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(54) Title: OCULOMEDIN AND GLAUCOMA

(57) Abstract: Nucleic acids encoding oculomedin polypeptide are disclosed. Also described are related nucleic acids encoding oculomedin polypeptides, oculomedin polypeptides; antibodies that bind to oculomedin polypeptides, methods of diagnosis of glaucoma; assays for agents that alter the activity of oculomedin polypeptide or which identify oculomedin binding partners or assess the strength of an interaction between oculomedin and an oculomedin binding partner, and the agents or binding partners identified by the assays; pharmaceutical compositions comprising the oculomedin nucleic acids, oculomedin polypeptides, or agents that alter the activity of the oculomedin polypeptides; as well as methods of therapy of glaucoma.

OCULOMEDIN AND GLAUCOMA

BACKGROUND OF THE INVENTION

Glaucoma is a group of ocular disorders, characterized by degeneration of the optic nerve. It is one of the leading causes of blindness worldwide. One major risk factor for developing glaucoma is family history: several different inherited forms of glaucoma have been described.

Primary congenital or infantile glaucoma (gene symbol:GLC3) is an inherited disorder that accounts for 0.01-0.04% of total blindness. It is characterized by an improper development of the aqueous outflow system of the eye, which leads to elevated intraocular pressure, enlargement of the globe or cornea (i.e., buphthalmos), damage to the optic nerve, and eventual visual impairment. Pathogenesis of GLC3 remains elusive despite efforts to identify a single anatomic defect. At least two chromosomal locations associated with the disease have been identified: one locus at 2p21 (GLC3A) (Sarfarazi, M. *et al.*(1995) *Genomics* 30:171-177; and a second locus at 1p36 (GLC3B) (Akarsu, A.N. *et al.* (1996) *Hum. Mol. Gen.* 5(8):1199-1203). Other specific loci, including a region of 6p and chromosome 11, have been excluded (Akarsu, A.N. *et al.* (1996) *Am. J. Med. Genet.* 61:290-292).

Primary open angle glaucoma (gene symbol: GLC3) is a common disorder characterized by atrophy of the optic nerve resulting in visual field loss and eventual blindness. GLC3 has been divided into two major groups, based on age of onset and differences in clinical presentation.

Juvenile-onset primary open angle glaucoma (GLC3A) usually manifests in late childhood or early adulthood. The progression of GLC3A is rapid and severe with high intraocular pressure, is poorly responsive to medical treatment, and is such that it usually requires ocular surgery. GLC3A was initially mapped to the q21-q31 region of chromosome 1 (Sheffield, V.C. *et al.* (1996) *Hum. Mol. Genet.* 4:1837-1844); mutations in the gene for trabecular meshwork inducible glucocorticoid response (TIGR) protein (Mechelen), located at chromosome 1q24, have been

identified as associated with GLC3A glaucoma (Stone, E.M. *et al.* (1997) *Science 275*:668-670; Stoilova, D. *et al.* (1997) *Opthamalic Genetics 18(3)*:109-118; Adam, M.F. *et al.* (1997) *Hum. Mol. Genet. 6*:2091-2097; Michels-Rautenstrauss, K.G., *et al.* (1998) *Hum. Genet.* 102:103-106; Mansergh, F.C. *et al.* (1998) *Hum. Mutat. 11*:244-251).

Adult- or late-onset primary open angle glaucoma (GLC1B) is the most common type of glaucoma. It is milder and develops more gradually than juvenile-onset primary open angle glaucoma, with variable onset usually after the age of 40. GLC1B is associated with slight to moderate elevation of intraocular pressure, and often responds satisfactorily to regularly monitored medical treatment. However, because the disease progresses gradually and painlessly, it may not be detected until a late stage when irreversible damage to the optic nerve has already occurred. Linkage, haplotype and clinical data have assigned a locus for GLC1B to the 2cen-q13 region as well as a new locus 3q21-q22 (Stoilova, D. *et al.* (1996) *Genomics* 36:142-150). Further evidence has identified several additional loci for primary open angle glaucoma. GLC1C, an adult-onset POAG gene, has been mapped to 3q (Wirtz, M.K. *et al.* (1997) *Am. J. Hum. Genet.* 60:296-304); GLC1D has been mapped to 8q23 (Trifan, O.C. *et al.* (1998) *Am. J. Ophthalmol. 126*:17-28); GLC1E has been mapped to 10p15-p14 (Sarfarazi, M. *et al.* (1998) *Am. J. Hum. Genet.* 62: 641-652).

Because of the insidious nature of glaucoma, a need remains for a better and earlier means to diagnose or predict the likelihood of development of glaucoma, so that preventative or palliative measures can be taken before significant damage to the optical nerve occurs.

SUMMARY OF THE INVENTION

As described herein, a gene encoding a novel protein, oculomedin, has been identified in human eye trabecular cells. The sequence of the cDNA encoding the oculomedin protein identified as described herein is shown in Figure 1 (SEQ ID NO: 1); the predicted amino acid sequence is shown in Figure 2 (SEQ ID NO: 2). Accordingly, this invention pertains to an isolated nucleic acid molecule encoding

oculomedin, or encoding an active derivative or fragment thereof. In particular embodiments, the isolated nucleic acid molecule encodes a polypeptide with the same amino acid sequence as the endogenous oculomedin (e.g., a polypeptide with the same amino acid sequence as SEQ ID NO:2). In another embodiment, the isolated nucleic acid molecule has the same nucleotide sequence as the cDNA encoding the endogenous oculomedin (e.g., SEQ ID NO:1), or as the endogenous gene encoding oculomedin. The invention also relates to DNA constructs comprising the nucleic acid molecules described herein operatively linked to a regulatory sequence (e.g., in a vector); to recombinant host cells, such as bacterial cells, fungal cells, plant cells, insect cells and mammalian cells, comprising the nucleic acid molecules described herein operatively linked to a regulatory sequence; and to methods for preparing oculomedin polypeptides or active derivative or fragments thereof, by culturing such recombinant host cells.

The invention additionally pertains to an isolated oculomedin polypeptide (e.g., SEQ ID NO:2), or an active derivative or fragment thereof. In another embodiment, the polypeptide is a derivative possessing substantial sequence identity with the endogenous oculomedin polypeptide. The invention also pertains to an antibody, or an antigen-binding fragment thereof, which selectively binds to oculomedin or an active derivative or fragment thereof, or to a mutant oculomedin polypeptide or an active derivative or fragment thereof. In a particular embodiment, the antibody is a monoclonal antibody; in another particular embodiment, the antibody is a polyclonal antibody. The invention also relates to a method for assaying the presence of oculomedin in a cell, e.g., in a tissue sample, comprising contacting cells with an antibody which specifically binds to oculomedin polypeptide.

The invention also pertains to methods of diagnosing glaucoma in an individual, as well as kits useful in the methods of diagnosis. The methods include detecting the presence of a polymorphism (e.g., a mutation) in the oculomedin gene, or detecting alterations in expression or composition of the oculomedin polypeptide. The alterations can be quantitative, qualitative, or both quantitative and qualitative.

The presence of a polymorphism in the gene, or of an alteration in expression or in composition of the oculomedin polypeptide, is indicative of glaucoma.

The invention further relates to an assay for identifying agents which alter (e.g., enhance or inhibit) the activity or expression of oculomedin polypeptide, as well as for identifying oculomedin binding partners and for assessing the strength of an interaction between oculomedin and an oculomedin binding partner. For example, a cell or fraction thereof containing oculomedin polypeptide, or an active fragment or derivative thereof, can be contacted with an agent to be tested, and the level of oculomedin expression or activity can be assessed. Agents that enhance or inhibit oculomedin polypeptide expression or activity, or which are oculomedin binding partners, are also included in the current invention, as are methods of altering (enhancing or inhibiting) oculomedin polypeptide expression or activity by contacting a cell containing the oculomedin gene and/or polypeptide, or by contacting the oculomedin polypeptide, with an agent that enhances or inhibits expression or activity of the oculomedin gene or polypeptide.

Additionally, the invention pertains to pharmaceutical compositions comprising the oculomedin nucleic acids of the invention, the oculomedin polypeptides of the invention, and/or the agents that alter activity of oculomedin. The invention further pertains to methods of treating glaucoma, by administering oculomedin nucleic acids of the invention, oculomedin polypeptides of the invention, the agents that alter activity of oculomedin polypeptide, or compositions comprising the oculomedin nucleic acids, oculomedin polypeptides, and/or the agents that alter activity of oculomedin. Methods of treating glaucoma also comprise use of antisense therapy, ribozymes, and homologous recombination. The invention further pertains to transgenic animals comprising a nucleic acid of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the cDNA (SEQ ID NO:1) encoding oculomedin.

Figure 2 depicts amino acid sequence (SEQ ID NO:2) of oculomedin.

The foregoing and other objects, features and advantages of the invention will be apparent from the following more particular description of preferred embodiments of the invention, as illustrated in the accompanying drawings.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a gene induced by mechanical stretching in human trabecular cells of the eye. As described herein, Applicants have isolated and sequenced a nucleic acid encoding a polypeptide, oculomedin, which is expressed in human trabecular cells of the eye and also in the retina, although not in other tissues, including the brain. Trabecular cells of the eye form a mesh-like structure called trabecular meshwork, which is located at the iridocorneal angle. The trabecular meshwork is the main site of aqueous outflow and thus plays an important role in regulation of intraocular pressure (Yuo, B.Y.J.T., (1996) *Surv. Ophthalmol. 40*:379-390). Glaucoma is caused mainly by elevation of the intraocular pressure. Resistance of aqueous outflow at trabecular meshwork is considered as responsible for the development of high intraocular pressure (Yuo, B.Y.J.T. (1996) *Surv. Ophthalmol. 40*:379-390). Oculomedin is expressed in trabecular cells as well as in the retina, in a manner that is similar to the expression of trabecular meshwork inducible glucocorticoid response (TIGR) gene. Mutations in the TIGR gene, such as a change in a single nucleotide resulting in a single amino acid change, have been associated with glaucoma (see, e.g., Stone, E.M. *et al.* (1997) *Science 275*:668-670; Stoilova, D. *et al.* (1997) *Opthalmic. Genet. 18*:109-118; Adam, M.F. *et al.*(1997) *Hum. Mol. Genet. 6*:2091-2097; Kee, C. and Ahn, B.H. (1997) *Korean. J. Ophthalmol. 11*:75-78; Richards, J.E. *et al.* (1998) *Ophthalmology 105*:1698-1707; Mansergh, F.C. *et al.* (1998) *Hum. Mutat. 11*:244-251). Therefore, it is expected that a mutation in the oculomedin gene and/or protein would similarly be associated with glaucoma.

NUCLEIC ACIDS OF THE INVENTION

Accordingly, the invention pertains to an isolated nucleic acid molecule encoding mammalian, e.g., primate or human, oculomedin. The terms, "TISR gene," "oculomedin gene," "OCLM gene," "nucleotide encoding oculomedin polypeptide," as well as "oculomedin polynucleotide" all refer to an isolated nucleic acid molecule that encodes an oculomedin polypeptide as described herein. Nucleic acid molecules of the present invention can be RNA (e.g., mRNA), or DNA, such as cDNA and genomic DNA. DNA molecules can be double-stranded or single-stranded; single stranded RNA or DNA can be either the coding (sense) strand or the non-coding (antisense) strand. Preferably, the nucleic acid molecule comprises at least about 25 contiguous nucleotides, more preferably at least about 50 contiguous nucleotides, and even more preferably at least about 125 contiguous nucleotides. The nucleic acid molecule can be only that polynucleotide which encodes at least a fragment of the amino acid sequence of the oculomedin polypeptide (e.g., the polynucleotide encoding SEQ ID NO:2); alternatively, the nucleic acid molecule can include at least a fragment of the oculomedin amino acid coding sequence along with additional non-coding sequences such as introns and non-coding 3' and 5' sequences (including regulatory sequences, for example) (e.g., SEQ ID NO:1). Additionally, the nucleic acid molecule can contain a marker sequence, for example, a nucleotide sequence which encodes a polypeptide, to assist in isolation or purification of the oculomedin polypeptide. Such sequences include, but are not limited to, those which encode a glutathione-S-transferase (GST) fusion protein and those which encode a hemagglutinin A (HA) peptide marker from influenza. In a preferred embodiment, the nucleic acid molecule has the sequence shown in Figure 1 (SEQ ID NO:1).

In a preferred embodiment, the nucleic acid molecule contains nucleotides encoding an oculomedin polypeptide; or contains a nucleotide sequence encoding an active derivative or active fragment of an oculomedin polypeptide; or encodes a polypeptide which is at least about 90% identical (i.e., a polypeptide which has substantial sequence identity) to the polypeptides described herein.

The nucleic acid molecules of the invention are "isolated" or "substantially purified;" as used herein, an "isolated" or "substantially purified" nucleic acid molecule or nucleotide sequence is intended to mean a nucleic acid molecule or nucleotide sequence which is not flanked by nucleotide sequences which normally (in nature) flank the gene or nucleotide sequence (as in genomic sequences) and/or has been completely or partially purified from other transcribed sequences (e.g., as in an RNA library). For example, an isolated nucleic acid of the invention may be substantially isolated with respect to the complex cellular milieu in which it naturally occurs. In some instances, the isolated material will form part of a composition (for example, a crude extract containing other substances), buffer system or reagent mix. In other circumstance, the material may be purified to essential homogeneity, for example as determined by PAGE or column chromatography such as HPLC. Thus, an isolated nucleic acid molecule or nucleotide sequence can include a nucleic acid molecule or nucleotide sequence which is synthesized chemically or by recombinant means. Therefore, recombinant DNA contained in a vector are included in the definition of "isolated" as used herein. Also, isolated nucleotide sequences include recombinant DNA molecules in heterologous organisms, as well as partially or substantially purified DNA molecules in solution. *In vivo* and *in vitro* RNA transcripts of the DNA molecules of the present invention are also encompassed by "isolated" nucleotide sequences. Such isolated nucleotide sequences are useful in the manufacture of the encoded polypeptide, as probes for isolating homologous sequences (e.g., from other mammalian species), for gene mapping (e.g., by *in situ* hybridization with chromosomes), or for detecting expression of the oculomedin gene in tissue (e.g., human tissue), such as by Northern blot analysis.

The present invention also pertains to nucleotide sequences which are not necessarily found in nature but which encode polypeptides described herein. Thus, DNA molecules which comprise a sequence which is different from the naturally-occurring nucleotide sequence but which, due to the degeneracy of the genetic code, encode an oculomedin polypeptide of the present invention are the subject of this invention. The invention also encompasses variations of the nucleotide sequences of

the invention, such as those encoding portions, active fragments or active derivatives of the polypeptides as described below. Such variations can be naturally-occurring, such as in the case of allelic variation, or non-naturally-occurring, such as those induced by various mutagens and mutagenic processes. Intended variations include, but are not limited to, addition, deletion and substitution of one or more nucleotides which can result in conservative or non-conservative amino acid changes, including additions and deletions. Preferably, the nucleotide or amino acid variations are silent or conserved; that is, they do not alter the characteristics or activity of the oculomedin polypeptide.

