(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
23 October 2003 (23.10.2003)

PCT

(10) International Publication Number
WO 03/087367 A2

(51) International Patent Classification[7]: C12N 15/11, A61K 31/713, C12Q 1/68, C12N 15/86, 5/10, A01K 67/027

(21) International Application Number: PCT/EP03/04002

(22) International Filing Date: 16 April 2003 (16.04.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
02008761.5 18 April 2002 (18.04.2002) EP
60/431,173 5 December 2002 (05.12.2002) US

(71) Applicant *(for all designated States except US)*: **LYNKEUS BIOTECH GMBH** [DE/DE]; Friedrich-Bergius-Ring 15, 97076 Würzburg (DE).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **SCHULTE, Ralf, Wilhelm** [DE/DE]; Schellenbergstrasse 17, 81547 München (DE). **GÖHRING, Frank** [DE/DE]; Robert-Kirchhoff-strasse 35, 97076 Würzburg (DE).

(74) Agent: **KNAUTHE EGGERS**; Postfach 31 01 40, 80102 München (DE).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/087367 A2

(54) Title: MEANS AND METHODS FOR THE SPECIFIC INHIBITION OF GENES IN CELLS AND TISSUE OF THE CNS AND/OR EYE

(57) Abstract: Described is a method for the specific modulation of the expression of target genes in cells and/or tissues of the CNS and/or eye, wherein a composition comprising one or more doubled stranded oligoribonucleotides (dsRNA) is introduced into the cell, tissue or organism outside the blood-brain or blood-retina barriers. Furthermore, a method for the identification and validation of the function of a gene is provided, wherein the method provides a test cell, test tissue or test organism, which allow information to be gained on the function of the target gene. In addition, compositions and kits are described useful for those methods. In particular, components and methods for the diagnostic use and/or therapy of disorders related to the CNS and/or eye are provided which are based on RNA interference.

Means and Methods for the Specific Inhibition of Genes in Cells and Tissue of the CNS and/or Eye

The present invention relates to the specific modulation of the expression of genes in cells and tissues of the CNS and/or the eye. In particular, the present invention relates to the use of one or more double-stranded oligoribonucleotides (dsRNA) for the preparation of a composition for the specific modulation of the expression of one or more target genes in cells and/or tissues of the CNS and/or eye of a subject, wherein said composition is designed to be applied outside the blood-brain or blood-retina barriers. The instant invention further relates to methods for the identification and validation, respectively, of the function of a gene comprising the mentioned use of dsRNA for providing a test cell, test tissue or test organism and comparing the resulting phenotype with that of a suitable control, thus allowing information on the function of the gene to be gained. In addition, the present invention relates to cells, tissue and non-human organisms obtainable by the method of the invention, wherein said organisms preferably display of phenotype of a CNS or eye disease. Furthermore, the present invention relates to the use of RNA interference technique for the diagnosis and/or therapy of disorders related to CNS and/or eye and to method of identification and isolation of drugs capable of specific modulation of the expression of a target gene in cells and/or tissues of the eye making use of the afore-mentioned methods.

Several documents are cited throughout the text of this specification. Each of the documents cited herein (including any manufacturer's specifications, instructions, etc.) are hereby incorporated herein by reference; however, there is no admission that any document cited is indeed prior art as to the present invention.

The human eye is an organ of extraordinary complexity, the specific function of particular structures and tissues of which are coordinated in such a way as to ensure optimal process of seeing, from impact of the light ray on the lens to transformation into electrical impulses and the transmission into the areas of the brain responsible for conscious perception. Particularly, tissues at the back of the eye such as the multi-layer retina, in which functionally highly specialized types of cells mediate the transformation of light energy to electrical impulses, and also the retinal pigment epithelium (RPE) are characterized by an extremely high

metabolic activity. The active supply of photoreceptors with nutrients from the blood circulation and the simultaneous removal and processing of degradation products of the visual process, takes place via the RPE, which in turn is separated from the blood vessels of the choriocapillaris by Bruch's membrane. The exchange of substances via the RPE and Bruch's membrane is controlled and specific, and on the basis of this functional analogy to the blood-brain barrier one refers to a blood-retina barrier in this case.
The activity of numerous, often specifically expressed genes is necessary for controlling and carrying out the phototransduction process in the cells of the retina and the metabolic exchange across the blood-retina barrier, and furthermore also for maintaining the structure and functional integrity of numerous components of the tissues of the back of the eye. This unique and highly developed system is therefore very susceptible to numerous genetic defects, expressed in a broad phenotypical range of retinal diseases.

Like the human central nervous system the human eye is an organ characterized by high complexity and the coordinated functioning of numerous specific structures and tissues. Both are protected by barriers (tear secretion, enzymes, transport mechanisms, blood-retina and blood-CNS barrier) against harmful environmental influences. Like the blood-brain barrier, the blood-retina barrier also represents a physiological barrier for the uptake of medication by the inner part of the eye, and makes pharmacological therapy of ocular diseases very difficult indeed – if at all possible - at the present state of technology.

Medication currently available on the market for the treatment of disorders of the CNS including ophthalmological diseases is therefore almost exclusively available for treatment of clinical symptoms often associated with side effects due to the high doses necessary. A causal therapy of the CNS, and particularly of the back sections of the eye, was not possible apart from the injections. Furthermore, the current state of information on the complex molecular metabolic interrelationship underlying the etiology of retinal diseases of multi-factorial origin is only limited. Consequently, medicaments available on the market are suitable to treat the symptoms of such diseases only.

In view of the need of therapeutic means for the treatment of diseases related to CNS and/or the eye, the technical problem of the present invention is to provide means and methods for the identification and modulation of genes involved in disorders of the CNS and/or the eye.

More specifically, the technical problem of present invention is to provide non-invasive methods for the controlled modulation of target genes and gene products in the mammalian CNS and/or eye while overcoming the blood-brain and/or blood retina barrier without injuring it.

This is also relevant for example, for the application of so-called single-stranded antisense oligonucleotides for the inhibition of expression of target genes, whose application to the eye necessitates intravitreal injection. Overcoming the blood-retina barrier thus represents a technical problem in the therapy of ocular diseases by specific inhibition of protein expression in the eye tissue.

The solution to said technical problem is achieved by providing the embodiments characterized in the claims, and described further below.

Thus, the present invention relates to the use of one or more double-stranded oligoribonucleotides (dsRNA) for the preparation of a composition for the specific modulation of the expression of one or more target genes in cells and/or tissues of the CNS and/or eye of a subject, wherein said composition is designed to be applied outside the blood-brain or blood-retina barriers. Likewise, the present invention relates to a method for the specific modulation of the expression of target genes in cells and/or tissues of the CNS and/or eye, wherein a composition comprising one or more double-stranded oligoribonucleotides (dsRNA) is introduced into a cell, tissue or organism outside the blood-brain or blood-retina barriers; wherein preferably said method results in the provision of a non-human organism comprising cells and/or tissue containing said dsRNA, and wherein said organism or the corresponding test cells or tissue are maintained under conditions allowing the degradation of the corresponding mRNA of one or more target genes by RNA interference.

The present invention is based on the surprising finding that in contrast to wide-spread professional opinion active substances of the invention are able to cross the blood-retina barrier as a physiological barrier, enabling a systemic or local application on the outer side of the eye for specific treatment of a disease in the back of the eye.
Due to the high similarity in function as well as cellular and molecular architecture of the blood-retina and blood-brain barrier the methods and pharmaceutical compositions provided

by the present invention are also be expected to cross the blood-brain barrier and thereby applicable to the treatment of diseases of the CNS.

Thus, the present invention provides improved methods as well as components for the treatment of CNS and/or eye diseases.

The fundamental idea of the present invention concerns a method for the specific inhibition of the expression of target genes in cells and tissues of the CNS and/or eye, by

- delivery of one or more double-stranded oligoribonucleotides (dsRNA) outside the blood-retina or blood-brain barrier,
- the double-stranded oligoribonucleotides (dsRNA) crossing the respective barrier and modulation of the expression of the corresponding mRNA of one or more target genes by RNA interference.

Hence, in accordance with the present invention the compositions comprising a dsRNA capable of modulating a target gene or gene product in the CNS or the eye are preferably designed to be administered without any substantial, i.e. substantially effective amount of delivery-enhancing agents facilitating passage of compounds through the blood-brain barrier and/or without the necessity of applying invasive methods and devices; see, e.g., those compounds , methods and devices described in US2002183683 and WO03/000018.
The method for specific inhibition of genes by double-stranded oligoribonucleotides (dsRNA) is known from WO 01/75164. The disclosure of this application is hereby incorporated by reference into the present description. This application describes that double-stranded oligoribonucleotides (dsRNA) induce specific degradation of mRNA after delivery to the target cells. The specificity of this process is mediated by the complementarity of one of the two dsRNA strands to the mRNA of the target gene. The process of gene-specific, post-transcriptional switching off of genes by dsRNA molecules is referred to as RNA interference (RNAi). This term was originally developed by Fire and co-workers to describe the observation that delivery of dsRNA molecules to the threadworm Caenorhabditis *elegans* blocks gene expression (Fire et al., 1999). Subsequently, RNAi could also be demonstrated in plants, protozoa, insects (Kasschau and Carrington 1998) and recently also in mammalian cells (Caplen et al., 2001; Elbashir et al., 2001). The mechanism by which RNAi suppresses gene expression is not yet fully understood. Studies of non-mammalian cells have shown that dsRNA molecules are transformed into small interfering RNA molecules (siRNA molecules)

by endogenous ribonucleases (Bernstein et al., 2001; Grishok et al., 2001; Hamilton and Baulcombe, 1999; Knight and Bass, 2001; Zamore et al., 2000). The 21 to 23 bp long siRNA molecules are thus the actual mediators of the degradation of the mRNA of the target gene.
For the specific inhibition of a target gene, it suffices that a double-stranded oligoribonucleotide exhibits a sequence of 21 to 23 nucleotides (base pairs) in length identical to the target gene; see, e.g., Elbashir et al., Methods 26 (2002), 199-213 and Martinez et al., Cell 110 (2002), 563-574

The technical problem of a targeted application of active substances to the CNS and the eye, particularly to the back of the eye is due to the structure of the human eye and brain, whose barriers prevent active substance from reaching the target tissue. Current treatments are associated with considerable side effects and expected to have long-term consequences. Direct application, e.g. by injection into the back of the eye, is very unpleasant for the person concerned, especially when repeated or chronic treatment is necessary. Furthermore, direct application into the bulb is associated with considerable side effects and the medium-term occurrence of secondary disorders respectively, such as cataract and glaucoma. Systemic application on the other hand generally gives rise to side effects outside the target organs *eye* and brain – often without significant quantities of active substance being detectable in the target tissue. Even with sufficient target specificity, which would minimize the risk of unwanted side effects of systemic application, this method of application remains inefficient, since the target tissue and target cells are located beyond the blood-brain or blood-retina barrier and the active substance is not able to reach its site of activity because of the stringent activity of this barrier. This problem has been solved by the present invention.

