: 4.8%

Sense strand siRNA: UUUUUUGUAUU1801UUUAAt (SEQ ID NO:171)

Antisense strand siRNA: UAAA1081AAUACAAAAAAAt (SEQ ID NO:172)

Position in gene sequence: 1990

GC content: 47.6%

Sense strand siRNA: AGUGCUGGGAUUACAGGCUtt (SEQ ID NO:173)

Antisense strand siRNA: AGCCUGUAAUCCCAGCACUtt (SEQ ID NO:174)

Position in gene sequence: 2034

GC content: 38.1%

Sense strand siRNA: UGUCCUAGAGAGUUUUGUGtt (SEQ ID NO:175)

Antisense strand siRNA: CACAAAACUCUCUAGGACAtt (SEQ ID NO:176)

Position in gene sequence: 2060

GC content: 14.3%

Sense strand siRNA: UUCUUUAUGUAUAUUUGUAtt (SEQ ID NO:177)

Antisense strand siRNA: UACAAUAUACAUAAGAAtt (SEQ ID NO:178)

Position in gene sequence: 2099

GC content: 38.1%

Sense strand siRNA: AGUGCUUUAAGUGUGGAGAtt (SEQ ID NO:179)

Antisense strand siRNA: UCUCCACACUUAAGCACUtt (SEQ ID NO:180)

Position in gene sequence: 2109

GC content: 33.3%

Sense strand siRNA: GUGUGGAGAGUCAAUUAAAAtt (SEQ ID NO:181)

Antisense strand siRNA: UUUAAUUGACUCUCCACACtt (SEQ ID NO:182)

Position in gene sequence: 2123

GC content: 23.8%

Sense strand siRNA: UUAAACACCUUUACUCUUAAtt (SEQ ID NO:183)

Antisense strand siRNA: UAAGAGUAAAGGUGUUUAAAtt (SEQ ID NO:184)

Position in gene sequence: 2127

GC content: 28.6%

Sense strand siRNA: ACACCUUUACUCUUAAGAAAAtt (SEQ ID NO:185)

Antisense strand siRNA: UUUCUAAGAGUAAAGGUGUtt (SEQ ID NO:186)

Position in gene sequence: 2145

GC content: 38.1%

Sense strand siRNA: AUACGGAUUC2041GGCAGtt (SEQ ID NO:187)

Antisense strand siRNA: CUGCC1402GAAUCCGUAUtt (SEQ ID NO:188)

Position in gene sequence: 2175

GC content: 28.6%

Sense strand siRNA: UAUUGGUUUCUCUUUGGUAtt (SEQ ID NO:189)

Antisense strand siRNA: UACCAAAGAGAAACCAAUAtt (SEQ ID NO:190)

Position in gene sequence: 2200

GC content: 23.8%

Sense strand siRNA: UAAAAGUUUAUCCGUAUGUtt (SEQ ID NO:191)

Antisense strand siRNA: ACAUACGGAUAAACUUUUAtt (SEQ ID NO:192)

Position in gene sequence: 2203

GC content: 23.8%

Sense strand siRNA: AAGUUUAUCCGUAUGU210tt (SEQ ID NO:193)

Antisense strand siRNA: 012ACAUACGGAUAAACUUtt (SEQ ID NO:194)

Position in gene sequence: 2205

GC content: 28.6%

Sense strand siRNA: GUUUAUCCGUAUGU2101Ctt (SEQ ID NO:195)

Antisense strand siRNA: G1012ACAUACGGAUAAACtt (SEQ ID NO:196)

Position in gene sequence: 2228

GC content: 28.6%

Sense strand siRNA: CGGAUUUGUGGAAAAAAAAAAAtt (SEQ ID NO:197)

Antisense strand siRNA: UUUUUUUUCCACAAAUCCGtt (SEQ ID NO:198)

Position in gene sequence: 2241

GC content: 0%

Sense strand siRNA: AAAAAAAAAAAAAAAAAAAAAAtt (SEQ ID NO:199)

Antisense strand siRNA: UUUUUUUUUUUUUUUUUUUUtt (SEQ ID NO:200)

Position in gene sequence: 2243

GC content: 0%

Sense strand siRNA: AAAAAAAAAAAAAAAAAAAAAAtt (SEQ ID NO:199)

Antisense strand siRNA: UUUUUUUUUUUUUUUUUUUUtt (SEQ ID NO:200)

Position in gene sequence: 2245

GC content: 0%

Sense strand siRNA: AAAAAAAAAAAAAAAAAAAAtt (SEQ ID NO:199)

Antisense strand siRNA: UUUUUUUUUUUUUUUUUUtt (SEQ ID NO:200)

[0061] In some embodiments, an shRNA (small hairpin RNA or short hairpin RNA) molecule has a sequence that is at least 85%, 90%, 95%, or 100% complementary (or any range derivable therein) to any of the sequences identified in the table below (or to a sense strand identified in the table above), which can be used to target that sequence identified in the table. In some embodiments it is specifically contemplated that an shRNA is provided in a vector.

PSPH shRNA in ORF, UTR5m and UTR3

No.	Start	Target sequence(DNA)	Region	GC%
1	892	GCAGTGCCTTTCAAAGCTGCT (SEQ ID NO:201)	ORF	52.39
2	957	GCAGAGACTCATAGCAGAGCA (SEQ ID NO:202)	ORF	52.39
3	997	GGCATAAGGGAGCTGGTAAGT (SEQ ID NO:203)	ORF	52.39
4	1019	GCCTACAGGAGCGAAATGTTC (SEQ ID NO:204)	ORF	52.39
5	1068	GAGTATGTAGAGCATGTTGC (SEQ ID NO:205)	ORF	42.86
6	1108	GCAACCAATGTATTGCCAAT (SEQ ID NO:206)	ORF	38.1
7	1123	GCCAATAGGCTGAAATCTAC (SEQ ID NO:207)	ORF	42.86
8	1130	GGCTGAAATTCTACTTTAACG (SEQ ID NO:208)	ORF	38.1
9	1131	GCTGAAATFCTACTTTAACGG (SEQ ID NO:209)	ORF	38.1
10	1270	GCCACAGATATGGAGCCTGT (SEQ ID NO:210)	ORF	52.39

No.	Start	Target sequence(DNA)	Region	GC%
1	115	GCGTTTGTTCCTTCATTG (SEQ ID NO:211)	UTR5	47.62
2	234	GCCTAGCGAAGATGAAGATAG (SEQ ID NO:212)	UTR5	47.62
3	239	GCGAAGATGAAGATAGATGC (SEQ ID NO:213)	UTR5	42.86
4	266	GAAAGTGTGGATACCTTTAG (SEQ ID NO:214)	UTR5	42.86
5	306	GATGGGAGTTGAGACGTAAGA (SEQ ID NO:215)	UTR5	47.62
6	309	GGGAGTTGAGACGTAAGAACC (SEQ ID NO:216)	UTR5	52.39
7	438	GCAGATCTCAGAGAGCGTTC (SEQ ID NO:217)	UTR5	52.39
8	584	GGCTCTTGCTCTTGCAGAATC (SEQ ID NO:218)	UTR5	52.39
9	585	GCTCTTGCTCTTGCAGAATCC (SEQ ID NO:219)	UTR5	52.39
10	597	GCAGAATCCACAGGCTCTTCT (SEQ ID NO:220)	UTR5	47.62

No.	Start	Target sequence(DNA)	Region	G/C%
1	1995	GTGTGGAGAGTCAATTAAACA (SEQ ID NO:221)	UTR3	38.1
2	2041	GGCAGCCTTCAGTGAATATTG (SEQ ID NO:222)	UTR3	47.62
3	2042	GCAGCCTTCAGTGAATATTGG (SEQ ID NO:223)	UTR3	47.62
4	2045	GCCTTCAGTGAAATTGGTTT (SEQ ID NO:224)	UTR3	38.1

ORF (open reading frame)
UTR-5 (Untranslated Region -5)
UTR3 (Untranslated Region -3)

[0062] In some embodiments, the strand or strands of dsRNA are 100 bases (or basepairs) or less, in which case they may also be referred to as “siRNA.” In specific embodiments the strand or strands of the dsRNA are less than 70 bases in length. With respect to those embodiments, the dsRNA strand or strands may be from 5-70, 10-65, 20-60, 30-55, 40-50 bases or basepairs in length. A dsRNA that has a complementarity region equal to or less than 30 basepairs (such as a single stranded hairpin RNA in which the stem or complementary portion is less than or equal to 30 basepairs) or one in which the strands are 30 bases or fewer in length is specifically contemplated, as such molecules evade a mammalian’s cell antiviral response. Thus, a hairpin dsRNA (one strand) may be 70 or fewer bases in length with a complementary region of 30 basepairs or fewer. In some cases, a dsRNA may be processed in the cell into siRNA.

[0063] Chemically synthesized siRNAs are found to work optimally when they are in cell culture at concentrations of 25-100 nM, but concentrations of about 100 nM have achieved effective suppression of expression in mammalian cells. siRNAs have been most effective in mammalian cell culture at about 100 nM. In several instances, however, lower concentrations of chemically synthesized siRNA have been used (Caplen *et al.*, 2000; Elbashir *et al.*, 2001).

[0064] PCT publications WO 99/32619 and WO 01/68836 suggest that RNA for use in siRNA may be chemically or enzymatically synthesized. Both of these texts are incorporated herein in their entirety by reference. The contemplated constructs provide templates that produce RNAs that contain nucleotide sequences identical to a portion of the target gene. Typically the length of identical sequences provided is at least 25 bases, and may be as many as 400 or more bases in length. Longer dsRNAs may be digested to 21-25mer lengths with endogenous nuclease complex that converts long dsRNAs to siRNAs *in vivo*. No distinction is made between the expected properties of chemical or enzymatically synthesized dsRNA in its use in RNA interference.

[0065] Similarly, WO 00/44914, incorporated herein by reference, suggests that single strands of RNA can be produced enzymatically or by partial/total organic synthesis. U.S. Patent 5,795,715 reports the simultaneous transcription of two complementary DNA sequence strands in a single reaction mixture, wherein the two transcripts are immediately hybridized.

[0066] Methods and compositions for making and/or using particularly siRNAs, in addition to the methods disclosed herein, are disclosed in a number of references, for example US Patent Publications 20090258926, 20090258424, 20090210955, 20090149644, 20090093026, 20090061487, 20090012021, 20080249046, 20080249039, 20080188429, 20080182813, 20080146788, 20080112916, 20060172925, which are hereby incorporated by reference in their entireties.

[0067] These references provide specific information regarding formulations, modifications of siRNAs or shRNAs, delivery compositions, and vector designs for expressing siRNA.

2. Vectors

[0068] Vectors of the present invention are designed, primarily, to transform cells with a therapeutic or preventative phosphoserine phosphatase inhibitor encoding a phosphoserine phosphatase inhibitor nucleic acid sequence under the control of a eukaryotic promoter (*i.e.*, constitutive, inducible, repressable, tissue specific). Also, the vectors may contain a selectable marker if, for no other reason, to facilitate their manipulation *in vitro*. However, selectable markers may play an important role in producing recombinant cells.

[0069] Composition and method embodiments include administering therapeutic compositions to a patient.

[0070] Any nucleic acid molecule of the invention may be comprised in a vector. The term "vector" is used to refer to a carrier nucleic acid molecule into which a nucleic acid sequence can be inserted for introduction into a cell where it can be replicated. A nucleic acid sequence can be "exogenous," which means that it is foreign to the cell into which the vector is being introduced or that the sequence is homologous to a sequence in the cell but in a position within the host cell nucleic acid in which the sequence is ordinarily not found. Vectors include plasmids, cosmids, viruses (bacteriophage, animal viruses, and plant viruses), and artificial chromosomes (*e.g.*, YACs). One of skill in the art would be well equipped to construct a vector through standard recombinant techniques, which are described in Sambrook *et al.*, (2001) and Ausubel *et al.*, 1996, both incorporated herein by reference.

[0071] The term "expression vector" refers to a vector containing a nucleic acid sequence coding for at least part of a gene product capable of being transcribed. In some cases, RNA molecules are then translated into a protein, polypeptide, or peptide. Expression vectors can contain a variety of "control sequences," which refer to nucleic acid sequences necessary for the transcription and possibly translation of an operably linked coding sequence in a particular host organism. In addition to control sequences that govern transcription and translation, vectors and expression vectors may contain nucleic acid sequences that serve other functions as well and are well known to those of ordinary skill in the art. The term "gene product" generally refers

[0072] One method for delivery of the recombinant DNA involves the use of an adenovirus expression vector. "Adenovirus expression vector" is meant to include those constructs containing adenovirus sequences sufficient to (a) support packaging of the construct and (b) to ultimately express a recombinant gene construct that has been cloned therein. The adenovirus vector may be replication defective, or at least conditionally defective, the nature of the adenovirus vector is not believed to be crucial to the successful practice of the invention. The adenovirus may be of any of the 42 different known serotypes or subgroups A-F. Adenovirus type 5 of subgroup C is the some starting material in order to obtain the conditional replication-defective adenovirus vector for use in the present invention. As stated above, the typical vector according to the present invention is replication defective and will not have an adenovirus E1 region. Thus, it will be most convenient to introduce the transforming construct at the position from which the E1-coding sequences have been removed. However, the position of insertion of the construct within the adenovirus sequences is not critical to the invention. The polynucleotide encoding the gene of interest may also be inserted in lieu of the deleted E3 region in E3 replacement vectors as described by Karlsson *et al.* (1986) or in the E4 region where a helper cell line or helper virus complements the E4 defect.

[0073] The retroviruses are a group of single-stranded RNA viruses characterized by an ability to convert their RNA to double-stranded DNA in infected cells by a process of reverse-transcription (Coffin, 1990). In order to construct a retroviral vector, a nucleic acid encoding a gene of interest is inserted into the viral genome in the place of certain viral sequences to produce a virus that is replication-defective. In order to produce virions, a packaging cell line containing the gag, pol,

and env genes but without the LTR and packaging components is constructed (Mann *et al.*, 1983).

[0074] Other viral vectors include adeno-associated virus (AAV) (described in U.S. Patent 5,139,941 and U.S. Patent 4,797,368, each incorporated herein by reference), vaccinia virus, other poxviruses, lentivirus, Epstein Barr viruses, and picornaviruses.

3. Protamine Delivery of Nucleic Acids

[0075] Protamine may also be used to form a complex with an expression construct. Such complexes may then be formulated with the lipid compositions described above for administration to a cell. Protamines are small highly basic nucleoproteins associated with DNA. Their use in the delivery of nucleic acids is described in U.S. Patent No. 5,187,260, which is incorporated by reference.

4. Lipid Formulations for Nucleic Acid Delivery

[0076] In a further embodiment of the invention, a nucleic acid may be entrapped in a liposome or lipid formulation. Liposomes are vesicular structures characterized by a phospholipid bilayer membrane and an inner aqueous medium. Multilamellar liposomes have multiple lipid layers separated by aqueous medium. They form spontaneously when phospholipids are suspended in an excess of aqueous solution. The lipid components undergo self-rearrangement before the formation of closed structures and entrap water and dissolved solutes between the lipid bilayers (Ghosh and Bachhawat, 1991). Also contemplated is a gene construct complexed with Lipofectamine (Gibco BRL).

[0077] Advances in lipid formulations have improved the efficiency of gene transfer *in vivo* (Smyth-Templeton *et al.*, 1997; WO 98/07408). A novel lipid formulation composed of an equimolar ratio of 1,2-bis(oleoyloxy)-3-(trimethyl ammonio)propane (DOTAP) and cholesterol significantly enhances systemic *in vivo* gene transfer, approximately 150-fold. The DOTAP:cholesterol lipid formulation is said to form a unique structure termed a “sandwich liposome”. This formulation is reported to “sandwich” DNA between an invaginated bi-layer or ‘vase’ structure. Beneficial characteristics of these lipid structures include a positive colloidal

stabilization by cholesterol, two dimensional DNA packing and increased serum stability.

[0078] In further embodiments, the liposome is further defined as a nanoparticle. A “nanoparticle” is defined herein to refer to a submicron particle. The submicron particle can be of any size. For example, the nanoparticle may have a diameter of from about 0.1, 1, 10, 100, 300, 500, 700, 1000 nanometers or greater. The nanoparticles that are administered to a subject may be of more than one size.

[0079] Any method known to those of ordinary skill in the art can be used to produce nanoparticles. In some embodiments, the nanoparticles are extruded during the production process. Information pertaining to the production of nanoparticles can be found in U.S. Patent App. Pub. No. 20050143336, U.S. Patent App. Pub. No. 20030223938, U.S. Patent App. Pub. No. 20030147966, each of which is herein specifically incorporated by reference into this section.

[0080] In certain embodiments, an anti-inflammatory agent is administered with the lipid to prevent or reduce inflammation secondary to administration of a lipid:nucleic acid complex. For example, the anti-inflammatory agent may be a non-steroidal anti-inflammatory agent, a salicylate, an anti-rheumatic agent, a steroid, or an immunosuppressive agent.