Other alterations of the nucleic acid molecules of the invention can include, for example, labelling, methylation, internucleotide modifications such as uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, carbamates), charged linkages (e.g., phosphorothioates, phosphorodithioates), pendent moieties (e.g., polypeptides), intercalators (e.g., acridine, psoralen), chelators, alkylators, and modified linkages (e.g., alpha anomeric nucleic acids). Also included are synthetic molecules that mimic nucleic acid molecules in the ability to bind to a designated sequences via hydrogen bonding and other chemical interactions. Such molecules include, for example, those in which peptide linkages substitute for phosphate linkages in the backbone of the molecule.

The invention described herein also relates to fragments of the isolated nucleic acid molecules described herein. The term "fragment" is intended to encompass a portion of a nucleotide sequence described herein which is from at least about 25 contiguous nucleotides to at least about 50 contiguous nucleotides, or longer, in length. One or more introns can also be present. Such fragments are useful as probes, e.g., for diagnostic methods, and also as primers. Particularly preferred primers and probes selectively hybridize to a nucleic acid molecule encoding a oculomedin polypeptide described herein. For example, fragments which encode antigenic regions of a oculomedin polypeptide described herein are useful. Additionally, fragments which retain oculomedin activity are particularly useful.

The invention also pertains to nucleic acid molecules which hybridize under high stringency hybridization conditions, such as for selective hybridization, to a nucleotide sequence described herein (e.g., nucleic acid molecules which specifically hybridize to a nucleotide sequence encoding polypeptides described herein, and, optionally, have an activity of the polypeptide). Hybridization probes are oligonucleotides which bind in a base-specific manner to a complementary strand of nucleic acid. Suitable probes include polypeptide nucleic acids, as described in Nielsen *et al.* (*Science* 254, 1497-1500 (1991)).

Such nucleic acid molecules can be detected and/or isolated by specific hybridization (e.g., under high stringency conditions). "Stringency conditions" for hybridization is a term of art which refers to the incubation and wash conditions, e.g., conditions of temperature and buffer concentration, which permit hybridization of a particular nucleic acid to a second nucleic acid; the first nucleic acid may be perfectly (i.e., 100%) complementary to the second, or the first and second may share some degree of complementarity which is less than perfect (e.g., 70%, 75%, 85%, 95%). For example, certain high stringency conditions can be used which distinguish perfectly complementary nucleic acids from those of less complementarity.

"High stringency conditions", "moderate stringency conditions" and "low stringency conditions" for nucleic acid hybridizations are explained on pages 2.10.1-2.10.16 and pages 6.3.1-6 in *Current Protocols in Molecular Biology* (Ausubel, F.M. *et al.*, "*Current Protocols in Molecular Biology*", John Wiley & Sons, (1998), the teachings of which are hereby incorporated by reference). The exact conditions which determine the stringency of hybridization depend not only on ionic strength (e.g., 0.2XSSC, 0.1XSSC), temperature (e.g., room temperature, 42°C, 68°C) and the concentration of destabilizing agents such as formamide or denaturing agents such as SDS, but also on factors such as the length of the nucleic acid sequence, base composition, percent mismatch between hybridizing sequences and the frequency of occurrence of subsets of that sequence within other non-identical sequences. Thus, high, moderate or low stringency conditions can be determined empirically.

By varying hybridization conditions from a level of stringency at which no hybridization occurs to a level at which hybridization is first observed, conditions which will allow a given sequence to hybridize (e.g., selectively) with the most similar sequences in the sample can be determined.

Exemplary conditions are described in Krause, M.H. and S.A. Aaronson, *Methods in Enzymology, 200*:546-556 (1991). Also, in, Ausubel, *et al.*, "*Current Protocols in Molecular Biology*", John Wiley & Sons, (1998), which describes the determination of washing conditions for moderate or low stringency conditions. Washing is the step in which conditions are usually set so as to determine a minimum level of complementarity of the hybrids. Generally, starting from the lowest temperature at which only homologous hybridization occurs, each °C by which the final wash temperature is reduced (holding SSC concentration constant) allows an increase by 1% in the maximum extent of mismatching among the sequences that hybridize. Generally, doubling the concentration of SSC results in an increase in T_m of ~17°C. Using these guidelines, the washing temperature can be determined empirically for high, moderate or low stringency, depending on the level of mismatch sought.

For example, a low stringency wash can comprise washing in a solution containing 0.2XSSC/0.1% SDS for 10 min at room temperature; a moderate stringency wash can comprise washing in a prewarmed solution (42°C) solution containing 0.2XSSC/0.1% SDS for 15 min at 42°C; and a high stringency wash can comprise washing in prewarmed (68°C) solution containing 0.1XSSC/0.1%SDS for 15 min at 68°C. Furthermore, washes can be performed repeatedly or sequentially to obtain a desired result as known in the art. Equivalent conditions can be determined by varying one or more of the parameters given as an example, as known in the art, while maintaining a similar degree of identity or similarity between the target nucleic acid molecule and the primer or probe used.

Such hybridizable nucleotide sequences are useful as probes and primers, e.g., for diagnostic applications. As used herein, the term "primer" refers to a single-stranded oligonucleotide which acts as a point of initiation of template-directed DNA synthesis under appropriate conditions (*e.g.*, in the presence of four different

nucleoside triphosphates and an agent for polymerization, such as, DNA or RNA polymerase or reverse transcriptase) in an appropriate buffer and at a suitable temperature. The appropriate length of a primer depends on the intended use of the primer, but typically ranges from 15 to 30 nucleotides. Short primer molecules generally require cooler temperatures to form sufficiently stable hybrid complexes with the template. A primer need not reflect the exact sequence of the template, but must be sufficiently complementary to hybridize with a template. The term "primer site" refers to the area of the target DNA to which a primer hybridizes. The term "primer pair" refers to a set of primers including a 5' (upstream) primer that hybridizes with the 5' end of the DNA sequence to be amplified and a 3' (downstream) primer that hybridizes with the complement of the 3' end of the sequence to be amplified.

The invention also pertains to nucleotide sequences which have a substantial identity with the nucleotide sequences described herein; particularly preferred are nucleotide sequences which have at least about 70% identity, and more preferably at least about 80% identity, and even more preferably at least about 90% identity, with nucleotide sequences described herein. Particularly preferred in this instance are nucleotide sequences encoding polypeptides having an activity of an oculomedin polypeptide described herein. For example, in one embodiment, the nucleotide sequence is a TISR nucleotide encoding a polypeptide as described below.

To determine the percent identity of two nucleotide sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the sequence of a first nucleotide sequence). The nucleotides at corresponding nucleotide positions are then compared. When a position in the first sequence is occupied by the same nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity = # of identical positions/total # of positions x 100).

The determination of percent identity between two sequences can be accomplished using a mathematical algorithm. A preferred, non-limiting example of

a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin *et al.*, *Proc. Natl. Acad. Sci. USA*, *90*:5873-5877 (1993). Such an algorithm is incorporated into the NBLAST program which can be used to identify sequences having the desired identity to nucleotide sequences of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, *Nucleic Acids Res, 25*:3389-3402 (1997). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., NBLAST) can be used. See http://www.ncbi.nlm.nih.gov. In one embodiment, parameters for sequence comparison can be set at W=12. Parameters can also be varied (e.g., W=5 or W=20). The value "W" determines how many continuous nucleotides must be identical for the program to identify two sequences as containing regions of identity.

The nucleotide sequences of the nucleic acid molecules described herein (e.g.,SEQ ID NO:1) can be amplified by methods known in the art. For example, this can be accomplished by e.g., PCR. *See generally PCR Technology: Principles and Applications for DNA Amplification* (ed. H.A. Erlich, Freeman Press, NY, NY, 1992); *PCR Protocols: A Guide to Methods and Applications* (eds. Innis, *et al.*, Academic Press, San Diego, CA, 1990); Mattila *et al.*, *Nucleic Acids Res.* 19, 4967 (1991); Eckert *et al.*, *PCR Methods and Applications* 1, 17 (1991); *PCR* (eds. McPherson *et al.*, IRL Press, Oxford); and U.S. Patent 4,683,202.

Other suitable amplification methods include the ligase chain reaction (LCR) (see Wu and Wallace, *Genomics* 4, 560 (1989), Landegren *et al.*, *Science* 241, 1077 (1988), transcription amplification (Kwoh *et al.*, *Proc. Natl. Acad. Sci. USA* 86, 1173 (1989)), and self-sustained sequence replication (Guatelli *et al.*, *Proc. Nat. Acad. Sci. USA,* 87, 1874 (1990)) and nucleic acid based sequence amplification (NASBA). The latter two amplification methods involve isothermal reactions based on isothermal transcription, which produce both single stranded RNA (ssRNA) and double stranded DNA (dsDNA) as the amplification products in a ratio of about 30 or 100 to 1, respectively.

The amplified DNA can be radiolabeled and used as a probe for screening a library or other suitable vector to identify homologous nucleotide sequences.

Corresponding clones can be isolated, DNA can be obtained following *in vivo* excision, and the cloned insert can be sequenced in either or both orientations by art recognized methods, to identify the correct reading frame encoding a protein of the appropriate molecular weight. For example, the direct analysis of the nucleotide sequence of homologous nucleic acid molecules of the present invention can be accomplished using either the dideoxy chain termination method or the Maxam - Gilbert method (see Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual* (2nd Ed., CSHP, New York 1989); Zyskind *et al.*, *Recombinant DNA Laboratory Manual*, (Acad. Press, 1988)). Using these or similar methods, the protein(s) and the DNA encoding the protein can be isolated, sequenced and further characterized.

The invention also provides expression vectors containing a nucleotide sequence encoding a oculomedin polypeptide or active derivative or fragment thereof, operably linked to at least one regulatory sequence. Many such vectors are commercially available, and other suitable vectors can be readily prepared by the skilled artisan. "Operatively linked" is intended to meant that the nucleotide sequence is linked to a regulatory sequence in a manner which allows expression of the nucleotide sequence. Regulatory sequences are art-recognized and are selected to produce a oculomedin polypeptide or active derivative thereof. Accordingly, the term "regulatory sequence" includes promoters, enhancers, and other expression control elements which are described in Goeddel, *Gene Expression Technology: Methods in Enzymology 185*, Academic Press, San Diego, CA (1990). For example, the native regulatory sequences or regulatory sequences native to the transformed host cell can be employed. The design of the expression vector can depend on such factors as the choice of the host cell to be transformed and/or the type of protein desired to be expressed. For instance, the polypeptides encoded by the nucleic acid molecules of the present invention can be produced by ligating the cloned gene or cDNA, or a portion thereof, into a vector suitable for expression in either prokaryotic cells, eukaryotic cells or both (see, for example, Broach, *et al.*, *Experimental Manipulation of Gene Expression*, ed. M. Inouye (Academic Press, 1983) p. 83; *Molecular Cloning: A Laboratory Manual*, 2nd Ed., ed. Sambrook *et al.* (Cold Spring Harbor Laboratory Press, 1989) Chapters 16 and 17). If desired, the

expression constructs can contain one or more selectable markers, including, but not limited to, the gene that encodes dihydrofolate reductase and the genes that confer resistance to neomycin, tetracycline, ampicillin, chloramphenicol, kanamycin and streptomycin resistance. Vectors can also include other sequences, such as sequences necessary to render the vector capable of replicating in a host cell. For example, the vector can include one or more of the following: an autonomously replicating sequence (ARS), expression control sequences, ribosome-binding sites, RNA splice sites, polyadenylation sites, transcriptional terminator sequences, secretion signals and mRNA stabilizing sequences. Vectors can be plasmid, viral, or others known in the art, used for replication and/or expression in mammalian cells. Expression of a sequence can be by any promoter known in the art to act in mammalian, preferably human, cells. Such promoters can be inducible or constitutive. Representative promoters include but are not limited to: the SV40 early promoter region (Bernoist and Chambon (1981) *Nature* 290:304-310), the promoter contained in the 3' long terminal repeat of Reus sarcoma virus (Yamamoto *et al.* (1980), *Cell* 22:787-797), the herpes thymidine kinase promoter (Wagner *et al.* (1981), *Proc. Natl. Acad. Sci. USA* 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster *et al,* (1982) *Nature* (296:39-42), etc.

Prokaryotic and eukaryotic host cells transformed by the described vectors are also provided by this invention. For instance, cells which can be transformed with the vectors of the present invention include, but are not limited to, bacterial cells such as *E. coli* (e.g., *E. coli* K12 strains), *Streptomyces*, *Pseudomonas*, *Serratia marcescens* and *Salmonella typhimurium*, insect cells (baculovirus), including *Drosophila*, fungal cells, such as yeast cells, plant cells and mammalian cells, such as thymocytes, Chinese hamster ovary cells (CHO), and COS cells. The host cells can be transformed by the described vectors by various methods (e.g., electroporation, transfection using calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection, infection where the vector is an infectious agent such as a retroviral genome, and other methods), depending on the type of cellular host. The nucleic acid molecules of the present invention can be produced, for example, by replication

in such a host cell, as described above. Alternatively, the nucleic acid molecules can also be produced by chemical synthesis.

OCULOMEDIN POLYPEPTIDES

This invention also pertains to an isolated polypeptide, e.g., a protein, which is a novel oculomedin polypeptide, and to a polypeptide encoded by nucleotide sequences described herein. The term "polypeptide" refers to a polymer of amino acids, and not to a specific length; thus, peptides, oligopeptides and proteins are included within the definition of a polypeptide. The polypeptides of the invention can be partially or substantially purified (e.g., purified to homogeneity). For example, the polypeptides of the present invention can be isolated or purified from recombinant cell culture by a variety of processes. A polypeptide that is "isolated" is substantially free of naturally associated components, such as by separation from the components which accompany it in its natural state. Thus, a polypeptide which is chemically synthesized, or synthesized in a cellular system different from the cell in which it naturally originates will be substantially free of naturally associated components, and thus, is considered to be "isolated". Methods of isolation include, but are not limited to, anion or cation exchange chromatography, ethanol precipitation, polyacrylamide gel electrophoresis, affinity chromatography and high performance liquid chromatography (HPLC). The particular method used will depend upon the properties of the polypeptide and the selection of the host cell; appropriate methods will be readily apparent to those skilled in the art.

According to the invention, the amino acid sequence of the polypeptide can be that of the naturally-occurring oculomedin polypeptide (e.g., SEQ ID NO:2) or can comprise alterations therein. Polypeptides comprising alterations are referred to herein as "derivatives" of the native oculomedin polypeptide. Such alterations include conservative or non-conservative amino acid substitutions, additions and deletions of one or more amino acids; however, such alterations should preserve at least one activity of the oculomedin polypeptide, i.e., the altered or mutant polypeptide should be an active derivative of the naturally-occurring polypeptide.

