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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 September 2013 (19.09.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/138374 A2

(51) International Patent Classification:
*C12N 15/113* (2010.01)

(21) International Application Number:
PCT/US2013/030589

(22) International Filing Date:
12 March 2013 (12.03.2013)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/611,225 15 March 2012 (15.03.2012) US
61/614,664 23 March 2012 (23.03.2012) US

(71) Applicants: **CURNA, INC.** [US/US]; 4400 Biscayna Blvd., Miami, FL 33137 (US). **THE SCRIPPS RESEARCH INSTITUTE** [US/US]; 10550 North Torrey Pines Road, La Jolla, CA 92037 (US).

(72) Inventors: **FAGHIHI, Mohammad, Ali**; 1501 NW 10th Ave., BRB-407, Miami, FL 33136 (US). **COITO, Carlos**; 4518 Corniche Circle #26, West Palm Beach, FL 33417 (US).

(74) Agent: **GUISE, Jeffrey, W.**; Wilson Sonsini Goodrich & Rosati, 650 Page Mill Road, Palo Alto, CA 94304 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

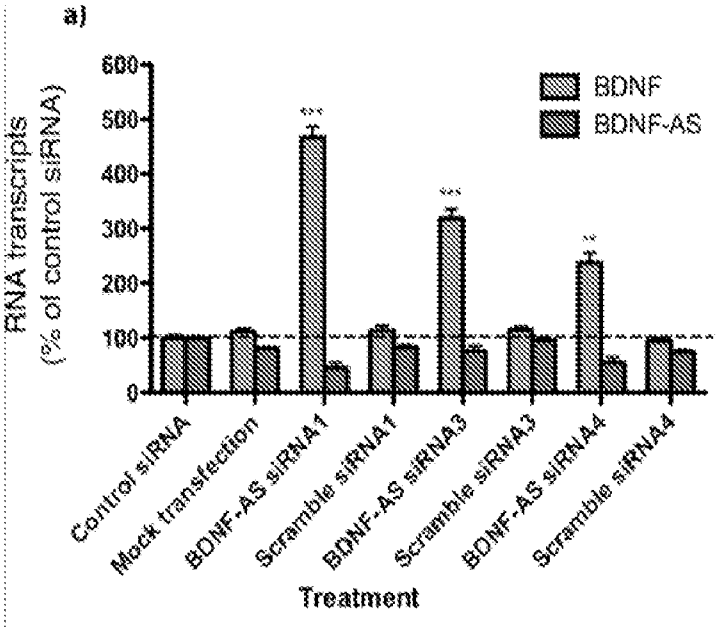
(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (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 (Rule 48.2(g))*

— *with sequence listing part of description (Rule 5.2(a))*

(54) Title: TREATMENT OF BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF) RELATED DISEASES BY INHIBITION OF NATURAL ANTISENSE TRANSCRIPT TO BDNF




(57) Abstract: The present invention relates to antisense oligonucleotides that modulate the expression of and/or function of Brain derived neurotrophic factor (BDNF), in particular, by targeting natural antisense polynucleotides of Brain derived neurotrophic factor (BDNF). The invention also relates to the identification of these antisense oligonucleotides and their use in treating diseases and disorders associated with the expression of BDNF.

WO 2013/138374 A2

# TREATMENT OF BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF) RELATED DISEASES BY INHIBITION OF NATURAL ANTISENSE TRANSCRIPT TO BDNF

## CROSS-REFERENCE TO RELATED APPLICATION

**[0001]** This application claims the benefit of U.S. Prov. Pat. App. Ser. No. 61/611,225, filed on March 15, 2012; and U.S. Prov. Pat. App. Ser. No. 61/614,664, filed on March 23, 2012, both of which are incorporated herein by reference in their entirety.

## FIELD OF THE INVENTION

**[0002]** Embodiments of the invention comprise oligonucleotides modulating expression and/or function of BDNF and associated molecules.

## BACKGROUND

**[0003]** DNA-RNA and RNA-RNA hybridization are important to many aspects of nucleic acid function including DNA replication, transcription, and translation. Hybridization is also central to a variety of technologies that either detect a particular nucleic acid or alter its expression. Antisense nucleotides, for example, disrupt gene expression by hybridizing to target RNA, thereby interfering with RNA splicing, transcription, translation, and replication. Antisense DNA has the added feature that DNA-RNA hybrids serve as a substrate for digestion by ribonuclease H, an activity that is present in most cell types. Antisense molecules can be delivered into cells, as is the case for oligodeoxynucleotides (ODNs), or they can be expressed from endogenous genes as RNA molecules. The FDA recently approved an antisense drug, VITRAVENE™ (for treatment of cytomegalovirus retinitis), reflecting that antisense has therapeutic utility.

**[0004]** WO 2010/093904 and its US counterpart US/2011/0319475 disclose BDNF as a target for modulation using oligonucleotides recited therein. There is a need for continued development with respect to natural antisense targets and newly developed oligonucleotides that complement such targets and modulate BDNF protein expression to potentially treat or be used in research associated with treating BDNF related diseases and conditions.

## SUMMARY

**[0005]** This Summary is provided to present a summary of the invention to briefly indicate the nature and substance of the invention. It is submitted with the understanding that it will not be used to interpret or limit the scope or meaning of the claims.

**[0006]** In one embodiment, the invention provides methods for inhibiting the action of a natural antisense transcript by using antisense oligonucleotide(s) targeted to any region of the natural antisense transcript resulting in up-regulation of the corresponding BDNF sense gene in mammalian

organisms. It is also contemplated herein that inhibition of the natural antisense transcripts recited herein can be achieved by siRNA, ribozymes and small molecules, which are considered to be within the scope of the present invention.

**[0007]** One embodiment provides a method of modulating function and/or expression of a BDNF polynucleotide in biological systems, including, but not limited to, patient cells or tissues *in vivo* or *in vitro* comprising contacting said biological system or said cells or tissues with an antisense oligonucleotide of about 5 to about 30 nucleotides in length wherein said oligonucleotide has at least 50% sequence identity to a reverse complement of a polynucleotide comprising 5 to 30 consecutive nucleotides within nucleotides 1 to 1279 of SEQ ID NO: 3 or 1 to 1478 of SEQ ID NO: 4 or 1 to 1437 of SEQ ID NO: 5 or 1 to 2322 of SEQ ID NO: 6 or 1 to 2036 of SEQ ID NO: 7 or 1 to 2364 of SEQ ID NO: 8 or 1 to 3136 of SEQ ID NO: 9 or 1 to 906 of SEQ ID NO: 10 or 1 to 992 of SEQ ID NO: 11 thereby modulating function and/or expression of the BDNF polynucleotide in said biological system including said patient cells or tissues *in vivo* or *in vitro*, with the proviso that the oligonucleotides having SEQ ID NOS 50-55 are excluded.

**[0008]** In an embodiment, an oligonucleotide recited above targets a natural antisense sequence of BDNF polynucleotides present in a biological system, for example, nucleotides set forth in SEQ ID NOS: 3 to 11, and any variants, alleles, homologs, mutants, derivatives, fragments and complementary sequences thereto. Examples of such antisense oligonucleotides are set forth as SEQ ID NOS: 12 to 49.

**[0009]** In another embodiment, the invention comprises a method of modulating the function or expression of a BDNF polynucleotide in a biological system comprising contacting said biological system with at least one antisense oligonucleotide that targets a natural antisense transcript of the BDNF polynucleotide comprising 5 to 30 consecutive nucleotides within nucleotides 1 to 1279 of SEQ ID NO: 3 or 1 to 1478 of SEQ ID NO: 4 or 1 to 1437 of SEQ ID NO: 5 or 1 to 2322 of SEQ ID NO: 6 or 1 to 2036 of SEQ ID NO: 7 or 1 to 2364 of SEQ ID NO: 8 or 1 to 3136 of SEQ ID NO: 9 or 1 to 906 of SEQ ID NO: 10 or 1 to 992 of SEQ ID NO: 11 thereby modulating the function and/or expression of the BDNF polynucleotide in said biological system.

**[0010]** In another embodiment, the invention comprises a method of modulating the function or expression of a BDNF polynucleotide in a biological system comprising contacting said biological system with at least one antisense oligonucleotide that targets a region of a natural antisense transcript of the BDNF polynucleotide comprising 5 to 30 consecutive nucleotides within nucleotides 1 to 1279 of SEQ ID NO: 3 or 1 to 1478 of SEQ ID NO: 4 or 1 to 1437 of SEQ ID NO: 5 or 1 to 2322 of SEQ ID NO: 6 or 1 to 2036 of SEQ ID NO: 7 or 1 to 2364 of SEQ ID NO: 8 or 1 to 3136 of SEQ ID NO: 9 or 1 to 906 of SEQ ID NO: 10 or 1 to 992 of SEQ ID NO: 11 thereby modulating the function and/or expression of the BDNF polynucleotide in said biological system.

**[0011]** In an embodiment, the invention comprises a method of increasing the function and/or expression of a BDNF polynucleotide having SEQ ID NO. 1 and 2 in a biological system comprising contacting said biological system with at least one antisense oligonucleotide that targets a natural antisense transcript of said BDNF polynucleotide comprising 5 to 30 consecutive nucleotides within nucleotides 1 to 1279 of SEQ ID NO: 3 or 1 to 1478 of SEQ ID NO: 4 or 1 to 1437 of SEQ ID NO: 5 or 1 to 2322 of SEQ ID NO: 6 or 1 to 2036 of SEQ ID NO: 7 or 1 to 2364 of SEQ ID NO: 8 or 1 to 3136 of SEQ ID NO: 9 or 1 to 906 of SEQ ID NO: 10 or 1 to 992 of SEQ ID NO: 11 thereby increasing the function and/or expression of said BDNF polynucleotide or expression product thereof.

**[0012]** In another embodiment, the invention comprises a method of method of increasing the function and/or expression of a BDNF polynucleotide having SEQ ID NO. 1 and 2 in a biological system comprising contacting said biological system with at least one antisense oligonucleotide that targets a natural antisense transcript of said BDNF polynucleotide thereby increasing the function and/or expression of said BDNF polynucleotide or expression product thereof wherein the natural antisense transcripts are selected from SEQ ID NOS. 3 to 11.

**[0013]** In another embodiment, the invention comprises a method of method of increasing the function and/or expression of a BDNF polynucleotide having SEQ ID NO. 1 and 2 in a biological system comprising contacting said biological system with at least one antisense oligonucleotide that targets a natural antisense transcript of said BDNF polynucleotide thereby increasing the function and/or expression of said BDNF polynucleotide or expression product thereof wherein the natural antisense transcripts are selected from SEQ ID NOS. 3 to 11 and wherein the antisense oligonucleotides are selected from at least one of SEQ ID NOS. 12 to 49.

**[0014]** In an embodiment, a composition comprises one or more antisense oligonucleotides which bind to sense and/or antisense BDNF polynucleotides.

**[0015]** In an embodiment, the oligonucleotides comprise one or more modified or substituted nucleotides.

**[0016]** In an embodiment, the oligonucleotides comprise one or more modified bonds.

**[0017]** In yet another embodiment, the modified nucleotides comprise modified bases comprising phosphorothioate, methylphosphonate, peptide nucleic acids, 2'-O-methyl, fluoro- or carbon, methylene or other locked nucleic acid (LNA) molecules. Preferably, the modified nucleotides are locked nucleic acid molecules, including α-L-LNA.

**[0018]** In an embodiment, the oligonucleotides are administered to a patient subcutaneously, intramuscularly, intravenously or intraperitoneally.

**[0019]** In an embodiment, the oligonucleotides are administered in a pharmaceutical composition. A treatment regimen comprises administering the antisense compounds at least once to patient;

however, this treatment can be modified to include multiple doses over a period of time. The treatment can be combined with one or more other types of therapies.

**[0020]** In an embodiment, the oligonucleotides are encapsulated in a liposome or attached to a carrier molecule (e.g. cholesterol, TAT peptide).

**[0021]** In an embodiment, the present invention comprises the use of SEQ ID NOS 50-55 as oligonucleotides targeting the natural antisense transcripts (NATs) to modulate the expression of a BDNF polynucleotide wherein said NATs are selected from the group consisting of SEQ ID NOS. 3 to 11. In another embodiment, the present invention comprises the use of SEQ ID NOS 50-55 as oligonucleotides targeting the natural antisense transcripts (NATs) to modulate the expression of a BDNF polynucleotide wherein said NATs are selected from the group consisting of SEQ ID NOS. 3, 4, 5, 7, 8, 9, 10 and 11.

**[0022]** Other aspects are described *infra.*

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0023]** Figures 1a-e show antisense-mediated regulation of sense mRNA and protein. Figure 1a shows that after transfection of several human and mouse cell lines with three siRNA oligonucleotides, targeted to non-overlapping regions of the BDNF-AS transcript, knockdown and upregulation of BDNF transcript occurred. Figure 1b shows time course study data after administration of BDNF-AS-targeted siRNA in the endogenous expression of both BDNF and BDNF-AS transcripts. The data shows that over the course of time BDNF-AS is downregulated and then BDNF expression is upregulated and is reversible. Figure 1c shows that BDNF protein, measured by ELISA, was significantly increased with two siRNAs targeting BDNF-AS transcript, but not with scrambled siRNAs or a control nontargeting siRNA. Figure 1d shows protein levels of BDNF following administration of various siRNAs using ELISA and/or western blotting. Figure 1e shows the fold change percentage of BDNF compared to mock control versus increasing concentrations of oligonucleotide ($10^{-12}$ to $10^{-6}$ M).

**[0024]** Figure 2 shows Bdnf upregulation increases neuronal outgrowth.

**[0025]** Figure 3 shows Bdnf-AS regulates Bdnf mRNA and protein in vivo.

**[0026]** Figure 4 shows Blocking of Bdnf-AS, in vivo, causes an increase in neuronal survival and proliferation.

**[0027]** Figure 5 shows BDNF-AS knockdown leads to BDNF mRNA upregulation.

**[0028]** Figure 6 shows Posttranscriptional regulation of Bdnf expression.

**[0029]** Figure 7 shows Inhibition of the human BDNF-AS transcript by hBDNFAntagoNAT.

**[0030]** Figure 8 shows Inhibition of the mouse Bdnf-AS transcript in N2a cells, by AntagoNATs.

**[0031]** Figure 9 shows BDNF-AS knockdown neither changes the level of TrkB nor BDNF neighboring genes (Let7C and KIF18A) in both directions: LIN7C and KIF18A are genes located 3' downstream and 5' upstream of BDNF, respectively.

**[0032]** Sequence Listing Description: SEQ ID NO: 1: Homo sapiens Brain derived neurotrophic factor (BDNF), transcript variant 3, mRNA. (NCBI Accession No.: NM_170735); SEQ ID NO: 2: Mus musculus brain derived neurotrophic factor (Bdnf), transcript variant 1, mRNA (NCBI Accession No.: NM_007540); SEQ ID NO: 3: Natural BDNF antisense sequence (transcript variant BT1A;NR_033313.1); SEQ ID NO: 4: Natural BDNF antisense sequence (transcript variant BT2A; NR_033314.1); SEQ ID NO: 5: Natural BDNF antisense sequence (transcript variant BT1B; NR_033315.1); SEQ ID NO: 6: Natural BDNF antisense sequence (transcript variant BT2B; NR_002832.2); SEQ ID NO: 7: Natural BDNF antisense sequence (transcript variant BT1C; NR_033312.1); SEQ ID NO: 8: Natural BDNF antisense sequence (BDNF-AS variant); SEQ ID NO: 9: Natural BDNF antisense sequence; SEQ ID NO: 10: Mouse natural BDNF antisense sequence (Mouse BDNF-AS variant 1); SEQ ID NO: 11: Mouse natural BDNF antisense sequence (Mouse BDNF-AS variant 2); SEQ ID NOs: 12 to 55: Antisense oligonucleotides; SEQ ID NO: 56 to 59: Reverse complement of the antisense oligonucleotides 12 to 15 respectively; SEQ ID NO: 60 to 64: Reverse complement of the antisense oligonucleotides 42 to 46 respectively; SEQ ID NO: 65 and 66: Assay sequences. LNA (2'-O,4'-C methylene locked nucleic acid): +A* or +T* or +C* or +G*; 2'OM (2'-O-methyl): mU* or mA* or mC* or mG*; PS (phosphothioate): T* or A* or G* or c*; RNA: rU or rA or rG or rC.

## DETAILED DESCRIPTION

**[0033]** Several aspects of the invention are described below with reference to example applications for illustration. It should be understood that numerous specific details, relationships, and methods are set forth to provide a full understanding of the invention. One having ordinary skill in the relevant art, however, will readily recognize that the invention can be practiced without one or more of the specific details or with other methods. The present invention is not limited by the ordering of acts or events, as some acts may occur in different orders and/or concurrently with other acts or events. Furthermore, not all illustrated acts or events are required to implement a methodology in accordance with the present invention.

**[0034]** All genes, gene names, and gene products disclosed herein are intended to correspond to homologs from any species for which the compositions and methods disclosed herein are applicable. Thus, the terms include, but are not limited to genes and gene products from humans and mice. It is understood that when a gene or gene product from a particular species is disclosed, this disclosure is intended to be exemplary only, and is not to be interpreted as a limitation unless the context in which it appears clearly indicates. Thus, for example, for the genes disclosed herein, which in some

embodiments relate to mammalian nucleic acid and amino acid sequences are intended to encompass homologous and/or orthologous genes and gene products from other animals including, but not limited to other mammals, fish, amphibians, reptiles, and birds. In an embodiment, the genes or nucleic acid sequences are human.

*Definitions*

**[0035]** The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. As used herein, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms "including", "includes", "having", "has", "with", or variants thereof are used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term "comprising."

**[0036]** The term "about" or "approximately" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, i.e., the limitations of the measurement system. For example, "about" can mean within 1 or more than 1 standard deviation, per the practice in the art. Alternatively, "about" can mean a range of up to 20%, preferably up to 10%, more preferably up to 5%, and more preferably still up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, preferably within 5-fold, and more preferably within 2-fold, of a value. Where particular values are described in the application and claims, unless otherwise stated the term "about" meaning within an acceptable error range for the particular value should be assumed.

**[0037]** As used herein, the term "mRNA" means the presently known mRNA transcript(s) of a targeted gene, and any further transcripts which may be elucidated.

**[0038]** By "antisense oligonucleotides" or "antisense compound" is meant an RNA or DNA molecule that binds to another RNA or DNA (target RNA, DNA). For example, if it is an RNA oligonucleotide it binds to another RNA target by means of RNA-RNA interactions and alters the activity of the target RNA. An antisense oligonucleotide can upregulate or downregulate expression and/or function of a particular polynucleotide. The definition is meant to include any foreign RNA or DNA molecule which is useful from a therapeutic, diagnostic, or other viewpoint. Such molecules include, for example, antisense RNA or DNA molecules, interference RNA (RNAi), micro RNA, decoy RNA molecules, siRNA, enzymatic RNA, therapeutic editing RNA and agonist and antagonist RNA, antisense oligomeric compounds, antisense oligonucleotides, external guide sequence (EGS) oligonucleotides, alternate splicers, primers, probes, and other oligomeric compounds that hybridize to at least a portion of the target nucleic acid. As such, these compounds

may be introduced in the form of single-stranded, double-stranded, partially single-stranded, or circular oligomeric compounds.

**[0039]** In the context of this invention, the term "oligonucleotide" refers to an oligomer or polymer of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) or mimetics thereof. The term "oligonucleotide", also includes linear or circular oligomers of natural and/or modified monomers or linkages, including deoxyribonucleosides, ribonucleosides, substituted and alpha-anomeric forms thereof, peptide nucleic acids (PNA), locked nucleic acids (LNA), phosphorothioate, methylphosphonate, and the like. Oligonucleotides are capable of specifically binding to a target polynucleotide by way of a regular pattern of monomer-to-monomer interactions, such as Watson-Crick type of base pairing, Hoögsteen or reverse Hoögsteen types of base pairing, or the like.

**[0040]** The oligonucleotide may be "chimeric", that is, composed of different regions. In the context of this invention "chimeric" compounds are oligonucleotides, which contain two or more chemical regions, for example, DNA region(s), RNA region(s), PNA region(s) etc. Each chemical region is made up of at least one monomer unit, i.e., a nucleotide in the case of an oligonucleotides compound. These oligonucleotides typically comprise at least one region wherein the oligonucleotide is modified in order to exhibit one or more desired properties. The desired properties of the oligonucleotide include, but are not limited, for example, to increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid. Different regions of the oligonucleotide may therefore have different properties. The chimeric oligonucleotides of the present invention can be formed as mixed structures of two or more oligonucleotides, modified oligonucleotides, oligonucleosides and/or oligonucleotide analogs as described above.

**[0041]** The oligonucleotide can be composed of regions that can be linked in "register", that is, when the monomers are linked consecutively, as in native DNA, or linked via spacers. The spacers are intended to constitute a covalent "bridge" between the regions and have in preferred cases a length not exceeding about 100 carbon atoms. The spacers may carry different functionalities, for example, having positive or negative charge, carry special nucleic acid binding properties (intercalators, groove binders, toxins, fluorophors etc.), being lipophilic, inducing special secondary structures like, for example, alanine containing peptides that induce alpha-helices.

**[0042]** As used herein "BDNF" and "Brain derived neurotrophic factor" are inclusive of all family members, mutants, alleles, fragments, species, coding and noncoding sequences, sense and antisense polynucleotide strands, etc.

**[0043]** As used herein, the words 'Brain derived neurotrophic factor', 'Brain-derived neurotrophic factor' and BDNF, are considered the same in the literature and are used interchangeably in the present application.

**[0044]** As used herein, the term "oligonucleotide specific for" or "oligonucleotide which targets" refers to an oligonucleotide having a sequence (i) capable of forming a stable complex with a portion of the targeted gene, or (ii) capable of forming a stable duplex with a portion of a mRNA transcript of the targeted gene. Stability of the complexes and duplexes can be determined by theoretical calculations and/or in vitro assays. Exemplary assays for determining stability of hybridization complexes and duplexes are described in the Examples below.

**[0045]** As used herein, the term "target nucleic acid" encompasses DNA, RNA (comprising premRNA and mRNA) transcribed from such DNA, and also cDNA derived from such RNA, coding, noncoding sequences, sense or antisense polynucleotides. The specific hybridization of an oligomeric compound with its target nucleic acid interferes with the normal function of the nucleic acid. This modulation of function of a target nucleic acid by compounds, which specifically hybridize to it, is generally referred to as "antisense". The functions of DNA to be interfered include, for example, replication and transcription. The functions of RNA to be interfered, include all vital functions such as, for example, translocation of the RNA to the site of protein translation, translation of protein from the RNA, splicing of the RNA to yield one or more mRNA species, and catalytic activity which may be engaged in or facilitated by the RNA. The overall effect of such interference with target nucleic acid function is modulation of the expression of an encoded product or oligonucleotides.

**[0046]** RNA interference "RNAi" is mediated by double stranded RNA (dsRNA) molecules that have sequence-specific homology to their "target" nucleic acid sequences. In certain embodiments of the present invention, the mediators are 5-25 nucleotide "small interfering" RNA duplexes (siRNAs). The siRNAs are derived from the processing of dsRNA by an RNase enzyme known as Dicer. siRNA duplex products are recruited into a multi-protein siRNA complex termed RISC (RNA Induced Silencing Complex). Without wishing to be bound by any particular theory, a RISC is then believed to be guided to a target nucleic acid (suitably mRNA), where the siRNA duplex interacts in a sequence-specific way to mediate cleavage in a catalytic fashion. Small interfering RNAs that can be used in accordance with the present invention can be synthesized and used according to procedures that are well known in the art and that will be familiar to the ordinarily skilled artisan. Small interfering RNAs for use in the methods of the present invention suitably comprise between about 1 to about 50 nucleotides (nt). In examples of non limiting embodiments, siRNAs can comprise about 5 to about 40 nt, about 5 to about 30 nt, about 10 to about 30 nt, about 15 to about 25 nt, or about 20-25 nucleotides.

**[0047]** Selection of appropriate oligonucleotides is facilitated by using computer programs that automatically align nucleic acid sequences and indicate regions of identity or homology. Such programs are used to compare nucleic acid sequences obtained, for example, by searching databases

such as GenBank or by sequencing PCR products. Comparison of nucleic acid sequences from a range of species allows the selection of nucleic acid sequences that display an appropriate degree of identity between species. In the case of genes that have not been sequenced, Southern blots are performed to allow a determination of the degree of identity between genes in target species and other species. By performing Southern blots at varying degrees of stringency, as is well known in the art, it is possible to obtain an approximate measure of identity. These procedures allow the selection of oligonucleotides that exhibit a high degree of complementarity to target nucleic acid sequences in a subject to be controlled and a lower degree of complementarity to corresponding nucleic acid sequences in other species. One skilled in the art will realize that there is considerable latitude in selecting appropriate regions of genes for use in the present invention.

**[0048]** By "enzymatic RNA" is meant an RNA molecule with enzymatic activity (Cech, (1988) *J. American. Med. Assoc.* 260, 3030-3035). Enzymatic nucleic acids (ribozymes) act by first binding to a target RNA. Such binding occurs through the target binding portion of an enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through base pairing, and once bound to the correct site, acts enzymatically to cut the target RNA.

**[0049]** By "decoy RNA" is meant an RNA molecule that mimics the natural binding domain for a ligand. The decoy RNA therefore competes with natural binding target for the binding of a specific ligand. For example, it has been shown that over-expression of HIV trans-activation response (TAR) RNA can act as a "decoy" and efficiently binds HIV tat protein, thereby preventing it from binding to TAR sequences encoded in the HIV RNA. This is meant to be a specific example. Those in the art will recognize that this is but one example, and other embodiments can be readily generated using techniques generally known in the art.

**[0050]** As used herein, the term "monomers" typically indicates monomers linked by phosphodiester bonds or analogs thereof to form oligonucleotides ranging in size from a few monomeric units, e.g., from about 3-4, to about several hundreds of monomeric units. Analogs of phosphodiester linkages include: phosphorothioate, phosphorodithioate, methylphosphornates, phosphoroselenoate, phosphoramidate, and the like, as more fully described below.

**[0051]** The term “nucleotide” covers naturally occurring nucleotides as well as nonnaturally occurring nucleotides. It should be clear to the person skilled in the art that various nucleotides which previously have been considered "non-naturally occurring" have subsequently been found in nature. Thus, "nucleotides" includes not only the known purine and pyrimidine heterocycles-containing molecules, but also heterocyclic analogues and tautomers thereof. Illustrative examples of other types of nucleotides are molecules containing adenine, guanine, thymine, cytosine, uracil, purine, xanthine, diaminopurine, 8-oxo- N6-methyladenine, 7-deazaxanthine, 7-deazaguanine,

N4,N4-ethanocytosin, N6,N6-ethano-2,6- diaminopurine, 5-methylcytosine, 5-(C3-C6)-alkynylcytosine, 5-fluorouracil, 5-bromouracil, pseudoisocytosine, 2-hydroxy-5-methyl-4-triazolopyridin, isocytosine, isoguanin, inosine and the "non-naturally occurring" nucleotides described in Benner *et al.*, U.S. Pat No. 5,432,272. The term "nucleotide" is intended to cover every and all of these examples as well as analogues and tautomers thereof. Especially interesting nucleotides are those containing adenine, guanine, thymine, cytosine, and uracil, which are considered as the naturally occurring nucleotides in relation to therapeutic and diagnostic application in humans. Nucleotides include the natural 2'-deoxy and 2'- hydroxyl sugars, e.g., as described in Kornberg and Baker, DNA Replication, 2nd Ed. (Freeman, San Francisco, 1992) as well as their analogs.

**[0052]** "Analogs" in reference to nucleotides includes synthetic nucleotides having modified base moieties and/or modified sugar moieties (see e.g., described generally by Scheit, Nucleotide Analogs, John Wiley, New York, 1980; Freier & Altmann, (1997) *Nucl. Acid. Res.*, 25(22), 4429-4443, Toulmé, J.J., (2001) *Nature* Biotechnology 19:17-18; Manoharan M., (1999) *Biochemica et Biophysica Acta* 1489:117-139; Freier S. M., (1997) *Nucleic Acid Research*, 25:4429-4443, Uhlman, E., (2000) *Drug Discovery & Development*, 3: 203-213, Herdewin P., (2000) *Antisense & Nucleic Acid Drug Dev.*, 10:297-310); 2'-O, 3`-C-linked [3.2.0] bicycloarabinonucleosides. Such analogs include synthetic nucleotides designed to enhance binding properties, e.g., duplex or triplex stability, specificity, or the like.

**[0053]** As used herein, "hybridization" means the pairing of substantially complementary strands of oligomeric compounds. One mechanism of pairing involves hydrogen bonding, which may be Watson-Crick, Hoögsteen or reversed Hoögsteen hydrogen bonding, between complementary nucleoside or nucleotide bases (nucleotides) of the strands of oligomeric compounds. For example, adenine and thymine are complementary nucleotides which pair through the formation of hydrogen bonds. Hybridization can occur under varying circumstances.

**[0054]** An antisense compound is "specifically hybridizable" when binding of the compound to the target nucleic acid interferes with the normal function of the target nucleic acid to cause a modulation of function and/or activity, and there is a sufficient degree of complementarity to avoid non-specific binding of the antisense compound to non-target nucleic acid sequences under conditions in which specific binding is desired, i.e., under physiological conditions in the case of in vivo assays or therapeutic treatment, and under conditions in which assays are performed in the case of in vitro assays.

**[0055]** As used herein, the phrase "stringent hybridization conditions" or "stringent conditions" refers to conditions under which a compound of the invention will hybridize to its target sequence, but to a minimal number of other sequences. Stringent conditions are sequence-dependent and will

be different in different circumstances and in the context of this invention, "stringent conditions" under which oligomeric compounds hybridize to a target sequence are determined by the nature and composition of the oligomeric compounds and the assays in which they are being investigated. In general, stringent hybridization conditions comprise low concentrations (<0.15M) of salts with inorganic cations such as Na+ or K+ (i.e., low ionic strength), temperature higher than 20°C - 25° C. below the Tm of the oligomeric compound:target sequence complex, and the presence of denaturants such as formamide, dimethylformamide, dimethyl sulfoxide, or the detergent sodium dodecyl sulfate (SDS). For example, the hybridization rate decreases 1.1% for each 1% formamide. An example of a high stringency hybridization condition is 0.1X sodium chloride-sodium citrate buffer (SSC)/0.1% (w/v) SDS at 60° C. for 30 minutes.

**[0056]** "Complementary," as used herein, refers to the capacity for precise pairing between two nucleotides on one or two oligomeric strands. For example, if a nucleobase at a certain position of an antisense compound is capable of hydrogen bonding with a nucleobase at a certain position of a target nucleic acid, said target nucleic acid being a DNA, RNA, or oligonucleotide molecule, then the position of hydrogen bonding between the oligonucleotide and the target nucleic acid is considered to be a complementary position. The oligomeric compound and the further DNA, RNA, or oligonucleotide molecule are complementary to each other when a sufficient number of complementary positions in each molecule are occupied by nucleotides which can hydrogen bond with each other. Thus, "specifically hybridizable" and "complementary" are terms which are used to indicate a sufficient degree of precise pairing or complementarity over a sufficient number of nucleotides such that stable and specific binding occurs between the oligomeric compound and a target nucleic acid.

**[0057]** It is understood in the art that the sequence of an oligomeric compound need not be 100% complementary to that of its target nucleic acid to be specifically hybridizable. Moreover, an oligonucleotide may hybridize over one or more segments such that intervening or adjacent segments are not involved in the hybridization event (e.g., a loop structure, mismatch or hairpin structure). The oligomeric compounds of the present invention comprise at least about 70%, or at least about 75%, or at least about 80%, or at least about 85%, or at least about 90%, or at least about 95%, or at least about 99% sequence complementarity to a target region within the target nucleic acid sequence to which they are targeted. For example, an antisense compound in which 18 of 20 nucleotides of the antisense compound are complementary to a target region, and would therefore specifically hybridize, would represent 90 percent complementarity. In this example, the remaining non-complementary nucleotides may be clustered or interspersed with complementary nucleotides and need not be contiguous to each other or to complementary nucleotides. As such, an antisense compound which is 18 nucleotides in length having 4 (four) non-complementary nucleotides which

are flanked by two regions of complete complementarity with the target nucleic acid would have 77.8% overall complementarity with the target nucleic acid and would thus fall within the scope of the present invention. Percent complementarity of an antisense compound with a region of a target nucleic acid can be determined routinely using BLAST programs (basic local alignment search tools) and PowerBLAST programs known in the art. Percent homology, sequence identity or complementarity, can be determined by, for example, the Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison Wis.), using default settings, which uses the algorithm of Smith and Waterman (*Adv. Appl. Math.*, (1981) 2, 482-489).

**[0058]** As used herein, the term "Thermal Melting Point (Tm)" refers to the temperature, under defined ionic strength, pH, and nucleic acid concentration, at which 50% of the oligonucleotides complementary to the target sequence hybridize to the target sequence at equilibrium. Typically, stringent conditions will be those in which the salt concentration is at least about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short oligonucleotides (e.g., 10 to 50 nucleotide). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide.

**[0059]** As used herein, "modulation" means either an increase (stimulation) or a decrease (inhibition) in the expression of a gene.

**[0060]** The term "variant", when used in the context of a polynucleotide sequence, may encompass a polynucleotide sequence related to a wild type gene. This definition may also include, for example, "allelic," "splice," "species," or "polymorphic" variants. A splice variant may have significant identity to a reference molecule, but will generally have a greater or lesser number of polynucleotides due to alternate splicing of exons during mRNA processing. The corresponding polypeptide may possess additional functional domains or an absence of domains. Species variants are polynucleotide sequences that vary from one species to another. Of particular utility in the invention are variants of wild type gene products. Variants may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in polypeptides whose structure or function may or may not be altered. Any given natural or recombinant gene may have none, one, or many allelic forms. Common mutational changes that give rise to variants are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

**[0061]** The resulting polypeptides generally will have significant amino acid identity relative to each other. A polymorphic variant is a variation in the polynucleotide sequence of a particular gene between individuals of a given species. Polymorphic variants also may encompass "single nucleotide polymorphisms" (SNPs,) or single base mutations in which the polynucleotide sequence varies by

one base. The presence of SNPs may be indicative of, for example, a certain population with a propensity for a disease state, that is susceptibility versus resistance.

**[0062]** Derivative polynucleotides include nucleic acids subjected to chemical modification, for example, replacement of hydrogen by an alkyl, acyl, or amino group. Derivatives, e.g., derivative oligonucleotides, may comprise non-naturally-occurring portions, such as altered sugar moieties or inter-sugar linkages. Exemplary among these are phosphorothioate and other sulfur containing species which are known in the art. Derivative nucleic acids may also contain labels, including radionucleotides, enzymes, fluorescent agents, chemiluminescent agents, chromogenic agents, substrates, cofactors, inhibitors, magnetic particles, and the like.

**[0063]** A "derivative" polypeptide or peptide is one that is modified, for example, by glycosylation, pegylation, phosphorylation, sulfation, reduction/alkylation, acylation, chemical coupling, or mild formalin treatment. A derivative may also be modified to contain a detectable label, either directly or indirectly, including, but not limited to, a radioisotope, fluorescent, and enzyme label.

**[0064]** As used herein, the term "animal" or "patient" is meant to include, for example, humans, sheep, elks, deer, mule deer, minks, mammals, monkeys, horses, cattle, pigs, goats, dogs, cats, rats, mice, birds, chicken, reptiles, fish, insects and arachnids.

**[0065]** "Mammal" covers warm blooded mammals that are typically under medical care (e.g., humans and domesticated animals). Examples include feline, canine, equine, bovine, and human, as well as just human.

**[0066]** "Treating" or "treatment" covers the treatment of a disease-state in a mammal, and includes: (a) preventing the disease-state from occurring in a mammal, in particular, when such mammal is predisposed to the disease-state but has not yet been diagnosed as having it; (b) inhibiting the disease-state, e.g., arresting it development; and/or (c) relieving the disease-state, e.g., causing regression of the disease state until a desired endpoint is reached. Treating also includes the amelioration of a symptom of a disease (e.g., lessen the pain or discomfort), wherein such amelioration may or may not be directly affecting the disease (e.g., cause, transmission, expression, etc.).

**[0067]** As used herein, "cancer" refers to all types of cancer or neoplasm or malignant tumors found in mammals, including, but not limited to: leukemias, lymphomas, melanomas, carcinomas and sarcomas. The cancer manifests itself as a "tumor" or tissue comprising malignant cells of the cancer. Examples of tumors include sarcomas and carcinomas such as, but not limited to: fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell

carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma. Additional cancers which can be treated by the disclosed composition according to the invention include but not limited to, for example, Hodgkin's Disease, Non-Hodgkin's Lymphoma, multiple myeloma, neuroblastoma, breast cancer, ovarian cancer, lung cancer, rhabdomyosarcoma, primary thrombocytosis, primary macroglobulinemia, small-cell lung tumors, primary brain tumors, stomach cancer, colon cancer, malignant pancreatic insulanoma, malignant carcinoid, urinary bladder cancer, gastric cancer, premalignant skin lesions, testicular cancer, lymphomas, thyroid cancer, neuroblastoma, esophageal cancer, genitourinary tract cancer, malignant hypercalcemia, cervical cancer, endometrial cancer, adrenal cortical cancer, and prostate cancer.

**[0068]** As used herein a "Neurological disease or disorder" refers to any disease or disorder of the nervous system and/or visual system. "Neurological disease or disorder" include disease or disorders that involve the central nervous system (brain, brainstem and cerebellum), the peripheral nervous system (including cranial nerves), and the autonomic nervous system (parts of which are located in both central and peripheral nervous system). A Neurological disease or disorder includes but is not limited to acquired epileptiform aphasia; acute disseminated encephalomyelitis; adrenoleukodystrophy; age-related macular degeneration; agenesis of the corpus callosum; agnosia; Aicardi syndrome; Alexander disease; Alpers' disease; alternating hemiplegia; Alzheimer's disease; Vascular dementia; amyotrophic lateral sclerosis; anencephaly; Angelman syndrome; angiomatosis; anoxia; aphasia; apraxia; arachnoid cysts; arachnoiditis; Anronl-Chiari malformation; arteriovenous malformation; Asperger syndrome; ataxia telegiectasia; attention deficit hyperactivity disorder; autism; autonomic dysfunction; back pain; Batten disease; Behcet's disease; Bell's palsy; benign essential blepharospasm; benign focal; amyotrophy; benign intracranial hypertension; Binswanger's disease; blepharospasm; Bloch Sulzberger syndrome; brachial plexus injury; brain abscess; brain injury; brain tumors (including glioblastoma multiforme); spinal tumor; Brown-Sequard syndrome; Canavan disease; carpal tunnel syndrome; causalgia; central pain syndrome; central pontine myelinolysis; cephalic disorder; cerebral aneurysm; cerebral arteriosclerosis; cerebral atrophy; cerebral gigantism; cerebral palsy; Charcot-Marie-Tooth disease; chemotherapy-induced neuropathy and neuropathic pain; Chiari malformation; chorea; chronic inflammatory demyelinating

polyneuropathy; chronic pain; chronic regional pain syndrome; Coffin Lowry syndrome; coma, including persistent vegetative state; congenital facial diplegia; corticobasal degeneration; cranial arteritis; craniosynostosis; Creutzfeldt-Jakob disease; cumulative trauma disorders; Cushing's syndrome; cytomegalic inclusion body disease; cytomegalovirus infection; dancing eyes-dancing feet syndrome; DandyWalker syndrome; Dawson disease; De Morsier's syndrome; Dejerine-Klumke palsy; dementia; dermatomyositis; diabetic neuropathy; diffuse sclerosis; dysautonomia; dysgraphia; dyslexia; dystonias; early infantile epileptic encephalopathy; empty sella syndrome; encephalitis; encephaloceles; encephalotrigeminal angiomatosis; epilepsy; Erb's palsy; essential tremor; Fabry's disease; Fahr's syndrome; fainting; familial spastic paralysis; febrile seizures; Fisher syndrome; Friedreich's ataxia; fronto-temporal dementia and other "tauopathies"; Gaucher's disease; Gerstmann's syndrome; giant cell arteritis; giant cell inclusion disease; globoid cell leukodystrophy; Guillain-Barre syndrome; HTLV-1-associated myelopathy; Hallervorden-Spatz disease; head injury; headache; hemifacial spasm; hereditary spastic paraplegia; heredopathia atactic a polyneuritiformis; herpes zoster oticus; herpes zoster; Hirayama syndrome; HIVassociated dementia and neuropathy (also neurological manifestations of AIDS); holoprosencephaly; Huntington's disease and other polyglutamine repeat diseases; hydranencephaly; hydrocephalus; hypercortisolism; hypoxia; immune-mediated encephalomyelitis; inclusion body myositis; incontinentia pigmenti; infantile phytanic acid storage disease; infantile refsum disease; infantile spasms; inflammatory myopathy; intracranial cyst; intracranial hypertension; Joubert syndrome; Keams-Sayre syndrome; Kennedy disease Kinsboume syndrome; Klippel Feil syndrome; Krabbe disease; Kugelberg-Welander disease; kuru; Lafora disease; Lambert-Eaton myasthenic syndrome; Landau-Kleffner syndrome; lateral medullary (Wallenberg) syndrome; learning disabilities; Leigh's disease; Lennox-Gustaut syndrome; Lesch-Nyhan syndrome; leukodystrophy; Lewy body dementia; Lissencephaly; locked-in syndrome; Lou Gehrig's disease (i.e., motor neuron disease or amyotrophic lateral sclerosis); lumbar disc disease; Lyme disease--neurological sequelae; Machado-Joseph disease; macrencephaly; megalencephaly; Melkersson-Rosenthal syndrome; Menieres disease; meningitis; Menkes disease; metachromatic leukodystrophy; microcephaly; migraine; Miller Fisher syndrome; mini-strokes; mitochondrial myopathies; Mobius syndrome; monomelic amyotrophy; motor neuron disease; Moyamoya disease; mucopolysaccharidoses; milti-infarct dementia; multifocal motor neuropathy; multiple sclerosis and other demyelinating disorders; multiple system atrophy with postural hypotension; muscular dystrophy; myasthenia gravis; myelinoclastic diffuse sclerosis; myoclonic encephalopathy of infants; myoclonus; myopathy; myotonia congenital; narcolepsy; neurofibromatosis; neuroleptic malignant syndrome; neurological manifestations of AIDS; neurological sequelae of lupus; neuromyotonia; neuronal

ceroid lipofuscinosis; neuronal migration disorders; Niemann-Pick disease; O'Sullivan-McLeod syndrome; occipital neuralgia; occult spinal dysraphism sequence; Ohtahara syndrome; olivopontocerebellar atrophy; opsoclonus myoclonus; optic neuritis; orthostatic hypotension; overuse syndrome; paresthesia; a neurodegenerative disease or disorder (Parkinson's disease, Huntington's disease, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), dementia, multiple sclerosis and other diseases and disorders associated with neuronal cell death); paramyotonia congenital; paraneoplastic diseases; paroxysmal attacks; Parry Romberg syndrome; Pelizaeus-Merzbacher disease; periodic paralyses; peripheral neuropathy; painful neuropathy and neuropathic pain; persistent vegetative state; pervasive developmental disorders; photic sneeze reflex; phytanic acid storage disease; Pick's disease; pinched nerve; pituitary tumors; polymyositis; porencephaly; post-polio syndrome; postherpetic neuralgia; postinfectious encephalomyelitis; postural hypotension; Prader- Willi syndrome; primary lateral sclerosis; prion diseases; progressive hemifacial atrophy; progressive multifocalleukoencephalopathy; progressive sclerosing poliodystrophy; progressive supranuclear palsy; pseudotumor cerebri; Ramsay-Hunt syndrome (types I and 11); Rasmussen's encephalitis; reflex sympathetic dystrophy syndrome; Refsum disease; repetitive motion disorders; repetitive stress injuries; restless legs syndrome; retrovirus-associated myelopathy; Rett syndrome; Reye's syndrome; Saint Vitus dance; Sandhoff disease; Schilder's disease; schizencephaly; septo-optic dysplasia; shaken baby syndrome; shingles; Shy-Drager syndrome; Sjogren's syndrome; sleep apnea; Soto's syndrome; spasticity; spina bifida; spinal cord injury; spinal cord tumors; spinal muscular atrophy; Stiff-Person syndrome; stroke; Sturge-Weber syndrome; subacute sclerosing panencephalitis; subcortical arteriosclerotic encephalopathy; Sydenham chorea; syncope; syringomyelia; tardive dyskinesia; Tay-Sachs disease; temporal arteritis; tethered spinal cord syndrome; Thomsen disease; thoracic outlet syndrome; Tic Douloureux; Todd's paralysis; Tourette syndrome; transient ischemic attack; transmissible spongiform encephalopathies; transverse myelitis; traumatic brain injury; tremor; trigeminal neuralgia; tropical spastic paraparesis; tuberous sclerosis; vascular dementia (multi-infarct dementia); vasculitis including temporal arteritis; Von Hippel-Lindau disease; Wallenberg's syndrome; Werdnig-Hoffman disease; West syndrome; whiplash; Williams syndrome; Wildon's disease; and Zellweger syndrome.

**[0069]** A “proliferative disease or disorder” includes, but is not limited to, hematopoietic neoplastic disorders involving hyperplastic/neoplastic cells of hematopoietic origin arising from myeloid, lymphoid or erythroid lineages, or precursor cells thereof. These include, but are not limited to erythroblastic leukemia, acute promyeloid leukemia (APML), chronic myelogenous leukemia (CML), lymphoid malignancies, including, but not limited to, acute lymphoblastic

leukemia (ALL), which includes B-lineage ALL and T-lineage ALL, chronic lymphocytic leukemia (CLL), prolymphocytic leukemia (PLL), hairy cell leukemia (HLL) and Waldenstrom's macroglobulinemia (WM). Additional forms of malignant lymphomas include, but are not limited to, non-Hodgkin lymphoma and variants thereof, peripheral T cell lymphomas, adult T cell leukemia/lymphoma (ATL), cutaneous T-cell lymphoma (CTCL), large granular lymphocytic leukemia (LGF), Hodgkin's disease and Reed-Sternberg disease.

**[0070]** An "Inflammation" refers to systemic inflammatory conditions and conditions associated locally with migration and attraction of monocytes, leukocytes and/or neutrophils. Examples of inflammation include, but are not limited to, Inflammation resulting from infection with pathogenic organisms (including gram-positive bacteria, gram-negative bacteria, viruses, fungi, and parasites such as protozoa and helminths), transplant rejection (including rejection of solid organs such as kidney, liver, heart, lung or cornea, as well as rejection of bone marrow transplants including graft-versus-host disease (GVHD)), or from localized chronic or acute autoimmune or allergic reactions. Autoimmune diseases include acute glomerulonephritis; rheumatoid or reactive arthritis; chronic glomerulonephritis; inflammatory bowel diseases such as Crohn's disease, ulcerative colitis and necrotizing enterocolitis; hepatitis; sepsis; alcoholic liver disease; non-alcoholic steatosis; granulocyte transfusion associated syndromes; inflammatory dermatoses such as contact dermatitis, atopic dermatitis, psoriasis; systemic lupus erythematosus (SLE), autoimmune thyroiditis, multiple sclerosis, and some forms of diabetes, or any other autoimmune state where attack by the subject's own immune system results in pathologic tissue destruction. Allergic reactions include allergic asthma, chronic bronchitis, acute and delayed hypersensitivity. Systemic inflammatory disease states include inflammation associated with trauma, burns, reperfusion following ischemic events (e.g. thrombotic events in heart, brain, intestines or peripheral vasculature, including myocardial infarction and stroke), sepsis, ARDS or multiple organ dysfunction syndrome. Inflammatory cell recruitment also occurs in atherosclerotic plaques. Inflammation includes, but is not limited to, Non-Hodgkin's lymphoma, Wegener's granulomatosis, Hashimoto's thyroiditis, hepatocellular carcinoma, thymus atrophy, chronic pancreatitis, rheumatoid arthritis, reactive lymphoid hyperplasia, osteoarthritis, ulcerative colitis, papillary carcinoma, Crohn's disease, ulcerative colitis, acute cholecystitis, chronic cholecystitis, cirrhosis, chronic sialadenitis, peritonitis, acute pancreatitis, chronic pancreatitis, chronic Gastritis, adenomyosis, endometriosis, acute cervicitis, chronic cervicitis, lymphoid hyperplasia, multiple sclerosis, hypertrophy secondary to idiopathic thrombocytopenic purpura, primary IgA nephropathy, systemic lupus erythematosus, psoriasis, pulmonary emphysema, chronic pyelonephritis, and chronic cystitis.

*Polynucleotide and Oligonucleotide Compositions and Molecules*

**[0071]** *Targets:* In one embodiment, the targets comprise nucleic acid sequences of Brain derived neurotrophic factor (BDNF), including without limitation sense and/or antisense noncoding and/or coding sequences associated with BDNF. PCT Pub. No. WO 2010/093904 and U.S. Pat. App. Pub. No. 2011/0319475, both titled "Treatment of Brain Derived Neurotrophic Factor (BDNF) Related Diseases by Inhibition of Natural Antisense Transcript to BDNF" and incorporated by reference herein in their entirety, disclose BDNF as a target for modulation using oligonucleotides as recited therein.

**[0072]** Neurotrophins are a class of structurally related growth factors that promote neural survival and differentiation. They stimulate neurite outgrowth, suggesting that they can promote regeneration of injured neurons, and act as target-derived neurotrophic factors to stimulate collateral sprouting in target tissues that produce the neurotrophi. Brain-derived neurotrophic factor (BDNF) was initially characterized as a basic protein present in brain extracts and capable of increasing the survival of dorsal root ganglia. When axonal communication with the cell body is interrupted by injury, Schwann cells produce neurotrophic factors such as nerve growth factor (NGF) and BDNF. Neurotrophins are released from the Schwann cells and dispersed diffusely in gradient fashion around regenerating axons, which then extend distally along the neurotrophins' density gradient. Local application of BDNF to transected nerves in neonatal rats has been shown to prevent massive death of motor neurons that follows axotomy. The mRNA titer of BDNF increases to several times the normal level four days after auxotomy and reaches its maximum at 4 weeks. Moreover, BDNF has been reported to enhance the survival of cholinergic neurons in culture.

**[0073]** In an embodiment, antisense oligonucleotides are used to prevent or treat diseases or disorders associated with BDNF family members. Exemplary Brain derived neurotrophic factor (BDNF) mediated diseases and disorders which can be treated with the antisensense oligonucleotides of the invention and/or with cell/tissues regenerated from stem cells obtained using and/or having the antisense compounds comprise: a disease or disorder associated with abnormal function and/or expression of BDNF, a neurological disease or disorder, a disease or a disorder associated with defective neurogenesis; a neurodegenerative disease or disorder (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis etc.); a neuropsychiatric disorder (depression, schizophrenia, schizofreniform disorder, schizoaffective disorder, and delusional disorder; anxiety disorders such as panic disorder, phobias (including agoraphobia), an obsessive-compulsive disorder, a posttraumatic stress disorder, a bipolar disorder, anorexia nervosa, bulimia nervosa), an autoimmune disorder (e.g., multiple sclerosis) of the central nervous system, memory loss, a long term or a short term memory disorder, benign forgetfulness, a childhood learning disorder, close head injury, an attention deficit disorder, neuronal reaction to viral infection, brain damage, narcolepsy, a sleep disorder (e.g., circadian rhythm disorders, insomnia and

narcolepsy); severance of nerves or nerve damage, severance of cerebrospinal nerve cord (CNS) and a damage to brain or nerve cells, a neurological deficit associated with AIDS, a motor and tic disorder characterized by motor and/or vocal tics (e.g., Tourette's disorder, chronic motor or vocal tic disorder, transient tic disorder, and stereotypic movement disorder), a substance abuse disorder (e.g., substance dependence, substance abuse and the sequalae of substance abuse/dependence, such as substance-induced psychological disorder, substance withdrawal and substance-induced dementia or amnestic disorder), traumatic brain injury, tinnitus, neuralgia (e.g., trigeminal neuralgia) pain (e.g chronic pain, chronic inflammatory pain, pain associated with arthritis, fibromyalgia, back pain, cancer-associated pain, pain associated with digestive disease, pain associated with Crohn's disease, pain associated with autoimmune disease, pain associated with endocrine disease, pain associated with diabetic neuropathy, phantom limb pain, spontaneous pain, chronic post-surgical pain, chronic temporomandibular pain, causalgia, post-herpetic neuralgia, AIDS-related pain, complex regional pain syndromes type I and II, trigeminal neuralgia, chronic back pain, pain associated with spinal cord injury, pain associated with drug intake and recurrent acute pain, neuropathic pain), inappropriate neuronal activity resulting in neurodysthesias in a disease such as diabetes, an MS and a motor neuron disease, ataxias, muscular rigidity (spasticity), temporomandibular joint dysfunction, Reward deficiency syndrome (RDS), neurotoxicity caused by alcohol or substance abuse (e.g., ecstacy, methamphetamine etc.), mental retardation or cognitive impairment (e.g., nonsyndromic X-linked mental retardation, fragile X syndrome, Down's syndrome, autism), aphasia, Bell's palsy, Creutzfeldt-jacob disease, encephalitis, age related macular degeneration, ondine syndrome, WAGR syndrome, hearing loss, Rett syndrome, epilepsy, spinal cord injury, stroke, hypoxia, ischemia, brain injury, diabetic neuropathy, peripheral neuropathy, nerve transplantation complications, motor neuron disease, peripheral nerve injury, obesity, a metabolic syndrome, cancer, asthma, an atopic disease, inflammation, allergy, eczema, a neuro-oncological disease or disorder, neuro-immunological disease or disorder and neuro-otological disease or disorder; and a disease or disorder associated with aging and senescence.

**[0074]** The present invention provides a mechanism by which endogenous NATs suppress transcription of their sense gene counterparts. The invention provides that endogenous gene expression can be upregulated, in a locus specific manner by the removal or inhibition of the NATs, which are transcribed from most transcriptional units.

**[0075]** One embodiment of the present invention provides examples of functional ncRNAs that regulate protein output, the phenomenon applicable to many other genomic loci.

**[0076]** The Brain-derived Neurotrophic Factor (BDNF) is a member of the "neurotrophin" family of growth factors, essential for neuronal growth, maturation differentiation and maintenance. BDNF is also essential for neuronal plasticity and shown to be involved in learning, and memory processes.

The BDNF locus is on chromosome 11 and shows active transcription from both strands, which leads to transcription of a noncoding NATs.

**[0077]** The present invention characterizes the regulatory role of this antisense RNA molecule, BDNF-AS that exerts a potent reciprocal and dynamic regulation over the expression of sense BDNF mRNA and protein, both in vitro and in vivo.

**[0078]** One embodiment of the present invention provides a strategy for upregulation of mRNA expression, using antisense RNA transcript inhibitory molecules, which are termed as AntagoNATs. AntagoNATs are described, e.g., in PCT Pub. No. WO 2012/068340, incorporated herein by reference in its entirety.

**[0079]** The number of ncRNAs in eukaryotic genomes have been shown to increase as a function of developmental complexity and there is, for example, a great deal of diversity in ncRNAs expressed in the nervous system. Over the past few years, there have been reports on functional NATs and showed their potential involvement in human disorders, including Alzheimer's disease, Parkinson's diseaseand Fragile X syndrome. Moreover, it has been reported that upregulation of CD97 sense gene can be attained by knockdown of its antisense RNA transcript. Upregulation of progesterone receptor (PR), and other endogenous transcripts was reported following targeting of promoter-derived noncoding RNAs. Transcriptional activation of p21 gene and Oct4 promoter were reported following NATs depletion. Antisense RNA-induced chromatin remodeling seems to be a feasible and dynamic mode of action for many low copy number NATs. If so, antisense RNA might predominantly exert local effects to maintain or modify chromatin structure, ultimately activating or suppressing sense gene expression.

**[0080]** PCR2 is a protein complex that consists of four core subunits: Eed, Suz12, RbAp48 and the catalytic Ezh2, that catalyzes the trimethylation of histone H3-lysine. (H3K27met3). Recent studies provide evidence for direct RNA-potein interaction between Ezh2 and many ncRNA transcripts. Other studies of X inactivation and HOX gene cluster show RNA transcripts to be involved in the PRC2-mediated induction of H3K27met3, repressive chromatin marks. PRC2 transcriptome profiling has identified over 9,000 PRC2-interacting RNAs in embryonic stem cells, many of them categorized as antisense RNA transcripts. Epigenetic silencing of p15 and DM1 genes were reported to involve heterochromatin formation by its antisense RNA. The traditional binary division of chromatin into hetero- or eu-chromatin categories might not be complete as recent work has shown that there are five principal chromatin types that are more dynamic and flexible than originally believed. Likely applicable to a large number of gene loci, NATs can be manipulated in order to obtain a locus-specific alteration in chromatin modification. As examples, it is shown that cleavage (by siRNA) or inhibition (by AntagoNATs) of the antisense transcripts of BDNF genes leads to the upregulation of corresponding mRNAs.

**[0081]** Neurotrophins belong to a class of secreted growth factors that enhance the survival, development, differentiation and function of neurons and BDNF is an important molecular mediator of synaptic plasticity. BDNF is suggested to synchronize neuronal and glial maturation, participate in axonal and dendritic differentiation and protect and enhance neuronal cell survival. Neurotrophin expression levels are impaired in neurodegenerative and in psychiatric and neurodevelopmental disorders. The upregulation of neurotrophins is believed to have beneficial effects on several neurological disorders. AntagoNATs can be used as a therapeutic strategy to inhibit BDNF-AS and consequently enhance neuronal proliferation and survival in a variety of disease states. It cannot be excluded that the herein described approach to upregulate the synthesis of endogenous BDNF molecules, presumed to contain natural modifications and to represent all known splice forms, will prove to be distinct, and perhaps superior, to administrating synthetic BDNF molecules.

**[0082]** In an embodiment, modulation of BDNF by one or more antisense oligonucleotides is administered to a patient in need thereof, to prevent or treat any disease or disorder related to BDNF abnormal expression, function, activity as compared to a normal control.

**[0083]** In an embodiment, the oligonucleotides are specific for natural antisense transcripts of BDNF recited herein, which includes, without limitation noncoding regions. The BDNF targets comprise variants of BDNF; mutants of BDNF, including SNPs; noncoding sequences of BDNF; alleles, fragments and the like. Preferably the oligonucleotide is an antisense RNA molecule.

**[0084]** In accordance with embodiments of the invention, the target nucleic acid molecule is not limited to BDNF polynucleotides alone but extends to any of the isoforms, receptors, homologs, non-coding regions and the like of BDNF.