[0081] Synthesis of DOTAP:Chol nanoparticles is by any method known to those of ordinary skill in the art. For example, the method can be in accordance with that set forth in Chada *et al.*, 2003, or Templeton *et al.*, 1997, both of which are herein specifically incorporated by reference. DOTAP:Chol-DNA complexes were prepared fresh two to three hours prior to injection in mice.

[0082] One of ordinary skill in the art would be familiar with use of liposomes or lipid formulation to entrap nucleic acid sequences. Liposomes are vesicular structures characterized by a phospholipid bilayer membrane and an inner aqueous medium. Multilamellar liposomes have multiple lipid layers separated by aqueous medium. They form spontaneously when phospholipids are suspended in an excess of aqueous solution. The lipid components undergo self-rearrangement before the formation of closed structures and entrap water and dissolved solutes between the lipid bilayers (Ghosh and Bachhawat, 1991). Also contemplated is a gene construct complexed with Lipofectamine (Gibco BRL).

B. Proteins and Polypeptides

[0083] Embodiments concern methods and compositions involving a phosphoserine phosphatase inhibitor that is a polypeptide. In certain embodiments, the phosphoserine phosphatase polypeptide inhibitors are used in the treatment or prevention of OSA. The terms “protein” and “polypeptide” are used interchangeably herein and they both cover what is understood as a “peptide” (a polypeptide molecule having 100 or fewer amino acid residues). In certain embodiments, the phosphoserine phosphatase inhibitor is a protein, polypeptide, or peptide; in particular embodiments, the phosphoserine phosphatase inhibitor is protein or polypeptide that is an antibody.

[0084] As will be understood by those of skill in the art, modification and changes may be made in the structure of a phosphoserine phosphatase inhibitor polypeptide or peptide, and still produce a molecule having like or otherwise desirable characteristics. For example, certain amino acids may be substituted for other amino acids or include deletions, additions, or truncations in the protein sequence without appreciable loss of interactive binding capacity with structures. Since it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid sequence substitutions can be made in a protein sequence (or, of course, its underlying DNA coding sequence) and nevertheless obtain a protein with similar inhibitory properties. It is thus contemplated by the inventors that various changes may be made in the sequence of phosphoserine phosphatase inhibitor polypeptides or peptides (or underlying DNA) without appreciable loss of their biological utility or activity.

[0085] It is also well understood that where certain residues are shown to be particularly important to the biological or structural properties of a protein or peptide, *e.g.*, residues in the binding site of an antibody, such residues may not generally be exchanged.

[0086] Some embodiments of the present invention pertain to methods and compositions involving an inhibitor of phosphoserine phosphatase, wherein the inhibitor is an antibody that binds phosphoserine phosphatase.

[0087] As used herein, the term "antibody" refers to any form of antibody or fragment thereof that exhibits the desired biological activity. Thus, it is used in the

broadest sense and specifically covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), and antibody fragments so long as they exhibit the desired biological activity. An antibody inhibitor may be considered a neutralizing antibody.

[0088] Included within the definition of an antibody that binds phosphoserine phosphatase is a phosphoserine phosphatase antibody binding fragment. As used herein, the term "phosphoserine phosphatase binding fragment" or "binding fragment thereof" encompasses a fragment or a derivative of an antibody that still substantially retain its biological activity of inhibiting phosphoserine phosphatase activity. Therefore, the term "antibody fragment" or phosphoserine phosphatase binding fragment refers to a portion of a full length antibody, generally the antigen binding or variable region thereof. Examples of antibody fragments include Fab, Fab', F(ab').sub.2, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules, *e.g.*, sc-Fv; and multispecific antibodies formed from antibody fragments. Typically, a binding fragment or derivative retains at least 50% of its PBEF inhibitory activity. Preferably, a binding fragment or derivative retains about or at least about 60%, 70%, 80%, 90%, 95%, 99% or 100% of its phosphoserine phosphatase inhibitory activity. It is also intended that a phosphoserine phosphatase binding fragment can include conservative amino acid substitutions that do not substantially alter its biologic activity.

[0089] Embodiments may concern a "humanized antibody," which refers to forms of antibodies that contain sequences from non-human (*e.g.*, murine) antibodies as well as human antibodies. Such antibodies are chimeric antibodies which contain minimal sequence derived from non-human immunoglobulin. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin.

C. Small Molecules

[0090] The embodiments concern phosphoserine phosphatase inhibitors that are small molecules, which refers to a small compound that is biologically active but is not a polymer. It does refer to a monomer. In certain embodiments, the small molecule may be okadaic acid or a pharmaceutically acceptable salt, amide or ester thereof. The salt may be an alkali metal or alkaline earth metal salt such as the sodium or potassium salt. The amide may be the simple acid amide, $-\text{CONH}_2$, or a mono- or di-alkyl amide such as an amide denoted by the formula $-\text{CONR}_1\text{R}_2$ wherein R_1 is hydrogen or $\text{C}_1\text{-C}_4$ alkyl and R_2 is $\text{C}_1\text{-C}_4$ alkyl. Alternatively, the amide may be an amide with an amino acid, for example an amide between okadaic acid and glycine or lysine. The amide with glycine is glycookadaic acid. The amide between okadaic acid and an amino acid may be in the form of a pharmaceutically acceptable salt. The ester may be a $\text{C}_1\text{-C}_4$ alkyl ester such as the methyl or ethyl ester of okadaic acid. An alternative inhibitor for use in the invention is calyculin A or a derivative thereof. Any functional derivative capable of mimicking TNF or IL-1 or of inducing induction of the Egr-1 gene may be used. The derivative may be the acid or amines of calyculin A. The following protein phosphatase inhibitors may also be used in embodiments described herein: okadaic acid, calyculin A, dinophysistoxin-1, okadaic acid tetramethyl ether, acanthifolicin, 7-0-palmitoylokadaic acid, 7-0-docosahexaenoylokadaic acid, glycookadaic acid, okadylamine, okadanol, nor-okadanol, okadaic acid glycol, and okadaic acid spiroketal II. Other embodiments concern the following: cantharidic Acid (potent inhibitor of PP2A ($\text{IC}_{50} = 50 \text{ nM}$)); cypermethrin (potent inhibitor of PP2B ($\text{IC}_{50} = 40 \text{ pM}$)); DARPP-32 (potent inhibitor of PP1 ($\text{IC}_{50} = 1 \text{ nM}$)); Deltamethrin (potent inhibitor of PP2B ($\text{IC}_{50} = 100 \text{ pM}$)); eIF-2a Inhibitor (blocks the activity of PP1 and its nonenzymatic cofactor GADD34); endothall (specific inhibitor of PP2A ($\text{IC}_{50} = 90 \text{ nM}$)); Fenvalerate (potent inhibitor of PP2B ($\text{IC}_{50} = 2 - 4 \text{ nM}$)); Microcystin-LR, *Microcystis aeruginosa* (potent inhibitor of PP1 ($\text{IC}_{50} = 1.7 \text{ nM}$) and PP2A ($\text{IC}_{50} = 40 \text{ pM}$)); NIPP-1 (potent and specific inhibitor of PP1 ($\text{K}_i = 1 - 10 \text{ pM}$)); Okadaic Acid (potent inhibitor of PP1 ($\text{IC}_{50} = 10 - 15 \text{ nM}$) and PP2A ($\text{IC}_{50} = 100 \text{ pM}$)); Protein Phosphatase 2A Inhibitor II PP2A (potent inhibitor of all forms of PP2A ($\text{IC}_{50} = \sim 100 \text{ pM}$)); Protein Phosphatase Inhibitor 2 (the regulatory subunit of the cytosolic type 1 PP1 that inhibits the activity of the free catalytic subunit of PP1 ($\text{IC}_{50} = 1 \text{ nM}$)); Tautomycin

(selective PP1 inhibitor with ~38-fold greater potency compared to PP2A ($IC_{50} = 1.6$ nM for PP1 and 62 nM for PP2A)); Tautomycin (inhibits PP1 ($IC_{50} = 1$ nM), PP2A ($IC_{50} = 10$ nM), and smooth muscle endogenous phosphatase ($IC_{50} = 6$ nM)).

D. Formulations and Modes of Administration

[0091] Embodiments concern substances that can be used to prevent or treat conditions or diseases. In particular, the present invention concerns phosphoserine phosphatase inhibitors as preventative and therapeutic agents. Methods may be employed with respect to individuals who have been diagnosed with a particular inflammatory condition or disease or who are deemed to be at risk for an inflammatory condition or disease.

[0092] It is contemplated that compositions may be administered to a patient within about 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 minutes, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 hours, 1, 2, 3, 4, 5, 6, 7 days, 1, 2, 3, 4, 5 weeks, and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months of being diagnosed OSA, identified as having symptoms of OSA, or identified as at risk for OSA.

[0093] In certain embodiments, a course of treatment will last 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90 days or more. It is contemplated that one agent may be given on day 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, and/or 90, any combination thereof, and another agent is given on day 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, and/or 90, or any combination thereof. Within a single day (24-hour period), the patient may be given one or multiple administrations of the agent(s). Moreover, after a course of treatment,

it is contemplated that there is a period of time at which no other treatment is administered. This time period may last 1, 2, 3, 4, 5, 6, 7 days, and/or 1, 2, 3, 4, 5 weeks, and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months or more, depending on the condition of the patient, such as their prognosis, strength, health, *etc.*

[0094] In particular embodiments, compositions may be administered 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more times, and/or they may be administered every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 hours, or 1, 2, 3, 4, 5, 6, 7 days, or 1, 2, 3, 4, 5 weeks, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months, or any range or combination derivable therein.

[0095] Compounds and compositions may be administered to a patient intravenously, intradermally, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrapleurally, intratracheally, intranasally, intravitreally, intravaginally, intrarectally, topically, intratumorally, intramuscularly, intraperitoneally, subcutaneously, subconjunctival, intravesicularly, mucosally, intrapericardially, intraumbilically, intraocularly, orally, directly, topically, locally, by inhalation, by injection, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, *via* a catheter, *via* nebulizer, *via* aerosol, or *via* a lavage. In certain embodiments, adenoid and/or tonsillar cells or tissue is directly administered a phosphoserine phosphatase inhibitor.

[0096] In certain embodiments, the composition is administered orally. Examples of other routes of administration include intravitreal administration, intralesional administration, intratumoral administration, topical administration to the surface of the eye, topical application to the surface of a tumor, direct application to a neovascular membrane, subconjunctival administration, periocular administration, retrobulbar administration, subtenon administration, intracameral administration, subretinal administration, posterior juxtasceral administration, and suprachoroidal administration.

[0097] In some embodiments, pharmaceutical compositions are administered to a subject. Different aspects of the present invention involve administering an effective amount of a composition to a subject. Such compositions will generally be dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium.

[0098] The phrases “pharmaceutically acceptable” or “pharmacologically acceptable” refer to molecular entities and compositions that do not produce an adverse, allergic, or other untoward reaction when administered to an animal, or human. As used herein, “pharmaceutically acceptable carrier” includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredients, its use in immunogenic and therapeutic compositions is contemplated. Supplementary active ingredients, such as other anti-cancer agents, can also be incorporated into the compositions.

[0099] In addition to the compounds formulated for parenteral administration, such as those for intravenous or intramuscular injection, other pharmaceutically acceptable forms include, *e.g.*, tablets or other solids for oral administration (which may include enterically coated formulations); time release capsules; sustained release forms, and any other form currently used, including inhalants and the like.

[00100] The active compounds of the present invention can be formulated for parenteral administration, *e.g.*, formulated for injection via the intravenous, intramuscular, sub-cutaneous, or even intraperitoneal routes. Typically, such compositions can be prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for use to prepare solutions or suspensions upon the addition of a liquid prior to injection can also be prepared; and, the preparations can also be emulsified.

[00101] Solutions of the active compounds as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. A solution may be 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2,

8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9.0, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9% or more of the PBEF inhibitor, or any range derivable therein.

[00102] The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil, or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that it may be easily injected. It also should be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi.

[00103] The proteinaceous compositions may be formulated into a neutral or salt form. Pharmaceutically acceptable salts, include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

[00104] The carrier also can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion, and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[00105] Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various other ingredients enumerated above, as required, followed by filtered sterilization.

Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques, which yield a powder of the active ingredient, plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[00106] Such compositions would normally be administered as pharmaceutically acceptable compositions that include physiologically acceptable carriers, buffers or other excipients. As used herein, the term “pharmaceutically acceptable” refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem complications commensurate with a reasonable benefit/risk ratio. The term “pharmaceutically acceptable carrier,” means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting a chemical agent.

[00107] For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered, if necessary, and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous, and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage could be dissolved in isotonic NaCl solution and either added to hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, Remington's Pharmaceutical Sciences, 1990). Some variation in dosage will necessarily occur depending on the condition of the subject. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

[00108] An effective amount of therapeutic or prophylactic composition is determined based on the intended goal. The term “unit dose” or “dosage” refers to physically discrete units suitable for use in a subject, each unit containing a

predetermined quantity of the composition calculated to produce the desired responses discussed above in association with its administration, *i.e.*, the appropriate route and regimen. The quantity to be administered, both according to number of treatments and unit dose, depends on the protection desired.

[00109] Precise amounts of the composition also depend on the judgment of the practitioner and are peculiar to each individual. Factors affecting dose include physical and clinical state of the subject, route of administration, intended goal of treatment (alleviation of symptoms versus cure), and potency, stability, and toxicity of the particular composition.

[00110] Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically or prophylactically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above/

1. Solid or Semi-Solid Formulations

[00111] The pharmaceutical compositions may be formulated as a solid or semi-solid. Solid and semi-solid formulations refer to any formulation other than aqueous formulations. One of ordinary skill in the art would be familiar with formulation of agents as a solid or semi-solid.

[00112] [0304] Examples include a gel, a matrix, a foam, a cream, an ointment, a lozenge, a lollipop, a gum, a powder, a gel strip, a film, a hydrogel, a dissolving strip, a paste, a toothpaste, or a solid stick. Some of these formulations are discussed in greater detail as follows.

2. Gel

[00113] A gel is defined herein as an apparently solid, jelly-like material formed from a colloidal solution. A colloidal solution is a solution in which finely divided particles which are dispersed within a continuous medium in a manner that prevents them from being filtered easily or settled rapidly. Methods pertaining to the formulation of gels are set forth in U.S. Pat. No. 6,828,308, U.S. Pat. No. 6,280,752, U.S. Pat. No. 6,258,830, U.S. Pat. No. 5,914,334, U.S. Pat. No. 5,888,493, and U.S. Pat. No. 5,571,314, each of which is herein specifically incorporated by reference in its entirety.

3. Topical Gel

[00114] Some of the pharmaceutical compositions set forth herein are formulated as a topical gel. For example, a nucleic acid expression construct may be formulated as a hydrophobic gel based pharmaceutical formulation. A hydrophobic gel may be formulated, for example, by mixing a pentamer cyclomethicone component (Dow Corning 245 fluid.TM.) with a liquid suspension of a nucleic acid expression construct, hydrogenated castor oil, octyl palmitate and a mixture of cyclomethicone and dimethiconol in an 8:2 ratio. Preferably, the pentamer cyclomethicone component is approximately 40% of the gel, the liquid nucleic acid expression construct component is approximately 30.0% of the gel, the hydrogenated castor oil component is approximately 10% of the gel, the octyl palmitate component is approximately 10.0% of the gel and the cyclomethicone/dimethiconol component is approximately 10.0% of the gel. Each component listed above may be mixed together while heated at approximately 80-90.degree. C. under vacuum. Upon lowering the temperature to, for example, 37.degree. C., the nucleic acid expression construct component may then be added and the final gel composition should be allowed to cool to an ambient temperature. The final concentration of the nucleic acid expression construct in the hydrophobic gel formulation will depend on the type of construct employed and the administrative goal.

4. Oral Gel Formulation

[00115] An oral gel pharmaceutical formulation for delivery of a nucleic acid expression construct or other inhibitor may also be prepared using any method known to those of ordinary skill in the art. Such a pharmaceutical formulation may be applied to the oral cavity. Such a gel may be created, for example, by mixing water, potassium sorbate, sodium benzoate, disodium EDTA, hyaluronic acid and maltodextrin. After dissolution of the aforementioned ingredients, polyvinylpyrrolidone may be added under stirring and vacuum, for example 30 mm Hg until complete solvation. Other ingredients, such as hydroxyethylcellulose and sweeteners such as sodium saccharin may be stirred into the mixture while still under vacuum until complete solvation. Next, hydrogenated castor oil, benzalkonium chloride, and a mixture of propylene glycol and glycyrrhetinic acid may be stirred into the mixture, under the same conditions and in the order listed, until complete dissolution of the components. The mixture may form a gel by being stirred under

vacuum for an additional 30 minutes. Table 4 provides a list of the aforementioned components in preferable concentrations.