Medication for the treatment of ophthalmological diseases currently on the market is almost exclusively available for treatment of clinical symptoms of the front of the eye, since the relatively easy application of eye drops is possible in this case. A causal therapy, particularly of the back sections of the eye, is not possible with conventional pharmaceutical compositions apart from the injections associated with side effects described earlier.
CNS related disorders are currently treated mainly by systemic application. In some cases surgery or stimulation by implanted probes, as for example in Parkinson disease, can relieve the symptoms but do not treat the cause of the disease.
As a solution to this technical problem the present invention provides a method for the specific intervention in diseases of the CNS or the back of the eye on a molecular level,

without requiring direct application to the target tissue. The present invention opens up comprehensively the broad not yet or only unsatisfactorily addressable therapeutic field of diseases of the CNS, the inner eye and the back of the eye, in particular the inner segment of the eye ball. The intervention is based on an inhibition of genes expressed specifically or predominantly in the target tissues of CNS or the eye, respectively, characterized by the ability of the required active substances to cross the blood-brain and/or the blood-retina barrier, allowing systemic or local application outside the respective barrier.

Examples for CNS disorders are, for example, Alzheimer's disease, Parkinson disease, depression, bipolar disorder, schizophrenia, amnesia, migraine-headache, stroke, insomnia, alcohol abuse, anxiety, obsessive compulsive disorder, cerebral acquired human immunodeficiency syndrome, chronic pain and many others.

The compositions of the invention may be administered locally or systemically e.g., intravenously. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like. Furthermore, the pharmaceutical composition of the invention may comprise further agents such as interleukins or interferons depending on the intended use of the pharmaceutical composition.
In accordance with the present invention the pharmaceutical compositions are administered to a subject in an effective dose of between about 0,1 µg to about 10 mg units/day and/or units/kg body weigth, preferably between about 0,1 and 2.0 mg. The appropiate dose can also be determined as descibed further below and in the examples.

In a preferred embodiment the use according to the present invention is directed to the treatment of a discorder such as of the CNS described above. Most preferably, the disorder to be treated is related to eye. Such disorders include chorioretinitis and herpes retinitis, which

may be considered as acquired forms of retinal disease, the majority of retinal disease disorders are reduced to a genetic predisposition. These include for example primary retinal detachment (ablatio retinae), retinal blastoma, retinal astrocytoma (Bourneville-Pringle), angiomatosis retinae (Hippel-Lindau), Coat's disease (exudative retinitis), Eale's disease, central serous retinopathy, ocular albinism, retinitis pigmentosa, retinitis punctata albescens, Usher syndrome, Leber's congenital amaurosis, cone dystrophy, vitelliform macular degeneration (Best's disease), juvenile retinoschisis, North Carolina macular dystrophy, Sorsby's fundus dystrophy, Doyne's honey comb retinal dystrophy (Malattia Leventinese), Stargardt's disease, Wagner vitreoretinal degeneration or Age-related macular degeneration (AMD) as well as single-gene retinopathies like Morbus Best or Morbus Stargardt. Various genetic defects are known which lead or predispose to this wide range of eye disease phenotypes.

Therefore, in a preferred embodiment of the methods and uses of the present invention cells of the retinal pigment epithelium (RPE), neurosensory retina and/or choriodea are particularly preferred. In a particularly embodiment of the present invention, the disorder to be treated is wet age-related macular degeneration (AMD) or diabetic retinopathy.

The following description deals with AMD as example for a complex eye disease with a genetic component. The example shall illustrate the associated technical problems with reference to the study of molecular causes and the development of diagnostic and pharmacological intervention strategies.

AMD, which can be thought as a sub-type of retinal degeneration, is the most common cause of visual morbidity in the developed world with a prevalence increasing from 9% in persons over 52 years to more than 25% in persons over the age of 75 (Paetkau et al. 1978, Leibowitz et al. 1980, Banks and Hutton 1981, Ghafour et al. 1983, Hyman 1987, Hyman et al. 1983, Grey et al. 1989, Yap and Weatherill 1989, Heiba et al. 1994).

AMD is a complex disease caused by exogenous as well as endogenous factors (Meyers and Zachary 1988; Seddon et al. 1997). In addition to environmental factors, several personal risk factors such as hypermetropia, light skin and iris colour, elevated serum cholesterol levels, hypertension or cigarette smoking have been suggested (Hyman et al. 1983, Klein et al. 1993, Sperduto and Hiller 1986, The Eye Disease Case-Control Study Group 1992, Bressler and Bressler 1995). A genetic component for AMD has been documented by several groups (Gass 1973, Piguet et al. 1993, Silvestri et al. 1994) and has lead to the hypothesis that the disease

may be triggered by environmental/individual factors in those persons who are genetically predisposed. The number of genes which, when mutated, can confer susceptibility to AMD is not known but may be numerous.

With recent physical approaches for the treatment of AMD such as laser photocoagulation, photodynamic therapy (using verteprofin, trade name Visudyne ®, Novartis), irradiation or surgical therapies, success was only achieved with a moderate percentage of the patients. genetic heterogeneity make it difficult to apply conventional approaches for the identification of genes predisposing to AMD. Due to the complexity of the clinical phenotype, it may be assumed that the number of genes is large, which, when mutated contribute to AMD susceptibility.

Hence, the methods, uses and compositions of the present invention described herein represent an important improvement and alternative therapeutic intervention for the treatment of this particular disease as well as of others. For those embodiments the pharmaceutical compositions are preferably designed to be effective in (and applied to) the posterior segment of the eye, preferably in a form designed to be applied outside the retinal region of the blood-retina barrier.

As mentioned before, the compound used in accordance with the present invention is dsRNA, which usually substantially consists of ribonucleotides which preferbly contain a portion of double-stranded oligoribonucleotides (dsRNA). Desirably, the region of the double stranded RNA that is present in a double stranded conformation includes at least 5, 10, 20, 30, 50, 75, 100 or 200. Preferably, the double stranded region includes between 15 and 30 nucleotides, most preferably 20 to 25 and particularly preferred 21 to 23 nucleotides. Secondary structure prediction and in vitro accessibility of mRNA as tools in the selection of target sites is described for example in Amarzguioui, Nucleic Acids Res. 28 (2000), 4113-4124. Minimising the secondary structure of DNA targets by incorporation of a modified deoxynucleoside: implications for nucleic acid analysis by hybridisation is described in Nguyen, Nucleic Acids Res. 28 (2000), 3904-3909.
The dsRNA molecule can also contain a terminal 3'-hydroxyl group and may represent an analogue of naturally occurring RNA, differing from the nucleotide sequence of said gene or gene product by addition, deletion, substitution or modification of one or more nucleotides. General processes of introducing an RNA into a living cell to inhibit gene expression of a

target gene in that cell comprising RNA with double-stranded structure, i.e. dsRNA or RNAi are known to the person skilled in the art and are described, for in WO99/32619, WO01/68836, WO01/77350, WO00/44895, WO02/055692 and WO02/055693, the disclosure content of which is hereby incorporated by reference.
Preferably, the target gene subject of the RNA interference is predominantly or more preferably specifically expressed in said cell and/or tissue of the CNS and/or eye.

Another technical problem consists in the identification of genes, which cause CNS and retinal diseases as well as in the validation of these genes as targets for diagnosis and for pharmacological intervention. Conventional experimental strategies are often difficult to apply. The low penetration and/or incidence or the occurrence of symptoms only late in life, as for AMD, which is generally diagnosed in the 7^{th} decade of life, hampers the identification of genes involved in the etiology. Positional cloning is often not possible since linkage studies lead to conflicting results or are altogether impossible due to the small number of patients and afflicted families. Similarly, linkage studies might be inconclusive when the group of patients to be studied includes individuals who suffer from a disorder different to the disease whose etiology is to be elucidated. This might happen if two or more disorders have identical or very similar symptoms, which are difficult to diagnose differentially as is the case for many CNS disorders. Schizophrenia e.g. is thought to be caused by several different etiologies with a large number of genes involved.

Hence, in a further aspect the present invention relates to a method for the specific modulation of the expression of target genes in cells and/or tissues of the CNS and/or eye, wherein a composition comprising one or more double-stranded oligoribonucleotides (dsRNA) is introduced into a cell, tissue or organism outside the blood-brain or blood-retina barriers. These cells, tissues or organisms can on one hand be used for the validation of identified target genes and on the other hand for the identification of such target genes itself by the following steps:

- degradation of the corresponding mRNA of one or more target genes
- providing and maintenance of a test cell or test tissue, in which the corresponding mRNA of one or more target genes is/are degraded, and preferably
- observation and comparison of the generated phenotype of the produced test cell or test tissue with that of a suitable control cell or control tissue, in order to obtain information on the functions of the genes.