**[0085]** In an embodiment, an oligonucleotide targets a natural antisense sequence (natural antisense to the coding and non-coding regions) of BDNF targets, including, without limitation, variants, alleles, homologs, mutants, derivatives, fragments and complementary sequences thereto. Preferably the oligonucleotide is an antisense RNA or DNA molecule.

**[0086]** In an embodiment, the oligomeric compounds of the present invention also include variants in which a different base is present at one or more of the nucleotide positions in the compound. For example, if the first nucleotide is an adenine, variants may be produced which contain thymidine, guanosine, cytidine or other natural or unnatural nucleotides at this position. This may be done at any of the positions of the antisense compound. These compounds are then tested using the methods described herein to determine their ability to inhibit expression of a target nucleic acid.

**[0087]** In some embodiments, homology, sequence identity or complementarity, between the antisense compound and target is from about 50% to about 60%. In some embodiments, homology, sequence identity or complementarity, is from about 60% to about 70%. In some embodiments, homology, sequence identity or complementarity, is from about 70% to about 80%. In some

embodiments, homology, sequence identity or complementarity, is from about 80% to about 90%. In some embodiments, homology, sequence identity or complementarity, is about 90%, about 92%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100%.

**[0088]** An antisense compound is specifically hybridizable when binding of the compound to the target nucleic acid interferes with the normal function of the target nucleic acid to cause a loss of activity, and there is a sufficient degree of complementarity to avoid non-specific binding of the antisense compound to non-target nucleic acid sequences under conditions in which specific binding is desired. Such conditions include, i.e., physiological conditions in the case of in vivo assays or therapeutic treatment, and conditions in which assays are performed in the case of in vitro assays.

**[0089]** An antisense compound, whether DNA, RNA, chimeric, substituted etc, is specifically hybridizable when binding of the compound to the target DNA or RNA molecule interferes with the normal function of the target DNA or RNA to cause a loss of utility, and there is a sufficient degree of complementarily to avoid non-specific binding of the antisense compound to non-target sequences under conditions in which specific binding is desired, i.e., under physiological conditions in the case of in vivo assays or therapeutic treatment, and in the case of in vitro assays, under conditions in which the assays are performed.

**[0090]** In an embodiment, targeting of BDNF including without limitation, antisense sequences which are identified and expanded, using for example, PCR, hybridization etc., one or more of the sequences set forth as SEQ ID NOS: 3 to 11, and the like, modulate the expression or function of BDNF. In one embodiment, expression or function is up-regulated as compared to a control. In an embodiment, expression or function is down-regulated as compared to a control.

**[0091]** In an embodiment, oligonucleotides comprise nucleic acid sequences set forth as SEQ ID NOS: 12 to 49 including antisense sequences which are identified and expanded, using for example, PCR, hybridization etc. These oligonucleotides can comprise one or more modified nucleotides, shorter or longer fragments, modified bonds and the like. Examples of modified bonds or internucleotide linkages comprise phosphorothioate, phosphorodithioate or the like. In an embodiment, the nucleotides comprise a phosphorus derivative. The phosphorus derivative (or modified phosphate group) which may be attached to the sugar or sugar analog moiety in the modified oligonucleotides of the present invention may be a monophosphate, diphosphate, triphosphate, alkylphosphate, alkanephosphate, phosphorothioate and the like. The preparation of the above-noted phosphate analogs, and their incorporation into nucleotides, modified nucleotides and oligonucleotides, per se, is also known and need not be described here.

**[0092]** The specificity and sensitivity of antisense is also harnessed by those of skill in the art for therapeutic uses. Antisense oligonucleotides have been employed as therapeutic moieties in the treatment of disease states in animals and man. Antisense oligonucleotides have been safely and

effectively administered to humans and numerous clinical trials are presently underway. It is thus established that oligonucleotides can be useful therapeutic modalities that can be configured to be useful in treatment regimes for treatment of cells, tissues and animals, especially humans.

**[0093]** In embodiments of the present invention oligomeric antisense compounds, particularly oligonucleotides, bind to target nucleic acid molecules and modulate the expression and/or function of molecules encoded by a target gene. The functions of DNA to be interfered comprise, for example, replication and transcription. The functions of RNA to be interfered comprise all vital functions such as, for example, translocation of the RNA to the site of protein translation, translation of protein from the RNA, splicing of the RNA to yield one or more mRNA species, and catalytic activity which may be engaged in or facilitated by the RNA. The functions may be up-regulated or inhibited depending on the functions desired.

**[0094]** The antisense compounds, include, antisense oligomeric compounds, antisense oligonucleotides, external guide sequence (EGS) oligonucleotides, alternate splicers, primers, probes, and other oligomeric compounds that hybridize to at least a portion of the target nucleic acid. As such, these compounds may be introduced in the form of single-stranded, double-stranded, partially single-stranded, or circular oligomeric compounds.

**[0095]** Targeting an antisense compound to a particular nucleic acid molecule, in the context of this invention, can be a multistep process. The process usually begins with the identification of a target nucleic acid whose function is to be modulated. This target nucleic acid may be, for example, a cellular gene (or mRNA transcribed from the gene) whose expression is associated with a particular disorder or disease state, or a nucleic acid molecule from an infectious agent. In the present invention, the target nucleic acid encodes Brain derived neurotrophic factor (BDNF).

**[0096]** The targeting process usually also includes determination of at least one target region, segment, or site within the target nucleic acid for the antisense interaction to occur such that the desired effect, e.g., modulation of expression, will result. Within the context of the present invention, the term "region" is defined as a portion of the target nucleic acid having at least one identifiable structure, function, or characteristic. Within regions of target nucleic acids are segments. "Segments" are defined as smaller or sub-portions of regions within a target nucleic acid. "Sites," as used in the present invention, are defined as positions within a target nucleic acid.

**[0097]** In an embodiment, the antisense oligonucleotides bind to the natural antisense sequences of Brain derived neurotrophic factor (BDNF) and modulate the expression and/or function of BDNF (SEQ ID NO: 1 and 2). Examples of antisense sequences include SEQ ID NOS: 3 to 55.

**[0098]** In an embodiment, the antisense oligonucleotides bind to one or more segments of Brain derived neurotrophic factor (BDNF) polynucleotides and modulate the expression and/or function of

BDNF. The segments comprise at least five consecutive nucleotides of the BDNF sense or antisense polynucleotides.

**[0099]** In an embodiment, the antisense oligonucleotides are specific for natural antisense sequences of BDNF wherein binding of the oligonucleotides to the natural antisense sequences of BDNF modulate expression and/or function of BDNF.

**[00100]** In an embodiment, oligonucleotide compounds comprise sequences set forth as SEQ ID NOS: 12 to 49, antisense sequences which are identified and expanded, using for example, PCR, hybridization etc These oligonucleotides can comprise one or more modified nucleotides, shorter or longer fragments, modified bonds and the like. Examples of modified bonds or internucleotide linkages comprise phosphorothioate, phosphorodithioate or the like. In an embodiment, the nucleotides comprise a phosphorus derivative. The phosphorus derivative (or modified phosphate group) which may be attached to the sugar or sugar analog moiety in the modified oligonucleotides of the present invention may be a monophosphate, diphosphate, triphosphate, alkylphosphate, alkanephosphate, phosphorothioate and the like. The preparation of the above-noted phosphate analogs, and their incorporation into nucleotides, modified nucleotides and oligonucleotides, per se, is also known and need not be described here.

**[00101]** Since, as is known in the art, the translation initiation codon is typically 5'-AUG (in transcribed mRNA molecules; 5'-ATG in the corresponding DNA molecule), the translation initiation codon is also referred to as the "AUG codon," the "start codon" or the "AUG start codon". A minority of genes has a translation initiation codon having the RNA sequence 5'-GUG, 5'-UUG or 5'-CUG; and 5'-AUA, 5'-ACG and 5'-CUG have been shown to function in vivo. Thus, the terms "translation initiation codon" and "start codon" can encompass many codon sequences, even though the initiator amino acid in each instance is typically methionine (in eukaryotes) or formyl methionine (in prokaryotes). Eukaryotic and prokaryotic genes may have two or more alternative start codons, any one of which may be preferentially utilized for translation initiation in a particular cell type or tissue, or under a particular set of conditions. In the context of the invention, "start codon" and "translation initiation codon" refer to the codon or codons that are used in vivo to initiate translation of an mRNA transcribed from a gene encoding Brain derived neurotrophic factor (BDNF), regardless of the sequence(s) of such codons. A translation termination codon (or "stop codon") of a gene may have one of three sequences, i.e., 5'-UAA, 5'-UAG and 5'-UGA (the corresponding DNA sequences are 5'-TAA, 5'- TAG and 5'-TGA, respectively).

**[00102]** The terms "start codon region" and "translation initiation codon region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation initiation codon. Similarly, the terms "stop codon region" and "translation termination codon region" refer to a portion of such an mRNA

or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation termination codon. Consequently, the "start codon region" (or "translation initiation codon region") and the "stop codon region" (or "translation termination codon region") are all regions that may be targeted effectively with the antisense compounds of the present invention.

**[00103]** The open reading frame (ORF) or "coding region," which is known in the art to refer to the region between the translation initiation codon and the translation termination codon, is also a region which may be targeted effectively. Within the context of the present invention, a targeted region is the intragenic region encompassing the translation initiation or termination codon of the open reading frame (ORF) of a gene.

**[00104]** Another target region includes the 5' untranslated region (5'UTR), known in the art to refer to the portion of an mRNA in the 5' direction from the translation initiation codon, and thus including nucleotides between the 5' cap site and the translation initiation codon of an mRNA (or corresponding nucleotides on the gene). Still another target region includes the 3' untranslated region (3'UTR), known in the art to refer to the portion of an mRNA in the 3' direction from the translation termination codon, and thus including nucleotides between the translation termination codon and 3' end of an mRNA (or corresponding nucleotides on the gene). The 5' cap site of an mRNA comprises an N7-methylated guanosine residue joined to the 5'-most residue of the mRNA via a 5'-5' triphosphate linkage. The 5' cap region of an mRNA is considered to include the 5' cap structure itself as well as the first 50 nucleotides adjacent to the cap site. Another target region for this invention is the 5' cap region.

**[00105]** Although some eukaryotic mRNA transcripts are directly translated, many contain one or more regions, known as "introns," which are excised from a transcript before it is translated. The remaining (and therefore translated) regions are known as "exons" and are spliced together to form a continuous mRNA sequence. In one embodiment, targeting splice sites, i.e., intron-exon junctions or exon-intron junctions, is particularly useful in situations where aberrant splicing is implicated in disease, or where an overproduction of a particular splice product is implicated in disease. An aberrant fusion junction due to rearrangement or deletion is another embodiment of a target site. mRNA transcripts produced via the process of splicing of two (or more) mRNAs from different gene sources are known as "fusion transcripts". Introns can be effectively targeted using antisense compounds targeted to, for example, DNA or pre-mRNA.

**[00106]** In an embodiment, the antisense oligonucleotides bind to coding and/or non-coding regions of a target polynucleotide and modulate the expression and/or function of the target molecule.

**[00107]** In an embodiment, the antisense oligonucleotides bind to natural antisense polynucleotides and modulate the expression and/or function of the target molecule.

**[00108]** In an embodiment, the antisense oligonucleotides bind to sense polynucleotides and modulate the expression and/or function of the target molecule.

**[00109]** Alternative RNA transcripts can be produced from the same genomic region of DNA. These alternative transcripts are generally known as "variants". More specifically, "pre-mRNA variants" are transcripts produced from the same genomic DNA that differ from other transcripts produced from the same genomic DNA in either their start or stop position and contain both intronic and exonic sequence.

**[00110]** Upon excision of one or more exon or intron regions, or portions thereof during splicing, pre-mRNA variants produce smaller "mRNA variants". Consequently, mRNA variants are processed pre-mRNA variants and each unique pre-mRNA variant must always produce a unique mRNA variant as a result of splicing. These mRNA variants are also known as "alternative splice variants". If no splicing of the pre-mRNA variant occurs then the pre-mRNA variant is identical to the mRNA variant.

**[00111]** Variants can be produced through the use of alternative signals to start or stop transcription. Pre-mRNAs and mRNAs can possess more than one start codon or stop codon. Variants that originate from a pre-mRNA or mRNA that use alternative start codons are known as "alternative start variants" of that pre-mRNA or mRNA. Those transcripts that use an alternative stop codon are known as "alternative stop variants" of that pre-mRNA or mRNA. One specific type of alternative stop variant is the "polyA variant" in which the multiple transcripts produced result from the alternative selection of one of the "polyA stop signals" by the transcription machinery, thereby producing transcripts that terminate at unique polyA sites. Within the context of the invention, the types of variants described herein are also embodiments of target nucleic acids.

**[00112]** The locations on the target nucleic acid to which the antisense compounds hybridize are defined as at least a 5-nucleotide long portion of a target region to which an active antisense compound is targeted.

**[00113]** While the specific sequences of certain exemplary target segments are set forth herein, one of skill in the art will recognize that these serve to illustrate and describe particular embodiments within the scope of the present invention. Additional target segments are readily identifiable by one having ordinary skill in the art in view of this disclosure.

**[00114]** Target segments 5-100 nucleotides in length comprising a stretch of at least five (5) consecutive nucleotides selected from within the illustrative preferred target segments are considered to be suitable for targeting as well.

**[00115]** Target segments can include DNA or RNA sequences that comprise at least the 5 consecutive nucleotides from the 5'-terminus of one of the illustrative preferred target segments (the remaining nucleotides being a consecutive stretch of the same DNA or RNA beginning immediately

upstream of the 5'-terminus of the target segment and continuing until the DNA or RNA contains about 5 to about 100 nucleotides). Similarly preferred target segments are represented by DNA or RNA sequences that comprise at least the 5 consecutive nucleotides from the 3'-terminus of one of the illustrative preferred target segments (the remaining nucleotides being a consecutive stretch of the same DNA or RNA beginning immediately downstream of the 3'-terminus of the target segment and continuing until the DNA or RNA contains about 5 to about 100 nucleotides). One having skill in the art armed with the target segments illustrated herein will be able, without undue experimentation, to identify further preferred target segments.

**[00116]** Once one or more target regions, segments or sites have been identified, antisense compounds are chosen which are sufficiently complementary to the target, i.e., hybridize sufficiently well and with sufficient specificity, to give the desired effect.

**[00117]** In embodiments of the invention the oligonucleotides bind to an antisense strand of a particular target. The oligonucleotides are at least 5 nucleotides in length and can be synthesized so each oligonucleotide targets overlapping sequences such that oligonucleotides are synthesized to cover the entire length of the target polynucleotide. The targets also include coding as well as non coding regions.

**[00118]** In one embodiment, it is preferred to target specific nucleic acids by antisense oligonucleotides. Targeting an antisense compound to a particular nucleic acid is a multistep process. The process usually begins with the identification of a nucleic acid sequence whose function is to be modulated. This may be, for example, a cellular gene (or mRNA transcribed from the gene) whose expression is associated with a particular disorder or disease state, or a non coding polynucleotide such as for example, non coding RNA (ncRNA).

**[00119]** RNAs can be classified into (1) messenger RNAs (mRNAs), which are translated into proteins, and (2) non-protein-coding RNAs (ncRNAs). ncRNAs comprise microRNAs, antisense transcripts and other Transcriptional Units (TU) containing a high density of stop codons and lacking any extensive "Open Reading Frame". Many ncRNAs appear to start from initiation sites in 3' untranslated regions (3'UTRs) of protein-coding loci. ncRNAs are often rare and at least half of the ncRNAs that have been sequenced by the FANTOM consortium seem not to be polyadenylated. Most researchers have for obvious reasons focused on polyadenylated mRNAs that are processed and exported to the cytoplasm. Recently, it was shown that the set of non-polyadenylated nuclear RNAs may be very large, and that many such transcripts arise from so-called intergenic regions. The mechanism by which ncRNAs may regulate gene expression is by base pairing with target transcripts. The RNAs that function by base pairing can be grouped into (1) cis encoded RNAs that are encoded at the same genetic location, but on the opposite strand to the RNAs they act upon and therefore display perfect complementarity to their target, and (2) trans-encoded RNAs that are

encoded at a chromosomal location distinct from the RNAs they act upon and generally do not exhibit perfect base-pairing potential with their targets.

**[00120]** Without wishing to be bound by theory, perturbation of an antisense polynucleotide by the antisense oligonucleotides described herein can alter the expression of the corresponding sense messenger RNAs. However, this regulation can either be discordant (antisense knockdown results in messenger RNA elevation) or concordant (antisense knockdown results in concomitant messenger RNA reduction). In these cases, antisense oligonucleotides can be targeted to overlapping or non-overlapping parts of the antisense transcript resulting in its knockdown or sequestration. Coding as well as non-coding antisense can be targeted in an identical manner and that either category is capable of regulating the corresponding sense transcripts – either in a concordant or disconcordant manner. The strategies that are employed in identifying new oligonucleotides for use against a target can be based on the knockdown of antisense RNA transcripts by antisense oligonucleotides or any other means of modulating the desired target.

**[00121]** *Strategy 1*: In the case of discordant regulation, knocking down the antisense transcript elevates the expression of the conventional (sense) gene. Should that latter gene encode for a known or putative drug target, then knockdown of its antisense counterpart could conceivably mimic the action of a receptor agonist or an enzyme stimulant.

**[00122]** *Strategy 2*: In the case of concordant regulation, one could concomitantly knock down both antisense and sense transcripts and thereby achieve synergistic reduction of the conventional (sense) gene expression. If, for example, an antisense oligonucleotide is used to achieve knockdown, then this strategy can be used to apply one antisense oligonucleotide targeted to the sense transcript and another antisense oligonucleotide to the corresponding antisense transcript, or a single energetically symmetric antisense oligonucleotide that simultaneously targets overlapping sense and antisense transcripts.

**[00123]** According to the present invention, antisense compounds include antisense oligonucleotides, ribozymes, external guide sequence (EGS) oligonucleotides, siRNA compounds, single- or double-stranded RNA interference (RNAi) compounds such as siRNA compounds, and other oligomeric compounds which hybridize to at least a portion of the target nucleic acid and modulate its function. As such, they may be DNA, RNA, DNA-like, RNA-like, or mixtures thereof, or may be mimetics of one or more of these. These compounds may be single-stranded, doublestranded, circular or hairpin oligomeric compounds and may contain structural elements such as internal or terminal bulges, mismatches or loops. Antisense compounds are routinely prepared linearly but can be joined or otherwise prepared to be circular and/or branched. Antisense compounds can include constructs such as, for example, two strands hybridized to form a wholly or partially double-stranded compound or a single strand with sufficient self-complementarity to allow

for hybridization and formation of a fully or partially double-stranded compound. The two strands can be linked internally leaving free 3' or 5' termini or can be linked to form a continuous hairpin structure or loop. The hairpin structure may contain an overhang on either the 5' or 3' terminus producing an extension of single stranded character. The double stranded compounds optionally can include overhangs on the ends. Further modifications can include conjugate groups attached to one of the termini, selected nucleotide positions, sugar positions or to one of the internucleoside linkages. Alternatively, the two strands can be linked via a non-nucleic acid moiety or linker group. When formed from only one strand, dsRNA can take the form of a self-complementary hairpin-type molecule that doubles back on itself to form a duplex. Thus, the dsRNAs can be fully or partially double stranded. Specific modulation of gene expression can be achieved by stable expression of dsRNA hairpins in transgenic cell lines, however, in some embodiments, the gene expression or function is up regulated. When formed from two strands, or a single strand that takes the form of a self-complementary hairpin-type molecule doubled back on itself to form a duplex, the two strands (or duplex-forming regions of a single strand) are complementary RNA strands that base pair in Watson-Crick fashion.

**[00124]** Once introduced to a system, the compounds of the invention may elicit the action of one or more enzymes or structural proteins to effect cleavage or other modification of the target nucleic acid or may work via occupancy-based mechanisms. In general, nucleic acids (including oligonucleotides) may be described as "DNA-like" (i.e., generally having one or more 2'-deoxy sugars and, generally, T rather than U bases) or "RNA-like" (i.e., generally having one or more 2'-hydroxyl or 2'-modified sugars and, generally U rather than T bases). Nucleic acid helices can adopt more than one type of structure, most commonly the A- and B-forms. It is believed that, in general, oligonucleotides which have B-form-like structure are "DNA-like" and those which have A-formlike structure are "RNA-like." In some (chimeric) embodiments, an antisense compound may contain both A- and B-form regions.

**[00125]** In an embodiment, the desired oligonucleotides or antisense compounds, comprise at least one of: antisense RNA, antisense DNA, chimeric antisense oligonucleotides, antisense oligonucleotides comprising modified linkages, interference RNA (RNAi), short interfering RNA (siRNA); a micro, interfering RNA (miRNA); a small, temporal RNA (stRNA); or a short, hairpin RNA (shRNA); small RNA-induced gene activation (RNAa); small activating RNAs (saRNAs), or combinations thereof.

**[00126]** dsRNA can also activate gene expression, a mechanism that has been termed "small RNA-induced gene activation" or RNAa. dsRNAs targeting gene promoters induce potent transcriptional activation of associated genes. RNAa was demonstrated in human cells using synthetic dsRNAs,

termed "small activating RNAs" (saRNAs). It is currently not known whether RNAa is conserved in other organisms.

**[00127]** Small double-stranded RNA (dsRNA), such as small interfering RNA (siRNA) and microRNA (miRNA), have been found to be the trigger of an evolutionary conserved mechanism known as RNA interference (RNAi). RNAi invariably leads to gene silencing via remodeling chromatin to thereby suppress transcription, degrading complementary mRNA, or blocking protein translation. However, in instances described in detail in the examples section which follows, oligonucleotides are shown to increase the expression and/or function of the Brain derived neurotrophic factor (BDNF) polynucleotides and encoded products thereof. dsRNAs may also act as small activating RNAs (saRNA). Without wishing to be bound by theory, by targeting sequences in gene promoters, saRNAs would induce target gene expression in a phenomenon referred to as dsRNA-induced transcriptional activation (RNAa).

**[00128]** In a further embodiment, the "preferred target segments" identified herein may be employed in a screen for additional compounds that modulate the expression of Brain derived neurotrophic factor (BDNF) polynucleotides. "Modulators" are those compounds that decrease or increase the expression of a nucleic acid molecule encoding BDNF and which comprise at least a 5-nucleotide portion that is complementary to a preferred target segment. The screening method comprises the steps of contacting a preferred target segment of a nucleic acid molecule encoding sense or natural antisense polynucleotides of BDNF with one or more candidate modulators, and selecting for one or more candidate modulators which decrease or increase the expression of a nucleic acid molecule encoding BDNF polynucleotides, e.g. SEQ ID NOS: 12 to 49. Once it is shown that the candidate modulator or modulators are capable of modulating (e.g. either decreasing or increasing) the expression of a nucleic acid molecule encoding BDNF polynucleotides, the modulator may then be employed in further investigative studies of the function of BDNF polynucleotides, or for use as a research, diagnostic, or therapeutic agent in accordance with the present invention.

**[00129]** Targeting the natural antisense sequence preferably modulates the function of the target gene. For example, the BDNF gene (e.g. accession number NM_170735 and NM_007540). In an embodiment, the target is an antisense polynucleotide of the BDNF gene. In an embodiment, an antisense oligonucleotide targets sense and/or natural antisense sequences of BDNF polynucleotides (e.g. accession number NM_170735 and NM_007540), variants, alleles, isoforms, homologs, mutants, derivatives, fragments and complementary sequences thereto. Preferably the oligonucleotide is an antisense molecule and the targets include coding and noncoding regions of antisense and/or sense BDNF polynucleotides.

**[00130]** The preferred target segments of the present invention may be also be combined with their respective complementary antisense compounds of the present invention to form stabilized double-stranded (duplexed) oligonucleotides.

**[00131]** Such double stranded oligonucleotide moieties have been shown in the art to modulate target expression and regulate translation as well as RNA processing via an antisense mechanism. Moreover, the double-stranded moieties may be subject to chemical modifications. For example, such double-stranded moieties have been shown to inhibit the target by the classical hybridization of antisense strand of the duplex to the target, thereby triggering enzymatic degradation of the target.

**[00132]** In an embodiment, an antisense oligonucleotide targets Brain derived neurotrophic factor (BDNF) polynucleotides (e.g. accession number NM_170735 and NM_007540), variants, alleles, homologs, mutants, derivatives, fragments and complementary sequences thereto. Preferably the oligonucleotide is an antisense molecule.

**[00133]** In accordance with embodiments of the invention, the target nucleic acid molecule is not limited to BDNF alone but extends to any polynucleotide variant thereof and any polynucleotide that produces, affects, impacts or results in or relates to a BDNF expression product and/or any isoforms thereof.

**[00134]** In an embodiment, an oligonucleotide targets a natural antisense sequence of BDNF polynucleotides, for example, polynucleotides set forth as SEQ ID NOS: 3 to 11, and any variants, alleles, homologs, mutants, derivatives, fragments and complementary sequences thereto. Examples of antisense oligonucleotides are set forth as SEQ ID NOS: 12 to 49.

**[00135]** In one embodiment, the oligonucleotides are complementary to or bind to nucleic acid sequences of BDNF antisense, including without limitation noncoding sense and/or antisense sequences associated with BDNF polynucleotides and modulate expression and/or function of BDNF molecules.

**[00136]** In an embodiment, the oligonucleotides are complementary to or bind to nucleic acid sequences of BDNF natural antisense, set forth as SEQ ID NOS: 3 to 11, and modulate expression and/or function of BDNF molecules.

**[00137]** In an embodiment, oligonucleotides comprise sequences of at least 5 consecutive nucleotides of SEQ ID NOS: 12 to 49 and modulate expression and/or function of BDNF molecules.

**[00138]** The polynucleotide targets comprise BDNF, including family members thereof, variants of BDNF; mutants of BDNF, including SNPs; noncoding sequences of BDNF; alleles of BDNF; species variants, fragments and the like. Preferably the oligonucleotide is an antisense molecule.

**[00139]** In an embodiment, the oligonucleotide targeting BDNF polynucleotides, comprise: antisense RNA, interference RNA (RNAi), short interfering RNA (siRNA); micro interfering RNA

(miRNA); a small, temporal RNA (stRNA); or a short, hairpin RNA (shRNA); small RNA-induced gene activation (RNAa); or, small activating RNA (saRNA).

**[00140]** In an embodiment, targeting of Brain derived neurotrophic factor (BDNF) polynucleotides, e.g. SEQ ID NOS: 3 to 55 modulate the expression or function of these targets. In one embodiment, expression or function is up-regulated as compared to a control. In an embodiment, expression or function is down-regulated as compared to a control.

**[00141]** In an embodiment, antisense compounds comprise sequences set forth as SEQ ID NOS: 12 to 49. These oligonucleotides can comprise one or more modified nucleotides, shorter or longer fragments, modified bonds and the like.

**[00142]** In an embodiment, SEQ ID NOS: 12 to 49 comprise one or more LNA nucleotides. Table 1 shows exemplary antisense oligonucleotides useful in the methods of the invention.

***Table 1:***

| Sequence ID | Antisense Sequence Name | Sequence |
|---|---|---|
| SEQ ID NO:12 | CUR-2046 (Antisense) | ArArCrArArArCrArArCrUrGrGrUrGrArGrCrCrUrGrG |
| SEQ ID NO:13 | CUR-2047 Antisense) | rUrGrArGrCrCrUrArArGrArUrArCrArUrUrGrCrUrCrU |
| SEQ ID NO:14 | CUR-2048 (Antisense) | rGrUrGrCrUrGrUrUrGrUrArArGrArUrUrArGrCrCrArC |
| SEQ ID NO:15 | CUR-2049 (Antisense) | rArArUrGrArCrArUrGrUrUrUrGrUrArGrGrGrArGrCrC |
| SEQ ID NO:16 | CUR-2050 | +C*mC*mA*+G*mG*mU*+G*mU*mG*mC*+G*mG*mA*+C |
| SEQ ID NO:17 | CUR-2051 | +C*mC*mA*+U*mG*mG*+G*mA*mC*mU*+C*mU*mG*+G |
| SEQ ID NO:18 | CUR-2052 | +A*mG*mA*+G*mC*mG*+U*mG*mA*mA*+U*mG*mG*+G |
| SEQ ID NO:19 | CUR-2053 | +C*mC*mC*+A*mA*mG*+G*mC*mA*mG*+G*mU*mU*+C |
| SEQ ID NO:20 | CUR-2054 | +A*mA*mG*+A*mU*mG*+C*mU*mU*mG*+A*mC*mA*+U |
| SEQ ID NO:21 | CUR-2055 | +C*mA*mU*+U*mG*mG*+C*mU*mG*mA*+C*mA*mC*+U |
| SEQ ID NO:22 | CUR-2056 | +U*mU*mC*+G*mA*mA*+C*mA*mC*mG*+U*mG*mA*+U |
| SEQ ID NO:23 | CUR-2057 | +A*mG*mA*+A*mG*mA*+G*mC*mU*mG*+U*mU*mG*+G |
| SEQ ID NO:24 | CUR-2058 | +A*mU*mG*+A*mG*mG*+A*mC*mC*mA*+G*mA*mA*+A |

| | | |
|---|---|---|
| SEQ ID NO:25 | CUR-2059 | +G*mU*mU*+C*mG*mG*+C*mC*mC*mA*+A*mU*mG*+A |
| SEQ ID NO:26 | CUR-2060 | +A*mG*mA*+A*mA*mA*+C*mA*mA*mU*+A*mA*mG*+G |
| SEQ ID NO:27 | CUR-2061 | +A*mC*mG*+C*mA*mG*+A*mC*mU*mU*+G*mU*mA*+C |
| SEQ ID NO:28 | CUR-2062 | +A*mC*mG*+U*mC*mC*+A*mG*mG*mG*+U*mG*mA*+U |
| SEQ ID NO:29 | CUR-2063 | +G*mC*mU*+C*mA*mG*+U*mA*mG*mU*+C*mA*mA*+G |
| SEQ ID NO:30 | CUR-2064 | +U*mG*mC*+C*mU*mU*+U*mG*mG*mA*+G*mC*mC*+U |
| SEQ ID NO:31 | CUR-2065 | +C*mC*mU*+C*mU*mU*+C*mU*mC*mU*+U*mU*mC*+U |
| SEQ ID NO:32 | CUR-2066 | +C*+C*+C*G*G*T*A*T*C*C*A*A*A*+G*+G*+C |
| SEQ ID NO:33 | CUR-2067 | +G*+T*+A*T*T*A*G*C*G*A*G*T*G*+G*+G*+T |
| SEQ ID NO:34 | CUR-2068 | +G*+T*+C*T*A*T*G*A*G*G*G*T*T*+C*+G*+G |
| SEQ ID NO:35 | CUR-2069 | +C*+C*+T*C*C*T*C*T*A*C*T*C*T*+T*+T*+C |
| SEQ ID NO:36 | CUR-2070 | +G*+G*+C*A*G*G*T*T*C*G*A*G*A*+G*+G*+T |
| SEQ ID NO:37 | CUR-2071 | +T*+T*+C*C*T*T*C*C*C*A*C*A*G*+T*+T*+C |
| SEQ ID NO:38 | CUR-2072 | +C*+G*+G*T*T*G*C*A*T*G*A*A*G*+G*+C*+G |
| SEQ ID NO:39 | CUR-2073 | +T*+G*+G*C*T*G*G*C*G*A*T*T*C*+A*+T*+A |
| SEQ ID NO:40 | CUR-2074 | +C*+A*+A*C*A*T*A*T*C*A*G*G*A*+G*+C*+C |
| SEQ ID NO:41 | CUR-2075 | +T*+G*+T*A*T*T*C*C*C*A*G*A*A*+C*+T*+T |
| SEQ ID NO:42 | CUR-2076 (Antisense) | rUrArUrGrGrUrUrArUrUrUrCrArUrArCrUrUrCrGrGrUrUrGrCrArUrG |
| SEQ ID NO:43 | CUR-2077 (Antisense) | rArGrArArGrUrArArArCrGrUrCrCrArCrGrGrArCrArArGrGrCrArArC |
| SEQ ID NO:44 | CUR-2078 (Antisense) | rArUrUrUrCrUrArCrGrArGrArCrCrArArGrUrGrUrArArUrCrCrCrArU |
| SEQ ID NO:45 | CUR-2079 (Antisense) | rUrArArGrGrArCrGrCrGrGrArCrUrUrGrUrArCrArCrUrUrCrCrGrGrG |
| SEQ ID NO:46 | CUR-2080 (Antisense) | rArGrArArArGrArArArArGrUrUrCrUrArArCrCrUrGrUrUrCrUrGrUrGrU |

| | | |
|---|---|---|
| SEQ ID NO:47 | CUR-2081 | +G*+A*+T*T*T*C*A*G*A*G*C*C*G*+C*+A*+G |
| SEQ ID NO:48 | CUR-2082 | +G*+A*+C*A*C*A*T*C*C*A*T*C*C*+C*+A*+G |
| SEQ ID NO:49 | CUR-2083 | +C*+C*+T*C*G*T*C*A*T*G*T*C*T*+G*+T*+G |
| SEQ ID NO:50 | CUR-0071 | C*+T*+T*G*A*A*T*T*G*T*T*T*+G*+T*+A |
| SEQ ID NO:51 | CUR-0072 | A*+G*+T*T*G*C*A*A*G*A*G*T*+T*+G*+G |
| SEQ ID NO:52 | CUR-0073 | A*+T*+C*T*G*T*T*C*T*G*C*T*+G*+T*+C |
| SEQ ID NO:53 | CUR-0074 | C*+A*+T*A*T*T*C*T*T*G*G*A*+C*+G*+A |
| SEQ ID NO:54 | CUR-0075 | T*+G*+T*G*C*T*G*T*T*G*T*A*+A*+G*+A |
| SEQ ID NO:55 | CUR-0076 | T*+G*+A*C*A*G*A*G*G*A*G*T*+A*+T*+T |

**[00143]** The modulation of a desired target nucleic acid can be carried out in several ways known in the art. For example, antisense oligonucleotides, siRNA etc. Enzymatic nucleic acid molecules (e.g., ribozymes) are nucleic acid molecules capable of catalyzing one or more of a variety of reactions, including the ability to repeatedly cleave other separate nucleic acid molecules in a nucleotide base sequence-specific manner. Such enzymatic nucleic acid molecules can be used, for example, to target virtually any RNA transcript.

**[00144]** Because of their sequence-specificity, trans-cleaving enzymatic nucleic acid molecules show promise as therapeutic agents for human disease. Enzymatic nucleic acid molecules can be designed to cleave specific RNA targets within the background of cellular RNA. Such a cleavage event renders the mRNA non-functional and abrogates protein expression from that RNA. In this manner, synthesis of a protein associated with a disease state can be selectively inhibited.

**[00145]** In general, enzymatic nucleic acids with RNA cleaving activity act by first binding to a target RNA. Such binding occurs through the target binding portion of an enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through complementary base pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct synthesis of an encoded protein. After an enzymatic nucleic acid has bound and cleaved its RNA target, it is released from that RNA to search for another target and can repeatedly bind and cleave new targets.

**[00146]** Several approaches such as in vitro selection (evolution) strategies (Orgel, (1979) Proc. R. Soc. London, B 205, 435) have been used to evolve new nucleic acid catalysts capable of catalyzing a variety of reactions, such as cleavage and ligation of phosphodiester linkages and amide linkages.

**[00147]** The development of ribozymes that are optimal for catalytic activity would contribute significantly to any strategy that employs RNA-cleaving ribozymes for the purpose of regulating gene expression. The hammerhead ribozyme, for example, functions with a catalytic rate (kcat) of about 1 min-1 in the presence of saturating (10 mM) concentrations of Mg2+ cofactor. An artificial "RNA ligase" ribozyme has been shown to catalyze the corresponding self-modification reaction with a rate of about 100 min-1. In addition, it is known that certain modified hammerhead ribozymes that have substrate binding arms made of DNA catalyze RNA cleavage with multiple turn-over rates that approach 100 min-1. Finally, replacement of a specific residue within the catalytic core of the hammerhead with certain nucleotide analogues gives modified ribozymes that show as much as a 10-fold improvement in catalytic rate. These findings demonstrate that ribozymes can promote chemical transformations with catalytic rates that are significantly greater than those displayed in vitro by most natural self-cleaving ribozymes. It is then possible that the structures of certain selfcleaving ribozymes may be optimized to give maximal catalytic activity, or that entirely new RNA motifs can be made that display significantly faster rates for RNA phosphodiester cleavage.

**[00148]** Intermolecular cleavage of an RNA substrate by an RNA catalyst that fits the "hammerhead" model was first shown in 1987 (Uhlenbeck, O. C. (1987) Nature, 328: 596-600). The RNA catalyst was recovered and reacted with multiple RNA molecules, demonstrating that it was truly catalytic.

**[00149]** Catalytic RNAs designed based on the "hammerhead" motif have been used to cleave specific target sequences by making appropriate base changes in the catalytic RNA to maintain necessary base pairing with the target sequences. This has allowed use of the catalytic RNA to cleave specific target sequences and indicates that catalytic RNAs designed according to the "hammerhead" model may possibly cleave specific substrate RNAs in vivo.

**[00150]** RNA interference (RNAi) has become a powerful tool for modulating gene expression in mammals and mammalian cells. This approach requires the delivery of small interfering RNA (siRNA) either as RNA itself or as DNA, using an expression plasmid or virus and the coding sequence for small hairpin RNAs that are processed to siRNAs. This system enables efficient transport of the pre-siRNAs to the cytoplasm where they are active and permit the use of regulated and tissue specific promoters for gene expression.

**[00151]** In an embodiment, an oligonucleotide or antisense compound comprises an oligomer or polymer of ribonucleic acid (RNA) and/or deoxyribonucleic acid (DNA), or a mimetic, chimera, analog or homolog thereof. This term includes oligonucleotides composed of naturally occurring

nucleotides, sugars and covalent internucleoside (backbone) linkages as well as oligonucleotides having non-naturally occurring portions which function similarly. Such modified or substituted oligonucleotides are often desired over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for a target nucleic acid and increased stability in the presence of nucleases.

**[00152]** According to the present invention, the oligonucleotides or "antisense compounds" include antisense oligonucleotides (e.g. RNA, DNA, mimetic, chimera, analog or homolog thereof), ribozymes, external guide sequence (EGS) oligonucleotides, siRNA compounds, single- or double-stranded RNA interference (RNAi) compounds such as siRNA compounds, saRNA, aRNA, and other oligomeric compounds which hybridize to at least a portion of the target nucleic acid and modulate its function. As such, they may be DNA, RNA, DNA-like, RNA-like, or mixtures thereof, or may be mimetics of one or more of these. These compounds may be single-stranded, double-stranded, circular or hairpin oligomeric compounds and may contain structural elements such as internal or terminal bulges, mismatches or loops. Antisense compounds are routinely prepared linearly but can be joined or otherwise prepared to be circular and/or branched. Antisense compounds can include constructs such as, for example, two strands hybridized to form a wholly or partially double-stranded compound or a single strand with sufficient self-complementarity to allow for hybridization and formation of a fully or partially double-stranded compound. The two strands can be linked internally leaving free 3' or 5' termini or can be linked to form a continuous hairpin structure or loop. The hairpin structure may contain an overhang on either the 5' or 3' terminus producing an extension of single stranded character. The double stranded compounds optionally can include overhangs on the ends. Further modifications can include conjugate groups attached to one of the termini, selected nucleotide positions, sugar positions or to one of the internucleoside linkages. Alternatively, the two strands can be linked via a non-nucleic acid moiety or linker group. When formed from only one strand, dsRNA can take the form of a self-complementary hairpin-type molecule that doubles back on itself to form a duplex. Thus, the dsRNAs can be fully or partially double stranded. Specific modulation of gene expression can be achieved by stable expression of dsRNA hairpins in transgenic cell lines. When formed from two strands, or a single strand that takes the form of a self-complementary hairpin-type molecule doubled back on itself to form a duplex, the two strands (or duplex-forming regions of a single strand) are complementary RNA strands that base pair in Watson-Crick fashion.

**[00153]** Once introduced to a system, the compounds of the invention may elicit the action of one or more enzymes or structural proteins to effect cleavage or other modification of the target nucleic acid or may work via occupancy-based mechanisms. In general, nucleic acids (including oligonucleotides) may be described as "DNA-like" (i.e., generally having one or more 2'-deoxy

sugars and, generally, T rather than U bases) or "RNA-like" (i.e., generally having one or more 2'-hydroxyl or 2'-modified sugars and, generally U rather than T bases). Nucleic acid helices can adopt more than one type of structure, most commonly the A- and B-forms. It is believed that, in general, oligonucleotides which have B-form-like structure are "DNA-like" and those which have A-formlike structure are "RNA-like." In some (chimeric) embodiments, an antisense compound may contain both A- and B-form regions.

**[00154]** The antisense compounds in accordance with this invention can comprise an antisense portion from about 5 to about 80 nucleotides (i.e. from about 5 to about 80 linked nucleosides) in length. This refers to the length of the antisense strand or portion of the antisense compound. In other words, a single-stranded antisense compound of the invention comprises from 5 to about 80 nucleotides, and a double-stranded antisense compound of the invention (such as a dsRNA, for example) comprises a sense and an antisense strand or portion of 5 to about 80 nucleotides in length. One of ordinary skill in the art will appreciate that this comprehends antisense portions of 5, 6, 7,8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80 nucleotides in length, or any range therewithin.

**[00155]** In one embodiment, the antisense compounds of the invention have antisense portions of 10 to 50 nucleotides in length. One having ordinary skill in the art will appreciate that this embodies oligonucleotides having antisense portions of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 nucleotides in length, or any range therewithin. In some embodiments, the oligonucleotides are 15 nucleotides in length.

**[00156]** In one embodiment, the antisense or oligonucleotide compounds of the invention have antisense portions of 12 or 13 to 30 nucleotides in length. One having ordinary skill in the art will appreciate that this embodies antisense compounds having antisense portions of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 nucleotides in length, or any range therewithin.

**[00157]** In an embodiment, the oligomeric compounds of the present invention also include variants in which a different base is present at one or more of the nucleotide positions in the compound. For example, if the first nucleotide is an adenosine, variants may be produced which contain thymidine, guanosine or cytidine at this position. This may be done at any of the positions of the antisense or dsRNA compounds. These compounds are then tested using the methods described herein to determine their ability to inhibit expression of a target nucleic acid.

**[00158]** In some embodiments, homology, sequence identity or complementarity, between the antisense compound and target is from about 40% to about 60%. In some embodiments, homology,

sequence identity or complementarity, is from about 60% to about 70%. In some embodiments, homology, sequence identity or complementarity, is from about 70% to about 80%. In some embodiments, homology, sequence identity or complementarity, is from about 80% to about 90%. In some embodiments, homology, sequence identity or complementarity, is about 90%, about 92%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100%.

**[00159]** In an embodiment, the antisense oligonucleotides, such as for example, nucleic acid molecules set forth in SEQ ID NOS: 12 to 49 comprise one or more substitutions or modifications. In one embodiment, the nucleotides are substituted with locked nucleic acids (LNA).

**[00160]** In an embodiment, the oligonucleotides target one or more regions of the nucleic acid molecules sense and/or antisense of coding and/or non-coding sequences associated with BDNF and the sequences set forth as SEQ ID NOS: 1 to 11. The oligonucleotides are also targeted to overlapping regions of SEQ ID NOS: 1 to 11.

**[00161]** Certain preferred oligonucleotides of this invention are chimeric oligonucleotides. "Chimeric oligonucleotides" or "chimeras," in the context of this invention, are oligonucleotides which contain two or more chemically distinct regions, each made up of at least one nucleotide. These oligonucleotides typically contain at least one region of modified nucleotides that confers one or more beneficial properties (such as, for example, increased nuclease resistance, increased uptake into cells, increased binding affinity for the target) and a region that is a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of antisense modulation of gene expression. Consequently, comparable results can often be obtained with shorter oligonucleotides when chimeric oligonucleotides are used, compared to phosphorothioate deoxyoligonucleotides hybridizing to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art. In one an embodiment, a chimeric oligonucleotide comprises at least one region modified to increase target binding affinity, and, usually, a region that acts as a substrate for RNAse H. Affinity of an oligonucleotide for its target (in this case, a nucleic acid encoding ras) is routinely determined by measuring the Tm of an oligonucleotide/target pair, which is the temperature at which the oligonucleotide and target dissociate; dissociation is detected spectrophotometrically. The higher the Tm, the greater is the affinity of the oligonucleotide for the target.

**[00162]** Chimeric antisense compounds of the invention may be formed as composite structures of two or more oligonucleotides, modified oligonucleotides, oligonucleosides and/or oligonucleotides mimetics as described above. Such; compounds have also been referred to in the art as hybrids or

gapmers. Representative United States patents that teach the preparation of such hybrid structures comprise, but are not limited to, US patent nos. 5,013,830; 5,149,797; 5, 220,007; 5,256,775; 5,366,878; 5,403,711; 5,491,133; 5,565,350; 5,623,065; 5,652,355; 5,652,356; and 5,700,922, each of which is herein incorporated by reference.

**[00163]** In an embodiment, the region of the oligonucleotide which is modified comprises at least one nucleotide modified at the 2' position of the sugar, most preferably a 2'-Oalkyl, 2'-O-alkyl-O-alkyl or 2'-fluoro-modified nucleotide. In other an embodiment, RNA modifications include 2'-fluoro, 2'-amino and 2' O-methyl modifications on the ribose of pyrimidines, abasic residues or an inverted base at the 3' end of the RNA. Such modifications are routinely incorporated into oligonucleotides and these oligonucleotides have been shown to have a higher Tm (i.e., higher target binding affinity) than; 2'-deoxyoligonucleotides against a given target. The effect of such increased affinity is to greatly enhance RNAi oligonucleotide inhibition of gene expression. RNAse H is a cellular endonuclease that cleaves the RNA strand of RNA:DNA duplexes; activation of this enzyme therefore results in cleavage of the RNA target, and thus can greatly enhance the efficiency of RNAi inhibition. Cleavage of the RNA target can be routinely demonstrated by gel electrophoresis. In an embodiment, the chimeric oligonucleotide is also modified to enhance nuclease resistance. Cells contain a variety of exo- and endo-nucleases which can degrade nucleic acids. A number of nucleotide and nucleoside modifications have been shown to make the oligonucleotide into which they are incorporated more resistant to nuclease digestion than the native oligodeoxynucleotide. Nuclease resistance is routinely measured by incubating oligonucleotides with cellular extracts or isolated nuclease solutions and measuring the extent of intact oligonucleotide remaining over time, usually by gel electrophoresis. Oligonucleotides which have been modified to enhance their nuclease resistance survive intact for a longer time than unmodified oligonucleotides. A variety of oligonucleotide modifications have been demonstrated to enhance or confer nuclease resistance. Oligonucleotides which contain at least one phosphorothioate modification are presently more preferred. In some cases, oligonucleotide modifications which enhance target binding affinity are also, independently, able to enhance nuclease resistance.

**[00164]** Specific examples of some preferred oligonucleotides envisioned for this invention include those comprising modified backbones, for example, phosphorothioates, phosphotriesters, methyl phosphonates, short chain alkyl or cycloalkyl intersugar linkages or short chain heteroatomic or heterocyclic intersugar linkages. Most preferred are oligonucleotides with phosphorothioate backbones and those with heteroatom backbones, particularly CH2 --NH--O--CH2, CH,--N(CH3)--O--CH2 [known as a methylene(methylimino) or MMI backbone], CH2 --O--N (CH3)--CH2, CH2 –N (CH3)--N (CH3)--CH2 and O--N (CH3)--CH2 --CH2 backbones, wherein the native phosphodiester backbone is represented as O--P--O--CH,). The amide backbones disclosed by De

Mesmaeker et al. (1995) Acc. Chem. Res. 28:366-374 are also preferred. Also preferred are oligonucleotides having morpholino backbone structures (Summerton and Weller, U.S. Pat. No. 5,034,506). In other an embodiment, such as the peptide nucleic acid (PNA) backbone, the phosphodiester backbone of the oligonucleotide is replaced with a polyamide backbone, the nucleotides being bound directly or indirectly to the aza nitrogen atoms of the polyamide backbone. Oligonucleotides may also comprise one or more substituted sugar moieties. Preferred oligonucleotides comprise one of the following at the 2' position: OH, SH, SCH3, F, OCN, OCH3, O(CH2)n CH3, O(CH2)n NH2 or O(CH2)n CH3 where n is from 1 to about 10; C1 to C10 lower alkyl, alkoxyalkoxy, substituted lower alkyl, alkaryl or aralkyl; Cl; Br; CN; CF3 ; OCF3; O--, S--, or N-alkyl; O--, S--, or N-alkenyl; SOCH3; SO2 CH3; ONO2; NO2; N3; NH2; heterocycloalkyl; heterocycloalkaryl; aminoalkylamino; polyalkylamino; substituted silyl; an RNA cleaving group; a reporter group; an intercalator; a group for improving the pharmacokinetic properties of an oligonucleotide; or a group for improving the pharmacodynamic properties of an oligonucleotide and other substituents having similar properties. A preferred modification includes 2'-methoxyethoxy [2'-O-CH2 CH2 OCH3, also known as 2'-O-(2-methoxyethyl)]. Other preferred modifications include 2'-methoxy (2'-O--CH3), 2'- propoxy (2'-OCH2 CH2CH3) and 2'-fluoro (2'-F). Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide and the 5' position of 5' terminal nucleotide. Oligonucleotides may also have sugar mimetics such as cyclobutyls in place of the pentofuranosyl group.

**[00165]** Oligonucleotides may also include, additionally or alternatively, nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleotides include adenine (A), guanine (G), thymine (T), cytosine (C) and uracil (U). Modified nucleotides include nucleotides found only infrequently or transiently in natural nucleic acids, e.g., hypoxanthine, 6-methyladenine, 5-Me pyrimidines, particularly 5-methylcytosine (also referred to as 5-methyl-2' deoxycytosine and often referred to in the art as 5-Me-C), 5-hydroxymethylcytosine (HMC), glycosyl HMC and gentobiosyl HMC, as well as synthetic nucleotides, e.g., 2-aminoadenine, 2-(methylamino)adenine, 2-(imidazolylalkyl)adenine, 2-(aminoalklyamino)adenine or other heterosubstituted alkyladenines, 2-thiouracil, 2-thiothymine, 5-bromouracil, 5-hydroxymethyluracil, 8-azaguanine, 7-deazaguanine, N6 (6-aminohexyl)adenine and 2,6-diaminopurine. A "universal" base known in the art, e.g., inosine, may be included. 5-Me-C substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C. and are presently preferred base substitutions.

**[00166]** Another modification of the oligonucleotides of the invention involves chemically linking to the oligonucleotide one or more moieties or conjugates which enhance the activity or cellular

uptake of the oligonucleotide. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety, a cholesteryl moiety, an aliphatic chain, e.g., dodecandiol or undecyl residues, a polyamine or a polyethylene glycol chain, or Adamantane acetic acid. Oligonucleotides comprising lipophilic moieties, and methods for preparing such oligonucleotides are known in the art, for example, U.S. Pat. Nos. 5,138,045, 5,218,105 and 5,459,255.

**[00167]** It is not necessary for all positions in a given oligonucleotide to be uniformly modified, and in fact more than one of the aforementioned modifications may be incorporated in a single oligonucleotide or even at within a single nucleoside within an oligonucleotide. The present invention also includes oligonucleotides which are chimeric oligonucleotides as hereinbefore defined.

**[00168]** In another embodiment, the nucleic acid molecule of the present invention is conjugated with another moiety including but not limited to abasic nucleotides, polyether, polyamine, polyamides, peptides, carbohydrates, lipid, or polyhydrocarbon compounds. Those skilled in the art will recognize that these molecules can be linked to one or more of any nucleotides comprising the nucleic acid molecule at several positions on the sugar, base or phosphate group.

**[00169]** The oligonucleotides used in accordance with this invention may be conveniently and routinely made through the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including Applied Biosystems. Any other means for such synthesis may also be employed; the actual synthesis of the oligonucleotides is well within the talents of one of ordinary skill in the art. It is also well known to use similar techniques to prepare other oligonucleotides such as the phosphorothioates and alkylated derivatives. It is also well known to use similar techniques and commercially available modified amidites and controlled-pore glass (CPG) products such as biotin, fluorescein, acridine or psoralen-modified amidites and/or CPG (available from Glen Research, Sterling VA) to synthesize fluorescently labeled, biotinylated or other modified oligonucleotides such as cholesterol-modified oligonucleotides.

**[00170]** In accordance with the invention, use of modifications such as the use of LNA monomers to enhance the potency, specificity and duration of action and broaden the routes of administration of oligonucleotides comprised of current chemistries such as MOE, ANA, FANA, PS etc. This can be achieved by substituting some of the monomers in the current oligonucleotides by LNA monomers. The LNA modified oligonucleotide may have a size similar to the parent compound or may be larger or preferably smaller. It is preferred that such LNA-modified oligonucleotides contain less than about 70%, more preferably less than about 60%, most preferably less than about 50% LNA monomers and that their sizes are between about 5 and 25 nucleotides, more preferably between about 12 and 20 nucleotides.

**[00171]** Preferred modified oligonucleotide backbones comprise, but not limited to, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates comprising 3'alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates comprising 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included.

**[00172]** Representative United States patents that teach the preparation of the above phosphorus containing linkages comprise, but are not limited to, US patent nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5, 177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455, 233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563, 253; 5,571,799; 5,587,361; and 5,625,050, each of which is herein incorporated by reference.

**[00173]** Preferred modified oligonucleotide backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These comprise those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH2 component parts.

**[00174]** Representative United States patents that teach the preparation of the above oligonucleosides comprise, but are not limited to, US patent nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264, 562; 5, 264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596, 086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623, 070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439, each of which is herein incorporated by reference.

**[00175]** In other preferred oligonucleotide mimetics, both the sugar and the internucleoside linkage, i.e., the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, an oligonucleotide mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar-backbone of

an oligonucleotide is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative United States patents that teach the preparation of PNA compounds comprise, but are not limited to, US patent nos. 5,539,082; 5,714,331; and 5,719,262, each of which is herein incorporated by reference . Further teaching of PNA compounds can be found in Nielsen, et al. (1991) Science 254, 1497-1500.

**[00176]** In an embodiment of the invention the oligonucleotides with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular- CH2-NH-O-CH2-,-CH2-N (CH3)-O-CH2-known as a methylene (methylimino) or MMI backbone,- CH2-O-N (CH3)-CH2-,-CH2N(CH3)-N(CH3) CH2-and-O-N(CH3)-CH2-CH2- wherein the native phosphodiester backbone is represented as-O-P-O-CH2- of the above referenced US patent no. 5,489,677, and the amide backbones of the above referenced US patent no. 5,602,240. Also preferred are oligonucleotides having morpholino backbone structures of the above-referenced US patent no. 5,034,506.

**[00177]** Modified oligonucleotides may also contain one or more substituted sugar moieties. Preferred oligonucleotides comprise one of the following at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S-or N-alkynyl; or O alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C to CO alkyl or $C_2$ to $C_{10}$ alkenyl and alkynyl. Particularly preferred are O (CH2)n OmCH3, O(CH2)n,OCH3, O(CH2)nNH2, O(CH2)nCH3, O(CH2)nONH2, and O(CH2)nON(CH3)2 where n and m can be from 1 to about 10. Other preferred oligonucleotides comprise one of the following at the 2' position: C to CO, (lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH3, OCN, Cl, Br, CN, CF3, OCF3, SOCH3, SO2CH3, ONO2, NO2, N3, NH2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. A preferred modification comprises 2'-methoxyethoxy (2'-O-CH2CH2OCH3, also known as 2'-O-(2- methoxyethyl) or 2'-MOE) i.e., an alkoxyalkoxy group. A further preferred modification comprises 2'-dimethylaminooxyethoxy, i.e. , a O(CH2)2ON(CH3)2 group, also known as 2'-DMAOE, as described in examples herein below, and 2'- dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'- DMAEOE), i.e., 2'-O-CH2-O-CH2-N (CH2)2.

**[00178]** Other preferred modifications comprise 2'-methoxy (2'-O CH3), 2'-aminopropoxy (2'-O CH2CH2CH2NH2) and 2'-fluoro (2'-F). Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Oligonucleotides may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar.

Representative United States patents that teach the preparation of such modified sugar structures comprise, but are not limited to, US patent nos. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514, 785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646, 265; 5,658,873; 5,670,633; and 5,700,920, each of which is herein incorporated by reference.

**[00179]** Oligonucleotides may also comprise nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleotides comprise the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleotides comprise other synthetic and natural nucleotides such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudo-uracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylquanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine.

**[00180]** Further, nucleotides comprise those disclosed in United States Patent No. 3,687,808, those disclosed in 'The Concise Encyclopedia of Polymer Science And Engineering', pages 858-859, Kroschwitz, J.I., ed. John Wiley & Sons, 1990, those disclosed by Englisch et al., 'Angewandle Chemie, International Edition', 1991, 30, page 613, and those disclosed by Sanghvi, Y.S., Chapter 15, 'Antisense Research and Applications', pages 289-302, Crooke, S.T. and Lebleu, B. ea., CRC Press, 1993. Certain of these nucleotides are particularly useful for increasing the binding affinity of the oligomeric compounds of the invention. These comprise 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and 0-6 substituted purines, comprising 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C (Sanghvi, Y.S., Crooke, S.T. and Lebleu, B., eds, 'Antisense Research and Applications', CRC Press, Boca Raton, 1993, pp. 276-278) and are presently preferred base substitutions, even more particularly when combined with 2'-Omethoxyethyl sugar modifications.

**[00181]** Representative United States patents that teach the preparation of the above noted modified nucleotides as well as other modified nucleotides comprise, but are not limited to, US patent nos. 3,687,808, as well as 4,845,205; 5,130,302; 5,134,066; 5,175, 273; 5, 367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,596,091; 5,614,617; 5,750,692, and 5,681,941, each of which is herein incorporated by reference.

**[00182]** Another modification of the oligonucleotides of the invention involves chemically linking to the oligonucleotide one or more moieties or conjugates, which enhance the activity, cellular distribution, or cellular uptake of the oligonucleotide.

**[00183]** Such moieties comprise but are not limited to, lipid moieties such as a cholesterol moiety, cholic acid, a thioether, e.g., hexyl-S-tritylthiol, a thiocholesterol, an aliphatic chain, e.g., dodecandiol or undecyl residues, a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate, a polyamine or a polyethylene glycol chain, or Adamantane acetic acid, a palmityl moiety, or an octadecylamine or hexylamino-carbonyl-t oxycholesterol moiety.

**[00184]** Representative United States patents that teach the preparation of such oligonucleotides conjugates comprise, but are not limited to, US patent nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552, 538; 5,578,717, 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486, 603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762, 779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082, 830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5, 245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391, 723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5, 565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599, 928 and 5,688,941, each of which is herein incorporated by reference.