For example, the mutation(s) can preferably preserve the three dimensional configuration of the binding and/or catalytic site of the native polypeptide. Alternatively, the fragment retains ligand-binding activity, immunological activity, or other biological activities. Immunological activities include both immunogenic function, as well as sharing of immunological epitopes for binding. Biological activities include the ability of oculomedin to be induced by stretching of trabecular cells. The presence or absence of oculomedin activity or activities can be determined by various standard functional assays including, but not limited to, assays for DNA binding activity, assays for regulatory effects of oculomedin on the expression of other genes, and assays for cellular effects of oculomedin on development of glaucoma. Moreover, amino acids which are essential for the function of the oculomedin polypeptide can be identified by methods known in the art. Particularly useful methods include site-directed mutagenesis and alanine-scanning mutagenesis (for example, Cunningham and Wells, *Science 244*:1081-1085 (1989)), crystallization and nuclear magnetic resonance. The altered polypeptides produced by these methods can be tested for particular biologic activities, including immunogenicity and antigenicity.

Specifically, appropriate amino acid alterations can be made on the basis of several criteria, including hydrophobicity, basic or acidic character, charge, polarity, size, the presence or absence of a functional group (e.g., -SH or a glycosylation site), and aromatic character. Assignment of various amino acids to similar groups based on the properties above will be readily apparent to the skilled artisan; further appropriate amino acid changes can also be found in Bowie *et al.* (*Science 247*:1306-1310(1990)). For example, conservative amino acid replacements can be those that take place within a family of amino acids that are related in their side chains. Genetically encoded amino acids are generally divided into four families: (1) acidic-aspartate, glutamate; (2) basic-lysine, arginine, histidine; (3) nonpolar-alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar-glycine, asparagine, glutamine, cystine, serine, threonine, tyrosine. Phenylalanine, tryptophan and tyrosine are sometimes classified jointly as aromatic amino acids. For example, it is reasonable to expect that an isolated

replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine or a similar conservative replacement of an amino acid with a structurally related amino acid will not have a major effect on activity or functionality. Other alterations of the polypeptides of the invention include, for example, glycosylations, acetylations, carboxylations, phosphorylations, ubiquitination, labelling (e.g., with radionuclides), enzymatic modifications, incorporation of analogs of an amino acid (including, e.g, natural amino acids), substituted linkages, and other modifications known in the art, both naturally and non-naturally occurring.

Additionally included in the invention are active fragments of the oculomedin polypeptides described herein, as well as fragments of the active derivatives described above. An "active fragment," as referred to herein, is a portion of polypeptide (or a portion of an active derivative) that retains the polypeptide's activity, as described above. The term "fragment" is intended to encompass a portion of a polypeptide described herein which is from at least about 12 contiguous amino acids to at least about 20 contiguous amino acids, more preferably at least about 25 amino acids, even more preferably at least about 30 amino acids, and yet more preferably at least about 35 amino acids. The fragment retains a biological activity of the polypeptide, as described above.

Also included in the invention are polypeptides which are at least about 70% identical to the novel oculomedin polypeptide described herein, preferably about 90% identical, and even more preferably about 95% identical. However, polypeptides exhibiting lower levels of identity are also useful, particular if they exhibit high, e.g., at least about 80%, identity over one or more particular domains of the polypeptide. For example, polypeptides sharing high degrees of identity over domains necessary for particular activities, including binding and enzymatic activity, are included herein.

The oculomedin polypeptide can also be a fusion protein comprising all or a portion of the oculomedin amino acid sequence fused to an additional component. Representative fusion partners include immunoglobulins, bacterial β-galactosidase,

trpE, protein A, β-lactamase, α-amylase, alcohol dehydrogenase, and yeast α mating factor. Additional components, such as radioisotopes and antigenic tags, can be selected to assist in the isolation or purification of the polypeptide or to extend the half life of the polypeptide; for example, a hexahistidine tag would permit ready purification by nickel chromatography. Furthermore, polypeptides of the present invention can be progenitors of the oculomedin polypeptide; progenitors are molecules which are cleaved to form an active oculomedin polypeptide.

Polypeptides described herein can also be isolated from naturally-occurring sources, chemically synthesized or recombinantly produced. For example, a nucleic acid molecule described herein can be used to produce a recombinant form of the encoded polypeptide via microbial or eukaryotic cellular processes. Ligating the polynucleotide sequence into a gene construct, such as an expression vector, and transforming or transfecting into hosts, either eukaryotic (yeast, avian, insect, plant or mammalian) or prokaryotic (bacterial cells), are standard procedures used in producing other well known proteins. Similar procedures, or modifications thereof, can be employed to prepare recombinant polypeptides according to the present invention by microbial means or tissue-culture technology. The polypeptides of the present invention can be isolated or purified (e.g., to homogeneity) from cell culture by a variety of processes. These include, but are not limited to, anion or cation exchange chromatography, ethanol precipitation, affinity chromatography and high performance liquid chromatography (HPLC). The particular method used will depend upon the properties of the polypeptide; appropriate methods will be readily apparent to those skilled in the art. For example, with respect to protein or polypeptide identification, bands identified by gel analysis can be isolated and purified by HPLC, and the resulting purified protein can be sequenced. Alternatively, the purified polypeptide can be enzymatically digested by methods known in the art to produce polypeptide fragments which can be sequenced. The sequencing can be performed, for example, by the methods of Wilm *et al.* (*Nature 379*(6564):466-469 (1996)). The polypeptide may be isolated by conventional means of protein biochemistry and purification to obtain a substantially pure product, *i.e.*, 80, 95 or 99% free of cell component contaminants, as described in

Jacoby, *Methods in Enzymology* Volume 104, Academic Press, New York (1984); Scopes, *Protein Purification, Principles and Practice*, 2nd Edition, Springer-Verlag, New York (1987); and Deutscher (ed), *Guide to Protein Purification, Methods in Enzymology*, Vol. 182 (1990).

Oculomedin polypeptides of the present invention can be used as a molecular weight marker on SDS-PAGE gels or on molecular sieve gel filtration columns using art-recognized methods. In addition, the oculomedin polypeptides can be used in the preparation of antibodies.

ANTIBODIES

The present invention also relates to antibodies which bind to a oculomedin polypeptide. For instance, polyclonal and monoclonal antibodies, including non-human and human antibodies, humanized antibodies, chimeric antibodies and antigen-binding fragments thereof (*Current Protocols in Immunology*, John Wiley & Sons, N.Y. (1994); EP Application 173,494 (Morrison); International Patent Application WO86/01533 (Neuberger); and U.S. Patent No. 5,225,539 (Winters)) which bind to a described polypeptide, are within the scope of the invention. A mammal, such as a mouse, rat, hamster or rabbit, can be immunized with an immunogenic form of the oculomedin polypeptide (e.g., the oculomedin polypeptide or a peptide comprising an antigenic fragment of the polypeptide which is capable of eliciting an antibody response). Techniques for conferring immunogenicity on a protein or peptide include conjugation to carriers or other techniques well known in the art. The protein or polypeptide can be administered in the presence of an adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassays can be used with the immunogen as antigen to assess the levels of antibody.

Following immunization, anti-polypeptide antisera can be obtained, and if desired, polyclonal antibodies can be isolated from the serum. Monoclonal antibodies can also be produced by standard techniques which are well known in the art (Kohler and Milstein, *Nature 256*:495-497 (1975); Kozbar et al., *Immunology*

Today 4:72 (1983); and Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96 (1985)). The term "antibody" as used herein is intended to include fragments thereof, such as Fab and $F(ab)_2$. Antibodies described herein can be used to inhibit the activity of the oculomedin polypeptides described herein, particularly *in vitro* and in cell extracts, using methods known in the art. Additionally, such antibodies, in conjunction with a label, such as a radioactive label, can be used to assay for the presence of the expressed protein in a cell from, e.g., a tissue sample. Such antibodies can also be used in an immunoabsorption process, such as an ELISA, to isolate an oculomedin polypeptide. Tissue samples which can be assayed include primate, particularly human, tissues, e.g., differentiated and non-differentiated cells. Examples include tissues of the eye (e.g., retina, trabecular meshwork cells, ciliary epithelial cells, etc.)

METHODS OF DIAGNOSIS AND KITS FOR DIAGNOSIS

The nucleic acids, polypeptides and antibodies described herein can be used methods of diagnosis of glaucoma, as well as in kits useful for diagnosis of glaucoma.

In one embodiment of the invention, diagnosis of glaucoma is made by detecting a polymorphism in the oculomedin gene. The polymorphism can be a mutation in the oculomedin gene, such as the insertion or deletion of a single nucleotide, or of more than one nucleotide, resulting in a frame shift mutation; the change of at least one nucleotide, resulting in a change in the encoded amino acid; the change of at least one nucleotide, resulting in the generation of a premature stop codon; the deletion of several nucleotides, resulting in a deletion of one or more amino acids encoded by the nucleotides; the insertion of one or several nucleotides, such as by unequal recombination or gene conversion, resulting in an interruption of the coding sequence of the gene; duplication of all or a part of the gene; transposition of all or a part of the gene; or rearrangement of all or a part of the gene. More than one such mutation may be present in a single gene. Such sequence changes cause a mutation in the polypeptide encoded by the oculomedin gene. For example, if the mutation is a frame shift mutation, the frame shift can result in a

change in the encoded amino acids, and/or can result in the generation of a premature stop codon, causing generation of a truncated polypeptide. Alternatively, a polymorphism associated with glaucoma can be a synonymous mutation in one or more nucleotides (i.e., a mutation that does not result in a change in the polypeptide encoded by the oculomedin gene). Such a polymorphism may alter splicing sites, affect the stability or transport of mRNA, or otherwise affect the transcription or translation of the gene. A oculomedin gene that has any of the mutations described above is referred to herein as a "mutant gene."

In a first method of diagnosing glaucoma, hybridization methods, such as Southern analysis, are used (see Current Protocols in Molecular Biology, Ausubel, F. *et al.*, eds., John Wiley & Sons, including all supplements through 1999). For example, a test sample of genomic DNA, RNA, or cDNA, is obtained from an individual suspected of having (or carrying a defect for) glaucoma (the "test individual"). The individual can be an adult, child, or fetus. The test sample can be from any source which contains genomic DNA, such as a blood sample, sample of amniotic fluid, sample of cerebrospinal fluid, or tissue sample from skin, muscle, buccal or conjunctival mucosa, placenta, gastrointestinal tract or other organs. A test sample of DNA from fetal cells or tissue can be obtained by appropriate methods, such as by amniocentesis or chorionic villus sampling. The DNA, RNA, or cDNA sample is then examined to determine whether a polymorphism in the oculomedin gene is present. The presence of the polymorphism can be indicated by hybridization of the gene in the genomic DNA, RNA, or cDNA to a nucleic acid probe. A "nucleic acid probe", as used herein, can be a DNA probe or an RNA probe; the nucleic acid probe contains at least one polymorphism in the oculomedin gene. The probe can be any of the nucleic acid molecules described above (e.g., the gene, a fragment, a vector comprising the gene, etc.)

To diagnose glaucoma, a hybridization sample is formed by contacting the test sample containing a oculomedin gene, with at least one nucleic acid probe. The hybridization sample is maintained under conditions which are sufficient to allow specific hybridization of the nucleic acid probe to the oculomedin gene. "Specific hybridization", as used herein, indicates exact hybridization (e.g., with no

mismatches). Specific hybridization can be performed under high stringency conditions or moderate stringency conditions, for example, as described above. In a particularly preferred embodiment, the hybridization conditions for specific hybridization are high stringency.

Specific hybridization, if present, is then detected using standard methods. If specific hybridization occurs between the nucleic acid probe and the oculomedin gene in the test sample, then the oculomedin gene has the polymorphism that is present in the nucleic acid probe. More than one nucleic acid probe can also be used concurrently in this method. Specific hybridization of any one of the nucleic acid probes is indicative of a polymorphism in the oculomedin gene, and is therefore diagnostic for glaucoma.

In another hybridization method, Northern analysis (see Current Protocols in Molecular Biology, Ausubel, F. *et al.*, eds., John Wiley & Sons, *supra*) is used to identify the presence of a polymorphism associated with glaucoma. For Northern analysis, a test sample of RNA is obtained from the individual by appropriate means. Specific hybridization of a nucleic acid probe, as described above, to RNA from the individual is indicative of a polymorphism in the oculomedin gene, and is therefore diagnostic for glaucoma.

For representative examples of use of nucleic acid probes, see, for example, U.S. Patents No. 5,288,611 and 4,851,330.

Alternatively, a peptide nucleic acid (PNA) probe can be used instead of a nucleic acid probe in the hybridization methods described above. PNA is a DNA mimic having a peptide-like, inorganic backbone, such as N-(2-aminoethyl)glycine units, with an organic base (A, G, C, T or U) attached to the glycine nitrogen via a methylene carbonyl linker (see, for example, Nielsen, P.E. *et al., Bioconjugate Chemistry*, 1994, 5, American Chemical Society, p. 1 (1994). The PNA probe can be designed to specifically hybridize to a gene having a polymorphism associated with glaucoma. Hybridization of the PNA probe to the oculomedin gene is diagnostic for a glaucoma.

In another method of the invention, mutation analysis by restriction digestion can be used to detect mutant genes, or genes containing polymorphisms, if the

mutation or polymorphism in the gene results in the creation or elimination of a restriction site. A test sample containing genomic DNA is obtained from the individual. Polymerase chain reaction (PCR) can be used to amplify the oculomedin gene (and, if necessary, the flanking sequences) in the test sample of genomic DNA from the test individual. RFLP analysis is conducted as described (see Current Protocols in Molecular Biology, *supra*). The digestion pattern of the relevant DNA fragment indicates the presence or absence of the mutation or polymorphism in the oculomedin gene, and therefore indicates the presence or absence of glaucoma.

Sequence analysis can also be used to detect specific polymorphisms in the oculomedin gene. A test sample of DNA or RNA is obtained from the test individual. PCR or other appropriate methods can be used to amplify the gene, and/or its flanking sequences, if desired. The sequence of the oculomedin gene, or a fragment of the gene, or cDNA, or fragment of the cDNA, or mRNA, or fragment of the mRNA, is determined, using standard methods. The sequence of the gene, gene fragment, cDNA, cDNA fragment, mRNA, or mRNA fragment is compared with the known nucleic acid sequence of the gene, cDNA (e.g., SEQ ID NO:1) or mRNA, as appropriate. The presence of a polymorphism in the oculomedin gene indicates that the individual has glaucoma.

Allele-specific oligonucleotides can also be used to detect the presence of a polymorphism in the oculomedin gene, through the use of dot-blot hybridization of amplified oligonucleotides with allele-specific oligonucleotide (ASO) probes (see, for example, Saiki, R. *et al.*, (1986), *Nature (London) 324*:163-166). An "allele-specific oligonucleotide" (also referred to herein as an "allele-specific oligonucleotide probe") is an oligonucleotide of approximately 10-50 base pairs, preferably approximately 15-30 base pairs, that specifically hybridizes to the oculomedin gene, and that contains a polymorphism associated with glaucoma. An allele-specific oligonucleotide probe that is specific for particular polymorphisms in the oculomedin gene can be prepared, using standard methods (see Current Protocols in Molecular Biology, *supra*). To identify polymorphisms in the gene that are associated with glaucoma, a test sample of DNA is obtained from the individual. PCR can be used to amplify all or a fragment of the oculomedin gene, and its

flanking sequences. The DNA containing the amplified oculomedin gene (or fragment of the gene) is dot-blotted, using standard methods (see Current Protocols in Molecular Biology, *supra*), and the blot is contacted with the oligonucleotide probe. The presence of specific hybridization of the probe to the amplified oculomedin gene is then detected. Specific hybridization of an allele-specific oligonucleotide probe to DNA from the individual is indicative of a polymorphism in the oculomedin gene, and is therefore indicative of glaucoma.