In a preferred embodiment and as described in the examples this means that the test cell or tissue provided is derived from the animal to which the composition mentioned above has been applied to. Assaying the test cell or tissue can thus be done in vivo or in vitro, for example after the subject cells or tissue have been isolated from the animal, in particular, mammalian animals are preferred.

Means and methods for identifying nucleotide acid sequences that modulate the function of a cell, the expression of a gene in a cell, or the biological activity of a target polypeptide in a cell, which are based on a sole cell culture based method are described for example in EP 1 229 134 A2. However, the technical details concerning selection of doubled stranded RNA, RNA expression vectors, etc. can be obtained from the prior art such as mentioned European patent application EP 1 229 134 A2 and adapted to the method of the invention, which is performed on the basis of an animal system, i.e. wherein the test cell or the test tissue is preferably comprised in a non-human animal, at least when applying the one or more double stranded RNA molecules. Target gene function can be followed by observing a responsive change in the phenotype of said cell, tissue or animal, when appying the dsRNA, wherein said phenotype is preferably related to a disorder of the CNS and/or eye.

In a preferred embodiment of the present invention, the use or method described earlier said specific modulation of the expression being an inhibition of target gene expression. Said one or more of said target genes preferably encode a cellular mRNA. As mentioned before, the target cells and/or tissues are cells and/or tissues of the eye.

In a embodiment of the present invention said cells or tissues are cells or tissues of the inner segment of the eye ball, preferably retinal cells, and particularly preferred cells of the retinal pigment epithelium (RPE) or neurosensory retina cells.

Furthermore, the present invention relates to a drug or pharmaceutical composition, which contains one or more double-stranded oligoribonucleotides (dsRNA), that by means of RNA interference, inhibits the expression of the corresponding mRNA of one or more target genes, whose restricted functions cause an eye disease, and which are applied outside the blood-retina barrier, particularly outside the eye.
At the same time, the side effects associated with direct application, e.g. by injection, based on the structure of the human eye and which are very unpleasant for the persons concerned

especially on repeated or chronic treatment, and associated with long-term consequences such as e.g. cataract and glaucoma, are reduced in accordance with the invention. Double-stranded oligoribonucleotides (dsRNA) are used in the present invention, which cross the blood-retina barrier after application, in order to elicit inhibition of the target genes in the target cells by RNA interference of the corresponding mRNA molecules. The present invention includes further a drug of dsRNA molecules for the specific treatment of genetically caused eye diseases. The present invention opens up the broad so far not or only unsatisfactorily addressable therapeutic field of diseases of the inner eye and the back of the eye.

Based on the specific functions of the cells of retinal tissue and the RPE, it is presumed that genes, whose aberrant function cause diseases in the back of the eye, are specifically expressed in the tissues and cells of the back of the eye, thus representing preferred targets for drug interventions. The effect of modulating gene expression will be maximal with no or very little side effects if genes are targeted that are specifically expressed in the tissue of brain and/or eye. Therefore the present invention includes embodiments of the uses or methods described above, wherein one or more of said target genes are predominantly expressed in said cell and/or tissue, or wherein the expression of one or more of said target genes is specific for said cell and/or tissue.

The solution of the technical problem underlying the present invention consists of the provision of a method for the specific inhibition of genes, whose aberrant functions are causally associated with CNS or eye diseases of monogenic or multifactorial origin. AMD for example may be taken as one form of degenerative retinal disease.

As mentioned before, for the specific inhibition of a target gene, it suffices that a double-stranded oligoribonucleotide comprises a sequence of 21 to 23 nucleotides (base pairs) in length identical or substantial identical to the target gene. The use or method described above, wherein said dsRNA molecules are between 21 and 23 nucleotides in length is therefore a preferred embodiment of the invention. Said dsRNA molecules can contain a terminal 3'-hydroxyl group, have been chemically synthesized and/or represent an analogue of naturally occurring RNA. Said dsRNA analogues can also differ from the corresponding naturally occurring RNA by addition, deletion, substitution or modification of one or more nucleotides. In a preferred embodiment said dsRNA molecules inhibit the corresponding target genes by "posttranscriptional silencing".

The central idea of the present invention is surprising in so far as dsRNA molecules of a length of 21 to 23 nucleotides, are able to cross the blood-retina barrier, and specifically inactivate target genes in the tissues of the back of the eye, after systemic application, for example by intravenous injection. This overcoming the blood-retina barrier is all the more remarkable, because no experiment could demonstrate overcoming this barrier by dsRNA so far. Due to the high similarity in function as well as cellular and molecular architecture of the blood-retina and blood-brain barrier the methods and pharmaceutical compositions provided by the present invention will also be able to cross the blood-brain barrier and thereby applicable to the treatment of diseases of the CNS.

The nucleotides can not only be applied as "naked" dsRNA, preferred are embodiments, wherein said dsRNA molecules are encoded by a vector.

Vectors and recombinant nucleic acid molecules that encode dsRNA or appropriate engineered RNA precursors that expressed in a cell are processed by the cell to produce target small interfering RNAs (siRNAs) that selectively silence target genes (by cleaning specific mRNAs) using the cells own RNA interference (RNAi) as described in the literature, for example in WO 03/006477. Appropriate regulatory sequences with which expression can be selectively controlled both temporarily and specially i.e., at particular times and/or in particular tissue, organs or cells are known to the persons skilled in the art and are also described inter alia in WO 03/006477, that disclosure content or which is hereby incorporated by reference.

Particularly preferred are those, wherein the expression of said dsRNA is under control of a cell and/or tissue specific promoter. Vectors that can be used in accordance with the teaching of the present invention are known to the person skilled in the art; see, e.g., heritable and inducible genetic interference by double-stranded RNA encoded by transgenes described in Tavernarakis et al., Nat. Genet. 24 (2000), 180-183. Further vectors and methods for gene transfer and generation of transgenic animals are described in the prior art; see, e.g., adeno-associated virus related vectors described in Qing et al., Virol. 77 (2003), 2741-2746; human immunodeficiency virus type 2 (HIV-2) vector-mediated in vivo gene transfer into adult rabbit retina described in Cheng et al. Curr. Eye Res. 24 (2002), 196-201, long-term transgene expression in the RPE after gene transfer with a high-capacity adenoviral vector described in Kreppel et al., Invest. Ophthalmol. Vis. Sci. 43 (2002), 1965-1970 and non-invasive observation of repeated adenoviral GFP gene delivery to the anterior segment of the monkey eye in vivo described in Borras et al., J. Gene Med. 3 (2001) , 437-449.

CNS gene transfer has also been described in Leone et al., Curr. Opin. Mol. Ther. 1 (1999), 487-492.

Additionally, the dsRNAs can be introduced into the cells or tissues bound to other molecules and/or combined with one or more suitable carriers. Such a carrier can be a micellar structure, preferably a liposome, a coat protein, derived from a virus such as the cytomegalovirus (CMV) or produced synthetically, adeno-associated virus (AAV) or adenovirus. The dsRNA can also be bound to cationic porphyrins, cationic polyamines, polymeric DNA-binding cations or fusogenic peptides. Packaging of the dsRNA into coat proteins or liposomes and/or associating it with carriers will not only improve the targeting but also elongate the half-life. Preferred are carriers and/or the dsRNA-binding molecules, selected such that the dsRNA molecules are delivered continuously to the target cells or target tissues over a defined period of time after application. Thereby peak concentrations of dsRNA, which might lead to side effects or simply be ineffective, can be avoided. Also preferred are carriers that are specific for the cells and/or tissues defined above. Such carriers are well known to the person skilled in the art; see, e.g., Adams et al., J. Biomater. Sci. Polym. Ed. 13 (2002), 991-1006, for the effects of acyl chain length on the micelle properties; Dass, J. Pharm. Pharmacol. 54 (2002), 3-27, for cationic liposomes and cyclodextrins; Yang and Hsieh, Pharm. Res. 18 (2001), 922-927, for protamine sulfate enhancing the transduction efficiency of recombinant adeno-associated virus-mediated gene delivery.

The method described in this invention is distinguished from the prior art by the fact that it could be shown for the first time that dsRNA molecules, preferably of the length specified above, can be detected inside the eye after systemic or local application outside the eyeball. The detection is based on the specific inhibition of specified target genes in cells or tissues of the inner eye by RNA interference.

Needless to say that in the above-described screening methods, induction of an interferon response may not desired as this could lead to cell death, anti-proliferation and possibly to prevention of gene silencing. Means and Methods how to prevent an interferon response during gene silencing are known to the persons skilled in the art, and are described inter alia in example 7 of EP 1 229 134 A2, the disclosure content of which is hereby incorporated by reference.

In a preferred embodiment of the methods and uses of the present invention the composition is in a form designed to be introduced into the cells or tissue of the CNS or eye by a suitable carrier, characterized by the application occurring outside the blood-CNS and/or blood-retina barriers, for instance as eye drops. It can also be administered systemically, iontophoretically or by retrobulbar injection.

Iontophoresis has been defined as the active introduction of ionised molecules into tissues by means of an electric current. The technique has been used to enhance drug delivery into tissues underlying the donor electrode (e.g. skin) as well as to the general blood circulation, thus providing systemic delivery of a drug to the entire body. Iontophoresis devices require at least two electrodes, both being in electrical contact with some portion of a biological membrane surface of the body. One electrode commonly referred to as the "donor" or "active" electrode, is the electrode from which the biologically active substance, such as a drug or prodrug, is delivered into the body. Another electrode having an opposite polarity functions to complete the electric circuit between the body and the electrical power source. This electrode is commonly referred to as the "receptor" or "passive" electrode. During iontophoresis, an electrical potential is applied over the electrodes, in order to create an electrical current to pass through the drug solution and the adjacent tissue. Iontophoresis has been described for the treatment of blood-vessel related disorders (e.g. restenosis), bladder, uterus, urethra and prostate disorders. U.S. Patent Nos. 6,219,557; 5,588,961; 5,843016; 5,486,160; 5,222,936; 5,232,441; 5,401,239 and 5,728,068 disclose different types of iontophoresis catheters for insertion into hollow, tubular organs (bladder, urethra and prostate) or into blood vessels. US 2002183683 suggests the method for delivery of active substances into the CNS.