**[00185]** *Drug discovery:* The compounds of the present invention can also be applied in the areas of drug discovery and target validation. The present invention comprehends the use of the compounds and preferred target segments identified herein in drug discovery efforts to elucidate relationships that exist between Brain derived neurotrophic factor (BDNF) polynucleotides and a disease state, phenotype, or condition. These methods include detecting or modulating BDNF polynucleotides comprising contacting a sample, tissue, cell, or organism with the compounds of the present invention, measuring the nucleic acid or protein level of BDNF polynucleotides and/or a related phenotypic or chemical endpoint at some time after treatment, and optionally comparing the measured value to a non-treated sample or sample treated with a further compound of the invention. These methods can also be performed in parallel or in combination with other experiments to determine the function of unknown genes for the process of target validation or to determine the validity of a particular gene product as a target for treatment or prevention of a particular disease, condition, or phenotype.

*Assessing Up-regulation or Inhibition of Gene Expression:*

**[00186]** Transfer of an exogenous nucleic acid into a host cell or organism can be assessed by directly detecting the presence of the nucleic acid in the cell or organism. Such detection can be

achieved by several methods well known in the art. For example, the presence of the exogenous nucleic acid can be detected by Southern blot or by a polymerase chain reaction (PCR) technique using primers that specifically amplify nucleotide sequences associated with the nucleic acid. Expression of the exogenous nucleic acids can also be measured using conventional methods including gene expression analysis. For instance, mRNA produced from an exogenous nucleic acid can be detected and quantified using a Northern blot and reverse transcription PCR (RT-PCR).

**[00187]** Expression of RNA from the exogenous nucleic acid can also be detected by measuring an enzymatic activity or a reporter protein activity. For example, antisense modulatory activity can be measured indirectly as a decrease or increase in target nucleic acid expression as an indication that the exogenous nucleic acid is producing the effector RNA. Based on sequence conservation, primers can be designed and used to amplify coding regions of the target genes. Initially, the most highly expressed coding region from each gene can be used to build a model control gene, although any coding or non coding region can be used. Each control gene is assembled by inserting each coding region between a reporter coding region and its poly(A) signal. These plasmids would produce an mRNA with a reporter gene in the upstream portion of the gene and a potential RNAi target in the 3' non-coding region. The effectiveness of individual antisense oligonucleotides would be assayed by modulation of the reporter gene. Reporter genes useful in the methods of the present invention include acetohydroxyacid synthase (AHAS), alkaline phosphatase (AP), beta galactosidase (LacZ), beta glucoronidase (GUS), chloramphenicol acetyltransferase (CAT), green fluorescent protein (GFP), red fluorescent protein (RFP), yellow fluorescent protein (YFP), cyan fluorescent protein (CFP), horseradish peroxidase (HRP), luciferase (Luc), nopaline synthase (NOS), octopine synthase (OCS), and derivatives thereof. Multiple selectable markers are available that confer resistance to ampicillin, bleomycin, chloramphenicol, gentamycin, hygromycin, kanamycin, lincomycin, methotrexate, phosphinothricin, puromycin, and tetracycline. Methods to determine modulation of a reporter gene are well known in the art, and include, but are not limited to, fluorometric methods (e.g. fluorescence spectroscopy, Fluorescence Activated Cell Sorting (FACS), fluorescence microscopy), antibiotic resistance determination.

**[00188]** BDNF protein and mRNA expression can be assayed using methods known to those of skill in the art and described elsewhere herein. For example, immunoassays such as the ELISA can be used to measure protein levels. BDNF ELISA assay kits are available commercially, e.g., from R&D Systems (Minneapolis, MN).

**[00189]** In embodiments, BDNF expression (e.g., mRNA or protein) in a sample (e.g., cells or tissues in vivo or in vitro) treated using an antisense oligonucleotide of the invention is evaluated by comparison with BDNF expression in a control sample. For example, expression of the protein or nucleic acid can be compared using methods known to those of skill in the art with that in a mock-

treated or untreated sample. Alternatively, comparison with a sample treated with a control antisense oligonucleotide (e.g., one having an altered or different sequence) can be made depending on the information desired. In another embodiment, a difference in the expression of the BDNF protein or nucleic acid in a treated vs. an untreated sample can be compared with the difference in expression of a different nucleic acid (including any standard deemed appropriate by the researcher, e.g., a housekeeping gene) in a treated sample vs. an untreated sample.

**[00190]** Observed differences can be expressed as desired, e.g., in the form of a ratio or fraction, for use in a comparison with control. In embodiments, the level of BDNF mRNA or protein, in a sample treated with an antisense oligonucleotide of the present invention, is increased or decreased by about 1.25-fold to about 10-fold or more relative to an untreated sample or a sample treated with a control nucleic acid. In embodiments, the level of BDNF mRNA or protein is increased or decreased by at least about 1.25-fold, at least about 1.3-fold, at least about 1.4-fold, at least about 1.5-fold, at least about 1.6-fold, at least about 1.7-fold, at least about 1.8-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 4.5-fold, at least about 5-fold, at least about 5.5-fold, at least about 6-fold, at least about 6.5-fold, at least about 7-fold, at least about 7.5-fold, at least about 8-fold, at least about 8.5-fold, at least about 9-fold, at least about 9.5-fold, or at least about 10-fold or more.

*Kits, Research Reagents, Diagnostics, and Therapeutics*

**[00191]** The compounds of the present invention can be utilized for diagnostics, therapeutics, and prophylaxis, and as research reagents and components of kits. Furthermore, antisense oligonucleotides, which are able to inhibit gene expression with exquisite specificity, are often used by those of ordinary skill to elucidate the function of particular genes or to distinguish between functions of various members of a biological pathway.

**[00192]** For use in kits and diagnostics and in various biological systems, the compounds of the present invention, either alone or in combination with other compounds or therapeutics, are useful as tools in differential and/or combinatorial analyses to elucidate expression patterns of a portion or the entire complement of genes expressed within cells and tissues.

**[00193]** As used herein the term "biological system" or "system" is defined as any organism, cell, cell culture or tissue that expresses, or is made competent to express products of the Brain derived neurotrophic factor (BDNF) genes. These include, but are not limited to, humans, transgenic animals, cells, cell cultures, tissues, xenografts, transplants and combinations thereof.

**[00194]** As one non limiting example, expression patterns within cells or tissues treated with one or more antisense compounds are compared to control cells or tissues not treated with antisense compounds and the patterns produced are analyzed for differential levels of gene expression as they pertain, for example, to disease association, signaling pathway, cellular localization, expression

level, size, structure or function of the genes examined. These analyses can be performed on stimulated or unstimulated cells and in the presence or absence of other compounds that affect expression patterns.

**[00195]** Examples of methods of gene expression analysis known in the art include DNA arrays or microarrays, SAGE (serial analysis of gene expression), READS (restriction enzyme amplification of digested cDNAs), TOGA (total gene expression analysis), protein arrays and proteomics, expressed sequence tag (EST) sequencing, subtractive RNA fingerprinting (SuRF), subtractive cloning, differential display (DD), comparative genomic hybridization, FISH (fluorescent in situ hybridization) techniques and mass spectrometry methods.

**[00196]** The compounds of the invention are useful for research and diagnostics, because these compounds hybridize to nucleic acids encoding Brain derived neurotrophic factor (BDNF). For example, oligonucleotides that hybridize with such efficiency and under such conditions as disclosed herein as to be effective BDNF modulators are effective primers or probes under conditions favoring gene amplification or detection, respectively. These primers and probes are useful in methods requiring the specific detection of nucleic acid molecules encoding BDNF and in the amplification of said nucleic acid molecules for detection or for use in further studies of BDNF. Hybridization of the antisense oligonucleotides, particularly the primers and probes, of the invention with a nucleic acid encoding BDNF can be detected by means known in the art. Such means may include conjugation of an enzyme to the oligonucleotide, radiolabeling of the oligonucleotide, or any other suitable detection means. Kits using such detection means for detecting the level of BDNF in a sample may also be prepared.

**[00197]** The specificity and sensitivity of antisense are also harnessed by those of skill in the art for therapeutic uses. Antisense compounds have been employed as therapeutic moieties in the treatment of disease states in animals, including humans. Antisense oligonucleotide drugs have been safely and effectively administered to humans and numerous clinical trials are presently underway. It is thus established that antisense compounds can be useful therapeutic modalities that can be configured to be useful in treatment regimes for the treatment of cells, tissues and animals, especially humans.

**[00198]** For therapeutics, an animal, preferably a human, suspected of having a disease or disorder which can be treated by modulating the expression of BDNF polynucleotides is treated by administering antisense compounds in accordance with this invention. For example, in one non-limiting embodiment, the methods comprise the step of administering to the animal in need of treatment, a therapeutically effective amount of BDNF modulator. The BDNF modulators of the present invention effectively modulate the activity of the BDNF or modulate the expression of the BDNF protein. In one embodiment, the activity or expression of BDNF in an animal is inhibited by about 10% as compared to a control. Preferably, the activity or expression of BDNF in an animal is

inhibited by about 30%. More preferably, the activity or expression of BDNF in an animal is inhibited by 50% or more. Thus, the oligomeric compounds modulate expression of Brain derived neurotrophic factor (BDNF) mRNA by at least 10%, by at least 50%, by at least 25%, by at least 30%, by at least 40%, by at least 50%, by at least 60%, by at least 70%, by at least 75%, by at least 80%, by at least 85%, by at least 90%, by at least 95%, by at least 98%, by at least 99%, or by 100% as compared to a control.

**[00199]** In one embodiment, the activity or expression of Brain derived neurotrophic factor (BDNF) in an animal is increased by about 10% as compared to a control. Preferably, the activity or expression of BDNF in an animal is increased by about 30%. More preferably, the activity or expression of BDNF in an animal is increased by 50% or more. Thus, the oligomeric compounds modulate expression of BDNF mRNA by at least 10%, by at least 50%, by at least 25%, by at least 30%, by at least 40%, by at least 50%, by at least 60%, by at least 70%, by at least 75%, by at least 80%, by at least 85%, by at least 90%, by at least 95%, by at least 98%, by at least 99%, or by 100% as compared to a control.

**[00200]** For example, the increase or reduction of the expression of Brain derived neurotrophic factor (BDNF) may be measured in serum, blood, adipose tissue, liver or any other body fluid, tissue or organ of the animal. Preferably, the cells contained within said fluids, tissues or organs being analyzed contain a nucleic acid molecule encoding BDNF peptides and/or the BDNF protein itself.

**[00201]** The compounds of the invention can be utilized in pharmaceutical compositions by adding an effective amount of a compound to a suitable pharmaceutically acceptable diluent or carrier. Use of the compounds and methods of the invention may also be useful prophylactically.

*Conjugates*

**[00202]** Another modification of the oligonucleotides of the invention involves chemically linking to the oligonucleotide one or more moieties or conjugates that enhance the activity, cellular distribution or cellular uptake of the oligonucleotide. These moieties or conjugates can include conjugate groups covalently bound to functional groups such as primary or secondary hydroxyl groups. Conjugate groups of the invention include intercalators, reporter molecules, polyamines, polyamides, polyethylene glycols, polyethers, groups that enhance the pharmacodynamic properties of oligomers, and groups that enhance the pharmacokinetic properties of oligomers. Typicalconjugate groups include cholesterols, lipids, phospholipids, biotin, phenazine, folate, phenanthridine, anthraquinone, acridine, fluoresceins, rhodamines, coumarins, and dyes. Groups that enhance the pharmacodynamic properties, in the context of this invention, include groups that improve uptake, enhance resistance to degradation, and/or strengthen sequence-specific hybridization with the target nucleic acid. Groups that enhance the pharmacokinetic properties, in the context of this invention, include groups that improve uptake, distribution, metabolism or

excretion of the compounds of the present invention. Representative conjugate groups are disclosed in International Patent Application No. PCT/US92/09196, filed Oct. 23, 1992, and U.S. Pat. No. 6,287,860, which are incorporated herein by reference. Conjugate moieties include, but are not limited to, lipid moieties such as a cholesterol moiety, cholic acid, a thioether, e.g., hexyl-5-tritylthiol, a thiocholesterol, an aliphatic chain, e.g., dodecandiol or undecyl residues, a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-Hphosphonate, a polyamine or a polyethylene glycol chain, or Adamantane acetic acid, a palmityl moiety, or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety. Oligonucleotides of the invention may also be conjugated to active drug substances, for example, aspirin, warfarin, phenylbutazone, ibuprofen, suprofen, fenbufen, ketoprofen, (S)-(+)-pranoprofen, carprofen, dansylsarcosine, 2,3,5-triiodobenzoic acid, flufenamic acid, folinic acid, a benzothiadiazide, chlorothiazide, a diazepine, indomethicin, a barbiturate, a cephalosporin, a sulfa drug, an antidiabetic, an antibacterial or an antibiotic.

**[00203]** Representative United States patents that teach the preparation of such oligonucleotides conjugates include, but are not limited to, U.S. Pat. Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391,723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941.

*Formulations*

**[00204]** The compounds of the invention may also be admixed, encapsulated, conjugated or otherwise associated with other molecules, molecule structures or mixtures of compounds, as forexample, liposomes, receptor-targeted molecules, oral, rectal, topical or other formulations, for assisting in uptake, distribution and/or absorption. Representative United States patents that teach the preparation of such uptake, distribution and/or absorption-assisting formulations include, but are not limited to, U.S. Pat. Nos. 5,108,921; 5,354,844; 5,416,016; 5,459,127; 5,521,291; 5,543,165; 5,547,932; 5,583,020; 5,591,721; 4,426,330; 4,534,899; 5,013,556; 5,108,921; 5,213,804; 5,227,170; 5,264,221; 5,356,633; 5,395,619; 5,416,016; 5,417,978; 5,462,854; 5,469,854; 5,512,295; 5,527,528; 5,534,259; 5,543,152; 5,556,948; 5,580,575; and 5,595,756, each of which is herein incorporated by reference.

**[00205]** Although, the antisense oligonucleotides do not need to be administered in the context of a vector in order to modulate a target expression and/or function, embodiments of the invention relates

to expression vector constructs for the expression of antisense oligonucleotides, comprising promoters, hybrid promoter gene sequences and possess a strong constitutive promoter activity, or a promoter activity which can be induced in the desired case.

**[00206]** In an embodiment, invention practice involves administering at least one of the foregoing antisense oligonucleotides with a suitable nucleic acid delivery system. In one embodiment, that system includes a non-viral vector operably linked to the polynucleotide. Examples of such nonviral vectors include the oligonucleotide alone (e.g. any one or more of SEQ ID NOS: 12 to 49) or in combination with a suitable protein, polysaccharide or lipid formulation.

**[00207]** Additionally suitable nucleic acid delivery systems include viral vector, typically sequence from at least one of an adenovirus, adenovirus-associated virus (AAV), helper-dependent adenovirus, retrovirus, or hemagglutinatin virus of Japan-liposome (HVJ) complex. Preferably, the viral vector comprises a strong eukaryotic promoter operably linked to the polynucleotide e.g., a cytomegalovirus (CMV) promoter.

**[00208]** Additionally preferred vectors include viral vectors, fusion proteins and chemical conjugates. Retroviral vectors include Moloney murine leukemia viruses and HIV-based viruses. One preferred HIV-based viral vector comprises at least two vectors wherein the gag and pol genes are from an HIV genome and the env gene is from another virus. DNA viral vectors are preferred. These vectors include pox vectors such as orthopox or avipox vectors, herpesvirus vectors such as a herpes simplex I virus (HSV) vector, Adenovirus Vectors and Adeno-associated Virus Vectors.

**[00209]** The antisense compounds of the invention encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other compound which, upon administration to an animal, including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof.

**[00210]** The term "pharmaceutically acceptable salts" refers to physiologically and pharmaceutically acceptable salts of the compounds of the invention: i.e., salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto. For oligonucleotides, preferred examples of pharmaceutically acceptable salts and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein by reference.

**[00211]** The present invention also includes pharmaceutical compositions and formulations that include the antisense compounds of the invention. The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including ophthalmic and to mucous membranes including vaginal and rectal delivery), pulmonary, e.g., by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal), oral or parenteral. Parenteral administration includes intravenous,

intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial, e.g., intrathecal or intraventricular, administration.

**[00212]** For treating tissues in the central nervous system, administration can be made by, e.g., injection or infusion into the cerebrospinal fluid. Administration of antisense RNA into cerebrospinal fluid is described, e.g., in U.S. Pat. App. Pub. No. 2007/0117772, "Methods for slowing familial ALS disease progression," incorporated herein by reference in its entirety.

**[00213]** When it is intended that the antisense oligonucleotide of the present invention be administered to cells in the central nervous system, administration can be with one or more agents capable of promoting penetration of the subject antisense oligonucleotide across the blood-brain barrier. Injection can be made, e.g., in the entorhinal cortex or hippocampus. Delivery of neurotrophic factors by administration of an adenovirus vector to motor neurons in muscle tissue is described in, e.g., U.S. Pat. No. 6,632,427, "Adenoviral-vector-mediated gene transfer into medullary motor neurons," incorporated herein by reference. Delivery of vectors directly to the brain, e.g., the striatum, the thalamus, the hippocampus, or the substantia nigra, is known in the art and described, e.g., in U.S. Pat. No. 6,756,523, "Adenovirus vectors for the transfer of foreign genes into cells of the central nervous system particularly in brain," incorporated herein by reference. Administration can be rapid as by injection or made over a period of time as by slow infusion or administration of slow release formulations.

**[00214]** The subject antisense oligonucleotides can also be linked or conjugated with agents that provide desirable pharmaceutical or pharmacodynamic properties. For example, the antisense oligonucleotide can be coupled to any substance, known in the art to promote penetration or transport across the blood-brain barrier, such as an antibody to the transferrin receptor, and administered by intravenous injection. The antisense compound can be linked with a viral vector, for example, that makes the antisense compound more effective and/or increases the transport of the antisense compound across the blood-brain barrier. Osmotic blood brain barrier disruption can also be accomplished by, e.g., infusion of sugars including, but not limited to, meso erythritol, xylitol, D(+) galactose, D(+) lactose, D(+) xylose, dulcitol, myo-inositol, L(-) fructose, D(-) mannitol, D(+) glucose, D(+) arabinose, D(-) arabinose, cellobiose, D(+) maltose, D(+) raffinose, L(+) rhamnose, D(+) melibiose, D(-) ribose, adonitol, D(+) arabitol, L(-) arabitol, D(+) fucose, L(-) fucose, D(-) lyxose, L(+) lyxose, and L(-) lyxose, or amino acids including, but not limited to, glutamine, lysine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glycine, histidine, leucine, methionine, phenylalanine, proline, serine, threonine, tyrosine, valine, and taurine. Methods and materials for enhancing blood brain barrier penetration are described, e.g., in U. S. Patent No. 4,866,042, "Method for the delivery of genetic material across the blood brain barrier," 6,294,520, "Material for passage

through the blood-brain barrier," and 6,936,589, "Parenteral delivery systems," all incorporated herein by reference in their entirety.

**[00215]** The subject antisense compounds may be admixed, encapsulated, conjugated or otherwise associated with other molecules, molecule structures or mixtures of compounds, for example, liposomes, receptor-targeted molecules, oral, rectal, topical or other formulations, for assisting in uptake, distribution and/or absorption. For example, cationic lipids may be included in the formulation to facilitate oligonucleotide uptake. One such composition shown to facilitate uptake is LIPOFECTIN (available from GIBCO-BRL, Bethesda, MD).

**[00216]** Oligonucleotides with at least one 2'-O-methoxyethyl modification are believed to be particularly useful for oral administration. Pharmaceutical compositions and formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms, gloves and the like may also be useful.

**[00217]** The pharmaceutical formulations of the present invention, which may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

**[00218]** The compositions of the present invention may be formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, gel capsules, liquid syrups, soft gels, suppositories, and enemas. The compositions of the present invention may also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions may further contain substances that increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

**[00219]** Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, foams and liposome-containing formulations. The pharmaceutical compositions and formulations of the present invention may comprise one or more penetration enhancers, carriers, excipients or other active or inactive ingredients.

**[00220]** Emulsions are typically heterogeneous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1 μm in diameter. Emulsions may contain additional components in addition to the dispersed phases, and the active drug that may be present as a solution in either the aqueous phase, oily phase or itself as a separate phase. Microemulsions are included as

an embodiment of the present invention. Emulsions and their uses are well known in the art and are further described in U.S. Pat. No. 6,287,860.

**[00221]** Formulations of the present invention include liposomal formulations. As used in the present invention, the term "liposome" means a vesicle composed of amphiphilic lipids arranged in a spherical bilayer or bilayers. Liposomes are unilamellar or multilamellar vesicles which have a membrane formed from a lipophilic material and an aqueous interior that contains the composition to be delivered. Cationic liposomes are positively charged liposomes that are believed to interact with negatively charged DNA molecules to form a stable complex. Liposomes that are pH-sensitive or negatively-charged are believed to entrap DNA rather than complex with it. Both cationic and noncationic liposomes have been used to deliver DNA to cells.

**[00222]** Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids. When incorporated into liposomes, these specialized lipids result in liposomes with enhanced circulation lifetimes relative to liposomeslacking such specialized lipids. Examples of sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome comprises one or more glycolipids or is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. Liposomes and their uses are further described in U.S. Pat. No. 6,287,860.

**[00223]** The pharmaceutical formulations and compositions of the present invention may also include surfactants. The use of surfactants in drug products, formulations and in emulsions is well known in the art. Surfactants and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein by reference.

**[00224]** In one embodiment, the present invention employs various penetration enhancers to effect the efficient delivery of nucleic acids, particularly oligonucleotides. In addition to aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance the permeability of lipophilic drugs. Penetration enhancers may be classified as belonging to one of five broad categories, i.e., surfactants, fatty acids, bile salts, chelating agents, and non-chelating nonsurfactants. Penetration enhancers and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein by reference.

**[00225]** One of skill in the art will recognize that formulations are routinely designed according to their intended use, i.e. route of administration.

**[00226]** Preferred formulations for topical administration include those in which the oligonucleotides of the invention are in admixture with a topical delivery agent such as lipids, liposomes, fatty acids, fatty acid esters, steroids, chelating agents and surfactants. Preferred lipids and liposomes include neutral (e.g. dioleoyl-phosphatidyl DOPE ethanolamine, dimyristoylphosphatidyl choline DMPC, distearolyphosphatidyl choline) negative (e.g.

dimyristoylphosphatidyl glycerol DMPG) and cationic (e.g. dioleoyltetramethylaminopropyl DOTAP and dioleoyl-phosphatidyl ethanolamine DOTMA).

**[00227]** For topical or other administration, oligonucleotides of the invention may be encapsulated within liposomes or may form complexes thereto, in particular to cationic liposomes. Alternatively, oligonucleotides may be complexed to lipids, in particular to cationic lipids. Preferred fatty acids and esters, pharmaceutically acceptable salts thereof, and their uses are further described in U.S. Pat. No. 6,287,860.

**[00228]** Compositions and formulations for oral administration include powders or granules, microparticulates, nanoparticulates, suspensions or solutions in water or non-aqueous media, capsules, gel capsules, sachets, tablets or minitablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable. Preferred oral formulations are those in which oligonucleotides of the invention are administered in conjunction with one or more penetration enhancers surfactants and chelators. Preferred surfactants include fatty acids and/or esters or salts thereof, bile acids and/or salts thereof. Preferred bile acids/salts and fatty acids and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein by reference. Also preferred are combinations of penetration enhancers, for example, fatty acids/salts in combination with bile acids/salts. A particularly preferred combination is the sodium salt of lauric acid, capric acid and UDCA. Further penetration enhancers include polyoxyethylene-9-lauryl ether, polyoxyethylene-20-cetyl ether. Oligonucleotides of the invention may be delivered orally, in granular form including sprayed dried particles, or complexed to form micro or nanoparticles. Oligonucleotide complexing agents and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein by reference.

**[00229]** Compositions and formulations for parenteral, intrathecal or intraventricular administration may include sterile aqueous solutions that may also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

**[00230]** Certain embodiments of the invention provide pharmaceutical compositions containing one or more oligomeric compounds and one or more other chemotherapeutic agents that function by a non-antisense mechanism. Examples of such chemotherapeutic agents include but are not limited to cancer chemotherapeutic drugs such as daunorubicin, daunomycin, dactinomycin, doxorubicin, epirubicin, idarubicin, esorubicin, bleomycin, mafosfamide, ifosfamide, cytosine arabinoside, bischloroethyl- nitrosurea, busulfan, mitomycin C, actinomycin D, mithramycin, prednisone, hydroxyprogesterone, testosterone, tamoxifen, dacarbazine, procarbazine, hexamethylmelamine, pentamethylmelamine, mitoxantrone, amsacrine, chlorambucil, methylcyclohexylnitrosurea, nitrogen mustards, melphalan, cyclophosphamide, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-

azacytidine, hydroxyurea, deoxycoformycin, 4-hydroxyperoxycyclo-phosphoramide, 5-fluorouracil (5-FU), 5-fluorodeoxyuridine (5-FUdR), methotrexate (MTX), colchicine, taxol, vincristine, vinblastine, etoposide (VP-16), trimetrexate, irinotecan, topotecan, gemcitabine, teniposide, cisplatin and diethylstilbestrol (DES). When used with the compounds of the invention, such chemotherapeutic agents may be used individually (e.g., 5-FU and oligonucleotide), sequentially (e.g., 5-FU and oligonucleotide for a period of time followed by MTX and oligonucleotide), or in combination with one or more other such chemotherapeutic agents (e.g., 5-FU, MTX and oligonucleotide, or 5-FU, radiotherapy and oligonucleotide). Anti-inflammatory drugs, including but not limited to nonsteroidal anti-inflammatory drugs and corticosteroids, and antiviral drugs, including but not limited to ribivirin, vidarabine, acyclovir and ganciclovir, may also be combined in compositions of the invention. Combinations of antisense compounds and other non-antisense drugs are also within the scope of this invention. Two or more combined compounds may be used together or sequentially.

**[00231]** In another related embodiment, compositions of the invention may contain one or more antisense compounds, particularly oligonucleotides, targeted to a first nucleic acid and one or more additional antisense compounds targeted to a second nucleic acid target. For example, the first target may be a particular antisense sequence of Brain derived neurotrophic factor (BDNF), and the second target may be a region from another nucleotide sequence. Alternatively, compositions of the invention may contain two or more antisense compounds targeted to different regions of the same Brain derived neurotrophic factor (BDNF) nucleic acid target. Numerous examples of antisense compounds are illustrated herein and others may be selected from among suitable compounds known in the art. Two or more combined compounds may be used together or sequentially.

*Dosing:*

**[00232]** The formulation of therapeutic compositions and their subsequent administration (dosing) is believed to be within the skill of those in the art. Dosing is dependent on severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary depending on the relative potency of individual oligonucleotides, and can generally be estimated based on EC50s found to be effective *in vitro* and *in vivo* animal models. In general, dosage is from 0.01 μg to 10 mg per kg of body weight, and may be given once or more daily, weekly, monthly or yearly, or even once every 2 to 20 years. Persons of ordinary skill in the art can easily estimate repetition rates for dosing based on measured residence times and concentrations of the drug in bodily fluids or tissues. Following successful treatment, it

may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state, wherein the oligonucleotide is administered in maintenance doses, ranging from 0.01 μg to 10 mg per kg of body weight, once or more daily, to once every 2-20 years.

**[00233]** In embodiments, a patient is treated with a dosage of drug that is at least about 1, at least about 2, at least about 3, at least about 4, at least about 5, at least about 6, at least about 7, at least about 8, at least about 9, at least about 10, at least about 15, at least about 20, at least about 25, at least about 30, at least about 35, at least about 40, at least about 45, at least about 50, at least about 60, at least about 70, at least about 80, at least about 90, or at least about 10 mg/kg body weight. Certain injected dosages of antisense oligonucleotides are described, e.g., in U.S. Pat. No. 7,563,884, "Antisense modulation of PTP1B expression," incorporated herein by reference in its entirety.

**[00234]** While various embodiments of the present invention have been described above, it should be understood that they have been presented by way of example only, and not limitation. Numerous changes to the disclosed embodiments can be made in accordance with the disclosure herein without departing from the spirit or scope of the invention. Thus, the breadth and scope of the present invention should not be limited by any of the above described embodiments.

**[00235]** All documents mentioned herein are incorporated herein by reference. All publications and patent documents cited in this application are incorporated by reference for all purposes to the same extent as if each individual publication or patent document were so individually denoted. By their citation of various references in this document, Applicants do not admit any particular reference is "prior art" to their invention. Embodiments of inventive compositions and methods are illustrated in the following examples.

## EXAMPLES

**[00236]** The following non-limiting Examples serve to illustrate selected embodiments of the invention. It will be appreciated that variations in proportions and alternatives in elements of the components shown will be apparent to those skilled in the art and are within the scope of embodiments of the present invention.

*Example 1: Design of antisense oligonucleotides specific for a nucleic acid molecule antisense to a Brain derived neurotrophic factor (BDNF) and/or a sense strand of BDNF polynucleotide*

**[00237]** As indicated above the term "oligonucleotide specific for" or "oligonucleotide targets" refers to an oligonucleotide having a sequence (i) capable of forming a stable complex with a portion of the targeted gene, or (ii) capable of forming a stable duplex with a portion of an mRNA transcript of the targeted gene.

**[00238]** Selection of appropriate oligonucleotides is facilitated by using computer programs (e.g. IDT AntiSense Design, IDT OligoAnalyzer) that automatically identify in each given sequence subsequences of 19-25 nucleotides that will form hybrids with a target polynucleotide sequence with

a desired melting temperature (usually 50-60°C) and will not form self-dimers or other complex secondary structures.

**[00239]** Selection of appropriate oligonucleotides is further facilitated by using computer programs that automatically align nucleic acid sequences and indicate regions of identity or homology. Such programs are used to compare nucleic acid sequences obtained, for example, by searching databases such as GenBank or by sequencing PCR products. Comparison of nucleic acid sequences from a range of genes and intergenic regions of a given genome allows the selection of nucleic acid sequences that display an appropriate degree of specificity to the gene of interest. These procedures allow the selection of oligonucleotides that exhibit a high degree of complementarity to target nucleic acid sequences and a lower degree of complementarity to other nucleic acid sequences in a given genome. One skilled in the art will realize that there is considerable latitude in selecting appropriate regions of genes for use in the present invention.

**[00240]** An antisense compound is "specifically hybridizable" when binding of the compound to the target nucleic acid interferes with the normal function of the target nucleic acid to cause a modulation of function and/or activity, and there is a sufficient degree of complementarity to avoid non-specific binding of the antisense compound to non-target nucleic acid sequences under conditions in which specific binding is desired, i.e., under physiological conditions in the case of *in vivo* assays or therapeutic treatment, and under conditions in which assays are performed in the case of in vitro assays.

**[00241]** The hybridization properties of the oligonucleotides described herein can be determined by one or more *in vitro* assays as known in the art. For example, the properties of the oligonucleotides described herein can be obtained by determination of binding strength between the target natural antisense and a potential drug molecules using melting curve assay.

**[00242]** The binding strength between the target natural antisense and a potential drug molecule (Molecule) can be estimated using any of the established methods of measuring the strength of intermolecular interactions, for example, a melting curve assay.

**[00243]** Melting curve assay determines the temperature at which a rapid transition from double-stranded to single-stranded conformation occurs for the natural antisense/Molecule complex. This temperature is widely accepted as a reliable measure of the interaction strength between the two molecules.

**[00244]** A melting curve assay can be performed using a cDNA copy of the actual natural antisense RNA molecule or a synthetic DNA or RNA nucleotide corresponding to the binding site of the Molecule. Multiple kits containing all necessary reagents to perform this assay are available (e.g. Applied Biosystems Inc. MeltDoctor kit). These kits include a suitable buffer solution containing one of the double strand DNA (dsDNA) binding dyes (such as ABI HRM dyes, SYBR Green,

SYTO, etc.). The properties of the dsDNA dyes are such that they emit almost no fluorescence in free form, but are highly fluorescent when bound to dsDNA.

**[00245]** To perform the assay the cDNA or a corresponding oligonucleotide are mixed with Molecule in concentrations defined by the particular manufacturer's protocols. The mixture is heated to 95 °C to dissociate all pre-formed dsDNA complexes, then slowly cooled to room temperature or other lower temperature defined by the kit manufacturer to allow the DNA molecules to anneal. The newly formed complexes are then slowly heated to 95 °C with simultaneous continuous collection of data on the amount of fluorescence that is produced by the reaction. The fluorescence intensity is inversely proportional to the amounts of dsDNA present in the reaction. The data can be collected using a real time PCR instrument compatible with the kit (e.g.ABI's StepOne Plus Real Time PCR System or lightTyper instrument, Roche Diagnostics, Lewes, UK).

**[00246]** Melting peaks are constructed by plotting the negative derivative of fluorescence with respect to temperature (-d(Fluorescence)/dT) on the y-axis) against temperature (x-axis) using appropriate software (for example lightTyper (Roche) or SDS Dissociation Curve, ABI). The data is analyzed to identify the temperature of the rapid transition from dsDNA complex to single strand molecules. This temperature is called Tm and is directly proportional to the strength of interaction between the two molecules. Typically, Tm will exceed 40 °C.

*Design of modified AntagoNAT molecules:*

**[00247]** A number of DNA based antisense oligonucleotides were designed and tested, termed AntagoNATs, targeting noncoding Bdnf-AS and other antisense transcripts. Various AntagoNATs were designed ranging from 12 to 20 nucleotides in length with or without full phosphorothioate modification plus/minus 2-O'-methyl RNA or LNA modified nucleotides. highest efficacy was observed on Bdnf mRNA level with 16-nucleotide phosphorothioate gapmer with three LNA-modified nucleotides at each end (XXXnnnnnnnnnnXXX). For blocking interactions between human BDNF sense-antisense transcripts, 14-nucleotide mixmers containing both LNA and 2-O'-methyl RNA molecules were used. Although these 2-O'-methyl RNA-modified oligonucleotides are suggested to only block the RNA, marginal downregulation of targeted RNAs was observed in this experiment (Figure 11). Sequences of various AntagoNATs, as well as all other siRNAs, primers and probes used for these studies are listed in Table 1.

*Example 2: Modulation of BDNF polynucleotides*

**[00248]** All antisense oligonucleotides used in Example 2 were designed as described in Example 1. The manufacturer (IDT Inc. of Coralville, IA) was instructed to manufacture the designed phosphothioate bond oligonucleotides and provided the designed phosphothioate analogs shown in Table 1. The asterisk designation between nucleotides indicates the presence of phosphothioate bond. The oligonucleotides required for the experiment in Example 2 can be synthesized using any

appropriate state of the art method, for example the method used by IDT: on solid support, such as a 5 micron controlled pore glass bead (CPG), using phosphoramidite monomers (normal nucleotides with all active groups protected with protection groups, e.g. trityl group on sugar, benzoyl on A and C and N-2-isobutyryl on G). Protection groups prevent the unwanted reactions during oligonucleotide synthesis. Protection groups are removed at the end of the synthesis process. The initial nucleotide is linked to the solid support through the 3'carbon and the synthesis proceeds in the 3' to 5'direction. The addition of a new base to a growing oligonucleotide chain takes place in four steps: 1) the protection group is removed from the 5' oxygen of the immobilized nucleotide using trichloroacetic acid; 2) the immobilized and the next-in-sequence nucleotides are coupled together using tetrazole; the reaction proceeds through a tetrazolyl phosphoramidite intermediate; 3) the unreacted free nucleotides and reaction byproducts are washed away and the unreacted immobilized oligonucleotides are capped to prevent their participation in the next round of synthesis; capping is achieved by acetylating the free 5' hydroxyl using acetic anhydride and N-methyl imidazole; 4) to stabilize the bond between the nucleotides the phosphorus is oxidized using iodine and water, if a phosphodiester bond is to be produced, or Beaucage reagent (3H-1,2-benzodithiol-3-one-1,1-dioxide), if a phosphothioate bond is desired. By alternating the two oxidizing agents, a chimeric backbone can be constructed. The four step cycle described above is repeated for every nucleotide in the sequence. When the complete sequence is synthesized, the oligonucleotide is cleaved from the solid support and deprotected using ammonium hydroxide at high temperature. Protection groups are washed away by desalting and the remaining oligonucleotides are lyophilized.

*Treatment of Hek293 cells with different siRNA to quantify the amount of BDNF mRNA*

**[00249]** 1. Hek293 cells from ATCC (cat# CRL-1573) were grown in MEM/EBSS (Hyclone cat #SH30024) +10% FBS+ penicillin+streptomycin at 37°C and 5% $CO_2$. One day before the experiment the cells were replated at the density of $5x10^5$/well into 6 well plates and incubated at 37°C and 5% $CO_2$.

2. On the day of the experiment the media in the 6 well plates was changed to fresh MEM/EBSS +10% FBS.

3. All BDNF-AntagoNAT (oligonucleotide antisense of BDNF-AS) were diluted to the concentration of 20 uM and the BDNF-AS siRNA (siRNA complementary of BDNF-AS at 10uM; both oligonucleotide compounds are manufactured by IDT. To dose one well, 2 µl of this solution was incubated with 400 µl of Opti-MEM media (Gibco cat#31985-070) and 4 ul of Lipofectamine 2000 (Invitrogen cat# 11668019) at room temperature for 20 min and applied drop wise to one well of the 6 well plates with HepG2 cells. Similar mixture including 2 µl of water instead of the oligonucleotide solution was used for the mock-transfected controls.

4. After 3-18 h of incubation at 37°C and 5% $CO_2$ the media was changed to fresh MEM/EBSS +10% FBS+ penicillin+streptomycin.

5. 48h after addition of antisense oligonuclotides was performed. The media was then removed and RNA was extracted from the cells using SV Total RNA Isolation System from Promega (cat # Z3105) or RNeasy Total RNA Isolation kit from Qiagen (cat# 74181) following the manufacturers' instructions.

6. 200-400 ng of extracted RNA was added to the reverse transcription reaction performed using random hexamers, 2.5 mM mixture of dNTP, $MgCl_2$ and appropriate buffer. The cDNA (20-40ng) from this reverse transcription reaction was used to monitor gene expression by real time PCR using ABI Taqman Gene Expression Mix (cat#4369510) and 300nM of forward and reverse primers, and 200nM of probe in a final reaction volume of 15µl. The primers/probes were designed using FileBuilder software (Applied Biosystem). Primers were strand specific for sense-antisense pairs and the probes covered exon boundaries to eliminate the chance of genomic DNA amplification. The ABI assay for human BDNF was Applied Biosystems Taqman Gene Expression Assay: Hs00542425_s1 (BDNF) by Applied Biosystems Inc., Foster City CA). The following PCR cycle was used: 50°C for 2 min, 95°C for 10 min, 40 cycles of (95°C for 15 seconds, 60°C for 1 min) using GeneAmp 7900 Machine (Applied Biosystems). Fold change in gene expression after treatment with antisense oligonucleotides was calculated based on the difference in 18S-normalized dCt values between treated and mock-transfected samples.

7. Detection oligos for BDNF-AS:

ABI assay ID Hs00417345_m1

Context sequence GCACACCTGGAGATACTCTATTATA (SEQ ID No.: 65)

8. Detection oligos for BDNF:

ABI assay ID Hs00542425_s1

CCTGCAGAATGGCCTGGAATTACAA (SEQ ID No.: 66)

Detection oligos for BDNF-AS: ABI assay ID Hs00417345_m1

Context sequence GCACACCTGGAGATACTCTATTATA (SEQ ID No.: 65)

Detection oligos for BDNF: ABI assay ID Hs00542425_s1

CCTGCAGAATGGCCTGGAATTACAA (SEQ ID No.: 66)

9. The results are based on cycle threshold (Ct) values. The calculated differences between the Ct values for experimental and references genes (18S RNA) as ddCt and graphed as a percentage of each RNA to calibrator sample.

*Results:* Transfection of several human and mouse cells lines including HEK293T cells with different siRNA that target non-overlapping regions of the BDNF-AS transcript show a 2-6 fold upregulation of the BDNF transcript (Figure 1a and Figure 6) at 48h. The up-regulation of BDNF

was not related to the choice of endogenous controls (Figure 5a-b). The up-regulation of did not affect the regulation of other BDNF neighboring genes (Figure 9).

**[00250]** Figure 5 shows BDNF-AS knockdown leads to BDNF mRNA upregulation. Knockdown of BDNF-AS, using siRNAs-1 (10 nM) targeting the non-overlapping region of the BDNF-AS transcript, caused a 6-fold upregulation of BDNF (sense) mRNA (****= $P < 0.0001$). Results depicted here were obtained from experiments in HEK293T cells, using beta actin (left panel) or 18S rRNA (right panel) as endogenous controls and the mock transfection as a reference sample. This experiment is intended to show that choice of endogenous controls or reference calibrator sample does not change the observed upregulation of BDNF mRNA.

**[00251]** Figure 6 shows Posttranscriptional regulation of Bdnf expression. Transfected N2a cells with combination of mBdnf-AntagoNAT9 targeting mouse Bdnf-AS transcript and Drosha siRNA targeting Drosha protein, which is involved in microRNA (miRNA) processing. Bdnf mRNA upregulation was observed following treatment of cells with mBdnf-ANtagoNAT9 (*** = p value <0.0001). Addition of Drosha siRNA marginally increased Bdnf transcript over mBdnf-AntagoNAT9 treatment (*= p value <0.05). This experiment may suggest involvement of other post-transcriptional mechanisms, such as miRNAs in regulation of Bdnf transcript.

**[00252]** Figure 9 shows BDNF-AS knockdown neither changes the level of TrkB nor BDNF neighboring genes (Let7C and KIF18A) in both directions: LIN7C and KIF18A are genes located 3' downstream and 5' upstream of BDNF, respectively. Neurotrophic tyrosine kinase, receptor, type 2 (TrkB) encodes a membrane-bound receptor for BDNF and is located on a different chromosome (Chr-9) as BDNF. It was determined whether these genes were altered upon depletion of the BDNF-AS transcript. HEK293T cells were transfected with control siRNA or BDNF-AS siRNA and measured several transcript levels. It was observed that the BDNF-AS transcript was downregulated and that BDNF mRNA was upregulated as indicated elsewhere in this manuscript. It was found that the knockdown of BDNFAS has no effect on TrkB expression or on the neighboring genes Let7C and KIF18A. These data suggest that upon BDNF-AS depletion, there is a locus-specific alteration of BDNF expression.

*Treatment of Hek293 cells with one siRNA in a time course of 0-96h to quantify the amount of BDNF and BDNF-AS*

**[00253]** The methodology followed was same as in Treatment of Hek293 cells with siRNA but this time the cells are harvested at 0 to 96h after addition of the oligos.

*Results:* The time course of BDNF and BDNF-AS expression shows an optimum up-regulation of BDNF due to the siRNA at 48h simultaneously to an optimum downregulation of the BDNF-AS (Figure 1b).

*Treatment of Hek293 cells with different hBDNF-AntagoNATs to quantify the amount of BDNF and BDNF-AS*

**[00254]** The methodology followed was same as in Treatment of Hek293 cells with siRNA but this time the cells are treated with AntagoNATs.

*Results:* The BDNF-AS transcript contains a 225-nucleotide overlapping region that has full complementarity to the BDNF mRNA. TheRNA-RNA interactions may be responsible for the discordant regulation of BDNF by its antisense transcript. To determine the regulatory role of BDNF-AS on BDNF mRNA, the gapmers (AntagoNATs) containing both LNA and 2'OMe RNA modifications were utilized to block the interaction between sense and antisense transcripts. The overlapping region was covered by tiling hBDNF-AatagoNATs. It was found that the use of hBDNF-AntagoNATs upregulates the BDNF mRNA. A marginal downregulation of BDNF-AS transcript was observed, which was not expected for 2'OMe-RNA containing blocking oligos. The 16 hBDNF-AntagoNATs were tested and it was found that blocking the first half of the BDNF-AS overlapping region has a greater effect on the upregulation of BDNF mRNA. Specifically, hBDNF-AntagoNAT1 and hBDNFAntagoNAT4 caused significant upregulation of BDNF mRNA. Unlike synthetic siRNAs, antisense oligonucleotides are single-stranded and can be shorter in length; therefore, reducing non-specific (off-target) binding effects. Single-stranded locked nucleic acid (LNA)-modified oligonucleotides are generally more effective, in vivo, compared to unmodified siRNAs (Figure 7).

*Treatment of mouse N2a cells with different mBDNF-AntagoNATs to quantify the amount of BDNF and BDNF-AS*

**[00255]** The methodology followed was same as in Treatment of Hek293 cells with different hBDNF-AntagoNATs to quantify the amount of BDNF and BDNF-AS but this time the cells are N2a cells. Furthermore, the following PCR cycle was used: 50°C for 2 min, 95°C for 10 min, 50 cycles of (95°C for 15 seconds, 60°C for 1 min) using GeneAmp 7900 Machine (Applied Biosystems).

**[00256]** Results Figure 8 shows Inhibition of the mouse Bdnf-AS transcript in N2a cells, by AntagoNATs: that blocking of the overlap region between human BDNF sense and antisense transcripts upregulates BDNF mRNA levels. It was then determined if a similar regulatory mechanism exists in a mouse cell line and tested 11 mBdnf-AntagoNATs that target the mouse Bdnf-AS transcript. mBdnf-AntagoNATs contain a phosphorothioate backbone and three LNA-modified nucleotides at both 3' and 5' ends. Control oligonucleotides have a similar backbone and modifications, but do not target any sequence in the mammalian genomes. Two mBdnf-AntagoNATs (mBdnf-AntagoNA3 and mBdnf-AntagoNAT-9) were able to increase Bdnf mRNA levels in N2a cells. In sum, blocking the mouse Bdnf-AS transcript with single-stranded

AntagoNATs (16-mer) caused an upregulation of Bdnf mRNA levels in mouse N2a cells. These data suggest that the antisense transcript of Bdnf exerts a suppressive effect on Bdnf mRNA.

*Treatment of Hek293 cells with different siRNA to quantify the BDNF protein*

**[00257]** The methodology followed was same as in Treatment of Hek293 cells with different siRNA to quantify the amount of BDNF mRNA, except at step 5 where, 48h after addition of siRNA was performed. The media was then removed and cells were disrupted and their levels of BDNF protein was quantified by ELISA (Figure 1c) and western blot (Figure 1d).

**[00258]** *Western blot:* HEK293T cells were transfected with 10 nM of BDNF-AS, or control siRNA. The cells were disrupted, 48 h post transfection, with 200 µl of Laemmli sample buffer (Biorad) containing 350 mM DTT. 20 µl of the lysate was separated on a 10% SDS PAGE and transferred it to a nitrocellulose membrane overnight. Then the incubated the membrane with primary antibody for MecP2 (Abcam), BDNF (Promega, catalog number G164B) and secondary antibody conjugated to HRP. After addition of HRP substrate, the chemiluminescent signal was detected with X-ray film. The same membrane was stripped and reused it for detection of β-Actin as a loading control.

**[00259]** *ELISA:* Cells were transfected with 20 nM of BDNF-AS siRNA or control siRNA. The cell supernatant was collected for ELISA experiments. Alternatively, total protein was extracted from mouse brain tissues embedded in protein extraction buffer plus protease inhibitors (BCA kit, Fisher) and homogenized with the bioruptor and metal beads. Total protein was measured using BCA protein assay kit (Pierce catalog number 23227) and sample loads were normalized to total protein concentrations. the ELISA kits were purchased for human BDNF from Promega (catalog number G7611) or mouse Bdnf Millipore (catalog number CYT306) and ELISA was performed following the supplier's protocol. Average absorbance was subtracted of three repeats at 450 nm from background and normalized it to the control sample.

*Treatment of Hek293 cells (not sure) with different concentrations of mBDNF-AntagoNAT9 to quantify the BDNF mRNA*

**[00260]** The methodology followed was same as in Treatment of Hek293 cells with different siRNA to quantify the amount of BDNF mRNA except at step 3, where all mBDNF-AntagoNAT9 was diluted to different concentration such as the final of 11 different concentrations were applied to the cells (1:3 serial dilutions ranging from 300nM to 5pM) using the same proportional amounts of Lipofectamine 2000 (Invitrogen cat# 11668019) as in Treatment of Hek293 cells with different siRNA to quantify the amount of BDNF mRNA, using the in a same volume of Opti-MEM media (Gibco cat#31985-070). This was performed at room temperature for 20 min and applied dropwise to one well of the 6 well plates with HepG2 cells. Similar mixture including water instead of the oligonucleotide solution was used for the mock-transfected controls.

*Results:* As shown here in Figure 1e, there is a dose-dependent up-regulation of BDNF when BDNF-AS is targeted by mBDNF-AntagoNAT9.

**[00261]** Figure 1 shows Antisense-mediated regulation of sense mRNA and protein. (A) Knockdown of brain derived neurotrophic factor (BDNF) natural antisense transcript, BDNF-AS, in HEK293T cells (n=12 per treatment) with each of three unique siRNAs (10 nM) targeting the non-overlapping region of BDNF-AS transcript, caused 2-6 fold upregulation of BDNF (sense) mRNA (n=6 for each data point/treatment ***= $P < 0.001$, **= $P < 0.01$). Similar results were obtained from experiments using Human cortical neuron (HCN), glioblastoma (MK059) cells, mouse N2a cells and neurospheres "data not shown". Scrambled sequences, mock transfection and control siRNAs were used as controls. Control siRNA for this and other experiments is an inert siRNA (CCUCUCCACGCGCAGUACATT) that does not target any known sequence in the mammalian genome. All measurements were normalized to the 18S rRNA and graphed as a percentage of each mRNA to the negative siRNA control sample.

**[00262]** (B) Changes in BDNF and BDNF-AS transcripts were assessed over a period of time, following BDNF-AS knockdown (n=6 for each data point/treatment). siRNA knockdown of human BDNF-AS resulted in efficient and consistent downregulation of BDNF-AS, starting at 6 h and continuing on to 72 h. BDNF mRNA levels rose at 18 h, remaining high for more than 72 h, reversing to pre-treatment levels at 96 h. Note that the peak at 48 h is consistent and reproducible. Although BDNF-AS knockdown begins after 6 h, upregulation of BDNF started 18 h post-treatment. This time lag between the depletion of BDNF-AS and the increase of BDNF mRNA shows the sequential order of events indicating that the cells require time to adapt to the removal of the antisense transcript before upregulating BDNF.

**[00263]** (C) siRNA-mediated knockdown of BDNF-AS transcript caused an increase in BDNF protein levels measured by ELISA. Cells were transfected with 10 nM of two active siRNAs for BDNF-AS, scrambled siRNAs or a control siRNA for 48 hours. The supernatants of these cells were concentrated and analyzed for BDNF protein by ELISA, using a commercially available kit. BDNF protein was significantly increased (n=6 per treatment, ***=$P < 0.0001$, **= $P<0.001$) with siRNA targeting BDNF-AS transcript.

**[00264]** (D) Western blots confirmed that knockdown of the non-protein-coding BDNF-AS, with BDNF-AS siRNA1, but not control non-targeting siRNA transcript increased BDNF protein levels without changing the levels of beta-actin. Collectively, these data suggest that there is a discordant relationship between the sense and antisense BDNF transcripts in which BDNF-AS suppresses the expression of BDNF mRNA and protein. Removal of this negative regulatory effect, by BDNF-AS knockdown, causes upregulation of BDNF mRNA and protein levels.

**[00265]** (E) Dose-dependent increases in Bdnf following Bdnf-AS depletion: dose response experiments were performed using 11 different concentrations (1:3 serial dilutions ranging from 300nM to 5pM) of mBdnf-AntagoNAT9 (n=6 per data point/treatment) and a dose-dependent increase was observed in Bdnf mRNA levels at 1-300 nM concentration with an EC50 of 6.6 nM.

*Treatment of hippocampal neurospheres with siRNA*

**[00266]** *Dissecting mouse hippocampal neural stem cells in neurospheres:* neuronal stem cells were separated from the hippocampus of mouse pups, P0-P1. The hippocampi were mechanically separated to single cells, collected by short spins and grown in a mixture of DMEM and F12, containing glutamine, antibiotics, B27 solution and 0.001 mM concentration of both EGF and FGF. After 3-4 days floating neurospheres formed. 100,000 cells were plated in 24-well plates coated with poly-L-Lysine (PLL). The plating of neurosphere cells onto PLL will start the differentiation process. On the third day post-plating, growth factors were removed from the medium and allowed the cells to grow for 4 more days (7 days post-plating). By this time, the cell culture had a mix of neural cell lineages consisting of astrocytes, neurons, oligodendrocytes and their progenitors making it more similar to mature brain tissue. The expression of Bdnf and Bdnf-AS was measured in floating neurospheres as well as in 3 and 7 days post-plating cultures. Knockdown experiments were performed, using either 50 nM siRNAs or 20 nM antisense oligonucleotides targeting Bdnf-AS transcript, at 3 or 7 days post-plating. Neural stem cells are also seeded in immunocytochemistry chambers, (18,000 cell per well) in a total volume of 80 µl. Neurospheres were then transfected, using the same protocol, to assess the functional effects of Bdnf-AS knockdown on murine primary cells. After 48-72 h, cells were fixed with paraformaldehyde (4%) for 20 min and washed with 1X PBS several times. After blocking with FBS, neurospheres were incubated with primary antibody (Monoclonal Rabbit β tubulin III, TUJ1) at a 1:2000 concentration overnight. Fixed cells were incubated with secondary antibody, labeled with Alexafluor 568 (goat anti-rabbit IgG, 2mg/ml, at concentration of 1:5000). Nuclei were stained with Hoechst stain. Images were obtained by immunofluorescence antigen detection microscopy.

*Targeting of BDNF-AS by AntagoNATs:*

**[00267]** The term AntagoNAT is used here to describe single-stranded oligonucleotide molecules that inhibit sense-antisense interactions (with different modifications, see supplementary methods). Single–stranded gapmer were designed, oligonucleotides, 14 nucleotides in length, with 2'O-Methyl RNA and/or locked nucleic acid (LNA) modifications. Using this strategy, we tiled the entire overlapping region between human BDNF-AS and BDNF transcripts and identified several efficacious AntagoNATs capable of upregulating of BDNF mRNA. hBDNF-AntagoNAT1 and hBDNF-AntagoNAT4, targeting the first part of the overlapping region, produced the largest

response. The data suggests that blockage of BDNF antisense RNA, by single-stranded AntagoNATs, is sufficient in causing an increase in BDNF mRNA.

**[00268]** Then single-stranded gapmer were designed, LNA-modified, 15 DNA oligonucleotides (AntagoNATs) 16-nucleotides in length with phosphorothioate backbone, complementary to mouse Bdnf-AS. Two AntagoNATs (mBdnf-AntagoNAT3 and mBdnf-AntagoNAT9) consistently showed a statistically significant increase in Bdnf mRNA levels in mouse N2a cells (Fig. 7).

**[00269]** Figure 7 shows Inhibition of the human BDNF-AS transcript by hBDNFAntagoNAT: The BDNF-AS transcript contains a 225-nucleotide overlapping region that has full complementarity to the BDNF mRNA. RNA-RNA interactions may be responsible for the discordant regulation of BDNF by its antisense transcript. To determine the regulatory role of BDNF-AS on BDNF mRNA, gapmers (AntagoNATs) containing both LNA and 2'OMe RNA modification were utilized to block the interaction between sense and antisense transcripts. overlapping region was covered by tiling hBDNF-AatagoNATs. It was found that the use of hBDNF-AntagoNATs upregulates the BDNF mRNA. Marginal downregulation of BDNF-AS transcript was observed, which was not expected for 2'OMe-RNA containing blocking oligos. 16 hBDNF-AntagoNATs (14-mers each with the sequences provided below) were tested and it was found that blocking the first half of the BDNF-AS overlapping region has a greater effect on the upregulation of BDNF mRNA. Specifically, hBDNF-AntagoNAT1 and hBDNFAntagoNAT4 caused significant upregulation of BDNF mRNA. Unlike synthetic siRNAs, antisense oligonucleotides are single-stranded and can be shorter in length; therefore, reducing non-specific (off-target) binding effects. Single-stranded locked nucleic acid (LNA)-modified oligonucleotides are generally more effective, in vivo, compared to unmodified siRNAs.Bdnf upregulation increases neuronal outgrowth.

*Bdnf upregulation increases neuronal outgrowth:*

**[00270]** Consistent with many previous reports that indicate stimulatory effects of Bdnf on neuronal outgrowth and adult neurogenesis16-17, it was found that an increase in the endogenous Bdnf level due to the knockdown of Bdnf-AS transcript resulted in increased neuronal cell number and in neurite outgrowth and maturation at 3 and 7 days post-plating in neurospheres (Fig. 5 a-d). These data suggest that the upregulation of endogenous Bdnf, due to inhibition of antisense RNA, induces neuronal differentiation in neuronal progenitor cells and might cause a mature phenotype in nascent neurons.

**[00271]** *Results:* Figure 2 shows Bdnf upregulation increases neuronal outgrowth (A-B) Immunocytochemistry images of hippocampal neurospheres treated with either control siRNA (A) or Bdnf-AS siRNA (B) 3 d post-platting. (C-D) Immunocytochemistry images of neuronal

maturation and neurite outgrowth in hippocampal neurospheres treated with either control siRNA (C) or Bdnf-AS siRNA (D) 7 d post-plating. Treatment of cells with siRNA targeting the Bdnf-AS transcript resulted in increased neuronal cell number as well as increase in neurite outgrowth and maturation, both at 3d or 7d postplating neurospheres. B-tubulin III stained red, GFAP stained green and DAPI stained blue.

*Delivery intracerebroventicular (ICV) of mBDNF-* AntagoNAT9 using osmotic mini-pumps knockdown BDNF-AS and up-regulate BDNF

**Mouse studies**: 10 eight-week-old male C57BL/6 mice were used for *in vivo* experiments. The mice were prepared with chronic indwelling cannulae in the dorsal third ventricle implanted subcutaneously with osmotic mini-pumps that delivered continuous infusions (0.11 microliter/h) of synthetic antisense oligonucleotide directed against *Bdnf*-AS (m*Bdnf*-AntagoNAT9) or control oligonucleotide (inert sequence that does not exist in human or mouse) at a dose of 1.5 mg/kg/d for 4 weeks. Tubing was connected to the exit port of the osmotic minipump and tunneled subcutaneously to the indwelling cannula, such that the treatments were delivered directly into the brain. At 5 d post-implantation all animals received daily intra-peritoneal (IP) injection of BrdU (80 mg/kg), for five consecutive days. At the 28th day post-surgery, the animals were sacrificed and three tissues were excised (hippocampus, frontal cortex and cerebellum) from each mouse brain for quantitative RNA measurements.