[00116] Alternatively, a commercially available oral gel formulation comprising the aforementioned components, such as Gelclair.RTM. (Helsinn Healthcare, Switzerland), may be employed. TABLE-US-00004

Component	% by weight
Sodium hyaluronate	0.1
Glycyrrhetic acid	0.06
Polyvinylpyrrolidone	9.0
Maltodextrin	6.00
Propylene glycol	2.94
Potassium sorbate	0.3
Hydroxyethyl cellulose	1.5
Hydrogenated castor oil	PEG-40 0.27
Disodium EDTA	0.1
Benzalkonium chloride	0.5
Sodium saccharin	0.1
Depurated water	78.60

[00117] The gel may subsequently be combined with one or more nucleic acid expression constructs according to described embodiments. For example, 15 ml of the aforementioned gel may be mixed with 30-50 ml of a liquid suspension of a nucleic acid expression construct. The concentration of the nucleic acid expression construct both in the liquid suspension and in the gel formulation will depend on the type of expression construct employed and the therapeutic use.

5. Matrix

[00118] A matrix is defined herein as a surrounding substance within which something else is contained, such as a pharmaceutical ingredient. Methods pertaining to the formulation of a conducting silicone matrix is set forth in U.S. Pat. No. 6,119,036, which is herein specifically incorporated by reference in its entirety. Also referenced are methods pertaining to formulation of a collagen based matrix, as in Doukas *et al.*, 2001., and Gu *et al.*, 2004.

6. Foam

[00119] A foam is defined herein as is a composition that is formed by trapping many gas bubbles in a liquid. Methods pertaining to the formulation and administration of foams are set forth in U.S. Pat. No. 4,112,942, U.S. Pat. No. 5,652,194, U.S. Pat. No. 6,140,355, U.S. Pat. No. 6,258,374, and U.S. Pat. No. 6,558,043, each of which is herein specifically incorporated by reference in its entirety. A typical foam pharmaceutical formulation may, for example, be constructed by introducing a gas into a gel or aqueous pharmaceutical composition such that bubbles of the gas are within the pharmaceutical composition.

7. Cream and Lotion

[00120] A cream is defined herein as semi-solid emulsion, which is defined herein to refer to a composition that includes a mixture of one or more oils and water. Lotions and creams are considered to refer to the same type of formulation. Methods pertaining to the formulation of creams are set forth in U.S. Pat. No. 6,333,194, U.S. Pat. No. 6,620,451, U.S. Pat. No. 6,261,574, U.S. Pat. No. 5,874,094, and U.S. Pat. No. 4,372,944, each of which is herein specifically incorporated by reference in its entirety.

8. Ointment

[00121] An ointment is defined herein as a viscous semisolid preparation used topically on a variety of body surfaces. Methods pertaining to the formulation of ointments are set forth in U.S. Pat. No. 5,078,993, U.S. Pat. No. 4,868,168, and U.S. Pat. No. 4,526,899, each of which is herein specifically incorporated by reference in its entirety.

[00122] By way of example, an ointment pharmaceutical formulation may comprise approximately 23.75 w/v % isostearyl benzoate, 23.85 w/v % bis(2-ethylhexyl)malate, 10.00 w/v % cyclomethicone, 5.00 w/v % stearyl alcohol, 10.00 w/v % microporous cellulose, 15.00 w/v % ethylene/vinyl acetate copolymer, 0.1 w/v % butylparaben, 0.1 w/v % propylparaben and 2.20 w/v % of the nucleic acid.

9. Gel Strip

[00123] A gel strip is defined herein as a thin layer of gel with elastic properties. The gel may or may not be formulated with an adhesive. The gel may be formulated to slowly dissolve over time. For example, a gel designed for oral application may be designed to dissolve following application.

[00124] Another oral delivery system suitable for use in accordance with the present invention is a dissolvable strip. An example of such a device is the Cool Mint Listerine PocketPaks.RTM. Strips, a micro-thin starch-based film impregnated with ingredients found in Listerine.RTM. Antiseptic (Thymol, Eucalyptol, Methyl Salicylate, Menthol). Non-active strip ingredients include pullulan, flavors, aspartame, potassium acesulfame, copper gluconate, polysorbate 80, carrageenan, glyceryl oleate, locust bean gum, propylene glycol and xanthan gum.

10. Film

[00125] A film is defined herein as a thin sheet or strip of flexible material, such as a cellulose derivative or a thermoplastic resin, coated with a selected pharmaceutical ingredient.

11. Lollipop

[00126] A lollipop is a lozenge attached to one end of a stick that is used as a handle. A pharmaceutical film, lozenge, or lollipop of the present invention may be composed of ingredients, which may include, for example, xanthan gum, locust bean gum, carrageenan and pullulan. The ingredients may be hydrated in purified water and then stored overnight at 4°C., after which, coloring agents, copper gluconate, sweeteners, flavorants and polyoxyethylene sorbitol esters such as polysorbate 80 and Atmos 300.TM. (ICI Co.), and the nucleic acid expression construct may be added to the mixture. A film preparation of the present invention may be made for example, by pouring the aforementioned mixture into a mold and cast as a film, which may then be dried drying and cut into a desired size, depending on desired dosage of the pharmaceutical composition. A film may also be formulated without the addition of sweeteners or flavorants, for example, if the formulation is not contemplated for oral application.

12. Lozenge

[00127] Solid lozenges are well known in the drug delivery field. A lozenge is a small solid of a therapeutic agent and other agents such as binders and sweeteners, that is designed to slowly dissolve when placed in the mouth of a subject. A lozenge may contain other ingredients known in such dosage forms such as acidity regulators, opacifiers, stabilizing agents, buffering agents, flavorings, sweeteners, coloring agents and preservatives. For example, solid formulations may be prepared as lozenges by heating the lozenge base (*e.g.*, a mixture of sugar and liquid glucose) under vacuum to remove excess water and the remaining components are then blended into the mixture. The resulting mixture is then drawn into a continuous cylindrical mass from which the individual lozenges are formed. The lozenges are then cooled, subjected to a visual check and packed into suitable packaging.

[00128] One form of suitable packaging is a blister pack of a water-impermeable plastics material (*e.g.*, polyvinylchloride) closed by a metallic foil. The

patient removes the lozenge by applying pressure to the blister to force the lozenge to rupture and pass through the metal foil seal. Lozenges will normally be sucked by the patient to release the drug. Masticable solid dose formulations may be made by the methods used to prepare chewable candy products or chewing gums. For example, a chewable solid dosage form may be prepared from an extruded mixture of sugar and glucose syrup to which the drug has been added with optional addition of whipping agents, humectants, lubricants, flavors and colorings. See *Pharmaceutical Dosage Forms* (1989). A lollipop (or film or lozenge) of the present invention may be composed of ingredients, which may include, for example, xanthan gum, locust bean gum, carrageenan and pullulan. The ingredients may, for example, be hydrated in purified water and then stored overnight at 4.degree. C., after which, coloring agents, copper gluconate, sweeteners, flavorants and polyoxyethylene sorbitol esters such as polysorbate 80 and Atmos 300.TM. (ICI Co.), and the nucleic acid expression construct may be added to the mixture.

13. Hydrogel

[00129] A hydrogel is defined herein as a network of polymer chains that are sometimes found as a colloidal gel in which water is the dispersion medium. Using the teachings of the specification and the knowledge of those skilled in the art, one can also compose a pharmaceutical formulation as hydrogel such that it may be complexed with a nucleic acid expression construct for topical delivery to a subject. An example of a hydrogel formulation for the delivery of nucleic acids in a viral vector is shown below.

14. Dissolving Strip

[00130] A dissolving strip is defined herein as a film contemplated to dissolve in the presence of an aqueous environment such as a body cavity.

15. Paste and Toothpaste

[00131] A paste is defined herein as a substance that behaves as a solid until a sufficiently large load or stress is applied, at which point it flows like a fluid. A toothpaste is defined herein as a paste or gel used to clean and improve the aesthetic appearance of teeth. A paste dentifrice that may include water, binders, abrasives, flavoring agents, foaming agents, and humectants. Methods pertaining to the formulation of toothpastes are set forth in U.S. Pat. No. 4,627,979, U.S. Pat. No.

6,508,647, U.S. Patent Appn. 20020045148, and U.S. Patent Appn. 20040018155, each of which is herein specifically incorporated by reference in its entirety.

16. Gum

[00132] The present invention also contemplates gum-based pharmaceutical formulation of the present invention may be constructed for oral delivery of a nucleic acid to a subject. By way of example, gum base pellets may be frozen to increase hardness and mechanically ground into a powder form. Subsequently, the gum powder may be elevated to room temperature and mixed with a sweetener, such as fructose or aspartame, comprising approximately 20-65% by weight of the gum-sweetener composition. The gum-sweetener composition may then be supplemented with a liquid suspension of a nucleic acid of the present invention. For instance, the amount of the liquid suspension of the nucleic acid may be approximately equal to 2% by weight of the gum-sweetener composition. The mixture of the gum-sweetener composition and the nucleic acid may then be pressed into a desired shape and administered to a subject. Other methods of formulating a therapeutic agent in a gum are contemplated by the present invention, and are well-known to those of ordinary skill in the art.

17. Diluents and Carriers

[00133] In certain defined embodiments, oral pharmaceutical compositions will comprise an inert diluent and/or assimilable edible carrier, and/or they may be enclosed in hard and/or soft shell gelatin capsule, and/or they may be compressed into tablets, and/or they may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compounds may be incorporated with excipients and/or used in the form of ingestible tablets, buccal tables, troches, capsules, elixirs, suspensions, syrups, wafers, and/or the like.

[00134] Solid forms suitable for solution in, or suspension in, liquid prior to topical use are also contemplated by the present embodiments.

[00135] The solid and semisolid formulations of the present invention may contain the following: a binder, as gum tragacanth, acacia, cornstarch, and/or gelatin; excipients, such as dicalcium phosphate; a disintegrating agent, such as corn starch, potato starch, alginic acid and/or the like; a lubricant, such as magnesium stearate; a fragrance, and/or a sweetening agent, such as sucrose, lactose and/or

saccharin may be added and/or a flavoring agent, such as peppermint, oil of wintergreen, and/or cherry flavoring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings and/or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, and/or capsules may be coated with shellac, sugar and/or both. Preservatives, dyes, and flavorings known to those of ordinary skill in the art are contemplated.

[00136] The solid and semisolid formulations of the present invention contemplated for use on skin surfaces may include other ingredients, which are commonly blended in compositions for cosmetic purposes. For example, such cosmetic ingredients include: waxes, oils, humectants, preservatives, antioxidants, ultraviolet absorbers, ultraviolet scattering agents, polymers, surface active agents, colorants, pigments, powders, drugs, alcohols, solvents, fragrances, flavors, etc, are contemplated. Specific examples of cosmetic compositions include, but are not limited to: make-up cosmetics such as lipstick, lip-gloss, lip balm, skin blemish concealer, and lotion. Methods pertaining to cosmetic formulations designed for use as pharmaceutical carriers are set forth in U.S. Pat. No. 6,967,023, U.S. Pat. No. 6,942,878, U.S. Pat. No. 6,881,776, U.S. Pat. No. 6,939,859 and U.S. Pat. No. 6,673,863, each of which is herein specifically incorporated by reference in its entirety.

18. Aqueous Formulations

[00137] Certain of the pharmaceutical compositions of the present invention can be formulated as aqueous compositions. Aqueous compositions of the present invention comprise an effective amount of the nucleic acid, dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium.

[00138] Administration of certain embodiments of the pharmaceutical compositions set forth herein will be via any common route so long as the target tissue is available via that route. For example, this includes gastric, oral, nasal, or topical. Such compositions would normally be administered as pharmaceutically acceptable compositions that include physiologically acceptable carriers, buffers or other excipients. Examples of other excipients include fragrances and flavorants.

[00139] The formulation may be in a liquid form or suspension. A typical composition for such purpose comprises a pharmaceutically acceptable carrier. For instance, the composition may contain 10 mg, 25 mg, 50 mg or up to about 100 mg of human serum albumin per ml of phosphate buffered saline. Other pharmaceutically acceptable carriers include aqueous solutions, non-toxic excipients, including salts, preservatives, buffers and the like. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oil and injectable organic esters such as ethyloleate. Aqueous carriers include water, alcoholic/aqueous solutions, saline solutions, parenteral vehicles such as sodium chloride, Ringer's dextrose, etc. Preservatives include antimicrobial agents, anti-oxidants, chelating agents and inert gases. The pH and exact concentration of the various components of the pharmaceutical composition are adjusted according to well-known parameters.

[00140] Examples of aqueous compositions for oral administration include a mouthwash, mouthrinse, a coating for application to the mouth via an applicator, or mouthspray. Mouthwash formulations are well-known to those of skill in the art. Formulations pertaining to mouthwashes and oral rinses are discussed in detail, for example, in U.S. Pat. No. 6,387,352, U.S. Pat. No. 6,348,187, U.S. Pat. No. 6,171,611, U.S. Pat. No. 6,165,494, U.S. Pat. No. 6,117,417, U.S. Pat. No. 5,993,785, U.S. Pat. No. 5,695,746, U.S. Pat. No. 5,470,561, U.S. Pat. No. 4,919,918, U.S. Patent Appn. 20040076590, U.S. Patent Appn. 20030152530, and U.S. Patent Appn. 20020044910, each of which is herein specifically incorporated by reference into this section of the specification and all other sections of the specification.

[00141] Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate and/or the like. These compositions take the form of solutions such as mouthwashes and mouthrinses. Such compositions and/or preparations should contain at least 0.1% of active compound. The percentage of the compositions and/or preparations may, of course, be varied and/or may conveniently be between about 2 to about 75% of the weight of the unit, and/or preferably between 25-60%. The amount of active compounds in such therapeutically useful compositions is such that a suitable dosage will be obtained.

[00142] For oral administration the inhibitors, particularly phosphoserine phosphatase siRNAs may be incorporated with excipients and used in

the form of non-ingestible mouthwashes and dentifrices. A mouthwash may be prepared incorporating the active ingredient in the required amount in an appropriate solvent, such as a sodium borate solution (Dobell's Solution). Alternatively, the active ingredient may be incorporated into an antiseptic wash containing sodium borate, glycerin and potassium bicarbonate. The active ingredient also may be dispersed in dentifrices, including: gels, pastes, powders and slurries. The compositions of the present invention may be formulated in a neutral or salt form. Pharmaceutically-acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

[00143] For oral administration the nucleic acids may also be incorporated with dyes to aid in the detection of hypertrophic cells such as toluidene blue O dye and used in the form of non-digestible mouthwashes, oral rinses and dentifrices. A mouthwash may be prepared incorporating the active ingredient in the required amount in an orally administered dye composition, such as a composition of toluidene blue O dye, a buffer, a flavorant, a preservative, acetic acid, ethyl alcohol and water. Methods and formulations pertaining to the use of Toluidene Blue O dye in the staining of precancerous and cancerous lesions may be found in, for example, U.S. Pat. No. 4,321,251, U.S. Pat. No. 5,372,801, U.S. Pat. No. 6,086,852, and U.S. Patent Appn. 20040146919, each of which is specifically incorporated by reference in its entirety.

[00144] By way of example, the pharmaceutical formulation may be administered to a subject using the following steps: 1) the subject gargles and swishes approximately 15 ml of a rinse solution comprising 1% acetic acid and sodium benzoate preservative in water for 20 seconds followed by expectoration, 2) the subject gargles and swishes approximately 15 ml of water for 20 seconds followed by expectoration, 3) the subject gargles and swishes approximately 30 ml of the pharmaceutical formulation for 60 seconds followed by expectoration, 4) step 1 is repeated twice, and 5) step 2 is repeated twice. Other methods of administering these

compositions are contemplated, and are well-known to those of ordinary skill in the art.

[00145] Observations of the oral cavity may be conducted under appropriate magnification and appropriate light immediately after application of the pharmaceutical formulation to examine the oral cavity for the presence of dyed precancerous and cancerous cells. Subsequent observations of the oral cavity may be conducted after a period of time to allow for transduction of the cells of the oral cavity with a nucleic acid of the present invention. Such observations may be conducted under appropriate magnification and appropriate light.

[00146] Examples of aqueous compositions for application to topical surfaces include emulsions or pharmaceutically acceptable carriers such as solutions of the active compounds as free base or pharmacologically acceptable salts, active compounds mixed with water and a surfactant, and emulsions. Emulsions are typically heterogenous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1 μm in diameter (Rosoff, 1988; Block, 1988). Emulsions are often biphasic systems comprising of two immiscible liquid phases intimately mixed and dispersed with each other. In general, emulsions may be either water in oil (w/o) or of the oil in water (o/w) variety. Methods pertaining to emulsions that may be used with the methods and compositions of the present invention set forth in U.S. Pat. No. 6,841,539 and U.S. Pat. No. 5,830,499, each of which is herein specifically incorporated by reference in its entirety. Aqueous compositions for application to the skin may also include dispersions in glycerol, liquid polyethylene glycols and mixtures thereof. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

[00147] The use of liposomes and/or nanoparticles is also contemplated in the present invention. The formation and use of liposomes is generally known to those of skill in the art, and is also described below. Liposomes are also discussed elsewhere in this specification.