In any of the uses or methods described herein the subject or organism can be a vertebrate, preferably a mammal or a human. The described methods can be applied to cells and/or tissues of vertebrate origin and particularly of mammalian origin. Human cells and/or tissues are preferred.

The current knowledge about pathological metabolic interrelationships based on restricted or lacking function of a single or a number of causative genes, such as for AMD or other retinal disease patterns, is not sufficient for the medical treatment of such diseases. Suitable animal or cell culture models for such diseases are not available, due to the complexity of the disorders and the lack of simple methods for intervention and manipulation of the eye or the CNS

Using the methods provided by this invention, animal models may easily be generated, which reproduce the symptoms of diseases of the inner eye and/or CNS of predominantly genetic origin. These animal models are suitable to initiate the development of specific pharmaceutical products for ophthalmology and CNS-related diseases and can be used in the validation of products.

The method, illustrated below by examples of the procedure thus, is suitable for the provision of cell culture as well as animal models with which targets, whose restricted function cause diseases of the eye and/or the CNS, can be identified and validated. The method is moreover suitable for the specific intervention in eye diseases on a molecular level, without necessitating direct application to the back of the eye. The specificity of RNAi for the inhibition of genes expressed specifically in target cells minimizes the risk of unwanted side effects.

In a still further embodiment, the present invention relates to a transgenic non-human animal which due to the presence of one or more dsRNA molecules, displays an aberrant expression of one or more target genes, and which obtained by the methods described above, especially when said animal reproduces a disorder of the CNS and/or the eye.

A method for the production of a transgenic non-human animal, which is also encompassed by the present invention, for example transgenic mouse, comprises introduction of a polynucleotide or targeting vector encoding said polypeptide into a germ cell, an embryonic cell, stem cell or an egg or a cell derived therefrom. The non-human animal can be used in accordance with a screening method of the invention described herein. Production of transgenic embryos and screening of those can be performed, e.g., as described by A. L. Joyner Ed., Gene Targeting, A Practical Approach (1993), Oxford University Press. A general method for making transgenic non-human animals is described in the art, see for example WO 94/24274. For making transgenic non-human organisms (which include homologously targeted non-human animals), embryonal stem cells (ES cells) are preferred. Murine ES cells, such as AB-1 line grown on mitotically inactive SNL76/7 cell feeder layers (McMahon and Bradley, Cell 62: 1073-1085 (1990)) essentially as described (Robertson, E. J. (1987) in Teratocarcinomas and Embryonic Stem Cells: A Practical Approach. E. J. Robertson, ed. (Oxford: IRL Press), p. 71-112) may be used for homologous gene targeting. Other suitable ES lines include, but are not limited to, the E14 line (Hooper et al., Nature 326: 292-295 (1987)), the D3 line (Doetschman et al., J. Embryol. Exp. Morph. 87: 27-45 (1985)), the CCE line (Robertson et al., Nature 323: 445-448 (1986)), the AK-7 line (Zhuang et al., Cell 77:

875-884 (1994)). The success of generating a mouse line from ES cells bearing a specific targeted mutation depends on the pluripotence of the ES cells (i. e., their ability, once injected into a host developing embryo, such as a blastocyst or morula, to participate in embryogenesis and contribute to the germ cells of the resulting animal). The blastocysts containing the injected ES cells are allowed to develop in the uteri of pseudopregnant nonhuman females and are born as chimeric mice. The resultant transgenic mice are chimeric for cells having either the recombinase or reporter loci and are backcrossed and screened for the presence of the correctly targeted transgene (s) by PCR or Southern blot analysis on tail biopsy DNA of offspring so as to identify transgenic mice heterozygous for either the recombinase or reporter locus/loci.

It might be also desirable to inactivate target gene expression or function at a certain stage of development and/or life of the transgenic animal. This can be achieved by using, for example, tissue specific (see supra), developmental and/or cell regulated and/or inducible promoters which drive the expression of, e.g., an antisense or ribozyme directed against the RNA transcript encoding the target gene mRNA; see also supra. A suitable inducible system is for example tetracycline-regulated gene expression as described, e.g., by Gossen and Bujard (Proc. Natl. Acad. Sci. 89 USA (1992), 5547-5551) and Gossen et al. (Trends Biotech. 12 (1994), 58-62). Similar, the expression of a mutant target gene may be controlled by such regulatory elements. Preferably, the presence of the transgenes in cells of the transgenic animals leads to to various physiological, developmental and/or morphological changes, preferably to conditions related to disorders of the CNS and/or eye such as those described above.

For the method of the present invention, in particular, mammlian animals are preferred, especially mice and rats. Corresponding animal systems that can be adapted in accordance with the present invention are known to person skilled in the art; see, e.g., molecular biological approaches to neurological disorders including knockout and transgenic mouse models described in Shibata et al., Neuropathology 22 (2002), :337-349. However, the widely used zebra fish may also be used since this model system has also been shown to provide valuable predicitve results; see, e.g. Gerlai et al., Pharmacol. Biochem. Behav. 67 (2000), 773-782.

In another embodiment of the present invention, said transgenic non-human animal is used for a process in the discovery of drugs for the treatment of a disorder of the CNS and/or the eye.

Preferred non-human transgenic animals are mammals, for example mice. Particularly preferred are transgenic organisms, especially if the organism displays the phenotype of an eye disease. The phenotype of a disease of the inner segment of the eye ball, a retinal disease and particularly a degenerative retinal disease is preferred. The organism can be for example mouse, rat or zebra fish; se supra.

A preferred embodiment of this invention is a pharmaceutical composition useful for the treatment of disease as defined above, comprising a composition disclosed in the description of this invention. Also included in the embodiments of this invention is a diagnostic composition useful for detecting a gene or gene expression involved in diseases of the CNS and/or eye, comprising a composition as defined in the description or a cell, tissue or an organism described above.

In contrast to the cited literature in which the use of siRNA and other RNA based molecules is described for cell culture only, experiments performed in accordance with the present invention surprisingly demonstrate that dsRNA molecules of a length of 21 to 23 nucleotides are capable of, after systemic application, for example by intravenous injection, to cross the blood-retina barrier, and specifically inactivate target genes in the tissues of the back of the eye. This overcoming the blood-retina barrier is all the more remarkable, because no experiment could demonstrate overcoming the blood-brain barrier by dsRNA so far. The methods and uses of the invention, explained below by means of examples, are thus suitable for the provision of animal models with which targets, the restricted function of which causing diseases of the eye, can be identified and validated. Those methods are moreover suitable for the specific intervention in CNS and eye diseases on a molecular level, without necessitating direct application to the site of, for example affected cells or tissue. The specificity of selected inhibitors such as preferably RNAi for the inhibition of genes expressed specifically in target cells minimizes the risk of unwanted side effects.

The dosage regimen of the pharmaceutical compositions in all of the above described methods and uses of the present invention will be determined by the attending physician and clinical factors. As is well known in the medical arts, dosages for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. A typical dose can be, for example, in the range of

0.001 μg to 10 mg (or of nucleic acid for expression or for inhibition of expression in this range); however, doses below or above this exemplary range are envisioned, especially considering the aforementioned factors. Generally, the regimen as a regular administration of the pharmaceutical composition should be in the range of 0.01 μg to 10 mg units per day. If the regimen is a continuous infusion, it should also be in the range of 0.01 μg to 10 mg units per kilogram of body weight per minute, respectively. Progress can be monitored by periodic assessment. Dosages will vary but a preferred dosage for intravenous administration of nucleics acids is from approximately 10^6 to 10^{12} copies of the nucleic acid molecule.

Therapeutic or diagnostic compositions of the invention are administered to an individual in an effective dose sufficient to treat or diagnose disorders in which modulation of a target gene or gene product is indicated. The effective amount may vary according to a variety of factors such as the individual's condition, weight, sex and age. Other factors include the mode of administration. The pharmaceutical compositions may be provided to the individual by a variety of routes such as by intracoronary, intraperitoneal, subcutaneous, intravenous, transdermal, intrasynovial, intramuscular or oral routes. In addition, co-administration or sequential administration of other agents may be desirable.

A therapeutically effective dose refers to that amount of compounds described in accordance with the present invention needed to ameliorate the symptoms or condition. Therapeutic efficacy and toxicity of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50.

Additionally the present invention provides a method for the identification and isolation of a drug capable of specific modulation of the expression of a target gene in cells and/or tissues of the eye, comprising the steps:

- contacting a cell or tissue or a non-human organism described above with a compound to be screened and;
- determining if the compound antagonizes or agonizes the effect of said one or more double-stranded oligoribonucleotides (dsRNA) molecule

Preferred is an embodiment, which further comprises comparing the non-human organism treated with said compound – or the test cell or tissue - with a non-treated control, wherein reversion or amelioration of the phenotype as defined above is indicative for suitable drug or lead compound for a drug for the treatment of a disease related to the eye.

The test substances which can be tested and identified according to a method of the invention may be expression libraries, e.g., cDNA expression libraries, peptides, proteins, nucleic acids, antibodies, small organic compounds, hormones, peptidomimetics, PNAs, aptamers or the like (Milner, Nature Medicine 1 (1995), 879-880; Hupp, Cell 83 (1995), 237-245; Gibbs, Cell 79 (1994), 193-198 and references cited supra). The test substances to be tested also can be so called "fast seconds" of known drugs. The invention also relates to further contacting the test cells with a second test substance or mixture of test substances in the presence of the first test substance.