*Knockdown of Bdnf-AS increases Bdnf in vivo:*

**[00272]** Osmotic mini-pumps for intracerebroventricular (ICV) delivery of mBdnf-AntagoNAT9 to C57BL/6 mice were utilized. mBdnf-AntagoNAT9 were then selected, which is targeting a non-overlapping region of mouse Bdnf-AS, over other active AntagoNATs, based on its high efficacy to increase in Bdnf mRNA in vitro. After 28 days of continuous AntagoNAT infusion, Bdnf mRNA levels were increased across forebrain regions adjacent to the third ventricle in mice treated with mBdnf-AntagoNAT9 as compared to levels unaltered by an inert control oligonucleotide (Fig. 3a,b). Bdnf and Bdnf-AS transcripts were unaltered in the hypothalamus, a structure that is not immediately adjacent to the third ventricle (Fig. 3c). Moreover, it was found that AntagoNAT-mediated blockade of Bdnf-AS results increased Bdnf protein levels (Fig 3 d,e). These findings correspond with the in vitro data described above and indicate that the blockade of Bdnf-AS results in the increase of Bdnf mRNA and protein expression in vivo.

*RNA extraction and RT-PCR of the mouse brain samples:* Mice were euthanized after 28 days and the brains were excised. One hemibrain from each mouse was fixed in 4% formaldehyde overnight for histological studies. Another hemibrain was excised for RNA quantitative measurement from the hippocampus, frontal cortex and cerebellum. RNA was extracted after homogenization in Trizol reagent (Invitrogen, 15596-026) according to the manufacturer's protocol. The aqueous phase was

separated and added an equal volume of 70% ethanol before passing the samples through Qiagen RNeasy columns (QIAGEN, 74106) and those RNA samples were subjected to on-column DNAse treatment for removal of DNA contamination. 400 ng of each sample was used for the first strand cDNA synthesis and RT-PCR measurements were carried out. The percentile changes were plotted in RNA levels, for individual tissues as compared to control mice, in each graph.

**[00273]** *Results:* Figure 3 shows Bdnf-AS regulates Bdnf mRNA and protein in vivo; (A-C) Using osmotic mini-pumps, mBdnf-AntagoNAT9 (CAACATATCAGGAGCC) or control oligonucleotide (CCACGCGCAGTACATG) was infused constantly over a period of 28 d, into the third ventricle of mouse brain (n=5 per treatment group *= $P < 0.05$, **= $P < 0.01$, ***= $P < 0.0001$) . mBdnf-AntagoNAT9 directed against Bdnf-AS but not the control oligonucleotide resulted in an increase in Bdnf levels in the hippocampus (A) and frontal cortex (B). In the hypothalamus (C) both transcripts were unchanged, as was expected for a tissue that is not directly connected to the third ventricle of the brain. (D-E) BDNF protein levels were assessed by ELISA and found that mBdnf-AntagoNAT9 treatment results in an increase in BDNF protein, both in the hippocampus (D) and frontal cortex (E), as compared to control oligonucleotide treated mice.

*Delivery intracerebroventicular (ICV) ofmBDNF- AntagoNAT9 using osmotic mini-pumps knockdown BDNF-AS and up-regulate BDNF*

**[00274]** BrdU was injected in the mice treated with mBdnf-AntagoNAT9 in the first week of the study for 5 days. After 28 days of continuous AntagoNAT infusion, histological examination of brain tissues was performed and neuronal proliferation was quantified and survival using Ki67 and BrdU markers, respectively. In mice treated with mBdnf- AntagoNAT9, an increase was observed in Ki67 positive (proliferating) cells as compared to control treated mice (Fig 4a,b). the number of Ki67 positive cells was quantified and a significant increase in cell proliferation was found in mice treated with mBdnf-AntagoNAT9 compared to control oligonucleotide (Fig. 4c). In mice treated with mBdnf-AntagoNAT9, there was a significant increase in BrdU incorporation (surviving cells) as compared to the control oligonucleotide- treated mice (Fig. 4d). There were no differences in hippocampal volume between control and mBdnf-AntagoNAT9 treated mice (Fig. 4e). These findings demonstrate that Bdnf-AS regulates Bdnf levels in vivo.

**[00275]** *Results:* Figure 4 shows Blocking of Bdnf-AS, in vivo, causes an increase in neuronal survival and proliferation; (A-B) mice were treated with mBdnf-AntagoNAT9 or control oligos. After 28 d of continuous mBdnf-AntagoNAT9 infusion, histological examination of brain tissues was performed, using Ki67. Ki67 is the marker of proliferating cells in hippocampus and an increase in the number of proliferating cells was observed in mice received Bdnf- AntagoNAT treatment compare to mice received control oligos. In mice treated with mBdnf-AntagoNAT9 (B), there was an increase in Ki67 positive cells (proliferating cells), as compared to control treated mice

(A). (C) Mice treated with mBdnf-AntagoNAT9 had a significant increase in the number of Ki67 positive cells as compared to control treated mice. (D) In mice treated with mBdnf-AntagoNAT9, there was a significant increase in the number of surviving cells (BrdU positive) as compared to control oligonucleotide treated mice. (E) There were no differences in hippocampal volume between control and mBdnf- AntagoNAT9 treated mice. Together these data (n=5 per treatment group *= $P < 0.05$, ***= $P < 0.001$) demonstrates that Bdnf-AS regulates Bdnf levels in vivo and that blocking Bdnf sense-antisense interactions results in an increase in neuronal lineage, proliferation and survival.

**[00276]** Although the invention has been illustrated and described with respect to one or more implementations, equivalent alterations and modifications will occur to others skilled in the art upon the reading and understanding of this specification and the annexed drawings. In addition, while a particular feature of the invention may have been disclosed with respect to only one of several implementations, such feature may be combined with one or more other features of the other implementations as may be desired and advantageous for any given or particular application.

**[00277]** The Abstract of the disclosure will allow the reader to quickly ascertain the nature of the technical disclosure. It is submitted with the understanding that it will not be used to interpret or limit the scope or meaning of the following claims.

## CLAIMS

What is claimed is:

1. A method of modulating a function of and/or the expression of a Brain derived neurotrophic factor (BDNF) polynucleotide in a biological system comprising: contacting said system with at least one antisense oligonucleotide 5 to 30 nucleotides in length wherein said at least one oligonucleotide has at least 50% sequence identity to a reverse complement of a natural antisense of a Brain derived neurotrophic factor (BDNF) polynucleotide; thereby modulating a function of and/or the expression of the Brain derived neurotrophic factor (BDNF) polynucleotide , with the proviso that the oligonucleotides having SEQ ID NOS 50-55 are excluded..

2. A method of modulating a function of and/or the expression of a Brain derived neurotrophic factor (BDNF) polynucleotide in a biological system according to claim 1 comprising: contacting said biological system with at least one antisense oligonucleotide 5 to 30 nucleotides in length wherein said at least one oligonucleotide has at least 50% sequence identity to a reverse complement of a polynucleotide comprising 5 to 30 consecutive nucleotides within the natural antisense transcript nucleotides 1 to 1279 of SEQ ID NO: 3 or 1 to 1478 of SEQ ID NO: 4 or 1 to 1437 of SEQ ID NO: 5 or 1 to 2322 of SEQ ID NO: 6 or 1 to 2036 of SEQ ID NO: 7 or 1 to 2364 of SEQ ID NO: 8 or 1 to 3136 of SEQ ID NO: 9 or 1 to 906 of SEQ ID NO: 10 or 1 to 992 of SEQ ID NO: 11 , with the proviso that the oligonucleotides having SEQ ID NOS 50-55 are excluded. thereby modulating a function of and/or the expression of the Brain derived neurotrophic factor (BDNF) polynucleotide.

3. A method of modulating a function of and/or the expression of a Brain derived neurotrophic factor (BDNF) polynucleotide in patient cells or tissues *in vivo* or *in vitro* comprising: contacting said cells or tissues with at least one antisense oligonucleotide 5 to 30 nucleotides in length wherein said oligonucleotide has at least 50% sequence identity to an antisense oligonucleotide to the Brain derived neurotrophic factor (BDNF) polynucleotide; thereby modulating a function of and/or the expression of the Brain derived neurotrophic factor (BDNF) polynucleotide in patient cells or tissues *in vivo* or *in vitro,* , with the proviso that the oligonucleotides having SEQ ID NOS 50-55 are excluded.

4. A method of modulating a function of and/or the expression of a Brain derived neurotrophic factor (BDNF) polynucleotide in patient cells or tissues according to claim 3 comprising: contacting said biological system with at least one antisense oligonucleotide 5 to 30 nucleotides in length wherein said at least one oligonucleotide has at least 50% sequence identity to a reverse complement of a polynucleotide comprising 5 to 30 consecutive nucleotides within the natural antisense transcript nucleotides 1 to 1279 of SEQ ID NO: 3 or 1 to 1478 of SEQ ID NO: 4 or 1 to 1437 of SEQ ID NO: 5 or 1 to 2322 of SEQ ID NO: 6 or 1 to 2036 of SEQ ID NO: 7 or 1 to 2364 of SEQ ID

NO: 8 or 1 to 3136 of SEQ ID NO: 9 or 1 to 906 of SEQ ID NO: 10 or 1 to 992 of SEQ ID NO: 11, , with the proviso that the oligonucleotides having SEQ ID NOS 50-55 are excluded, thereby modulating a function of and/or the expression of the Brain derived neurotrophic factor (BDNF) polynucleotide.

5. A method of modulating a function of and/or the expression of a Brain derived neurotrophic factor (BDNF) polynucleotide in a biological system comprising: contacting said system with at least one antisense oligonucleotide that targets a region of a natural antisense oligonucleotide of the Brain derived neurotrophic factor (BDNF) polynucleotide; thereby modulating a function of and/or the expression of the Brain derived neurotrophic factor (BDNF) polynucleotide , with the proviso that the oligonucleotides having SEQ ID NOS 50-55 are excluded..

6. The method of claim 5, wherein a function of and/or the expression of the Brain derived neurotrophic factor (BDNF) is increased *in vivo* or *in vitro* with respect to a control.

7. The method of claim 5, wherein the at least one antisense oligonucleotide targets a natural antisense sequence of a Brain derived neurotrophic factor (BDNF) polynucleotide.

8. The method of claim 5, wherein the at least one antisense oligonucleotide targets a nucleic acid sequence comprising coding and/or non-coding nucleic acid sequences of a Brain derived neurotrophic factor (BDNF) polynucleotide.

9. The method of claim 5, wherein the at least one antisense oligonucleotide targets overlapping and/or non-overlapping sequences of a Brain derived neurotrophic factor (BDNF) polynucleotide.

10. The method of claim 5, wherein the at least one antisense oligonucleotide comprises one or more modifications selected from: at least one modified sugar moiety, at least one modified internucleoside linkage, at least one modified nucleotide, and combinations thereof.

11. The method of claim 10, wherein the one or more modifications comprise at least one modified sugar moiety selected from: a 2'-O-methoxyethyl modified sugar moiety, a 2'-methoxy modified sugar moiety, a 2'-O-alkyl modified sugar moiety, a bicyclic sugar moiety, and combinations thereof.

12. The method of claim 10, wherein the one or more modifications comprise at least one modified internucleoside linkage selected from: a phosphorothioate, 2'- Omethoxyethyl (MOE), 2'-fluoro, alkylphosphonate, phosphorodithioate, alkylphosphonothioate, phosphoramidate, carbamate, carbonate, phosphate triester, acetamidate, carboxymethyl ester, and combinations thereof.

13. The method of claim 10, wherein the one or more modifications comprise at least one modified nucleotide selected from: a peptide nucleic acid (PNA), a locked nucleic acid (LNA), an arabino-nucleic acid (FANA), an analogue, a derivative, and combinations thereof.

14. The method of claim 1, wherein the at least one oligonucleotide comprises at least one oligonucleotide sequences set forth as SEQ ID NOS: 12 to 49.

15. A method of modulating a function of and/or the expression of a Brain derived neurotrophic factor (BDNF) gene in mammalian cells or tissues *in vivo* or *in vitro* comprising: contacting said cells or tissues with at least one short interfering RNA (siRNA) oligonucleotide 5 to 30 nucleotides in length, said at least one siRNA oligonucleotide being specific for an antisense polynucleotide of a Brain derived neurotrophic factor (BDNF) polynucleotide, wherein said at least one siRNA oligonucleotide has at least 50% sequence identity to a complementary sequence of at least about five consecutive nucleic acids of the antisense and/or sense nucleic acid molecule of the Brain derived neurotrophic factor (BDNF) polynucleotide; and, modulating a function of and/or the expression of Brain derived neurotrophic factor (BDNF) in mammalian cells or tissues *in vivo* or *in vitro*.

16. The method of claim 15, wherein said oligonucleotide has at least 80% sequence identity to a sequence of at least about five consecutive nucleic acids that is complementary to the antisense and/or sense nucleic acid molecule of the Brain derived neurotrophic factor (BDNF) polynucleotide.

17. An oligonucleotide 5 to 30 nucleotides in length wherein said oligonucleotide has at least 50% sequence identity to a reverse complement of a polynucleotide comprising 5 to 30 consecutive nucleotides within the natural antisense transcript nucleotides 1 to 1279 of SEQ ID NO: 3 or 1 to 1478 of SEQ ID NO: 4 or 1 to 1437 of SEQ ID NO: 5 or 1 to 2322 of SEQ ID NO: 6 or 1 to 2036 of SEQ ID NO: 7 or 1 to 2364 of SEQ ID NO: 8 or 1 to 3136 of SEQ ID NO: 9 or 1 to 906 of SEQ ID NO: 10 or 1 to 992 of SEQ ID NO: 11, with the proviso that the oligonucleotides having SEQ ID NOS 50-55 are excluded, and optionally further comprising at least one modification wherein the at least one modification is selected from: at least one modified sugar moiety; at least one modified internucleotide linkage; at least one modified nucleotide, and combinations thereof; wherein said oligonucleotide is an antisense compound which hybridizes to and modulates the function and/or expression of a Brain derived neurotrophic factor (BDNF) gene *in vivo* or *in vitro* as compared to a normal control.

18. The oligonucleotide according to claim 17 wherein said oligonucleotide is 5 to 30 nucleotides in length and has at least 50% sequence identity to the reverse complement of 5-30 consecutive nucleotides within a natural antisense transcript of the BDNF gene.

19. The oligonucleotide of claim 18, wherein the at least one modification comprises an internucleotide linkage selected from the group consisting of: phosphorothioate, alkylphosphonate, phosphorodithioate, alkylphosphonothioate, phosphoramidate, carbamate, carbonate, phosphate triester, acetamidate, carboxymethyl ester, and combinations thereof.

20. The oligonucleotide of claim 18, wherein said oligonucleotide comprises at least one phosphorothioate internucleotide linkage.

21. The oligonucleotide of claim 18, wherein said oligonucleotide comprises a backbone of phosphorothioate internucleotide linkages.

22. The oligonucleotide of claim 18, wherein the oligonucleotide comprises at least one modified nucleotide, said modified nucleotide selected from: a peptide nucleic acid, a locked nucleic acid (LNA), analogue, derivative, and a combination thereof.

23. The oligonucleotide of claim 18, wherein the oligonucleotide comprises a plurality of modifications, wherein said modifications comprise modified nucleotides selected from: phosphorothioate, alkylphosphonate, phosphorodithioate, alkylphosphonothioate, phosphoramidate, carbamate, carbonate, phosphate triester, acetamidate, carboxymethyl ester, and a combination thereof.

24. The oligonucleotide of claim 18, wherein the oligonucleotide comprises a plurality of modifications, wherein said modifications comprise modified nucleotides selected from: peptide nucleic acids, locked nucleic acids (LNA), analogues, derivatives, and a combination thereof.

25. The oligonucleotide of claim 18, wherein the oligonucleotide comprises at least one modified sugar moiety selected from: a 2'-O-methoxyethyl modified sugar moiety, a 2'-methoxy modified sugar moiety, a 2'-O-alkyl modified sugar moiety, a bicyclic sugar moiety, and a combination thereof.

26. The oligonucleotide of claim 18, wherein the oligonucleotide comprises a plurality of modifications, wherein said modifications comprise modified sugar moieties selected from: a 2'-O-methoxyethyl modified sugar moiety, a 2'-methoxy modified sugar moiety, a 2'-O-alkyl modified sugar moiety, a bicyclic sugar moiety, and a combination thereof.

27. The oligonucleotide of claim 18, wherein the oligonucleotide is of at least about 5 to 30 nucleotides in length and hybridizes to an antisense and/or sense strand of a Brain derived neurotrophic factor (BDNF) polynucleotide wherein said oligonucleotide has at least about 60% sequence identity to a complementary sequence of at least about five consecutive nucleic acids of the antisense and/or sense coding and/or noncoding nucleic acid sequences of the Brain derived neurotrophic factor (BDNF) polynucleotide.

28. The oligonucleotide of claim 18, wherein the oligonucleotide has at least about 80% sequence identity to a complementary sequence of at least about five consecutive nucleic acids of the antisense and/or sense coding and/or noncoding nucleic acid sequence of the Brain derived neurotrophic factor (BDNF) polynucleotide.

29. The oligonucleotide of claim 18, wherein said oligonucleotide hybridizes to and modulates expression and/or function of at least one Brain derived neurotrophic factor (BDNF) polynucleotide *in vivo* or *in vitro*, as compared to a normal control.

30. The oligonucleotide of claim 18, wherein the oligonucleotide comprises the sequences set forth as SEQ ID NOS: 12 to 49.

31. A pharmaceutical composition comprising one or more oligonucleotides specific for one or more Brain derived neurotrophic factor (BDNF) polynucleotides according to claim 17 and a pharmaceutically acceptable excipient.

32. The composition of claim 31, wherein the oligonucleotides have at least about 40% sequence identity as compared to any one of the nucleotide sequences set forth as SEQ ID NOS: 12 to 49.

33. The composition of claim 31, wherein the oligonucleotides comprise nucleotide sequences set forth as SEQ ID NOS: 12 to 49.

34. The composition of claim 33, wherein the oligonucleotides set forth as SEQ ID NOS: 12 to 49 comprise one or more modifications or substitutions.

35. The composition of claim 34, wherein the one or more modifications are selected from: phosphorothioate, methylphosphonate, peptide nucleic acid, locked nucleic acid (LNA) molecules, and combinations thereof.

36. A method of preventing or treating a disease associated with at least one Brain derived neurotrophic factor (BDNF) polynucleotide and/or at least one encoded product thereof, comprising: administering to a patient a therapeutically effective dose of at least one antisense oligonucleotide 5 to 30 nucleotides in length wherein said at least one oligonucleotide has at least 50% sequence identity to a reverse complement of a polynucleotide comprising 5 to 30 consecutive nucleotides within the natural antisense transcript nucleotides 1 to 1279 of SEQ ID NO: 3 or 1 to 1478 of SEQ ID NO: 4 or 1 to 1437 of SEQ ID NO: 5 or 1 to 2322 of SEQ ID NO: 6 or 1 to 2036 of SEQ ID NO: 7 or 1 to 2364 of SEQ ID NO: 8 or 1 to 3136 of SEQ ID NO: 9 or 1 to 906 of SEQ ID NO: 10 or 1 to 992 of SEQ ID NO: 11, , with the proviso that the oligonucleotides having SEQ ID NOS 50-55 are excluded and that binds to a natural antisense sequence of said at least one Brain derived neurotrophic factor (BDNF) polynucleotide and modulates expression of said at least one Brain derived neurotrophic factor (BDNF) polynucleotide; thereby preventing or treating the disease associated with the at least one Brain derived neurotrophic factor (BDNF) polynucleotide and/or at least one encoded product thereof.

37. The method of claim 36, wherein a disease associated with the at least one Brain derived neurotrophic factor (BDNF) polynucleotide is selected from: a disease or disorder associated with abnormal function and/or expression of BDNF, a neurological disease or disorder, a disease or a disorder associated with defective neurogenesis; a neurodegenerative disease or disorder (e.g.,

Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis etc.); a neuropsychiatric disorder (depression, schizophrenia, schizofreniform disorder, schizoaffective disorder, and delusional disorder; anxiety disorders such as panic disorder, phobias (including agoraphobia), an obsessive-compulsive disorder, a posttraumatic stress disorder, a bipolar disorder, anorexia nervosa, bulimia nervosa), an autoimmune disorder (e.g., multiple sclerosis) of the central nervous system, memory loss, a long term or a short term memory disorder, benign forgetfulness, a childhood learning disorder, close head injury, an attention deficit disorder, neuronal reaction to viral infection, brain damage, narcolepsy, a sleep disorder (e.g., circadian rhythm disorders, insomnia and narcolepsy); severance of nerves or nerve damage, severance of cerebrospinal nerve cord (CNS) and a damage to brain or nerve cells, a neurological deficit associated with AIDS, a motor and tic disorder characterized by motor and/or vocal tics (e.g., Tourette's disorder, chronic motor or vocal tic disorder, transient tic disorder, and stereotypic movement disorder), a substance abuse disorder (e.g., substance dependence, substance abuse and the sequalae of substance abuse/dependence, such as substance-induced psychological disorder, substance withdrawal and substance-induced dementia or amnestic disorder), traumatic brain injury, tinnitus, neuralgia (e.g., trigeminal neuralgia) pain (e.g chronic pain, chronic inflammatory pain, pain associated with arthritis, fibromyalgia, back pain, cancer-associated pain, pain associated with digestive disease, pain associated with Crohn's disease, pain associated with autoimmune disease, pain associated with endocrine disease, pain associated with diabetic neuropathy, phantom limb pain, spontaneous pain, chronic post-surgical pain, chronic temporomandibular pain, causalgia, post-herpetic neuralgia, AIDS-related pain, complex regional pain syndromes type I and II, trigeminal neuralgia, chronic back pain, pain associated with spinal cord injury, pain associated with drug intake and recurrent acute pain, neuropathic pain), inappropriate neuronal activity resulting in neurodysthesias in a disease such as diabetes, an MS and a motor neuron disease, ataxias, muscular rigidity (spasticity), temporomandibular joint dysfunction, Reward deficiency syndrome (RDS), neurotoxicity caused by alcohol or substance abuse (e.g., ecstacy, methamphetamine etc.), mental retardation or cognitive impairment (e.g., nonsyndromic X-linked mental retardation, fragile X syndrome, Down's syndrome, autism), aphasia, Bell's palsy, Creutzfeldt-jacob disease, encephalitis, age related macular degeneration, ondine syndrome, WAGR syndrome, hearing loss, Rett syndrome, epilepsy, spinal cord injury, stroke, hypoxia, ischemia, brain injury, diabetic neuropathy, peripheral neuropathy, nerve transplantation complications, motor neuron disease, peripheral nerve injury, obesity, a metabolic syndrome, cancer, asthma, an atopic disease, inflammation, allergy, eczema, a neuro-oncological disease or disorder, neuro-immunological disease or disorder and neuro-otological disease or disorder; and a disease or disorder associated with aging and senescence.

38. Use of an oligonucleotide selected from the group consisting of SEQ ID NOS 50-55 to target a natural antisense transcript ("NAT") of a BDNF polynucleotide to modulate the expression of the BDNF polynucleotide wherein said NATs are selected from the group consisting of SEQ ID NOS. 3 to 11.

39. Use of an oligonucleotide selected from the group consisting of SEQ ID NOS 50-55 to target a natural antisense transcript (NAT) of a BDNF polynucleotide to modulate the expression of the BDNF polynucleotide wherein said NAT is selected from the group consisting of SEQ ID NOS. 3, 4, 5, 7, 8, 9, 10 and 11.

FIG. 1

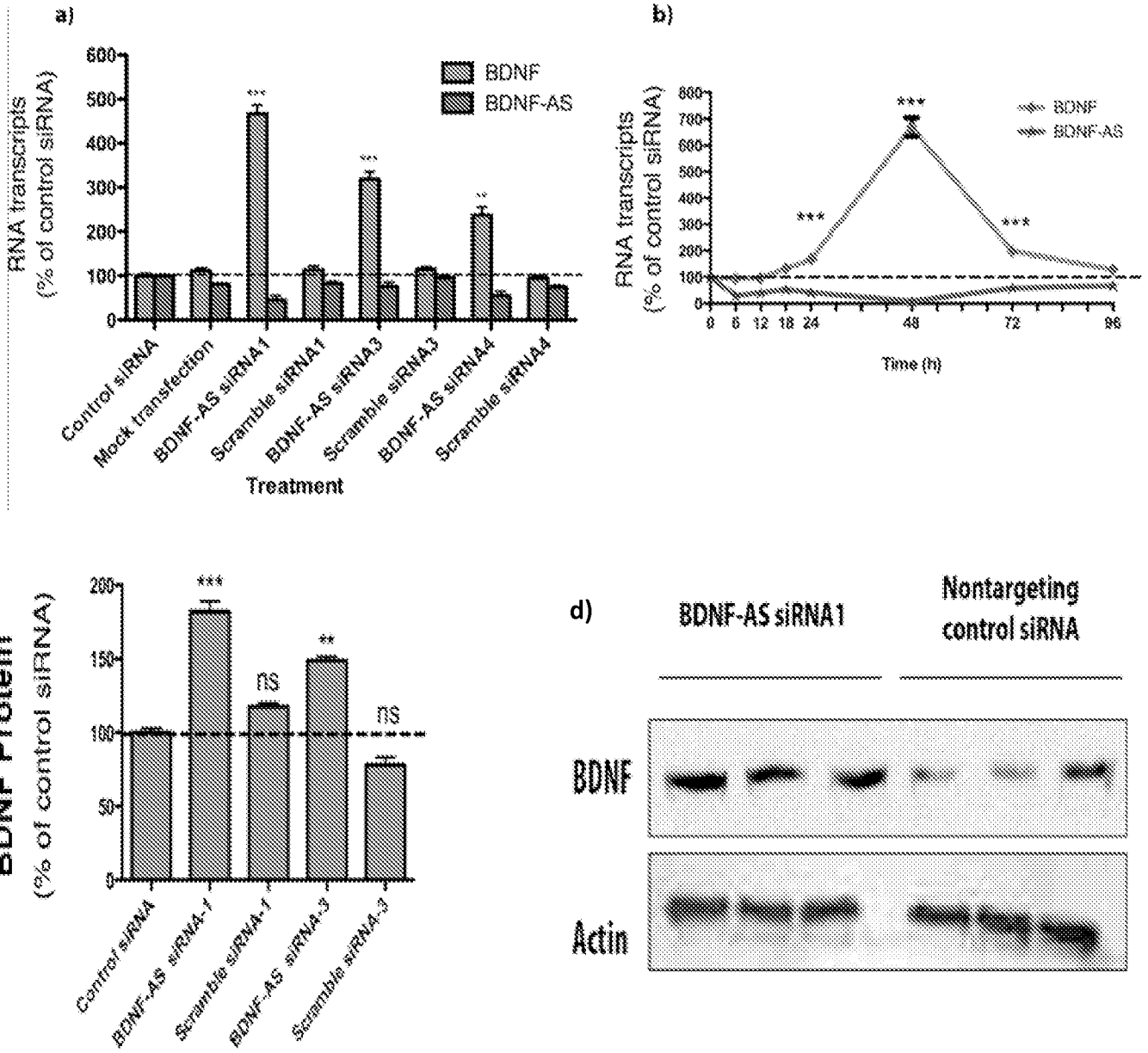

a)
RNA transcripts
(% of control siRNA)
BDNF
BDNF-AS
Control siRNA
Mock transfection
BDNF-AS siRNA1
Scramble siRNA1
BDNF-AS siRNA3
Scramble siRNA3
BDNF-AS siRNA4
Scramble siRNA4
Treatment
b)
RNA transcripts
(% of control siRNA)
BDNF
BDNF-AS
Time (h)
c)
BDNF Protein
(% of control siRNA)
Control siRNA
BDNF-AS siRNA-1
Scramble siRNA-1
BDNF-AS siRNA-3
Scramble siRNA-3
ns
d)
BDNF-AS siRNA1
Nontargeting control siRNA
BDNF
Actin

**FIG. 1**
**CON'T**

e)

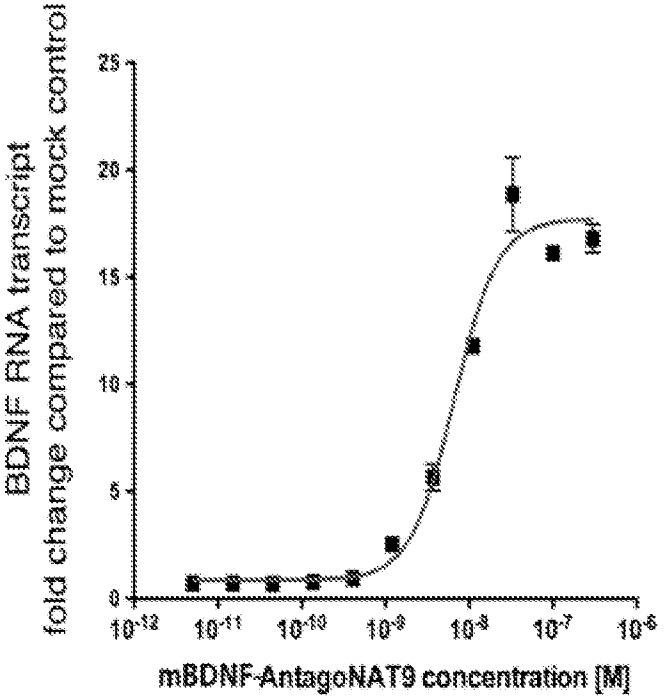
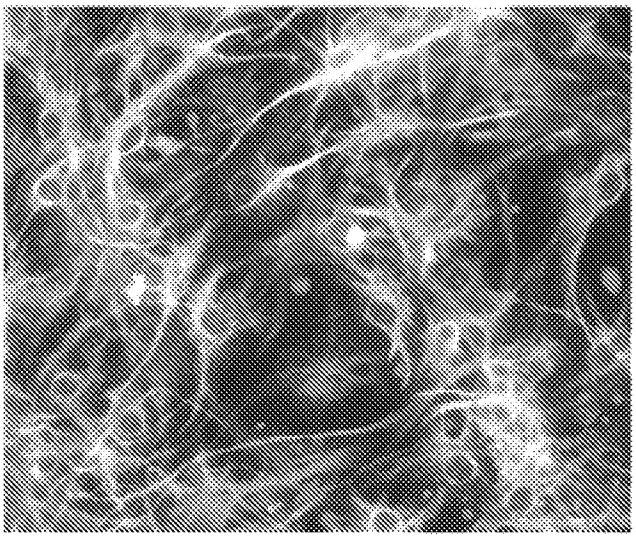

BDNF RNA transcript
fold change compared to mock control
25
20
15
10
5
0
$10^{-12}$
$10^{-11}$
$10^{-10}$
$10^{-9}$
$10^{-8}$
$10^{-7}$
$10^{-6}$
mBDNF-AntagoNAT9 concentration [M]

FIG. 2

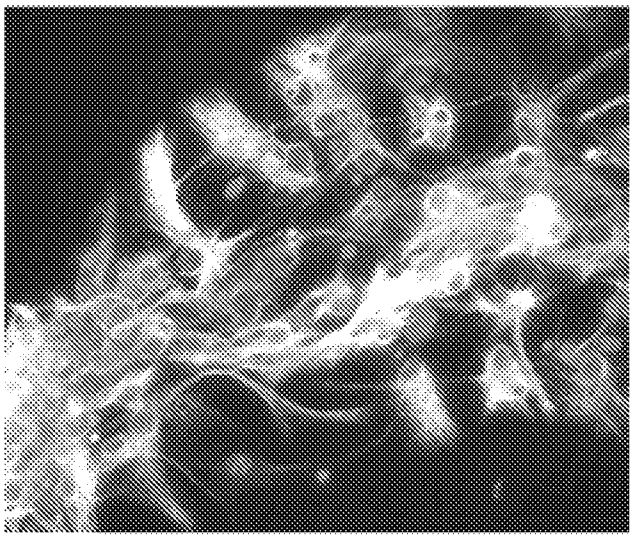
a)

Control siRNA (3d post-plating)



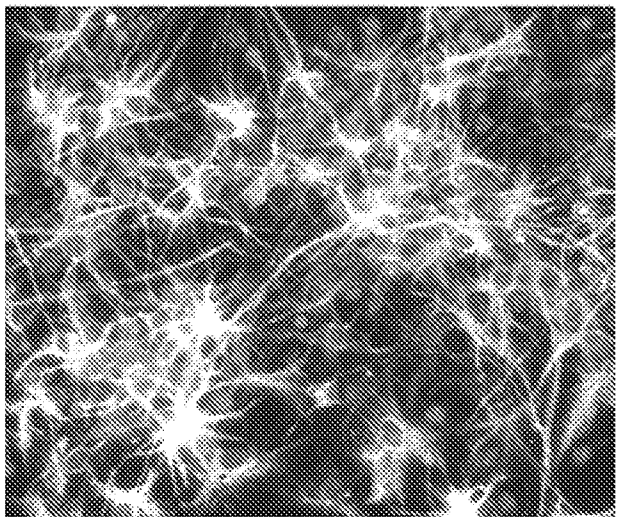
b)

BDNF-AS siRNA (3d post-plating)



c)

Control siRNA (7d post-plating)



d)

BDNF-AS siRNA (7d post-plating)

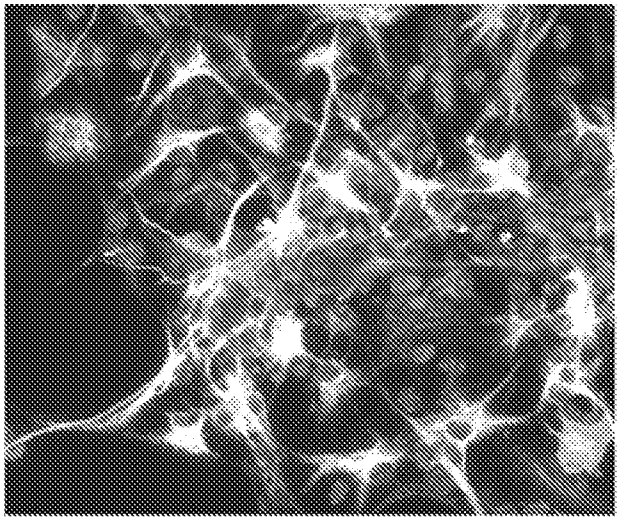


FIG. 3

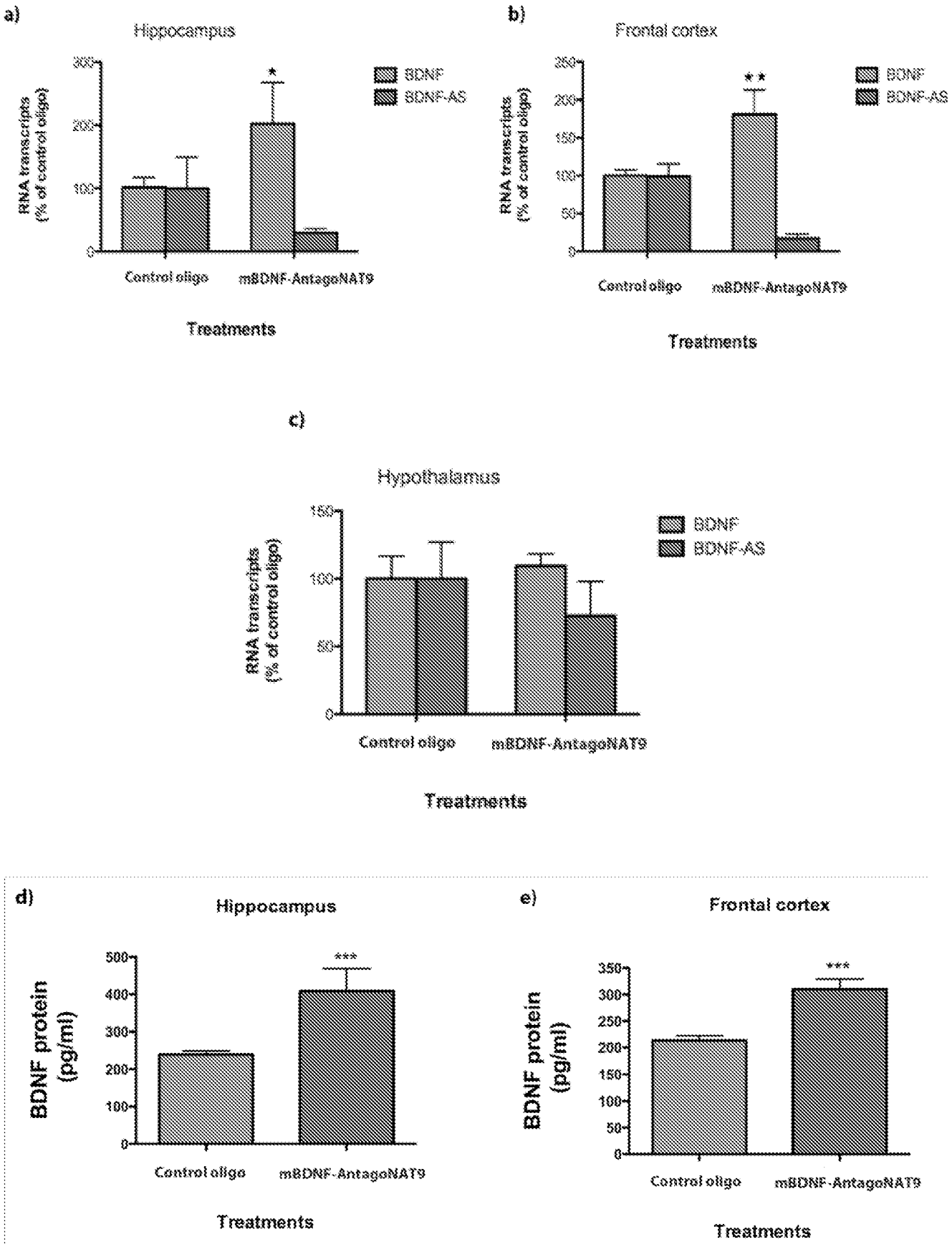

a)
Hippocampus
RNA transcripts (% of control oligo)
BDNF
BDNF-AS
*
Control oligo
mBDNF-AntagoNAT9
Treatments
b)
Frontal cortex
RNA transcripts (% of control oligo)
BDNF
BDNF-AS
**
Control oligo
mBDNF-AntagoNAT9
Treatments
c)
Hypothalamus
RNA transcripts (% of control oligo)
BDNF
BDNF-AS
Control oligo
mBDNF-AntagoNAT9
Treatments
d)
Hippocampus
BDNF protein (pg/ml)
***
Control oligo
mBDNF-AntagoNAT9
Treatments
e)
Frontal cortex
BDNF protein (pg/ml)
***
Control oligo
mBDNF-AntagoNAT9
Treatments

FIG. 4

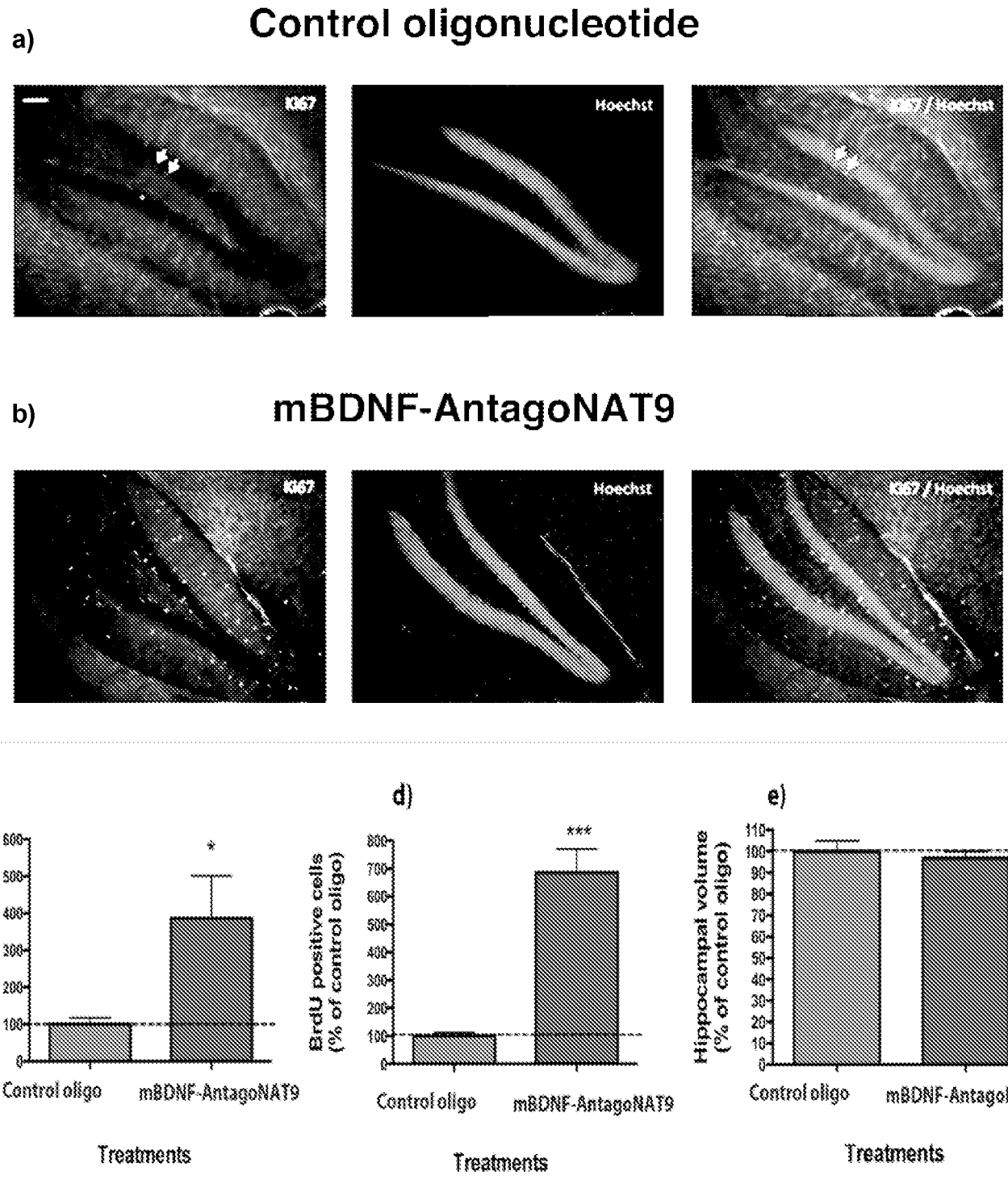

a)
Control oligonucleotide
Ki67
Hoechst
Ki67 / Hoechst
b)
mBDNF-AntagoNAT9
Ki67
Hoechst
Ki67 / Hoechst
c)
Ki67 positive cells (% of control oligo)
Control oligo
mBDNF-AntagoNAT9
Treatments
d)
BrdU positive cells (% of control oligo)
***
Control oligo
mBDNF-AntagoNAT9
Treatments
e)
Hippocampal volume (% of control oligo)
Control oligo
mBDNF-AntagoNAT9
Treatments

FIG. 5

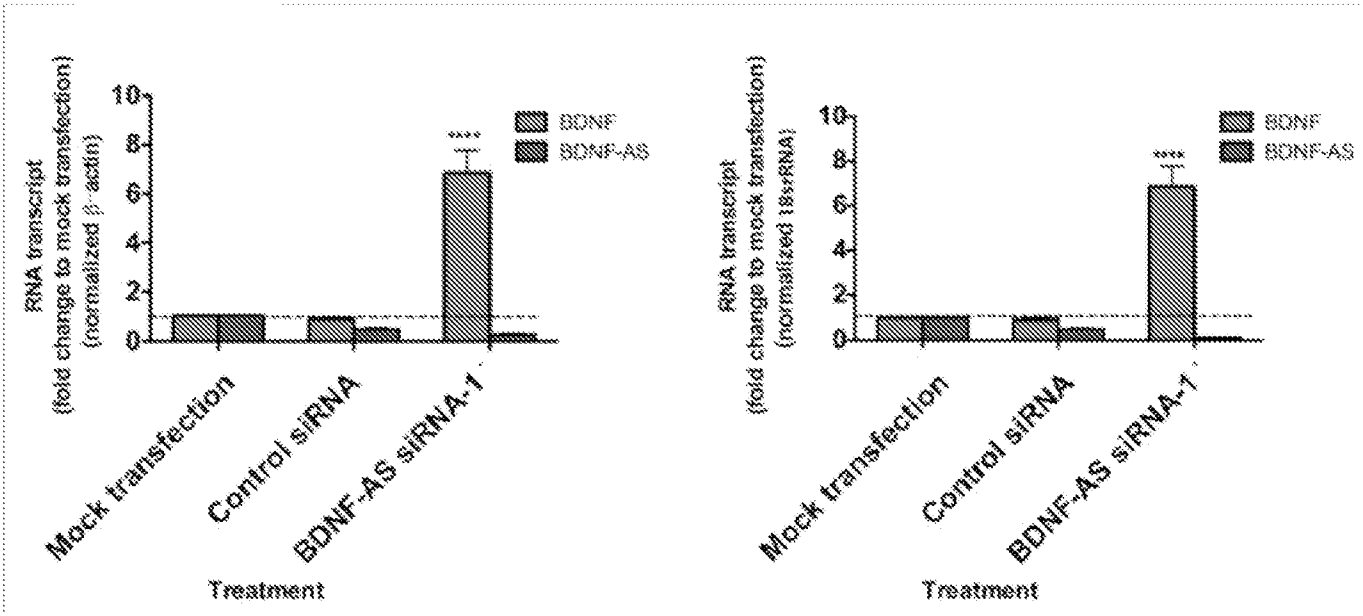

RNA transcript
(fold change to mock transfection)
(normalized β- actin)
10
8
6
4
2
0
BDNF
BDNF-AS
***
Mock transfection
Control siRNA
BDNF-AS siRNA-1
Treatment
RNA transcript
(fold change to mock transfection)
(normalized 18s rRNA)
10
8
6
4
2
0
BDNF
BDNF-AS
***
Mock transfection
Control siRNA
BDNF-AS siRNA-1
Treatment

FIG. 6

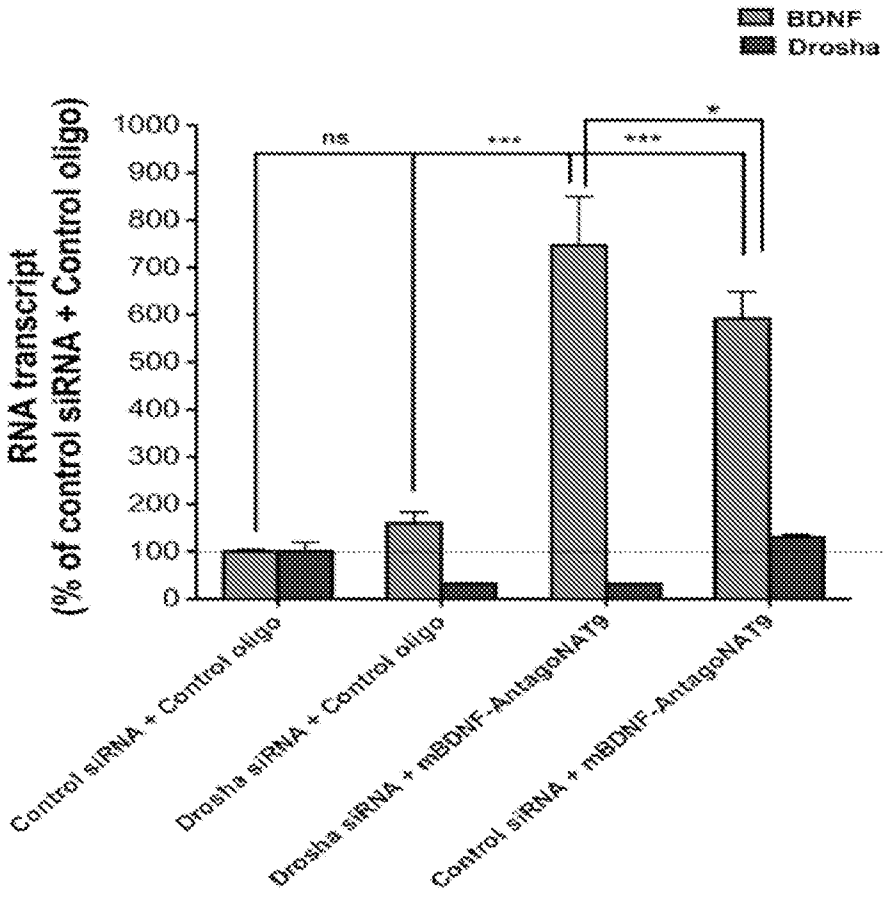

BDNF
Drosha
RNA transcript
(% of control siRNA + Control oligo)
1000
900
800
700
600
500
400
300
200
100
0
ns
***
***
*
Control siRNA + Control oligo
Drosha siRNA + Control oligo
Drosha siRNA + mBDNF-AntagoNAT9
Control siRNA + mBDNF-AntagoNAT9

FIG. 7

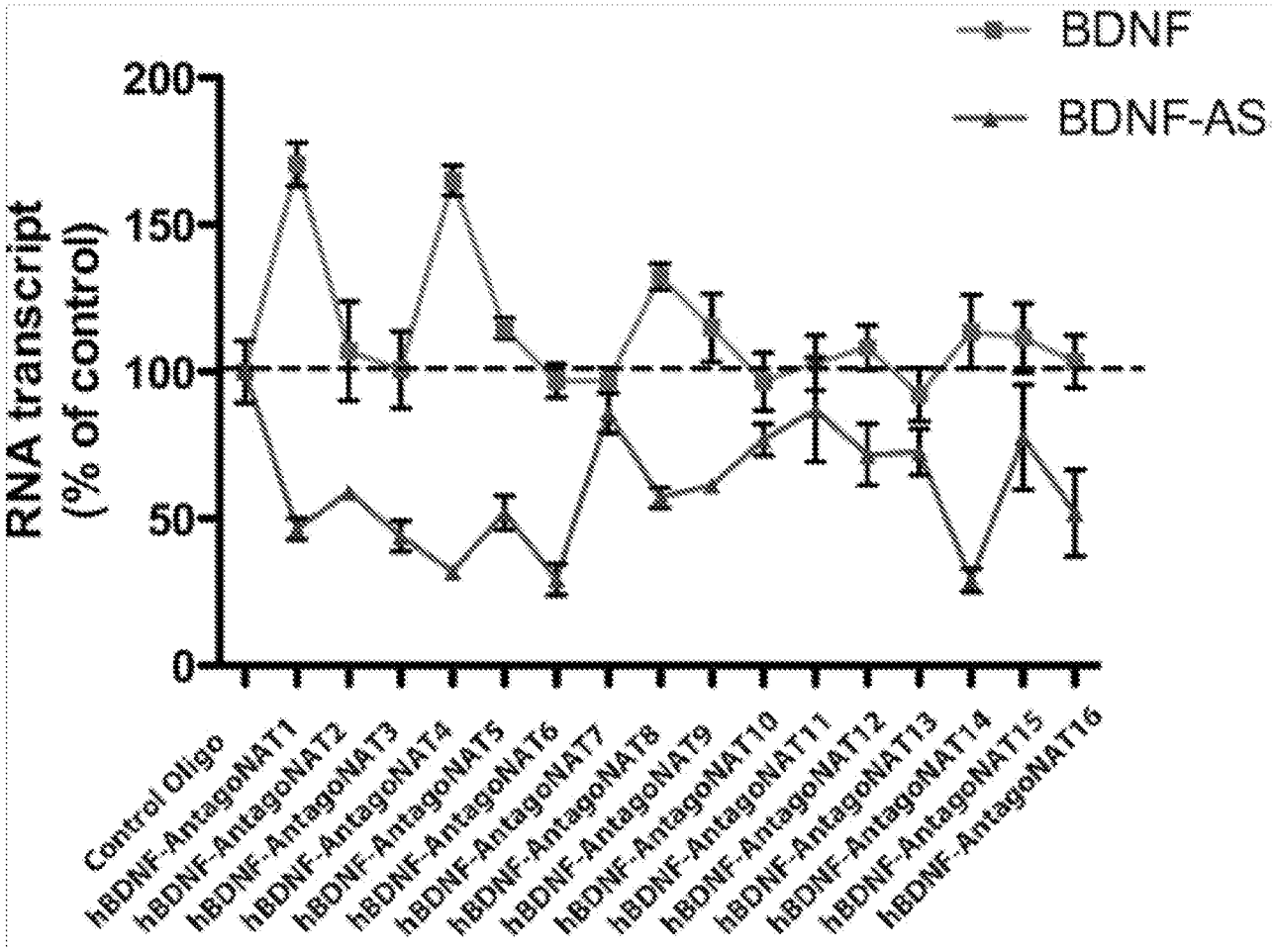

BDNF
BDNF-AS
RNA transcript
(% of control)
200
150
100
50
0
Control Oligo
hBDNF-AntagoNAT1
hBDNF-AntagoNAT2
hBDNF-AntagoNAT3
hBDNF-AntagoNAT4
hBDNF-AntagoNAT5
hBDNF-AntagoNAT6
hBDNF-AntagoNAT7
hBDNF-AntagoNAT8
hBDNF-AntagoNAT9
hBDNF-AntagoNAT10
hBDNF-AntagoNAT11
hBDNF-AntagoNAT12
hBDNF-AntagoNAT13
hBDNF-AntagoNAT14
hBDNF-AntagoNAT15
hBDNF-AntagoNAT16

FIG. 8

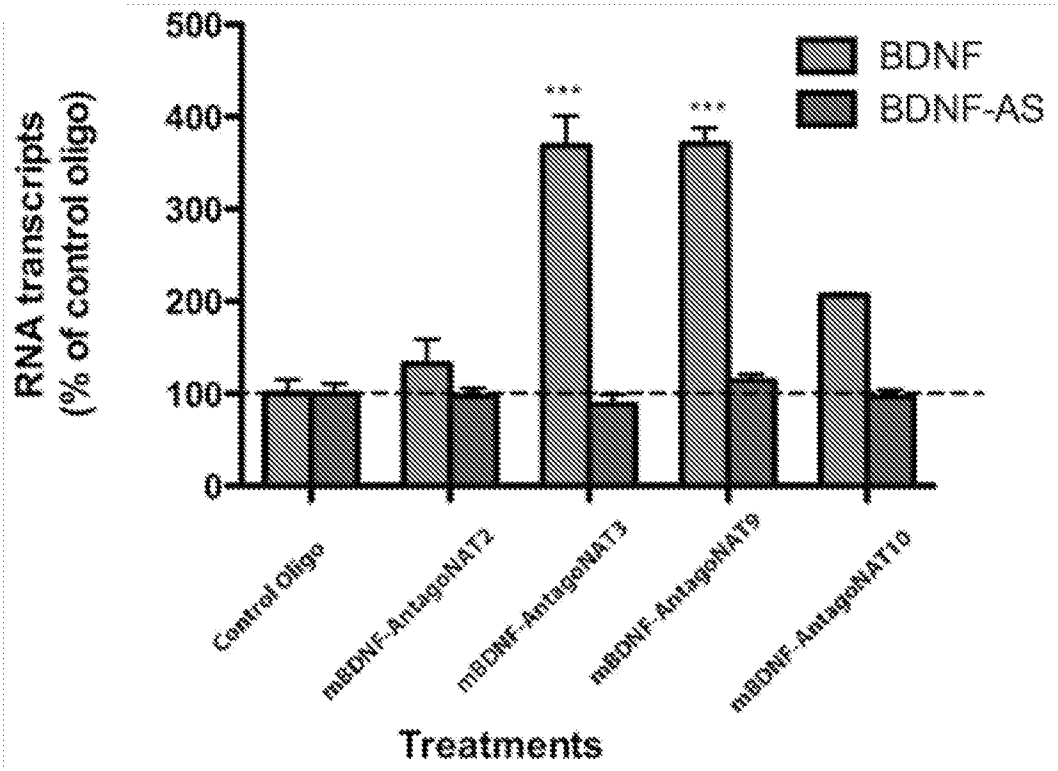
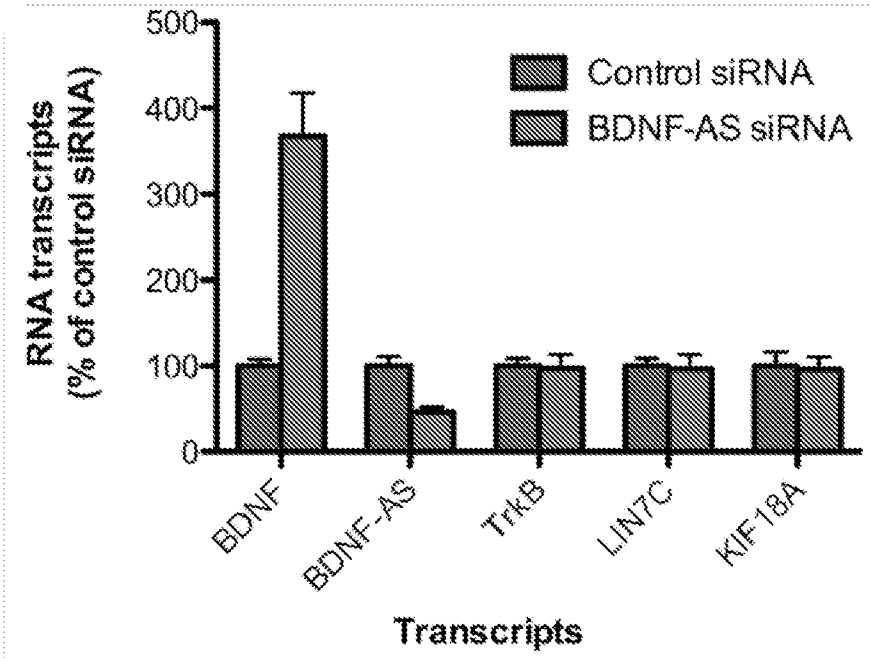

RNA transcripts
(% of control oligo)
500
400
300
200
100
0
BDNF
BDNF-AS
***
***
Control Oligo
mBDNF-AntagoNAT2
mBDNF-AntagoNAT3
mBDNF-AntagoNAT9
mBDNF-AntagoNAT10
Treatments

FIG. 9


RNA transcripts
(% of control siRNA)
500
400
300
200
100
0
Control siRNA
BDNF-AS siRNA
BDNF
BDNF-AS
TrkB
LIN7C
KIF18A
Transcripts

(19)中华人民共和国国家知识产权局

(12)发明专利申请

(10)申请公布号 CN 104583405 A
(43)申请公布日 2015.04.29

(21)申请号 201380024874.4
(22)申请日 2013.03.12
(30)优先权数据
61/611,225 2012.03.15 US
61/614,664 2012.03.23 US
(85)PCT国际申请进入国家阶段日
2014.11.12
(86)PCT国际申请的申请数据
PCT/US2013/030589 2013.03.12
(87)PCT国际申请的公布数据
WO2013/138374 EN 2013.09.19
(71)申请人 科纳公司
地址 美国佛罗里达州
申请人 斯克里普斯研究学院
(72)发明人 M·A·法格西西 C·科伊托
(74)专利代理机构 北京市金杜律师事务所 11256
代理人 陈文平 徐志明
(51)Int.Cl.
*C12N 15/113*(2006.01)