[00148] Nanocapsules can generally entrap compounds in a stable and reproducible way. To avoid side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1 μm) should be designed using polymers able to be degraded in vivo. Biodegradable polyalkyl-cyanoacrylate nanoparticles that

meet these requirements are contemplated for use in the present invention, and such particles may be easily made. Methods pertaining to the use of nanoparticles that may be used with the methods and compositions of the present invention include U.S. Pat. No. 6,555,376, U.S. Pat. No. 6,797,704, U.S. Patent Appn. 20050143336, U.S. Patent Appn. 20050196343 and U.S. Patent Appn. 20050260276, each of which is herein specifically incorporated by reference in its entirety.

[00149] One may also use solutions and/or sprays, hyposprays, aerosols and/or inhalants in the present invention for administration. One example is a spray for administration to the aerodigestive tract. The sprays are isotonic and/or slightly buffered to maintain a pH of 5.5 to 6.5. In addition, antimicrobial preservatives, similar to those used in ophthalmic preparations, and/or appropriate drug stabilizers, if required, may be included in the formulation. Methods pertaining to spray administration are set forth in U.S. Pat. No. 6,610,272 U.S. Pat. No. 6,551,578 U.S. Pat. No. 6,503,481, U.S. Pat. No. 5,250,298 and U.S. Pat. No. 5,158,761, each of which is specifically incorporated by reference into this section of the specification and all other sections of the specification.

[00150] Administration of certain embodiments of the aqueous pharmaceutical compositions set forth herein will be via any common route so long as the target tissue is available via that route. For example, this includes oral, nasal, mucosal, or topical. Such compositions would normally be administered as pharmaceutically acceptable compositions that include physiologically acceptable carriers, buffers or other excipients. Examples of other excipients include fragrances and flavorants.

19. Non-Ionic Surfactant Formulations

[00151] The pharmaceutical formulation may be a non-ionic surfactant for topical delivery. Such a formulation may be comprised of, for example, three separate components. The first component can be non-ionic lamellar layer forming surfactant. The second component can be another surfactant. The final component may be a nucleic acid expression construct, such as an adenoviral vector. The nucleic acid expression construct may be either lyophilized or suspended, for example, in distilled phosphate buffered saline and 10% glycerol at pH 7.4.

20. Emulsion Formulations

[00152] Using the teachings of the specification and the knowledge of those skilled in the art, one can also compose a pharmaceutical formulation as an emulsion for topical delivery of a nucleic acid. For instance, the nucleic acid expression construct may be a viral vector, such as an adenoviral vector. One example of an emulsion formulation for the delivery of nucleic acids in a viral vector is as follows: Poly(lactic-glycolic) acid (PLGA) may be dissolved in dichloromethane and mixed with an aqueous suspension of a viral vector. For instance, 1 ml of dichloromethane and 0.05 ml of an aqueous suspension of virus may be used. The solution may then be vortexed for approximately 30 seconds to form a water in oil emulsion. 1 ml of 1% poly vinyl alcohol may then be added to the emulsion and subsequently vortexed for an additional 30 seconds. After the second round of vortexing, the emulsion may then be added to 100 ml of a 0.1% poly vinyl alcohol solution and stirred for an additional 30 minutes. Next, the dichloromethane may be removed by applying a vacuum to the emulsion while stirring for 2.5 hours. After removal of the dichloromethane, the emulsion may then be filtered with 0.2 μm nylon filters and washed with 500 ml of phosphate buffered saline. In the case of emulsions containing viruses, a protective agent may be employed to prevent the denaturation of the viral proteins. Typical protective agents may include, for example, glycerol, sucrose and bovine serum albumin.

21. Nanoparticle Liposome Formulation

[00153] The present invention also includes nanoparticle liposome formulations for topical delivery of a nucleic acid expression construct. For instance, the liposome formulation may comprise DOTAP and cholesterol. An example of such a formulation containing a nucleic acid expression construct is shown below.

[00154] Cationic lipid (DOTAP) may be mixed with the neutral lipid cholesterol (Chol) at equimolar concentrations (Avanti Lipids). The mixed powdered lipids can be dissolved in HPLC-grade chloroform (Mallinckrodt, Chesterfield, Mo.) in a 1-L round-bottomed flask. After dissolution, the solution may be rotated on a Buchi rotary evaporator at 30.degree. C. for 30 min to make a thin film. The flask containing the thin lipid film may then be dried under a vacuum for 15 min. Once drying is complete, the film may be hydrated in 5% dextrose in water (D5W) to give a

final concentration of 20 mM DOTAP and 20 mM cholesterol, referred to as 20 mM DOTAP:Chol. The hydrated lipid film may be rotated in a water bath at 50.degree. C. for 45 min and then at 35.degree. C. for 10 min. The mixture may then be allowed to stand in the parafilm-covered flask at room temperature overnight, followed by sonication at low frequency (Lab-Line, TranSonic 820/H) for 5 min at 50.degree. C. After sonication, the mixture may be transferred to a tube and heated for 10 min at 50.degree. C., followed by sequential extrusion through Whatman (Kent, UK) filters of decreasing size: 1.0, 0.45, 0.2 and 0.1 .mu.m using syringes. Whatman Anotop filters, 0.2 .mu.m and 0.1 .mu.m, may be used. Upon extrusion, the liposomes can be stored under argon gas at 4°C.

22. Popsicle Formulation

[00155] Using the teachings of the specification and the knowledge of those skilled in the art, one can compose a pharmaceutical formulation for delivery of a nucleic acid expression construct as a popsicle for application to the oral cavity or gastrointestinal tract. A popsicle is defined herein as a frozen liquid formulation comprising a hand held applicator such as a stick or a sheath. For instance, the popsicle formulation may comprise a popsicle formulation and a suspension of the selected nucleic acid expression construct. Accordingly, a popsicle formulation may be composed of a frozen solution of a sugar (20% w/v), a flavorant (1.0% w/v), a colorant (0.5% w/v) and an aqueous solution containing a nucleic acid of the present invention (80% w/v). The components of the formulation may be mixed together in liquid form and subsequently frozen in a popsicle mold. Additional examples of popsicle formulations may be found for example in U.S. Pat. No. 5,194,269 and U.S. Pat. No. 5,660,866, each of which is herein specifically incorporated by reference in their entirety.

23. Nucleic Acid Uptake Enhancers

[00156] A "nucleic acid uptake enhancer" is defined herein to refer to any agent or composition of more than one agents that can be applied to the surface of a cell or contacted with the surface of a cell to facilitate uptake of a nucleic acid that is external to the cell. Exemplary agents include cationic lipids. Cationic lipids as nucleic acid uptake enhancers are discussed in greater detail in U.S. Pat. No. 6,670,332, U.S. Pat. No. 6,399,588, U.S. Pat. No. 6,147,055, U.S. Pat. No. 5,264,618,

U.S. Pat. No. 5,459,127, U.S. Pat. No. 5,994,317, and U.S. Pat. No. 5,861,397, each of which is herein specifically incorporated in its entirety. An example of a cationic lipid that can be applied in the methods and compositions of the present invention includes quaternary cytofectin (see U.S. Pat. No. 5,994,317 and U.S. Pat. No. 5,861,397).

E. Combination Treatments

[00157] In one aspect, it is contemplated that an inhibitor described herein may be used in conjunction with other OSA treatment or a different inhibitor. Alternatively, the therapy may precede or follow the other agent treatment by intervals ranging from minutes to weeks. In embodiments where the other agents and/or a proteins or polynucleotides are administered separately, one would generally ensure that a significant period of time did not expire between the time of each delivery, such that the agent and antigenic composition would still be able to exert an advantageously combined effect on the subject. In such instances, it is contemplated that one may administer both modalities within about 12-24 h of each other and, more preferably, within about 6-12 h of each other. In some situations, it may be desirable to extend the time period for administration significantly, however, where several days (2, 3, 4, 5, 6 or 7) to several weeks (1, 2, 3, 4, 5, 6, 7 or 8) lapse between the respective administrations.

[00158] Various combinations may be employed, for example, phosphoserine phosphatase inhibitor therapy is "A" and an inhibitor against another OSA biomarker is "B":

A/B/A	B/A/B	B/B/A	A/A/B	A/B/B	B/A/A	A/B/B/B	B/A/B/B
B/B/B/A	B/B/A/B	A/A/B/B	A/B/A/B	A/B/B/A	B/B/A/A		
B/A/B/A	B/A/A/B	A/A/A/B	B/A/A/A	A/B/A/A	A/A/B/A		

[00159] Administration of a therapeutic composition to a patient/subject will follow general protocols for the administration of such compounds, taking into account the toxicity, if any. It is expected that the treatment cycles would be repeated as necessary. It also is contemplated that various standard therapies, such as hydration, may be applied in combination with the described therapy.

F. Diagnostic Methods

[00160] A patient whose adenoid and/or tonsillar cells differentially express a OSA biomarker identifies the patient as having or at risk for OSA. It is contemplated that one or more standards may be generated and/or referred to in which normal levels of expression are defined or identified. For example, the standard may be the level of expression observed in patients who do not have OSA. That standard may then be referred to as a way of determining whether expression in a given patient is normal or above-normal. The type of standard generated will depend upon the assay or test employed to evaluate expression. Levels of RNA or protein may be assessed. In some embodiments of the invention, a score is assigned to a sample based on certain criteria and numbers within or above a certain number or range are deemed "above normal" or "below normal."

[00161] In certain embodiments, expression of an OSA biomarker is considered above normal if an assay indicates that a particular measurement, amount or level is at about or at most about 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 400%, 500%, 600%, 700%, 800%, 900% or above the measurement, amount or level observed in cells that have normal levels of the relevant OSA biomarker. In other words, for example, a cell with normal expression exhibit a level of transcripts that is x; the sample from the patient being tested may be 2.5x, in which case, in some embodiments that patient may be considered to have a below normal level of transcript and thus an above normal level of expression. Alternatively, in some embodiments, expression is considered above normal if an assay indicates that a particular measurement, amount or level is about or at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more standard deviations above the measurement, amount or level observed in cells that have normal levels of expression. In other cases, expression may be considered above normal if a measurement, amount or level indicative of expression is or is at most 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50 or more times greater than the measurement, amount, or level indicative of expression in normal cells.

[00162] In certain embodiments, expression of an OSA biomarker is considered below normal if an assay indicates that a particular measurement, amount or level is at about or at most about 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 400%, 500%, 600%, 700%, 800%, 900% or below the measurement,

amount or level observed in cells that have normal levels of the relevant OSA biomarker. In other words, for example, a cell with normal expression exhibit a level of transcripts that is x ; the sample from the patient being tested may be $2.5x$ less, in which case, in some embodiments that patient may be considered to have an above normal level of transcript and thus a below normal level of expression. Alternatively, in some embodiments, expression is considered below normal if an assay indicates that a particular measurement, amount or level is about or at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or fewer standard deviations above the measurement, amount or level observed in cells that have normal levels of expression. In other cases, expression may be considered below normal if a measurement, amount or level indicative of expression is or is at most 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50 or more times less than the measurement, amount, or level indicative of expression in normal cells.

[00163] Methods of the invention that involve evaluating the expression of an OSA biomarker in cells can be achieved by a number of ways that directly or indirectly provide information regarding their expression. Thus, ways of evaluating expression include, but are not limited to, assessing or measuring the corresponding protein, assessing or measuring the corresponding transcript, sequencing the corresponding transcript or genomic sequence, and assaying activity of the gene product of an OSA biomarker.

[00164] In some embodiments, methods involve evaluating expression in cells by assessing protein or transcript levels. The term “assessing” is used according to its ordinary and plain meaning to refer to “determining the extent of.” It is contemplated that methods may involve directly measuring or directly determining the level of expression. This may involve the use of specific machines or apparatuses. In addition, this will involve some type of chemical transformation. In certain embodiments, the level of protein or transcript is assessed by assaying (measuring) the amount of protein, transcript, or gene copy in the cells. In specific aspects of the invention, expression is evaluated by assessing an OSA biomarker protein. An anti-PBEF antibody can be used in some cases to assess an OSA biomarker protein. Such methods may involve using immunohistochemistry, Western blotting, ELISA, immunoprecipitation, or an antibody array. In particular embodiments, protein is assessed using immunohistochemistry. The use of immunohistochemistry allows for

quantitation and characterization of protein. It also allows an immunoreactive score for the sample to be determined. The term “immunoreactive score” (IRS) refers to a number that is calculated based on a scale reflecting the percentage of positive cells (on a scale of 1-4, where 0 = 0%, 1 = <10%, 2 = 10%-50%, 3 = >50%-80%, and 4 = >80%) multiplied by the intensity of staining (on a scale of 1-3, where 1= weak, 2= moderate, and 3= strong). Immunoreactive scores range from 0-12.

[00165] In some embodiments of the invention, expression is evaluated by assessing transcription. Transcription can be assessed by a variety of methods including those that involve amplifying transcripts or performing Northern blotting on transcripts. Amplification of transcripts can be utilized in quantitative polymerase chain reactions, which are well known to those of ordinary skill in the art. Alternatively, nuclease protection assays may be implemented to quantify transcripts. Other methods that take advantage of hybridization between a probe and target are also contemplated, such as fluorescence in situ hybridization (FISH) and RNA in situ hybridization (RISH). In another embodiment of the invention, RNA expression is measured using microarrays which can be manufactured containing either global genomic sequence content or disease-specific biomarkers.

[00166] Further embodiments of the invention involve evaluating expression by assaying the level of OSA biomarker activity. It is contemplated that levels are assayed from a sample from the patient. In embodiments of the invention a sample from a patient refers to a biological sample, which includes, but is not limited to a tissue biopsy or section, blood sample, lavage, swab, scrape, or other composition that may be extracted from the body and that contains cells. In particular embodiments, a sample contains adenoid and/or tonsillar cells.

[00167] Some embodiments concern polynucleotides and oligonucleotides, isolatable from cells, that are free from total genomic DNA and that are capable of expressing all or part of a protein or polypeptide. The polynucleotides or oligonucleotides may be identical or complementary to all or part of a nucleic acid sequence for an OSA biomarker. These nucleic acids may be used directly or indirectly to assess, evaluate, quantify, or determine expression.

[00168] A nucleic acid encoding all or part of an OSA biomarker sequence is contemplated for use with some embodiments. In certain embodiments

there is a nucleic acid that is identical or complementary to 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 441, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1010, 1020, 1030, 1040, 1050, 1060, 1070, 1080, 1090, 1095, 1100, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 9000, 10000, or more contiguous nucleotides, nucleosides, or base pairs (or any range derivable therein), including such sequences from SEQ ID NO:1 or SEQ ID NOs:3-224 (which means SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, through SEQ ID NO:224).

[00169] The various probes and primers designed around the nucleotide sequences of the present invention may be of any length, such as described above. By assigning numeric values to a sequence, for example, the first residue is 1, the second residue is 2, *etc.*, an algorithm defining all primers can be proposed: n to $n + y$, where n is an integer from 1 to the last number of the sequence and y is the length of the primer minus one, where $n + y$ does not exceed the last number of the sequence. Thus, for a 10-mer, the probes correspond to bases 1 to 10, 2 to 11, 3 to 12 ... and so on. For a 15-mer, the probes correspond to bases 1 to 15, 2 to 16, 3 to 17 ... and so on. For a 20-mer, the probes correspond to bases 1 to 20, 2 to 21, 3 to 22 ... and so on.

[00170] The use of a probe or primer of between 13 and 100 nucleotides, preferably between 17 and 100 nucleotides in length, or in some aspects of the invention up to 1-2 kilobases or more in length, allows the formation of a duplex molecule that is both stable and selective. Such probes or primers can be of lengths described above from any sequence provided herein, including the sequence information provided for the OSA biomarkers. Molecules having complementary sequences over contiguous stretches greater than 20 bases in length are generally preferred, to increase stability and/or selectivity of the hybrid molecules obtained.

One will generally prefer to design nucleic acid molecules for hybridization having one or more complementary sequences of 20 to 30 nucleotides, or even longer where desired. Such fragments may be readily prepared, for example, by directly synthesizing the fragment by chemical means or by introducing selected sequences into recombinant vectors for recombinant production.

[00171] Probes may be complementary (also referred to as “complementarity”) or identical to at least or at most 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 441, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, or 1000 contiguous bases, or any range derivable therein, of sequences (or their complements) disclosed herein. In some embodiments, the sequence is any of SEQ ID NO:1 or SEQ ID NOs:3-224. Alternatively, the sequence may be identical or complementary to a sequence of an OSA biomarker. Accordingly, the nucleotide sequences of the invention may be used for their ability to selectively form duplex molecules with complementary stretches of DNAs and/or RNAs or to provide primers for amplification of DNA or RNA from samples. Depending on the application envisioned, one would desire to employ varying conditions of hybridization to achieve varying degrees of selectivity of the probe or primers for the target sequence.