As mentioned above, the present invention provides convenient in vivo assays for identifying and obtaining drugs capable of modulating the gene activity, thereby being useful as a therapeutic agent for the treatment of diseases related to CNS disorders including (e.g.) Schizophrenia, Parkinson's Disease, Alzheimer's Disease, and eye diseases such as those described above. In accordance with this, the present invention provides also a use for compounds which have been known in the art, properly also known to be able to modulate target gene activity but which hitherto have not been suggested for medical use because of the lack of knowledge of phenotypic responses of an organism evoked by target gene activity or the lack of it.
One embodiment of this invention comprises a method for the production of a drug or prodrug identified by such a screening as a modulator or a derivative thereof, particularly if the substance has hitherto not been known as a drug for the treatment of a disorder of the CNS or the eye.

Substances are metabolized after their in vivo administration in order to be eliminated either by excretion or by metabolism to one or more active or inactive metabolites (Meyer, J. Pharmacokinet. Biopharm. 24 (1996), 449-459). Thus, rather than using the actual compound or drug identified and obtained in accordance with the methods of the present invention a corresponding formulation as a pro-drug can be used which is converted into its active form in the patient by his/her metabolism. Precautionary measures that may be taken for the

application of pro-drugs and drugs are described in the literature; see, for review, Ozama, J. Toxicol. Sci. 21 (1996), 323-329.

Furthermore, the present invention relates to the use of a compound identified, isolated and/or produced by any of these methods for the preparation of a composition for the treatment of said CNS and eye disorders. As a method for treatment the identified substance or the composition containing it can be administered to a subject suffering from such a disorder. Compounds identified, isolated and/or produced by the method described above can also be used as lead compounds in drug discovery and preparation of drugs or prodrugs.

The various steps recited above are generally known in the art. For example, computer programs for implementing these techniques are available; e.g., Rein, Computer-Assisted Modeling of Receptor-Ligand Interactions (Alan Liss, New York, 1989). Methods for the preparation of chemical derivatives and analogues are well known to those skilled in the art and are described in, for example, Beilstein, Handbook of Organic Chemistry, Springer edition New York Inc., 175 Fifth Avenue, New York, N.Y. 10010 U.S.A. and Organic Synthesis, Wiley, New York, USA. Furthermore, peptidomimetics and/or computer aided design of appropriate derivatives and analogues can be used, for example, according to the methods described above. Methods for the lead generation in drug discovery also include using proteins and detection methods such as mass spectrometry (Cheng et al. J. Am. Chem. Soc. 117 (1995), 8859–8860) and some nuclear magnetic resonance (NMR) methods (Fejzo et al., Chem. Biol. 6 (1999), 755–769; Lin et al., J. Org. Chem. 62 (1997), 8930–8931). They may also include or rely on quantitative structure-action relationship (QSAR) analyses (Kubinyi, J. Med. Chem. 41 (1993), 2553-2564, Kubinyi, Pharm. Unserer Zeit 23 (1994), 281-290) combinatorial biochemistry, classical chemistry and others (see, for example, Holzgrabe and Bechtold, Pharm. Acta Helv. 74 (2000), 149-155). Furthermore, examples of carriers and methods of formulation may be found in Remington's Pharmaceutical Sciences.

The present invention also relates to the use of a component selected from the group consisting of a composition, nucleic acid, non-human organism, host cell, cell line, tissue, organ, drug, carrier and/or vector for the specific modulation of expression of one or more target genes in cells and/or tissue of the CNS and/or eye, wherein said component comprises one or more dsRNA molecules which are applicable outside the blood-brain barrier or the retinal region of the blood-retina barrier. One or more of the components mentioned above

can be part of a kit for use in a method as defined herein above. The invention further relates to the use of the any one of these methods, cells or non-human organism in drug discovery or target gene isolation and/or validation as well as the use of RNA interference and the nucleic acid, non-human organism, host cell, cell line, tissue, organ, carrier and/or vector utilized for the diagnosis and/or therapy of disorders related to the CNS and/or eye.

The present invention also relates to kit compositions containing specific reagents such as those described herein-before. Kits containing oligonucleotides, dsRNA or vectors may be prepared. Such kits are used to detect for example the function of a target gene. Such characterization is useful for a variety of purposes including but not limited to forensic analyses, diagnostic applications, and epidemiological studies in accordance with the above-described methods of the present invention. The recombinant RNA molecules for example lend themselves to the formulation of kits suitable for the detection and typing of the target gene. Such a kit would typically comprise a compartmentalized carrier suitable to hold in close confinement at least one container. The carrier would further comprise reagents such as recombinant protein or antibodies suitable for detecting the expression or activity of the target gene or gene product. The carrier may also contain a means for detection such as labeled antigen or enzyme substrates or the like.

These and other embodiments are disclosed and included in the present description and in the examples. Literature regarding the materials, methods, applications and components, which can be used in accordance with the invention, may be obtained from public libraries and data bases, for example by using electronic devices. The public data base 'Medline' may for instance be used, which is supported by the National Center for Biotechnology Information and/or the National Library of Medicine at the National Institutes of Health. Other data bases and Internet addresses, such as the European Bioinformatics Institute (EBI), which is part of the European Molecular Biology Laboratory (EMBL), are known to the person skilled in the art, and can be found by using Internet search engines. A survey of patent information in biotechnology and a summary of relevant sources for patent information, which are useful for a retrospective search and current awareness are described in Berks, TIBTECH 12 (1994), 352-364.

The disclosure above describes the present invention in general. A more comprehensive understanding of the invention may be gained by reference to the following specific examples

and figure, which are merely provided for illustrative purposes and are not intended to limit the scope of the invention. The contents of all cited references (including literature references, granted patents, published patent applications as quoted in the text and manufacturer's descriptions and specifications, etc.) are hereby incorporated explicitly by reference; this is however no admission that any one of these documents is indeed prior art as to the present invention.

Unless stated otherwise, the present invention may be carried out by making use of conventional techniques of cell biology, cell culture, molecular biology, transgenetic biology, microbiology, recombinant DNA and RNA technology, which belong to the skill of the person skilled in the art. For a comprehensive description of such techniques in the literature, see for example: Molecular Cloning A Laboratory Manual, 2nd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press: 1989); DNA Cloning, Volumes I and II (D. N. Glover ed., 1985); Oligonucleotide Synthesis (M. J. Gait ed., 1984); Mullis et al. U.S. Pat. No. 4,683,195; Nucleic Acid Hybridization (B. D. Hames & S. J. Higgins eds. 1984); Transcription And Translation (B. D. Hames & S. J. Higgins eds. 1984); Culture Of Animal Cells (R. I. Freshney, Alan R. Liss, Inc., 1987); Immobilized Cells And Enzymes (IRL Press, 1986); B. Perbal, A Practical Guide To Molecular Cloning (1984); the treatise, Methods In Enzymology (Academic Press, Inc., N.Y.); Gene Transfer Vectors For Mammalian Cells (J. H. Miller and M. P. Calos eds., 1987, Cold Spring Harbor Laboratory); Methods In Enzymology, Vols. 154 and 155 (Wu et al. eds.), Immunochemical Methods In Cell And Molecular Biology (Mayer and Walker, eds., Academic Press, London, 1987); Handbook Of Experimental Immunology, Volumes I-IV (D. M. Weir and C. C. Blackwell, eds., 1986).

The figure shows:

Figure 1: eGFP-expression in retina and retinal pigment epithel (RPE) of systemically dsRNA-treated FVB.CG-TG(GFPU)5NAGY mice. The figure shows eGFP-expression in eye paraffin sections of dsRNA-treated FVB.Cg-Tg(GFPU)5Nagy mice. Expression in retina and retinal pigment epithel (RPE) of systemically dsRNA-treated FVB.CG-TG(GFPU)5NAGY mice is highest in the buffer control, slightly decreased in mice treated with non-silencing dsRNA and clearly decreased in eGFP-specific dsRNA treated mice (buffer control > 200 µg/kg BW non-silencing dsRNA > 100 µg/kg BW eGFP-specific dsRNA > 200 µg/kg BW eGFP-specific dsRNA).

EXAMPLES

As an example, inhibition of the expression of green fluorescent protein (eGFP) in the retinal pigment epithelium (RPE) and the retina of transgenic mice (FVB.Cg-Tg(GFPU)5Nagy, The Jackson Laboratory) by dsRNA molecules is analyzed.

Example 1 describes specific *post transcriptional gene silencing* by dsRNA of the target gene eGFP in the mouse animal model, during which the optimal dsRNA concentration for *post transcriptional gene silencing* on systemic application is to be determined (experimental procedure 1, results see table 1 and figure 1). The procedure involves the *in vivo* treatment of transgenic mice, which express the *enhanced* form of green fluorescent protein (eGFP) in their body cells, by systemic application of dsRNA oligoribonucleotide molecules against the target gene eGFP. Control animals are also treated systemically with non-silencing dsRNA molecules. For the purpose of *post transcriptional gene silencing,* the animals not under analgesic or anesthetic influence receive daily i.v. tail vein injections (1^{st} day of treatment: day 0, final day of treatment: day 20) of 100 or 200 µg eGFP-specific dsRNA/kg body weight (BW) and the control group of 200 µg non-silencing dsRNA/kg BW. A control group of animals treated with buffer (daily i.v. injection of 0.1 ml buffer into the tail vein) is also kept. Each group of experimental animals consists of 8 animals, the maximum injection volume/injection being 0.1 ml. On day 21, the animals are sacrificed by CO_2 inhalation.
The expression of green fluorescent protein in the eye of the mice is examined immunohistologically (spontaneous eGFP fluorescence: fluorescence microscopic evaluation; eGFP-specific immunofluorescence staining: fluorescence microscopic evaluation).