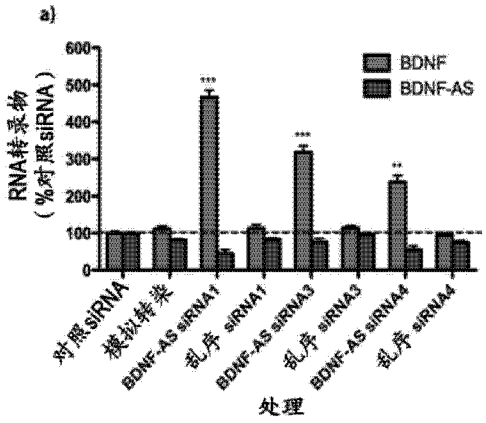
权利要求书5页 说明书48页
序列表37页 附图8页

(54)发明名称

通过抑制脑源神经营养因子(BDNF)的天然反义转录物治疗BDNF相关疾病

(57)摘要

本发明涉及调节脑源神经营养因子(BDNF)的表达和/或功能的反义寡核苷酸,特别是通过靶向于脑源神经营养因子(BDNF)的天然反义多核苷酸调节脑源神经营养因子(BDNF)的表达和/或功能。本发明还涉及这些反义寡核苷酸的鉴定及其治疗与BDNF的表达相关的疾病和障碍的用途。




CN 104583405 A

1. 一种调节生物系统中脑源神经营养因子 (BDNF) 多核苷酸的功能和 / 或表达的方法，包括 ：使所述生物系统与长度 5-30 个核苷酸的至少一种反义寡核苷酸接触，其中所述至少一种寡核苷酸与脑源神经营养因子 (BDNF) 多核苷酸的天然反义序列的反向互补序列具有至少 50％的序列同一性，从而调节所述脑源神经营养因子 (BDNF) 多核苷酸的功能和 / 或表达，条件是排除具有 SEQ ID NO:50-55 的寡核苷酸。

2. 根据权利要求 1 的调节生物系统中脑源神经营养因子 (BDNF) 多核苷酸的功能和 / 或表达的方法，包括 ：使所述生物系统与长度 5-30 个核苷酸的至少一种反义寡核苷酸接触，其中所述至少一种寡核苷酸与包含天然反义转录物 SEQ ID NO:3 的核苷酸 1-1279 或 SEQ ID NO:4 的核苷酸 1-1478 或 SEQ ID NO:5 的核苷酸 1-1437 或 SEQ ID NO:6 的核苷酸 1-2322 或 SEQ ID NO:7 的核苷酸 1-2036 或 SEQ ID NO:8 的核苷酸 1-2364 或 SEQ ID NO:9 的核苷酸 1-3136 或 SEQ ID NO:10 的核苷酸 1-906 或 SEQ ID NO:11 的核苷酸 1-992 内的 5-30 个连续核苷酸的多核苷酸的反向互补序列具有至少 50％的序列同一性，条件是排除具有 SEQ ID NO:50-55 的寡核苷酸，从而调节脑源神经营养因子 (BDNF) 多核苷酸的功能和 / 或表达。

3. 一种体内或体外调节患者细胞或组织中脑源神经营养因子 (BDNF) 多核苷酸的功能和 / 或表达的方法，包括 ：使所述细胞或组织与长度 5-30 个核苷酸的至少一种反义寡核苷酸接触，其中所述寡核苷酸与脑源神经营养因子 (BDNF) 多核苷酸的反义寡核苷酸具有至少 50％的序列同一性，从而体内或体外调节患者细胞或组织中脑源神经营养因子 (BDNF) 多核苷酸的功能和 / 或表达，条件是排除具有 SEQ ID NO:50-55 的寡核苷酸。

4. 根据权利要求 3 的调节患者细胞或组织中脑源神经营养因子 (BDNF) 多核苷酸的功能和 / 或表达的方法，包括 ：使所述生物系统与长度 5-30 个核苷酸的至少一种反义寡核苷酸接触，其中所述至少一种寡核苷酸与包含天然反义转录物 SEQ ID NO:3 的核苷酸 1-1279 或 SEQ ID NO:4 的核苷酸 1-1478 或 SEQ ID NO:5 的核苷酸 1-1437 或 SEQ ID NO:6 的核苷酸 1-2322 或 SEQ ID NO:7 的核苷酸 1-2036 或 SEQ ID NO:8 的核苷酸 1-2364 或 SEQ ID NO:9 的核苷酸 1-3136 或 SEQ ID NO:10 的核苷酸 1-906 或 SEQ ID NO:11 的核苷酸 1-992 内的 5-30 个连续核苷酸的多核苷酸的反向互补序列具有至少 50％的序列同一性，条件是排除具有 SEQ ID NO:50-55 的寡核苷酸，从而调节脑源神经营养因子 (BDNF) 多核苷酸的功能和 / 或表达。

5. 一种调节生物系统中的脑源神经营养因子 (BDNF) 多核苷酸的功能和 / 或表达的方法，包括 ：使所述系统与靶向于脑源神经营养因子 (BDNF) 多核苷酸的天然反义寡核苷酸的区域的至少一种反义寡核苷酸接触，从而调节脑源神经营养因子 (BDNF) 多核苷酸的功能和 / 或表达，条件是排除具有 SEQ ID NO:50-55 的寡核苷酸。

6. 根据权利要求 5 的方法，其中所述脑源神经营养因子 (BDNF) 的功能和 / 或表达相对于对照在体内或体外提高。

7. 根据权利要求 5 的方法，其中所述至少一种反义寡核苷酸靶向于脑源神经营养因子 (BDNF) 多核苷酸的天然反义序列。

8. 根据权利要求 5 的方法，其中所述至少一种反义寡核苷酸靶向于包含脑源神经营养因子 (BDNF) 多核苷酸的编码和 / 或非编码核酸序列的核酸序列。

9. 根据权利要求 5 的方法，其中所述至少一种反义寡核苷酸靶向于脑源神经营养因子

(BDNF) 多核苷酸的重叠和 / 或非重叠序列。

10. 根据权利要求 5 的方法，其中所述至少一种反义寡核苷酸包含选自以下的一种或多种修饰 ：至少一种修饰的糖部分、至少一种修饰的核苷间键、至少一种修饰的核苷酸及其组合。

11. 根据权利要求 10 的方法，其中所述一种或多种修饰包括选自以下的至少一种修饰的糖部分 ：2'-O- 甲氧基乙基修饰的糖部分、2'- 甲氧基修饰的糖部分、2'-O- 烷基修饰的糖部分、双环糖部分及其组合。

12. 根据权利要求 10 的方法，其中所述一种或多种修饰包括选自以下的至少一种修饰的核苷间键 ：硫代磷酸酯、2'-O 甲氧基乙基 (MOE)、2'- 氟、烷基磷酸酯、二硫代磷酸酯、烷基硫代膦酸酯、氨基磷酸酯、氨基甲酸酯、碳酸酯、磷酸三酯、氨基乙酸酯、羧甲基酯及其组合。

13. 根据权利要求 10 的方法，其中所述一种或多种修饰包括选自以下的至少一种修饰的核苷酸 ：肽核酸 (PNA)、锁核酸 (LNA)、阿拉伯糖核酸 (FANA)、其类似物、其衍生物及其组合。

14. 根据权利要求 1 的方法，其中所述至少一种寡核苷酸包含如 SEQ ID NO:12-49 所示的至少一个寡核苷酸序列。

15. 一种体内或体外调节哺乳动物细胞或组织中脑源神经营养因子 (BDNF) 基因的功能和 / 或表达的方法，包括 ：使所述细胞或组织与长度 5-30 个核苷酸的至少一种短干扰 RNA(siRNA) 寡核苷酸接触，其中所述至少一种 siRNA 寡核苷酸是对于脑源神经营养因子 (BDNF) 多核苷酸的反义多核苷酸特异性的，其中所述至少一种 siRNA 寡核苷酸与脑源神经营养因子 (BDNF) 多核苷酸的反义和 / 或有义核酸分子的至少约五个连续核酸的互补序列具有至少 50％的序列同一性 ；和在体内或体外调节哺乳动物细胞或组织中脑源神经营养因子 (BDNF) 的功能和 / 或表达。

16. 根据权利要求 15 的方法，其中所述寡核苷酸与至少约五个连续核酸的序列具有至少 80％的序列同一性，所述至少约五个连续核酸的序列与脑源神经营养因子 (BDNF) 多核苷酸的反义和 / 或有义核酸分子互补。

17. 一种长度 5-30 个核苷酸的寡核苷酸，其中所述寡核苷酸与多核苷酸的反向互补序列具有至少 50％的序列同一性，该多核苷酸包含天然反义转录物 SEQ ID NO:3 的核苷酸 1-1279 或 SEQ ID NO:4 的核苷酸 1-1478 或 SEQ ID NO:5 的核苷酸 1-1437 或 SEQ ID NO:6 的核苷酸 1-2322 或 SEQ ID NO:7 的核苷酸 1-2036 或 SEQ ID NO:8 的核苷酸 1-2364 或 SEQ ID NO:9 的核苷酸 1-3136 或 SEQ ID NO:10 的核苷酸 1-906 或 SEQ ID NO:11 的核苷酸 1-992 内的 5-30 个连续核苷酸，条件是排除具有 SEQ ID NO:50-55 的寡核苷酸，且任选地进一步包含至少一种修饰，其中所述至少一种修饰选自 ：至少一种修饰的糖部分、至少一种修饰的核苷间键、至少一种修饰的核苷酸及其组合 ；其中所述寡核苷酸是与脑源神经营养因子 (BDNF) 基因杂交且与正常对照相比在体内或体外调节脑源神经营养因子 (BDNF) 基因的功能和 / 或表达的反义化合物。

18. 根据权利要求 17 的寡核苷酸，其中所述寡核苷酸长度为 5-30 个核苷酸且与 BDNF 基因的天然反义转录物内的 5-30 个连续核苷酸的反向互补序列具有至少 50％的序列同一性。

19. 根据权利要求 18 的寡核苷酸，其中所述至少一种修饰包括选自以下的核苷间键 ：

硫代磷酸酯、烷基磷酸酯、二硫代磷酸酯、烷基硫代膦酸酯、氨基磷酸酯、氨基甲酸酯、碳酸酯、磷酸三酯、氨基乙酸酯、羧甲基酯及其组合。

20. 根据权利要求 18 的寡核苷酸，其中所述寡核苷酸包含至少一个硫代磷酸酯核苷间键。

21. 根据权利要求 18 的寡核苷酸，其中所述寡核苷酸包含硫代磷酸酯核苷间键的骨架。

22. 根据权利要求 18 的寡核苷酸，其中所述寡核苷酸包含至少一个修饰的核苷酸，所述修饰的核苷酸选自：肽核酸、锁核酸 (LNA)、其类似物、其衍生物及其组合。

23. 根据权利要求 18 的寡核苷酸，其中所述寡核苷酸包含多个修饰，其中所述修饰包括选自以下的修饰的核苷酸：硫代磷酸酯、烷基磷酸酯、二硫代磷酸酯、烷基硫代膦酸酯、氨基磷酸酯、氨基甲酸酯、碳酸酯、磷酸三酯、氨基乙酸酯、羧甲基酯及其组合。

24. 根据权利要求 18 的寡核苷酸，其中所述寡核苷酸包含多个修饰，其中所述修饰包括选自以下的修饰的核苷酸：肽核酸、锁核酸 (LNA)、其类似物、其衍生物及其组合。

25. 根据权利要求 18 的寡核苷酸，其中所述寡核苷酸包含选自以下的至少一种修饰的糖部分：2'-O- 甲氧基乙基修饰的糖部分、2'- 甲氧基修饰的糖部分、2'-O- 烷基修饰的糖部分、双环糖部分及其组合。

26. 根据权利要求 18 的寡核苷酸，其中所述寡核苷酸包含多个修饰，其中所述修饰包括选自以下的修饰的糖部分：2'-O- 甲氧基乙基修饰的糖部分、2'- 甲氧基修饰的糖部分、2'-O- 烷基修饰的糖部分、双环糖部分及其组合。

27. 根据权利要求 18 的寡核苷酸，其中所述寡核苷酸长度为至少约 5-30 个核苷酸且与脑源神经营养因子 (BDNF) 多核苷酸的反义和 / 或有义链杂交，其中所述寡核苷酸与脑源神经营养因子 (BDNF) 多核苷酸的反义和 / 或有义编码和 / 或非编码核酸序列的至少约五个连续核酸的互补序列具有至少约 60％的序列同一性。

28. 根据权利要求 18 的寡核苷酸，其中所述寡核苷酸与脑源神经营养因子 (BDNF) 多核苷酸的反义和 / 或有义编码和 / 或非编码核酸序列的至少约五个连续核酸的互补序列具有至少约 80％的序列同一性。

29. 根据权利要求 18 的寡核苷酸，其中所述寡核苷酸与至少一个脑源神经营养因子 (BDNF) 多核苷酸杂交且与正常对照相比在体内或体外调节至少一个脑源神经营养因子 (BDNF) 多核苷酸的表达和 / 或功能。

30. 根据权利要求 18 的寡核苷酸，其中所述寡核苷酸包含如 SEQ ID NO:12-49 所示的序列。

31. 一种药物组合物，包含根据权利要求 17 的对于一种或多种脑源神经营养因子 (BDNF) 多核苷酸特异性的寡核苷酸和药学上可接受的赋形剂。

32. 根据权利要求 31 的组合物，其中所述寡核苷酸与如 SEQ ID NO:12-49 所示的任何一个核苷酸序列相比具有至少约 40％的序列同一性。

33. 根据权利要求 31 的组合物，其中所述寡核苷酸包含如 SEQ ID NO:12-49 所示的核苷酸序列。

34. 根据权利要求 33 的组合物，其中所述如 SEQ ID NO:12-49 所示的寡核苷酸包含一个或多个修饰或置换。

35. 根据权利要求 34 的组合物，其中所述一个或多个修饰选自：硫代磷酸酯、甲基膦酸酯、肽核酸、锁核酸 (LNA) 分子及其组合。

36. 一种预防或治疗与至少一种脑源神经营养因子 (BDNF) 多核苷酸和 / 或其至少一种编码产物相关的疾病的方法，包括：向患者施用治疗有效剂量的长度 5-30 个核苷酸的至少一种反义寡核苷酸，其中所述至少一种寡核苷酸与包含天然反义转录物 SEQ ID NO:3 的核苷酸 1-1279 或 SEQ ID NO:4 的核苷酸 1-1478 或 SEQ ID NO:5 的核苷酸 1-1437 或 SEQ ID NO:6 的核苷酸 1-2322 或 SEQ ID NO:7 的核苷酸 1-2036 或 SEQ ID NO:8 的核苷酸 1-2364 或 SEQ ID NO:9 的核苷酸 1-3136 或 SEQ ID NO:10 的核苷酸 1-906 或 SEQ ID NO:11 的核苷酸 1-992 内的 5-30 个连续核苷酸的多核苷酸的反向互补序列具有至少 50％的序列同一性，条件是排除具有 SEQ ID NO:50-55 的寡核苷酸，并其结合所述至少一种脑源神经营养因子 (BDNF) 多核苷酸的天然反义序列和调节所述至少一种脑源神经营养因子 (BDNF) 多核苷酸的表达；从而预防或治疗与至少一种脑源神经营养因子 (BDNF) 多核苷酸和 / 或其至少一种编码产物相关的疾病。

37. 根据权利要求 36 的方法，其中与至少一种脑源神经营养因子 (BDNF) 多核苷酸相关的疾病选自：与 BDNF 的异常功能和 / 或表达相关的疾病或障碍、神经疾病或障碍、与神经发生缺陷相关的疾病或障碍、神经变性疾病或障碍（例如阿尔茨海默氏病、帕金森氏病、亨廷顿氏病、肌萎缩性侧索硬化等）、神经精神障碍（抑郁症、精神分裂症、精神分裂症样精神障碍、情感分裂性精神障碍和妄想性障碍、焦虑性障碍例如惊恐性障碍、恐怖症（包括广场恐怖症）、强迫性神经失调、创伤后应激障碍、双相性精神障碍、神经性厌食、神经性贪食）、中枢神经系统的自身免疫性疾病（例如，多发性硬化）、记忆丧失、长期或短期记忆障碍、良性健忘、儿童学习障碍、闭合性颅脑损伤、注意力缺陷障碍、对病毒感染的神经元反应、脑损伤、发作性睡病、睡眠障碍（例如，昼夜节律障碍、失眠症和发作性睡病）；神经中断或神经损伤、脑脊髓神经索 (CNS) 中断和脑或神经细胞损伤、与 AIDS 有关的神经功能缺损、以运动和 / 或发声性抽搐为特征的运动和抽搐障碍（例如，图雷特精神障碍、慢性运动或发声性抽搐障碍、短时抽搐性障碍和刻板型活动障碍）、物质滥用病症（例如，物质依赖、物质滥用以及物质滥用 / 依赖的后遗症，例如物质诱发的心理障碍、物质戒断和物质诱发的痴呆或遗忘症）、外伤性脑损伤、耳鸣、神经痛（例如，三叉神经痛）、疼痛（例如，慢性疼痛、慢性炎性痛、与关节炎有关的疼痛、纤维肌痛、背痛、癌症相关的疼痛、与消化性疾病有关的疼痛、与克罗恩氏病有关的疼痛、与自身免疫性疾病有关的疼痛、与内分泌疾病有关的疼痛、与糖尿病神经病变有关的疼痛、幻肢痛、自发性疼痛、慢性术后疼痛、慢性颞下颌疼痛、灼痛、疱疹后神经痛、AIDS 相关的疼痛、I 和 II 型复杂性区域疼痛综合征、三叉神经痛、慢性背痛、与脊髓损伤有关的疼痛、与药物摄入有关的疼痛和复发性急性疼痛、神经性疼痛）、在诸如糖尿病、MS 和运动神经元病的疾病中导致神经烦躁的不适当的神经元活性、共济失调、肌肉强直（痉挛状态）、颞下颚关节功能障碍、奖赏缺陷综合征 (RDS)、由酒精或物质滥用（例如，摇头丸、去氧麻黄碱等）引发的神经毒性、精神发育迟缓或认知缺损（例如，非综合征性 X 连锁精神发育迟缓、脆性 X 综合征、唐氏综合征、孤独症）、失语症、贝尔氏麻痹、克 - 雅病、脑炎、年龄相关性黄斑变性、ondine 综合征、WAGR 综合征、听力损失、雷特综合征、癫痫、脊髓损伤、中风、缺氧、缺血、脑损伤、糖尿病性神经病变、周围神经病、神经移植并发症、运动神经元病、周围神经损伤、肥胖症、代谢综合征、癌症、哮喘、特应性疾病、炎症、过敏症、湿疹、

神经肿瘤学疾病或病症、神经免疫学疾病或病症和神经耳科疾病或病症；以及与老化和衰老有关的疾病或病症。

38. 靶向于 BDNF 多核苷酸的天然反义转录物（“NAT”）的选自 SEQ ID NO:50-55 的寡核苷酸用于调节所述 BDNF 多核苷酸的表达的用途，其中所述 NAT 选自 SEQ ID NO:3-11。

39. 靶向于 BDNF 多核苷酸的天然反义转录物 (NAT) 的选自 SEQ ID NO:50-55 的寡核苷酸用于调节所述 BDNF 多核苷酸的表达的用途，其中所述 NAT 选自 SEQ ID NO:3、4、5、7、8、9、10 和 11。

# 通过抑制脑源神经营养因子（BDNF）的天然反义转录物治疗 BDNF 相关疾病

[0001] 相关申请的交叉引用

[0002] 本申请要求2012年3月15日提交的美国临时专利申请系列号61/611,225和2012年3月23日提交的美国临时专利申请系列号61/614,664的利益，这两者全文通过引用并入本文。

## 发明领域

[0003] 本发明的实施方案包括调节 BDNF 和相关分子的表达和/或功能的寡核苷酸。

## 背景技术

[0004] DNA-RNA 和 RNA-RNA 杂交对于核酸功能的许多方面（包括 DNA 复制、转录和翻译）而言为重要的。杂交对于探测特定核酸或者改变其表达的各种技术而言亦为主要的。反义核苷酸例如通过与靶 RNA 杂交来扰乱基因表达，从而干扰 RNA 剪接、转录、翻译和复制。反义 DNA 具有 DNA-RNA 杂合体充当核糖核酸酶 H 消化的底物的附加特征，该核糖核酸酶 H 消化活性存在于大多数细胞类型中。可将反义分子递送到细胞中，这与寡脱氧核苷酸 (ODN) 的情况一样，或者它们可作为 RNA 分子由内源基因表达。FDA 最近批准了一种反义药物，VITRAVENE™（用于治疗巨细胞病毒视网膜炎），这反映了反义物具有治疗应用。

[0005] WO 2010/093904 及其美国对应申请 US/2011/0319475 公开了 BDNF 作为使用本文所述的寡核苷酸调节的靶标。存在着针对天然反义靶标进行持续开发和与这种靶标互补并调节 BDNF 蛋白表达以潜在地治疗 BDNF 相关疾病和病症或用于与治疗 BDNF 相关疾病和病症有关的研究中的新开发寡核苷酸的需要。

[0006] 发明概述

[0007] 提供本概述以呈现本发明的概要，从而简要地指出本发明的性质和实质。在理解以下的情况下提出本概述：其不会用于解释或限制权利要求的范围或含义。

[0008] 在一个实施方案中，本发明提供通过使用靶向于天然反义转录物的任何区域的反义寡核苷酸来抑制天然反义转录物的作用，从而引起哺乳动物生物体中相应 BDNF 有义基因的上调的方法。本文也考虑本文所述的天然反义转录物的抑制可通过 siRNA、核酶和小分子来实现，其认为在本发明的范围之内。

[0009] 一个实施方案提供在体内或体外调节生物系统（包括，但不限于患者细胞或组织）中的 BDNF 多核苷酸的功能和/或表达的方法，所述方法包括用长度为约 5- 约 30 个核苷酸的反义寡核苷酸接触所述生物系统或所述细胞或组织，其中所述寡核苷酸与以下多核苷酸的反向互补序列具有至少 50％的序列同一性，所述多核苷酸包含在 SEQ ID NO:3 的核苷酸 1-1279 或 SEQ ID NO:4 的核苷酸 1-1478 或 SEQ ID NO:5 的核苷酸 1-1437 或 SEQ ID NO:6 的核苷酸 1-2322 或 SEQ ID NO:7 的核苷酸 1-2036 或 SEQ ID NO:8 的核苷酸 1-2364 或 SEQ ID NO:9 的核苷酸 1-3136 或 SEQ ID NO:10 的核苷酸 1-906 或 SEQ ID NO:11 的核苷酸 1-992 之内的 5-30 个连续核苷酸，从而在体内或体外调节所述生物系统包括所述患者

细胞或组织中BDNF多核苷酸的功能和/或表达，条件是排除具有SEQ ID NO:50-55的寡核苷酸。

[0010] 在一个实施方案中，上述的寡核苷酸靶向于生物系统中存在的BDNF多核苷酸的天然反义序列，例如SEQ ID NO:3-11所示的核苷酸，以及其任何变体、等位基因、同源物、突变体、衍生物、片段和互补序列。这类反义寡核苷酸的实例如SEQ ID NO:12-49所示。

[0011] 在另一个实施方案中，本发明包括调节生物系统中BDNF多核苷酸的功能或表达的方法，包括用靶向于BDNF多核苷酸的天然反义转录物的至少一个反义寡核苷酸接触所述生物系统，所述天然反义转录物包含在SEQ ID NO:3的核苷酸1-1279或SEQ ID NO:4的核苷酸1-1478或SEQ ID NO:5的核苷酸1-1437或SEQ ID NO:6的核苷酸1-2322或SEQ ID NO:7的核苷酸1-2036或SEQ ID NO:8的核苷酸1-2364或SEQ ID NO:9的核苷酸1-3136或SEQ ID NO:10的核苷酸1-906或SEQ ID NO:11的核苷酸1-992之内的5-30个连续核苷酸，从而调节所述生物系统中BDNF多核苷酸的功能和/或表达。

[0012] 在另一个实施方案中，本发明包括调节生物系统中BDNF多核苷酸的功能或表达的方法，包括用靶向于BDNF多核苷酸的天然反义转录物的区域的至少一种反义寡核苷酸接触所述生物系统，所述天然反义转录物包含在SEQ ID NO:3的核苷酸1-1279或SEQ ID NO:4的核苷酸1-1478或SEQ ID NO:5的核苷酸1-1437或SEQ ID NO:6的核苷酸1-2322或SEQ ID NO:7的核苷酸1-2036或SEQ ID NO:8的核苷酸1-2364或SEQ ID NO:9的核苷酸1-3136或SEQ ID NO:10的核苷酸1-906或SEQ ID NO:11的核苷酸1-992之内的5-30个连续核苷酸，从而调节所述生物系统中BDNF多核苷酸的功能和/或表达。

[0013] 在一个实施方案中，本发明包括提高生物系统中具有SEQ ID NO:1和2的BDNF多核苷酸的功能和/或表达的方法，包括用靶向于所述BDNF多核苷酸的天然反义转录物的至少一种反义寡核苷酸接触所述生物系统，所述天然反义转录物包含在SEQ ID NO:3的核苷酸1-1279或SEQ ID NO:4的核苷酸1-1478或SEQ ID NO:5的核苷酸1-1437或SEQ ID NO:6的核苷酸1-2322或SEQ ID NO:7的核苷酸1-2036或SEQ ID NO:8的核苷酸1-2364或SEQ ID NO:9的核苷酸1-3136或SEQ ID NO:10的核苷酸1-906或SEQ ID NO:11的核苷酸1-992之内的5-30个连续核苷酸，从而提高所述BDNF多核苷酸或其表达产物的功能和/或表达。

[0014] 在另一个实施方案中，本发明包括提高生物系统中具有SEQ ID NO:1和2的BDNF多核苷酸的功能和/或表达的方法，包括用靶向于所述BDNF多核苷酸的天然反义转录物的至少一种反义寡核苷酸接触所述生物系统，从而提高所述BDNF多核苷酸或其表达产物的功能和/或表达，其中所述天然反义转录物选自SEQ ID NO:3-11。

[0015] 在另一个实施方案中，本发明包括提高生物系统中具有SEQ ID NO:1和2的BDNF多核苷酸的功能和/或表达的方法，包括用靶向于所述BDNF多核苷酸的天然反义转录物的至少一种反义寡核苷酸接触所述生物系统，从而提高所述BDNF多核苷酸或其表达产物的功能和/或表达，其中所述天然反义转录物选自SEQ ID NO:3-11和其中所述反义寡核苷酸选自SEQ ID NO:12-49的至少一种。

[0016] 在一个实施方案中，组合物包含一种或多种与有义和/或反义BDNF多核苷酸结合的反义寡核苷酸。

[0017] 在一个实施方案中，所述寡核苷酸包含一个或多个经修饰或取代的核苷酸。

[0018] 在一个实施方案中，所述寡核苷酸包含一个或多个经修饰的键。

[0019] 在又另一个实施方案中，所述修饰的核苷酸包含经修饰的碱基，其包括硫代磷酸酯、甲基膦酸酯、肽核酸、2'-O- 甲基、氟或碳、亚甲基或其他锁核酸 (LNA) 分子。优选地，所述修饰的核苷酸为锁核酸分子，包括 α -L-LNA。

[0020] 在一个实施方案中，将所述寡核苷酸经皮下、肌内、静脉内或腹膜内施用于患者。

[0021] 在一个实施方案中，将所述寡核苷酸在药物组合物中施用。治疗方案包括至少一次向患者施用反义化合物；然而，可将此治疗修改成在一段时间内包含多个剂量。所述治疗可与一种或多种其它类型的疗法组合。

[0022] 在一个实施方案中，将所述寡核苷酸封装到脂质体中或连接于载体分子（例如胆固醇、TAT 肽）。

[0023] 在一个实施方案中，本发明包括 SEQ ID NO:50-55 作为靶向于天然反义转录物 (NAT) 的寡核苷酸调节 BDNF 多核苷酸的表达的用途，其中所述 NAT 选自 SEQ ID NO:3-11。在另一个实施方案中，本发明包括 SEQ ID NO:50-55 作为靶向于天然反义转录物 (NAT) 的寡核苷酸调节 BDNF 多核苷酸的表达的用途，其中所述 NAT 选自 SEQ ID NO:3、4、5、7、8、9、10 和 11。

[0024] 其它方面描述于下文。

[0025] 附图简要说明

[0026] 图 1a-e 显示有义 mRNA 和蛋白质的反义介导的调节。图 1a 显示，在用靶向于 BDNF-AS 转录物的非重叠区域的三种 siRNA 寡核苷酸转染几种人和小鼠细胞系后，发生 BDNF 转录物的敲减和上调。图 1b 显示 BDNF-AS 靶向的 siRNA 施用后 BDNF 和 BDNF-AS 转录物的内源表达的时程研究数据。数据显示随着时间过去，BDNF-AS 下调，且然后 BDNF 表达上调且是可逆的。图 1c 显示两种靶向于 BDNF-AS 转录物的 siRNA 显著增加通过 ELISA 测量的 BDNF 蛋白，而乱序的 siRNA 或对照的非靶向 siRNA 不显著增加 BDNF 蛋白。图 1d 显示施用各种 siRNA 后使用 ELISA 和 / 或蛋白质印迹测量的 BDNF 的蛋白质水平。图 1e 显示相对于提高的寡核苷酸浓度 ($10^{-12}$-$10^{-6}$M) 与模拟对照相比的百分倍数变化。

[0027] 图 2 显示 Bdnf 上调提高神经元突起生长 (neuronal outgrowth)。

[0028] 图 3 显示 Bdnf-AS 在体内调节 Bdnf mRNA 和蛋白质。

[0029] 图 4 显示体内 Bdnf-AS 的阻断引起神经元存活和增殖的增加。

[0030] 图 5 显示 BDNF-AS 敲减导致 BDNF mRNA 上调。

[0031] 图 6 显示 Bdnf 表达的转录后调节。

[0032] 图 7 显示 hBDNFAntagoNAT 抑制人 BDNF-AS 转录物。

[0033] 图 8 显示 AntagoNAT 抑制 N2a 细胞中的小鼠 Bdnf-AS 转录物。

[0034] 图 9 显示 BDNF-AS 敲减既不改变 TrkB 的水平也不改变两个方向的 BDNF 相邻基因 (Let7C 和 KIF18A)：LIN7C 和 KIF18A 分别是位于 BDNF3' 下游和 5' 上游的基因。

[0035] 序列表的说明：SEQ ID NO:1：人脑源神经营养因子 (BDNF)，转录物变体 3，mRNA. (NCBI 登录号：NM_170735)；SEQ ID NO:2：小鼠脑源神经营养因子 (Bdnf)，转录物变体 1，mRNA(NCBI 登录号：NM_007540)；SEQ ID NO:3：天然 BDNF 反义序列（转录物变体 BT1A；NR_033313.1)；SEQ ID NO:4：天然 BDNF 反义序列（转录物变体 BT2A；NR_033314.1)；SEQ ID NO:5：天然 BDNF 反义序列（转录物变体 BT1B；NR_033315.1)；SEQ ID NO:6：天然 BDNF

反义序列（转录物变体 BT2B ;NR_002832.2) ;SEQ ID NO:7 :天然 BDNF 反义序列（转录物变体 BT1C ;NR_033312.1) ;SEQ ID NO:8 :天然 BDNF 反义序列 (BDNF-AS 变体）;SEQ ID NO:9 :天然 BDNF 反义序列 ;SEQ ID NO:10 :小鼠天然 BDNF 反义序列（小鼠 BDNF-AS 变体 1) ;SEQ ID NO:11 :小鼠天然 BDNF 反义序列（小鼠 BDNF-AS 变体 2) ;SEQ ID NO:12-55 :反义寡核苷酸 ;SEQ ID NO:56-59 :分别反义寡核苷酸 12-15 的反向互补序列 ;SEQ ID NO:60-64 :分别反义寡核苷酸 42-46 的反向互补序列 ;SEQ ID NO:65 和 66 :分析序列。LNA(2'-O,4'-C 亚甲基锁核酸）:+A* 或 +T* 或 +C* 或 +G* ;2'OM(2'-O- 甲基）:mU* 或 mA* 或 mC* 或 mG* ;PS（硫代磷酸酯）:T* 或 A* 或 G* 或 c* ;RNA :rU 或 rA 或 rG 或 rC。

[0036] 详细说明

[0037] 参考用于说明的示例应用在下文中描述本发明的数个方面。应当理解的是，陈述许多具体细节、关系和方法来提供对本发明的充分理解。然而，在相关领域的普通技术人员将容易地认识到，可在不含一个或多个具体细节的情况下实施本发明或者可用其他方法来实施本发明。本发明不受行为或事件的排序限制，因为一些行为可以不同的顺序进行和 / 或与其他行为或事件同时进行。此外，并非所有说明性的行为或事件对实施本发明的方法都为必需的。

[0038] 本文公开的所有基因、基因名称和基因产物意图对应来自任何物种的同源物，对该物种而言本文公开的组合物和方法为适用的。因此，该术语包括但不限于来自人和小鼠的基因和基因产物。应当理解的是，当公开来自具体物种的基因或基因产物时，意图此公开仅为示范性的，并且除非其出现的上下文中明确指示，否则不应理解为限制。因此，例如，对于本文公开的在一些实施方案中有关哺乳动物核酸和氨基酸序列的基因而言，意图包括来自其他动物（包括，但不限于其他哺乳动物、鱼类、两栖动物、爬行动物和鸟类）的同源和 / 或直向同源基因和基因产物。在一个实施方案中，所述基因或核酸序列为人的基因或核酸序列。定义

[0039] 本文所用的术语仅以描述具体的实施方案为目的而不意图限制本发明。除非上下文另有明确指示，否则本文所用的单数形式“一”、“一个”和“所述”也意图包括复数形式。此外，就术语“包括的”、“包括”、“具有的”、“具有”、“含有”或其变型在详述和 / 或权利要求中所用的程度而言，这类术语意图以类似于术语“包含”的方式是包涵性的。

[0040] 术语“约”或“大约”意为在由本领域普通技术人员所确定的具体值的可接受误差范围之内，这部分取决于该值是如何测定或确定的，即，测量系统的限制。例如，按照本领域的实践，“约”可意为在 1 或大于 1 的标准偏差之内。或者，“约”可意为给定值的最多 20％，优选 10％，更优选 5％，和还更优选 1％的范围。或者，具体地关于生物系统或过程，该术语可意为在值的一个数量级之内，优选在值的 5 倍之内，更优选在 2 倍之内。当本申请和权利要求描述具体值时，除非另作说明，否则应假设术语“约”意为在具体值的可接受误差范围之内。

[0041] 本文所用的术语“mRNA”意为目前已知的靶向于基因的 mRNA 转录物，以及任何可阐明的其它转录物。

[0042] “反义寡核苷酸”或“反义化合物”意为与另一个 RNA 或 DNA（靶 RNA、DNA) 结合的 RNA 或 DNA 分子。例如，如果其为 RNA 寡核苷酸，则其通过 RNA-RNA 相互作用结合另一个 RNA 靶标并改变靶 RNA 的活性。反义寡核苷酸可上调或下调特定多核苷酸的表达和 / 或功能。

该定义意在包括从治疗、诊断或其他观点来看有用的任何外源 RNA 或 DNA 分子。这类分子包括例如反义 RNA 或 DNA 分子、干扰 RNA(RNAi)、微 RNA、诱饵 RNA 分子、siRNA、酶促 RNA、治疗性编辑 RNA(therapeutic editing RNA) 以及激动剂和拮抗剂RNA、反义寡聚化合物、反义寡核苷酸、外部指导序列 (EGS) 寡核苷酸、可变剪接物 (alternate splicer)、引物、探针以及其他与靶核酸的至少一部分杂交的寡聚化合物。因此,可将这些化合物以单链、双链、部分单链或环状寡聚化合物的形式引入。

[0043] 在本发明的情况中,术语“寡核苷酸”是指核糖核酸 (RNA) 或脱氧核糖核酸 (DNA) 或其模拟物的寡聚物或聚合物。术语“寡核苷酸”,也包括天然和 / 或经修饰单体或键 (linkage) 的线性或环状寡聚体,包括脱氧核糖核苷、核糖核苷、其取代和 α - 异头物形式、肽核酸 (PNA)、锁核酸 (LNA)、硫代磷酸酯、甲基膦酸酯等。寡核苷酸能够通过单体与单体相互作用的规律模式 ( 例如沃森 - 克里克 (Watson-Crick) 型碱基配对、胡格斯腾型 (Hoögsteen) 或反向胡格斯腾型碱基配对等 ) 特异地结合靶多核苷酸。

[0044] 寡核苷酸可为“嵌合的”,即,由不同的区组成。在本发明的情况中,“嵌合的”化合物为寡核苷酸,其包含两个或更多个化学区,例如,DNA 区、RNA 区、PNA 区等。每个化学区由至少一个单体单元 ( 即,在寡核苷酸化合物的情况下为核苷酸 ) 组成。这些寡核苷酸典型地包含至少一个区,其中所述寡核苷酸为经修饰的以表现出一种或多种所需特性。寡核苷酸的所需特性包括但不限于,例如增强的对核酸酶降解的抗性、增强的细胞摄取和 / 或增强的对靶核酸的结合亲和力。因此寡核苷酸的不同区可具有不同的特性。本发明的嵌合寡核苷酸可形成为两种或更多种如上所述的寡核苷酸、修饰寡核苷酸、寡聚核苷和 / 或寡核苷酸类似物的混合结构。

[0045] 寡核苷酸可由可“全符合状态 (in “register”)”地连接 ( 即此时单体像在天然 DNA 一样连续地连接 ) 或通过间隔物连接的区组成。所述间隔物意在构成区之间的共价“桥”,并在优选的情况下具有不超过约 100 个碳原子的长度。所述间隔物可携带不同的功能性,例如具有正或负电荷的、具有特殊的核酸结合特性 ( 嵌入剂、沟结合剂、毒素、荧光团等 )、为亲脂的、诱导特殊的二级结构如例如诱导 α - 螺旋的含丙氨酸的肽。

[0046] 本文所用的“BDNF”和“脑源神经营养因子”包括所有家族成员、突变体、等位基因、片段、种类 (species)、编码和非编码序列、有义和反义多核苷酸链等。

[0047] 本文所用的措词“脑源神经营养因子”、“脑来源的神经营养因子”和 BDNF 在文献中被认为是相同的且在本申请中可交换地使用。

[0048] 本文所用的术语“对 ..... 特异的寡核苷酸”或“靶向于 ..... 的寡核苷酸”是指具有以下序列的寡核苷酸 :(i) 能够与靶定基因的一部分形成稳定的复合体,或 (ii) 能够与靶定基因的 mRNA 转录物的一部分形成稳定的双链体。复合体和双链体的稳定性可通过理论计算和 / 或体外测定来确定。用于确定杂交复合体和双链体的稳定性的示例性测定法描述于下文实施例中。

[0049] 本文所用的术语“靶核酸”包括 DNA、从这类 DNA 转录的 RNA ( 包括前 mRNA 和 mRNA),以及从这类 RNA 衍生的 cDNA、编码序列、非编码序列、有义或反义多核苷酸。寡聚化合物与其靶核酸的特异性杂交干扰核酸的正常功能。这种通过特异地与靶核酸杂交的化合物对该靶核酸的功能调节,一般称为“反义”。待干扰的 DNA 功能包括例如复制和转录。待干扰的 RNA 功能,包括所有的生命机能,例如,RNA 向蛋白质翻译位点的易位、蛋白质自 RNA 的翻译、

产生一种或多种 mRNA 种类的 RNA 剪接，以及可由 RNA 参与或促进的催化活性。对靶核酸功能的这类干扰的整体效果为对编码产物或寡核苷酸表达的调节。

[0050] RNA 干扰“RNAi”由双链 RNA(dsRNA) 分子介导，该分子具有与其“靶”核酸序列的序列特异性同源性。在本发明的某些实施方案中，介体为 5-25 个核苷酸的“小干扰”RNA 双链体 (siRNA)。siRNA 通过称为切酶 (Dicer) 的 RNA 酶对 dsRNA 的加工得到。siRNA 双链体产物募集到叫做 RISC(RNA 诱导的沉默复合体 ) 的多蛋白 siRNA 复合体中。不希望受任何具体理论所约束，据认为随后 RISC 被引向靶核酸 ( 适当地为 mRNA)，其中 siRNA 双链体以序列特异性的方式相互作用来介导催化方式的切割。可依照本发明使用的小干扰 RNA 可根据本领域众所周知和普通技术人员熟悉的程序来合成和使用。用于本发明的方法中的小干扰 RNA 适当地包含约 1- 约 50 个核苷酸 (nt)。在非限制性实施方案的实例中，siRNA 可包含约 5- 约 40nt、约 5- 约 30nt、约 10- 约 30nt、约 15- 约 25nt、或约 20-25 个核苷酸。

[0051] 适当寡核苷酸的挑选通过使用电脑程序来辅助，该程序自动比对核酸序列并指示具有同一性或同源性的区。将这类程序用于比较通过例如搜索诸如 GenBank 等的数据库或通过测序 PCR 产物而获得的核酸序列。对来自一系列物种的核酸序列的比较，允许选择在物种之间显示适度同一性的核酸序列。在未测序基因的情况下，进行 DNA 印迹来确定在靶物种和其他物种的基因之间的同一性程度。如本领域众所周知的，通过在不同的严格程度下进行 DNA 印迹，可能获得同一性的近似衡量。这些程序允许选择对待控制的受试者中的靶核酸序列表现出高度的互补性并且对其他物种中的相应核酸序列表现出较低程度的互补性的寡核苷酸。本领域的技术人员将认识到，在挑选用于本发明的适当的基因区方面具有相当大的自由。

[0052] “酶促 RNA”意为具有酶活性的 RNA 分子 (Cech，(1988)J.American.Med.Assoc.260，3030-3035)。酶促核酸 ( 核酶 ) 通过首先结合靶 RNA 来起作用。这类结合通过酶促核酸的靶结合部分进行，所述靶结合部分保持紧密靠近起到切割靶 RNA 分子的作用的酶部分。因此，酶促核酸首先识别而后通过碱基配对结合靶 RNA，且一旦结合到正确的位点，即酶促切割靶 RNA。

[0053] “诱饵 RNA”意为模拟配体的天然结合域的 RNA 分子。因此诱饵 RNA 与天然结合靶标竞争与特异性配体的结合。例如，已显示 HIV 反式激活应答 (TAR)RNA 的过表达可充当“诱饵”并有效地结合 HIV tat 蛋白，从而阻止其结合到在 HIV RNA 中编码的 TAR 序列。这意指特定的实例。本领域的技术人员将认识到，这只是一个实例，而其他的实施方案可使用本领域一般已知的技术容易地产生。

[0054] 本文所用的术语“单体”通常指通过磷酸二酯键或其类似物连接以形成大小范围从少量单体单元 ( 例如从约 3-4) 到约数百个单体单元的寡核苷酸的单体。磷酸二酯键的类似物包括 :硫代磷酸酯、二硫代磷酸酯、甲基膦酸酯、硒代磷酸酯 (phosphoroselenoate)、氨基磷酸酯等，如下文更充分地描述的。

[0055] 术语“核苷酸”涵盖天然存在的核苷酸和非天然存在的核苷酸。本领域的技术人员应清楚的是，先前认为“非天然存在”的多种核苷酸后来已在自然中发现。因此，“核苷酸”不仅包括已知的含嘌呤和嘧啶杂环的分子，而且还包括其杂环类似物和互变异构体。其他类型的核苷酸的说明性实例为以下分子，其含有腺嘌呤、鸟嘌呤、胸腺嘧啶、胞嘧啶、尿嘧啶、嘌呤、黄嘌呤、二氨基嘌呤、8- 氧代 -N6- 甲基腺嘌呤、7- 脱氮杂黄嘌呤、7- 脱氮杂鸟嘌

呤、N4,N4- 桥亚乙基胞嘧啶 (ethanocytosin)、N6,N6- 桥亚乙基 -2,6- 二氨基嘌呤、5- 甲基胞嘧啶、5-(C3-C6)- 炔基胞嘧啶、5- 氟尿嘧啶、5- 溴尿嘧啶、假异胞嘧啶、2- 羟基 -5- 甲基 -4- 三唑并吡啶、异胞嘧啶、异鸟嘌呤、肌苷和在 Benner 等的美国专利第 5,432,272 号中描述的“非天然存在的”核苷酸。术语“核苷酸”意在涵盖这些实例以及其类似物和互变异构体中的每一个和全部。尤其令人关注的核苷酸为含腺嘌呤、鸟嘌呤、胸腺嘧啶、胞嘧啶和尿嘧啶的核苷酸,其被认为是有关人中的治疗和诊断应用的天然存在核苷酸。核苷酸包括天然 2’- 脱氧和 2’- 羟基糖,例如,如 Kornberg 和 Baker,DNA 复制 (DNA Replication),第 2 版 (Freeman,San Francisco,1992) 中所述的,以及其类似物。

[0056] 提及核苷酸的“类似物”包括具有经修饰的碱基部分和 / 或经修饰的糖部分的合成核苷酸 ( 参见例如,由 Scheit,核苷酸类似物 (Nucleotide Analogs),John Wiley,New York,1980 ;Freier 和 Altmann,(1997)Nucl.Acid.Res.,25(22),4429-4443,Toulmé,J.J.,(2001)Nature Biotechnology 19 :17-18 ;Manoharan M.,(1999)Biochemica et Biophysica Acta 1489 :117-139 ;Freier S.M.,(1997)Nucleic Acid Research,25 :4429-4443,Uhlman,E.,(2000)Drug Discovery&Development,3 :203-213,Herdewin P.,(2000)Antisense&Nucleic Acid Drug Dev.,10 :297-310 一般描述的 ) ;2’-O,3’-C- 连接的 [3.2.0] 二环阿糖核苷。这类类似物包括设计以增强结合特性的合成核苷酸,所述结合特性为例如双链体或三链体稳定性、特异性等。

[0057] 本文所用的“杂交”意为寡聚化合物的基本上互补链的配对。一种配对的机理涉及寡聚化合物的链的互补核苷或核苷酸碱基 ( 核苷酸 ) 之间的氢键合,其可为沃森 - 克里克、胡格斯腾型或反向胡格斯腾型氢键合。例如,腺嘌呤和胸腺嘧啶为互补的核苷酸,其通过形成氢键配对。杂交可在各种环境下发生。

[0058] 如果反义化合物与靶核酸的结合干扰靶核酸的正常功能而导致功能和 / 或活性的调节,并且在需要特异性结合的条件下 ( 即在体内测定或治疗性处理情况中的生理条件下,以及其中在体外测定情况下进行测定的条件下 ) 存在足够程度的互补性来避免所述反义化合物与非靶核酸序列的非特异性结合,反义化合物为“可特异地杂交的”。

[0059] 本文所用的短语“严格杂交条件”或“严格条件”是指以下条件 :在该条件下本发明的化合物与其靶序列杂交,但与最少数量的其他序列杂交。严格条件为序列依赖的且在不同环境下将不同,在本发明的情况中,在其下寡聚化合物与靶序列杂交的“严格条件”由寡聚化合物的性质和组成以及正在其中研究它们的试验来确定。一般而言,严格杂交条件包括低浓度 ( < 0.15M) 的含有诸如 Na+ 或 K+ 等无机阳离子的盐 ( 即,低离子强度 )、高于 20℃ -25℃而低于寡聚化合物 : 靶序列复合体的 Tm 的温度,以及变性剂的存在,例如甲酰胺、二甲基甲酰胺、二甲基亚砜,或去污剂十二烷基硫酸钠 (SDS)。例如,杂交率对于每 1% 甲酰胺降低 1.1%。高严格杂交条件的实例为 0.1X 氯化钠 - 柠檬酸钠缓冲液 (SSC)/0.1% (w/v)SDS、60℃下达 30 分钟。

[0060] 本文所用的“互补性”是指在一条或两条寡聚链上两个核苷酸之间精确配对的能力。例如,如果在反义化合物的某个位置上的核碱基能够与在靶核酸的某个位置上的核碱基氢键键合,所述靶核酸为 DNA、RNA 或寡核苷酸分子,则认为所述寡核苷酸和所述靶核酸之间氢键键合的位置为互补位置。当可彼此氢键键合的核苷酸占据了每个分子中足够数量的互补位置时,寡聚化合物和另外的 DNA、RNA 或寡核苷酸分子为彼此互补的。因此,“可特

异性杂交的”和“互补的”为用于表示在足够数量的核苷酸上有足够程度的精确配对或互补性以使得稳定和特异的结合发生在寡聚化合物和靶核酸之间的术语。

[0061] 本领域中理解，寡聚化合物的序列不需要与其可特异杂交的靶核酸的序列 100％互补。此外，寡核苷酸可在一个或多个区段上杂交，使得间插或邻近的区段不涉及杂交事件（例如，环结构、错配或发夹结构）。本发明的寡聚化合物包含与其靶向的靶核酸序列内的靶区域至少约 70％、或至少约 75％、或至少约 80％、或至少约 85％、或至少约 90％、或至少约 95％、或至少约 99％的序列互补。例如，其中反义化合物的 20 个核苷酸中有 18 个与靶区域互补且因而会特异地杂交的反义化合物，表示 90％互补性。在此实例中，余下的非互补核苷酸可与互补核苷酸是聚簇或散布的且不需要彼此邻接或邻接互补核苷酸。因此，长度为 18 个核苷酸的反义化合物具有 4（四）个非互补核苷酸，该非互补核苷酸位于与靶核酸完全互补的两个区的侧翼，所述反义化合物会具有与靶核酸 77.8％的总互补性，因此落入本发明的范围内。反义化合物与靶核酸区的互补性百分比可使用本领域已知的 BLAST 程序（基本局部比对搜索工具）和 PowerBLAST 程序常规地确定。百分同源性、序列同一性或互补性可通过例如 Gap 程序 (Wisconsin 序列分析包，Unix 操作系统版本 8，Genetics Computer Group，University Research Park，Madison Wis.）使用默认设置来确定，该程序使用 Smith 和 Waterman 的算法 (Adv. Appl. Math.，(1981)2，482-489)。

[0062] 本文所用的术语“解链温度 (Tm)”是指在限定的离子强度、pH 和核酸浓度下，平衡时 50％与靶序列互补的寡核苷酸与靶序列杂交的温度。典型地，对于短的寡核苷酸（例如，10-50 个核苷酸）而言严格条件为以下条件，其中盐浓度至少为约 0.01-1.0M Na 离子浓度（或其他盐），pH 7.0-8.3 且温度至少为约 30℃。严格条件也可通过外加诸如甲酰胺等去稳定剂来达到。

[0063] 本文所用的“调节”意为在基因表达方面的增加（刺激）或减少（抑制）。

[0064] 术语“变体”，当用于多核苷酸序列的情况下时，可包括与野生型基因相关的多核苷酸序列。此定义也可包括，例如，“等位基因的”、“剪接”、“物种”或“多态性”变体。剪接变体可具有与参比分子显著的同一性，但因为在 mRNA 加工期间外显子的可变剪接而通常具有更多或更少数量的多核苷酸。对应的多肽可具有附加的功能域或域的缺失。物种变体为在不同物种之间不同的多核苷酸序列。本发明中尤其实用的是野生型基因产物的变体。变体可由核酸序列中的至少一个突变产生并可导致产生改变的 mRNA 或者其结构或功能可能改变或不变的多肽。任何给定的天然或重组基因可不具有、或者具有一个或许多等位基因形式。产生变体的常见突变变化一般归因于核苷酸的自然缺失、添加或取代。这些变化类型中的每一个可单独或与其他类型联合发生，在给定序列中发生一次或多次。

[0065] 产生的多肽一般将具有相对于彼此的显著的氨基酸同一性。多态性变体为在给定物种的个体之间特定基因的多核苷酸序列的变化。多态性变体也可包括“单核苷酸多态性”(SNP) 或单碱基突变，其中多核苷酸序列因一个碱基而不同。SNP 的存在可指示例如具有疾病状态倾向（即与抗性相对的易感性）的某个群体。

[0066] 衍生物多核苷酸包括经过化学修饰的核酸，例如用烷基、酰基或氨基置换氢。衍生物（例如，衍生物寡核苷酸）可包含非天然存在的部分，例如改变的糖部分或糖间键。它们中示例性的是硫代磷酸酯及本领域已知的其他含硫的物质。衍生物核酸也可含有标记，包括放射性核苷酸、酶、荧光剂、化学发光剂、显色剂、底物、辅因子、抑制剂、磁性颗粒等等。

[0067] “衍生物”多肽或肽为经修饰的多肽或肽，例如，通过糖基化、聚乙二醇化、磷酸化作用、硫酸盐化作用、还原/烷基化、酰化、化学偶联或温和福尔马林处理。也可将衍生物修饰以含有可检测标记（直接地或间接地），包括但不限于放射性同位素、荧光和酶标记。

[0068] 本文所用的术语“动物”或“患者”意在包括例如人、绵羊、麋鹿、鹿、长耳鹿、貂、哺乳动物、猴、马、牛、猪、山羊、狗、猫、大鼠、小鼠、鸟、鸡、爬行动物、鱼、昆虫和蜘蛛类。

[0069] “哺乳动物”涵盖通常在医疗护理下的温血哺乳动物（例如，人和驯养动物）。实例包括猫科动物、犬科动物、马科动物、牛科动物和人，以及仅仅人。

[0070] “处理”或“治疗”涵盖对哺乳动物中疾病状态的处理，并包括：(a) 防止疾病状态出现于哺乳动物中，特别是当这类哺乳动物倾向于疾病状态但尚未诊断为患有该疾病状态时；(b) 抑制疾病状态，例如，阻止其发展；和/或 (c) 减轻疾病状态，例如，引起疾病状态的退行直到达到所需的终点。治疗也包括改善疾病的症状（例如，减少疼痛或不适），其中这类改善可直接或可非直接地影响疾病（例如，原因、传递、表达等）。

[0071] 本文所用的术语“癌症”是指在哺乳动物中发现的所有类型的癌症或肿瘤或恶性肿瘤，包括，但不限于：白血病、淋巴瘤、黑素瘤、癌瘤和肉瘤。癌症自身表现为包含癌症恶性细胞的“肿瘤”或组织。肿瘤的实例包括肉瘤或癌，例如但不限于：纤维肉瘤、黏液肉瘤、脂肪肉瘤、软骨肉瘤、骨肉瘤、脊索瘤、血管肉瘤、内皮肉瘤、淋巴管肉瘤、淋巴管内皮肉瘤、滑膜瘤、间皮瘤、尤因氏瘤 (Ewing′ s tumor)、平滑肌肉瘤、横纹肌肉瘤、结肠癌、胰腺癌、乳腺癌、卵巢癌、前列腺癌、鳞状细胞癌、基底细胞癌、腺癌、汗腺癌、皮脂腺癌、乳头状癌、乳头状腺癌、囊腺癌、髓样癌、支气管癌、肾细胞癌、肝细胞瘤、胆管癌、绒毛膜癌、精原细胞瘤、胚胎性癌、维尔姆斯氏肿瘤 (Wilms′ tumor)、子宫颈癌、睾丸肿瘤、肺癌、小细胞肺癌、膀胱癌、上皮癌、神经胶质瘤、星形细胞瘤、髓母细胞瘤、颅咽管瘤、室管膜瘤、松果体瘤、成血管细胞瘤、听神经瘤、少突神经胶质瘤、脑膜瘤、黑素瘤、成神经细胞瘤和视网膜母细胞瘤。可以通过根据本发明的所公开组合物治疗的另外的癌症包括，但不限于例如何杰金氏病、非何杰金氏淋巴瘤、多发性骨髓瘤、成神经细胞瘤、乳腺癌、卵巢癌、肺癌、横纹肌肉瘤、原发性血小板增多症、原发性巨球蛋白血症、小细胞肺癌、原发性脑肿瘤、胃癌、结肠癌、恶性胰腺瘤 (malignant pancreatic insulanoma)、恶性类癌瘤、膀胱癌、胃癌、癌前皮肤病变、睾丸癌、淋巴瘤、甲状腺癌、成神经细胞瘤、食管癌、泌尿生殖道癌、恶性高钙血症、宫颈癌、子宫内膜癌、肾上腺皮质癌和前列腺癌。

[0072] 如本文所用的，“神经疾病或障碍”是指神经系统和/或视觉系统的任何疾病或障碍。“神经疾病或障碍”包括累及中枢神经系统（脑、脑干和小脑）、外周神经系统（包括脑神经）和自主神经系统（其部分位于中枢和外周神经系统两者中）的疾病或障碍。神经疾病或障碍包括，但不限于获得性癫痫样失语症、急性播散性脑脊髓炎、脑白质肾上腺萎缩症、年龄相关性黄斑变性、胼胝体发育不全、认识不能、艾卡迪综合征、亚力山大病、阿尔佩斯病、交叉性肢体瘫痪、阿尔茨海默氏病、血管性痴呆、肌萎缩性侧索硬化、无脑畸形、Angelman 综合征、血管瘤病、缺氧症、失语症、精神性运动不能、蛛网膜囊肿、蛛网膜炎、Anronl-Chiari 畸形、动静脉畸形、阿斯佩各综合征、毛细血管扩张性共济失调 (ataxia telegiectasia)、注意力缺乏多动症、孤独症、自主神经功能障碍、背痛、巴藤病、贝切特氏病、贝耳氏麻痹、良性自发性睑痉挛、良性局灶性、肌萎缩、良性颅内高血压、宾斯旺格氏病、睑痉挛、Bloch Sulzberger 综合征、臂丛损伤、脑脓肿、脑损伤、脑肿瘤（包括多形性成胶