[00172] For applications requiring high selectivity, one will typically desire to employ relatively high stringency conditions to form the hybrids. For example, relatively low salt and/or high temperature conditions, such as provided by about 0.02 M to about 0.10 M NaCl at temperatures of about 50°C to about 70°C. Such high stringency conditions tolerate little, if any, mismatch between the probe or primers and the template or target strand and would be particularly suitable for isolating specific genes or for detecting specific mRNA transcripts. It is generally

appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide.

[00173] For certain applications, for example, site-directed mutagenesis, it is appreciated that lower stringency conditions are preferred. Under these conditions, hybridization may occur even though the sequences of the hybridizing strands are not perfectly complementary, but are mismatched at one or more positions. Conditions may be rendered less stringent by increasing salt concentration and/or decreasing temperature. For example, a medium stringency condition could be provided by about 0.1 to 0.25 M NaCl at temperatures of about 37°C to about 55°C, while a low stringency condition could be provided by about 0.15 M to about 0.9 M salt, at temperatures ranging from about 20°C to about 55°C. Hybridization conditions can be readily manipulated depending on the desired results.

[00174] In other embodiments, hybridization may be achieved under conditions of, for example, 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl₂, 1.0 mM dithiothreitol, at temperatures between approximately 20°C to about 37°C. Other hybridization conditions utilized could include approximately 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, at temperatures ranging from approximately 40°C to about 72°C.

[00175] In certain embodiments, it will be advantageous to employ nucleic acids of defined sequences of the present invention in combination with an appropriate means, such as a label, for determining hybridization. A wide variety of appropriate indicator means are known in the art, including fluorescent, radioactive, enzymatic or other ligands, such as avidin/biotin, which are capable of being detected. In preferred embodiments, one may desire to employ a fluorescent label or an enzyme tag such as urease, alkaline phosphatase or peroxidase, instead of radioactive or other environmentally undesirable reagents. In the case of enzyme tags, colorimetric indicator substrates are known that can be employed to provide a detection means that is visibly or spectrophotometrically detectable, to identify specific hybridization with complementary nucleic acid containing samples.

[00176] In general, it is envisioned that the probes or primers described herein will be useful as reagents in solution hybridization, as in PCR™, for detection of expression of corresponding genes, as well as in embodiments employing a solid

phase. In embodiments involving a solid phase, the test DNA (or RNA) is adsorbed or otherwise affixed to a selected matrix or surface. This fixed, single-stranded nucleic acid is then subjected to hybridization with selected probes under desired conditions. The conditions selected will depend on the particular circumstances (depending, for example, on the G+C content, type of target nucleic acid, source of nucleic acid, size of hybridization probe, *etc.*). Optimization of hybridization conditions for the particular application of interest is well known to those of skill in the art. After washing of the hybridized molecules to remove non-specifically bound probe molecules, hybridization is detected, and/or quantified, by determining the amount of bound label. Representative solid phase hybridization methods are disclosed in U.S. Patents 5,843,663, 5,900,481 and 5,919,626. Other methods of hybridization that may be used in the practice of the present invention are disclosed in U.S. Patents 5,849,481, 5,849,486 and 5,851,772. The relevant portions of these and other references identified in this section of the Specification are incorporated herein by reference.

[00177] Nucleic acids used as a template for amplification may be isolated from cells, tissues or other samples according to standard methodologies (Sambrook *et al.*, 2001). In certain embodiments, analysis is performed on whole cell or tissue homogenates or biological fluid samples without substantial purification of the template nucleic acid. The nucleic acid may be genomic DNA or fractionated or whole cell RNA. Where RNA is used, it may be desired to first convert the RNA to a complementary DNA.

[00178] The term “primer,” as used herein, is meant to encompass any nucleic acid that is capable of priming the synthesis of a nascent nucleic acid in a template-dependent process. Typically, primers are oligonucleotides from ten to twenty and/or thirty base pairs in length, but longer sequences can be employed. Primers may be provided in double-stranded and/or single-stranded form, although the single-stranded form is preferred.

[00179] Pairs of primers are designed to selectively hybridize to a nucleic acid corresponding to SEQ ID NO:1 or any other OSA biomarker identified in Table 1. These primer pairs are contacted with the template nucleic acid under conditions that permit selective hybridization. Depending upon the desired application, high stringency hybridization conditions may be selected that will only

allow hybridization to sequences that are completely complementary to the primers. In other embodiments, hybridization may occur under reduced stringency to allow for amplification of nucleic acids contain one or more mismatches with the primer sequences. Once hybridized, the template-primer complex is contacted with one or more enzymes that facilitate template-dependent nucleic acid synthesis. Multiple rounds of amplification, also referred to as “cycles,” are conducted until a sufficient amount of amplification product is produced.

[00180] The amplification product may be detected or quantified. In certain applications, the detection may be performed by visual means. Alternatively, the detection may involve indirect identification of the product via chemiluminescence, radioactive scintigraphy of incorporated radiolabel or fluorescent label or even via a system using electrical and/or thermal impulse signals (Bellus, 1994).

[00181] A number of template dependent processes are available to amplify the oligonucleotide sequences present in a given template sample. One of the best known amplification methods is the polymerase chain reaction (referred to as PCRTM) which is described in detail in U.S. Patents 4,683,195, 4,683,202 and 4,800,159, and in Innis *et al.*, 1988, each of which is incorporated herein by reference in their entirety.

[00182] A reverse transcriptase PCRTM amplification procedure may be performed to quantify the amount of mRNA amplified. Methods of reverse transcribing RNA into cDNA are well known (see Sambrook *et al.*, 2001). Alternative methods for reverse transcription utilize thermostable DNA polymerases. These methods are described in WO 90/07641. Polymerase chain reaction methodologies are well known in the art. Representative methods of RT-PCR are described in U.S. Patent 5,882,864.

[00183] Another method for amplification is ligase chain reaction (“LCR”), disclosed in European Application No. 320 308, incorporated herein by reference in its entirety. U.S. Patent 4,883,750 describes a method similar to LCR for binding probe pairs to a target sequence. A method based on PCRTM and oligonucleotide ligase assay (OLA), disclosed in U.S. Patent 5,912,148, may also be used. Alternative methods for amplification of target nucleic acid sequences that may

be used in the practice of the present invention are disclosed in U.S. Patents 5,843,650, 5,846,709, 5,846,783, 5,849,546, 5,849,497, 5,849,547, 5,858,652, 5,866,366, 5,916,776, 5,922,574, 5,928,905, 5,928,906, 5,932,451, 5,935,825, 5,939,291 and 5,942,391, GB Application No. 2 202 328, and in PCT Application No. PCT/US89/01025, each of which is incorporated herein by reference in its entirety. Qbeta Replicase, described in PCT Application No. PCT/US87/00880, may also be used as an amplification method in the present invention. In this method, a replicative sequence of RNA that has a region complementary to that of a target is added to a sample in the presence of an RNA polymerase. The polymerase will copy the replicative sequence which may then be detected.

[00184] An isothermal amplification method, in which restriction endonucleases and ligases are used to achieve the amplification of target molecules that contain nucleotide 5'-[alpha-thio]-triphosphates in one strand of a restriction site may also be useful in the amplification of nucleic acids in the present invention (Walker *et al.*, 1992). Strand Displacement Amplification (SDA), disclosed in U.S. Patent 5,916,779, is another method of carrying out isothermal amplification of nucleic acids which involves multiple rounds of strand displacement and synthesis, *i.e.*, nick translation.

[00185] Following any amplification or step such as primer extension, it may be desirable to separate the amplification or primer extension product from the template and/or the excess primer. In one embodiment, amplification products are separated by agarose, agarose-acrylamide or polyacrylamide gel electrophoresis using standard methods (Sambrook *et al.*, 2001). Separated amplification products may be cut out and eluted from the gel for further manipulation. Using low melting point agarose gels, the separated band may be removed by heating the gel, followed by extraction of the nucleic acid.

[00186] Separation of nucleic acids may also be effected by chromatographic techniques known in art. There are many kinds of chromatography which may be used in the practice of the present invention, including adsorption, partition, ion-exchange, hydroxylapatite, molecular sieve, reverse-phase, column, paper, thin-layer, and gas chromatography as well as HPLC.

[00187] In certain embodiments, the amplification products are visualized. A typical visualization method involves staining of a gel with ethidium bromide and visualization of bands under UV light. Alternatively, if the amplification products are integrally labeled with radio- or fluorometrically-labeled nucleotides, the separated amplification products can be exposed to x-ray film or visualized under the appropriate excitatory spectra.

[00188] In one embodiment, following separation of amplification products, a labeled nucleic acid probe is brought into contact with the amplified marker sequence. The probe preferably is conjugated to a chromophore but may be radiolabeled. In another embodiment, the probe is conjugated to a binding partner, such as an antibody or biotin, or another binding partner carrying a detectable moiety.

[00189] In particular embodiments, detection is by Southern blotting and hybridization with a labeled probe. The techniques involved in Southern blotting are well known to those of skill in the art (see Sambrook *et al.*, 2001). One example of the foregoing is described in U.S. Patent 5,279,721, incorporated by reference herein, which discloses an apparatus and method for the automated electrophoresis and transfer of nucleic acids. The apparatus permits electrophoresis and blotting without external manipulation of the gel and is ideally suited to carrying out methods according to the present invention.

[00190] Other methods of nucleic acid detection that may be used in the practice of the instant invention are disclosed in U.S. Patents 5,840,873, 5,843,640, 5,843,651, 5,846,708, 5,846,717, 5,846,726, 5,846,729, 5,849,487, 5,853,990, 5,853,992, 5,853,993, 5,856,092, 5,861,244, 5,863,732, 5,863,753, 5,866,331, 5,905,024, 5,910,407, 5,912,124, 5,912,145, 5,919,630, 5,925,517, 5,928,862, 5,928,869, 5,929,227, 5,932,413 and 5,935,791, each of which is incorporated herein by reference.

[00191] Reverse transcription (RT) of RNA to cDNA followed by relative quantitative PCR™ (RT-PCR) can be used to determine the relative concentrations of specific mRNA species isolated from a cell, such as an OSA-biomarker-encoding transcript. By determining that the concentration of a specific mRNA species varies, it is shown that the gene encoding the specific mRNA species is differentially expressed.

[00192] Specifically contemplated are chip-based DNA technologies such as those described by Hacia *et al.* (1996) and Shoemaker *et al.* (1996). Briefly, these techniques involve quantitative methods for analyzing large numbers of genes rapidly and accurately. By tagging genes with oligonucleotides or using fixed probe arrays, one can employ chip technology to segregate target molecules as high density arrays and screen these molecules on the basis of hybridization (see also, Pease *et al.*, 1994; and Fodor *et al.*, 1991). It is contemplated that this technology may be used in conjunction with evaluating the expression level of an OSA biomarker with respect to diagnostic methods of the invention.

III. SCREENING METHODS

[00193] Embodiments also concern methods for identifying OSA inhibitors, including an inhibitor of phosphoserine phosphatase. These assays may comprise random screening of large libraries of candidate substances; alternatively, the assays may be used to focus on particular classes of compounds selected with an eye towards structural attributes that are believed to make them more likely to modulate the function of one or more OSA biomarker gene or its gene product (transcript and/or protein). In certain embodiments, screening methods are applied to identify an inhibitor of phosphoserine phosphatase.

[00194] In some embodiments, one may assay for a measurable effect on the activity of the OSA biomarker protein. In other embodiments, one may assay for a measurable effect on the phenotype of a adenoid or tonsillar cell. In some cases, the cell is from an OSA patient. To identify an inhibitor, one generally will determine the activity of the OSA biomarker protein in the presence and absence of the candidate substance, wherein a modulator is defined as any substance that alters these characteristics. For example, a method generally comprises:

- (a) providing a candidate modulator;
- (b) admixing the candidate modulator with an isolated compound or cell expressing the compound;
- (c) measuring one or more characteristics of the compound or cell in step (b); and

- (d) comparing the characteristic measured in step (c) with the characteristic of the compound or cell in the absence of said candidate modulator,

wherein a difference between the measured characteristics indicates that said candidate modulator is, indeed, a modulator of the compound or cell.

[00195] Alternatively, cells or tissue with OSA may be compared to cells or tissue that are not subject to OSA in the presence of the candidate compound. Assays may be conducted in cell free systems, in isolated cells, or in organisms including transgenic animals.

[00196] It will, of course, be understood that all the screening methods are useful in themselves notwithstanding the fact that effective candidates may not be found. The invention provides methods for screening for such candidates, not solely methods of finding them.

[00197] As used herein the term “candidate substance” refers to any molecule that may be a “inhibitor”, *i.e.*, potentially affect the activity of an OSA biomarker protein directly.

[00198] One may acquire, from various commercial sources, small molecule libraries that are believed to meet the basic criteria for useful drugs in an effort to “brute force” the identification of useful compounds. Screening of such libraries, including combinatorially generated libraries (*e.g.*, peptide libraries), is a rapid and efficient way to screen large number of related (and unrelated) compounds for activity. Combinatorial approaches also lend themselves to rapid evolution of potential drugs by the creation of second, third and fourth generation compounds modeled of active, but otherwise undesirable compounds.

[00199] Candidate compounds may include fragments or parts of naturally-occurring compounds, or may be found as active combinations of known compounds, which are otherwise inactive. It is proposed that compounds isolated from natural sources, such as animals, bacteria, fungi, plant sources, including leaves and bark, and marine samples may be assayed as candidates for the presence of potentially useful pharmaceutical agents. It will be understood that the pharmaceutical agents to be screened could also be derived or synthesized from chemical compositions or man-made compounds. Thus, it is understood that the

candidate substance identified by the present invention may be peptide, polypeptide, polynucleotide, small molecule inhibitors or any other compounds that may be designed through rational drug design starting from known inhibitors or stimulators.

[00200] Other suitable modulators include antisense molecules, ribozymes, and antibodies (including single chain antibodies), each of which would be specific for the target molecule. Such compounds are well known to those of skill in the art. For example, an antisense molecule that bound to a translational or transcriptional start site, or splice junctions, would be ideal candidate inhibitors.

[00201] A quick, inexpensive and easy assay to run is an *in vitro* assay. Such assays generally use isolated molecules, can be run quickly and in large numbers, thereby increasing the amount of information obtainable in a short period of time. A variety of vessels may be used to run the assays, including test tubes, plates, dishes and other surfaces such as dipsticks or beads.

[00202] One example of a cell free assay is a binding assay. While not directly addressing function, the ability of a modulator to bind to a target molecule in a specific fashion is strong evidence of a related biological effect. For example, binding of a molecule to a target may, in and of itself, be inhibitory, due to steric, allosteric or charge-charge interactions. The target may be either free in solution, fixed to a support, expressed in or on the surface of a cell. Either the target or the compound may be labeled, thereby permitting determining of binding. Usually, the target will be the labeled species, decreasing the chance that the labeling will interfere with or enhance binding. Competitive binding formats can be performed in which one of the agents is labeled, and one may measure the amount of free label versus bound label to determine the effect on binding.

[00203] A technique for high throughput screening of compounds is described in WO 84/03564. Large numbers of small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. Bound polypeptide is detected by various methods.

IV. EXAMPLES

[00204] The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion. One skilled in the art will appreciate readily that the

present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those objects, ends and advantages inherent herein. The present examples, along with the methods described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses which are encompassed within the spirit of the invention as defined by the scope of the claims will occur to those skilled in the art.

EXAMPLE 1

MATERIALS AND METHODS

Patients

[00205] The study was approved by the University of Louisville Human Research Committee, and informed consent was obtained from the legal caregiver of each participant, with assent being obtained from children older than 7 years of age. Consecutive prepubertal non-obese children diagnosed with OSA at the University of Louisville Pediatric Sleep Research Center in Louisville, KY, invited to participate. Inclusion criteria were the presence of OSA according to polysomnographic criteria and age between 6 and 11 years. Exclusion criteria were any children with chronic medical condition, receiving medications, and any genetic or craniofacial syndromes. Age, gender, and ethnicity; use of medications, antihistamines, and presence of comorbidities were gathered from each participant. Details about polysomnographic and demographic characteristics of the children are provided in Table 2 below.

[00206] Children with recurrent tonsillar infection (RI) were selected based on a history of at least five tonsillar infections requiring administration of antibiotic courses over a period of less than 6 months, as well as the absence of any symptoms suggestive of OSA using a previously validated questionnaire (Montgomery-Downs *et al.*, 2004). Patients referred for RI were selected based on a history of at least five tonsillar infections in < 6 months and the absence of any symptoms suggestive of OSA using a previously validated questionnaire (Montgomery-Downs *et al.*, 2006). However, to further ascertain the absence of sleep-disordered breathing they were evaluated by an overnight sleep study.