Other methods of nucleic acid analysis can be used to detect polymorphisms in the oculomedin gene. Representative methods include direct manual sequencing (Church and Gilbert, (1988), *Proc. Natl. Acad. Sci. USA 81*:1991-1995; Sanger, F. *et al.* (1977) *Proc. Natl. Acad. Sci. 74*:5463-5467; Beavis *et al.* U.S. Pat. No. 5,288,644); automated fluorescent sequencing; single-stranded conformation polymorphism assays (SSCP); clamped denaturing gel electrophoresis (CDGE); denaturing gradient gel electrophoresis (DGGE) (Sheffield, V.C. *et al.* (19891) *Proc. Natl. Acad. Sci. USA 86*:232-236), mobility shift analysis (Orita, M. *et al.* (1989) *Proc. Natl. Acad. Sci. USA 86*:2766-2770), restriction enzyme analysis (Flavell *et al.* (1978) *Cell 15*:25; Geever, *et al.* (1981) *Proc. Natl. Acad. Sci. USA 78*:5081); heteroduplex analysis; chemical mismatch cleavage (CMC) (Cotton *et al.* (1985) *Proc. Natl. Acad. Sci. USA 85*:4397-4401); RNase protection assays (Myers, R.M. *et al.* (1985) *Science 230*:1242); use of polypeptides which recognize nucleotide mismatches, such as *E. coli* mutS protein; allele-specific PCR, for example.

In another embodiment of the invention, diagnosis of glaucoma can also be made by examining expression and/or composition of the oculomedin polypeptide. A test sample from an individual is assessed for the presence of an alteration in the expression and/or an alteration in composition of the polypeptide encoded by the oculomedin gene. An alteration in expression of a polypeptide encoded by a oculomedin gene can be, for example, an alteration in the quantitative polypeptide expression (i.e., the amount of polypeptide produced); an alteration in the composition of a polypeptide encoded by an oculomedin gene is an alteration in the qualitative polypeptide expression. Both such alterations can also be present. An "alteration" in the polypeptide expression or composition, as used herein, refers to an

alteration in expression or composition in a test sample, as compared with the expression or composition of polypeptide by a oculomedin gene in a control sample. A control sample is a sample that corresponds to the test sample (e.g., is from the same type of cells), and is from an individual who is not affected by glaucoma. An alteration in the expression or composition of the polypeptide in the test sample, as compared with the control sample, is indicative of glaucoma. Various means of examining expression or composition of the polypeptide encoded by the oculomedin gene can be used, including spectroscopy, colorimetry, electrophoresis, isoelectric focusing, and immunoassays (e.g., David *et al.*, U.S. Pat. No. 4,376,110) such as immunoblotting (see also Current Protocols in Molecular Biology, particularly chapter 10). For example, Western blotting analysis, using an antibody (e.g., as described above) that specifically binds to a polypeptide encoded by a mutant oculomedin gene, or an antibody that specifically binds to a polypeptide encoded by a non-mutant gene, can be used to identify the presence in a test sample of a polypeptide encoded by a polymorphic or mutant oculomedin gene, or the absence in a test sample of a polypeptide encoded by a non-polymorphic or non-mutant gene. The presence of a polypeptide encoded by a polymorphic or mutant gene, or the absence of a polypeptide encoded by a non-polymorphic or non-mutant gene, is diagnostic for glaucoma.

In one embodiment of this method, the level or amount of polypeptide encoded by a oculomedin gene in a test sample is compared with the level or amount of the polypeptide encoded by the oculomedin gene in a control sample. A level or amount of the polypeptide in the test sample that is higher or lower than the level or amount of the polypeptide in the control sample, such that the difference is statistically significant, is indicative of an alteration in the expression of the polypeptide encoded by the oculomedin gene, and is diagnostic for glaucoma. Alternatively, the composition of the polypeptide encoded by a oculomedin gene in a test sample is compared with the composition of the polypeptide encoded by the oculomedin gene in a control sample. A difference in the composition of the polypeptide in the test sample, as compared with the composition of the polypeptide in the control sample, is diagnostic for glaucoma. In another embodiment, both the

level or amount and the composition of the polypeptide can be assessed in the test sample and in the control sample. A difference in the amount or level of the polypeptide in the test sample, compared to the control sample; a difference in composition in the test sample, compared to the control sample; or both a difference in the amount or level, and a difference in the composition, is indicative of glaucoma.

Kits useful in the methods of diagnosis comprise components useful in any of the methods described herein, including for example, hybridization probes, restriction enzymes (e.g., for RFLP analysis), allele-specific oligonucleotides, antibodies which bind to mutant or to non-mutant (native) oculomedin polypeptide, means for amplification of nucleic acids comprising the oculomedin gene, or means for analyzing the nucleic acid sequence of the oculomedin gene or for analyzing the amino acid sequence of the oculomedin polypeptide, etc.

AGENTS THAT ALTER ACTIVITY OF THE OCULOMEDIN POLYPEPTIDE, OCULOMEDIN BINDING PARTNERS, AND ASSAYS THEREFOR

The present invention also relates to an assay for identifying agents which alter the activity of the oculomedin polypeptide. For example, a cell or cell lysate containing the oculomedin polypeptide, or an active fragment or derivative thereof (as described above), can be contacted with an agent to be tested; alternatively, the polypeptide can be contacted directly with the agent to be tested. The level (amount) of oculomedin activity is assessed, and is compared with the level of activity in a control (i.e., the level of activity of the oculomedin polypeptide or active fragment or derivative thereof in the absence of the agent to be tested). If the level of the activity in the presence of the agent differs, by an amount that is statistically significant, from the level of the activity in the absence of the agent, then the agent is an agent that alters the activity of oculomedin polypeptide. An increase in the level of oculomedin activity relative to a control, indicates that the agent is an agent that enhances (is an agonist of) oculomedin activity. Similarly, a decrease in the level of oculomedin activity relative to a control, indicates that the agent is an agent that

inhibits (is an antagonist of) oculomedin activity. In another embodiment, the level of activity of the oculomedin polypeptide or active derivative or fragment thereof in the presence of the agent to be tested, is compared with a control level that has previously been established. A level of the activity in the presence of the agent that differs from the control level by an amount that is statistically significant indicates that the agent alters oculomedin activity.

The present invention also relates to an assay for identifying agents which alter the expression of the oculomedin gene. For example, a cell or cell lysate containing a nucleic acid encoding oculomedin polypeptide (e.g., the oculomedin gene) can be contacted with an agent to be tested. The level and/or pattern of oculomedin expression (e.g., the level and/or pattern of mRNA or of protein expressed) is assessed, and is compared with the level and/or pattern of expression in a control (i.e., the level and/or pattern of the oculomedin expression in the absence of the agent to be tested). If the level and/or pattern in the presence of the agent differs, by an amount or in a manner that is statistically significant, from the level and/or pattern in the absence of the agent, then the agent is an agent that alters the expression of oculomedin gene. Enhancement of oculomedin expression indicates that the agent is an agonist of oculomedin activity. Similarly, inhibition of oculomedin expression indicates that the agent is an antagonist of oculomedin activity. In another embodiment, the level and/or pattern of the oculomedin polypeptide in the presence of the agent to be tested, is compared with a control level and/or pattern that has previously been established. A level and/or pattern in the presence of the agent that differs from the control level and/or pattern by an amount or in a manner that is statistically significant indicates that the agent alters oculomedin expression.

The present invention further relates to assays for identifying agents which alter the expression of the oculomedin gene or the oculomedin protein, using genes and/or proteins that function upstream (e.g., activators or repressors of the oculomedin gene) or downstream (e.g., proteins or genes downstream of the oculomedin polypeptide, such as an oculomedin binding partner, whether activated or repressed by oculomedin). For example, a cell or cell lysate containing the

desired gene or protein is contacted with the agent to be tested, and nucleic acid comprising the oculomedin gene, or oculomedin polypeptide, is then added. The level (amount) of activity, or the effect of oculomedin on downstream elements is assessed, and is compared with the level of activity in a control. If the level of the activity in the presence of the agent differs, by an amount that is statistically significant, from the level of the activity in the absence of the agent, then the agent is an agent that alters the activity of oculomedin polypeptide. An increase in the level of activity relative to a control, indicates that the agent is an agent that enhances (is an agonist of) oculomedin activity. Similarly, a decrease in the level of activity relative to a control, indicates that the agent is an agent that inhibits (is an antagonist of) oculomedin activity. In another embodiment, the level of activity in the presence of the agent to be tested, is compared with a control level that has previously been established. A level of the activity in the presence of the agent that differs from the control level by an amount that is statistically significant indicates that the agent alters oculomedin activity.

The present invention also relates to agents identified or identifiable by the assays described above. Agents identified or identifiable by the assays described herein may enhance (e.g., prolong or increase) or inhibit (e.g., shorten or decrease) the activity of the oculomedin polypeptide, or enhance or inhibit the expression of the oculomedin gene. The invention also pertains to methods of enhancing or inhibiting the activity of the oculomedin polypeptide, as well as to methods of enhancing or inhibiting the expression of the oculomedin gene. As used herein, "enhancing" is intended to encompass any increase in oculomedin expression or activity, whether brought about by increase in the activity of the polypeptide itself, or by increase in the amount of polypeptide (e.g., through increased expression of the gene) or polypeptide mimic present, or both. As used herein, "mimic" is intended to mean an agent which has the same activity as (or mimics) the oculomedin polypeptide and includes fragments or other variants of the oculomedin polypeptide as described above. As used herein, "inhibiting" is intended to encompass any decrease in oculomedin expression or activity, whether brought about by decrease in the activity of the polypeptide itself, by decrease in the amount

of polypeptide present, or by increase in the amount or activity of an inhibitor of oculomedin.

The invention further relates to assays to identify binding partners of oculomedin protein, as well as the binding partners themselves. If oculomedin (either alone or in a complex with other polypeptides) is capable of binding DNA and modifying transcription of a gene, a transcriptional based assay can be used, for example, in which an oculomedin responsive regulatory sequence is operably linked to a detectable marker gene. Monitoring the influence of agents on cells may be applied not only in basic drug screening, but also in clinical trials. In such clinical trials, the expression of a panel of genes can be used as a "read out" of a particular drug's therapeutic effect. In yet another aspect of the invention, the oculomedin polypeptides can be used to generate a "two hybrid" assay (see, for example, U.S. Pat. No. 5,283,317; Zervos *et al.* (1993) *Cell* 72:223-232; Madura *et al.* (1993) J *Biol Chem* 268:12046-12054; Bartel *et al.* (1993) *Biotechniques* 14:920-924; Iwabuchi *et al.* (1993) *Oncogene* 8:1693-1696; and Brent, PCT International Publication No. WO94/10300), for isolating coding sequences for other cellular proteins which bind to or interact with oculomedin ("oculomedin-binding proteins" or "oculomedin-bp"). Briefly, the two hybrid assay utilizes a system in which a functional transcriptional activator protein is generated from two separate fusion proteins. For example, the method can use of chimeric genes which express hybrid proteins. To illustrate, a first hybrid gene comprises the coding sequence for a DNA-binding domain of a transcriptional activator fused in frame to the coding sequence for a oculomedin polypeptide. The second hybrid protein encodes a transcriptional activation domain fused in frame to a test gene (e.g., from a cDNA library). If the bait and sample hybrid proteins are able to interact, e.g., form an oculomedin-dependent complex, they bring into close proximity the two domains of the transcriptional activator. This proximity is sufficient to cause transcription of the reporter gene which is operably linked to a transcriptional regulatory site responsive to the transcriptional activator, and expression of the reporter gene can be detected and used to score for the interaction of the oculomedin and test proteins.

Thus, an agent can be tested to determine whether it is an oculomedin binding partner, by contacting a first molecule comprising an oculomedin polypeptide operably linked to a heterologous DNA binding domain, with a second molecule comprising a test agent operably linked to a polypeptide transcriptional activation domain, and with a hybrid reporter gene comprising a nucleic acid encoding a reporter operably linked to a DNA sequence comprising a binding site for the heterologous DNA binding domain. The presence (or absence) of expression of the hybrid reporter gene is then assessed; the presence of expression indicates that the test agent is an oculomedin binding partner. The same methods can be used to assess the strength of an interaction between an oculomedin binding partner and oculomedin: the degree of expression of the hybrid reporter gene correlates directly with the strength of interaction between the oculomedin polypeptide and the oculomedin binding partner.

PHARMACEUTICAL COMPOSITIONS

The present invention also pertains to pharmaceutical compositions comprising nucleic acids described herein, particularly nucleotides encoding the polypeptides described herein; comprising polypeptides or proteins described herein; and/or comprising the agent that alters (e.g., enhances or inhibits) oculomedin polypeptide activity described herein. For instance, a polypeptide or protein, fragment, fusion protein or prodrug thereof, or a nucleotide or nucleic acid construct (vector) comprising a nucleotide of the present invention, or an agent that alters oculomedin polypeptide activity, can be formulated with a physiologically acceptable carrier or excipient to prepare a pharmaceutical composition. The carrier and composition can be sterile. The formulation should suit the mode of administration.

Suitable pharmaceutically acceptable carriers include but are not limited to water, salt solutions (e.g., NaCl), saline, buffered saline, alcohols, glycerol, ethanol, gum arabic, vegetable oils, benzyl alcohols, polyethylene glycols, gelatin, carbohydrates such as lactose, amylose or starch, dextrose, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid esters, hydroxymethylcellulose,

polyvinyl pyrolidone, etc., as well as combinations thereof. The pharmaceutical preparations can, if desired, be mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or aromatic substances and the like which do not deleteriously react with the active compounds.

The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, polyvinyl pyrollidone, sodium saccharine, cellulose, magnesium carbonate, etc.

Methods of introduction of these compositions include, but are not limited to, intradermal, intramuscular, intraperitoneal, intraocular, intravenous, subcutaneous, topical, oral and intranasal. In a preferred embodiment, the composition is introduced intraocularly (e.g., eye drops). Other suitable methods of introduction can also include gene therapy (as described below), rechargeable or biodegradable devices, particle acceleration devises ("gene guns") and slow release polymeric devices. The pharmaceutical compositions of this invention can also be administered as part of a combinatorial therapy with other agents.

The composition can be formulated in accordance with the routine procedures as a pharmaceutical composition adapted for administration to human beings. For example, compositions for intravenous administration typically are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile

pharmaceutical grade water, saline or dextrose/water. Where the composition is administered by injection, an ampule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

For topical application, nonsprayable forms, viscous to semi-solid or solid forms comprising a carrier compatible with topical application and having a dynamic viscosity preferably greater than water, can be employed. Suitable formulations include but are not limited to solutions, suspensions, emulsions, creams, ointments, powders, enemas, lotions, sols, liniments, salves, aerosols, etc., which are, if desired, sterilized or mixed with auxiliary agents, e.g., preservatives, stabilizers, wetting agents, buffers or salts for influencing osmotic pressure, etc. The agent may be incorporated into a cosmetic formulation. For topical application, also suitable are sprayable aerosol preparations wherein the active ingredient, preferably in combination with a solid or liquid inert carrier material, is packaged in a squeeze bottle or in admixture with a pressurized volatile, normally gaseous propellant, e.g., pressurized air.