质细胞瘤）、脊髓肿瘤、布朗－塞卡尔综合征、卡纳万病、腕管综合征、灼痛、中枢性痛综合征、脑桥中央髓鞘溶解、头部障碍、脑动脉瘤、脑动脉硬化、脑萎缩、大脑性巨人症、大脑性麻痹、夏－马－图三氏病、化疗诱导的神经痛和神经性疼痛、Chiari 畸形、舞蹈病、慢性炎性脱髓鞘性多神经病、慢性痛、慢性区域性疼痛综合征、科－勒二氏综合征、昏迷，包括持续性植物状态、先天性面瘫、皮质基底核退化症、颅动脉炎、颅缝早闭、克雅氏病、累积性创伤失调、柯兴综合征、巨细胞性包涵体病、巨细胞病毒感染、眼足舞蹈综合征、丹－沃二氏综合征、Dawson 病、德摩西埃综合征、Dejerine-Klumke 麻痹、痴呆、皮肌炎、糖尿病性神经病、弥漫性硬化症、家族性自主神经机能异常、书写困难、诵读困难、张力障碍、早期幼儿癫痫性脑病、空蝶鞍综合征、脑炎、脑膨出、脑三叉神经血管瘤病、癫痫、欧勃（氏）麻痹、特发性震颤、法布里氏病、法尔氏综合征、晕厥、家族性痉挛性麻痹、热性惊厥、菲希尔综合征、弗里德赖希氏共济失调、额颞叶性痴呆和其它“tau 蛋白病（tauopathies）”、高歇氏病、格斯特曼氏综合征、巨细胞性动脉炎、巨细胞性包涵体病、球样细胞脑白质营养不良、格－巴二氏综合征、HTLV-1- 相关脊髓病、哈－斯二氏病、颅脑损伤、头痛、半侧颜面痉挛、遗传性痉挛性截瘫、多神经炎型遗传性运动失调、耳部带状疱疹、带状疱疹、Hirayama 综合征、HIV 相关痴呆和神经病（也即 AIDS 的神经表现）、前脑无裂畸形、亨廷顿氏舞蹈病和其它多聚谷氨酰胺重复疾病 (polyglutamine repeat disease)、积水性无脑畸形、脑积水、皮质醇增多症、缺氧、免疫介导的脑脊髓炎、包涵体肌炎、色素失调症、婴儿植烷酸贮积病、婴儿型雷夫叙姆病 (infantile refsum disease)、婴儿痉挛、炎症性肌病、颅内囊肿、颅内高血压、朱伯特综合征 (Joubert syndrome)、卡恩斯塞尔综合征 (Kearns-sayre syndrome)、肯尼迪病 (Kennedy disease)、金斯布林纳综合征 (kinsbourne syndrome)、颈椎融合综合征 (Klippel Feil syndrome)、克拉伯病 (Krabbe disease)、库格尔贝格－韦兰德病 (Kugelberg-welander disease)、库鲁病 (kuru)、拉福拉病 (lafora disease)、朗－爱二氏肌无力综合征 (Lambert-eaton myasthenic syndrome)、获得性癫痫性失语 (Landau-Kleffner syndrome)、延髓外侧 (Wallenberg) 综合征、学习无能、利氏病 (leigh's disease)、Lennox-Gastaut 综合征 (Lennox-gastaut syndrome)、莱施－尼汉综合征 (Lesch-nyhan syndrome)、脑白质营养不良、路易体痴呆、无脑回、闭锁综合征 (locked-in syndrome)、路格里克氏病 (Lou Gehrig's disease)（即运动神经元病或肌萎缩性侧索硬化）、腰椎间盘病、莱姆病 (lyme disease)- 神经后遗症、马查多－约瑟夫病 (machado-joseph disease)、巨脑、巨脑畸形、梅尔克松－罗森塔尔综合征 (Melkersson-rosenthal syndrome)、梅尼埃病 (Meniere's disease)、脑膜炎、门克斯病 (Menkes disease)、异染性脑白质营养不良、小头畸形、偏头痛、米勒－费雪综合征 (Miller Fisher syndrome)、小中风、线粒体肌病、默比乌斯综合征 (mobius syndrome)、单肢肌萎缩、运动神经元病、烟雾病 (moyamoya disease)、粘多糖贮积病、多梗死性痴呆、多灶性运动神经病、多发性硬化和其它脱髓鞘疾病、多系统萎缩伴体位性低血压、肌肉萎缩症、重症肌无力、脱髓鞘弥漫性硬化、婴儿肌阵挛脑病、肌阵挛、肌病、先天性肌强直、发作性睡病、神经纤维瘤病、抗精神病药恶性综合征、AIDS 的神经表现、狼疮的神经后遗症、神经性肌强直、神经元蜡样质脂褐质沉积症、神经元移行异常、尼曼－匹克病 (niemann-pick disease)、O'sullivan-Mcleod 综合征、枕神经痛、隐性脊柱神经管闭合不全序列征、大田原综合征 (Ohtahara syndrome)、橄榄体脑桥小脑萎缩、视性眼阵挛－肌阵挛、视神经炎、直立性低血压、过劳综合征、感觉异常、神经变性

疾病或障碍（帕金森氏症、亨廷顿氏病、阿尔茨海默氏病、肌萎缩性侧索硬化 (ALS)、痴呆、多发性硬化及其它与神经元细胞死亡相关的疾病和障碍）、先天性肌强直病、副肿瘤疾病、阵发性发作 (paroxysmal attacks)、帕罗综合征 (Parry-Romberg syndrome)、佩－梅氏病 (Pelizaeus-Merzbacher disease)、周期性麻痹、周围神经病变、疼痛性神经病和神经性疼痛、持续性植物状态、广泛性发育障碍、旋光性喷嚏反射、植烷酸贮积病、皮克氏病、神经挟捏、垂体瘤、多小脑回 (pmg)、多肌炎、脑穿通畸形、脊髓灰质炎后综合征、带状疱疹后神经痛、感染后脑脊髓炎、体位性低血压、普－威二氏综合征 (Prader-Willi syndrome)、原发性侧索硬化、朊病毒病、进行性面部单侧萎缩、进行性多灶性脑白质病、进行性硬化性灰质萎缩、进行性核上麻痹、脑假瘤、拉姆齐－亨特综合征（I 型和 II 型）、拉斯马森 (Rasmussen) 脑炎、反射性交感神经营养不良综合征、雷夫叙姆病、反复性运动障碍、反复性应激损伤、多动腿综合征、逆转录病毒相关性脊髓病、雷特综合征、雷耶氏综合征、圣维特斯舞蹈病、桑德霍夫病、谢耳德病、脑裂、视隔发育不全 (septo-optic dysplasia)、惊吓婴儿综合征、带状疱疹、希－德综合征、斯耶格伦综合征、睡眠性呼吸暂停、索托斯综合征 (Soto’s syndrome)、痉挛状态、脊柱裂、脊髓损伤、脊髓瘤、脊髓性肌萎缩、僵人综合征 (Stiff-Person syndrome)、中风、斯－韦综合征、亚急性硬化性全脑炎、皮层下动脉硬化性脑病、西德纳姆舞蹈病、晕厥、脊髓空洞症、迟发性运动障碍、泰－萨病、颞动脉炎、脊髓栓系综合征 (tethered spinal cord syndrome)、肌强直性白内障、胸廓出口综合征、三叉神经痛、Todd 麻痹、图雷特综合症、短暂性脑缺血发作、传染性海绵状脑病、横贯性脊髓炎、外伤性脑损伤、震颤、三叉神经痛、热带痉挛性轻截瘫、结节性硬化症、血管性痴呆（多发梗塞性痴呆）、血管炎包括颞动脉炎、希－林二氏 (Von Hippel-Lindau) 病、瓦伦伯格综合征、韦－霍 (Werdnig-Hoffman) 病、韦斯特综合征、颈部扭伤 (whiplash)、威廉斯综合征、Wildon 病以及泽尔韦格综合征。

[0073] “增殖性疾病或障碍”包括，但不限于由骨髓、淋巴或红细胞谱系或其前体细胞产生的涉及造血系统源的增生性 / 赘生性细胞的造血系统肿瘤性疾病。这些包括，但不限于成红细胞白血病、急性前髓性细胞白血病 (APML)、慢性髓细胞性白血病 (CML)、淋巴样恶性肿瘤包括，但不限于急性成淋巴细胞性白血病 (ALL)（其包括 B- 谱系 ALL 和 T- 谱系 ALL）、慢性淋巴细胞性白血病 (CLL)、前淋巴细胞性白血病 (PLL)、毛细胞性白血病 (HLL) 和瓦尔登斯特伦氏巨球蛋白血症 (WM)。另外的恶性淋巴瘤形式包括，但不限于非霍杰金淋巴瘤及其变体、外周 T 细胞淋巴瘤、成人 T 细胞白血病 / 淋巴瘤 (ATL)、皮肤 T- 细胞淋巴瘤 (CTCL)、大颗粒淋巴细胞白血病 (LGF)、何杰金病和李特－斯顿伯格病 (Reed-Sternberg disease)。

[0074] “炎症”是指全身性炎性病症以及局部地与单核细胞、白细胞和 / 或中性粒细胞的迁移和吸引有关的病症。炎症的实例包括，但不限于由致病生物体（包括革兰氏阳性菌、革兰氏阴性菌、病毒、真菌以及寄生生物如原生动物和蠕虫）的感染、移植排斥（包括实质器官如肾、肝、心、肺或角膜的排斥以及骨髓移植物的排斥，包括移植物抗宿主病 (GVHD)）产生的炎症，或者由局限性慢性或急性自身免疫反应或变态反应产生的炎症。自身免疫性疾病包括急性肾小球肾炎；类风湿性或反应性关节炎；慢性肾小球肾炎；炎性肠病如克罗恩氏病、溃疡性结肠炎和坏死性小肠结肠炎；肝炎；脓毒症；酒精性肝病；非酒精性脂肪变性；与粒细胞输注有关的综合征；炎性皮肤病如接触性皮炎、特应性皮炎、银屑病；系统性红斑狼疮 (SLE)；自身免疫性甲状腺炎、多发性硬化以及糖尿病的某些形式、或任何其它自身免

疫状态（其中受试者自身免疫系统的攻击导致病理性组织破坏）。变态反应包括变应性哮喘、慢性支气管炎、急性和迟发型超敏反应。全身性炎性疾病状态包括与创伤、烧伤、缺血事件（例如在心、脑、肠或外周脉管系统中的血栓形成事件，包括心肌梗死和中风）后的再灌注、脓毒症、ARDS 或多器官功能障碍综合征相关的炎症。炎性细胞募集也在粥样硬化斑块中发生。炎症包括，但不限于非何杰金淋巴瘤、韦格纳肉芽肿病、桥本甲状腺炎、肝细胞癌、胸腺萎缩、慢性胰腺炎、类风湿性关节炎、反应性淋巴样增生、骨关节炎、溃疡性结肠炎、乳头状癌、克罗恩氏病、溃疡性结肠炎、急性胆囊炎、慢性胆囊炎、肝硬化、慢性涎腺炎、腹膜炎、急性胰腺炎、慢性胰腺炎、慢性胃炎、子宫内膜异位 (adenomyosis)、子宫内膜异位症 (endometriosis)、急性子宫颈炎、慢性子宫颈炎、淋巴样增生、多发性硬化、继发于特发性血小板减少性紫癜的肥大、原发性 IgA 肾病、系统性红斑狼疮、银屑病、肺气肿、慢性肾盂肾炎以及慢性膀胱炎。

[0075] 多核苷酸和寡核苷酸组成和分子

[0076] 靶标：在一个实施方案中，靶标包括脑源神经营养因子 (BDNF) 的核酸序列，包括但不限于与 BDNF 有关的有义和 / 或反义非编码和 / 或编码序列。PCT 公开号 WO 2010/093904 和美国专利申请公开号 2011/0319475（两者名称均为“Treatment of Brain Derived Neurotrophic Factor(BDNF)Related Diseases by Inhibition of Natural Antisense Transcript to BDNF”并通过引用全文并入本文）公开了 BDNF 为使用如其中所述的寡核苷酸调节的靶标。

[0077] 神经营养蛋白为一类结构上相关的生长因子，其促进神经生存和分化。它们刺激神经突生长，表明其可促进受损神经元的再生，并充当靶标 - 衍生神经营养因子以在产生神经营养蛋白的靶组织中刺激侧支出芽。脑源神经营养因子 (BDNF) 最初表征为存在于脑提取物中的碱性蛋白并能够增加背根神经节的存活。当与细胞体的轴突交流被损伤中断时，许旺细胞产生神经营养因子，例如神经生长因子 (NGF) 和 BDNF。神经营养蛋白从许旺细胞中释放且在再生轴突周围以梯度形式扩散式地分布，再生轴突然后顺着神经营养蛋白的密度梯度向远侧延伸。已显示局部施用 BDNF 至新生大鼠中的横断神经防止轴突切断术后运动神经元的大量死亡。BDNF 的 mRNA 效价在轴突切断术四天后增加到正常水平的数倍，并在 4 周时达到其最大值。此外，已报道 BDNF 增强培养物中胆碱能神经元的生存。

[0078] 在一个实施方案中，反义寡核苷酸用于预防和治疗与 BDNF 家族成员相关的疾病或障碍。可用本发明的反义寡核苷酸和 / 或用由使用反义化合物获得的和 / 或具有反义化合物的干细胞再生的细胞 / 组织治疗的示例性脑源神经营养因子 (BDNF) 介导疾病和障碍包括：与 BDNF 的异常功能和 / 或表达相关的疾病或障碍；神经疾病或障碍；与缺陷性神经发生有关的疾病或病症；神经变性疾病或障碍（例如，阿尔茨海默氏病、帕金森氏病、亨廷顿舞蹈病、肌萎缩侧索硬化等）；神经精神障碍（抑郁症、精神分裂症、精神分裂症样精神障碍 (schizofreniform disorder)、情感分裂性精神障碍和妄想性障碍；焦虑性障碍例如惊恐性障碍、恐怖症（包括广场恐怖症）、强迫性神经失调、创伤后应激障碍、双相性精神障碍、神经性厌食、神经性贪食）、中枢神经系统的自身免疫性障碍（例如，多发性硬化）、记忆丧失、长期或短期记忆障碍、良性健忘、儿童学习障碍、闭合性颅脑损伤、注意力缺陷障碍、对病毒感染的神经元反应、脑损伤、发作性睡病、睡眠障碍（例如，昼夜节律障碍、失眠症和发作性睡病）；神经中断或神经损伤、脑脊髓神经索 (CNS) 中断和脑或神经细胞损伤、

与AIDS有关的神经功能缺损、以运动和/或发声性抽搐(vocal tic)为特征的运动和抽搐障碍(例如,图雷特精神障碍(Tourette′ s disorder)、慢性运动或发声性抽搐障碍、短时抽搐性障碍和刻板型活动障碍)、物质滥用障碍(例如,物质依赖、物质滥用以及物质滥用/依赖的后遗症,例如物质诱发的心理障碍、物质戒断和物质诱发的痴呆或遗忘障碍)、外伤性脑损伤、耳鸣、神经痛(例如,三叉神经痛)、疼痛(例如,慢性疼痛、慢性炎性疼痛、与关节炎有关的疼痛、纤维肌痛、背痛、与癌症相关的疼痛、与消化性疾病有关的疼痛、与克罗恩氏病有关的疼痛、与自身免疫性疾病有关的疼痛、与内分泌疾病有关的疼痛、与糖尿病性神经病变有关的疼痛、幻肢痛、自发性疼痛、慢性术后疼痛、慢性颞下颌疼痛、灼痛、疱疹后神经痛、AIDS相关的疼痛、I和II型复杂性区域疼痛综合征、三叉神经痛、慢性背痛、与脊髓损伤有关的疼痛、与药物摄入有关的疼痛和复发性急性疼痛、神经病性疼痛)、在诸如糖尿病、MS和运动神经元病的疾病中导致neurodysthesias的不适当的神经元活性、共济失调、肌肉强直(痉挛状态)、颞下颚关节功能障碍、奖赏缺陷综合征(RDS)、由酒精或物质滥用(例如,摇头丸(ecstacy)、去氧麻黄碱等)引发的神经毒性、精神发育迟缓或认知缺损(例如,非综合征性X连锁精神发育迟缓、脆性X综合征、唐氏综合征、孤独症)、失语症、贝尔氏麻痹(Bell’s palsy)、克-雅氏病、脑炎、年龄相关性黄斑变性、ondine综合征、WAGR综合征、听力损失、雷特综合征、癫痫、脊髓损伤、中风、缺氧、缺血、脑损伤、糖尿病性神经病变、周围神经病、神经移植并发症、运动神经元病、周围神经损伤、肥胖症、代谢综合征、癌症、哮喘、特应性疾病、炎症、过敏症、湿疹、神经肿瘤学疾病或障碍、神经免疫学疾病或障碍和神经耳科疾病或障碍;以及与老化和衰老有关的疾病或障碍。

[0079] 本发明提供了内源NAT抑制其有义基因对应物的转录的机制。本发明提出内源基因表达可以通过去除或抑制从大多数转录单元转录的NAT以基因座特异性的方式上调。

[0080] 本发明的一个实施方案提供调节蛋白质输出(可适用于许多其它基因组基因座的现象)的功能性ncRNA的实例。

[0081] 脑源神经营养因子(BDNF)是对于神经元生长、成熟分化和维持必要的生长因子“神经营养因子”家族的成员。BDNF对于神经元可塑性也是必要的且显示为涉及学习和记忆过程。BDNF基因座是在染色体11上且显示两条链的活跃转录,其导致非编码NAT的转录。

[0082] 本发明鉴定了在体外和体内对有义BDNF mRNA和蛋白质的表达发挥有力的相互和动态调节作用的这一反义RNA分子(BDNF-AS)的调控作用。

[0083] 本发明的一个实施方案提供了使用反义RNA转录物抑制分子(称为AntagoNAT)上调mRNA表达的策略。AntagoNAT描述于例如PCT公开号WO 2012/068340中,其通过引用全文并入。

[0084] 真核生物基因组中ncRNA的数目已显示随发育复杂性而增加,且例如在神经系统中表达的ncRNA中存在显著的多样性。在过去几年中,已经有功能性NAT的报告且证明了其潜在地涉及人类障碍,包括阿尔茨海默氏病、帕金森氏病和脆性X染色体综合征。此外,已经报告CD97有义基因的上调可以通过敲减其反义转录物实现。孕酮受体(PR)的上调及其它内源转录物在启动子衍生的非编码RNA靶向后报告。p21基因和Oct4启动子的转录激活在NAT删除后报告。反义RNA诱导的染色质重建对于许多低拷贝数NAT似乎是一种可行的和动态的作用模式。如果是这样,反义RNA可以显著地发挥局部效应以维持或改变染色质结构,最终激活或抑制有义基因表达。

[0085] PCR2 是由四个核心亚基组成的蛋白质复合体 :Eed、Suz12、RbAp48 和催化性 Ezh2( 其催化组蛋白 H3- 赖氨酸的三甲基化 (H3K27met3))。最近的研究提供了 Ezh2 和许多 ncRNA 转录物之间的直接 RNA- 蛋白质相互作用的证据。X 灭活和 HOX 基因簇的其它研究显示 RNA 转录物参与 PRC2- 介导的 H3K27met3( 抑制性染色质标志 ) 的诱导。PRC2 转录组谱在胚胎干细胞中已经鉴定了超过 9,000 个 PRC2- 相互作用 RNA,它们中的许多分类为反义 RNA 转录物。p15 和 DM1 基因的后生沉默报告为通过其反义 RNA 涉及异染色质形成。染色质常规地二元分成异染色质和常染色质类别可能不是完全的,因为最近的工作证明存在五种主要的染色质类型,其比先前认为的具有更高的动态性和灵活性。类似地应用于大量的基因座,NAT 可以进行操作以获得染色质修饰中的基因座特异性改变。作为例子,BDNF 基因的反义转录物的切割 ( 通过 siRNA) 或抑制 ( 通过 AntagoNAT) 证明导致相应 mRNA 的上调。

[0086] 神经营养因子属于增强神经元的存活、发育、分化和功能的一类分泌型生长因子,且 BDNF 是突触可塑性的重要分子介质。BDNF 表明使神经元和胶质细胞成熟同步,参与轴突和树突分化及保护和增强神经元细胞存活。神经营养因子表达水平在神经变性障碍中和在精神和神经发育障碍中受损。神经营养因子的上调据认为对于几种神经障碍具有有益效果。AntagoNAT 可以用作抑制 BDNF-AS 的治疗策略并因此增强多种疾病状态中的神经元增殖和存活。不能排除上调内源 BDNF 分子 ( 推测包含天然修饰和代表所有已知的剪接形式 ) 的合成的本文描述的途径将证明与施用合成 BDNF 分子不同且也许优于施用合成 BDNF 分子。

[0087] 在一个实施方案中,一种或多种反义寡核苷酸对 BDNF 的调节施用于需要的患者以预防或治疗与相比于正常对照的 BDNF 异常表达、功能、活性相关的任何疾病或障碍。

[0088] 在一个实施方案中,寡核苷酸对于本文中所述的 BDNF 的天然反义转录物是特异性的,其包括,但不限于非编码区域。BDNF 靶标包括 BDNF 的变体 ;BDNF 的突变体,包括 SNP ;BDNF 的非编码序列 ;等位基因、片段等。优选所述寡核苷酸为反义 RNA 分子。

[0089] 依照本发明的实施方案,靶核酸分子不限于单独 BDNF 多核苷酸,而是扩展到 BDNF 的任何同种型、受体、同源物、非编码区等。

[0090] 在一个实施方案中,寡核苷酸靶向于 BDNF 靶标的天然反义序列 ( 针对编码和非编码区的天然反义物 ),所述 BDNF 靶标包括但不限于其变体、等位基因、同源物、突变体、衍生物、片段和互补序列。优选所述寡核苷酸为反义 RNA 或 DNA 分子。

[0091] 在一个实施方案中,本发明的寡聚化合物也包括变体,其中在所述化合物的一个或多个核苷酸位置上存在不同的碱基。例如,如果第一个核苷酸为腺嘌呤,则可产生在此位置含有胸苷、鸟苷、胞苷或其他天然或非天然核苷酸的变体。这可在所述反义化合物的任何位置上完成。然后使用本文所述的方法来检测这些化合物以确定其抑制靶核酸的表达的能力。

[0092] 在一些实施方案中,反义化合物与靶标之间的同源性、序列同一性或互补性为约 50% - 约 60%。在一些实施方案中,同源性、序列同一性或互补性为约 60% - 约 70%。在一些实施方案中,同源性、序列同一性或互补性为约 70% - 约 80%。在一些实施方案中,同源性、序列同一性或互补性为约 80% - 约 90%。在一些实施方案中,同源性、序列同一性或互补性为约 90%、约 92%、约 94%、约 95%、约 96%、约 97%、约 98%、约 99%或约 100%。

[0093] 反义化合物在以下情况时为可特异性杂交的 :所述化合物与靶核酸的结合干扰靶

核酸的正常功能而引起活性损失，并且在需要特异性结合的条件下存在足够程度的互补性以避免所述反义化合物与非靶核酸序列的非特异性结合。这类条件包括，即，在体内测定或治疗性处理情况中的生理条件，以及其中在体外测定情况下进行测定的条件。

[0094] 反义化合物，不论 DNA、RNA、嵌合的、取代的等等，在以下情况时为可特异性杂交的：所述化合物与靶 DNA 或 RNA 分子的结合干扰靶 DNA 或 RNA 的正常功能而引起效用损失，并且在需要特异性结合的条件下存在足够程度的互补性以避免所述反义化合物与非靶序列的非特异性结合，所述条件即在体内测定或治疗性处理情况中的生理条件，以及在体外测定情况下在其中进行测定的条件。

[0095] 在一个实施方案中，靶向于 BDNF 调节 BDNF 的表达或功能，BDNF 包括但不限于使用例如 PCR、杂交等鉴定和扩增的反义序列、一个或多个如 SEQ ID NO：3-11 所述的序列，等等。在一个实施方案中，表达或功能与对照相比为上调的。在一个实施方案中，表达或功能与对照相比为下调的。

[0096] 在一个实施方案中，寡核苷酸包括如 SEQ ID NO：12-49 所述的核酸序列，包括使用例如 PCR、杂交等鉴定和扩增的反义序列。这些寡核苷酸可包含一个或多个经修饰的核苷酸、较短或较长的片段、经修饰的键等。经修饰的键或核苷酸间键的实例包括硫代磷酸酯、二硫代磷酸酯等。在一个实施方案中，所述核苷酸包括磷衍生物。可连接到本发明的修饰寡核苷酸中的糖或糖类似物部分的磷衍生物（或经修饰的磷酸基）可为单磷酸酯、二磷酸酯、三磷酸酯、烷基磷酸酯、链烷磷酸酯、硫代磷酸酯等。上述磷酸酯类似物的制备，以及它们掺入到核苷酸、修饰的核苷酸和寡核苷酸中本身也为已知的且无需在此描述。

[0097] 反义物的特异性和敏感性也被本领域的技术人员用于治疗用途。已将反义寡核苷酸用作在动物和人的疾病状态治疗中的治疗部分。已将反义寡核苷酸安全和有效地施用给人，并且目前正在进行许多临床试验。因此已确定寡核苷酸可为有用的治疗形式，其可经配置以在用于治疗细胞、组织和动物尤其人的治疗方案中有用。

[0098] 在本发明的实施方案中，寡聚反义化合物（具体地寡核苷酸）结合到靶核酸分子并调节由靶基因编码的分子的表达和 / 或功能。待干扰的 DNA 功能包括例如复制和转录。待干扰的 RNA 功能包括所有的生命机能，例如 RNA 向蛋白质翻译位点的易位、蛋白质自 RNA 的翻译、产生一种或多种 mRNA 种类的 RNA 剪接，以及可由 RNA 参与或促进的催化活性。所述功能可被上调或受抑制，这取决于所需的功能。

[0099] 反义化合物包括反义寡聚化合物、反义寡核苷酸、外部指导序列 (EGS) 寡核苷酸、可变剪接物、引物、探针和与靶核酸的至少一部分杂交的其他寡聚化合物。因此，这些化合物可以单链、双链、部分单链或环状寡聚化合物的形式引入。

[0100] 在本发明的情况下，将反义化合物靶向于特定的核酸分子可为多步过程。所述过程通常以鉴定待调节其功能的靶核酸开始。此靶核酸可为，例如其表达与特定病症或疾病状态有关的细胞基因（或从基因转录的 mRNA），或来自传染剂的核酸分子。在本发明中，所述靶核酸编码脑源神经营养因子 (BDNF)。

[0101] 靶向过程通常也包括确定靶核酸内的至少一个靶区域、区段或位点以用于发生反义相互作用，使得产生所需的效应，例如，表达的调节。在本发明的情况中，术语“区域”定义为具有至少一个可识别结构、功能或特征的靶核酸的一部分。靶核酸区域内为区段。“区段”定义为在靶核酸内区域的较小或亚部分。本发明所用的“位点”定义为靶核酸内的位

置。

[0102] 在一个实施方案中，反义寡核苷酸结合到脑源神经营养因子 (BDNF) 的天然反义序列并调节 BDNF(SEQ ID NO :1 和 2) 的表达和 / 或功能。反义序列的实例包括 SEQ ID NO : 3-55。

[0103] 在一个实施方案中，反义寡核苷酸结合到脑源营养因子 (BDNF) 多核苷酸的一个或多个区段并调节 BDNF 的表达和 / 或功能。所述区段包含 BDNF 有义或反义多核苷酸的至少五个连续的核苷酸。

[0104] 在一个实施方案中，反义寡核苷酸对 BDNF 的天然反义序列而言为特异性的，其中所述寡核苷酸与 BDNF 的天然反义序列的结合调节 BDNF 的表达和 / 或功能。

[0105] 在一个实施方案中，寡核苷酸化合物包括如 SEQ ID NO :12-49 所述的序列、使用例如 PCR、杂交等鉴定和扩增的反义序列。这些寡核苷酸可包含一个或多个修饰核苷酸、较短或较长的片段、经修饰的键等。经修饰的键或核苷酸间键的实例包括硫代磷酸酯、二硫代磷酸酯等。在一个实施方案中，所述核苷酸包括磷衍生物。可连接到本发明的修饰寡核苷酸中的糖或糖类似物部分的磷衍生物 ( 或经修饰的磷酸基 ) 可为单磷酸酯、二磷酸酯、三磷酸酯、烷基磷酸酯、链烷磷酸酯、硫代磷酸酯等。上述磷酸酯类似物的制备，以及它们掺入到核苷酸、修饰的核苷酸和寡核苷酸中本身也为已知的且无需在此描述。

[0106] 由于如本领域已知，翻译起始密码子通常为 5′ -AUG( 在转录的 mRNA 分子中 ；在相应的 DNA 分子中为 5′ -ATG)，因而翻译起始密码子也称为“AUG 密码子”、“起始密码子”或“AUG 起始密码子”。少数基因具有翻译起始密码子，其具有 RNA 序列 5′ -GUG、5′ -UUG 或 5′ -CUG ；且 5′ -AUA、5′ -ACG 和 5′ -CUG 已显示在体内起作用。因此，术语“翻译起始密码子”和“起始密码子”可包括许多密码子序列，但在每个情况下起始氨基酸通常为甲硫氨酸 ( 在真核生物中 ) 或甲酰甲硫氨酸 ( 在原核生物中 )。真核和原核基因可具有两个或更多个备选起始密码子，其中的任何一个可优先地用于在特定细胞类型或组织中或在特定条件集下的翻译起始。在本发明的情况中，“起始密码子”和“翻译起始密码子”是指这样的一个或多个密码子，其在体内用于起始由编码脑源神经营养因子 (BDNF) 的基因转录的 mRNA 的翻译，与这类密码子的序列无关。基因的翻译终止密码子 ( 或“终止密码子”) 可具有三个序列中的一个，即 5′ -UAA、5′ -UAG 和 5′ -UGA( 对应的 DNA 序列分别为 5′ -TAA、5′ -TAG 和 5′ -TGA)。

[0107] 术语“起始密码子区”和“翻译起始密码子区”是指从翻译起始密码子开始在任一方向上 ( 即，5’或 3’) 包含约 25- 约 50 个连续的核苷酸的这类 mRNA 或基因的部分。类似地，术语“终止密码子区”和“翻译终止密码子区”是指从翻译终止密码子开始在任一方向上 ( 即，5’或 3’) 包含约 25- 约 50 个连续的核苷酸的这类 mRNA 或基因的部分。因此，“起始密码子区”( 或“翻译起始密码子区”) 和“终止密码子区”( 或“翻译终止密码子区”) 均为可用本发明的反义化合物有效地靶向于的区域。

[0108] 本领域已知的开放阅读框 (ORF) 或“编码区”是指在翻译起始密码子和翻译终止密码子之间的区域，也为可有效地靶向于的区域。在本发明的内容内，靶向的区域为包含基因开放阅读框 (ORF) 的翻译起始或终止密码子的基因内区。

[0109] 另一种靶区域包括本领域已知的 5’非翻译区 (5’UTR)，是指在翻译起始密码子的 5’方向上的 mRNA 的部分，因此包括在 mRNA 的 5’加帽位点和翻译起始密码子之间的核苷酸

（或基因上对应的核苷酸）。再一种靶区域包括本领域已知的3’非翻译区（3′ UTR），是指在翻译终止密码子3’方向上的mRNA的部分，因此包括在mRNA的翻译终止密码子和3’末端之间的核苷酸（或基因上对应的核苷酸）。mRNA的5’加帽位点包含经由5’-5’三磷酸酯键连接到mRNA的5’最末端残基的N7-甲基化鸟苷残基。认为mRNA的5’帽区包括5’帽结构本身以及邻近该帽位点的前50个核苷酸。用于本发明的另一种靶区域为5’帽区。

[0110] 尽管一些真核mRNA转录物为直接翻译的，但是许多包含一个或多个称为“内含子”的区域，其在翻译前被从转录物中切除。余下的（且因此翻译的）区称为“外显子”，并将其剪接在一起形成连续的mRNA序列。在一个实施方案中，靶向剪接位点（即，内含子-外显子连接处或外显子-内含子连接处）在疾病牵涉到异常剪接或疾病牵涉特定剪接产物过度产生的状况中特别有用。因重排或缺失所致的异常融合连接为靶位点的另一个实施方案。经由来自不同基因来源的两个（或更多个）mRNA的剪接过程产生的mRNA转录物称为“融合转录物”。内含子可使用靶向于例如DNA或前-mRNA的反义化合物来有效地靶向。

[0111] 在一个实施方案中，反义寡核苷酸结合到靶多核苷酸的编码和/或非编码区并调节靶分子的表达和/或功能。

[0112] 在一个实施方案中，反义寡核苷酸结合到天然反义多核苷酸并调节靶分子的表达和/或功能。

[0113] 在一个实施方案中，反义寡核苷酸结合到有义多核苷酸并调节靶分子的表达和/或功能。

[0114] 可变RNA转录物可产生自DNA的相同基因组区。这些可变转录物一般称为“变体”。更具体地，“前mRNA变体”为产生自相同的基因组DNA的转录物，其与产生自相同的基因组DNA的其他转录物在其起始或终止位置上不同且包含内含子和外显子序列二者。

[0115] 当剪接期间切除了一个或多个外显子或内含子区或其部分时，前mRNA变体产生更小的“mRNA变体”。因此，mRNA变体为经加工的前mRNA变体，并且由于剪接导致每种独特的前mRNA变体必须总是产生独特的mRNA变体。这些mRNA变体也称为“可变剪接变体”。如果未发生前mRNA变体的剪接，则前mRNA变体与mRNA变体完全相同。

[0116] 变体可通过使用可变信号启动或终止转录来产生。前mRNA和mRNA可具有多于一个起始密码子或终止密码子。起源于使用可变起始密码子的前mRNA或mRNA的变体称为该前mRNA或mRNA的“可变起始变体”。使用可变终止密码子的转录物称为该前mRNA或mRNA的“可变终止变体”。可变终止变体的一个具体类型为“聚腺苷酸变体”，其中所产生的多重转录物起因于转录机构对“聚腺苷酸终止信号”之一的可变选择，从而产生终止在独特的聚腺苷酸位点上的转录物。在本发明的情况内，本文所述的变体类型也为靶核酸的实施方案。

[0117] 将反义化合物与之杂交的靶核酸上的位置定义为活性反义化合物靶向于的靶区域的至少5个核苷酸长的部分。

[0118] 虽然将某些示例性靶区段的具体序列列举于此，但是本领域的技术人员会认识到，这些用于说明和描述在本发明范围内的具体实施方案。根据本公开内容，其他靶区段可由本领域普通技术人员容易地鉴定。

[0119] 长度为5-100个核苷酸并包含选自说明性优选靶区段之内的一段至少五(5)个连续的核苷酸的延伸的靶区段认为同样适合靶向。

[0120] 靶区段可包括DNA或RNA序列，其包含来自说明性优选靶区段之一的5’末端的至

少 5 个连续核苷酸（余下的核苷酸为相同 DNA 或 RNA 的连续延伸，其开始于靶区段 5’末端的紧接上游且持续直到该 DNA 或 RNA 包含约 5- 约 100 个核苷酸为止）。类似优选的靶区段由以下 DNA 或 RNA 序列表示，该序列包含来自说明性优选靶区段之一的 3’末端的至少 5 个连续核苷酸（余下的核苷酸为相同 DNA 或 RNA 的连续延伸，其开始于靶区段 3’末端的紧接下游且持续直到该 DNA 或 RNA 包含约 5- 约 100 个核苷酸为止）。本领域技术人员根据本文所说明的靶区段，无需过度试验就能够鉴定进一步优选的靶区段。

[0121] 一旦鉴定一个或多个靶区域、区段或位点，就选出与该靶标充分互补的反义化合物，即充分良好地且以足够的特异性杂交以得到所需的效果。

[0122] 在本发明的实施方案中，寡核苷酸与特定靶标的反义链结合。所述寡核苷酸长度为至少 5 个核苷酸且可为合成的，使得每个寡核苷酸靶向于重叠的序列，由此将寡核苷酸合成为覆盖靶多核苷酸的全长。靶标也包括编码区以及非编码区。

[0123] 在一个实施方案中，优选通过反义寡核苷酸来靶向于特定核酸。将反义化合物靶向于特定核酸为多步过程。该过程通常开始于鉴定其功能待调节的核酸序列。这可为，例如其表达与特定的病症或疾病状态有关的细胞基因（或从该基因转录的 mRNA），或非编码多核苷酸，例如非编码 RNA(ncRNA)。

[0124] 可将 RNA 归类为 (1) 信使 RNA(mRNA)，其被翻译成蛋白，和 (2) 非蛋白质编码的 RNA(ncRNA)。ncRNA 包括微 RNA、反义转录物和包含高密度的终止密码子并缺少任何广泛的“开放阅读框”的其他转录单元 (TU)。许多 ncRNA 似乎开始于蛋白编码基因座的 3’非翻译区 (3′ UTR) 中的起始位点。ncRNA 常常为罕见的且至少一半已由 FANTOM 协会测序的 ncRNA 似乎未聚腺苷酸化。大多数研究者因为明显的原因而关注经加工并输出到细胞质的聚腺苷酸化 mRNA。近来，已显示非聚腺苷酸化核 RNA 的群体可能非常巨大，且许多这类转录物产生于所谓的基因内区。ncRNA 可调节基因表达的机制为通过与靶转录物的碱基配对。通过碱基配对起作用的 RNA 可分组成 (1) 顺式编码 RNA，其在相同的基因位置、但在与其所作用的 RNA 相反的链上编码，因此显示对其靶标完美的互补性，和 (2) 反式编码 RNA，其在与其所作用的 RNA 不同的染色体位置上编码，一般不表现出与其靶标完美的碱基配对潜能。

[0125] 不希望受到理论的约束，通过本文所述的反义寡核苷酸来扰乱反义多核苷酸可改变相应有义信使 RNA 的表达。然而，此调节可能为非调和的（反义敲减导致信使 RNA 上升）或致的（反义敲减导致伴随的信使 RNA 下降）。在这些情况下，可将反义寡核苷酸靶向于反义转录物的重叠或非重叠部分，引起其敲减或隔离。编码以及非编码反义物可以相同的方式来靶向，并且任一种类别均能够调节相应有义转录物——以调和或非调和的方式。用于鉴定针对靶标使用的新寡核苷酸的策略可基于通过反义寡核苷酸或任何其他调节所需靶标的方法来敲减反义 RNA 转录物。

[0126] 策略 1：在非调和调节的情况下，敲减所述反义转录物提升常规（有义）基因的表达。若后者基因编码已知或假定的药物靶标，则其反义配对物的敲减可预想到地模拟受体激动剂或酶刺激剂的作用。

[0127] 策略 2：在调和调节的情况下，可以伴随地敲减反义和有义转录物两者，从而达到常规（有义）基因表达的协同下降。如果例如将反义寡核苷酸用于进行敲减，则此策略可用于将靶向的一种反义寡核苷酸应用于有义转录物和将另一种反义寡核苷酸应用于相应的反义转录物，或应用同时靶向于重叠的有义和反义转录物的单个有力对称的反义寡核苷

酸。

[0128] 根据本发明，反义化合物包括反义寡核苷酸、核酶、外部指导序列 (EGS) 寡核苷酸、siRNA 化合物、单链或双链 RNA 干扰 (RNAi) 化合物（例如 siRNA 化合物），以及与靶核酸的至少一部分杂交且调节其功能的其他寡聚化合物。因此，其可为 DNA、RNA、DNA 样、RNA 样、或其混合物，或可为这些中的一种或多种的模拟物。这些化合物可为单链、双链、环状或发夹寡聚化合物且可包含结构元件，例如内部或末端突起 (bulge)、错配或环。将反义化合物常规地制备为线性的，但可被连接或者另外制备成环状和 / 或分枝的。反义化合物可包括构建体，例如杂交以形成完全或部分双链化合物的两条链，或具有足够自身互补性以允许杂交并形成完全或部分双链化合物的单链。可将所述两条链内部连接而留下游离的 3’或 5’末端，或可将其连接形成连续的发夹结构或环。发夹结构可在 5’或 3’末端上包含突出端，从而产生单链特征的延伸。所述双链化合物任选可在末端上包含突出端。进一步的修饰可包括与末端之一、经挑选的核苷酸位置、糖位置或与核苷间键之一连接的缀合基团。或者，所述两条链可经由非核酸部分或连接基团来连接。当仅由一条链形成时，dsRNA 可呈自身互补的发夹型分子形式，其在其自身上对折形成双链体。因此，所述 dsRNA 可为完全或部分双链的。基因表达的特异性调节可通过在转基因细胞系中稳定表达 dsRNA 发夹来完成，然而，在一些实施方案中，基因表达或功能为上调的。当由两条链或呈其自身对折以形成双链体的自身互补发夹型分子形式的单链形成时，所述两条链（或单链的双链体形成区）为以沃森 - 克里克模式碱基配对的互补 RNA 链。

[0129] 一旦引入系统，本发明的化合物可引起一种或多种酶或结构蛋白的作用以实现靶核酸的切割或其他修饰，或可经由基于占据的机制来运作。一般而言，核酸（包括寡核苷酸）可描述为“DNA 样”（即，一般具有一个或多个 2’脱氧糖和一般地具有 T 而不是 U 碱基）或“RNA 样”（即，一般具有一个或多个 2’羟基或 2’修饰的糖和一般具有 U 而不是 T 碱基）。核酸螺旋可采取多于一种类型的结构，最常见地 A 和 B 型。据认为，一般而言，具有 B 型样结构的寡核苷酸为“DNA 样”而具有 A 型样结构的寡核苷酸为“RNA 样”。在一些（嵌合的）实施方案中，反义化合物可包含 A 型区和 B 型区两者。

[0130] 在一个实施方案中，所需的寡核苷酸或反义化合物包括以下的至少一种：反义 RNA、反义 DNA、嵌合反义寡核苷酸、包含经修饰的键的反义寡核苷酸、干扰 RNA(RNAi)、短干扰 RNA(siRNA)；微干扰 RNA(miRNA)；小时序 RNA(stRNA)；或短发夹 RNA(shRNA)；小 RNA 诱导的基因激活 (RNAa)；小激活 RNA(saRNA) 或其组合。

[0131] dsRNA 也可激活基因表达，这是已被称为“小 RNA 诱导的基因激活”或 RNAa 的机制。靶向于基因启动子的 dsRNA 诱导相关基因的有效转录激活。RNAa 在人细胞中使用合成 dsRNA（称为“小激活 RNA”(saRNA)）证实。目前未知 RNAa 在其他生物体中是否为保守的。

[0132] 已发现小双链 RNA(dsRNA)（例如小干扰 RNA(siRNA) 和微 RNA(miRNA)）是称为 RNA 干扰 (RNAi) 的进化保守机制的触发物。RNAi 总是经由重构染色质来导致基因沉默，从而抑制转录、降解互补 mRNA 或阻断蛋白翻译。然而，在下文实施例章节详述的例子中，显示寡核苷酸增加脑源神经营养因子 (BDNF) 多核苷酸和其编码产物的表达和 / 或功能。dsRNA 也可充当小激活 RNA(saRNA)。不希望受理论约束，通过靶向于基因启动子中的序列，saRNA 在称为 dsRNA 诱导的转录激活 (RNAa) 的现象中诱导靶基因表达。

[0133] 在另一个实施方案中，本文鉴定的“优选靶区段”可用于筛选调节脑源神经营养因

子 (BDNF) 多核苷酸表达的另外的化合物。“调节剂”为减少或增加编码 BDNF 的核酸分子的表达的化合物并包含与优选靶区段互补的至少 5 个核苷酸的部分。筛选方法包括以下步骤 :使编码 BDNF 的有义或天然反义多核苷酸的核酸分子的优选靶区段与一种或多种候选调节剂接触,以及选择一种或多种减少或增加编码 BDNF 多核苷酸的核酸分子表达的候选调节剂 ( 例如 SEQ ID NO :12-49)。一旦显示一种或多种候选调节剂能够调节 ( 例如减少或增加 ) 编码 BDNF 多核苷酸的核酸分子的表达,则可将所述调节剂用于 BDNF 多核苷酸功能的进一步调查研究,或用作依照本发明的研究、诊断或治疗剂。

[0134] 靶向于天然反义序列优选地调节靶基因的功能。例如,BDNF 基因 ( 例如登录号 NM_170735 和 NM_007540)。在一个实施方案中,靶标为 BDNF 基因的反义多核苷酸。在一个实施方案中,反义寡核苷酸靶向于 BDNF 多核苷酸 ( 例如登录号 NM_170735 和 NM_007540) 的有义和 / 或天然反义序列、其变体、等位基因、同种型、同源物、突变体、衍生物、片段和互补序列。优选所述寡核苷酸为反义分子且所述靶标包括反义和 / 或有义 BDNF 多核苷酸的编码和非编码区。

[0135] 本发明的优选靶区段也可与本发明的其相应互补反义化合物结合,以形成稳定的双链 ( 双链体 ) 寡核苷酸。

[0136] 本领域中已显示这类双链寡核苷酸部分经由反义机制来调节靶表达和调节翻译以及 RNA 加工。此外,所述双链部分可经受化学修饰。例如,已显示这类双链部分通过所述双链体的反义链与靶标的经典杂交来抑制该靶标,从而触发靶标的酶促降解。

[0137] 在一个实施方案中,反义寡核苷酸靶向于脑源神经营养因子 (BDNF) 多核苷酸 ( 例如登录号 NM_170735 和 NM_007540)、其变体、等位基因、同源物、突变体、衍生物、片段和互补序列。优选所述寡核苷酸为反义分子。

[0138] 依照本发明的实施方案,靶核酸分子不限于单独的 BDNF 而是延伸到其任何多核苷酸变体及产生、影响、作用或导致 BDNF 表达产物和或 / 其任何同种型或者与 BDNF 表达产物和或 / 其任何同种型相关的任何多核苷酸。

[0139] 在一个实施方案中,寡核苷酸靶向于 BDNF 多核苷酸的天然反义序列 ( 例如,如 SEQ ID NO :3-11 所示的多核苷酸 ),以及其任何变体、等位基因、同源物、突变体、衍生物、片段和互补序列。反义寡核苷酸的实例如 SEQ ID NO :12-49 所述。

[0140] 在一个实施方案中,所述寡核苷酸与 BDNF 反义物的核酸序列互补或结合,并调节 BDNF 分子的表达和 / 或功能,所述核酸序列包括但不限于与 BDNF 多核苷酸有关的非编码有义和 / 或反义序列。

[0141] 在一个实施方案中,所述寡核苷酸与如 SEQ ID NO :3-11 所示的 BDNF 天然反义物的核酸序列互补或结合,并调节 BDNF 分子的表达和 / 或功能。

[0142] 在一个实施方案中,寡核苷酸包含 SEQ ID NO :12-49 的至少 5 个连续核苷酸的序列且调节 BDNF 分子的表达和 / 或功能。

[0143] 多核苷酸靶标包括 BDNF( 包括其家族成员、BDNF 的变体 );BDNF 的突变体 ( 包括 SNP) ;BDNF 的非编码序列 ;BDNF 的等位基因 ;物种变体、片段等等。优选所述寡核苷酸为反义分子。

[0144] 在一个实施方案中,靶向于 BDNF 多核苷酸的寡核苷酸包括 :反义 RNA、干扰 RNA(RNAi)、短干扰 RNA(siRNA) ;微干扰 RNA(miRNA) ;小时序 RNA(stRNA) ;或短发夹

RNA(shRNA)；小 RNA 诱导的基因激活 (RNAa)；或小激活 RNA(saRNA)。

[0145] 在一个实施方案中，脑源神经营养因子 (BDNF) 多核苷酸的靶向 ( 例如 SEQ ID NO：3-55) 调节这些靶标的表达或功能。在一个实施方案中，表达或功能相比于对照为上调的。在一个实施方案中，表达或功能相比于对照为下调的。

[0146] 在一个实施方案中，反义化合物包括如 SEQ ID NO：12-49 所示的序列。这些寡核苷酸可包含一个或多个经修饰的核苷酸、更短或更长的片段、经修饰的键等等。

[0147] 在一个实施方案中，SEQ ID NO：12-49 包含一个或多个 LNA 核苷酸。表 1 显示可用于本发明的方法中的示例性反义寡核苷酸。

[0148] 表 1：

[0149]

| 序列 ID | 反义序列名称 | 序列 |
|---|---|---|
| SEQ ID NO:12 | CUR-2046 (反义) | ArArCrArArArCrArArCrUrGrGrUrGrArGrCrCrUrGrG |
| SEQ ID NO:13 | CUR-2047 (反义) | rUrGrArGrCrCrUrArArGrArUrArCrArUrUrGrCrUrCrU |
| SEQ ID NO:14 | CUR-2048 (反义) | rGrUrGrCrUrGrUrUrGrUrArArGrArUrUrArGrCrCrArC |
| SEQ ID NO:15 | CUR-2049 (反义) | rArArUrGrArCrArUrGrUrUrUrGrUrArGrGrGrArGrCrC |
| SEQ ID NO:16 | CUR-2050 | +C*mC*mA*+G*mG*mU*+G*mU*mG*mC*+G*mG*mA*+C |
| SEQ ID NO:17 | CUR-2051 | +C*mC*mA*+U*mG*mG*+G*mA*mC*mU*+C*mU*mG*+G |
| SEQ ID NO:18 | CUR-2052 | +A*mG*mA*+G*mC*mG*+U*mG*mA*mA*+U*mG*mG*+G |
| SEQ ID NO:19 | CUR-2053 | +C*mC*mC*+A*mA*mG*+G*mC*mA*mG*+G*mU*mU*+C |

[0150]

| | | |
|---|---|---|
| SEQ ID NO:20 | CUR-2054 | +A*mA*mG*+A*mU*mG*+C*mU*mU*mG*+A*mC*mA*+U |
| SEQ ID NO:21 | CUR-2055 | +C*mA*mU*+U*mG*mG*+C*mU*mG*mA*+C*mA*mC*+U |
| SEQ ID NO:22 | CUR-2056 | +U*mU*mC*+G*mA*mA*+C*mA*mC*mG*+U*mG*mA*+U |
| SEQ ID NO:23 | CUR-2057 | +A*mG*mA*+A*mG*mA*+G*mC*mU*mG*+U*mU*mG*+G |
| SEQ ID NO:24 | CUR-2058 | +A*mU*mG*+A*mG*mG*+A*mC*mC*mA*+G*mA*mA*+A |
| SEQ ID NO:25 | CUR-2059 | +G*mU*mU*+C*mG*mG*+C*mC*mC*mA*+A*mU*mG*+A |
| SEQ ID NO:26 | CUR-2060 | +A*mG*mA*+A*mA*mA*+C*mA*mA*mU*+A*mA*mG*+G |
| SEQ ID NO:27 | CUR-2061 | +A*mC*mG*+C*mA*mG*+A*mC*mU*mU*+G*mU*mA*+C |
| SEQ ID NO:28 | CUR-2062 | +A*mC*mG*+U*mC*mC*+A*mG*mG*mG*+U*mG*mA*+U |
| SEQ ID NO:29 | CUR-2063 | +G*mC*mU*+C*mA*mG*+U*mA*mG*mU*+C*mA*mA*+G |
| SEQ ID NO:30 | CUR-2064 | +U*mG*mC*+C*mU*mU*+U*mG*mG*mA*+G*mC*mC*+U |
| SEQ ID NO:31 | CUR-2065 | +C*mC*mU*+C*mU*mU*+C*mU*mC*mU*+U*mU*mC*+U |
| SEQ ID NO:32 | CUR-2066 | +C*+C*+C*G*G*T*A*T*C*C*A*A*A*+G*+G*+C |
| SEQ ID NO:33 | CUR-2067 | +G*+T*+A*T*T*A*G*C*G*A*G*T*G*+G*+G*+T |

[0151]

| | | |
|---|---|---|
| SEQ ID NO:34 | CUR-2068 | +G*+T*+C*T*A*T*G*A*G*G*G*T*T*+C*+G*+G |
| SEQ ID NO:35 | CUR-2069 | +C*+C*+T*C*C*T*C*T*A*C*T*C*T*+T*+T*+C |
| SEQ ID NO:36 | CUR-2070 | +G*+G*+C*A*G*G*T*T*C*G*A*G*A*+G*+G*+T |
| SEQ ID NO:37 | CUR-2071 | +T*+T*+C*C*T*T*C*C*C*A*C*A*G*+T*+T*+C |
| SEQ ID NO:38 | CUR-2072 | +C*+G*+G*T*T*G*C*A*T*G*A*A*G*+G*+C*+G |
| SEQ ID NO:39 | CUR-2073 | +T*+G*+G*C*T*G*G*C*G*A*T*T*C*+A*+T*+A |
| SEQ ID NO:40 | CUR-2074 | +C*+A*+A*C*A*T*A*T*C*A*G*G*A*+G*+C*+C |
| SEQ ID NO:41 | CUR-2075 | +T*+G*+T*A*T*T*C*C*C*A*G*A*A*+C*+T*+T |
| SEQ ID NO:42 | CUR-2076 (反义) | rUrArUrGrGrUrUrArUrUrUrCrArUrArCrUrUrCrGrGrUrUrGrCrArUrG |
| SEQ ID NO:43 | CUR-2077 (反义) | rArGrArArGrUrArArArCrGrUrCrCrArCrGrGrArCrArArGrGrCrArArC |
| SEQ ID NO:44 | CUR-2078 (反义) | rArUrUrUrCrUrArCrGrArGrArCrCrArArGrUrGrUrArArUrCrCrCrArU |
| SEQ ID NO:45 | CUR-2079 (反义) | rUrArArGrGrArCrGrCrGrGrArCrUrUrGrUrArCrArCrUrUrCrCrGrGrG |
| SEQ ID NO:46 | CUR-2080 (反义) | rArGrArArArGrArArArGrUrUrCrUrArArCrCrUrGrUrUrCrUrGrUrGrU |
| SEQ ID NO:47 | CUR-2081 | +G*+A*+T*T*T*C*A*G*A*G*C*C*G*+C*+A*+G |

[0152]

| | | |
|---|---|---|
| SEQ ID NO:48 | CUR-2082 | +G*+A*+C*A*C*A*T*C*C*A*T*C*C*+C*+A*+G |
| SEQ ID NO:49 | CUR-2083 | +C*+C*+T*C*G*T*C*A*T*G*T*C*T*+G*+T*+G |
| SEQ ID NO:50 | CUR-0071 | C*+T*+T*G*A*A*T*T*G*T*T*T*+G*+T*+A |
| SEQ ID NO:51 | CUR-0072 | A*+G*+T*T*G*C*A*A*G*A*G*T*+T*+G*+G |
| SEQ ID NO:52 | CUR-0073 | A*+T*+C*T*G*T*T*C*T*G*C*T*+G*+T*+C |
| SEQ ID NO:53 | CUR-0074 | C*+A*+T*A*T*T*C*T*T*G*G*A*+C*+G*+A |
| SEQ ID NO:54 | CUR-0075 | T*+G*+T*G*C*T*G*T*T*G*T*A*+A*+G*+A |
| SEQ ID NO:55 | CUR-0076 | T*+G*+A*C*A*G*A*G*G*A*G*T*+A*+T*+T |

[0153] 所需靶核酸的调节可以本领域已知的数种方式来进行。例如，反义寡核苷酸、siRNA 等。酶性核酸分子（例如，核酶）为能够催化一种或多种不同反应（包括以核苷酸碱基序列特异的方式重复切割其他单独核酸分子的能力）的核酸分子。这类酶性核酸分子可用于，例如，靶向于几乎任何 RNA 转录物。

[0154] 由于反式切割酶性核酸分子的序列特异性，其有望作为用于人类疾病的治疗剂。可将酶性核酸分子设计成在细胞 RNA 背景下切割特定的 RNA 靶标。这类切割事件致使 mRNA 无功能且终止从该 RNA 的蛋白质表达。以这种方式，可选择性地抑制与疾病状态有关的蛋白质的合成。

[0155] 一般而言，带有 RNA 切割活性的酶性核酸通过首先与靶 RNA 结合来起作用。这类结合通过酶性核酸的靶结合部分来进行，该酶性核酸的靶结合部分保持紧密靠近进行切割靶 RNA 的分子的酶促部分。因此，所述酶性核酸首先识别而后通过互补的碱基配对与靶 RNA 结合，且一旦与正确的位点结合，即酶促地作用以切割靶 RNA。这类靶 RNA 的策略性切割将破坏其指导编码蛋白质合成的能力。在酶性核酸结合和切割其 RNA 靶标之后，其从该 RNA 释放以寻找另一个靶标且可重复结合和切割新靶标。

[0156] 已使用诸如体外选择（进化）策略 (Orgel，(1979)Proc. R. Soc. London，B 205，435) 等数种途径来演化能够催化多种反应的新核酸催化剂，所述反应为例如磷酸二酯键和酰胺键的切割和连接。

[0157] 催化活性最佳的核酶的开发会显著地有助于以调节基因表达为目的而采用 RNA 切割核酶的任何策略。例如锤头核酶在 Mg2+ 辅因子的饱和 (10mM) 浓度存在下，以约 $1min^{-1}$ 的催化速率 (kcat) 起作用。已显示人造“RNA 连接酶”核酶以约 $100min^{-1}$ 的速率催化相应的自身修饰反应。此外，已知具有由 DNA 组成的底物结合臂的某些经修饰的锤头核酶以接近 $100min^{-1}$ 的多重转换速率 (multiple turn-over rate) 催化 RNA 切割。最终，用某些核苷酸类似物置换在锤头的催化核心内的特定残基产生显示出在催化速率上多达 10 倍改进的修饰核酶。这些研究结果证实核酶可以以显著高于大多数天然自身切割核酶体外展示的催化速率来促进化学转化。那么可能的是，可优化某些自身切割核酶的结构以产生最高的催化活性，或者可制备展示出显著更快的 RNA 磷酸二酯切割速率的全新 RNA 基序。

[0158] 符合“锤头”模型的 RNA 催化剂对 RNA 底物的分子间切割首先显示于 1987 年 (Uhlenbeck，O. C. (1987) Nature，328 :596-600)。将所述 RNA 催化剂回收且与多个 RNA 分子反应，证实其为真正催化性的。

[0159] 基于“锤头”基序设计的催化性 RNA 已通过在催化性 RNA 中作出适当的碱基改变以维持与靶序列的必要碱基配对，来用于切割特定靶序列。这允许使用催化性 RNA 来切割特定靶序列，并表明根据“锤头”模型设计的催化性 RNA 可能在体内切割特定底物 RNA。

[0160] RNA 干扰 (RNAi) 已成为调节哺乳动物和哺乳动物细胞中基因表达的强大工具。此方法要求使用表达质粒或病毒以及加工成 siRNA 的小发夹 RNA 的编码序列，将小干扰 RNA(siRNA) 作为 RNA 本身或作为 DNA 递送。此系统能够有效将前 siRNA 转运到它们在其中活跃的细胞质中，并允许使用经调节的和组织特异的启动子用于基因表达。

[0161] 在一个实施方案中，寡核苷酸或反义化合物包括核糖核酸 (RNA) 和 / 或脱氧核糖核酸 (DNA) 的寡聚体或多聚体、或其模拟物、嵌合体、类似物或同源物。此术语包括由天然存在核苷酸、糖和共价核苷间 ( 骨架 ) 键组成的寡核苷酸以及类似地起作用的具有非天然存在部分的寡核苷酸。由于所需性质，例如，增强的细胞摄取、对靶核酸增强的亲和力以及在核酸酶存在时增大的稳定性，常常需要这类经修饰或取代的寡核苷酸超过天然形式。