Polysomnographic assessment

[00207] Children were studied for up to 12 hours in a quiet, darkened room with an ambient temperature of 24°C in the company of one of their parents. No drugs were used to induce sleep. The following parameters were measured during the overnight sleep recordings: chest and abdominal wall movement by respiratory impedance or inductance plethysmography; heart rate by ECG; and air flow, which was triply monitored with a side-stream end-tidal capnograph that also provided breath-by-breath assessment of end-tidal carbon dioxide levels (PET_{CO₂}; BCI SC-300, Menomonee Falls, Wis), a nasal pressure cannula, and an oronasal thermistor. Arterial oxygen saturation (SpO₂) was assessed by pulse oximetry (Nellcor N 100; Nellcor Inc, Hayward, Calif), with simultaneous recording of the pulse waveform. The bilateral electro-oculogram, 8 channels of electroencephalogram, chin and anterior tibial electromyograms, and analog output from a body-position sensor (Braebon Medical Corp, Ogdensburg, NY) were also monitored. All measures were digitized with a commercially available polysomnography system (Rembrandt, MedCare Diagnostics, Amsterdam, Netherlands). Tracheal sound was monitored with a microphone sensor (Sleepmate, Midlothian, Va), and a digital time-synchronized video recording was performed.

[00208] The proportion of time spent in each sleep stage was expressed as percentage of total sleep time. Obstructive apnea was defined as the absence of airflow with continued chest wall and abdominal movement for duration of at least 2 breaths (Montgomery-Downs *et al.*, 2006; Standards and Indications for Cardiopulmonary Sleep Studies in Children, 1996). Hypopneas were defined as a decrease in oronasal flow of $\geq 50\%$ with a corresponding decrease in SpO₂ of 4% and/or arousal (Montgomery-Downs *et al.*, 2006). The obstructive apnea/hypopnea index was defined as the number of apneas and hypopneas per hour of total sleep time. The obstructive apnea index was defined as the number of apneas per hour of total sleep time. The diagnostic criteria for OSA included an obstructive apnea index >1 per hour of total sleep time and/or an obstructive apnea-hypopnea index >5 per hour of total sleep time with a nadir oxygen saturation value <92% (Montgomery-Downs *et al.*, 2006). The diagnosis of OSA was established by overnight polysomnography in the sleep laboratory and required the presence of an apnea-hypopnea index more than five events per hour of sleep (Goodwin *et al.*, 2003).

Body mass index

[00209] Height and weight were obtained using standard techniques from each child. BMI was then calculated (body mass/height²) and was expressed as BMI z-score using an online BMI z score calculator (<http://www.cdc.gov/epiinfo/>). Children with BMI z-score values exceeding 1.20 were classified as fulfilling the criteria for overweight/obesity (Kuczmarski *et al.*, 2000), and were excluded from this study.

Statistical analysis

[00210] Results are presented as mean $\pm$ SD unless stated otherwise. All analyses were conducted using statistical software (version 11.5; SPSS; Chicago, IL). Comparisons according to group assignment were made with independent *t* tests or analysis of variance followed by *post hoc* comparisons, with *p* values adjusted for unequal variances when appropriate (Levene test for equality of variances), or χ^2 analyses with Fisher's exact test (dichotomous outcomes). A two-tailed *P*-value < 0.05 was considered statistically significant.

Tonsillar tissue collection

[00211] Since tonsils cannot be obtained from normal children for obvious ethical reasons, consecutive children undergoing tonsillectomy at Kosair Children's Hospital for either OSA or RI were identified before surgery and recruited to the study. OSA and RI children were also required to have received their last dose of antibiotic therapy at least 6 wk before the day of the surgery. Children with OSA were excluded if they suffered from RI (based on aforementioned criteria). Children with known asthma, allergic rhinitis, history of allergies, and/or having received corticosteroid or leukotriene modifier therapy within 12 months from surgery were excluded (for both groups). Tonsils were removed by a pediatric ENT specialist, and a portion of each tonsil was stored in RNALater (Applied Biosystems/Ambion Woodward St. Austin, TX) as recommended by the manufacturer protocol, and stored at -80°C.

Tonsil Immunohistochemistry

[00212] Tonsils were placed overnight in a fixative containing 1% paraformaldehyde in PBS and 30% sucrose at 4°C. Post-fixed tissues were sectioned on a freezing microtome. Coronal sections (30 microns) of tonsils were initially incubated in 0.3% H₂O₂ for 30 minutes, washed several times in PBS, and blocked with a PBS/0.4% Triton X-100/0.5%TSA (Tyramide Signal Amplification, Perkin

Elmer Life Sciences, Boston, MA) blocking reagent/10% normal goat serum (Vector Laboratories, Burlingame CA) for 1 hour. Sections were then incubated with primary PSPH antisera (Abcam cta# ab58125; 1:1000) at 4 °C for 24 hours, and then washed in PBS 6 times for 5 minutes each wash. Sections were then incubated at room temperature for 1 hour in biotinylated anti-rabbit antibody (Vectastain Elite ABC kit, Burlingame CA; 1:600) in a PBS/0.5% TSA blocking reagent /10% goat serum solution. After 3 5-min washes, sections were incubated at room temperature with streptavidin-horseradish peroxidase diluted 1:100 in PBS/0.5% TSA blocking reagent. Subsequently, the sections were incubated with tetramethyl rhodamine tyramide (red) diluted 1:50 in amplification diluent (Perkin Elmer Life Sciences , Boston, MA) for 2 minutes. Sections were then washed in PBS, and mounted onto glass slides. Negative controls were prepared by either omitting the primary or the secondary antibody. Sections were prepared from 5 sets of tonsils and of adenoids from either OSA or RI groups, and were visualized using a fluorescent microscope by an investigator who was blinded to the sample source.

Mixed adenotonsillar primary cell culture system

[00213] Surgically removed tonsils and adenoids were placed in ice cold phosphate buffered saline (PBS) plus antibiotics and processing was started within 30 minutes after surgical excision under aseptic conditions. Briefly, tonsils were washed thoroughly with PBS, manually dissected into Petri dishes, and gently grounded with a syringe plunger through a 70 µm mesh screen to obtain a mixed cell suspension through mechanical dissociation. Red blood cells were removed by lysis buffer. Cells viability of all specimens was determined by trypan blue exclusion. Specimens with a viability of less than 70% were discarded. Cells cultures were established in standard medium RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS) plus antibiotics, which included streptomycin, fungisone, gentamycin, and penicillin to prevent bacterial and fungal contamination. Mixed cell suspensions were transferred onto 24-well or 96-round bottom-well plates at a concentration of 1×10^6 cells/well. Cells were cultured in a 5 % CO₂ incubator at 37°C for 48 hours. Cells were incubated to evaluate basal proliferation or treated with PSPH inhibitors such as okadaic acid, Calyculin A, and PPI2 at 10^{-6} M to 10^{-9} concentrations. Similarly, cells were transfected with a cocktail of 3 commercially available PSPH siRNA (Ambien; s11428, s11429, and s11430), and following optimization procedures, proliferation was assessed.

BrdU cell proliferation and apoptosis annexin V assays with flow cytometry

[00214] To detect global cell, T-cell and B-cell specific proliferation, we employed bromodeoxyuridine (BrdU) pulsed proliferation analysis using flow cytometry. All procedures were measured using the APC BrdU flow kit (BD Biosciences, San Diego, CA) as previously described (Kim *et al.*, 2009; Kheirandish-Gozal *et al.*, 2009) and as recommended by the manufacturer. In brief, at the end of 48 hours of cell culture in 24-well plates, cells were pulse-labeled with 1 mM BrdU for 4 hours. The cells were then washed with PBS, and BrdU labeled cells were stained with a 3-color antibody combination consisting of mouse anti-human CD45/PerCP Cy7, CD3/PE, and CD19/APC-Cy7 antibodies (BD Biosciences, San Diego, CA) in 50 µl staining buffer for 15 min on ice. Following binding, the cell-surface antibodies, cells were fixed and permeabilized with cytofix/cytoperm buffer. After this procedure, cells were suspended with DNase (300 µg/ml) for 1 hour at 37°C. The anti-BrdU APC antibody was added in perm/wash buffer and incubated for 20 min at room temperature. Isotype controls relevant for each of the antibodies were used to establish background fluorescence. Negative control was used as a sample that was untreated with BrdU and was not stained with specific fluorescence antibodies. Data were acquired on a FACS Aria flow cytometer using the FACS Diva 5.5 software (BD Biosciences, San Diego, CA). After gating of lymphocytes based on CD45+ cells, T-cell and B-cell numbers were calculated as CD3+/CD19- and CD3-/CD19+ cell populations, respectively. Proliferation of T-cells and B-cells was identified by counting CD3+/BrdU+ and CD19+/BrdU+ cell populations. A similar approach was undertaken using Annexin V mouse anti-human antibodies to quantify global, T-cell or B-cell specific apoptosis. The results were displayed as two color dot-plots and analyzed by FlowJo software (Tree Star, San Carlos, CA). All data are expressed as the percentage of positive cell from the total cell population.

RNA isolation and microarray hybridization

[00215] Total RNA from tonsils of 18 children with RI and 18 children with OSA was isolated using RNeasy Lipid Tissue Mini Kit with DNase treatment (Qiagen, Valencia, CA). Tissues were homogenized in 1 ml of QIAzol Lysis Reagent (Qiagen, Valencia, CA) using a PolyTron homogenizer. RNA integrity was assessed for each sample using the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA), cRNA was generated, fluorescently labeled with Cyanine 3-dCTP (Perkin Elmer, Boston, MA), and hybridized to the Agilent human whole-genome arrays

containing 44,000 transcripts. Microarrays were scanned (SureScan, Agilent Technologies) followed by image processing and filtering using Agilent Feature Extraction software. Further details are reported below.

[00216] The genes responsible for tonsillar hypertrophy in children with OSA were systematically identified. Initially, the filtered gene expression intensities of all 36 subjects (OSA and RI) were normalized and log-transformed. Intensities of multiple probes mapping to the same gene were averaged, resulting in ~31,000 unique gene expression values. Enriched pathways in tonsillar tissue from children with OSA were identified using gene set enrichment analysis (Subramanian *et al.*, 2005) (GSEA). Approximately 1800 gene sets were computationally sampled and a random permutation analysis of the subjects ($n = 1000$) was performed to determine enrichment of gene sets in each group at a false discovery rate cutoff $\leq 10\%$.

Gene interaction network analysis

[00217] Genes mapped to gene sets enriched in OSA subjects and involved in proliferative pathways were combined, and an interaction network was created using Ingenuity System's knowledge base (Calvano *et al.*, 2005) and several publicly available gene product relationship databases (Alfarano *et al.*, 2005; Peri *et al.*, 2004; Salwinski *et al.*, 2004). The interaction network, or interactome, was built around genes with the highest connectivity (seeds) using an iterative algorithm that systematically connects additional nodes to the initial seed. The topological characteristics of the interactome, *i.e.*, the number of nodes and connectivity, were extracted.

Ranking of network-associated genes based on their Significance Score

[00218] A score based on the topologic properties of the interactome and the differential expression levels of its nodes was developed to identify the genes most likely to be important in tonsillar hypertrophy. There is mounting evidence that the functional integrity of genetic networks is highly dependent on hubs of high connectivity (Gharib *et al.*, 2009; Luscombe *et al.*, 2004). Using the interactome's topology, we rank-ordered its members based on their connectivity. Since this method does not provide information on the relative differential expression of a given gene in the tonsillar tissue of children with OSA compared to RI, an integrated Significance Score was defined as:

[00219]
$$\text{Significance Score} = \text{Ln}[\text{Connectivity}] - \text{Ln}[P\text{-value}]$$

[00220] Where the *P*-value was based on a gene-by-gene inter-group comparison using the parametric *t*-test (two-sided, unequal variance). In general, the greater a gene's connectivity and the more significant its differential expression, the higher its score. However, genes with highly significant expression, even if sparsely connected will receive relatively high scores, as will genes with a modest degree of differential expression but high connectivity within the network. Statistical cutoff values were determined by performing a random permutation analysis on the subjects (*n* = 10,000) and obtaining a null frequency distribution for the Significance Score. Genes with scores above the 95th percentile of the null distribution were deemed significant (FIG. 4).

EXAMPLE 2

DISTINCT BIOLOGICAL PROCESSES ARE ACTIVATED IN TONSILLAR TISSUE OF CHILDREN WITH OSA

[00221] The following experiments described in Examples 2-7 using the Materials and Methods provided in the previous example yielded significant information regarding obstructive sleep apnea. Generally, the process involved using transcriptional profiling of tonsillar tissue in children with OSA or recurrent tonsillar infection (RI), using a gene set enrichment analysis to identify activated biological modules in OSA, generate gene product interaction network of the tonsillar proliferation module, do topological and statistical ranking of nodes in the proliferation interactome, perform targeted inhibition of candidate genes that reverse adenotonsillar proliferation *in vitro*.

[00222] The gene set enrichment analysis (Subramanian *et al.*, 2005) (GSEA) was applied to the gene expression profiles in tonsils of children with OSA (*n* = 18) and RI (*n* = 18). Out of approximately 1800 curated pathways sampled, 22 were enriched in the OSA phenotype at a false discovery rate $\leq 10\%$, whereas no gene sets were enriched in the RI group. The gene sets associated with OSA were functionally organized into five broad categories: proliferation, hypoxia, glutathione metabolism, cytochrome P450 activity, and fatty acid/steroid biosynthesis. The proliferation module contained the largest number of member genes (*n*=564), and given its potential biological relevance to tonsillar hypertrophy, became the focus of subsequent analyses. Members of this module were linked together based on previously reported gene product interactions, resulting in a complex network

comprised of 361 nodes and 2423 edges (Table 1). The fold-change in expression is identified below. Positive number identify increased expression in OSA.

Table 1. List of network-based candidate genes (significance score ≥ 4.86).

Gene Symbol	Connectivity	P-value	Log2[Fold Change]	Significance Score
CEACAM5	5	5.89E-05	0.7186	11.35
CEACAM6	3	1.68E-04	0.8680	9.79
SERPINB5	12	7.80E-04	0.3486	9.64
CEACAM1	5	3.28E-04	0.5106	9.63
LDLR	7	1.51E-03	0.2979	8.44
IVL	8	1.91E-03	0.6741	8.34
CD9	7	3.03E-03	0.4632	7.75
CDKN1A	45	1.99E-02	0.2636	7.72
IGFBP2	6	3.14E-03	0.4645	7.55
LCN2	3	1.63E-03	0.7940	7.52
HBEGF	13	7.33E-03	0.2373	7.48
DUSP1	14	1.00E-02	0.5998	7.24
ANXA9	1	7.67E-04	0.6070	7.17
CCL19	2	1.86E-03	-0.6579	6.98
CTGF	10	9.43E-03	0.2662	6.97
GJB2	2	2.10E-03	0.6750	6.86
ITGA2	9	1.03E-02	0.2343	6.77
ANXA2	15	1.75E-02	0.2833	6.75
PRSS3	5	6.55E-03	0.5838	6.64
KLKB1	4	5.60E-03	-0.9563	6.57
CITED2	4	5.76E-03	0.3185	6.54
ATF3	12	1.80E-02	0.2806	6.50
PLAT	19	2.91E-02	0.2961	6.48
PRSS2	3	4.93E-03	0.6736	6.41
SPRR1A	2	3.77E-03	0.8381	6.27
SDC1	13	2.73E-02	0.3663	6.17
PI3	3	6.56E-03	0.7578	6.13
TGFB1	111	2.48E-01	0.1828	6.10
LGALS7	3	6.93E-03	0.9365	6.07
SERPINB1	1	2.38E-03	0.3486	6.04
SPRR1B	3	7.79E-03	0.8352	5.95
IL1F6	3	7.86E-03	0.7158	5.94
CTSB	18	4.74E-02	0.3174	5.94
PSPH	1	2.68E-03	0.7013	5.92
DEFB4	4	1.13E-02	1.2882	5.87
IL1B	100	2.96E-01	-0.1812	5.82
ADM	6	1.85E-02	0.2979	5.78
KLF5	6	1.94E-02	0.3138	5.74
KLK7	3	1.03E-02	0.6823	5.67
KRT18	10	3.77E-02	0.2833	5.58
ITGA7	1	3.83E-03	-0.5538	5.57
JUNB	26	1.03E-01	0.3700	5.53
SLPI	10	4.08E-02	0.5080	5.50
PTGS2	32	1.32E-01	0.1372	5.49
CCK	4	1.67E-02	-0.5538	5.48

ELF3	6	2.69E-02	0.4063	5.41
SKP2	9	4.03E-02	0.3185	5.41
NR4A2	4	1.98E-02	0.3138	5.31
SPRR2G	1	5.05E-03	0.9666	5.29
CCND1	47	2.44E-01	0.1324	5.26
FOS	56	3.03E-01	0.2820	5.22
TGFB2	10	5.82E-02	-0.1193	5.15
CDCP1	9	5.29E-02	0.1007	5.14
BCL2	44	2.66E-01	0.1885	5.11
ATP1B1	6	3.64E-02	0.7186	5.10
CSTB	5	3.09E-02	0.3174	5.09
S100A9	12	7.56E-02	0.9580	5.07
IER2	3	1.96E-02	0.7940	5.03
HPSE	5	3.35E-02	0.5106	5.01
IL6	70	4.75E-01	0.5998	4.99
MXD1	3	2.06E-02	-0.9563	4.98
NDRG1	10	7.09E-02	0.6750	4.95
SGK	6	4.26E-02	0.9365	4.95
CD55	2	1.43E-02	-0.6579	4.94
FN1	47	3.37E-01	-0.1193	4.94
S100A10	5	3.61E-02	1.2882	4.93
HRAS	52	3.78E-01	0.1007	4.92
IL1A	32	2.35E-01	0.2771	4.92
PTHLH	12	9.17E-02	0.3486	4.87

Table 2. Demographic and polysomnographic characteristics of children with obstructive sleep apnea (OSA) and recurrent tonsillitis (RI).