Example 2 describes specific *post transcriptional gene silencing* by dsRNA of the target gene eGFP in the mouse animal model, during which the optimal time of efficacy (= the point in time at which the *gene silencing* effect is maximal) after a single systemic i.v. dsRNA injection into the tail vein, of *post transcriptional gene silencing* for systemic application is to be determined (Experimental procedure 2). The procedure involves the *in vivo* treatment of transgenic mice, which express the *enhanced* form of green fluorescent protein (eGFP) in their body cells, by systemic application of dsRNA oligoribonucleotide molecules against the target gene eGFP. The control animals are also treated systemically with non-silencing dsRNA molecules. For the purpose of *post transcriptional gene silencing,* the animals not under analgesic or anesthetic influence receive a single i.v. tail vein injection on day 0 of 200

µg eGFP-specific dsRNA/kg body weight (BW) and the control group of 200 µg non-silencing dsRNA/kg BW. Each group of experimental animals consists of 8 animals, the maximum injection volume/injection being 0.1 ml. The animals are sacrificed by CO2 inhalation on day 2, 3, 5, and 10 after i.v. injection.

The expression of green fluorescent protein in the eye of the mice is examined immunohistologically (spontaneous eGFP fluorescence: fluorescence microscopic evaluation; eGFP-specific immunofluorescence staining: fluorescence microscopic evaluation).

Example 3 describes specific *post transcriptional gene silencing* by dsRNA of the target gene eGFP in the mouse animal model, during which the optimal dsRNA concentration for *post transcriptional gene silencing* on local (retrobulbar) application is to be determined (Experimental procedure 3). The procedure involves the *in vivo* treatment of transgenic mice, which express the *enhanced* form of green fluorescent protein (eGFP) in their body cells, by retrobulbar application of dsRNA oligoribonucleotide molecules against the target gene eGFP. Control animals also receive a retrobulbar injection with non-silencing dsRNA molecules or buffer. For the purpose of *post transcriptional gene silencing,* the animals under analgesic and anesthetic influence receive a single retrobulbar injection (1st day of treatment: day 0) of 200 µg eGFP-specific dsRNA/kg body weight (BW) and the control groups of 200 µg non-silencing dsRNA/kg BW or buffer. Each group of experimental animals consists of 3 - 8 animals, the maximum injection volume/injection being 0.005 ml. The retrobulbar dsRNA injection is carried out both on the left and right eye. On day 3 or day 6, the animals are sacrificed by CO_2 inhalation.

The expression of green fluorescent protein in the eye of the mice is examined immunohistologically (spontaneous eGFP fluorescence: fluorescence microscopic evaluation; eGFP-specific immunofluorescence staining: fluorescence microscopic evaluation).

Example 4 describes specific *post transcriptional gene silencing* by dsRNA of the target gene eGFP in the mouse animal model, during which the optimal time of efficacy (= the point in time at which the *gene silencing* effect is maximal) after a single retrobulbar dsRNA injection, of *post transcriptional gene silencing* for local (retrobulbar) application is to be determined (Experimental procedure 4). The procedure involves the *in vivo* treatment of transgenic mice, which express the *enhanced* form of green fluorescent protein (eGFP) in their body cells, by retrobulbar injection of dsRNA oligoribonucleotide molecules against the target gene eGFP. Control animals also receive retrobulbar application of non-silencing

dsRNA molecules or buffer. For the purpose of *post transcriptional gene silencing,* the animals under analgesic and anesthetic influence receive a single retrobulbar injection on day 0 of 200 µg eGFP-specific dsRNA/kg body weight (BW) and the control groups of 200 µg non-silencing dsRNA/kg BW or buffer. Each group of experimental animals consists of 8 animals, the maximum injection volume/injection being 0.005 ml. The retrobulbar dsRNA injection is carried out both on the left and right eye. The animals are sacrificed by CO_2 inhalation on day 2, 3, 5, and 10 after retrobulbar injection.

The expression of green fluorescent protein in the eye of the mice is examined immunohistologically (spontaneous eGFP fluorescence: fluorescence microscopic evaluation; eGFP-specific immunofluorescence staining: fluorescence microscopic evaluation).

Example 5 describes specific *post transcriptional gene silencing* by dsRNA of the target gene eGFP in the mouse animal model, during which the optimal dsRNA activity for *post transcriptional gene silencing* on repeated local (retrobulbar) application is to be determined (Experimental procedure 5). The procedure involves the *in vivo* treatment of transgenic mice, which express the *enhanced* form of green fluorescent protein (eGFP) in their body cells, by retrobulbar application of dsRNA oligoribonucleotide molecules against the target gene eGFP. Control animals also receive a retrobulbar injection of non-silencing dsRNA molecules or buffer. For the purpose of *post transcriptional gene silencing,* the animals under analgesic and anesthetic influence receive a retrobulbar injection on days 0, 7 and 14 (1^{st} day of treatment: day 0, final day of treatment: day 14) of 200 µg eGFP-specific dsRNA/kg body weight (BW) and the control group of 200 µg non-silencing dsRNA/kg BW or 0.005 ml buffer. Each group of experimental animals consists of 8 animals, the maximum injection volume/injection being 0.005 ml. The retrobulbar dsRNA injection is carried out both on the left and right eye. On day 15, the animals are sacrificed by CO_2 inhalation.

The expression of green fluorescent protein in the eye of the mice is examined immunohistologically (spontaneous eGFP fluorescence: fluorescence microscopic evaluation; eGFP-specific immunofluorescence staining: fluorescence microscopic evaluation).

dsRNA constructs and plasmids:

For the design of the dsRNA molecules, sequences of the type AA(N19)TT (where N represents any nucleotide) were selected from the sequence of the target mRNA, in order to obtain 21 nucleotide (nt) long sense and antisense strands with symmetrical 3'-overhangs of two nucleotides in length. In the 3'-overhangs, 2'-deoxy-thymidine was used instead of

uridine. In order to ensure that the dsRNA molecules are exclusively directed against the target gene, the chosen dsRNA sequences are tested against the mouse genome in a BLAST analysis. The 21-nt RNA molecules are synthesized chemically and purified. For the duplex formation, 100 µg of the sense and antisense oligoribonucleotides each are mixed in 10 mM Tris/HCl, 20 mM NaCl (pH 7.0) and heated to 95°C and cooled to room temperature over a period of 18 hours. The dsRNA molecules are precipitated from ethanol and resuspended in sterile buffer (100 mM potassium acetate, 30 mM HEPES-KOH, 2 mM magnesium acetate, pH 7.4). The integrity and double strand character of the dsRNA are verified by gelelectrophoresis. Alternatively, the dsRNA molecules are obtained from commercial suppliers. The sequences of the target genes and the corresponding dsRNA molecules are as follows:

GFP dsRNA

DNA target sequence:	5′ G CAA GCT GAC CCT GAA GTT CA (SEQ ID NO 1) Coding region, 121-141 relative to the first nucleotide of the start codon (Acc. No. U55761)
dsRNA (sense)	5′ r(GCA AGC UGA CCC UGA AGU U) (SEQ ID NO 2)
dsRNA (antisense)	5′ r(AA CUU CAG GGU CAG CUU GC) (SEQ ID NO 3)

non-silencing dsRNA, control

DNA target sequence:	5′ AATTCTCCGAACGTGTCACGT	(SEQ ID NO 4)
dsRNA (sense)	5′ r(UUCUCCGAACGUGUCACGU)d(TT)	(SEQ ID NO 5)
dsRNA (antisense)	5′ r(ACGUGACACGUUCGGAGAA)d(TT)	(SEQ ID NO 6)

Analgesia and anesthesia of the mice:

For systemic application, the animals are immobilized and the dsRNAs are injected i.v. in the tail vein (maximal injection volume: 0.1 ml), where analgesia or anesthesia are refrained from, since this would put more stress on the animals than the i.v. injection itself. For retrobulbar injection (maximal injection volume: 0.005 ml) the animals are however subjected to short-term isofluorane inhalation anaesthesia and provided with Metamizole sodium for analgesic purposes. The animals are then kept in their accustomed animal cage surroundings. After completion of *in vivo* diagnosis (the end of each animal experiment is stated respectively in example 1 - 5) the animals are killed by CO_2 inhalation, enucleated and the eyes are studied histologically (immunohistology).

Study of eGFP expression in retinal pigment epithelium and retina:

After removal, the eyes are fixed in 4 % formalin/PBS solution for 24 hours. Using standard methods, the fixed samples are subsequently dehydrated in a series of increasing alcohol and embedded in paraffin. With the aid of a microtome, standard 5 to 12 µm serial slices are produced, stretched in a heated water bath and transferred to a polylysin-coated cover slip. The sections are then dried in an incubator for 2 hours at a temperature of 52 °C. The dried sections are deparaffinated in xylol, transferred to a decreasing series of alcohol followed by Tris/HCl pH 7.4. After blocking, the sections are incubated for 2 hours with primary anti-eGFP antiserum (polyclonal goat anti-eGFP antiserum, Santa Cruz No. sc-5384). Detection occurs by means of immunofluorescence staining by using a Cy2-conjugated rabbit anti-goat IgG (Dianova, No. 305-225-045). The samples are embedded and then mounted for microscopy with an Eclipse TE-2000-S microscope (Nikon), equipped with a 20x and 40x/1.3 objective. The spontaneous, eGFP-specific fluorescence in deparaffinated, untreated sections is analyzed using a fluorescence microscope.

Experimental procedures

Experimental procedure 1: Systemic siRN application. Determination of optimal dsRNA concentration for *post transcriptional gene silencing.*

Group	Substance	Number of animals
Control animals	Buffer	8
Negative control 200 µg dsRNA/kg BW	*non- silencing* dsRNA	8
200 µg dsRNA/kg BW	eGFP-specific dsRNA	8
100 µg dsRNA/kg BW	eGFP-specific dsRNA	8
Animals per experiment		32

For results see figure 1

Experimental procedure 2: Systemic siRNA application for the determination of the optimal time of efficacy of *post transcriptional gene silencing* (= point in time at which the *gene silencing* effect is maximal after single systemic dsRNA i.v. injection in the tail vein) on day 0.