[0162] 根据本发明，寡核苷酸或“反义化合物”包括反义寡核苷酸 ( 例如 RNA、DNA、其模拟物、嵌合体、类似物或同源物 )、核酶、外部指导序列 (EGS) 寡核苷酸、siRNA 化合物、单链或双链 RNA 干扰 (RNAi) 化合物例如 siRNA 化合物、saRNA、aRNA，以及与靶核酸的至少一部分杂交且调节其功能的其他寡聚化合物。因此，它们可为 DNA、RNA、DNA 样、RNA 样、或其混合物，或可为这些中的一种或多种的模拟物。这些化合物可为单链、双链、环状或发夹寡聚化合物且可包含结构元件，例如内部或末端突起、错配或环。将反义化合物常规地线性制备，但可以接合或者另外地制备成环状和 / 或分枝的。反义化合物可包括构建体，例如杂交以形成完全或部分双链化合物的两条链，或带有足够自身互补性以允许杂交并形成完全或部分双链化合物的单链。可将所述两条链内部连接而留下游离的 3’或 5’末端或可将其连接形成连续的发夹结构或环。发夹结构可在 5’或 3’末端上包含突出端，从而产生单链特征的延伸。双链化合物任选可在末端上包含突出端。进一步的修饰可包括与末端之一、经挑选的核苷酸位置、糖位置或核苷间键之一连接的缀合基团。可选地，所述两条链可经由非核酸部分或连接基团来连接。当形成自仅一条链时，dsRNA 可采取自身互补的发夹型分子的形式，其在其自身上对折以形成双链体。因此，所述 dsRNA 可为完全或部分双链的。基因表达的特异性调节可通过 dsRNA 发夹在转基因细胞系中的稳定表达来完成。当形成自两条链

或采取在其自身上对折以形成双链体的自身互补的发夹型分子形式的单链时，所述两条链（或单链的双链体形成区域）为以沃森 - 克里克模式碱基配对的互补 RNA 链。

[0163] 一旦引入系统，本发明的化合物可引起一种或多种酶或结构蛋白的作用以实现靶核酸的切割或其他修饰，或可经由基于占据的机制来工作。一般而言，核酸（包括寡核苷酸）可描述为“DNA 样”（即，一般具有一个或多个 2’ 脱氧糖和，一般地，T 而不是 U 碱基）或“RNA 样”（即，一般具有一个或多个 2’ 羟基或 2’ 修饰的糖和，一般 U 而不是 T 碱基）。核酸螺旋可采取多于一种类型的结构，最普通地 A 和 B 型。据认为，一般而言，具有 B 型样结构的寡核苷酸为“DNA 样”而具有 A 型样结构的寡核苷酸为“RNA 样”。在一些（嵌合的）实施方案中，反义化合物可包含 A 型和 B 型区域两者。

[0164] 依照本发明的反义化合物可包含长度约 5- 约 80 个核苷酸（即约 5- 约 80 个连接的核苷）的反义部分。这是指反义化合物的反义链或部分的长度。换言之，本发明的单链反义化合物包含 5- 约 80 个核苷酸，而本发明的双链反义化合物（例如，dsRNA）包含 5- 约 80 个核苷酸长度的有义和反义链或部分。本领域普通技术人员将认识到，这包括 5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24、25、26、27、28、29、30、31、32、33、34、35、36、37、38、39、40、41、42、43、44、45、46、47、48、49、50、51、52、53、54、55、56、57、58、59、60、61、62、63、64、65、66、67、68、69、70、71、72、73、74、75、76、77、78、79 或 80 个核苷酸长度、或其之内任何范围的反义部分。

[0165] 在一个实施方案中，本发明的反义化合物具有 10-50 个核苷酸长度的反义部分。本领域普通技术人员将认识到，这包括具有 10、11、12、13、14、15、16、17、18、19、20、21、22、23、24、25、26、27、28、29、30、31、32、33、34、35、36、37、38、39、40、41、42、43、44、45、46、47、48、49 或 50 个核苷酸长度、或其之内任何范围的反义部分的寡核苷酸。在一些实施方案中，寡核苷酸长度为 15 个核苷酸。

[0166] 在一个实施方案中，本发明的反义或寡核苷酸化合物具有 12 或 13-30 个核苷酸长度的反义部分。本领域普通技术人员将认识到，这包括具有 12、13、14、15、16、17、18、19、20、21、22、23、24、25、26、27、28、29 或 30 个核苷酸长度、或其之内任何范围的反义部分的反义化合物。

[0167] 在一个实施方案中，本发明的寡聚化合物也包括其中不同的碱基存在于化合物中的一个或多个核苷酸位置上的变体。例如，如果第一个核苷酸为腺苷，那么可产生在此位置包含胸苷、鸟苷或胞苷的变体。这可在反义或 dsRNA 化合物的任何位置上进行。然后使用本文所述的方法来测试这些化合物以确定其抑制靶核酸表达的能力。

[0168] 在一些实施方案中，反义化合物与靶标之间的同源性、序列同一性或互补性为约 40％ - 约 60％。在一些实施方案中，同源性、序列同一性或互补性为约 60％ - 约 70％。在一些实施方案中，同源性、序列同一性或互补性为约 70％ - 约 80％。在一些实施方案中，同源性、序列同一性或互补性为约 80％ - 约 90％。在一些实施方案中，同源性、序列同一性或互补性为约 90％、约 92％、约 94％、约 95％、约 96％、约 97％、约 98％、约 99％或约 100％。

[0169] 在一个实施方案中，反义寡核苷酸（例如，SEQ ID NO：12-49 中所示的核酸分子）包含一个或多个取代或修饰。在一个实施方案中，将核苷酸用锁核酸 (LNA) 取代。

[0170] 在一个实施方案中，寡核苷酸靶向于与 BDNF 有关的编码和 / 或非编码序列以及如 SEQ ID NO：1-11 所示序列的有义和 / 或反义的核酸分子的一个或多个区域。也将寡核苷

酸靶向于 SEQ ID NO :1-11 的重叠区域。

[0171] 本发明的某些优选的寡核苷酸为嵌合寡核苷酸。“嵌合寡核苷酸”或“嵌合体”,在本发明的背景中,为包含两个或更多个化学上不同区域的寡核苷酸,每个区域由至少一个核苷酸组成。这些寡核苷酸典型地包含赋予一种或多种有益特性(例如,对核酸酶的抗性增强、细胞摄入增加、对靶标的结合亲和力增强)的修饰核苷酸的至少一个区域,以及作为能够切割 RNA:DNA 或 RNA:RNA 杂合体的酶底物的区域。作为实例,核糖核酸酶 H 为细胞核酸内切酶,其切割 RNA:DNA 双链体的 RNA 链。核糖核酸酶 H 的活化因此导致 RNA 靶标的切割,从而大大地增强基因表达的反义调节效率。因此,当使用嵌合寡核苷酸时,与杂交到相同靶区域的硫代磷酸酯脱氧寡核苷酸相比,常常可用较短的寡核苷酸获得相当的结果。RNA 靶标的切割可通过凝胶电泳和必要时本领域已知的相关核酸杂交技术常规地检测。在一个实施方案中,嵌合寡核苷酸包含修饰成增加靶结合亲和力的至少一个区域,并且通常包含充当核糖核酸酶 H 的底物的区域。寡核苷酸对其靶标(在此情况下,编码 ras 的核酸)的亲和力通过测量寡核苷酸 / 靶标对的 Tm 来常规地确定,Tm 为寡核苷酸与靶标解离的温度;解离以分光光度法检测。Tm 越高,寡核苷酸对靶标的亲和力越大。

[0172] 本发明的嵌合反义化合物可作为如上所述的两个或更多个寡核苷酸、修饰寡核苷酸、寡聚核苷和 / 或寡核苷酸模拟物的复合结构而形成。本领域亦已将这类化合物称为杂合体或间体 (gapmer)。教导这类杂合结构制备的代表性美国专利包括但不限于,美国专利第 5,013,830、5,149,797、5,220,007、5,256,775、5,366,878、5,403,711、5,491,133、5,565,350、5,623,065、5,652,355、5,652,356 和 5,700,922 号,每个通过引用结合于本文中。

[0173] 在一个实施方案中,经修饰的寡核苷酸区域包含在糖的 2’位置上修饰的至少一个核苷酸,最优选 2’-O 烷基、2’-O- 烷基 -O- 烷基或 2’- 氟修饰的核苷酸。在其它的实施方案中,RNA 修饰包括在 RNA 3’末端的嘧啶、脱碱基残基或反向碱基的核糖上的 2’- 氟、2’- 氨基和 2’O- 甲基修饰。将这类修饰常规地掺入到寡核苷酸中,且已显示这些寡核苷酸针对给定靶标具有比 2’- 脱氧寡核苷酸更高的 Tm(即,更高的靶结合亲和力)。这种增加的亲和力的效应大大地增强基因表达的 RNAi 寡核苷酸抑制。核糖核酸酶 H 为切割 RNA:DNA 双链体的 RNA 链的细胞核酸内切酶;此酶的活化因此导致 RNA 靶标的切割,且因此可大大地增强 RNAi 抑制效率。RNA 靶标的切割可通过凝胶电泳来常规地证实。在一个实施方案中,也修饰嵌合寡核苷酸以增强核酸酶抗性。细胞包含可降解核酸的各种核酸外切酶和核酸内切酶。已显示许多核苷酸和核苷修饰使它们掺入的寡核苷酸比天然寡脱氧核苷酸对核酸酶消化有更高抗性。核酸酶抗性通过将寡核苷酸与细胞提取物或分离的核酸酶溶液一起孵育并测定随时间推移剩余的完好寡核苷酸的程度(通常地通过凝胶电泳)来常规地测定。已经修饰以增强其核酸酶抗性的寡核苷酸比未修饰寡核苷酸保持完整更长时间。已证实多种寡核苷酸修饰增强或赋予核酸酶抗性。包含至少一个硫代磷酸酯修饰的寡核苷酸为目前更优选的。在一些情况下,增强靶结合亲和力的寡核苷酸修饰也能够独立地增强核酸酶抗性。

[0174] 预想用于本发明的一些优选寡核苷酸的具体实例包括包含经修饰的骨架的那些,所述经修饰的骨架为例如硫代磷酸酯、磷酸三酯、甲基膦酸酯、短链烷基或环烷基糖间键或者短链杂原子或杂环糖间键。最优选的为带有硫代磷酸酯骨架和带有杂原子骨架的寡核苷酸,特别地 $CH_2$-NH-O-$CH_2$、CH,-N($CH_3$)-O-$CH_2$[称为亚甲基(甲亚氨基)或 MMI 骨架]、

$CH_2$-O-N($CH_3$)-$CH_2$、$CH_2$-N($CH_3$)-N($CH_3$)-$CH_2$和 O-N($CH_3$)-$CH_2$-$CH_2$骨架，其中天然磷酸二酯骨架表示为 O-P-O-CH。由 De Mesmaeker 等，(1995)Acc. Chem. Res. 28 :366-374 公开的酰胺骨架也为优选的。同样优选的为具有吗啉代骨架结构的寡核苷酸 (Summerton 和 Weller，美国专利第 5,034,506 号 )。在其他的实施方案中，将寡核苷酸的例如肽核酸 (PNA) 骨架、磷酸二酯骨架替换为聚酰胺骨架，核苷酸直接或间接地与聚酰胺骨架的氮杂氮原子结合。寡核苷酸也可包含一个或多个取代的糖部分。优选的寡核苷酸在 2’位置上包含下列中的一种 ：OH、SH、$SCH_3$、F、OCN、$OCH_3$、$O(CH_2)_nCH_3$、$O(CH_2)_nNH_2$或 $O(CH_2)_nCH_3$，其中 n 为 1- 约 10 ；C1-C10 低级烷基、烷氧基烷氧基、取代的低级烷基、烷芳基或芳烷基 ；Cl ；Br ；CN ；$CF_3$；$OCF_3$；O-、S-、或 N- 烷基 ；O-、S-、或 N- 烯基 ；$SOCH_3$；$SO_2CH_3$；$ONO_2$；$NO_2$；$N_3$；$NH_2$；杂环烷基 ；杂环烷芳基 ；氨基烷基氨基 ；聚烷基氨基 ；取代的甲硅烷基 ；RNA 切割基团 ；报道基团 ；嵌入剂 ；改进寡核苷酸药代动力学特性的基团 ；或改进寡核苷酸药效学特性的基团以及具有类似特性的其他取代基。优选的修饰包括 2’- 甲氧乙氧基 [2’ -O-$CH_2CH_2OCH_3$，也称为 2’ -O-(2- 甲氧乙基 )]。其他优选的修饰包括 2’- 甲氧基 (2’ -O-$CH_3$)、2’- 丙氧基 (2’ -O$CH_2CH_2CH_3$) 和 2’- 氟 (2’ -F)。类似的修饰也可在寡核苷酸的其他位置上进行，具体地在 3’ 末端核苷酸上糖的 3’位置和 5’末端核苷酸的 5’位置。寡核苷酸也可具有糖模拟物例如取代戊呋喃糖基基团的环丁基。

[0175] 寡核苷酸也可另外地或替代地包含核碱基 ( 本领域常常简称为“碱基”) 修饰或取代。本文所用的“未修饰的”或“天然的”核苷酸包括腺嘌呤 (A)、鸟嘌呤 (G)、胸腺嘧啶 (T)、胞嘧啶 (C) 和尿嘧啶 (U)。修饰的核苷酸包括在天然核酸中仅稀少或短暂地存在的核苷酸，例如，次黄嘌呤、6- 甲基腺嘌呤、5-Me 嘧啶、特别地 5- 甲基胞嘧啶 ( 也称为 5- 甲基 -2’脱氧胞嘧啶且常常在本领域中称为 5-Me-C)、5- 羟甲基胞嘧啶 (HMC)、糖基 HMC 和龙胆二糖基 HMC，以及合成核苷酸，例如，2- 氨基腺嘌呤、2-( 甲氨基 ) 腺嘌呤、2-( 咪唑基烷基 ) 腺嘌呤、2-( 氨烷基氨基 ) 腺嘌呤或其他杂取代的烷基腺嘌呤、2- 硫尿嘧啶、2- 硫胸腺嘧啶、5- 溴尿嘧啶、5- 羟基甲基尿嘧啶、8- 氮杂鸟嘌呤、7- 脱氮杂鸟嘌呤、N6(6- 氨己基 ) 腺嘌呤和 2,6- 二氨基嘌呤。可包括本领域已知的“通用的”碱基，例如，肌苷。已显示 5-Me-C 取代增强核酸双链体的稳定性达 0.6-1.2℃且为目前优选的碱基取代。

[0176] 本发明的寡核苷酸的另一种修饰涉及使一种或多种增强寡核苷酸的活性或细胞摄取的部分或缀合物与寡核苷酸化学连接。这类部分包括但不限于脂质部分，例如胆固醇部分、胆甾醇基部分，脂族链例如十二烷二醇或十一烷基残基，聚胺或聚乙二醇链，或者金刚烷乙酸。包含亲脂性部分的寡核苷酸以及用于制备这类寡核苷酸的方法为本领域已知的，例如美国专利第 5,138,045、5,218,105 和 5,459,255 号。

[0177] 无需将给定寡核苷酸中的所有位置一致地修饰，且实际上多于一种的上述修饰可掺入到单个寡核苷酸中或甚至在寡核苷酸内的单个核苷内。本发明也包括作为如上文中定义的嵌合寡核苷酸的寡核苷酸。

[0178] 在另一个实施方案中，本发明的核酸分子与另一个部分缀合，所述部分包括但不限于脱碱基核苷酸、聚醚、聚胺、聚酰胺、肽、碳水化合物、脂质或聚碳氢化合物。本领域技术人员将认识到，可将这些分子在糖、碱基或磷酸基的数个位置上连接到构成核酸分子的任何核苷酸中的一个或多个。

[0179] 依照本发明使用的寡核苷酸可通过众所周知的固相合成技术来便利和常规地制

备。用于这类合成的设备由包括 Applied Biosystems 在内的数个供应商销售。也可使用用于这类合成的任何其他方法；寡核苷酸的实际合成完全在本领域普通技术人员的才能之内。亦众所周知的是使用类似技术来制备其他寡核苷酸，例如硫代磷酸酯和烷基化衍生物。还众所周知的是使用类似技术和商购的经修饰的 amidites 和可控孔度玻璃 (CPG) 产品，例如生物素、荧光素、吖啶、或补骨脂素修饰的 amidites 和 / 或 CPG( 可从 Glen Research, Sterling VA 购买)，以合成荧光标记的、生物素化的或其他修饰的寡核苷酸，例如胆固醇修饰的寡核苷酸。

[0180] 依照本发明，使用修饰（例如使用 LNA 单体）以增加寡核苷酸的效能、特异性和作用持续时间并拓宽其施用途径，所述寡核苷酸由诸如 MOE、ANA、FANA、PS 等当前的化学物质组成。这可通过用 LNA 单体取代当前寡核苷酸中的一些单体来完成。LNA 修饰的寡核苷酸可具有类似于母体化合物的大小或者可更大或优选更小。优选这类 LNA 修饰寡核苷酸包含少于约 70％、更优选少于约 60％、最优选少于约 50％的 LNA 单体且其大小在约 5-25 个核苷酸之间，更优选在约 12-20 个核苷酸之间。

[0181] 优选的修饰寡核苷酸骨架包括但不限于硫代磷酸酯、手性硫代磷酸酯、二硫代磷酸酯、磷酸三酯、氨烷基磷酸三酯、甲基和其他烷基膦酸酯包括 3’ 烯基膦酸酯和手性膦酸酯、次磷酸酯、氨基磷酸酯包括 3’ - 氨基氨基磷酸酯和氨烷基氨基磷酸酯、硫羰基氨基磷酸酯、硫羰基烷基膦酸酯、硫羰基烷基磷酸三酯，以及具有正常 3’-5’ 键的硼烷磷酸酯 (boranophosphates)、这些的 2’-5’ 连接类似物，以及具有反极性的那些，其中核苷单元的相邻对为 3’-5’ 至 5’-3’ 或 2’-5’ 至 5’-2’ 连接的。也包括各种盐、混合盐和游离酸形式。

[0182] 教导制备上述含磷键的代表性的美国专利包括但不限于，美国专利第 3,687,808、4,469,863、4,476,301、5,023,243、5,177,196、5,188,897、5,264,423、5,276,019、5,278,302、5,286,717、5,321,131、5,399,676、5,405,939、5,453,496、5,455,233、5,466,677、5,476,925、5,519,126、5,536,821、5,541,306、5,550,111、5,563,253、5,571,799、5,587,361 和 5,625,050 号，每个通过引用结合于本文中。

[0183] 优选的修饰寡核苷酸骨架（其中不包含磷原子），具有由短链烷基或环烷基核苷间键、混合杂原子和烷基或环烷基核苷间键、或一种或多种短链杂原子或杂环核苷间键形成的骨架。这些包括具有吗啉代键的骨架（部分由核苷的糖部分形成）；硅氧烷骨架；硫化物、亚砜和砜骨架；甲乙酰基 (formacetyl) 和硫代甲乙酰基 (thioformacetyl) 骨架；亚甲基甲乙酰基和硫代甲乙酰基骨架；含烯骨架；氨基磺酸酯骨架；亚甲基亚氨基和亚甲基肼基骨架；磺酸酯或氨磺酰骨架；酰胺骨架；以及具有混合 N、O、S 和 $CH_2$ 组分部分的其他骨架。

[0184] 教导制备上述寡聚核苷的代表性的美国专利包括但不限于，美国专利第 5,034,506、5,166,315、5,185,444、5,214,134、5,216,141、5,235,033、5,264,562、5,264,564、5,405,938、5,434,257、5,466,677、5,470,967、5,489,677、5,541,307、5,561,225、5,596,086、5,602,240、5,610,289、5,602,240、5,608,046、5,610,289、5,618,704、5,623,070、5,663,312、5,633,360、5,677,437 和 5,677,439 号，每个通过引用结合于本文中。

[0185] 在其他优选的寡核苷酸模拟物中，将核苷酸单元的糖和核苷间键（即骨架）均用新基团替换。维持碱基单元用于与适当的核酸靶化合物杂交。一种这类寡聚化合物，即已显

示具有优秀的杂交特性的寡核苷酸模拟物，称为肽核酸 (PNA)。在 PNA 化合物中，寡核苷酸的糖骨架替换为含酰胺的骨架，具体地氨乙基氨基乙酸骨架。将核碱基保留并直接或间接地与骨架的酰胺部分的氮杂氮原子结合。教导制备 PNA 化合物的代表性的美国专利包括但不限于，美国专利第 5,539,082、5,714,331 和 5,719,262 号，每个通过引用结合于本文中。PNA 化合物的进一步教导可在 Nielsen 等，(1991)Science 254，1497-1500 中找到。

[0186] 在本发明的一个实施方案中，带有硫代磷酸酯骨架的寡核苷酸和带有杂原子骨架的寡聚核苷，具体地 $-CH_2-NH-O-CH_2-$，$-CH_2-N(CH_3)-O-CH_2-$（称为亚甲基（甲亚氨基）或 MMI 骨架），$-CH_2-O-N(CH_3)-CH_2-$、$-CH_2N(CH_3)-N(CH_3)CH_2-$ 和 $-O-N(CH_3)-CH_2-CH_2-$，其中天然磷酸二酯骨架表示为上文引用的美国专利第 5,489,677 号的 $-O-P-O-CH_2-$，以及上文引用的美国专利第 5,602,240 号的酰胺骨架。同样优选的为具有上文引用的美国专利第 5,034,506 号的吗啉代骨架结构的寡核苷酸。

[0187] 修饰寡核苷酸也可包含一个或多个经取代的糖部分。优选的寡核苷酸在 2’ 位置上包含下列中的一种 :OH ;F ;O-、S-、或 N- 烷基 ;O-、S-、或 N- 烯基 ;O-、S- 或 N- 炔基 ;或 O 烷基 -O- 烷基，其中所述烷基、烯基和炔基可为取代或未取代的 C 到 C0 烷基或 $C_2$ 到 $C_{10}$ 烯基和炔基。特别优选的为 $O(CH_2)_nO_mCH_3$、$O(CH_2)_n$、$OCH_3$、$O(CH_2)_nNH_2$、$O(CH_2)_nCH_3$、$O(CH_2)_nONH_2$ 和 $O(CH_2)_nON(CH_3)_2$，其中 n 和 m 可为 1- 约 10。其他优选的寡核苷酸在 2’ 位置上包含下列中的一种 :C 到 C0、低级烷基、取代的低级烷基、烷芳基、芳烷基、O- 烷芳基或 O- 芳烷基、SH、$SCH_3$、OCN、Cl、Br、CN、$CF_3$、$OCF_3$、$SOCH_3$、$SO_2CH_3$、$ONO_2$、$NO_2$、$N_3$、$NH_2$、杂环烷基、杂环烷芳基、氨烷基氨基、聚烷基氨基、取代的甲硅烷基、RNA 切割基团、报道基团、嵌入剂、用于改进寡核苷酸药代动力学特性的基团、或用于改进寡核苷酸药效学特性的基团，以及具有类似特性的其他取代基。优选的修饰包括 2’- 甲氧乙氧基 (2’ $-O-CH_2CH_2OCH_3$，也称为 2’-O-(2- 甲氧乙基）或 2’ -MOE)，即，烷氧基烷氧基基团。进一步优选的修饰包括 2’- 二甲基氨基氧基乙氧基，即 $O(CH_2)_2ON(CH_3)_2$ 基团，也称为 2’ -DMAOE（如下文实施例中所述），以及 2’- 二甲基氨基乙氧基乙氧基（本领域也称为 2’-O- 二甲基氨基乙氧基乙基或 2’ -DMAEOE)，即 2’ $-O-CH_2-O-CH_2-N(CH_2)_2$。

[0188] 其他优选的修饰包括 2’- 甲氧基 (2’ $-O\ CH_3$)、2’- 氨基丙氧基 (2’ $-OCH_2CH_2CH_2NH_2$) 和 2’- 氟 (2’ -F)。类似的修饰也可在寡核苷酸的其他位置上进行，具体地在 3’ 末端核苷酸上或 2’-5’ 连接的寡核苷酸中糖的 3’ 位置以及 5’ 末端核苷酸的 5’ 位置。寡核苷酸也可具有糖模拟物例如取代戊呋喃糖基糖的环丁基部分。教导制备这类经修饰的糖结构的代表性的美国专利包括但不限于，美国专利第 4,981,957、5,118,800、5,319,080、5,359,044、5,393,878、5,446,137、5,466,786、5,514,785、5,519,134、5,567,811、5,576,427、5,591,722、5,597,909、5,610,300、5,627,053、5,639,873、5,646,265、5,658,873、5,670,633 和 5,700,920 号，每个通过引用结合于本文中。

[0189] 寡核苷酸也可包含核碱基（本领域常常简称为“碱基”）修饰或取代。本文所用的“未修饰的”或“天然的”核苷酸包括嘌呤碱基腺嘌呤 (A) 和鸟嘌呤 (G)，以及嘧啶碱基胸腺嘧啶 (T)、胞嘧啶 (C) 和尿嘧啶 (U)。修饰的核苷酸包括其他合成和天然核苷酸，例如 5- 甲基胞嘧啶 (5-me-C)、5- 羟甲基胞嘧啶、黄嘌呤、次黄嘌呤、2- 氨基腺嘌呤、腺嘌呤和鸟嘌呤的 6- 甲基和其他烷基衍生物、腺嘌呤和鸟嘌呤的 2- 丙基和其他烷基衍生物、2- 硫尿嘧啶、2- 硫代胸腺嘧啶和 2- 硫代胞嘧啶、5- 卤代尿嘧啶和胞嘧啶、5- 丙炔基尿嘧啶和胞嘧啶、

6- 偶氮尿嘧啶、胞嘧啶和胸腺嘧啶、5- 尿嘧啶（假尿嘧啶）、4- 硫尿嘧啶、8- 卤代、8- 氨基、8- 巯基、8- 硫烷基、8- 羟基和其他 8- 取代的腺嘌呤和鸟嘌呤、5- 卤代具体地 5- 溴、5- 三氟甲基和其他 5- 取代的尿嘧啶和胞嘧啶、7- 甲基鸟嘌呤 (methylquanine) 和 7- 甲基腺嘌呤、8- 氮杂鸟嘌呤和 8- 氮杂腺嘌呤、7- 脱氮杂鸟嘌呤和 7- 脱氮杂腺嘌呤以及 3- 脱氮杂鸟嘌呤和 3- 脱氮杂腺嘌呤。

[0190] 此外，核苷酸包括公开于以下文献中的核苷酸：美国专利第 3,687,808 号、“高分子科学和工程的简明百科全书 (The Concise Encyclopedia of Polymer Science And Engineering)”，858-859 页，Kroschwitz，J.I.，编辑，John Wiley&Sons，1990、Englisch 等，′ Angewandle Chemie，International Edition′ ，1991，30，613 页和 Sanghvi，Y.S.，第 15 章，“反义研究和应用 (Antisense Research and Applications)”，289-302 页，Crooke，S.T. 和 Lebleu，B.ea.，CRC Press，1993。这些核苷酸中的某些对于增加本发明的寡聚化合物的结合亲和力特别地有用。这些包括 5- 取代嘧啶、6- 氮杂嘧啶和 N-2、N-6 和 0-6 取代的嘌呤，包括 2- 氨丙基腺嘌呤、5- 丙炔基尿嘧啶和 5- 丙炔基胞嘧啶。已显示 5- 甲基胞嘧啶取代增加核酸双链体的稳定性达 0.6-1.2℃ (Sanghvi，Y.S.，Crooke，S.T. 和 Lebleu，B.，编辑，“反义研究和应用”，CRC Press，Boca Raton，1993，276-278 页）且为目前优选的碱基取代，更特别地当与 2’-0- 甲氧基乙基糖修饰组合时。

[0191] 教导制备上述修饰核苷酸以及其他修饰核苷酸的代表性的美国专利包括但不限于，美国专利第 3,687,808、以及 4,845,205、5,130,302、5,134,066、5,175,273、5,367,066、5,432,272、5,457,187、5,459,255、5,484,908、5,502,177、5,525,711、5,552,540、5,587,469、5,596,091、5,614,617、5,750,692 和 5,681,941 号，每个通过引用结合于本文中。

[0192] 本发明的寡核苷酸的另一种修饰涉及使所述寡核苷酸与一种或多种部分或缀合物化学连接，其增强所述寡核苷酸的活性、细胞分布或细胞摄取。

[0193] 这类部分包括但不限于，脂质部分（例如胆固醇部分）、胆酸、硫醚（例如，己基 -S- 三苯甲基硫醇）、硫代胆固醇、脂族链（例如，十二烷二醇或十一烷基残基）、磷脂（例如，二 - 十六烷基 - 消旋 - 甘油或 1,2- 二 -0- 十六烷基 - 消旋 - 甘油 -3-H- 膦酸三乙铵）、聚胺或聚乙二醇链、或金刚烷乙酸、棕榈基部分、或十八胺或己基氨基 - 羰基 -t 羟胆固醇部分。

[0194] 教导制备这类寡核苷酸缀合物的代表性的美国专利包括但不限于，美国专利第 4,828,979、4,948,882、5,218,105、5,525,465、5,541,313、5,545,730、5,552,538、5,578,717、5,580,731、5,580,731、5,591,584、5,109,124、5,118,802、5,138,045、5,414,077、5,486,603、5,512,439、5,578,718、5,608,046、4,587,044、4,605,735、4,667,025、4,762,779、4,789,737、4,824,941、4,835,263、4,876,335、4,904,582、4,958,013、5,082,830、5,112,963、5,214,136、5,082,830、5,112,963、5,214,136、5,245,022、5,254,469、5,258,506、5,262,536、5,272,250、5,292,873、5,317,098、5,371,241、5,391,723、5,416,203、5,451,463、5,510,475、5,512,667、5,514,785、5,565,552、5,567,810、5,574,142、5,585,481、5,587,371、5,595,726、5,597,696、5,599,923、5,599,928 和 5,688,941 号，每个通过引用结合于本文中。

[0195] 药物开发：本发明的化合物也可应用于药物开发和靶标验证的领域。本发明包

括本文所鉴定的化合物和优选的靶区段在药物开发努力中阐明存在于脑源神经营养因子(BDNF)多核苷酸和疾病状态、表型或状况之间的关系的应用。这些方法包括检测或调节BDNF多核苷酸,包括使本发明的化合物与样品、组织、细胞或生物体接触,在处理后的某个时间测定BDNF多核苷酸的核酸或蛋白水平和/或相关的表型或化学终点,以及任选将该测定值与未处理样品或与用本发明的另一种化合物处理的样品比较。这些方法也可与其他试验平行或组合进行以确定未知基因的功能用于靶标验证过程,或确定特定基因产物作为用于治疗或预防特定疾病、状况或表型的靶标的有效性。

[0196] 评价基因表达的上调或抑制:

[0197] 外源核酸到宿主细胞或生物体中的转移可通过直接检测细胞或生物体中核酸的存在情况来评价。这类检测可通过本领域众所周知的数种方法来完成。例如,外源核酸的存在情况可通过DNA印迹或通过聚合酶链式反应(PCR)技术使用特异地扩增与所述核酸相关的核苷酸序列的引物来检测。外源核酸的表达也可使用包括基因表达分析在内的常规方法来测定。例如,由外源核酸产生的mRNA可使用RNA印迹和反转录PCR(RT-PCR)来检测和定量。

[0198] 来自外源核酸的RNA的表达也可通过测定酶活性或报道蛋白活性来检测。例如,反义调节活性可根据靶核酸表达的减少或增加间接地测定,以作为外源核酸正在产生效应物RNA的指示。基于序列保守性,可设计和使用引物来扩增靶基因的编码区。最初,可使用来自每个基因的最高表达的编码区来建立模型控制基因,虽然任何编码或非编码区均可使用。每个控制基因通过将每个编码区插入报道基因编码区和其聚腺苷酸信号之间来装配。这些质粒可产生在基因的上游部分具有报道基因以及在3'非编码区中具有潜在RNAi靶标的mRNA。单个反义寡核苷酸的有效性可通过报道基因的调节来测定。可用于本发明的方法中的报道基因包括乙酰羟酸合酶(AHAS)、碱性磷酸酶(AP)、β半乳糖苷酶(LacZ)、β葡糖醛酸酶(GUS)、氯霉素乙酰转移酶(CAT)、绿色荧光蛋白(GFP)、红色荧光蛋白(RFP)、黄色荧光蛋白(YFP)、青色荧光蛋白(CFP)、辣根过氧化物酶(HRP)、萤光素酶(Luc)、胭脂碱合酶(NOS)、章鱼碱合酶(OCS),以及其衍生物。多重选择标记为可利用的,其赋予对氨苄青霉素、博来霉素、氯霉素、庆大霉素、潮霉素、卡那霉素、林可霉素、甲氨蝶呤、草丁膦(phosphinothricin)、嘌呤霉素和四环素的抗性。确定报道基因调节的方法为本领域众所周知的,且包括但不限于,荧光测量法(例如荧光光谱法、荧光激活细胞分选术(FACS)、荧光显微法)、抗生素抗性测定。

[0199] BDNF蛋白和mRNA表达可使用本领域技术人员已知和本文别处所描述的方法测定。例如,免疫测定法(例如ELISA)可用来测定蛋白质水平。BDNF ELISA分析试剂盒可市购,例如,从R&D Systems(Minneapolis,MN)。

[0200] 在实施方案中,使用本发明反义寡核苷酸处理的样品(例如,体内或体外的细胞或组织)中的BDNF表达(例如,mRNA或蛋白质)通过与对照样品中的BDNF表达相比较来评价。例如,蛋白质或核酸的表达可使用本领域技术人员已知的方法与模拟处理或未处理样品中的蛋白质或核酸表达相比较。或者,与用对照反义寡核苷酸(例如,具有已改变或不同序列的反义寡核苷酸)处理的样品的比较可根据所需信息来进行。在另一个实施方案中,可将已处理样品对比未处理样品在BDNF蛋白或核酸表达方面的差异与已处理样品对比未处理样品在不同核酸(包括研究者认为适当的任何标准,例如,持家基因)的表达方面的差

异相比较。

[0201] 可将观察到的差异根据需要例如以比率或分数的形式表达，用于与对照比较。在实施方案中，在用本发明反义寡核苷酸处理的样品中，BDNF mRNA 或蛋白质水平相对于未处理样品或用对照核酸处理的样品增加或者减少约 1.25 倍－约 10 倍或更多。在实施方案中，BDNF mRNA 或蛋白质水平增加或减少至少约 1.25 倍、至少约 1.3 倍、至少约 1.4 倍、至少约 1.5 倍、至少约 1.6 倍、至少约 1.7 倍、至少约 1.8 倍、至少约 2 倍、至少约 2.5 倍、至少约 3 倍、至少约 3.5 倍、至少约 4 倍、至少约 4.5 倍、至少约 5 倍、至少约 5.5 倍、至少约 6 倍、至少约 6.5 倍、至少约 7 倍、至少约 7.5 倍、至少约 8 倍、至少约 8.5 倍、至少约 9 倍、至少约 9.5 倍、或至少约 10 倍或更多。

[0202] 试剂盒、研究试剂、诊断剂和治疗剂

[0203] 本发明的化合物可用于诊断、治疗和预防，并作为研究试剂和试剂盒的组分。此外，能够以精细的特异性抑制基因表达的反义寡核苷酸常常被普通技术人员用于阐明特定基因的功能或区分生物途径的各个成员之间的功能。

[0204] 对于用于试剂盒和诊断及各种生物系统中的应用，本发明的化合物（单独地或与其他化合物或治疗剂组合）可用作差异和 / 或组合分析中的工具，以阐明在细胞和组织内表达的基因的一部分或全部互补序列的表达模式。

[0205] 本文所用的术语“生物系统”或“系统”定义为表达或使得有能力表达脑源神经营养因子 (BDNF) 基因的产物的任何生物体、细胞、细胞培养物或组织。这些包括但不限于人、转基因动物、细胞、细胞培养物、组织、异种移植物、移植物及其组合。

[0206] 作为一个非限制性实例，将在用一种或多种反义化合物处理的细胞或组织内的表达模式与未用反义化合物处理的对照细胞或组织相比较，并针对基因表达的差异水平分析产生的模式，因为它们涉及，例如，所检测基因的疾病相关性、信号传导途径、细胞定位、表达水平、大小、结构或功能。这些分析可对刺激或未刺激的细胞以及在影响表达模式的其他化合物存在或不存在时进行。

[0207] 本领域已知的基因表达分析方法的实例包括 DNA 阵列或微阵列、SAGE（基因表达的系列分析）、READS（已消化 cDNA 的限制性酶扩增）、TOGA（总基因表达分析）、蛋白质阵列和蛋白质组学、表达序列标签 (EST) 测序、消减 (subtractive)RNA 指纹法 (SuRF)、消减克隆、差异显示 (DD)、比较基因组杂交、FISH（荧光原位杂交）技术和质谱分析法。

[0208] 本发明的化合物对于研究和诊断而言为有用的，因为这些化合物与编码脑源神经营养因子 (BDNF) 的核酸杂交。例如，作为有效的 BDNF 调节剂以本文公开的这类效率和在这类条件下杂交的寡核苷酸在有利于基因扩增或检测的条件下分别为有效的引物或探针。这些引物和探针可用于需要对编码 BDNF 的核酸分子特异检测的方法中，和可用于扩增所述核酸分子以用于检测或用于进一步研究 BDNF。本发明的反义寡核苷酸（具体地，引物和探针）与编码 BDNF 的核酸的杂交可通过本领域已知的方法来检测。这类方法可包括使酶与所述寡核苷酸缀合、放射性标记所述寡核苷酸或任何其他适当的检测方法。也可制备使用这类检测方法来检测样品中 BDNF 水平的试剂盒。

[0209] 反义物的特异性和灵敏度也由本领域技术人员掌握用于治疗用途。已将反义化合物在动物（包括人）的疾病状态的治疗中用作治疗部分。反义寡核苷酸药物已安全和有效地施用给人且许多临床试验目前正在进行。因此确认的是，反义化合物可为有用的治疗形

式，可将其配置以用于治疗细胞、组织和动物、尤其是人的治疗方案中。

[0210] 对于治疗而言，将怀疑具有可通过调节 BDNF 多核苷酸的表达来治疗的疾病或病症的动物（优选人）通过施用依照本发明的反义化合物来治疗。例如，在一个非限制性实施方案中，所述方法包括给需要治疗的动物施用治疗有效量的 BDNF 调节剂的步骤。本发明的 BDNF 调节剂有效地调节 BDNF 的活性或调节 BDNF 蛋白的表达。在一个实施方案中，动物中 BDNF 的活性或表达与对照相比抑制了约 10％。优选地，将动物中 BDNF 的活性或表达抑制约 30％。更优选地，将动物中 BDNF 的活性或表达抑制 50％或更多。因此，与对照相比，寡聚化合物将脑源神经营养因子 (BDNF)mRNA 的表达调节至少 10％、至少 50％、至少 25％、至少 30％、至少 40％、至少 50％、至少 60％、至少 70％、至少 75％、至少 80％、至少 85％、至少 90％、至少 95％、至少 98％、至少 99％、或 100％。

[0211] 在一个实施方案中，与对照相比，在动物中脑源神经营养因子 (BDNF) 的活性或表达增加约 10％。优选地，在动物中 BDNF 的活性或表达增加约 30％。更优选地，在动物中 BDNF 的活性或表达增加 50％或更多。因此，与对照比较，寡聚化合物调节 BDNF mRNA 的表达至少 10％、至少 50％、至少 25％、至少 30％、至少 40％、至少 50％、至少 60％、至少 70％、至少 75％、至少 80％、至少 85％、至少 90％、至少 95％、至少 98％、至少 99％或 100％。

[0212] 例如，脑源神经营养因子 (BDNF) 表达的提高或下降可在动物的血清、血液、脂肪组织、肝脏或任何其他体液、组织或器官中测定。优选地，包含于待分析的所述液体、组织或器官之内的细胞包含编码 BDNF 肽的核酸分子和 / 或 BDNF 蛋白本身。

[0213] 本发明的化合物可通过向合适的药学上可接受的稀释剂或载体中添加有效量的化合物来用于药物组合物中。本发明的化合物的应用和方法也可为预防上有用的。

[0214] 缀合物

[0215] 本发明的寡核苷酸的另一种修饰涉及将一种或多种增强寡核苷酸的活性、细胞分布或细胞摄取的部分或缀合物与寡核苷酸化学连接。这些部分或缀合物可包含与官能团（例如伯羟基或仲羟基）共价结合的缀合基团。本发明的缀合基团包括嵌入剂、报道分子、聚胺、聚酰胺、聚乙二醇、聚醚、增强寡聚体药效学特性的基团，以及增强寡聚体药代动力学特性的基团。典型的缀合基团包括胆固醇、脂质、磷脂、生物素、吩嗪、叶酸、菲啶、蒽醌、吖啶、荧光素、罗丹明、香豆素和染料。增强药效学特性的基团，在本发明的背景中，包括改善摄取、增强对降解的抗性和 / 或加强与靶核酸的序列特异性杂交的基团。增强药代动力学特性的基团，在本发明的背景中，包括改善本发明的化合物的摄取、分布、代谢或分泌的基团。代表性的缀合基团在提交于 1992 年 10 月 23 日的国际专利申请第 PCT/US92/09196 号和美国专利第 6,287,860 号中公开，所述文献通过引用结合于本文中。缀合部分包括但不限于，脂质部分（例如胆固醇部分）、胆酸、硫醚（例如，己基 -5- 三苯甲基硫醇）、硫代胆固醇、脂族链（例如，十二烷二醇或十一烷基残基）、磷脂（例如，二 - 十六烷基 - 消旋 - 甘油或 1,2- 二 -O- 十六烷基 - 消旋 - 甘油 -3-H- 膦酸三乙铵）、聚胺或聚乙二醇链、或金刚烷乙酸、棕榈基部分、或十八胺或己基氨基 - 羰基 - 羟胆固醇部分。也可将本发明的寡核苷酸与活性药物物质缀合，例如，阿司匹林、华法林、保泰松、布洛芬、舒洛芬、芬布芬、酮洛芬、(S)-(+) 普拉洛芬、卡洛芬、丹酰肌氨酸、2,3,5- 三碘苯甲酸、氟芬那酸、亚叶酸、苯并噻二嗪、氯噻嗪、二氮杂䓬、吲哚美辛 (indomethicin)、巴比妥酸盐、头孢菌素、磺胺类药物、抗糖尿病药、抗菌剂或抗生素。

[0216] 教导制备这类寡核苷酸缀合物的代表性的美国专利包括但不限于，美国专利第 4,828,979、4,948,882、5,218,105、5,525,465、5,541,313、5,545,730、5,552,538、5,578,717、5,580,731、5,580,731、5,591,584、5,109,124、5,118,802、5,138,045、5,414,077、5,486,603、5,512,439、5,578,718、5,608,046、4,587,044、4,605,735、4,667,025、4,762,779、4,789,737、4,824,941、4,835,263、4,876,335、4,904,582、4,958,013、5,082,830、5,112,963、5,214,136、5,082,830、5,112,963、5,214,136、5,245,022、5,254,469、5,258,506、5,262,536、5,272,250、5,292,873、5,317,098、5,371,241、5,391,723、5,416,203、5,451,463、5,510,475、5,512,667、5,514,785、5,565,552、5,567,810、5,574,142、5,585,481、5,587,371、5,595,726、5,597,696、5,599,923、5,599,928 和 5,688,941 号。

[0217] 制剂

[0218] 本发明的化合物也可与其他分子、分子结构或化合物的混合物掺混、封装、缀合或以其他方式关联，作为例如，脂质体、受体靶向分子、口服的、直肠的、局部的或其他制剂，用于帮助摄取、分布和 / 或吸收。教导制备这类摄取、分布和 / 或吸收辅助制剂的代表性的美国专利包括但不限于，美国专利第 5,108,921、5,354,844、5,416,016、5,459,127、5,521,291、5,543,165、5,547,932、5,583,020、5,591,721、4,426,330、4,534,899、5,013,556、5,108,921、5,213,804、5,227,170、5,264,221、5,356,633、5,395,619、5,416,016、5,417,978、5,462,854、5,469,854、5,512,295、5,527,528、5,534,259、5,543,152、5,556,948、5,580,575 和 5,595,756 号，每个通过引用结合于本文中。

[0219] 尽管，反义寡核苷酸不需要在载体的情况中施用以实现调节靶表达和 / 或功能的目的，但是本发明的实施方案涉及用于反义寡核苷酸表达的表达载体构建体，包括启动子、杂合启动子基因序列，并且拥有强组成型启动子活性，或可在所需情况下诱导的启动子活性。

[0220] 在一个实施方案中，本发明实施涉及用适合的核酸递送系统施用至少一种前述反义寡核苷酸。在一个实施方案中，该系统包含与多核苷酸可操作连接的非病毒载体。这类非病毒载体的实例包括单独的寡核苷酸（例如，SEQ ID NO :12-49 中的任何一个或多个）或与适合的蛋白、多糖或脂质制剂组合的寡核苷酸。

[0221] 其他适合的核酸递送系统包括病毒载体，典型地来自腺病毒、腺病毒伴随病毒 (AAV)、依赖辅助病毒的腺病毒、逆转录病毒或仙台病毒 - 脂质体 (HVJ) 复合体中的至少一种的序列。优选地，所述病毒载体包含与多核苷酸可操作连接的强真核启动子，例如，巨细胞病毒 (CMV) 启动子。

[0222] 另外优选的载体包括病毒载体、融合蛋白和化学缀合物。逆转录病毒载体包括莫洛尼鼠白血病病毒和基于 HIV 的病毒。一种优选的基于 HIV 的病毒载体包括至少两种载体，其中 gag 基因和 pol 基因来自 HIV 基因组而 env 基因来自另一种病毒。DNA 病毒载体为优选的。这些载体包括痘病毒载体（例如正痘病毒或禽痘病毒载体）、疱疹病毒载体（例如单纯疱疹 I 病毒 (HSV) 载体）、腺病毒载体和腺伴随病毒载体。

[0223] 本发明的反义化合物包括任何药学上可接受的盐、酯、或这类酯的盐、或任何其他化合物，其在施用给动物（包括人）后，能够提供（直接地或间接地）生物学活性的代谢物或其残留物。

[0224] 术语“药学上可接受的盐”是指本发明化合物的生理上和药学上可接受的盐：即，保留母体化合物的所需生物活性且不对其产生非所需的毒理学作用的盐。对于寡核苷酸而言，药学上可接受的盐的优选实例和其使用进一步描述于美国专利第6,287,860号中，其通过引用结合于本文中。

[0225] 本发明也包括包含本发明的反义化合物的药物组合物和制剂。本发明的药物组合物可以以若干方式来施用，这取决于是否需要局部或全身治疗以及待治疗的区域。施用可为局部的（包括眼的和至粘膜的包括阴道和直肠递送）、肺的（例如，通过吸入或吹入散剂或气雾剂，包括通过喷雾器）、气管内的、鼻内的、表皮的和经皮的、口服的或胃肠外的。胃肠外施用包括静脉内、动脉内、皮下、腹膜内或肌肉注射或输注；或颅内（例如，鞘内或脑室内）施用。

[0226] 对于治疗中枢神经系统中的组织而言，可通过例如注射或输注进入脑脊液进行施用。施用反义RNA进入脑脊液已在例如美国专利申请公开第2007/0117772号，“Methods for slowing familial ALS disease progression(减缓家族性ALS疾病进展的方法)”中描述，该申请通过引用以其整体结合于本文中。

[0227] 如果意图将本发明的反义寡核苷酸施用给中枢神经系统中的细胞，可与一种或多种能够促进所述反义寡核苷酸渗透穿过血脑屏障的物质一起施用。注射可在例如内嗅皮质或海马中进行。通过施用腺病毒载体至肌肉组织中的运动神经元而递送神经营养因子描述于，例如，美国专利第6,632,427号，“Adenoviral-vector-mediated gene transfer into medullary motor neurons(腺病毒载体介导基因转移进入髓质运动神经元)”，其通过引用结合于本文中。直接递送载体至脑（例如，纹状体、丘脑、海马或黑质）为本领域已知且描述于例如美国专利第6,756,523号，“Adenovirus vectors for the transfer of foreign genes into cells of the central nervous system particularly in brain(用于转移外源基因进入中枢神经系统细胞（具体地脑中）的腺病毒载体)”，其通过引用结合于本文中。施用可快速进行，如通过注射，或在一段时间内进行，如通过缓慢输注或施用缓释制剂。

[0228] 所述反义寡核苷酸也可与提供所需药学或药效学特性的物质连接或缀合。例如，反义寡核苷酸可与本领域已知的促进渗透或转运穿过血脑屏障的任何物质（例如转铁蛋白受体的抗体）偶连，并通过静脉内注射施用。反义化合物可与病毒载体连接，例如，使反义化合物更有效和/或增加反义化合物转运穿过血脑屏障的病毒载体。渗透性血脑屏障破坏也可通过例如输注糖或氨基酸来完成，所述糖包括但不限于，内消旋赤藓醇、木糖醇、D(+)半乳糖、D(+)乳糖、D(+)木糖、卫矛醇、肌醇、L(-)果糖、D(-)甘露醇、D(+)葡萄糖、D(+)阿拉伯糖、D(-)阿拉伯糖、纤维二糖、D(+)麦芽糖、D(+)蜜三糖、L(+)鼠李糖、D(+)蜜二糖、D(-)核糖、侧金盏花醇、D(+)阿拉伯糖醇、L(-)阿拉伯糖醇、D(+)岩藻糖、L(-)岩藻糖、D(-)来苏糖、L(+)来苏糖和L(-)来苏糖，所述氨基酸包括但不限于，谷氨酰胺、赖氨酸、精氨酸、天冬酰胺、天冬氨酸、半胱氨酸、谷氨酸、甘氨酸、组氨酸、亮氨酸、甲硫氨酸、苯丙氨酸、脯氨酸、丝氨酸、苏氨酸、酪氨酸、缬氨酸和牛磺酸。用于增强血脑屏障渗透的方法和材料描述于，例如，美国专利第4,866,042号，“Method for the delivery of genetic material across the blood brain barrier(用于递送遗传物质穿过血脑屏障的方法)”，第6,294,520号，“Material for passage through the blood-brain barrier(用于通过血脑屏障的材料)”，和第6,936,589号，“Parenteral delivery systems(胃肠外递送系

统）”，其全部通过引用以其整体结合于本文中。

[0229] 所述反义化合物可与其他分子、分子结构或化合物的混合物掺混、封装、缀合或以其他方式关联，例如脂质体、受体靶向分子、口服的、直肠的、局部的或其他制剂，用于帮助摄取、分布和/或吸收。例如，阳离子脂质可包含在制剂中以促进寡核苷酸摄取。一种显示出促进摄取的这类组合物为LIPOFECTIN（可从GIBCO-BRL，Bethesda，MD获得）。

[0230] 认为带有至少一个2’-O-甲氧基乙基修饰的寡核苷酸对于口服施用而言为特别有用的。用于局部施用的药物组合物和制剂可包括透皮贴剂、软膏剂、洗剂、乳膏剂、凝胶剂、滴剂、栓剂、喷雾剂、液体和散剂。常规药物载体、水性、粉末或油性基质、增稠剂等可为必要的或所需的。包被的避孕套、手套等也可为有用的。

[0231] 可适宜地以单位剂型存在的本发明药物制剂可根据药学工业中众所周知的常规技术来制备。这类技术包括使活性成分与药物载体或赋形剂结合的步骤。一般而言，制剂如下制备：使活性成分与液体载体或细碎的固体载体或两者均匀和紧密地结合，随后（在需要时）使产物成形。

[0232] 可将本发明的组合物制成许多可能剂型的任何一种，例如但不限于，片剂、胶囊剂、凝胶胶囊、液体糖浆、软凝胶、栓剂和灌肠剂。也可将本发明的组合物在水性、非水性或混合介质中制成混悬剂。水性混悬剂可进一步包含增加混悬剂粘度的物质，包括例如羧甲基纤维素钠、山梨醇和/或葡聚糖。所述混悬剂也可包含稳定剂。

[0233] 本发明的药物组合物包括但不限于，溶液剂、乳剂、泡沫剂和含脂质体的制剂。本发明的药物组合物和制剂可包含一种或多种渗透促进剂、载体、赋形剂或其他活性或非活性成分。

[0234] 乳剂典型地为一种液体以通常直径超过0.1μm的液滴形式分散在另一种液体中的非均质体系。乳剂可包含除分散相之外的其他组分，以及可作为在水相、油相中的溶液或其本身作为单独相存在的活性药物。微乳液包括为本发明的一个实施方案。乳剂及其使用为本领域众所周知的且进一步描述于美国专利第6,287,860号中。

[0235] 本发明的制剂包括脂质体制剂。本发明所用的术语“脂质体”意为由排列在一个或多个球形双层中的两亲脂质组成的囊泡。脂质体为具有由亲脂材料形成的膜和包含待递送组合物的水性内部的单层或多层囊泡。阳离子脂质体为带正电的脂质体，其认为与带负电的DNA分子相互作用以形成稳定的复合体。认为pH敏感的或带负电的脂质体诱捕DNA而不是与其复合。阳离子和非阳离子脂质体均已用来递送DNA到细胞。

[0236] 脂质体也包括“空间上稳定的”脂质体，该术语如本文所用是指包含一种或多种专门脂质的脂质体。当掺入到脂质体中时，这些专门脂质给脂质体带来相对于缺乏这类专门脂质的脂质体延长的循环存在期。空间上稳定的脂质体的实例为其中脂质体的囊泡形成脂质部分的部分包含一种或多种糖脂或衍生有一种或多种亲水聚合物（例如聚乙二醇(PEG)部分）的脂质体。脂质体及其使用进一步描述于美国专利第6,287,860号中。

[0237] 本发明的药物制剂和组合物也可包含表面活性剂。表面活性剂在药物产品、制剂和乳剂中的使用为本领域众所周知的。表面活性剂及其使用进一步描述于美国专利第6,287,860号中，其通过引用结合于本文中。

[0238] 在一个实施方案中，本发明使用各种渗透促进剂来实现核酸特别是寡核苷酸的有效递送。除了有助于非亲脂性药物穿过细胞膜的扩散之外，渗透促进剂还增加亲脂性药物

的渗透性。可将渗透促进剂归类为属于五大类的一种，即表面活性剂、脂肪酸、胆汁酸盐、螯合剂和非螯合非表面活性剂。渗透促进剂及其使用进一步描述于美国专利第 6,287,860 号，其通过引用结合于本文中。

[0239] 本领域的技术人员将认识到，制剂根据其预期用途（即给药途径）来常规地设计。

[0240] 用于局部给药的优选制剂包括其中本发明寡核苷酸与局部递送剂（例如，脂质、脂质体、脂肪酸、脂肪酸酯、甾类、螯合剂和表面活性剂）混合的制剂。优选的脂质和脂质体包括中性的（例如二油酰基 - 磷脂酰乙醇胺 DOPE、二肉豆蔻酰基磷脂酰胆碱 DMPC、二硬脂酰基磷脂酰胆碱）、阴性的（例如二肉豆蔻酰基磷脂酰甘油 DMPG）和阳离子的（例如二油酰基四甲基氨丙基 DOTAP 和二油酰基 - 磷脂酰基乙醇胺 DOTMA）。

[0241] 对于局部或其他给药而言，可将本发明的寡核苷酸封装在脂质体内或可与其（特别是与阳离子脂质体）形成复合体。或者，可将寡核苷酸与脂质（特别是阳离子脂质）复合。优选的脂肪酸和酯类、其药学上可接受的盐，以及它们的使用进一步描述于美国专利第 6,287,860 号中。

[0242] 用于口服给药的组合物和制剂包括散剂或颗粒剂、微粒、纳米颗粒、在水或非水性介质中的混悬剂或溶液剂、胶囊剂、凝胶胶囊、小药囊、片剂或小片。增稠剂、矫味剂、稀释剂、乳化剂、分散助剂或粘合剂可能为所需的。优选的口服制剂为其中将本发明的寡核苷酸与一种或多种渗透促进剂、表面活性剂和螯合剂结合施用的制剂。优选的表面活性剂包括脂肪酸和 / 或其酯或盐、胆汁酸和 / 或其盐。优选的胆汁酸 / 盐和脂肪酸及其使用进一步描述于美国专利第 6,287,860 号中，其通过引用结合于本文中。还优选的为渗透促进剂的组合，例如脂肪酸 / 盐与胆汁酸 / 盐的组合。特别优选的组合为月桂酸的钠盐、癸酸和 UDCA。另外的渗透促进剂包括聚氧化乙烯 -9- 月桂醚、聚氧化乙烯 -20- 鲸蜡醚。本发明的寡核苷酸可以以包括喷雾干燥颗粒的颗粒形式口服地递送，或复合以形成微米或纳米粒子。寡核苷酸复合剂及其使用进一步描述于美国专利第 6,287,860 号中，其通过引用结合于本文中。

[0243] 用于胃肠外、鞘内或室内给药的组合物和制剂可包括无菌水性溶液剂，其也可含有缓冲液、稀释剂和其他适合的添加剂，例如但不限于，渗透促进剂、载体化合物和其他药学上可接受的载体或赋形剂。

[0244] 本发明的某些实施方案提供药物组合物，所述药物组合物包含一种或多种寡聚化合物和一种或多种通过非反义机制来起作用的其他化学治疗剂。这类化学治疗剂的实例包括但不限于癌症化疗药物，例如柔红霉素、道诺霉素、更生霉素、多柔比星、表柔比星、伊达比星、依索比星、博来霉素、马磷酰胺、异环磷酰胺、胞嘧啶阿拉伯糖苷、双氯乙基 - 亚硝基脲、白消安、丝裂霉素 C、放线菌素 D、光神霉素、泼尼松、羟孕酮、睾酮、他莫昔芬、达卡巴嗪、丙卡巴肼、六甲蜜胺、五甲蜜胺、米托蒽醌、安吖啶、苯丁酸氮芥、甲基环己基亚硝基脲、氮芥、美法仑、环磷酰胺、6- 巯基嘌呤、6- 硫鸟嘌呤、阿糖胞苷、5- 氮杂胞苷、羟基脲、脱氧助间型霉素 (deoxycoformycin)、4- 羟基过氧环磷酰胺、5- 氟尿嘧啶 (5-FU)、5- 氟脱氧尿苷 (5-FUdR)、甲氨蝶呤 (MTX)、秋水仙碱、泰素、长春新碱、长春碱、依托泊苷 (VP-16)、三甲曲沙、伊立替康、拓泊替康、吉西他滨、替尼泊苷、顺铂和己烯雌酚 (DES)。当与本发明的化合物一起使用时，这类化学治疗剂可单独地（例如，5-FU 和寡核苷酸）、顺序地（例如，5-FU 和寡核苷酸持续一段时间，接着 MTX 和寡核苷酸）、或与一种或多种其他的这类化学治疗剂组合