	OSA (n=44)	RI (n=44)
Age (years)	6.0±1.6	5.5±2.3
Gender (F/M)	20/24	20/24
African American (n)	14	14
BMI z score	0.76±0.07	0.54±0.06
Total Sleep Time (TST; min)	487.5±9.4	482.9±8.3
SWS (%TST)	27.5±2.8	31.3±3.6
REM Sleep (%TST)	19.5±3.1	23.2±2.8
AHI (/hrTST)*	12.7±2.9	0.4±0.1
Nadir SaO₂ (%)*	83.5±3.4	95.7±0.3
Arousal Index (/hrTST)*	17.4±3.7	7.7±0.5

EXAMPLE 3**AN INTEGRATIVE SCORING STRATEGY IDENTIFIES PUTATIVE CANDIDATE GENES MEDIATING TONSILLAR HYPERTROPHY.**

[00223] Using the connectivity matrix of the proliferation interactome and the differential expression of its nodes, a significance scoring metric was developed that ranked the gene members (score range: 0.01–11.35). A random permutation analysis was then used to determine a 95% significance cut-off value of 4.87, resulting in the selection of 69 genes (Table 1). Many of these gene candidates were involved in inflammation signaling (*e.g.*, IL1B, IL1A, IL1F6, IL6, CCL19) and regulation (*e.g.*, JUNB, FOS), and tissue growth and remodeling (*e.g.*, TGFB1, TGFB2, HBEGF, CTGF, FN1). We have recently demonstrated that adenotonsillar tissue from children with sleep apnea express pro-inflammatory cytokines and is in a proliferative state (Kim *et al.*, 2009). Several members of the carcinoembryonic gene family were among the top-ranked hits in our analysis, including CEACAM1, CEACAM5, and CEACAM6. These molecules are critical mediators of cell-cell adhesion and determinants of tissue architecture, aberrant growth, and hypertrophy (Abou-Rjaily *et al.*, 2004; Benchimol *et al.*, 1989; Kuespert *et al.*, 2006). Two protein phosphatases, dual specificity phosphatase 1 (DUSP1) and phosphoserine phosphatase (PSPH), were among the statistically significant network genes. Since little is known about the role of protein phosphatases in promoting tonsillar hypertrophy, we proceeded to investigate whether PSPH and DUSP1 represented novel therapeutic targets.

EXAMPLE 4**SELECTIVE INHIBITION OF PHOSPHOSERINE PHOSPHATASE IS ANTI-PROLIFERATIVE**

[00224] PSPH protein expression was initially determined to be more abundant in children with OSA than those with RI, and seemed to localize primarily to germinal center within the tonsil structure. Next, mixed cellular tonsil and adenoid cultures (Serpero *et al.*, 2009) was then treated with the phosphatase inhibitors okadaic acid, calyculin A, and protein phosphatase inhibitor 2 (PPI2) at increasing concentrations. Exposure to okadaic acid (which inhibits both PSPH and DUSP1) and calyculin A reduced the proliferation of tonsillar/adenoid cell cultures harvested from children with OSA; however, this anti-proliferative effect was not seen with PPI2

(n=6 for all experiments) (FIG. 1). Much milder and non-significant effects were seen in tonsillar/adenoid cell cultures from children with recurrent infections (n=6 for all experiments). Similar findings emerged using PSPH and DUSP1 siRNA approaches in tonsil whole cell cultures (n=3/group). Therefore, these findings confirm that phosphatases orchestrate proliferative signaling pathways in upper airway lymphadenoid tissues of children with obstructive sleep apnea. Our expression profiling-based strategy implies that important mechanisms regulating adenotonsillar proliferation occur at the transcriptional level, and targeted perturbation of gene expression can alter the production and activity of its corresponding protein, reversing the lymphadenoid hypertrophy associated with pediatric OSA.

EXAMPLE 5

INHIBITION OF PHOSPHOSERINE PHOSPHATASE BLOCKS PROLIFERATION AND PROMOTES PROGRAMMED CELL DEATH

[00225] To determine proliferation and apoptosis in the mixed cell adenotonsillar cultures of OSA and RI children, we performed flow cytometric analysis using BRDu and Annexin V staining respectively (Kheirandish-Gozal *et al.*, 2009). Exposure of the cell cultures to 100 nM Calyculin dramatically reduced cellular proliferation (FIG. 2) and resulted in programmed cell death in approximately 70% of the cells collected from children with OSA, whereas apoptosis was present in 46% of control cells (FIG. 3). The selective difference in apoptosis was further characterized by differentiating T-cell (sorted by CD3+) and B-cell subtypes (sorted by CD19+). Treatment with 100 nM Calyculin caused cell death in 86% of T-cells compared to untreated rate of 39%, and in B-cell lymphocytes, exposure to Calyculin resulted in 71% cell death compared to 54% in untreated control cells (FIG. 3). These data imply that the anti-proliferative effect of targeting phosphoserine phosphatase is mediated, in part, through induction of apoptosis in adenotonsillar cells. Furthermore, programmed cell death appears to be more prominent in T-cells compared to B-cells.

EXAMPLE 6

QUANTITATIVE RT-PCR VALIDATION OF SELECTED CANDIDATE GENES

[00226] Differential expression of several candidate genes from our microarray experiments was confirmed using qRT-PCR (Table 3). Up-regulation of PSPH, CEACAM5, RANBP2 and SPRR2E in children with OSA was validated with

qRT-PCR in the original cohort of 36 subjects, and further confirmed in an additional cohort of 20 children (10 with OSA, 10 with RI). Further validation of additional genes was also performed in the original cohort (Table 3). All data are expressed as mean $\pm$ SD; data for the 18 initial subjects in each group and those for subsequent gene validation and proliferation studies were compared and found to be similar, and were therefore merged. * - $p < 0.001$

Table 3: Genes in microarray validated by qRT-PCR.

Gene list	Microarray		RT-PCR	
	Fold change	p-value	Fold change	p-value
CEACAM5	1.58	0.004	2.89	0.006
NGFB	0.99	0.89	1.06	0.3
PSPH	3.36	0.0006	1.53	0.04
RANBP2	1.05	0.81	1.52	0.02
NGFR	0.92	0.15	0.78	0.6
NR3C1- α	1.01	0.87	1.52	0.04
NTF3	1	0.99	1.43	0.1
SPRR2E	1.77	0.03	2.76	0.007

Fold change: OSA/RI; n=18/group for all genes and n=28/group for CEACAM 5, PSPH, RANBP2, and SPRR2E.

EXAMPLE 7

SIRNA AND SHRNA EXPERIMENTS

[00227] Mixed cell suspensions were transferred onto 24-well bottom-well plates at a concentration of 1×10^6 cells/well. Cells were cultured in a 5 % CO₂ incubator at 37°C for 48 hours. Cells were transfected using a shRNA against PSPH containing a GFP reporter (KH23333G ; SA Bioscience) or a cocktail combination of PSPH siRNAs (SIH623333ABCD; SA BioScience), and using a previously optimized plasmid-lipofectamine ratio consisting of mixture of 10 μ L (1-2 μ g/ μ L) of siRNA or shRNA and the Amaxa[®] Nucleofector[®] Technology. An appropriate GFP positive or GFP negative scrambled negative control was used for reference in both experiments. Transfection efficiency varied between 63-85% with a mean of 73.5% (FIG. 5). In addition experiments were also conducted with a cocktail of 3 commercially available PSPH siRNA (Ambion; s11428, s11429, and s11430). Since results were similar across all experiments, they were merged.

[00228] Bromodeoxyuridine (BrdU) pulsed proliferation analysis using flow cytometry was employed to detect global cell, T-cell-, and B-cell-specific proliferation. Annexin V mouse anti-human antibodies was used to quantify global, T-cell or B-cell specific apoptosis.

[00229] Significant reductions in proliferative rates occurred with PSPH siRNA (n=4) and shRNA (n=3) transfection compared to either control or scrambled peptides and reciprocally increased the percentage of apoptotic cells in mixed tonsil cell cultures obtained from children with demonstrated obstructive sleep apnea in their sleep studies (FIG. 6). Of note, this effect was more prominent in T cells compared to B cells.

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The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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CLAIMS

1. A method for inhibiting proliferation of adenoid or tonsillar cells comprising contacting the cells with an effective amount of a composition comprising a phosphatase inhibitor.
2. The method of claim 1, wherein the phosphatase inhibitor is a phosphoserine phosphatase inhibitor.
3. The method of claim 1, wherein the cells are hypertrophic.
4. The method of claim 3, wherein the hypertrophic cells contribute to obstructive sleep apnea.
5. The method of claim 1, wherein the phosphoserine phosphatase inhibitor is a small molecule, nucleic acid, or polypeptide.
6. The method of claim 5, wherein the phosphoserine phosphatase inhibitor is a nucleic acid.
7. The method of claim 6, wherein the nucleic acid is an siRNA against phosphoserine phosphatase.
8. The method of claim 7, wherein the composition contains a plurality of different siRNAs against phosphoserine phosphatase.
9. The method of claim 6, wherein the nucleic acid is a modified nucleic acid.
10. The method of claim 5, wherein the phosphoserine phosphatase inhibitor is a small molecule.
11. The method of claim 10, wherein the small molecule is okadaic acid, PPI2, or calyculin A.
12. A method for treating obstructive sleep apnea in a patient comprising administering an effective amount of a composition comprising a phosphatase

inhibitor to the patient, wherein the patient is suspected of having or has been diagnosed as having obstructive sleep apnea.

13. The method of claim 12, wherein the phosphatase inhibitor is a phosphoserine phosphatase inhibitor.

14. The method of claim 12, wherein the phosphoserine phosphatase inhibitor is a small molecule, nucleic acid, or polypeptide.

15. The method of claim 14, wherein the phosphoserine phosphatase inhibitor is a nucleic acid.

16. The method of claim 15, wherein the nucleic acid is an siRNA against phosphoserine phosphatase.

17. The method of claim 16, wherein the composition contains a plurality of different siRNAs against phosphoserine phosphatase.

18. The method of claim 15, wherein the nucleic acid is modified.

19. The method of claim 12, wherein the composition is administered multiple times.

20. The method of claim 12, wherein the composition is administered daily.

21. The method of claim 12, wherein the composition is formulated for oral, topical or mucosal administration.

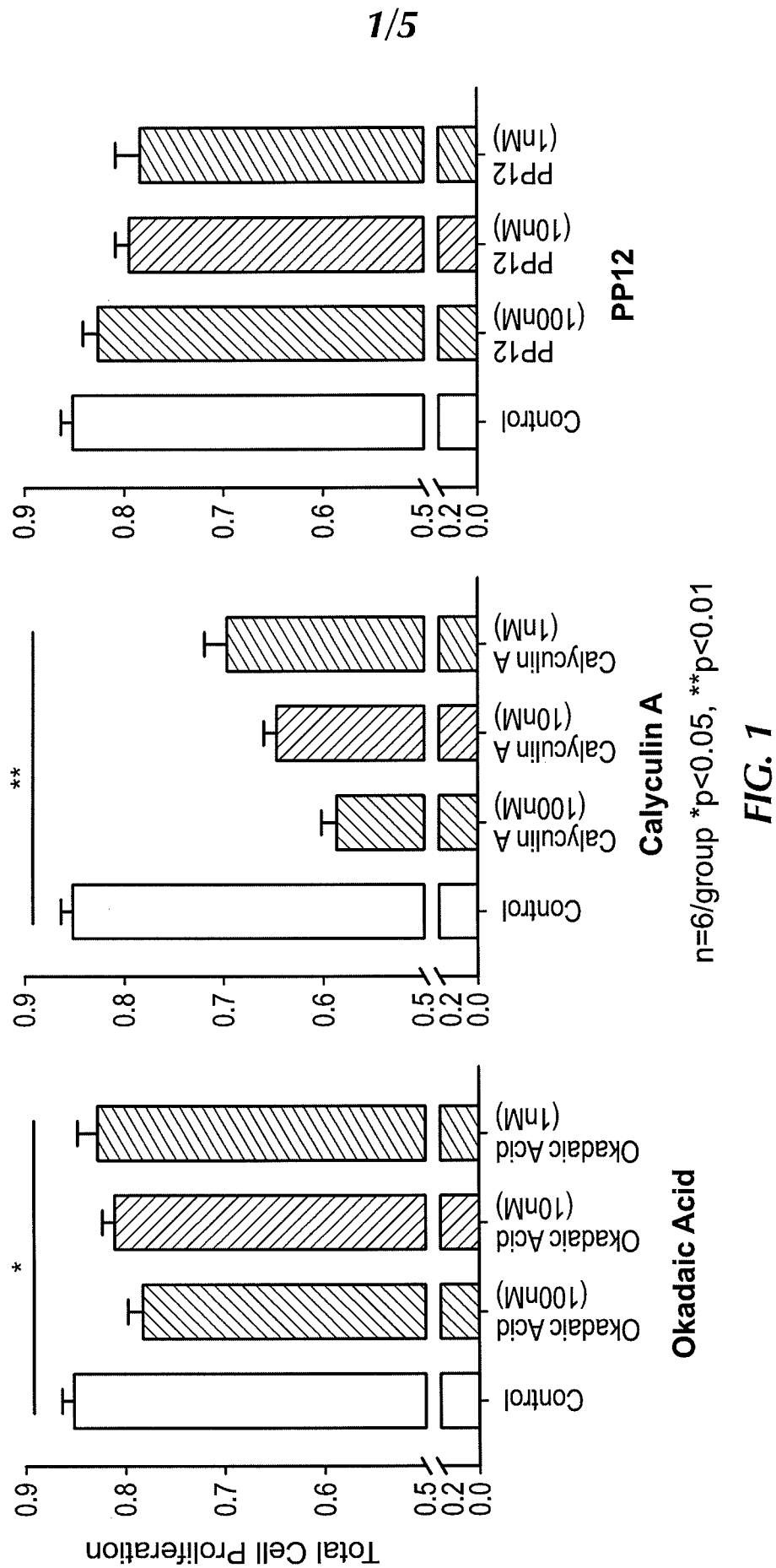
22. The method of claim 21, wherein the composition is a liquid, strip, lozenge, or lollipop.

23. The method of claim 21, wherein the composition is a mouthwash or spray.

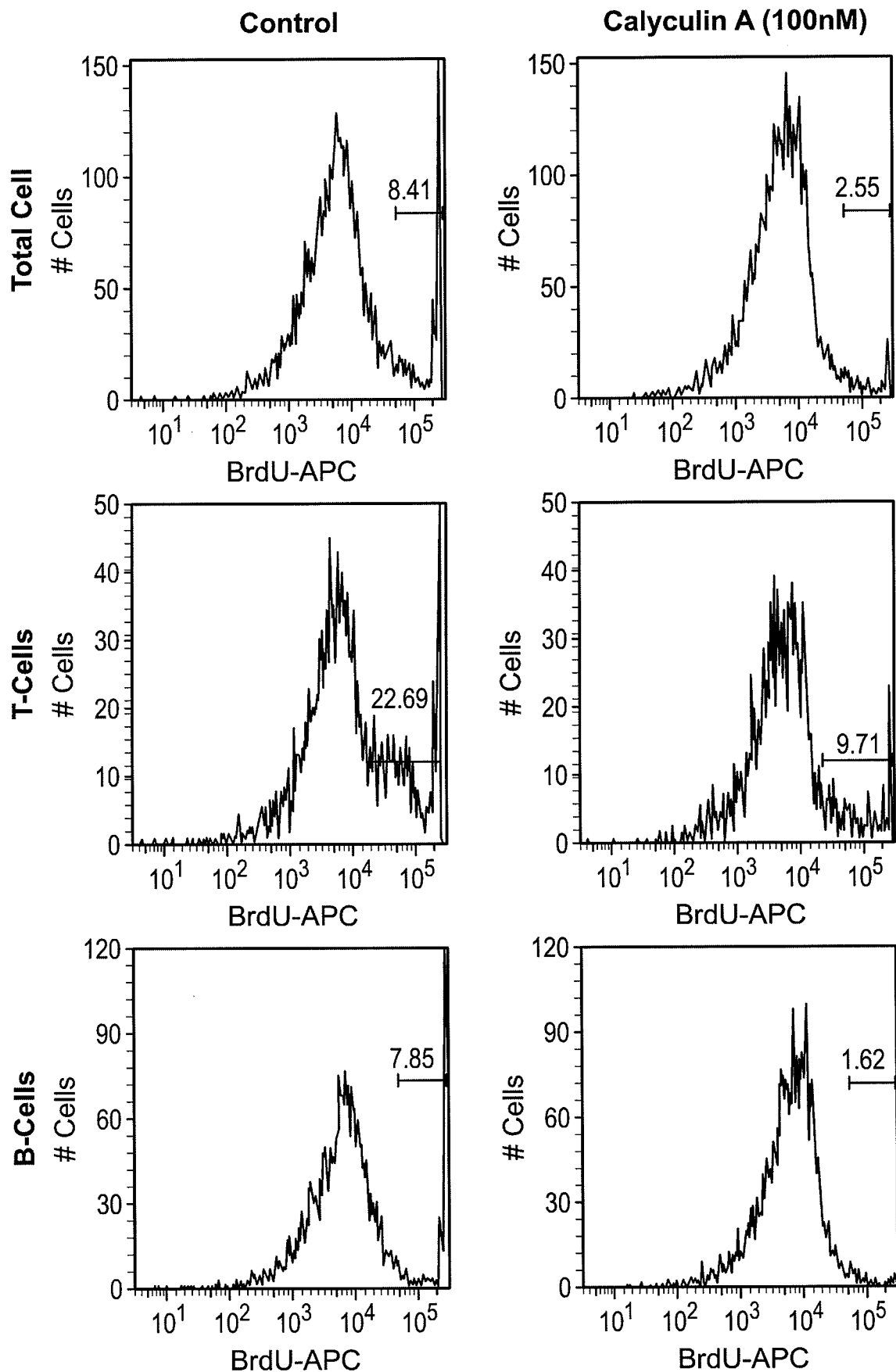
24. The method of claim 12, wherein the patient has been diagnosed with obstructive sleep apnea.