Agents described herein can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The agents are administered in a therapeutically effective amount. The amount of agents which will be therapeutically effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, *in vitro* or *in vivo* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of a practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use of sale for human administration. The pack or kit can be labeled with information regarding mode of administration, sequence of drug administration (e.g., separately, sequentially or concurrently), or the like. The pack or kit may also include means for reminding the patient to take the therapy. The pack or kit can be a single unit dosage of the combination therapy or it can be a plurality of unit dosages. In particular, the agents can be separated, mixed together in any combination, present in a single vial or tablet. Agents assembled in a blister pack or other dispensing means is preferred. For the purpose of this invention, unit dosage is intended to mean a dosage that is dependent on the individual pharmacodynamics of each agent and administered in FDA approved dosages in standard time courses.

METHODS OF THERAPY

The present invention also pertains to methods of treatment or therapy for glaucoma, using an oculomedin therapeutic agent. An "oculomedin therapeutic agent" is an agent that alters (e.g., enhances or inhibits) oculomedin polypeptide activity and/or oculomedin gene expression, as described herein (e.g., an oculomedin agonist or antagonist). The therapy is designed to inhibit, replace or supplement activity of the oculomedin polypeptide in an individual (for example, by administering a nucleic acid encoding the oculomedin polypeptide or a derivative or active fragment thereof; by administering the oculomedin polypeptide(s) or a derivative or active fragment thereof; and/or by administering an agent that alters the activity of the oculomedin polypeptide). The oculomedin therapeutic agent can be a nucleic acid (e.g., a gene, cDNA, mRNA, a nucleic acid encoding an oculomedin polypeptide or active fragment or derivative thereof, or an oligonucleotide); a protein, peptide, or peptidomimetic (e.g., an oculomedin polypeptide or an active

fragment or derivative thereof), an antibody (e.g., an antibody to a mutant oculomedin polypeptide, or an antibody to a non-mutant oculomedin polypeptide, as described above); a ribozyme; a small molecule or other agent that alters oculomedin polypeptide activity and/or gene expression (e.g., which upregulate or downregulate expression of the oculomedin gene). More than one oculomedin therapeutic agents can be used concurrently, if desired.

The oculomedin therapeutic agent(s) are administered in a therapeutically effective amount (i.e., an amount that is sufficient to treat the disease, such as by ameliorating symptoms associated with the disease, preventing or delaying the onset of the disease, and/or also lessening the severity or frequency of symptoms of the disease). The amount which will be therapeutically effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, *in vitro* or *in vivo* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of a practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

In one embodiment of the invention, a nucleic acid is used in the treatment of glaucoma. The term, "treatment" as used herein, refers not only to ameliorating symptoms associated with the disease, but also preventing or delaying the onset of the disease, and also lessening the severity or frequency of symptoms of the disease. In one embodiment, a nucleic acid of the invention (e.g., the nucleic acid encoding an oculomedin polypeptide, such as SEQ ID NO:1; or another nucleic acid that encodes the oculomedin polypeptide or a derivative or active fragment) can be used, either alone or in a pharmaceutical composition as described above. For example, the oculomedin gene or a cDNA encoding the oculomedin polypeptide, either by itself or included within a vector, can be introduced into cells (either *in vitro* or *in vivo*) such that the cells produce native oculomedin polypeptide. If necessary, cells that have been transformed with the gene or cDNA or a vector comprising the gene

or cDNA can be introduced (or re-introduced) into an individual affected with the disease. Thus, cells which, in nature, lack native oculomedin expression and activity, or have mutant oculomedin expression and activity, can be engineered to express oculomedin polypeptide (or, for example, an active fragment of the oculomedin polypeptide). In a preferred embodiment, nucleic acid encoding the oculomedin polypeptide, or an active fragment or derivative thereof, can be introduced into an expression vector, such as a viral vector, and the vector can be introduced into appropriate cells which lack native oculomedin expression in an animal. For example, for the treatment of glaucoma, the vector comprising the nucleic acid can be introduced intraocularly. In such methods, a cell population can be engineered to inducibly or constitutively express active oculomedin polypeptide. Other gene transfer systems, including viral and nonviral transfer systems, can be used. Alternatively, nonviral gene transfer methods, such as calcium phosphate coprecipitation, mechanical techniques (e.g., microinjection); membrane fusion-mediated transfer via liposomes; or direct DNA uptake, can also be used.

Alternatively, in another embodiment of the invention, a nucleic acid of the invention; a nucleic acid complementary to a nucleic acid of the invention; or a portion of such a nucleic acid (e.g., an oligonucleotide as described below), can be used in "antisense" therapy, in which a nucleic acid (e.g., an oligonucleotide) which specifically hybridizes to the mRNA and/or genomic DNA of the oculomedin gene is administered or generated *in situ*. The antisense nucleic acid that specifically hybridizes to the mRNA and/or DNA inhibits expression of the oculomedin protein, e.g., by inhibiting translation and/or transcription. Binding of the antisense nucleic acid can be by conventional base pair complementarity, or, for example, in the case of binding to DNA duplexes, through specific interaction in the major groove of the double helix.

An antisense construct of the present invention can be delivered, for example, as an expression plasmid as described above. When the plasmid is transcribed in the cell, it produces RNA which is complementary to a portion of the mRNA and/or DNA which encodes oculomedin protein. Alternatively, the antisense construct can be an oligonucleotide probe which is generated *ex vivo* and introduced

into cells; it then inhibits expression by hybridizing with the mRNA and/or genomic DNA of the oculomedin gene. In one embodiment, the oligonucleotide probes are modified oligonucleotides which are resistant to endogenous nucleases, e.g. exonucleases and/or endonucleases, thereby rendering them stable *in vivo*. Exemplary nucleic acid molecules for use as antisense oligonucleotides are phosphoramidate, phosphothioate and methylphosphonate analogs of DNA (see also U.S. Pat. Nos. 5,176,996; 5,264,564; and 5,256,775). Additionally, general approaches to constructing oligomers useful in antisense therapy are also described, for example, by Van der Krol *et al.* ((1988) *Biotechniques* 6:958-976); and Stein *et al.* ((1988) *Cancer Res* 48:2659-2668). With respect to antisense DNA, oligodeoxyribonucleotides derived from the translation initiation site, e.g. between the -10 and +10 regions of the oculomedin gene sequence, are preferred.

To perform antisense therapy, oligonucleotides (mRNA, cDNA or DNA) are designed that are complementary to mRNA encoding oculomedin. The antisense oligonucleotides bind to oculomedin mRNA transcripts and prevent translation. Absolute complementarity, although preferred, is not required. a sequence "complementary" to a portion of an RNA, as referred to herein, indicates that a sequence has sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double-stranded antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid, as described in detail above. Generally, the longer the hybridizing nucleic acid, the more base mismatches with an RNA it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures.

Nucleic acid molecules to be used in triple helix formation for the inhibition of transcription are preferably single stranded and composed of deoxyribonucleotides. The base composition of these oligonucleotides should promote triple helix formation via Hogsteen base pairing rules, which generally require sizable stretches of either purines or pyrimidines to be present on one strand

of a duplex. Nucleotide sequences may be pyrimidine-based, which will result in TAT and CGC triplets across the three associated strands of the resulting triple helix. The pyrimidine-rich molecules provide base complementarity to a purine-rich region of a single strand of the duplex in a parallel orientation to that strand. In addition, nucleic acid molecules may be chosen that are purine-rich, for example, containing a stretch of G residues. These molecules will form a triple helix with a DNA duplex that is rich in GC pairs, in which the majority of the purine residues are located on a single strand of the targeted duplex, resulting in CGC triplets across the three strands in the triplex. The potential sequences that can be targeted for triple helix formation may be increased by creating a "switchback" nucleic acid molecule which is synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with first one strand of a duplex and then the other, eliminating the necessity for a sizable stretch of either purines or pyrimidines to be present on one strand of a duplex.

In a preferred embodiment, oligonucleotides that are complementary to the 5' end of the message, e.g., the 5' untranslated sequence up to and including the AUG initiation codon, are used to inhibit translation. However, sequences complementary to the 3' untranslated sequences of mRNAs have recently been shown to be effective at inhibiting translation of mRNAs as well (Wagner, R. (1994) *Nature* 372:333); therefore, oligonucleotides complementary to either the 5' or 3' untranslated, non-coding regions of the oculomedin gene can also be used in an antisense approach to inhibit translation of endogenous oculomedin mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA can include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions can also be used in accordance with the invention. While antisense nucleotides complementary to the can region sequence can be used, those complementary to the transcribed untranslated region can also be used. Whether designed to hybridize to the 5', 3' or coding region of oculomedin mRNA, antisense nucleic acids are preferably at least six nucleotides in length, and are more preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In certain preferred embodiments, the oligonucleotide is at least 10 nucleotides, at least 18 nucleotides, at least 24 nucleotides, or at least 50 nucleotides.

If desired, *in vitro* studies can be performed to quantitate the ability of the antisense oligonucleotide to inhibit gene expression. These studies utilize controls that distinguish between antisense gene inhibition and nonspecific biological effects of oligonucleotides. These studies can compare levels of the target RNA or protein with that of an internal control RNA or protein. In a preferred embodiment, the control oligonucleotide is of approximately the same length as the test oligonucleotide and the nucleotide sequence of the oligonucleotide differs from the antisense sequence on so much so as to prevent specific hybridization to the target sequence.

The oligonucleotides used in antisense therapy can be DNA, RNA, or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotides can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotides can include other appended groups such as peptides (e.g. for targeting host cell receptors in vivo), or agents facilitating transport across the cell membrane (see, e.g., Letsinger *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:6553-6556; Lemaitre *et al.*, (1987), *Proc. Natl. Acad Sci. USA* 84:648-652; PCT International Publication No. W088/09810) or the blood-brain barrier (see, e.g., PCT International Publication No. W089/10134), or hybridization-triggered cleavage agents (see, e.g., Krol *et al.* (1988) *BioTechniques* 6:958-976) or intercalating agents. (See, e.g., Zon, (1988), *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The antisense oligonucleotide can comprise at least one (or more) modified base moiety which is selected from the group including but not limited to 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine,

2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5- oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. The antisense oligonucleotide can also comprise at least one modified sugar moiety selected from the group including but not limited to arabinose, 2-fluoroarabinose, xylulose, and hexose. In another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group consisting of a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof. In yet another embodiment, the antisense oligonucleotide is an α.-anomeric oligonucleotide. An α-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gautier *et al.*, (1987), *Nucl. Acids Res.* 15:6625-6641). The oligonucleotide is a 2'-0-methylribonucleotide (Inoue *et al.* (1987), *Nucl. Acids Res.* 15:6131-6148), or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett.* 215:327-330).

Oligonucleotides can be synthesized by standard methods known in the art and described herein (e.g. by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides can be synthesized by the method of Stein *et al.* ((1988) *Nucl. Acids Res.* 16:3209), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin *et al*, (1988*) Proc. Natl. Acad. Sci. USA.* 85:7448-7451), etc.

The antisense molecules are delivered to cells which express oculomedin *in vivo*. A number of methods can be used for delivering antisense DNA or RNA to cells; e.g., antisense molecules can be injected directly into the tissue site, or

modified antisense molecules, designed to target the desired cells (e.g., antisense linked to peptides or antibodies that specifically bind receptors or antigens expressed on the target cell surface) can be administered systematically. Alternatively, in a preferred embodiment, a recombinant DNA construct is utilized in which the antisense oligonucleotide is placed under the control of a strong promoter (e.g., pol III or pol II). The use of such a construct to transfect target cells in the patient results in the transcription of sufficient amounts of single stranded RNAs that will form complementary base pairs with the endogenous oculomedin transcripts and thereby prevent translation of the oculomedin mRNA. For example, a vector can be introduced *in vivo* such that it is taken up by a cell and directs the transcription of an antisense RNA. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art and described above. For example, a plasmid, cosmid, YAC or viral vector can be used to prepare the recombinant DNA construct which can be introduced directly into the tissue site (e.g., the trabecular meshwork). Alternatively, viral vectors can be used which selectively infect the desired tissue, in which case administration may be accomplished by another route (e.g., systematically).

Ribozyme molecules designed to catalytically cleave oculomedin mRNA transcripts can also be used to prevent translation of oculomedin mRNA and expression of oculomedin protein. (See, e.g., PCT International Publication No. W090/11364, and Sarver *et al.* (1990), *Science* 247:1222-1225). Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by an endonucleolytic cleavage. The composition of ribozyme molecules includes one or more sequences complementary to the target gene mRNA, and must include the catalytic sequence responsible for mRNA cleavage. For this sequence, see U.S. Pat. No. 5,093,246. Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the molecule of interest for ribozyme cleavage sites which include the following sequences, GUA, GUU and GUC. Once identified, short RNA

sequences of between approximately 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for predicted structural features, such as secondary structure, that can render the oligonucleotide sequence unsuitable. The suitability of candidate sequences can also be evaluated by testing their accessibility to hybridization with complementary oligonucleotides, using ribonuclease protection assays. Ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy oculomedin mRNAs. In another embodiment, hammerhead ribozymes are used. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA having the sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is described more fully in Haseloff and Gerlach, ((1988) *Nature* 334:585-591). Preferably the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the oculomedin mRNA, in order to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

The ribozymes used in the present invention can also include RNA endoribonucleases (hereinafter "Cech-type ribozymes") such as the one which occurs naturally in Tetrahymena Thermophila (known as the IVS, or L-19 IVS RNA) and which has been extensively described by Thomas Cech and collaborators (Zaug *et al* (1984) *Science* 224:574-578; Zaug and Cech, (1986) *Science* 231:470-475; Zaug *et al.* (1986) *Nature* 324:429-433; PCT International Publication No. WO88/04300.; Been and Cech (1986) *Cell* 47:207-216). The Cech-type ribozymes have an eight base pair active site which hybridizes to a target RNA sequence, after which cleavage of the target RNA takes place. The invention further encompasses those Cech-type ribozymes which target eight base-pair active site sequences that are present in oculomedin.

As in the antisense approach, the ribozymes can be composed of modified oligonucleotides (e.g. for improved stability, targeting, etc.) and are delivered to cells which express oculomedin in vivo (e.g., trabecular meshwork). A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive promoter, so that transfected cells will produce

sufficient quantities of the ribozyme to destroy endogenous oculomedin messages and inhibit translation. Because ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

Endogenous oculomedin gene expression can also be reduced by inactivating or "knocking out" the oculomedin gene or its promoter using targeted homologous recombination (e.g., see Smithies *et al.* (1985) *Nature* 317:230-234; Thomas & Capecchi (1987) *Cell* 51:503-512; Thompson *et al.* (1989) *Cell* 5:313-321). For example, a mutant, non-functional oculomedin gene (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous oculomedin gene (either the coding regions or regulatory regions of the oculomedin gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express oculomedin *in vivo*. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the oculomedin gene. The recombinant DNA constructs can be directly administered or targeted to the required site *in vivo* using appropriate vectors, as described above. Alternatively, expression of non-mutant oculomedin can be increased using a similar method: targeted homologous recombination can be used to insert a DNA construct comprising a non-mutant, functional oculomedin gene (e.g., a gene having SEQ ID NO:1) in place of a mutant oculomedin gene in the cell, as described above.