Group	Substance	Experiment ended after	Number of animals
Negative control (8 animals per point in time)	*non-silencing* dsRNA	2, 3, 5, 10 days	32
200 µg dsRNA/kg BW	eGFP-specific dsRNA	2 days	8
200 µg dsRNA/kg BW	eGFP-specific dsRNA	3 days	8
200 µg dsRNA/kg BW	eGFP-specific dsRNA	5 days	8
200 µg dsRNA/kg BW	eGFP-specific dsRNA	10 days	8
Animals per experiment			64

Experimental procedure 3: Retrobulbar siRNA application. Determination of optimal dsRNA concentration for *post transcriptional gene silencing.*
Single retrobulbar siRNA injection on day 0, experiment end on day 3 or day 6 (BW = body weight).

Group	Left eye	Right eye	Number of animals
Negative control 200 µg dsRNA/kg BW	*non-silencing* ds RNA	*non silencing* dsRNA	day 3: 4 day 6: 4 total: 8

Control	buffer	buffer	day 3: 3 day 6: 3 total: 6
200 µg dsRNA/kg BW	eGFP-specific dsRNA	eGFP-specific dsRNA	day 3: 8 day 6: 8 total: 16
Animals per experiment			30

Experimental procedure 4: Retrobulbar dsRNA injection for the determination of the optimal time of efficacy of *post transcriptional gene silencing* (= point in time at which the *gene silencing* effect is maximal after single retrobulbar dsRNA application on day 0).

Group	Left eye	Right eye	Experiment ended after	Number of animals
Negative control (8 animals per point in time)	*non-silencing* dsRNA	*non-silencing* dsRNA	2, 3, 5, 10 days	32
200 µg dsRNA/kg BW	eGFP-specific dsRNA	eGFP-specific dsRNA	2 days	8
200 µg dsRNA/kg BW	eGFP-specific dsRNA	eGFP-specific dsRNA	3 days	8
200 µg dsRNA/kg BW	eGFP-specific dsRNA	eGFP-specific dsRNA	5 days	8
200 µg dsRNA/kg BW	eGFP-specific dsRNA	eGFP-specific dsRNA	10 days	8
Animals per experiment				64

Experimental procedure 5: Repeated retrobulbar dsRNA injection for the determination of *post transcriptional gene silencing.*

Retrobulbar injection of 200 µg dsRNA/kg BW on day 0, 7, 14; on day 15 histolog. evaluation.

Group	Left eye	Right eye	Injection on day	Number of animals
Negative control (8 animals per point in time)	*non-silencing* dsRNA	*non-silencing* dsRNA	0, 7, 14	24
200 µg dsRNA/kg BW	eGFP-specific dsRNA	eGFP-specific dsRNA	0, 7, 14	24
Animals per experiment				48

References

Cited literature, the disclosure content of which is hereby incorporated by reference in the present description:

- Allikmets R. A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Stargardt macular dystrophy. Nat Genet. 17(1):122 (1997).
- Banks CN, Hutton WK. Blindness in New South Wales: an estimate of the prevalence and some of the contributing causes. Aust J Ophthalmol 9:285-288 (1981).
- Bernstein E, Caudy AA, Hammond SM, Hannon GJ. Role for a bidentate ribonuclease in the initiation step of RNA interference. Nature 409(6818):363-6 (2001).
- Bressler NM, Bressler SB. Preventative ophthalmology. Age-related macular degeneration. Ophthalmology 102:1206-1211(1995).
- Caplen N.J., Parrish S., Imani F., Fire A., and Morgan R.A. Specific inhibition of gene expression by small double-stranded RNAs in invertebrate and vertebrate systems. Proc. Natl. Acad. Sci. 09:9742-9747 (2001).
- Elbashir S.M., Harborth J., Lendeckel W., Yalcin A., Weber K., and Tuschel T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature 411: 494-498 (2001).
- Fire A., Xu S., Montgomery M.K., Kostas S.A., Driver S.E., and Mello C.C. Potent and specific genetic interference by double-stranded RNA in Coenorhabditis elegans. Nature 391:806-811(1998).
- Gass JD. Drusen and disciform macular detachment and degeneration. Arch Ophthalmol 90:206-217 (1973).
- Ghafour IM, Allan D, Foulds WS. Common causes of blindness and visual handicap in the west of Scotland. Br J Ophthalmol 67: 209-213 (1983).
- Grey RH, Burns-Cox CJ, Hughes A. Blind and partial sight registration in Avon. Br J Ophthalmol 73:88-94 (1989).
- Grishok A, Pasquinelli AE, Conte D, Li N, Parrish S, Ha I, Baillie DL, Fire A, Ruvkun G, Mello CC. Genes and mechanisms related to RNA interference regulate expression of the small temporal RNAs that control C. elegans developmental timing. Cell 106(1):23-34 (2001).
- Hamel CP, Tsilou E, Pfeffer BA, Hooks JJ, Detrick B, Redmond TM. Molecular cloning and expression of RPE65, a novel retinal pigment epithelium-specific

microsomal protein that is post-transcriptionally regulated in vitro. J Biol Chem. 268(21):15751-7(1993).

- Hamilton AJ and Baulcombe DC. A species of small antisense RNA in posttranscriptional gene silencing in plants. Science 286: 950-952(1999).
- Heiba IM, Elston RC, Klein BE, Klein R. Sibling correlations and segregation analysis of age-related maculopathy: the Beaver Dam Eye Study. Genet Epidemiol 11:51-67 (1994).
- Hyman LG, Lilienfeld AM, Ferris FL, Fine SL. Senile macular degeneration: a case-control study. Am J Epidemiol 118:213-227 (1983).
- Hyman L. Epidemiology of eye disease in the elderly. Eye 1: 330-341 (1987).
- Illing M., Molday L.L., Molday R.S. The 220-kDa rim protein of retinal rod outer segments is a member of the ABC transporter superfamily. J Biol Chem. 11;272(15):10303-10 (1997).
- Kasschau KD and Carrington JC. A counterdefensive strategy of plant viruses: suppression of posttranscriptional gene silencing. Cell 95:461-470(1998).
- Klein R, Klein BE, Franke T. The relationship of cardiovascular disease and its risk factors to age-related maculopathy. The Beaver Dam Eye Study. Ophthalmology 100:406-414 (1993).
- Knight SW and Bass BL. A role for the RNase III enzyme DCR-1 in RNA interference and germ line development in Caenorhabditis elegans. Science 2: 2269-2271(2001).
- Leibowitz HM, Krueger DE, Maunder LR, Milton RC, Kini MM, Kahn HA, Nickerson RJ, Pool J, Colton TL, Ganley JP, Loewenstein JI, Dawber TR. The Framingham Eye Study monograph: An ophthalmological and epidemiological study of cataract, glaucoma, diabetic retinopathy, macular degeneration, and visual acuity in a general population of 2631 adults, 1973-1975. Surv Ophthalmol 24(Suppl): 335-610 (1980).
- Lewis R.A., Shroyer N.F., Singh N., Allikmets R., Hutchinson A., Li Y., Lupski J.R., Leppert M., Dean M. Genotype/Phenotype analysis of a photoreceptor-specific ATP-binding cassette transporter gene, ABCR, in Stargardt disease. Am J Hum Genet. 1999 Feb;64(2):422-34 (1999).
- Meyers SM, Zachary AA. Monozygotic twins with age-related macular degeneration. Arch Ophthalmol 106:651-653 (1988).

- Okabe, M, Ikawa, M, Kominami, K, Nakanishi, T and Nishimune, Y. 'Green mice' as a source of ubiquitous green cells. FEBS Letters 407:313-9 (1997).
- Paetkau ME, Boyd TA, Grace M, Bach-Mills J, Winship B. Senile disciform macular degeneration and smoking. Can J Ophthalmol 13:67-71 (1978).
- Piguet B, Wells JA, Palmvang IB, Wormald R, Chisholm IH, Bird AC. Age-related Bruch's membrane change: a clinical study of the relative role of heredity and environment. Br J Ophthalmol 77:400-403 (1993).
- Schuler GD. Pieces of the puzzle: expressed sequence tags and the catalog of human genes. J Mol Med. 75(10):694-8 (1997)
- Seddon JM, Ajani UA, Mitchel BD. Familial aggregation of age-related maculopathy. Am J Ophthalmol 123: 199-206 (1997).
- Seeliger M.W., Grimm C., Stahlberg F., Friedburg C., Jaissle G., Zrenner E., Guo H., Reme C.E., Humphries P., Hoffmann F., Biel M., Fariss R.N., Redmond T.M., Wenzel A. New views on RPE65 deficiency: the rod system is the source of vision in a mouse model of Leber congenital amaurosis. Nat Genet. 29(1):70-4 (2001).
- Silvestri G, Johnston PB, Hughes AE. Is genetic predisposition an important risk factor in age-related macular degeneration? Eye 8:564-568 (1994).
- Sperduto RD, Hiller R. Systemic hypertension and age-related maculopathy in the Framingham Study. Arch Ophthalmol 104:216-219 (1986).
- The Eye Disease Case-Control Study Group. Risk factors for neovascular age-related macular. Arch Ophthalmol 110:1701-1708 (1992).
- Yap M, Weatherill J. Causes of blindness and partial sight in the Bradford Metropolitan District from 1980 to 1985. Ophthalmic Physiol Opt 9:289-292 (1989).
- Zamore PD, Tuschl T, Sharp PA, Bartel DP. RNAi: double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals. Cell 101: 25-33(2000).