25. The method of claim 12, wherein the patient is a pediatric patient.

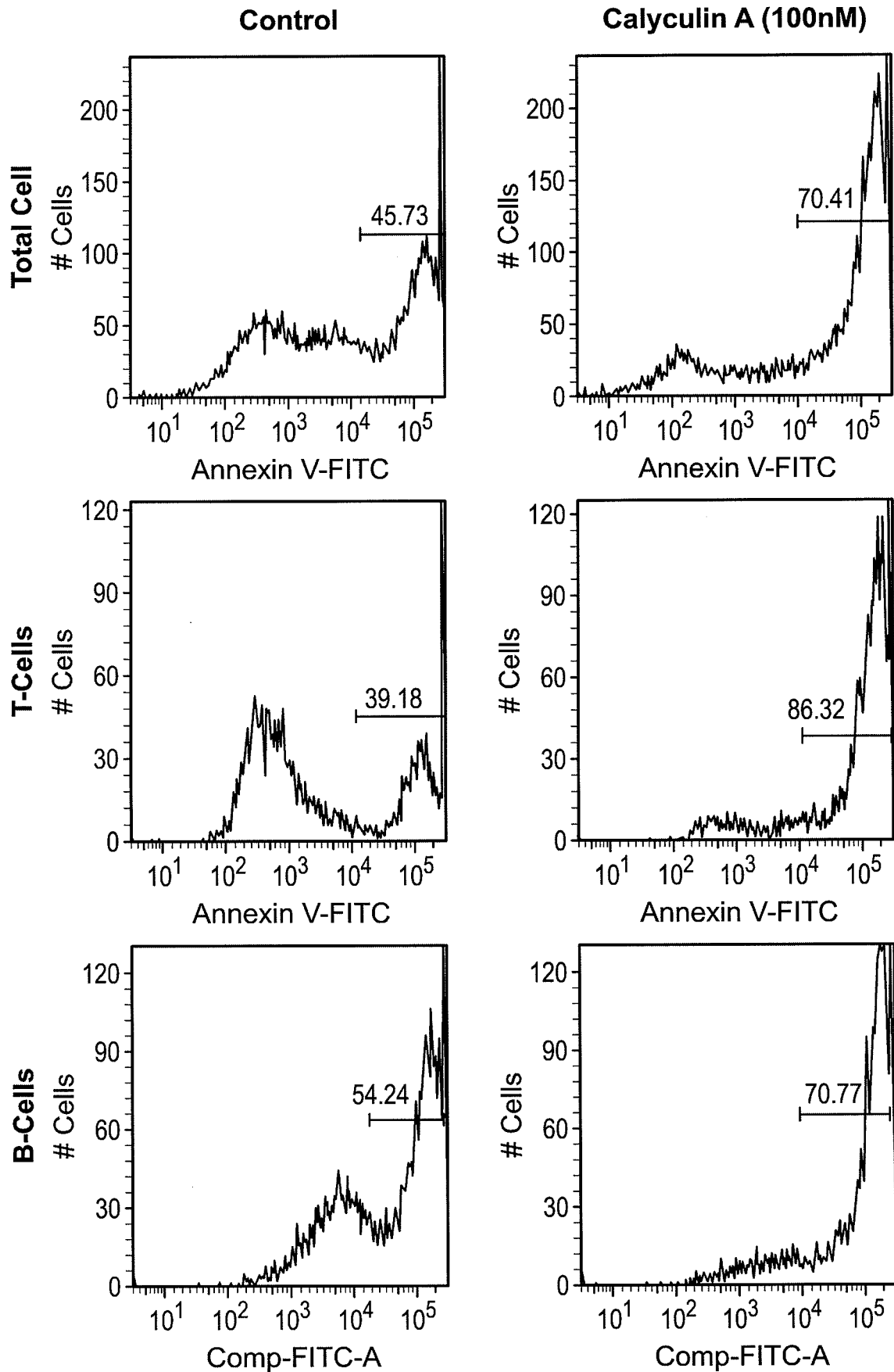
26. A method of diagnosing obstructive sleep apnea, or a risk thereof, in a subject comprising diagnosing a subject with obstructive sleep apnea based on the results of a test indicating a measurable difference in the expression level of at least one biomarker in a biological sample from the subject, wherein the at least one biomarker is a product of a gene selected from Table 1.
27. The method of claim 26, wherein the at least one biomarker is an mRNA.
28. The method of claim 26, wherein the at least one biomarker is a polypeptide of interest.
29. The method of claim 26, wherein the biological sample comprises lymphadenoid tissue.
30. The method of claim 29, wherein the lymphadenoid tissue is selected from the group consisting of tonsillar tissue and adenoid tissue.
31. The method of claim 26, wherein the subject is human.
32. The method of claim 26, further comprising ordering the test prior to diagnosing the subject.
33. A method of screening a candidate therapeutic for obstructive sleep apnea comprising:
- a) contacting hypertrophic adenoid and/or tonsillar cells with a candidate compound, wherein the candidate compound is an inhibitor of biomarker that is a product of a gene selected from Table 1, and
 - b) determining a change on the phenotype or activity of the cells based on the contact with the candidate compound, wherein the change identifies the candidate compound as a potential therapeutic for obstructive sleep apnea.



2/5

**FIG. 2**

3/5

**FIG. 3**

4/5

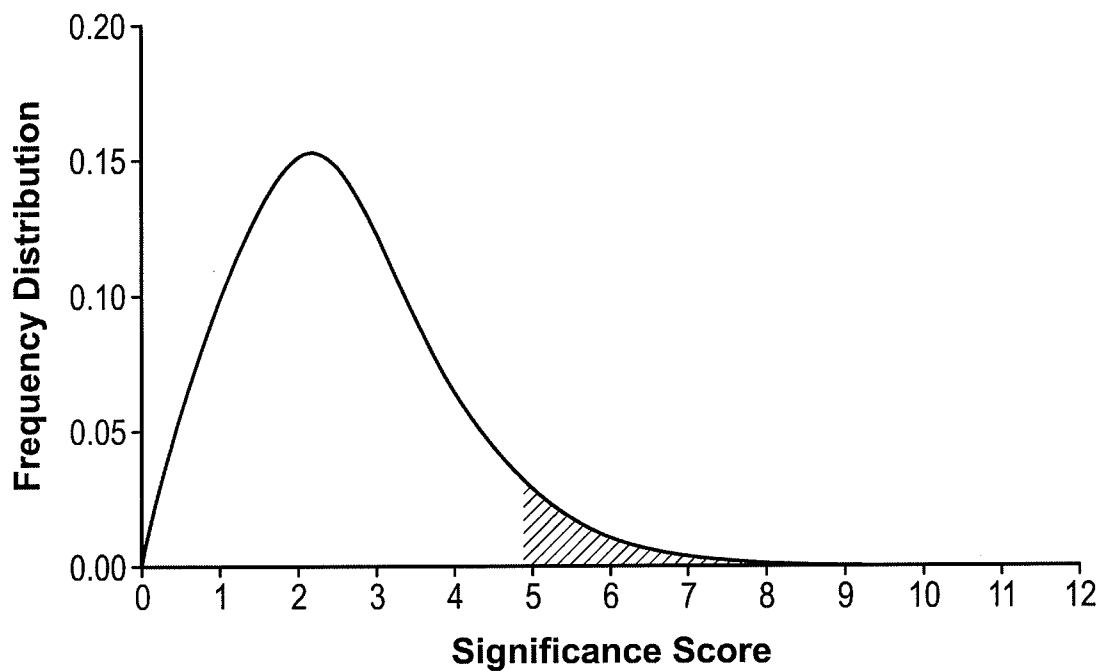


FIG. 4

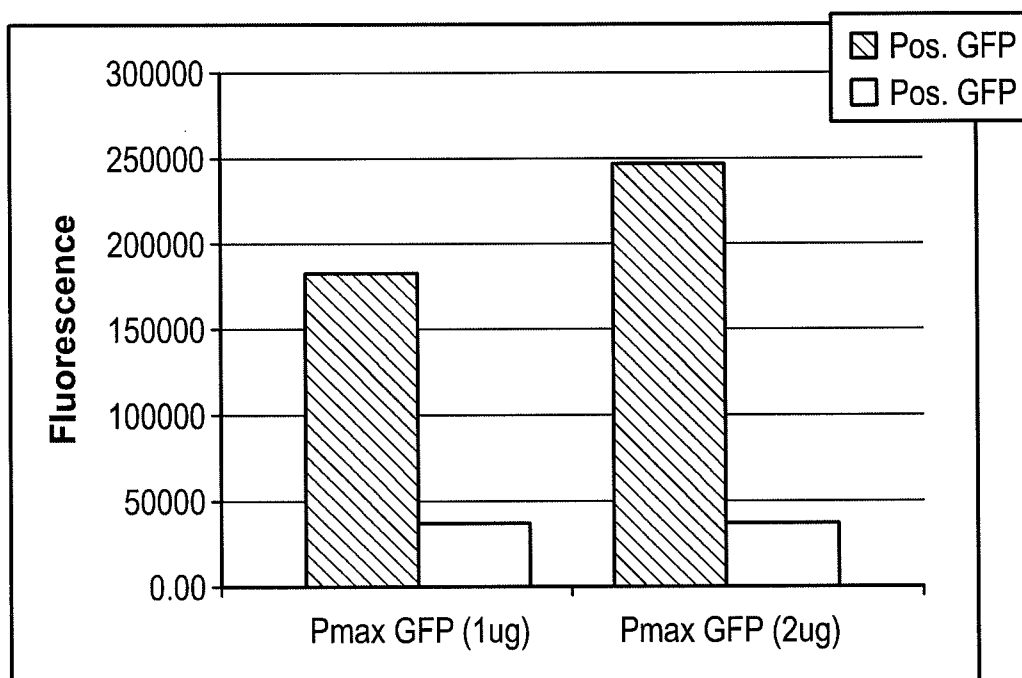


FIG. 5

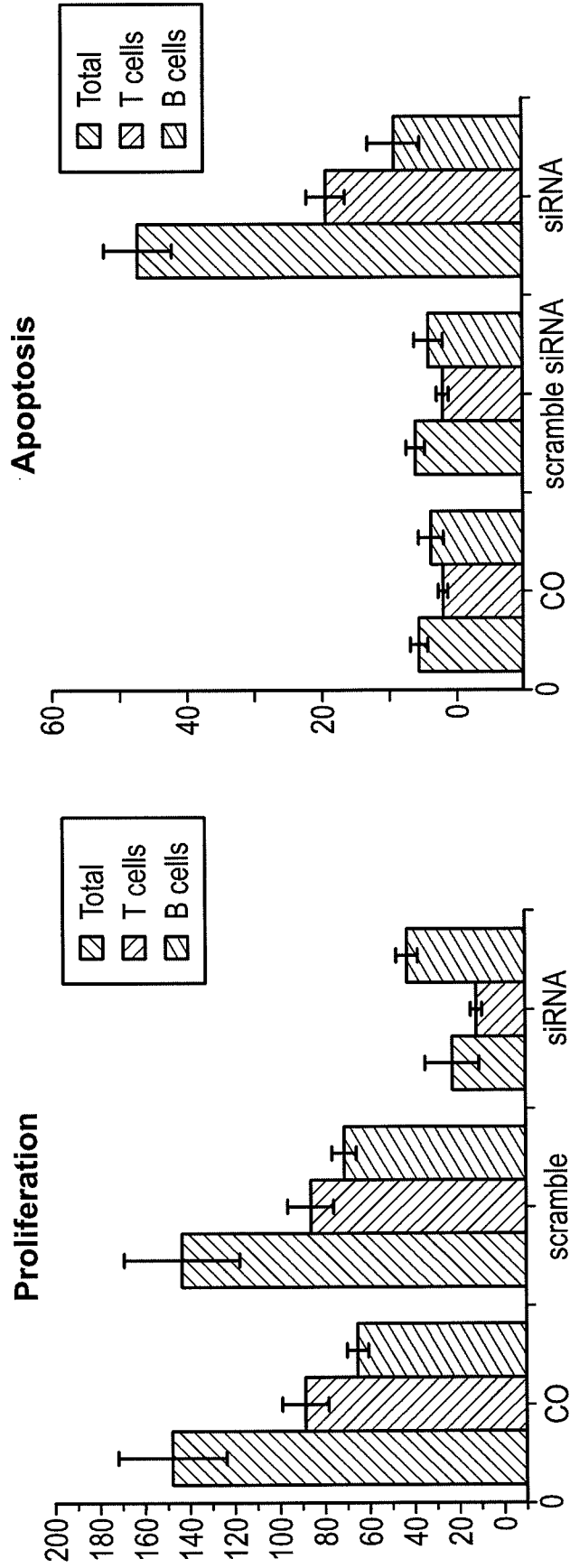


FIG. 6

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 09/62968

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/7088; G01N 33/48 (2010.01)

USPC - 514/44A; 436/86; 436/94

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

USPC: 514/44A; 436/86; 436/94

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

USPC: 514/44A; 436/86; 436/94

(text search)

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

PubWEST(PGPB,USPT,USOC,EPAB,JPAB); Google; PubMed

Search terms: obstructive sleep apnea, phosphatase, inhibitor, okadaic acid, hypertrophy, tonsil, adenoid, screening assay, contacting, diagnosis, kallikrein, siRNA, phosphoserine

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2005/0234000 A1 (MITCHELL et al.) 20 October 2005 (20.10.2005) para [0019], [0034], [0051], [0055]-[0057], [0059]-[0062], [0066], [0070]	12-18, 24-25 ----- 1-11, 19-23
Y	LI et al. Use of tonsil size in the evaluation of obstructive sleep apnoea. Arch. Dis. Child. August 2002 (08.2002), Vol. 87, No. 2, pages 156-159; abstract	1-11
Y	US 2006/0194840 A1 (GOZAL) 31 August 2006 (31.08.2006) para [0018], [0144], [0146], [0148], [0168]	19-23
Y	US 2006/0029980 A1 (GOZAL et al.) 9 February 2006 (09.02.2006) abstract; para [0003], [0032]-[0033]	26-33
Y	SUNG et al. Biomarkers for the lung cancer diagnosis and their advances in proteomics. BMB Rep. 30 September 2008 (30.09.2008), Vol. 40, No. 9, pages 615-625; abstract	26-33
Y	US 2008/0249294 A1 (HAEBERLI et al.) 9 October 2008 (09.10.2008) para [0127], [0254], [0490].	33



Further documents are listed in the continuation of Box C.



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document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&"

document member of the same patent family

Date of the actual completion of the international search

3 January 2010 (03.01.2010)

Date of mailing of the international search report

22 JAN 2010

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 09/62968

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2008/0027134 A1 (PETRY et al.) 31 January 2008 (31.01.2008)	1-33
A	US 2008/0103160 A1 (YI) 1 May 2008 (01.05.2008)	1-33
A	US 2004/0224899 A1 (GASTON et al.) 11 November 2004 (11.11.2004)	1-33
A	GOZAL et al. Systemic inflammation in non-obese children with obstructive sleep apnea. Sleep Med. March 2008 (03.2008), Vol. 9, No. 3, pages 254-259	1-33