Alternatively, endogenous oculomedin gene expression can be reduced by targeting deoxyribonucleotide sequences complementary to the regulatory region of the oculomedin gene (i.e., the oculomedin promoter and/or enhancers) to form triple helical structures that prevent transcription of the oculomedin gene in target cells in the body. (See generally, Helene, C. (1991) *Anticancer Drug Des.*, 6(6):569-84; Helene, C., *et al.* (1992) *Ann, N.Y. Acad. Sci.*, 660:27-36; and Maher, L. J. (1992) *Bioassays* 14(12):807-15). Likewise, the antisense constructs of the present invention, by antagonizing the normal biological activity of one of the oculomedin proteins, can be used in the manipulation of tissue, e.g. tissue differentiation, both *in vivo* and *for ex vivo* tissue cultures. Furthermore, the anti-sense techniques (e.g. microinjection of antisense molecules, or transfection with plasmids whose transcripts are anti-sense with regard to a oculomedin mRNA or gene sequence) can

be used to investigate role of oculomedin in developmental events, as well as the normal cellular function of oculomedin in adult tissue. Such techniques can be utilized in cell culture, but can also be used in the creation of transgenic animals.

In yet another embodiment of the invention, polypeptides and/or agents that alter (e.g., enhance or inhibit) oculomedin polypeptide activity, as described herein, can be used in the treatment or prevention of glaucoma. The polypeptides or agents can be delivered in a composition, as described above, or by themselves. They can be administered systemically, or can be targeted to a particular tissue (e.g.,eye tissue). The polypeptides and/or agents can be produced by a variety of means, including chemical synthesis; recombinant production; *in vivo* production (e.g., a transgenic animal, such as U.S. Pat. No. 4,873,316 to Meade *et al.*), for example, and can be isolated using standard means such as those described herein.

A combination of any of the above methods of treatment (e.g., administration of non-mutant oculomedin protein in conjunction with antisense therapy targeting mutant oculomedin mRNA), can also be used.

TRANSGENIC ANIMALS

Another aspect of the present invention concerns transgenic animals which are comprised of cells (of that animal) which contain a transgene of oculomedin and which preferably (though optionally) express an exogenous oculomedin polypeptide in one or more cells in the animal. The term, "transgene," as used herein, indicates a nucleic acid (encoding, e.g., an oculomedin polypeptide as described herein, or encoding an antisense transcript to an oculomedin gene), which is partly or entirely heterologous to the transgenic animal or cell into which it is introduced;, or is homologous to an endogenous gene of the transgenic animal or cell into which it is introduced, but which is designed to be inserted, or is inserted, into the animal's genome in such a way as to alter the genome of the cell into which it is inserted (e.g., at a location which differs from that of the natural gene or its insertion results in a knockout or an endogenous gene). "transgenic animal" refers to any animal, preferably a non-human mammal, bird or an amphibian, in which one or more of the cells of the animal contain heterologous nucleic acid introduced by way of human

intervention, such as by transgenic techniques known in the art. The nucleic acid is introduced into the cell, directly or indirectly by introduction into a precursor of the cell, by way of deliberate genetic manipulation, such as by microinjection or by infection with a recombinant virus. The term genetic manipulation does not include classical cross-breeding, or in vitro fertilization, but rather is directed to the introduction of a recombinant DNA molecule. This molecule may be integrated within a chromosome, or it may be extrachromosomally replicating DNA. An oculomedin transgene can encode the wild-type oculomedin polypeptide, as described above, or can encode homologs, active derivatives or active fragments thereof, or can also include an agonist or an antagonist of oculomedin. In preferred embodiments, the expression of the transgene is restricted to specific subsets of cells, tissues or developmental stages utilizing, for example, cis-acting sequences that control expression in the desired pattern. In the present invention, such mosaic expression of an oculomedin polypeptide can be useful for many forms of lineage analysis and can additionally provide a means to assess the effects of, for example, lack of oculomedin in small patches of tissue within an otherwise normal embryo. Toward this end, tissue-specific regulatory sequences and conditional regulatory sequences can be used to control expression of the transgene in certain spatial patterns. Moreover, temporal patterns of expression can be provided by, for example, conditional recombination systems or prokaryotic transcriptional regulatory sequences.

Genetic techniques allowing the expression of transgenes can be regulated via site-specific genetic manipulation *in vivo*, and are known to those skilled in the art. For instance, genetic systems are available which allow for the regulated expression of a recombinase that catalyzes the genetic recombination of a target sequence. As used herein, the phrase "target sequence" refers to a nucleotide sequence that is genetically recombined by a recombinase. The target sequence is flanked by recombinase recognition sequences and is generally either excised or inverted in cells expressing recombinase activity. Recombinase catalyzed recombination events can be designed such that recombination of the target sequence results in either the activation or repression of expression of, for example, an

oculomedin polypeptide as described herein. For example, excision of a target sequence which interferes with the expression of a recombinant oculomedin gene, such as one which encodes an antagonistic homolog or an antisense transcript, can be designed to activate expression of that gene. This interference with expression of the protein can result from a variety of mechanisms, such as spatial separation of the oculomedin gene from the promoter element or an internal stop codon. Moreover, the transgene can be made wherein the coding sequence of the gene is flanked by recombinase recognition sequences and is initially transfected into cells in a 3' to 5' orientation with respect to the promoter element. In such an instance, inversion of the target sequence will reorient the subject gene by placing the 5' end of the coding sequence in an orientation with respect to the promoter element which allow for promoter driven transcriptional activation.

The transgenic animals of the present invention all include within a plurality of their cells a transgene of the present invention, which transgene alters the phenotype of the "host cell" (e.g., with respect to regulation of cell growth, death and/or differentiation). Since it is possible to produce transgenic organisms of the invention utilizing one or more of the transgene constructs described herein, a general description will be given of the production of transgenic organisms by referring generally to exogenous genetic material. This general description can be adapted by those skilled in the art in order to incorporate specific transgene sequences into organisms utilizing the methods and materials described below.

In an illustrative embodiment, either the cre/loxP recombinase system of bacteriophage P1 (Lakso *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:6232-6236; Orban *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:6861-6865) or the FLP recombinase system of Saccharomyces cerevisiae (O'Gorman *et al.*. (1991) *Science* 251:1351-1355; PCT International Publication No. WO92/15694) can be used to generate *in vivo* site-specific genetic recombination systems. Cre recombinase catalyzes the site-specific recombination of an intervening target sequence located between loxP sequences. loxP sequences are 34 base pair nucleotide repeat sequences to which the Cre recombinase binds and are required for Cre recombinase mediated genetic recombination. The orientation of loxP sequences determines

whether the intervening target sequence is excised or inverted when Cre recombinase is present (Abremski *et al.* (1984) *J. Biol. Chem.* 259:1509-1514); catalyzing the excision of the target sequence when the loxP sequences are oriented as direct repeats and catalyzes inversion of the target sequence when loxP sequences are oriented as inverted repeats.

Accordingly, genetic recombination of the target sequence is dependent on expression of the Cre recombinase. Expression of the recombinase can be regulated by promoter elements which are subject to regulatory control, e.g., tissue-specific, developmental stage-specific, inducible or repressible by externally added agents. This regulated control will result in genetic recombination of the target sequence only in cells where recombinase expression is mediated by the promoter element. Thus, the activation of expression of a recombinant oculomedin polypeptide can be regulated via control of recombinase expression.

Use of the cre/loxP recombinase system to regulate expression of a recombinant oculomedin polypeptide requires the construction of a transgenic animal containing transgenes encoding both the Cre recombinase and the subject polypeptide. Animals containing both the Cre recombinase and a recombinant oculomedin gene can be generated through the construction of "double" transgenic animals. A convenient method for providing such animals is to mate two transgenic animals each containing a transgene, e.g., an oculomedin gene and recombinase gene. One advantage derived from initially constructing transgenic animals containing an oculomedin transgene in a recombinase-mediated expressible format derives from the possibility that the subject protein, whether agonistic or antagonistic, can be deleterious upon expression in the transgenic animal. In such an instance, a founder population, in which the subject transgene is silent in all tissues, can be propagated and maintained. Individuals of this founder population can be crossed with animals expressing the recombinase in, for example, one or more tissues and/or a desired temporal pattern. Thus, the creation of a founder population in which, for example, an antagonistic oculomedin transgene is silent will allow the study of progeny from that founder in which disruption of oculomedin mediated

induction in a particular tissue or at certain developmental stages would result in, for example, a lethal phenotype.

Similar conditional transgenes can be provided using prokaryotic promoter sequences which require prokaryotic proteins to be simultaneous expressed in order to facilitate expression of the oculomedin transgene. Exemplary promoters and the corresponding trans-activating prokaryotic proteins are given in U.S. Pat. No. 4,833,080. Moreover, expression of the conditional transgenes can be induced by gene therapy-like methods wherein a gene encoding the trans-activating protein, e.g. a recombinase or a prokaryotic protein, is delivered to the tissue and caused to be expressed, such as in a cell-type specific manner. By this method, an oculomedin transgene could remain silent into adulthood until "turned on" by the introduction of the trans-activator.

In one embodiment, the transgenic non-human animals of the invention are produced by introducing transgenes into the germline of the non-human animal. Embryonal target cells at various developmental stages can be used to introduce transgenes. Different methods are used depending on the stage of development of the embryonal target cell. The specific line(s) of any animal used to practice this invention are selected for general good health, good embryo yields, good pronuclear visibility in the embryo, and good reproductive fitness. In addition, the haplotype is a significant factor. For example, when transgenic mice are to be produced, strains such as C57BL/6 or FVB lines are often used (Jackson Laboratory, Bar Harbor, Me.). Preferred strains are those with H-2^b, H-2^d or H-2^q haplotypes such as C57BL/6 or DBA/1. The line(s) used to practice this invention may themselves be transgenics, and/or may be knockouts (i.e., obtained from animals which have one or more genes partially or completely suppressed). In one embodiment, the transgene construct is introduced into a single stage embryo. The zygote is the best target for micro-injection. In the mouse, the male pronucleus reaches the size of approximately 20 micrometers in diameter which allows reproducible injection of 1-2 pl of DNA solution. The use of zygotes as a target for gene transfer has a major advantage in that in most cases the injected DNA will be incorporated into the host gene before the first cleavage (Brinster *et al.* (1985)*Proc. Natl. Acad.Sci. USA* 82:4438-4442).

As a consequence, all cells of the transgenic animal will carry the incorporated transgene. This will in general also be reflected in the efficient transmission of the transgene to offspring of the founder since 50% of the germ cells will harbor the transgene.

Normally, fertilized embryos are incubated in suitable media until the pronuclei appear. At about this time, the nucleotide sequence comprising the transgene is introduced into the female or male pronucleus as described below. In some species such as mice, the male pronucleus is preferred. It is most preferred that the exogenous genetic material be added to the male DNA complement of the zygote prior to its being processed by the ovum nucleus or the zygote female pronucleus. It is thought that the ovum nucleus or female pronucleus releases molecules which affect the male DNA complement, perhaps by replacing the protamines of the male DNA with histones, thereby facilitating the combination of the female and male DNA complements to form the diploid zygote. Thus, it is preferred that the exogenous genetic material be added to the male complement of DNA or any other complement of DNA prior to its being affected by the female pronucleus. For example, the exogenous genetic material is added to the early male pronucleus, as soon as possible after the formation of the male pronucleus, which is when the male and female pronuclei are well separated and both are located close to the cell membrane. Alternatively, the exogenous genetic material could be added to the nucleus of the sperm after it has been induced to undergo decondensation. Sperm containing the exogenous genetic material can then be added to the ovum or the decondensed sperm could be added to the ovum with the transgene constructs being added as soon as possible thereafter.

Introduction of the transgene nucleotide sequence into the embryo may be accomplished by any means known in the art such as, for example, microinjection, electroporation, or lipofection. Following introduction of the transgene nucleotide sequence into the embryo, the embryo may be incubated in vitro for varying amounts of time, or reimplanted into the surrogate host, or both. In vitro incubation to maturity is within the scope of this invention. One common method is to incubate

the embryos in vitro for about 1-7 days, depending on the species, and then reimplant them into the surrogate host.

For the purposes of this invention a zygote is essentially the formation of a diploid cell which is capable of developing into a complete organism. Generally, the zygote will be comprised of an egg containing a nucleus formed, either naturally or artificially, by the fusion of two haploid nuclei from a gamete or gametes. Thus, the gamete nuclei must be ones which are naturally compatible, i.e., ones which result in a viable zygote capable of undergoing differentiation and developing into a functioning organism. Generally, a euploid zygote is preferred. If an aneuploid zygote is obtained, then the number of chromosomes should not vary by more than one with respect to the euploid number of the organism from which either gamete originated.

In addition to biological considerations, physical considerations also play a role in the amount (e.g., volume) of exogenous genetic material which can be added to the nucleus of the zygote or to the genetic material which forms a part of the zygote nucleus. If no genetic material is removed, then the amount of exogenous genetic material which can be added is limited by the amount which will be absorbed without being physically disruptive. Generally, the volume of exogenous genetic material inserted will not exceed about 10 picoliters. The physical effects of addition must not be so great as to physically destroy the viability of the zygote. The biological limit of the number and variety of DNA sequences will vary depending upon the particular zygote and functions of the exogenous genetic material and will be readily apparent to one skilled in the art, because the genetic material, including the exogenous genetic material, of the resulting zygote must be biologically capable of initiating and maintaining the differentiation and development of the zygote into a functional organism. The number of copies of the transgene constructs which are added to the zygote is dependent upon the total amount of exogenous genetic material added and will be the amount which enables the genetic transformation to occur. Theoretically only one copy is required; however, generally, numerous copies are utilized, for example, approximately 1,000-20,000 copies of the transgene construct, in order to insure that one copy is functional. As regards the present

invention, there will often be an advantage to having more than one functioning copy of each of the inserted exogenous DNA sequences to enhance the phenotypic expression of the exogenous DNA sequences. Any technique which allows for the addition of the exogenous genetic material into nucleic genetic material can be utilized so long as it is not destructive to the cell, nuclear membrane or other existing cellular or genetic structures. In one embodiment, the exogenous genetic material is preferentially inserted into the nucleic genetic material by microinjection. Microinjection of cells and cellular structures is known and is used in the art.

Reimplantation of embryos is performed using standard methods. Usually, the surrogate host is anesthetized, and the embryos are inserted into the oviduct. The number of embryos implanted into a particular host will vary by species, but will usually be comparable to the number of off spring the species naturally produces.