Claims

1. Use of one or more double-stranded oligoribonucleotides (dsRNA) for the preparation of a composition for the specific modulation of the expression of one or more target genes in cells and/or tissues of the CNS and/or eye of a subject, wherein said composition is designed to be applied outside the blood-brain or blood-retina barriers.

2. A method for the specific modulation of the expression of target genes in cells and/or tissues of the CNS and/or eye, wherein a composition comprising one or more double-stranded oligoribonucleotides (dsRNA) is introduced into a cell, tissue or organism outside the blood-brain or blood-retina barriers.

3. The method of claim 2, wherein said method results in the provision of a test cell, test tissue or test organism, which can be preferably maintained under conditions allowing the degradation of the corresponding mRNA of one or more of target genes by RNA interference.

4. The method of claim 3 for the identification or validation of the function of a gene, further comprising comparing the resulting phenotype produced in the test cell, test tissue or test organism with that of a suitable control, thus allowing information on the function of the gene to be gained.

5. The use or method of any one of claims 1 to 4, wherein said specific modulation of the expression is an inhibition of target gene expression.

6. The use or method of any one of claims 1 to 5, wherein one or more of said target genes encode a cellular mRNA.

7. The use or method of any one of claims 1 or 6, wherein the cells and/or tissues are cells and/or tissues of the eye.

8. The use or method of any one of claims 1 to 7, wherein said cells or tissues are cells or tissues of the inner segment of the eye ball.

9. The use or method of claim 8, wherein said cells are retinal cells.

10. The use or method of claim 9, wherein said cells are cells of the retinal pigment epithelium (RPE) or neurosensory retina cells.

11. The use or method of any one of claims 1 to 10, wherein one or more of said target genes are predominantly expressed in said cell and/or tissue.

12. The use or method of any one of claims 1 to 11, wherein the expression of one or more of said target genes is specific for said cell and/or tissue.

13. The use or method of any one of claims 1 to 12, wherein said dsRNA molecules are between 21 and 23 nucleotides in length.

14. The use or method of any one of claims 1 to 13, wherein said dsRNA molecules contain a terminal 3'-hydroxyl group.

15. The use or method of any one of claims 1 to 14, wherein said dsRNA molecules have been chemically synthesized.

16. The use or method of any one of claims 1 to 15, wherein said dsRNA molecules represent an analogue of naturally occurring RNA.

17. The use or method of any one of claims 1 to 16, wherein said dsRNA analogues differ from the corresponding naturally occurring RNA by addition, deletion, substitution or modification of one or more nucleotides.

18. The use or method of any one of claims 1 to 17, wherein said dsRNA molecules inhibit the corresponding target genes by "posttranscriptional silencing".

19. The use or method of any one of claims 1 to 18, wherein said dsRNA molecules are encoded by a vector.

20. The use or method of any one of claims 19, wherein the expression said dsRNA is under control of a cell and/or tissue specific promoter.

21. The use or method of any one of claims 1 to 20, wherein the dsRNAs are introduced into the cells or tissues bound to other molecules and/or combined with one or more suitable carriers.

22. The use or method of claim 21, wherein the carrier is a micellar structure, preferably a liposome, a coat protein, derived from a virus such as the cytomegalovirus (CMV) or produced synthetically, adeno-associated virus (AAV) or adenovirus.

23. The use or method of claim 21 or 22, wherein the dsRNA is bound to cationic porphyrins, cationic polyamines, polymeric DNA-binding cations or fusogenic peptides.

24. The use or method of any one of claims 21 to 23, wherein the carrier and/or the dsRNA-binding molecules were selected such that the dsRNA molecules are delivered continuously to the target cells or target tissues over a defined period of time after application.

25. The use or method of any one of claims 21 to 24, wherein said carrier is specific for said cells and/or tissues as defined in any one of claims 7 to 12.

26. The use or method of any use of claims 1 to 25, wherein said composition is in form to be applied outside the eye ball, preferably by iontophoresis, retrobulbar or systemic application or as eye drops.

27. The use or method of any one of claims 1 to 25, wherein the subject or organism is a vertebrate.

28. The use or method of any one of claims 1 to 25, wherein the subject or organism is a mammal, preferably human.

29. The method of any one of claims 2 to 26, wherein the cells and/or tissues are of vertebrate origin.

30. The method of any one of claims 2 to 26 or 29, wherein the cells and/or tissues are of mammalian origin.

31. The method of claim 30, wherein the cells and/or tissues are of human origin.

32. A cell or tissue obtainable by a method of any one of claims 2 to 25 or 29 to 31, wherein the expression of a target genes is modulated.

33. An non-human organism obtainable by a method of any one of claims 2 to 27, comprising a cell or tissue of claim 32.

34. The organism of claim 33 which is a transgenic organism.

35. The organism of claim 33 or 34, wherein the organism displays the phenotype of an eye disease.

36. The organism of claim 35, wherein the organism displays the phenotype of a disease of the inner segment of the eye ball.

37. The organism of any one of claims 33 to 36, wherein the organism displays the phenotype of a retinal disease.

38. The organism of any one of claims 33 to 37, wherein the organism displays the phenotype of a degenerative retinal disease.

39. The organism of any one of claims 33 to 38 is a mouse, rat or zebra fish.

40. A pharmaceutical composition useful for the treatment of disease as defined in any one of claims 35 to 38, comprising a composition as defined in any one of claims 1 to 26.

41. A diagnostic composition for detecting a gene or gene expression involved in diseases of the CNS and/or eye, comprising a composition as defined in any one of claims 1 to 26, a cell or tissue of claim 32 or an organism of any one of claims 33 to 39.

42. A method of identification and isolation of a drug capable of specific modulation of the expression of a target gene in cells and/or tissues of the eye, comprising the steps:
(a) contacting a cell or tissue of claim 32 or a non-human organism of any one of claims 33 to 39 with a compound to be screened and;
(b) determining if the compound antagonizes or agonizes the effect of said one or more double-stranded oligoribonucleotides (dsRNA) molecules.

43. The method of claim 42, further comprising comparing the non-human organism treated with said compound with a non-treated control, wherein reversion or amelioration of the phenotype as defined in any one of claims 35 to 38 is indicative for a drug or lead compound for a drug for the treatment of a disease related to the eye.

44. Use of a component selected from the group consisting of a composition, nucleic acid, non-human organism, host cell, cell line, tissue, organ, drug, carrier and/or vector for the specific modulation of expression of one or more target genes in cells and/or tissue of the CNS and/or eye, wherein said component comprises one or more dsRNA molecules which are applicable outside the blood-brain barrier or the retinal region of the blood-retina barrier.

45. A kit for use in a method of any one of claims 2 to 27, 29 to 31, or 42 to 43, comprising at least one component as defined in claim 44.

46. The use of the method of any one of claims 2 to 31, cell of claim 32, or non-human organism of any one of claims 33 to 39 in drug discovery or target gene isolation and/or validation.

47. Use of RNA interference for the diagnosis and/or therapy of disorders related to the CNS and/or eye, or a nucleic acid, non-human organism, host cell, cell line, tissue, organ, carrier and/or vector for such use.

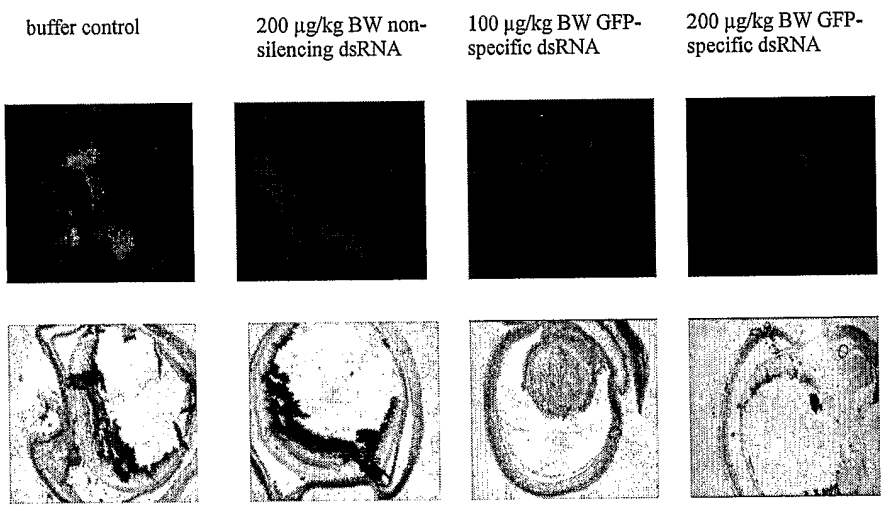
fluorescence
buffer control
200 µg/kg BW non-silencing dsRNA
100 µg/kg BW GFP-specific dsRNA
200 µg/kg BW GFP-specific dsRNA
bright-field

Figure 1

SEQUENCE LISTING

<110> Lynkeus Biotech GmbH

<120> Means and Methods for the Specific Inhibition of Genes in Cells and Tissue of the CNS and/or the Eye

<130> LY01A05/P-WO

<150> EP02008761.5

<151> 2002-04-18

<150> US 60/431,173

<151> 2002-12-05

<160> 6

<170> PatentIn version 3.1

<210> 1

<211> 21

<212> DNA

<213> Artificial

<400> 1
gcaagctgac cctgaagttc a 21

<210> 2

<211> 19

<212> RNA

<213> Artificial

<400> 2
gcaagcugac ccugaaguu 19

<210> 3

<211> 19

<212> RNA

<213> Artificial

<400> 3
aacuucaggg ucagcuugc 19

<210> 4

<211> 21

<212> DNA

<213> Artificial

<400> 4
aattctccga acgtgtcacg t 21

<210> 5

<211> 21

<212> DNA/RNA

<213> Artificial

<400> 5
uucuccgaac gugucacgut t 21

<210> 6

<211> 21

<212> DNA/RNA

<213> Artificial

<400> 6
acgugacacg uucggagaat t 21