Transgenic offspring of the surrogate host may be screened for the presence and/or expression of the transgene by a variety of methods. Screening is often accomplished by Southern blot or Northern blot analysis, using a probe that is complementary to at least a portion of the transgene. Western blot analysis using an antibody against the protein encoded by the transgene can be employed as an alternative or additional method for screening for the presence of the transgene product. Typically, DNA is prepared from tail tissue and analyzed by Southern analysis or PCR for the transgene. Alternatively, the tissues or cells believed to express the transgene at the highest levels are tested for the presence and expression of the transgene using Southern analysis or PCR, although any tissues or cell types may be used for this analysis. Alternative or additional methods for evaluating the presence of the transgene include, without limitation, suitable biochemical assays such as enzyme and/or immunological assays, histological stains for particular marker or enzyme activities, flow cytometric analysis, and the like. Analysis of the blood can also be useful to detect the presence of the transgene product in the blood, as well as to evaluate the effect of the transgene on the levels of various types of blood cells and other blood constituents.

Progeny of the transgenic animals may be obtained by mating the transgenic animal with a suitable partner, or by in vitro fertilization of eggs and/or sperm

obtained from the transgenic animal. Where mating with a partner is to be performed, the partner may or may not be transgenic and/or a knockout; where it is transgenic, it may contain the same or a different transgene, or both. Alternatively, the partner may be a parental line. Where in vitro fertilization is used, the fertilized embryo may be implanted into a surrogate host or incubated in vitro, or both. Using either method, the progeny may be evaluated for the presence of the transgene using methods described above, or other appropriate methods.

The transgenic animals produced in accordance with the present invention will include exogenous genetic material. As set out above, the exogenous genetic material can, in certain embodiments, be a DNA sequence which results in the production of an oculomedin polypeptide, or derivative or active fragment thereof, an agonistic or antagonistic polypeptide, and antisense transcript. Further, in such embodiments the sequence can comprise a transcriptional control element, e.g., a promoter, which preferably allows the expression of the transgene product in a specific type of cell.

Retroviral infection can also be used to introduce transgene into a non-human animal. The developing non-human embryo can be cultured in vitro to the blastocyst stage. During this time, the blastomeres can be targets for retroviral infection (Jaenich, R. (1976) *Proc. Natl. Acad. Sci. USA* 73:1260-1264). Efficient infection of the blastomeres is obtained by enzymatic treatment to remove the zona peliucida (see. e.g., Manipulating the Mouse Embryo, Hogan eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1986). The viral vector system used to introduce the transgene is typically a replication-defective retrovirus carrying the transgene (Jahner *et al.* (1985) *Proc. Natl. Acad. Sci. USA* 82:6927-6931; Van der Putten *et al.* (1985) *Proc. Natl. Acad. Sci. USA* 82:6148-6152). Transfection is easily and efficiently obtained by culturing the blastomeres on a monolayer of virus-producing cells (Van der Putten, supra; Stewart *et al.* (1987) *EMBO J.* 6:383-388). Alternatively, infection can be performed at a later stage. Virus or virus-producing cells can be injected into the blastocoele (Jahner *et al.* (1982) *Nature* 298:623-628). Most of the founders will be mosaic for the transgene since incorporation occurs only in a subset of the cells which formed the transgenic

non-human animal. Further, the founder may contain various retroviral insertions of the transgene at different positions in the genome which generally will segregate in the offspring. In addition, it is also possible to introduce transgenes into the germ line by intrauterine retroviral infection of the midgestation embryo (Jahner *et al.* (1982) supra).

A third type of target cell for transgene introduction is the embryonal stem cell (ES). ES cells are obtained from pre-implantation embryos cultured in vitro and fused with embryos (Evans *et al.* (1981) *Nature* 292:154-156; Bradley *et al.* (1984) *Nature* 309:255-258; Gossler *et al.* (1986) *Proc. Natl. Acad. Sci. USA* 83:9065-9069; and Robertson *et al.* (1986) *Nature* 322:445-448). Transgenes can be efficiently introduced into the ES cells by DNA transfection or by retrovirus-mediated transduction. Such transformed ES cells can thereafter be combined with blastocysts from a non-human animal. The ES cells thereafter colonize the embryo and contribute to the germ line of the resulting chimeric animal. For review see Jaenisch, R. (1988) *Science* 240:1468-1474.

In one embodiment, gene targeting, which is a method of using homologous recombination to modify an animal's genome, can be used to introduce changes into cultured embryonic stem cells. By targeting An oculomedin gene in ES cells, these changes can be introduced into the germline of animals to generate chimeras. The gene targeting procedure is accomplished by introducing into tissue culture cells a DNA targeting construct that includes a segment homologous to a target locus (e.g., comprising the oculomedin gene), and which also includes an intended sequence modification to the oculomedin genomic sequence (e.g., insertion, deletion, point mutation). The treated cells are then screened for accurate targeting to identify and isolate those which have been properly targeted. Gene targeting in embryonic stem cells is a means for disrupting an oculomedin gene function through the use of a targeting transgene construct designed to undergo homologous recombination with one or more oculomedin genomic sequences. The targeting construct can be arranged so that, upon recombination with an element of an oculomedin gene, a positive selection marker is inserted into (or replaces) coding sequences of the gene. The inserted sequence functionally disrupts the oculomedin gene, while also

providing a positive selection trait. Exemplary oculomedin targeting constructs are described in more detail below.

Generally, the embryonic stem cells (ES cells) used to produce the knockout animals will be of the same species as the knockout animal to be generated. Thus for example, mouse embryonic stem cells will usually be used for generation of knockout mice. Embryonic stem cells are generated and maintained using methods well known to the skilled artisan such as those described by Doetschman *et al.* (1985) *J. Embryol. Exp. Mol.* 87:27-45). Any line of ES cells can be used, however, the line chosen is typically selected for the ability of the cells to integrate into and become part of the germ line of a developing embryo so as to create germ line transmission of the knockout construct. Thus, any ES cell line that is believed to have this capability is suitable for use herein. One mouse strain that is typically used for production of ES cells, is the 129J strain. Another ES cell line is murine cell line D3 (American Type Culture Collection, catalog no. CKL 1934). Still another preferred ES cell line is the WW6 cell line (Ioffe *et al.* (1995) *Proc. Natl. Acad. Sci. USA* 92:7357-7361). The cells are cultured and prepared for knockout construct insertion using methods well known to the skilled artisan, such as those set forth by Robertson in: Teratocarcinomas and Embryonic Stem Cells: A Practical Approach, E. J. Robertson, ed. IRL Press, Washington, D.C. (1987)); by Bradley *et al.* (1986) Current Topics in Devel. Biol. 20:357-371); and by Hogan *et al.* (Manipulating the Mouse Embryo: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1986)).

Insertion of the knockout construct into the ES cells can be accomplished using a variety of methods well known in the art including for example, electroporation, microinjection, and calcium phosphate treatment. A preferred method of insertion is electroporation. Each knockout construct to be inserted into the cell must first be in the linear form. Therefore, if the knockout construct has been inserted into a vector, linearization is accomplished by digesting the DNA with a suitable restriction endonuclease selected to cut only within the vector sequence and not within the knockout construct sequence. For insertion, the knockout construct is added to the ES cells under appropriate conditions for the insertion

method chosen, as is known to the skilled artisan. Where more than one construct is to be introduced into the ES cell, each knockout construct can be introduced concurrently or consecutively.

If the ES cells are to be electroporated, the ES cells and knockout construct DNA are exposed to an electric pulse using an electroporation machine and following the manufacturer's guidelines for use. After electroporation, the ES cells are typically allowed to recover under suitable incubation conditions. The cells are then screened for the presence of the knockout construct. Screening can be accomplished using a variety of methods. Where the marker gene is an antibiotic resistance gene, for example, the ES cells may be cultured in the presence of an otherwise lethal concentration of antibiotic. Those ES cells that survive have presumably integrated the knockout construct. If the marker gene is other than an antibiotic resistance gene, a Southern blot of the ES cell genomic DNA can be probed with a sequence of DNA designed to hybridize only to the marker sequence. Alternatively, PCR can be used. Finally, if the marker gene is a gene that encodes an enzyme whose activity can be detected (e.g., .beta.-galactosidase), the enzyme substrate can be added to the cells under suitable conditions, and the enzymatic activity can be analyzed. One skilled in the art will be familiar with other useful markers and the means for detecting their presence in a given cell. All such markers are contemplated as being included within the scope of the invention.

The knockout construct may integrate into several locations in the ES cell genome, and may integrate into a different location in each ES cell's genome due to the occurrence of random insertion events. The desired location of insertion is in a complementary position to the DNA sequence to be knocked out, e.g., the oculomedin coding sequence, transcriptional regulatory sequence, etc. Typically, less than about 1-5% of the ES cells that take up the knockout construct will actually integrate the knockout construct in the desired location. To identify those ES cells with proper integration of the knockout construct, total DNA can be extracted from the ES cells using standard methods, and the DNA can then be probed on a Southern blot with a probe or probes designed to hybridize in a specific pattern to genomic DNA digested with particular restriction enzyme(s). Alternatively, or additionally,

the genomic DNA can be amplified by PCR with probes specifically designed to amplify DNA fragments of a particular size and sequence (i.e., only those cells containing the knockout construct in the proper position will generate DNA fragments of the proper size).

After suitable ES cells containing the knockout construct in the proper location have been identified, the cells can be inserted into an embryo. Insertion can be accomplished in a variety of ways known to the skilled artisan, a preferred method is by microinjection. For microinjection, about 10-30 cells are collected into a micropipet and injected into embryos that are at the proper stage of development (e.g., blastocyte) to permit integration of the foreign ES cell containing the knockout construct into the developing embryo. The suitable stage of development for the embryo used for insertion of ES cells is generally species dependent; for example, for mice it is about 3.5 days. The embryos are obtained by perfusing the uterus of pregnant females. Suitable methods for accomplishing this are known to the skilled artisan, and are set forth by, e.g., Bradley et al. (supra). While any embryo of the right stage of development is suitable for use, preferred embryos are male. In mice, the preferred embryos also have genes coding for a coat color that is different from the coat color encoded by the ES cell genes. In this way, the offspring can be screened easily for the presence of the knockout construct by looking for mosaic coat color (indicating that the ES cell was incorporated into the developing embryo). Thus, for example, if the ES cell line carries the genes for white fur, the embryo selected will carry genes for black or brown fur.

After the ES cell has been introduced into the embryo, the embryo may be implanted into the uterus of a pseudopregnant foster mother for gestation. While any foster mother may be used, the foster mother is typically selected for her ability to breed and reproduce well, and for her ability to care for the young. Such foster mothers are typically prepared by mating with vasectomized males of the same species. The stage of the pseudopregnant foster mother is important for successful implantation, and it is species dependent. For mice, this stage is about 2-3 days pseudopregnant.

Offspring that are born to the foster mother may be screened initially for mosaic coat color where the coat color selection strategy as described above. Alternatively or in addition, DNA from tail tissue of the offspring can be screened for the presence of the knockout construct using standard methods, such as Southern blots and/or PCR as described above. Alternatively, other means of identifying and characterizing the knockout offspring can be used. For example, Northern blots can be used to probe the mRNA for the presence or absence of transcripts encoding either the gene knocked out, the marker gene, or both. In addition, Western blots can be used to assess the level of expression of the oculomedin gene knocked out in various tissues of the offspring by probing the Western blot with an antibody against the particular oculomedin polypeptide, or an antibody against the marker gene product, where this gene is expressed. Finally, in situ analysis (such as fixing the cells and labeling with antibody) and/or FACS (fluorescence activated cell sorting) analysis of various cells from the offspring can be conducted using suitable antibodies to look for the presence or absence of the knockout construct gene product.

Offspring that appear to be mosaics can be crossed to each other, if they are believed to carry the knockout construct in their germ line, in order to generate homozygous knockout animals. Homozygotes can be identified by Southern blotting of equivalent amounts of genomic DNA from mice that are the product of this cross, as well as mice that are known heterozygotes and wild type mice.

Yet other methods of making knock-out or disruption transgenic animals are also generally known (see, for example, Manipulating the Mouse Embryo, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986)). Recombinase dependent knockouts can also be generated, e.g. by homologous recombination to insert target sequences, such that tissue specific and/or temporal control of inactivation of an oculomedin-gene can be controlled by recombinase sequences (described herein).

Animals containing more than one knockout construct and/or more than one transgene expression construct can prepared in several ways. The preferred manner of preparation is to generate a series of animals, each containing one of the desired

transgenic phenotypes. Such animals are bred together through a series of crosses, backcrosses and selections, to ultimately generate a single animal containing all desired knockout constructs and/or expression constructs, where the animal is otherwise congenic (genetically identical) to the wild type except for the presence of the knockout construct(s) and/or transgene(s).

The following Exemplification is offered for the purpose of illustrating the present invention and are not to be construed to limit the scope of this invention. The teachings of all references cited herein are hereby incorporated herein by reference in their entirety.

EXEMPLIFICATION: Identification of Oculomedin, a Protein Induced by Stretching of Trabecular Cells of the Eye

MATERIALS AND METHODS

Human trabecular cell culture Trabeculum tissue fragments excised during trabeculectomy in patients with primary open-angle glaucoma were placed in wells of a 24-well multidish containing Dulbecco's modified Eagle's medium (DMEM) supplemented with 15% fetal calf serum, 100 mg/L ampicillin and 100 mg/L streptomycin, and incubated under a humidified atmosphere of 5% carbon dioxide and 95% air at 37°C. Cells, growing out of the tissue fragments usually 2 weeks after the start of culture, were transferred to a 6-well multidish and then to 6-cm petri dishes (Matsuo, T. and Matsuo, N., *Br. J. Ophthalmol. 60*:561-566 (1996)).

Cyclic mechanical stretching Three 6-cm petri dishes with confluent trabecular cells were placed securely on the apparatus for cyclic mechanical stretching, with a piston under the dish moving up and down as reported previously (Matsuo, T. *et al., Jpn. J. Ophthalmol. 40*:289-296 (1996)). The piston was moved at a cycle of 30 seconds to stretch the dish bottom at the maximum magnitude of a strain of 4500 microstrains (4.5 mm/m) for 24 hours. Three dishes for a non-stretched control were placed in the same incubator for the same period.

Isolation of mRNA and subtraction Trabecular cells of 3 stretched and non-stretched dishes were dislodged by incubation with Hanks' balanced salt solution containing 0.25% trypsin and 1 mM EDTA (Gibco BRL, Gaithersburg, MD, USA). Poly(A)+ RNA was isolated (Micro-FastTrack mRNA Isolation Kit, Invitrogen, Carlsbad, CA, USA) and cDNA was synthesized. Subtraction of non-stretched control cell cDNA from stretched cell cDNA was done with a polymerase chain reaction (PCR)-based method (PCR Select cDNA Subtraction Kit, Clontech Laboratories, Palo Alto, CA, USA). Briefly, stretched cell cDNA and non-stretched control cell cDNA were digested with a restriction enzyme, RsaI, to obtain shorter, blunt-ended DNA. Two different adaptors (adaptor 1 and adaptor 2) were then ligated to 5' end of each strand of stretched cDNA after the digestion. The adaptor 1-ligated stretched cell cDNA and adaptor 2-ligated stretched cell cDNA were separately hybridized at 68°C for 8 hours with an excess of non-stretched control cell cDNA after denaturation at 98°C for 1.5 minutes. The two primary hybridization samples were then mixed together without denaturation and hybridized at 68°C overnight with an excess of denatured non-stretched control cell cDNA. Differentially expressed sequences in stretched cell cDNA were amplified by two rounds of PCR. The first PCR amplification was based on suppression PCR to amplify only cDNA with different adaptors at both ends, which were further enriched by the second PCR amplification with nested primers.

Isolation and sequencing of stretch-specific cDNA clones and Northern blot analysis Stretch-specific sequences amplified by PCR were cloned into a vector, pCR-Script SK(+) phagemid (pCR-Script SK(+) Cloning Kit, Stratagene, La Jolla, CA, USA). Plasmids were isolated with Qiagen Plasmid Mini Kit (Qiagen, Hilden, Germany) and then sequenced with ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit and ABI PRISM 310 Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA), using primers, pUC/M13 Primers, Forward and Reverse (Promega, Madison, WI, USA). Northern blot analysis was first done with dot blot. Isolated cDNA clones were labeled with alkaline phosphatase (AlkPhos Direct, Amersham Life Science, Buckinghamshire, England) and hybridized in each well of BIO-DOT Microfiltration Apparatus (Bio-Rad Laboratories, Richmond, CA,

USA) with mRNA which were isolated from stretched and non-stretched control trabecular cells and blotted on a sheet of nylon membrane (Hibond N+, Amersham Life Science, Buckinghamshire, England). Signals were detected with chemiluminescence (CDP-Star Detection Reagent, Amersham Life Science, Buckinghamshire, England) on Hyperfilm MP (Amersham Life Science, Buckinghamshire, England).

Full-length cDNA isolation Human eye cDNA library cloned in λ gt10 (Clontech Laboratories, Palo Alto, CA, USA) was screened with isolated stretch-specific sequences which were labeled with alkaline phosphatase as described above. Phage DNA was purified from plate lysates with Qiagen Lambda Mini Kit (Qiagen, Hilden, Germany), and inserts of isolated λ clones were amplified by PCR using primers, λ gt10 forward and reverse sequencing primers (Clontech Laboratories, Palo Alto, CA, USA). The PCR-amplified sequences were cloned into a vector, pCR TOPO vector (TOPO TA Cloning Kit, , Invitrogen, Carlsbad, CA, USA) and sequenced. Stretched cell-derived and non-stretched control cell-derived mRNA, mixed with RNA Sample Loading Buffer (5 Prime-3 Prime Inc., Boulder, CO, USA) were separated on a 1% agarose mini-gel containing formaldehyde with MOPS buffer, and transferred by blotting to a nylon membrane (Hibond N+) with Vacuum Blotting Unit and Pump (2016 VACUGENE, LKB Bromma). The blot was cross-linked with ultraviolet irradiation (Spectrolinker, Stratagene, La Jolla, CA, USA) and hybridized with alkaline phosphatase-labeled full length cDNA as described above. The expression of isolated genes in other tissues was examined by Northern blot analysis using Human 12-Lane Multiple Tissue Northern (MTN) Blot (Clontech Laboratories, Palo Alto, CA, USA) and also a blot with mRNA isolated from human retina.

RESULTS

Ten clones were detected by dot blot screening to be expressed more predominantly in mechanically-stretched trabecular cells than in control cells. Northern blot analysis confirmed that one clone with an insert of 210 base pairs was selectively expressed only in the stretched cells. A full-length cDNA with 1116 base

pairs (GenBank Accession Number, AF142063) was isolated from the human eye cDNA library by screening with the insert. Northern blot analysis with this full-length cDNA as a probe detected the expression of mRNA with about 1770 base length, only in the stretched cells.

The gene was also expressed in the human retina, but its expression was not detected in the human brain, heart, skeletal muscle, colon, thymus, spleen, kidney, liver, small intestine, placenta, lung, or peripheral blood leukocytes..

The cDNA had an Alu repetitive element in the 5' untranslated region. Search in the GenBank did not detect any expressed sequence tags (ESTs) which showed homology with the cDNA. An open reading frame in the cDNA encoded a putative small protein with 44 amino acids. The predicted amino acids showed 70% homology, 48% identity (13/27) and 70% similarity (19/27), with a domain of rat neurokinin B precursor (neuromedin K), although the homologous region is not the domain which is conserved among tachykinin peptides (Bonner, T.L. *et al., Brain Res. 388*:243-249 (1987)). Based on this homology, the putative protein was named 'oculomedin'.

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the spirit and scope of the invention as defined by the appended claims.

CLAIMS

What is claimed is:

1. Isolated nucleic acid molecule which encodes a oculomedin polypeptide or an active derivative or fragment thereof.

2. Isolated nucleic acid molecule of Claim 1, wherein the oculomedin polypeptide has the amino acid sequence of SEQ ID NO:2.

3. Isolated nucleic acid molecule of Claim 1, wherein said nucleic acid molecule has the nucleotide sequence of SEQ ID NO:1.

4. A DNA construct comprising the isolated nucleic acid molecule of Claim 1 operatively linked to a regulatory sequence.

5. The DNA construct of Claim 4, wherein the construct is replicable in a host cell.

6. A recombinant host cell comprising the isolated nucleic acid molecule of Claim 1 operatively linked to a regulatory sequence.

7. A method for preparing oculomedin polypeptide, or an active derivative or fragment thereof, comprising culturing the recombinant host cell of Claim 6.

8. Isolated oculomedin polypeptide, or an active derivative or fragment.

9. Isolated oculomedin protein of Claim 8, wherein the polypeptide is a derivative possessing substantial sequence identity with the endogenous oculomedin polypeptide.

10. Isolated oculomedin protein of Claim 8, wherein the polypeptide has the amino acid sequence of SEQ ID NO:2.

11. An antibody, or an antigen-binding fragment thereof, which selectively binds to the isolated oculomedin polypeptide or active derivative or fragment thereof according to Claim 8.

12. A method for assaying the presence of a mammalian oculomedin polypeptide in a cell, comprising contacting said cell with an antibody of Claim 11.

13. The method of Claim 12, wherein said cell is in a tissue sample.

14. An antibody, or an antigen-binding fragment thereof, which selectively binds to an isolated mutant oculomedin polypeptide or an active derivative or fragment thereof.

15. A method of diagnosing glaucoma in an individual, comprising detecting a polymorphism in the oculomedin gene, wherein the presence of the polymorphism in the gene is indicative of glaucoma.

16. A method of diagnosing glaucoma, comprising detecting an alteration in the expression or composition of oculomedin polypeptide, wherein the presence of an alteration in expression or composition of the polypeptide is indicative of glaucoma.

17. The method of Claim 16, wherein the alteration is a qualitative alteration in the composition of the oculomedin polypeptide.

18. The method of Claim 16, wherein the alteration is a quantitative alteration in expression of the oculomedin polypeptide.

19. The method of Claim 16, wherein the alteration is both a qualitative alteration in the composition and a quantitative alteration in the expression of the oculomedin protein.

20. A method of identifying an agent which alters activity of the oculomedin polypeptide of Claim 8, comprising the steps of:

a) contacting the oculomedin polypeptide or an active derivative or fragment thereof, with an agent to be tested;

b) assessing the level of activity of the oculomedin polypeptide or active derivative or fragment thereof; and

c) comparing the level of activity with a level of activity of the oculomedin polypeptide or active derivative or fragment thereof in the absence of the agent,

wherein if the level of activity of the oculomedin polypeptide or active derivative or fragment thereof in the presence of the agent differs, by an amount that is statistically significant, from the level in the absence of the agent, then the agent is an agent that alters activity of the oculomedin polypeptide.

21. The method of Claim 20, wherein the agent inhibits activity of the oculomedin polypeptide.

22. The method of Claim 20, wherein the agent enhances activity of the oculomedin polypeptide.

23. An agent which alters activity of oculomedin polypeptide, identified according to the method of Claim 20.

24. A method of altering activity of oculomedin polypeptide of Claim 8, comprising contacting the oculomedin polypeptide with an agent that alters activity of the oculomedin polypeptide.

25. A method of identifying an agent which alters expression of the oculomedin gene, comprising the steps of:

a) contacting a cell containing a nucleic acid of Claim 1 with an agent to be tested;

b) assessing the level of expression of the nucleic acid; and

c) comparing the level of expression with a level of expression of the nucleic acid in the absence of the agent,

wherein if the level of expression of the nucleotide in the presence of the agent differs, by an amount that is statistically significant, from the level of expression in the absence of the agent, then the agent is an agent that alters expression of the oculomedin gene.

26. The method of Claim 25, wherein the agent inhibits expression of the oculomedin gene.

27. The method of Claim 25, wherein the agent enhances expression of the oculomedin gene.

28. An agent which alters expression of oculomedin gene, identified according to the method of Claim 25.

29. A method of altering expression of oculomedin gene, comprising contacting a cell containing the oculomedin gene an agent that alters expression of the oculomedin gene.

30. A pharmaceutical composition comprising an isolated nucleic acid molecule of Claim 1.

31. A pharmaceutical composition comprising an oculomedin polypeptide of Claim 8.

32. A pharmaceutical composition comprising an agent that alters activity of oculomedin polypeptide or alters expression of oculomedin gene.

33. A method of treating glaucoma in an individual, comprising administering to the individual, isolated nucleic acid molecule of Claim 1, in a therapeutically effective amount.

34. A method of treating glaucoma in an individual, comprising administering to the individual, oculomedin polypeptide of Claim 8 in a therapeutically effective amount.

35. A method of treating glaucoma in an individual, comprising administering to the individual an oculomedin therapeutic agent in a therapeutically effective amount.

36. The method of Claim 35, wherein the oculomedin therapeutic agent is an oculomedin agonist.

37. The method of Claim 35, wherein the oculomedin therapeutic agent is an oculomedin antagonist.

38. The method of Claim 35, wherein the oculomedin therapeutic agent alters oculomedin polypeptide activity.

39. The method of Claim 35, wherein the oculomedin therapeutic agent alters oculomedin gene expression.

40. The method of Claim 35, wherein the oculomedin therapeutic agent is selected from the group consisting of: a nucleic acid, protein, peptide, peptidomimetic, ribozyme, small molecule, and antibody.

41. A method of determining whether a test agent is an oculomedin binding partner, comprising:

a) contacting a first molecule comprising an oculomedin polypeptide operably linked to a heterologous DNA binding domain with a second molecule comprising a test agent operably linked to a polypeptide transcriptional activation domain and with a hybrid reporter gene comprising a nucleic acid encoding a reporter operably linked to a DNA sequence comprising a binding site for the heterologous DNA binding domain; and

b) detecting the presence of expression of the hybrid reporter gene,

wherein presence of expression indicates that the test agent is an oculomedin binding partner.

42. A method of assessing the strength of an interaction between an oculomedin polypeptide and an oculomedin binding partner, comprising:

a) contacting a first molecule comprising an oculomedin polypeptide operably linked to a heterologous DNA binding domain with a second molecule comprising an oculomedin binding partner operably linked to a polypeptide transcriptional activation domain and with a hybrid reporter gene comprising a nucleic acid encoding a reporter operably linked to a DNA sequence comprising a binding site for the heterologous DNA binding domain; and

b) assessing expression of the hybrid reporter gene,

wherein the degree of expression of the hybrid reporter gene is indicative of the strength of interaction between the oculomedin polypeptide and the oculomedin binding partner.

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FIG. 1

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FIG. 2

SEQUENCE LISTING

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/23516

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/12 C12N15/11 C07K14/47 C07K16/18 C12Q1/68
A61K38/17 A61P27/06 A01K67/027 G01N33/50 G01N33/53

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)
IPC 7 C12N C07K

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SATO Y ET AL.: "A novel gene (oculomedin) induced by mechanical stretching in human trabecular cells of the eye" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 259, no. 2, 7 June 1999 (1999-06-07), pages 349-351, XP002139471 Note: 100.0% nt sequence identity with SEQ ID NO:1 in 999 bp overlap, 100.0% aa sequence identity with SEQ ID NO:2 in 44 aa overlap. the whole document	1-22, 25-27, 30,31, 33,34, 41,42

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 June 2000	26/06/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	van de Kamp, M

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

INTERNATIONAL SEARCH REPORT

Int. ational Application No
PCT/US 99/23516

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 20131 A (UNIV IOWA RES FOUND) 14 May 1998 (1998-05-14) page 4, line 16 -page 6, line 4 page 47-82 claims 11-31	15-22, 25-27, 33,34, 41,42
A	BONNER T I ET AL.: "A cDNA encoding the precursor of the rat neuropeptide, neurokinin B" MOLECULAR BRAIN RESEARCH, vol. 2, 1987, pages 243-249, XP002076988 cited in the application Note: 48.2% aa sequence identity with SEQ ID NO:2 in 27 aa overlap. abstract; figure 1	1,4-9, 11-14, 30,31
A	WO 98 55612 A (ZYMOGENETICS INC) 10 December 1998 (1998-12-10) page 27, line 24 -page 31, line 28 page 54, line 27 -page 58, line 12 page 61, line 3 -page 63, line 2 page 65, line 31 -page 66, line 4	20-22, 25-27, 41,42
A	WO 99 47923 A (MACKINNON RODERICK ;UNIV ROCKEFELLER (US)) 23 September 1999 (1999-09-23) Note: 36.4% aa sequence identity of SEQ ID NO:9 with SEQ ID NO:2 in 22 aa overlap. page 3-21	20-22, 41,42
A	CRAIG J E ET AL.: "Glaucoma genetics: where are we? Where will we go?" CURRENT OPINION IN OPHTHALMOLOGY, vol. 10, no. 2, April 1999 (1999-04), pages 126-134, XP000914582 abstract	1,8,15, 16,20, 25,30, 31,33,34
A	STEWART W C: "Perspectives in the medical treatment of glaucoma" CURRENT OPINION IN OPHTHALMOLOGY, vol. 10, no. 2, April 1999 (1999-04), pages 99-108, XP000914581 abstract	1,8,15, 16,20, 25,30, 31,33,34

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Remark (1): Although claim 15-19, in sofar as they are concerned with in vivo methods, are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Remark (2): Although claims 33 and 34 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.2

Claims Nos.: 23,24,28,29,32,35-40

Remark (3): Claims 23, 24, 28, 29, 32, and 35-40 refer to agents or oculomedin therapeutic agents that alter the activity of the oculomedin polypeptide, or that alter the expression of the oculomedin gene, without giving a true technical characterisation. Moreover, no specific compounds are defined in the application. In consequence, the scope of said claims is ambiguous and vague, and their subject-matter is not sufficiently disclosed and supported (Art. 5 and 6 PCT). No search can be carried out for such purely speculative claims whose wording is, in fact, a mere recitation of the results to be achieved.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ational Application No
PCT/US 99/23516

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9820131 A	14-05-1998	US 5925748 A	20-07-1999
		US 5885776 A	23-03-1999
		AU 5436498 A	29-05-1998
		EP 0942975 A	22-09-1999
WO 9855612 A	10-12-1998	AU 7699198 A	21-12-1998
WO 9947923 A	23-09-1999	AU 3198899 A	11-10-1999