（例如，5-FU、MTX 和寡核苷酸，或者 5-FU、放射疗法和寡核苷酸）使用。抗炎药（包括但不限于非甾体抗炎药和皮质类固醇）和抗病毒药物（包括但不限于利巴韦林、阿糖腺苷、阿昔洛韦和更昔洛韦）也可组合到本发明的组合物中。反义化合物和其他非反义药物的组合也在本发明的范围之内。两种或更多种组合的化合物可一起或顺序使用。

[0245] 在另一个相关的实施方案中，本发明的组合物可包含靶向于第一核酸的一种或多种反义化合物（特别是寡核苷酸），以及靶向于第二核酸靶标的一种或多种其他反义化合物。例如，第一靶标可为脑源神经营养因子 (BDNF) 的特定反义序列，和第二靶标可为来自另一个核苷酸序列的区域。或者，本发明的组合物可包含靶向于相同脑源神经营养因子 (BDNF) 核酸靶标的不同区域的两种或更多种反义化合物。本文举例说明了许多反义化合物的实例而其他的可选自本领域已知的适合化合物。两种或更多种组合化合物可一起或顺序使用。

[0246] 给药：

[0247] 认为治疗组合物的制剂和其随后的施用（给药）在本领域技术人员的技术之内。给药取决于要治疗的疾病状态的严重性和反应性，而疗程从数天持续到数月，或直到实现治愈或达到疾病状态的减轻。最佳给药方案可根据患者体内药物蓄积的测量来计算。普通技术人员可容易地确定最适剂量、给药方法和重复率。最适剂量可根据单个寡核苷酸的相对功效而不同，一般可基于在体外和动物模型中体内发现有效的 EC50 来估计。一般而言，剂量为 0.01 μg-10mg/kg 体重，且可每天、每周、每月或每年给药一次或多次，或甚至每 2-20 年一次。本领域普通技术人员可基于测定体液或组织中药物的停留时间和浓度来容易地估计给药的重复率。成功治疗之后，可能需要对患者进行维持疗法以预防疾病状态的复发，其中所述寡核苷酸以维持剂量来施用，范围为 0.01 μg-10mg/kg 体重，每天一次或多次到每 2-20 年一次。

[0248] 在实施方案中，使用下列剂量的药物治疗患者，所述剂量为至少约 1、至少约 2、至少约 3、至少约 4、至少约 5、至少约 6、至少约 7、至少约 8、至少约 9、至少约 10、至少约 15、至少约 20、至少约 25、至少约 30、至少约 35、至少约 40、至少约 45、至少约 50、至少约 60、至少约 70、至少约 80、至少约 90、或至少约 100mg/kg 体重。反义寡核苷酸的某些注射剂量描述于，例如，美国专利第 7,563,884 号，“Antisense modulation of PTP1B expression(PTP1B 表达的反义调节）”，通过引用以其整体结合于本文中。

[0249] 虽然上文已描述了本发明的各种实施方案，但是应理解的是，其仅以实例的方式提供，而并非限制。对公开的实施方案的许多改变可依照本文的公开内容来进行，而不会背离本发明的精神或范围。因此，本发明的广度和范围不应受到任何上述的实施方案所限制。

[0250] 本文提及的所有文件都通过引用结合到本文中。本申请引用的所有出版物和专利文件都为所有目的而通过引用结合，其程度如同单独地指出各个出版物或专利文件通过引用结合到本文中一样。至于在本文件中对不同参考文献的引用，申请人并不承认任何具体参考文献对其发明而言为“现有技术”。本发明的组合物和方法的实施方案举例说明于下列实施例中。

## 实施例

[0251] 下列非限制性的实施例用于举例说明本发明的所选实施方案。应理解的是，所示

组分的比例变化和要素替换对本领域的技术人员而言为显而易见的且在本发明的实施方案的范围之内。

[0252] 实施例 1 :对脑源神经营养因子 (BDNF) 反义的核酸分子和 / 或 BDNF 多核苷酸有义链特异性的反义寡核苷酸的设计

[0253] 如上指出的术语“对 ..... 特异性的寡核苷酸”或“寡核苷酸靶向于 .....”是指具有以下序列的寡核苷酸 :(i) 能够与靶向基因的一部分形成稳定的复合体,或 (ii) 能够与靶向基因的 mRNA 转录物的一部分形成稳定的双链体。

[0254] 适当寡核苷酸的选择通过使用计算机程序 ( 例如 IDT AntiSense Design, IDT OligoAnalyzer) 来促进,该计算机程序在各给定的序列中自动确定以所需的解链温度 ( 通常 50-60℃ ) 与靶多核苷酸序列形成杂合体且不形成自身二聚体或其它复杂二级结构的 19-25 个核苷酸的子序列。

[0255] 适当寡核苷酸的选择进一步通过使用计算机程序来促进,该计算机程序自动比对核酸序列并指出同一性或同源性区域。这类程序用于例如通过搜索诸如 GenBank 的数据库或通过测序 PCR 产物而比较获得的核酸序列。来自给定基因组的一系列基因和基因间区域的核酸序列的比较允许选择显示出对目标基因的适当特异性程度的核酸序列。这些程序允许选择对靶核酸序列表现高度的互补性而对在给定基因组中的其它核酸序列表现较低程度的互补性的寡核苷酸。本领域技术人员将认识到,在选择用于本发明的适当的基因区域上具有相当大的自由。

[0256] 如果所述化合物与靶核酸的结合干扰靶核酸的正常功能以导致功能和 / 或活性的调节,并且在要求特异性结合的条件下 ( 即在体内测定或治疗处理情况中的生理条件下,以及在其中在体外分析情况中进行测定的条件下 ) 具有足够程度的互补性以避免所述反义化合物与非靶核酸序列的非特异性结合,则反义化合物为“可特异杂交的”。

[0257] 本文所述的寡核苷酸的杂交特性可通过本领域已知的一种或多种体外测定法来确定。例如,本文所述的寡核苷酸的特性可通过使用解链曲线测定法确定靶天然反义物和潜在药物分子之间的结合强度来获得。

[0258] 靶天然反义物和潜在药物分子 ( 分子 ) 之间的结合强度可使用任何已建立的测定分子间相互作用强度的方法例如解链曲线测定法来评估。

[0259] 解链曲线测定法确定天然反义物 / 分子复合体发生从双链构象到单链构象的迅速转变时的温度。此温度被广泛接受为两个分子之间相互作用强度的可靠衡量。

[0260] 解链曲线测定法可使用实际的天然反义 RNA 分子的 cDNA 拷贝或对应于分子的结合位点的合成 DNA 或 RNA 核苷酸来进行。包含进行此测定的所有必需试剂的多种试剂盒为可得的 ( 例如 Applied Biosystems Inc. MeltDoctor 试剂盒 )。这些试剂盒包含含有双链 DNA(dsDNA) 结合染料 ( 例如 ABI HRM 染料、SYBR Green、SYTO, 等等 ) 之一的适宜缓冲溶液。dsDNA 染料的特性为使得其在游离形式几乎不发射荧光,但当与 dsDNA 结合时为高度荧光的。

[0261] 为进行所述测定,将所述 cDNA 或相应寡核苷酸以由具体制造商的方案限定的浓度与分子混合。将所述混合物加热到 95℃以解离所有预先形成的 dsDNA 复合体,然后缓慢冷却到室温或由试剂盒制造商确定的其他较低温度以使 DNA 分子退火。随后将新形成的复合体缓慢加热到 95℃,同时连续地收集由反应产生的荧光量的数据。荧光强度反比于反应

中存在的dsDNA的量。数据可使用与所述试剂盒相容的实时PCR仪器(例如ABI's StepOne Plus Real Time PCR System或LightTyper仪器,Roche Diagnostics,Lewes,UK)来收集。

[0262] 熔融峰通过使用适当软件(例如LightTyper(Roche)或SDS Dissociation Curve,ABI)对温度(x-轴)绘制荧光相对于温度的负导数(在y-轴上的-d(荧光)/dT)的图形来构建。分析数据以确定从dsDNA复合体迅速转变到单链分子的温度。此温度称为Tm且正比于两个分子之间的相互作用的强度。典型地,Tm将超过40℃。

[0263] 修饰的AntagoNAT分子的设计:

[0264] 设计和测试了多个基于DNA的反义寡核苷酸,称为AntagoNAT,其靶向非编码Bdnf-AS和其它反义转录物。设计了长度范围12-20个核苷酸的各种AntagoNAT,其具有或没有完全硫代磷酸酯修饰+/-2-O'-甲基RNA或LNA修饰的核苷酸。最高的效力在各末端具有三个LNA-修饰的核苷酸的16-核苷酸硫代磷酸酯间体(XXXnnnnnnnnnnXXX)的Bdnf mRNA水平上观察到。为阻断人BDNF有义-反义转录物之间的相互作用,使用包含LNA和2-O'-甲基RNA分子两者的14-核苷酸混合体(mixmer)。虽然这些2-O'-甲基RNA-修饰的寡核苷酸表明为仅阻断RNA,但靶向RNA的边缘下调在该试验中观察到(图11)。各种AntagoNAT的序列以及用于这些研究的所有其它siRNA、引物和探针列于表1中。

[0265] 实施例2:BDNF多核苷酸的调节

[0266] 用于实施例2中的所有反义寡核苷酸如实施例1中描述的设计。制造商(Coralville,IA的IDT Inc.)指导制造设计的硫代磷酸酯键寡核苷酸并提供表1中所示的设计的硫代磷酸酯类似物。核苷酸之间的星号标记指示硫代磷酸酯键的存在。实施例2中的试验所需的寡核苷酸可以采用亚磷酰胺单体(所有活性基团用保护基保护的正常核苷酸,例如糖上的三苯甲基、A和C上的苯甲酰基及G上的N-2-异丁酰基)使用任何合适的现有技术方法(例如IDT使用的方法)在固体载体如5微米可控微孔玻璃珠(CPG)上合成。保护基防止寡核苷酸合成过程中不希望的反应。保护基在合成过程结束时除去。初始核苷酸通过3'碳连接到固体载体上且合成沿3'-5'方向进行。新碱基添加到生长的寡核苷酸链上以四个步骤发生:1)保护基使用三氯乙酸从固定的核苷酸的5'氧上除去;2)固定的核苷酸和序列中下一个核苷酸使用四唑偶联在一起;反应通过四唑基亚磷酰胺中间体进行;3)洗掉未反应的游离核苷酸和反应副产物且未反应的固定寡核苷酸加帽以防止它们参与下一轮的合成;加帽使用乙酸酐和N-甲基咪唑通过使游离的5'羟基乙酰化而实现;4)为使核苷酸之间的键稳定,如果要产生磷酸二酯键,磷使用碘和水氧化,或者如果希望硫代磷酸酯键,磷使用Beaucage试剂(3H-1,2-苯并二硫醇-3-酮-1,1-二氧化物)氧化。通过交替两种氧化剂,可以构建嵌合骨架。以上描述的四步骤循环对于序列中的每一核苷酸重复。当合成完整的序列时,寡核苷酸从固体载体切除并在高温下使用氢氧化铵脱保护。保护基通过脱盐洗掉且残留的寡核苷酸进行冻干。

[0267] Hek293细胞用不同的siRNA处理以定量BDNF mRNA的量

[0268] 1.来自ATCC的Hek293细胞(cat#CRL-1573)在37℃和5% $CO_2$下在MEM/EBSS(Hyclone cat#SH30024)+10% FBS+青霉素+链霉素中生长。试验前一天,细胞以$5x10^5$/孔的密度重接种到6孔板中并在37℃和5% $CO_2$下孵育。

[0269] 2.在试验这一天,6孔板中的培养基更换为新鲜MEM/EBSS+10% FBS。

[0270] 3.所有BDNF-AntagoNAT(BDNF-AS的反义寡核苷酸)稀释到20uM的浓度且

BDNF-AS siRNA(BDNF-AS 互补的 siRNA,10uM;两种寡核苷酸化合物通过 IDT 制造。为对一个孔加药,2μl 的这一溶液与 400μl 的 Opti-MEM 培养基 (Gibco cat#31985-070) 和 4ul 的 Lipofectamine 2000(Invitrogen cat#11668019) 在室温下孵育 20min 并逐滴施加到具有 HepG2 细胞的 6 孔板的一个孔。包括 2μl 的水而不是寡核苷酸溶液的类似混合物用于模拟转染的对照。

[0271] 4. 在 37℃和 5% $CO_2$下孵育 3-18h 后,培养基更换为新鲜 MEM/EBSS+10% FBS+ 青霉素 + 链霉素。

[0272] 5.48h 后进行反义寡核苷酸的添加。培养基然后移除且 RNA 使用来自 Promega 的 SV Total RNA Isolation System(cat#Z3105) 或来自 Qiagen 的 RNeasy Total RNA Isolation 试剂盒 (cat#74181) 按照制造商的说明从细胞提取。

[0273] 6.200-400ng 的提取 RNA 添加到使用无规六聚体、2.5mM 的 dNTP 混合物、$MgCl_2$和适宜的缓冲液进行的反转录反应中。来自这一反转录反应的 cDNA(20-40ng) 用于使用 ABI Taqman Gene Expression Mix(cat#4369510) 和 300nM 的正向和反向引物及 15μl 最终反应体积中的 200nM 探针通过实时 PCR 监测基因表达。引物 / 探针使用 FileBuilder 软件 (Applied Biosystem) 设计。引物是对有义 - 反义对特异性的链且探针覆盖外显子边界以排除基因组 DNA 扩增的可能。用于人 BDNF 的 ABI 分析是 Applied Biosystems Taqman Gene Expression Assay:Applied Biosystems Inc.,Foster City CA 的 Hs00542425_s1(BDNF)。使用以下的 PCR 循环:50℃下 2min,95℃下 10min,40 个循环的 (95℃下 15 秒,60℃下 1min),使用 GeneAmp 7900 仪器 (Applied Biosystems)。反义寡核苷酸处理后基因表达的倍数变化基于处理的和模拟转染的样品之间 18S- 标准化的 dCt 值的差异计算。

[0274] 7.BDNF-AS 的检测寡聚物:

[0275] ABI 分析 ID Hs00417345_m1

[0276] 背景序列 GCACACCTGGAGATACTCTATTATA(SEQ ID No:65)

[0277] 8.BDNF 的检测寡聚物:

[0278] ABI 分析 ID Hs00542425_s1

[0279] CCTGCAGAATGGCCTGGAATTACAA(SEQ ID No:66)

[0280] BDNF-AS 的检测寡聚物:ABI 分析 ID Hs00417345_m1

[0281] 背景序列 GCACACCTGGAGATACTCTATTATA(SEQ ID No:65)

[0282] BDNF 的检测寡聚物:ABI 分析 ID Hs00542425_s1

[0283] CCTGCAGAATGGCCTGGAATTACAA(SEQ ID No:66)

[0284] 9. 结果是基于循环阈 (Ct) 值。实验和参照基因 (18S RNA) 的 Ct 值之间的计算差异为 ddCt 且作为各 RNA 对校准样品的百分比作图。

[0285] 结果:几种人和小鼠细胞系 ( 包括 HEK293T 细胞 ) 用靶向 BDNF-AS 转录物的非重叠区域的不同 siRNA 转染在 48h 时显示 BDNF 转录物的 2-6 倍上调 ( 图 1a 和图 6)。BDNF 的上调与内源对照的选择不相关 ( 图 5a-b)。上调不影响其它 BDNF 相邻基因的调节 ( 图 9)。

[0286] 图 5 显示 BDNF-AS 敲减导致 BDNF mRNA 上调。BDNF-AS 使用靶向 BDNF-AS 转录物的非重叠区域的 siRNAs-1(10nM) 敲减引起 BDNF( 有义 )mRNA 的 6- 倍上调 (**** = P<0.0001)。此处描述的结果使用 β 肌动蛋白 ( 左图 ) 或 18S rRNA( 右图 ) 作为内源对照和使用模拟转染作为参照样品从 HEK293T 细胞的试验获得。这一试验意在显示内源对照或

参照校准样品的选择不改变所观察的 BDNF mRNA 的上调。

[0287] 图 6 显示 Bdnf 表达的转录后调节。N2a 细胞用靶向小鼠 Bdnf-AS 转录物的 mBdnf-AntagoNAT9 和靶向 Drosha 蛋白（其参与微 RNA(miRNA) 处理）的 Drosha siRNA 的组合转染。Bdnf mRNA 上调在用 mBdnf-ANtagoNAT9 处理细胞后观察到 (*** = p 值 <0.0001)。添加 Drosha siRNA 相对于 mBdnf-AntagoNAT9 处理边缘地增加 Bdnf 转录物 (* = p 值 <0.05)。这一试验可以表明其它转录后机制如 miRNA 参与 Bdnf 转录物的调节。

[0288] 图 9 显示 BDNF-AS 敲减不改变 TrkB 或沿两个方向的 BDNF 相邻基因 (Let7C 和 KIF18A) 的水平 :LIN7C 和 KIF18A 分别是位于 BDNF 的 3’下游和 5’上游的基因。神经营养酪氨酸激酶受体 2 型 (TrkB) 编码 BDNF 的膜结合受体且作为 BDNF 位于不同染色体 (Chr-9) 上。确定了这些基因是否在 BDNF-AS 转录物删除时改变。HEK293T 细胞用对照 siRNA 或 BDNF-AS siRNA 转染并测量几种转录物水平。观察到 BDNF-AS 转录物下调且 BDNF mRNA 如这一手稿 (manuscript) 中其它地方所示的上调。据发现 BDNF AS 的敲减对 TrkB 表达或对相邻基因 Let7C 和 KIF18A 没有影响。这些数据表明在 BDNF-AS 删除时，BDNF 表达存在基因座特异性的改变。

[0289] Hek293 细胞在 0-96h 的时程中用一种 siRNA 处理以定量 BDNF 和 BDNF-AS 的量

[0290] 以下的方法与 Hek293 细胞用 siRNA 处理相同，但此次细胞在添加寡聚物后 0-96h 时收获。

[0291] 结果 :BDNF 和 BDNF-AS 表达的时程显示在 48h 时与 BDNF-AS 的最佳下调同时的由 siRNA 导致的 BDNF 的最佳上调（图 1b)。

[0292] Hek293 细胞用不同 hBDNF-AntagoNAT 处理以定量 BDNF 和 BDNF-AS 的量

[0293] 以下的方法与 Hek293 细胞用 siRNA 处理相同，但此次细胞用 AntagoNAT 处理。

[0294] 结果 :BDNF-AS 转录物包含 225- 核苷酸的重叠区域，其具有与 BDNF mRNA 的完全互补性。RNA-RNA 相互作用可能通过其反义转录物负责 BDNF 的不调和调节。为确定 BDNF-AS 对 BDNF mRNA 的调节作用，包含 LNA 和 2’OMe RNA 修饰两者的间体 (AntagoNAT) 用于阻断有义和反义转录物之间的相互作用。重叠区域被铺砌的 hBDNF-AatagoNAT 覆盖。据发现 hBDNF-AntagoNAT 的使用上调 BDNF mRNA。观察到 BDNF-AS 转录物的边缘下调，其对于包含阻断寡聚物的 2’OMe-RNA 未预料到。测试了 16 个 hBDNF-AntagoNAT 并发现阻断 BDNF-AS 重叠区域的前一半对于 BDNF mRNA 的上调具有更大的作用。具体地，hBDNF-AntagoNAT1 和 hBDNFAntagoNAT4 引起 BDNF mRNA 的显著上调。与合成 siRNA 不同，反义寡核苷酸是单链的且长度上可以更短 ;因此，降低了非特异性（脱靶）结合效应。单链的锁核酸 (LNA)- 修饰的寡核苷酸与未修饰的 siRNA 相比在体内一般是更有效的（图 7)。

[0295] 小鼠 N2a 细胞用不同 mBDNF-AntagoNAT 处理以定量 BDNF 和 BDNF-AS 的量

[0296] 以下的方法与用不同 hBDNF-AntagoNAT 处理 Hek293 细胞以定量 BDNF 和 BDNF-AS 的量相同，但这次细胞是 N2a 细胞。此外，使用以下 PCR 循环 :50℃下 2min，95℃下 10min，50 个循环的 (95℃下 15 秒，60℃下 1min)，使用 GeneAmp 7900 仪器 (Applied Biosystems)。

[0297] 结果 :图 8 显示 N2a 细胞中小鼠 Bdnf-AS 转录物被 AntagoNAT 抑制 :人 BDNF 有义和反义转录物之间重叠区域的阻断上调 BDNF mRNA 水平。然后确定是否相似的调控机制存在于小鼠细胞系中且测试靶向于小鼠 Bdnf-AS 转录物的 11 个 mBdnf-AntagoNAT。mBdnf-AntagoNAT 包含硫代磷酸酯骨架和在 3’和 5’两个末端处的三个 LNA- 修饰的核苷

酸。对照寡核苷酸具有相似的骨架和修饰，但不靶向哺乳动物基因组中的任何序列。两个mBdnf-AntagoNAT(mBdnf-AntagoNA3 和 mBdnf-AntagoNAT-9) 能够提高 N2a 细胞中的 Bdnf mRNA 水平。总之，用单链 AntagoNAT(16 聚体）阻断小鼠 Bdnf-AS 转录物引起小鼠 N2a 细胞中 Bdnf mRNA 水平的上调。这些数据表明 Bdnf 的反义转录物对 Bdnf mRNA 发挥抑制性作用。

[0298] Hek293 细胞用不同 siRNA 处理以定量 BDNF 蛋白

[0299] 以下方法与用不同 siRNA 处理 Hek293 细胞以定量 BDNF mRNA 的量相同，除了在步骤 5 中 48h 后添加 siRNA。然后移除培养基且破坏细胞和通过 ELISA（图 1c) 和蛋白质印迹（图 1d) 定量其 BDNF 蛋白的水平。

[0300] 蛋白质印迹 :HEK293T 细胞用 10nM 的 BDNF-AS 或对照 siRNA 转染。在转染后 48h 用 200μl 的包含 350mM DTT 的 Laemmli 样品缓冲液 (Biorad) 破坏细胞。20μl 的溶解产物在 10％ SDS PAGE 上分离并将其转移到硝基纤维素膜上过夜。然后膜与 MecP2 的初级抗体 (Abcam)、BDNF(Promega, 目录号 G164B) 和与 HRP 偶联的二级抗体孵育。在添加 HRP 底物后，化学发光信号用 X- 射线薄膜检测。剥离相同的膜且将其重新用于检测作为加样对照的 β - 肌动蛋白。

[0301] ELISA :细胞用 20nM 的 BDNF-AS siRNA 或对照 siRNA 转染。收集细胞上清液用于 ELISA 实验。或者，从埋置在蛋白质提取缓冲液 + 蛋白酶抑制剂 (BCA 试剂盒，Fisher) 中并用生物破碎器 (bioruptor) 和金属珠匀浆的小鼠脑组织提取总蛋白质。总蛋白质使用 BCA 蛋白质分析试剂盒 (Pierce 目录号 23227) 测量且样品加载量相对于总蛋白质浓度标准化。ELISA 试剂盒对于人 BDNF 从 Promega（目录号 G7611) 购得或对于小鼠 Bdnf 从 Millipore（目录号 CYT306) 购得，且按照供应商的方案进行 ELISA。从背景减去 450nm 下三次重复的平均吸光度并使其相对于对照样品标准化。

[0302] Hek293 细胞（不确定）用不同浓度的 mBDNF-AntagoNAT9 处理以定量 BDNF mRNA

[0303] 以下方法与 Hek293 细胞用不同 siRNA 处理以定量 BDNF mRNA 的量相同，除了步骤 3 中所有 mBDNF-AntagoNAT9 稀释到不同浓度，如最终的 11 个不同浓度使用与 Hek293 细胞用不同 siRNA 处理以定量 BDNF mRNA 的量相同比例量的 Lipofectamine 2000(Invitrogen cat#11668019) 施用于细胞（从 300nM 到 5pM 范围的 1:3 系列稀释），在相同体积的 Opti-MEM 培养基 (Gibco cat#31985-070) 中使用。这在室温下进行 20min 并逐滴施用于具有 HepG2 细胞的 6 孔板的一个孔。包括水而不是寡核苷酸溶液的相似混合物用于模拟转染的对照。

[0304] 结果 :如图 1e 中所示，当 BDNF-AS 被 mBDNF-AntagoNAT9 靶向时，存在 BDNF 的剂量依赖性的上调。

[0305] 图 1 显示有义 mRNA 和蛋白质的反义介导的调节。(A) 脑源神经营养因子 (BDNF) 天然反义转录物 BDNF-AS 在 HEK293T 细胞（每处理 n = 12) 用靶向于 BDNF-AS 转录物的非重叠区域的三个独特 siRNA 中的各 siRNA(10nM) 敲减引起 BDNF（有义）mRNA 的 2-6 倍上调（对于各数据点 n = 6/ 处理，*** = P<0.001，** = P<0.01)。类似的结果从使用人皮层神经元 (HCN)、成胶质细胞瘤 (MK059) 细胞、小鼠 N2a 细胞和神经球（数据未显示）的实验获得。错配序列、模拟转染和对照 siRNA 用作对照。用于这一实验和其它实验的对照 siRNA 是不靶向哺乳动物基因组中任何已知序列的惰性 siRNA(CCUCUCCACGCGCAGUACATT)。所有测

量值相对于 18S rRNA 标准化并作为各 mRNA 相对于阴性 siRNA 对照样品的百分比作图。

[0306] (B)BDNF 和 BDNF-AS 转录物的变化在 BDNF-AS 敲减后的一段时间内评估（对于各数据点 n = 6/ 处理）。人 BDNF-AS 的 siRNA 敲减导致在 6h 时开始并持续到 72h 的有效和一致的 BDNF-AS 下调。BDNF mRNA 水平在 18h 时上升，保持高水平超过 72h，96h 时逆转到处理前水平。注意到 48h 时的峰值是一致的和可再现的。虽然 BDNF-AS 敲减在 6h 后开始，BDNF 的上调在处理后 18h 开始。BDNF-AS 的耗竭和 BDNF mRNA 的增加之间的这种时间延迟显示事件的序列顺序，其指示细胞需要时间在上调 BDNF 之前适应于反义转录物的去除。

[0307] (C)siRNA- 介导的 BDNF-AS 转录物敲减引起通过 ELISA 测量的 BDNF 蛋白质水平的提高。细胞用 10nM 的两种 BDNF-AS 的活性 siRNA、乱序 siRNA 或对照 siRNA 转染 48 小时。这些细胞的上清液浓缩并使用商购可得的试剂盒通过 ELISA 分析 BDNF 蛋白。BDNF 蛋白利用靶向于 BDNF-AS 转录物的 siRNA 显著提高 (n = 6/ 处理，*** = P<0.0001，** = P<0.001)。

[0308] (D) 蛋白质印迹确认非蛋白质编码的 BDNF-AS 用 BDNF-AS siRNA1 敲减提高 BDNF 蛋白水平但不改变 β- 肌动蛋白的水平，但不是对照非靶向 siRNA 转录物。总起来说，这些数据表明在有义和反义 BDNF 转录物之间存在不调和的关系，其中 BDNF-AS 抑制 BDNF mRNA 和蛋白质的表达。通过 BDNF-AS 敲减去除这一负调控作用引起 BDNF mRNA 和蛋白质水平的上调。

[0309] (E)Bdnf-AS 删除后 Bdnf 的剂量依赖性提高：使用 11 种不同浓度 (1:3 系列稀释，范围 300nM-5pM) 的 mBdnf-AntagoNAT9(n = 6/ 数据点 / 处理）进行剂量反应试验且在 1-300nM 浓度下到观察到 Bdnf mRNA 水平的剂量依赖性的提高，EC50 为 6.6nM。

[0310] 海马神经球用 siRNA 处理

[0311] 切开神经球中的小鼠海马神经干细胞：神经干细胞从小鼠幼仔 (P0-P1) 的海马分离。海马机械分离成单细胞，通过短时旋转收集并在 DMEM 和 F12 的混合物（包含谷氨酰胺、抗生素、B27 溶液及 0.001mM 浓度的 EGF 和 FGF 两者）中生长。3-4 天后，形成漂浮神经球。100,000 个细胞接种在聚 L- 赖氨酸 (PLL) 涂覆的 24- 孔板中。神经球细胞接种到 PLL 上将开始分化过程。在接种后第三天，从培养基移除生长因子并允许细胞再生长 4 天（接种后 7 天）。此时，细胞培养物具有由星形胶质细胞、神经元、少突细胞及其祖细胞组成的神经细胞谱系的混合物，使得其与成熟脑组织更相似。Bdnf 和 Bdnf-AS 的表达在漂浮神经球中以及在接种后 3 和 7 的培养物中测量。在接种后 3 或 7 天使用靶向 Bdnf-AS 转录物的 50nM siRNA 或 20nM 反义寡核苷酸进行敲减实验。神经干细胞也接种在总体积 80 μ l 的免疫细胞化学室中 (18,000 细胞 / 孔）。然后神经球使用相同的方案转染以评估 Bdnf-AS 敲减对鼠原代细胞的功能效应。在 48-72h 后，细胞用低聚甲醛 (4％）固定 20min 并用 1X PBS 洗涤几次。在用 FBS 封闭后，神经球与 1:2000 浓度的一级抗体（单克隆兔 β 微管蛋白 III,TUJ1) 孵育过夜。固定的细胞与 Alexafluor 568 标记的二级抗体（山羊抗兔 IgG，2mg/ml，浓度 1:5000) 孵育。细胞核用 Hoechst 染色剂染色。通过免疫荧光抗原检测显微术获得图像。

[0312] AntagoNAT 靶向于 BDNF-AS：

[0313] 术语 AntagoNAT 在本文中用于描述抑制有义 - 反义相互作用的单链寡核苷酸分子（具有不同修饰，参见补充方法）。设计了单链间体，寡核苷酸，长度 14 个核苷酸，具有 2’O- 甲基 RNA 和 / 或锁核酸 (LNA) 修饰。采用这一策略，我们铺砌了人 BDNF-AS 和 BDNF

转录物之间的整个重叠区域并鉴定了几种能够上调 BDNF mRNA 的有效 AntagoNAT。靶向于重叠区域的第一部分的 hBDNF-AntagoNAT1 和 hBDNF-AntagoNAT4 产生最大的反应。数据表明 BDNF 反义 RNA 通过单链 AntagoNAT 阻断足以引起 BDNF mRNA 的增加。

[0314] 然后设计单链间体，LNA- 修饰的 15 个 DNA 寡核苷酸 (AntagoNAT)，长度 16 核苷酸，具有硫代磷酸酯骨架，与小鼠 Bdnf-AS 互补。两种 AntagoNAT(mBdnf-AntagoNAT3 和 mBdnf-AntagoNAT9) 始终显示在小鼠 N2a 细胞中 Bdnf mRNA 水平的统计学显著的提高（图 7）。

[0315] 图 7 显示 hBDNFAntagoNAT 对人 BDNF-AS 转录物的抑制 :BDNF-AS 转录物包含 225- 核苷酸的重叠区域，其具有与 BDNF mRNA 的完全互补性。RNA-RNA 相互作用可能负责其反义转录物对 BDNF 的不调和调节。为确定 BDNF-AS 对 BDNF mRNA 的调节作用，包含 LNA 和 2’ OMe RNA 修饰两者的间体 (AntagoNAT) 用于阻断有义和反义转录物之间的相互作用。重叠区域通过铺砌 hBDNF-AatagoNAT 覆盖。据发现 hBDNF-AntagoNAT 的使用上调 BDNF mRNA。观察到 BDNF-AS 转录物的边缘下调，这对于含阻断寡聚物的 2’ OMe-RNA 是没有预期的。16 个 hBDNF-AntagoNAT（各具有以下提供的序列的 14 聚体）进行测试且发现阻断 BDNF-AS 重叠区域的前一半对于 BDNF mRNA 上调具有更大作用。特别地，hBDNF-AntagoNAT1 和 hBDNFAntagoNAT4 导致 BDNF mRNA 的显著上调。与合成 siRNA 不同，反义寡核苷酸是单链的且长度可以更短 ;因此，降低非特异性（脱靶）结合效应。单链的锁核酸 (LNA)- 修饰的寡核苷酸与未修饰的 siRNA 相比在体内一般是更有效的。Bdnf 上调增加神经元外生长。

[0316] Bdnf 上调增加神经元外生长 :

[0317] 与表明 Bdnf 对神经元外生长和成人神经发生的刺激作用的许多先前的报告 16-17 一致，发现由于 Bdnf-AS 转录物的敲减造成的内源 Bdnf 水平的提高导致提高的神经元细胞数目及导致神经突外生长和神经球中接种后 3 和 7 天的成熟（图 5a-d)。这些数据表明由于反义 RNA 的抑制造成的内源 Bdnf 上调诱导神经元祖细胞的神经元分化且可以引起新生神经元的成熟表型。

[0318] 结果 :图 2 显示 Bdnf 上调增加神经元外生长。(A-B) 接种后 3d 用对照 siRNA(A) 或 Bdnf-AS siRNA(B) 处理的海马神经球的免疫细胞化学图像。(C-D) 接种后 7d 用对照 siRNA(C) 或 Bdnf-AS siRNA(D) 处理的海马神经球中神经元成熟和神经突外生长的免疫细胞化学图像。细胞用靶向 Bdnf-AS 转录物的 siRNA 处理在接种后 3d 或 7d 神经球中都导致提高的神经元细胞数目以及神经突外生长和成熟的增加。β - 微管蛋白 III 染成红色，GFAP 染成绿色和 DAPI 染成蓝色。

[0319] 使用渗透压微型泵脑室内 (ICV) 递送 mBDNF-AntagoNAT9 敲减 BDNF-AS 和上调 BDNF

[0320] 小鼠研究 :10 只八周龄雄性 C57BL/6 小鼠用于体内试验。小鼠用背侧第三室中的长期植入插管制备，皮下植入以 1.5mg/kg/d 的剂量连续输注 (0.11 微升 /h) 递送针对 Bdnf-AS 的合成反义寡核苷酸 (mBdnf-AntagoNAT9) 或对照寡核苷酸（不存在于人或小鼠中的惰性序列）4 周的渗透压微型泵。管道与渗透压微型泵的出口连接并皮下通向植入的插管，以使得治疗直接递送到脑中。在植入后 5d，所有动物每日接受 BrdU(80mg/kg) 的腹膜内 (IP) 注射，连续五天。在手术后第 28 天，处死动物并从各小鼠脑切除三种组织（海马、额皮质和小脑）用于定量 RNA 测量。

[0321] Bdnf-AS 的敲减在体内提高 Bdnf：

[0322] 利用用于脑室内 (ICV) 递送 mBdnf-AntagoNAT9 到 C57BL/6 小鼠的渗透压微型泵。然后基于在体内增加 Bdnf mRNA 的高效力选择靶向于小鼠 Bdnf-AS 的非重叠区域的 mBdnf-AntagoNAT9，其优于其它活性 AntagoNAT。在连续 AntagoNAT 输注的 28 天后，在用 mBdnf-AntagoNAT9 处理的小鼠中 Bdnf mRNA 水平与惰性对照寡核苷酸的未改变水平相比在邻近第三脑室的前脑区域中提高（图 3a,b）。Bdnf 和 Bdnf-AS 转录物在下丘脑（不与第三脑室直接相邻的结构）中未改变（图 3c）。而且，发现 AntagoNAT- 介导的 Bdnf-AS 阻断导致提高的 Bdnf 蛋白质水平（图 3d,e）。这些发现与以上描述的体外数据对应且表明 Bdnf-AS 的阻断导致体内 Bdnf mRNA 和蛋白质表达的增加。

[0323] 小鼠脑样品的 RNA 提取和 RT-PCR：小鼠在 28 天后处死且切除脑。各小鼠的半个脑在 4％甲醛中固定过夜用于组织学研究。另一半的脑切碎用于海马、额皮质和小脑的 RNA 定量测量。RNA 按照制造商的方案在 Trizol 试剂 (Invitrogen,15596-026) 中匀浆后提取。分离水性相并在使样品通过 Qiagen RNeasy 柱 (QIAGEN,74106) 之前添加等体积的 70％乙醇，且这些 RNA 样品进行柱上 DNA 酶处理以除去 DNA 污染。400ng 的各样品用于第一链 cDNA 合成并进行 RT-PCR 测量。在各个图形中对于单个组织的 RNA 水平相对于对照小鼠的百分变化作图。

[0324] 结果：图 3 显示 Bdnf-AS 体内调节 Bdnf mRNA 和蛋白质；(A-C) 使用渗透压微型泵，mBdnf-AntagoNAT9(CAACATATCAGGAGCC) 或对照寡核苷酸 (CCACGCGCAGTACATG) 在 28d 的时间内恒定地输注到小鼠脑的第三脑室中 (n ＝ 5/ 处理组，* ＝ P<0.05,** ＝ P<0.01,*** ＝ P<0.001)。针对 Bdnf-AS 的 mBdnf-AntagoNAT9 导致海马 (A) 和额皮质 (B) 中 Bdnf 水平的提高，但对照寡核苷酸没有。在下丘脑 (C) 中，两种转录物未改变，如对于与脑的第三脑室不直接连接的组织所预期的。(D-E)BDNF 蛋白质水平通过 ELISA 评估且发现 mBdnf-AntagoNAT9 处理导致在海马 (D) 和额皮质 (E) 中与对照寡核苷酸处理的小鼠相比 BDNF 蛋白质的增加。

[0325] 使用渗透压微型泵脑室内 (ICV) 递送 mBDNF-AntagoNAT9 敲减 BDNF-AS 和上调 BDNF

[0326] BrdU 在研究的第一周注射到用 mBdnf-AntagoNAT9 处理的小鼠中，持续 5 天。在连续 AntagoNAT 输注的 28 天后，进行脑组织的组织学检验并分别使用 Ki67 和 BrdU 标志物定量神经元的增殖和存活。在用 mBdnf-AntagoNAT9 处理的小鼠中，与对照处理小鼠相比观察到 Ki67 阳性（增殖）细胞的增加（图 4a,b）。定量 Ki67 阳性细胞的数量且在 mBdnf-AntagoNAT9 处理的小鼠中发现与对照寡核苷酸相比的细胞增殖的显著增加（图 4c）。在用 mBdnf-AntagoNAT9 处理的小鼠中，与对照寡核苷酸处理的小鼠相比存在 BrdU 掺入（存活的细胞）的显著增加（图 4d）。对照和 mBdnf-AntagoNAT9 处理小鼠之间没有海马体积的差异（图 4e）。这些发现表明 Bdnf-AS 在体内调节 Bdnf 水平。

[0327] 结果：图 4 显示 Bdnf-AS 体内阻断导致神经元存活和增殖的提高；(A-B) 小鼠用 mBdnf-AntagoNAT9 或对照寡聚物处理。在 mBdnf-AntagoNAT9 输注的 28d 后，使用 Ki67 进行脑组织的组织学检验。Ki67 是海马中增殖细胞的标志物且与接受对照寡聚物的小鼠相比，在接受 Bdnf-AntagoNAT 处理的小鼠中观察到增殖细胞数目的增加。在 mBdnf-AntagoNAT9 处理的小鼠中 (B)，与对照处理的小鼠 (A) 相比，存在 Ki67 阳性细胞（增殖细胞）的增加。

(C) 用 mBdnf-AntagoNAT9 处理的小鼠与对照处理的小鼠相比具有 Ki67 阳性细胞数目的显著增加。(D) 在用 mBdnf-AntagoNAT9 处理的小鼠中，与对照寡核苷酸处理的小鼠相比，存在存活细胞 (BrdU 阳性) 数目的显著增加。(E) 对照和 mBdnf-AntagoNAT9 处理的小鼠之间没有海马体积的差异。这些数据 (每处理组 n = 5，* = P<0.05，*** = P<0.001) 一起证明 Bdnf-AS 在体内调节 Bdnf 水平且阻断 Bdnf 有义 - 反义相互作用导致神经元谱系、增殖和存活的增加。

[0328] 尽管已就一个或多个实施方式举例说明并描述本发明，但在阅读和理解本说明书和附图后，本领域技术人员将会想到等价改变和修饰。此外，虽然本发明的具体特征可能仅就几个实施方式中的一个公开，但这类特征可与其他实施方式的一个或多个其他特征组合，如对于任何给定或具体应用而言可为所需和有利的。

[0329] 本公开内容的摘要将允许读者快速确定本技术公开内容的性质。在理解以下的情况下将其提出：其将不用于解释或限制随附权利要求的范围或含义。

[0001]

序列表

<110> CURNA, INC.

<110> THE SCRIPPS RESEARCH INSTITUTE

<120> 通过脑源神经营养因子(BDNF)的天然反义转录物治疗 BDNF 相关疾病

<130> US61/614,664

<150> US61/614,664
<151> 2012-03-23

<160> 66

<170> PatentIn version 3.5

<210> 1
<211> 4755
<212> DNA
<213> 智人

<400> 1
cacacacaca cacacacaca gagagaacat ctctagtaaa aagaaaagtt gagctttctt 60

agctagatgt gtgtattagc cagaaaaagc caaggagtga agggttttag agaactggag 120

gagataaagt ggagtctgca tatgggaggc atttgaaatg gacttaaatg tctttttaat 180

gctgactttt tcagttttct ccttaccaga cacattgttt tcatgacatt agccccaggc 240

atagacacat cattaaaatg aacatgtcaa aaaatgattt ctgtttagaa ataagcaaaa 300

cattttcagt tgtgaccacc caggtgtaga ataaagaaca gtggaattgg gagccctgag 360

ttctaacata aactttcttc atgacataag gcaagtcttc tatggccttt ggtttcctta 420

cctgtaaaac aggatggctc aatgaaatta tctttcttct ttgctataat agagtatctc 480

tgtgggaaga ggaaaaaaaa agtcaattta aaggctcctt atagttcccc aactgctgtt 540

ttattgtgct attcatgcct agacatcaca tagctagaaa ggcccatcag acccctcagg 600

ccactgctgt tcctgtcaca cattcctgca aaggaccatg ttgctaactt gaaaaaaatt 660

[0002]

actattaatt acacttgcag ttgttgctta gtaacattta tgattttgtg tttctcgtga 720

cagcatgagc agagatcatt aaaaattaaa cttacaaagc tgctaaagtg ggaagaagga 780

gaacttgaag ccacaatttt tgcacttgct tagaagccat ctaatctcag gttatatgct 840

agatcttggg ggcaaacact gcatgtctct ggtttatatt aaaccacata cagcacacta 900

ctgacactga tttgtgtctg gtgcagctgg agtttatcac caagacataa aaaaaccttg 960

accctgcaga atggcctgga attacaatca gatgggccac atggcatccc ggtgaaagaa 1020

agccctaacc agttttctgt cttgtttctg ctttctccct acagttccac caggtgagaa 1080

gagtgatgac catccttttc cttactatgg ttatttcata ctttggttgc atgaaggctg 1140

cccccatgaa agaagcaaac atccgaggac aaggtggctt ggcctaccca ggtgtgcgga 1200

cccatgggac tctggagagc gtgaatgggc ccaaggcagg ttcaagaggc ttgacatcat 1260

tggctgacac tttcgaacac gtgatagaag agctgttgga tgaggaccag aaagttcggc 1320

ccaatgaaga aaacaataag gacgcagact tgtacacgtc cagggtgatg ctcagtagtc 1380

aagtgccttt ggagcctcct cttctctttc tgctggagga atacaaaaat tacctagatg 1440

ctgcaaacat gtccatgagg gtccggcgcc actctgaccc tgcccgccga ggggagctga 1500

gcgtgtgtga cagtattagt gagtgggtaa cggcggcaga caaaaagact gcagtggaca 1560

tgtcgggcgg gacggtcaca gtccttgaaa aggtccctgt atcaaaaggc caactgaagc 1620

aatacttcta cgagaccaag tgcaatccca tgggttacac aaaagaaggc tgcaggggca 1680

tagacaaaag gcattggaac tcccagtgcc gaactaccca gtcgtacgtg cgggccctta 1740

ccatggatag caaaaagaga attggctggc gattcataag gatagacact tcttgtgtat 1800

gtacattgac cattaaaagg ggaagatagt ggatttatgt tgtatagatt agattatatt 1860

gagacaaaaa ttatctattt gtatatatac ataacagggt aaattattca gttaagaaaa 1920

aaataatttt atgaactgca tgtataaatg aagtttatac agtacagtgg ttctacaatc 1980

[0003]

tatttattgg acatgtccat gaccagaagg gaaacagtca tttgcgcaca acttaaaaag 2040

tctgcattac attccttgat aatgttgtgg tttgttgccg ttgccaagaa ctgaaaacat 2100

aaaaagttaa aaaaaataat aaattgcatg ctgctttaat tgtgaattga taataaactg 2160

tcctctttca gaaaacagaa aaaaacacac acacacacaa caaaaatttg aaccaaaaca 2220

ttccgtttac attttagaca gtaagtatct tcgttcttgt tagtactata tctgttttac 2280

tgcttttaac ttctgatagc gttggaatta aaacaatgtc aaggtgctgt tgtcattgct 2340

ttactggctt aggggatggg ggatgggggg tatatttttg tttgttttgt gttttttttt 2400

cgtttgtttg ttttgttttt tagttcccac agggagtaga gatggggaaa gaattcctac 2460

aatatatatt ctggctgata aaagatacat ttgtatgttg tgaagatgtt tgcaatatcg 2520

atcagatgac tagaaagtga ataaaaatta aggcaactga acaaaaaaat gctcacactc 2580

cacatcccgt gatgcacctc ccaggccccg ctcattcttt gggcgttggt cagagtaagc 2640

tgcttttgac ggaaggacct atgtttgctc agaacacatt ctttcccccc ctccccctct 2700

ggtctcctct ttgttttgtt ttaaggaaga aaaatcagtt gcgcgttctg aaatatttta 2760

ccactgctgt gaacaagtga acacattgtg tcacatcatg acactcgtat aagcatggag 2820

aacagtgatt ttttttaga acagaaaaca acaaaaaata accccaaaat gaagattatt 2880

ttttatgagg agtgaacatt tgggtaaatc atggctaagc ttaaaaaaaa ctcatggtga 2940

ggcttaacaa tgtcttgtaa gcaaaaggta gagccctgta tcaacccaga aacacctaga 3000

tcagaacagg aatccacatt gccagtgaca tgagactgaa cagccaaatg gaggctatgt 3060

ggagttggca ttgcatttac cggcagtgcg ggaggaattt ctgagtggcc atcccaaggt 3120

ctaggtggag gtggggcatg gtatttgaga cattccaaaa cgaaggcctc tgaaggaccc 3180

ttcagaggtg gctctggaat gacatgtgtc aagctgcttg gacctcgtgc tttaagtgcc 3240

tacattatct aactgtgctc aagaggttct cgactggagg accacactca agccgactta 3300

[0004]

tgcccaccat cccacctctg gataattttg cataaaattg gattagcctg gagcaggttg 3360

ggagccaaat gtggcatttg tgatcatgag attgatgcaa tgagatagaa gatgtttgct 3420

acctgaacac ttattgcttt gaaactagac ttgaggaaac cagggtttat cttttgagaa 3480

cttttggtaa gggaaaaggg aacaggaaaa gaaaccccaa actcaggccg aatgatcaag 3540

gggacccata ggaaatcttg tccagagaca agacttcggg aaggtgtctg gacattcaga 3600

acaccaagac ttgaaggtgc cttgctcaat ggaagaggcc aggacagagc tgacaaaatt 3660

ttgctcccca gtgaaggcca cagcaacctt ctgcccatcc tgtctgttca tggagagggt 3720

ccctgcctca cctctgccat tttgggttag gagaagtcaa gttgggagcc tgaaatagtg 3780

gttcttggaa aaatggatcc ccagtgaaaa ctagagctct aagcccattc agcccatttc 3840

acacctgaaa atgttagtga tcaccacttg gaccagcatc cttaagtatc agaaagcccc 3900

aagcaattgc tgcatcttag tagggtgagg gataagcaaa agaggatgtt caccataacc 3960

caggaatgaa gataccatca gcaaagaatt tcaatttgtt cagtctttca tttagagcta 4020

gtctttcaca gtaccatctg aatacctctt tgaaagaagg aagactttac gtagtgtaga 4080

tttgttttgt gttgtttgaa aatattatct ttgtaattat ttttaatatg taaggaatgc 4140

ttggaatatc tgctatatgt caactttatg cagcttcctt ttgagggaca aatttaaaac 4200

aaacaacccc ccatcacaaa cttaaaggat tgcaagggcc agatctgtta agtggtttca 4260

taggagacac atccagcaat tgtgtggtca gtggctcttt tacccaataa gatacatcac 4320

agtcacatgc ttgatggttt atgttgacct aagatttatt ttgttaaaat ctctctctgt 4380

tgtgttcgtt cttgttctgt tttgttttgt tttttaaagt cttgctgtgg tctctttgtg 4440

gcagaagtgt ttcatgcatg gcagcaggcc tgttgctttt ttatggcgat tcccattgaa 4500

aatgtaagta aatgtctgtg gccttgttct ctctatggta aagatattat tcaccatgta 4560

aaacaaaaaa caatatttat tgtattttag tatatttata taattatgtt attgaaaaaa 4620

[0005]

attggcatta aaacttaacc gcatcagaac ctattgtaaa tacaagttct atttaagtgt 4680

actaattaac atataatata tgttttaaat atagaatttt taatgttttt aaatatattt 4740

tcaaagtaca taaaa 4755

<210> 2
<211> 4302
<212> DNA
<213> 小鼠

<400> 2
taaagcagta gccggctggt gcagaaaagc aacaagttcc ccagcggtct tcccgcccta 60

gcttgacaag gcgaagggtt tcttacctgg cgacagggaa atctcctgag ccgagctcat 120

ctttgccaga gccccaggtg tgacctgagc agtgggcaaa ggatcggcgt gcaaattgga 180

ttatttttat gggggtactc tgaaactccc tcactttctc tgggaacttt ttgtgctagg 240

gctcagtgac aggcgttgag aaagctgctt caggaaacgc ccgctatata gcagggcaat 300

tggacagtca ttggtaacct cgctcattca ttagaatcac gtaagaactc aaagggaaac 360

gtgtctctca gaatgagggc gtttgcgtaa atctataggt ttttcaacat cgatgccagt 420

tgctttgtct tctgtagtcg ccaaggtgga tgagagttga agctttgcgg atattgcgaa 480

gggttattag attcataagt cacaccaagt ggtgggcgat ccactgagca aagccgaact 540

tctcacatga tgacttcaaa caagacacat taccttcctg catctgttgg ggagacaaga 600

ttttaagaca ctgagtctcc aggacagcaa agccacaatg ttccaccagg tgagaagagt 660

gatgaccatc cttttcctta ctatggttat ttcatacttc ggttgcatga aggcggcgcc 720

catgaaagaa gtaaacgtcc acggacaagg caacttggcc tacccaggtg tgcggaccca 780

tgggactctg gagagcgtga atgggcccag ggcaggttcg agaggtctga cgacgacatc 840

actggctgac acttttgagc acgtcatcga agagctgctg gatgaggacc agaaggttcg 900

[0006]

gcccaacgaa gaaaaccata aggacgcgga cttgtacact tcccgggtga tgctcagcag 960

tcaagtgcct ttggagcctc ctctactctt tctgctggag gaatacaaaa attacctgga 1020

tgccgcaaac atgtctatga gggttcggcg ccactccgac cctgcccgcc gtggggagct 1080

gagcgtgtgt gacagtatta gcgagtgggt cacagcggca gataaaaaga ctgcagtgga 1140

catgtctggc gggacggtca cagtcctaga gaaagtcccg gtatccaaag gccaactgaa 1200

gcagtatttc tacgagacca agtgtaatcc catgggttac accaaggaag gctgcagggg 1260

catagacaaa aggcactgga actcgcaatg ccgaactacc caatcgtatg ttcgggccct 1320

tactatggat agcaaaaaga gaattggctg gcgattcata aggatagaca cttcctgtgt 1380

atgtacactg accattaaaa ggggaagata gtggatttat gttgtataga ttatattgag 1440

acaaaattat ctatttgtat atatacataa cagggtaaat tattcagtta agaaaaaata 1500

attttatgaa ctgcatgtat aaatgaagtt tatacagtac agtggttcta caatctattt 1560

attggacata tccatgacct gaaaggaaac agtcatttgc gcacaacttt aaaagtctgc 1620

attacattcc tcgataatgt tgtggtttgt tgccgttgcc aagaattgaa aacaaaaagt 1680

ttaaaaaaaa taataataaa ttgcatgctg ctttaattgt gaattgataa taaactgtcc 1740

ctctttcaga aaacagatta aaaaaacaaa aaacaaaaaa aaaaaaacaa aaaacaaaaa 1800

caaaaattgg aaccaaaaca ttccgtttac attttagaca ctaagtatct tcgttcttgt 1860

tagtactctg ttttactgct ttcgacttct gatagcgttg gaattaaaac aatgtcaagg 1920

tgctgttgtc attgctttac tggcgtaagg gacggggaat gggaggggta gatttctgtt 1980

tgttttgtgt tttatttttgt ttgtttgttt gttttgtttt ttagttccac ccggagtagg 2040

gatggagaaa atttcttcac tatccattct ggttgataaa gcgttacatt tgtatgttgt 2100

aaagatgttt gcaaaatcca atcagatgac tggaaaacaa ataaaaatta aggcaactga 2160

ataaaatgct cacactccac tgcccatgat gtatctccct ggtccccctc agctcactct 2220

[0007]

tctggcatgg gtcagggaaa attgctttta ttggaaagac cagcatttgt tcaaagcata 2280

ctctttccct ccctcctccc attttggtcc cttctttttg ttttgtttta agaaagaaaa 2340

ttaagttgcg cgctttaaaa tattttacta ctgctacaaa cagatgaaca atgtgtgtca 2400

ttttatgaca ctcatggaaa acagtgattt ttttttaccc taaagaaaaa caaataaaaa 2460

taacccaaaa tattcttttt ttaaaaggca taaatattgg gtaaattgta atatggccta 2520

acagtgtttg cagataaaag ttattgtata cacccagata cttagataag agcagggatc 2580

cacactgcca ttgaaatagg actgaatggc cctgcggagg ctaagtggag ctgacatact 2640

atttcctggc agtgcaggag gaatttctga gtggccatcc taaggtctag gatggaggtg 2700

gggaatggta cttgagacat tcctaaagga aggctcggaa gcacccttca gagcaggctc 2760

tggaatgatg tgtcaagttg cttaggcctt ctgctttaag tgcctacatt acctaacagt 2820

gctcaagagg ttctcgattg gagaaccaca ctcaaatcca tttatagcct ccatcccatt 2880

tctaaataat tgtgtataaa gttggattaa cctggagcaa ctttggatcc aaatatggca 2940

cagcaataat gatattaatg cagcatgatg ggaaatgttt gctgtgaaga gaattgattt 3000

gctttgagct tagacttcag gaagcctagg ttttttattt ttttattttt gagacatttt 3060

ggtaaaagga aaaaaagaaa acaaacaaac aaacaaacaa aaccagaaaa agcatcaaaa 3120

ctcaggcaga atgagcaatg tctgaaaggg ctagaaaaac aagacatagc aaggtgcttt 3180

cactgtgaaa gagacaagaa cacaggagga aatattgctt cagtgaagag cacagacggc 3240

tcctgccaat ttattacaag agtcccgtct gtactttacc ctttggggtt agaagtcaag 3300

ttggaagcct gaatgaatgg acccaatgag aactagtgtt aagcccattt ccctagtcag 3360

gtttttttca agcgtgaatg tgttagtggt tactctcctg ggttcctgag catcagaaaa 3420

aaaaaaaaaa agaggcaaac aatcgcttca tcttaggagt ggaaaggaaa cagaagtgga 3480

cgtccgctgt gactcaggga gtgaagatac catcagcaaa tagtttcttt tttgttcatt 3540

[0008]

cgttcctttc gagttagcct gtcttttgga ataccactga atatgctgtt tttgaaagac 3600

ttcatgtagc atagattgtt ttgtgccgtt taccaaatta acctttgtca tcgtttttta 3660

acctattcag gaatgcttgg aatatctgct ctatgttaac tttttgcagc ttcattctga 3720

gagacattag tcaaacaaac aaaaggatcc ccatcacaat cttacagtac tgcaagggcc 3780

aggtctgtta atcggcttca caggagacat cagcaattgt gtggtcagtg gctggctctc 3840

ttacccacta agatacatca tagctacatg ttggtggttt atgttgacct gagatttatt 3900

tgttaaaatc tcttcttcgt ttctgttcgt tctggttctg ttctgttctg ttctgttctg 3960

ttttggtttt aaagtcttgc tgtggtctct tgttggcaga aatgttttat gcatggcagc 4020

aggcctgttg ctttttttata gtgattccca ttgaaactgt aagtaaatgt ctgtggcctt 4080

gttctctcta tggtaaagat attattcacc atgtaaaaca agaaaaaata tttattgtat 4140

tttagtatat ttatataatt atgttattga aaaaattggc attaaaactt aaccacatca 4200

gaagcctatt gtaaatacag gttctattta agtgtaccaa ttaacatata atatatgttt 4260

taaatataga atttttaatg tttttaaata tattttcaaa gt 4302

<210> 3
<211> 1279
<212> DNA
<213> 智人

<400> 3
atcgcgagat caggaaggtg gccgagtgtg tcgccgcggc catcaggcac ttctccttcc 60

tgcccttgta tgaagaagga tgtgtttgct tcccccttgtg ccatgattgt aaatttcctg 120

aggcctcctc agccctgcag aactggctag agcaatgtat cttaggctca cttaaggaag 180

ctgtagagat gagcccaagg agggaaacca gaagagcccc ccaggctcac cagttgtttg 240

ttggctccct acaaacatgt cattcaagtg gctaatctta caacagcaca aattcatcta 300

accagaaaga gaagaggagg ctccaaaggc acttgactac tgagcatcac cctggacgtg 360

[0009]

tacaagtctg cgtccttatt gttttcttca ttgggccgaa ctttctggtc ctcatccaac 420

agctcttcta tcacgtgttc gaaagtgtca gccaatgatg tcaagcctct tgaacctgcc 480

ttgggcccat tcacgctctc cagagtccca tgggtccgca cacctggaga tactctatta 540

tagcaaagaa gaaagataat ttcattgagc catcctgttt tacaggattt tccctcctgg 600

tgagtcaaaa tgaacaagaa ataccccagg acctcccttc cctccttggc cattaatgag 660

atgaaggcaa ttaactcaca tagtataaat gaatcatttg aggtgatgac tgcattttag 720

gcaaatgatg actttcttgg ttccattggt ttgcaagtaa aagttacaca cattgaaaag 780

acactgaaac agatttccta aatgcttcat tttctggatg caccaatgtt gacctactat 840

acatgttaaa tggttttaaa atatcacctt aaaataaagg aaacttccag ctactaactc 900

agctctgaat gggctatgaa aggctccaaa ggtatgtgaa aaattactgt tattttgctt 960

taaaaaatgt gatgtctaag agtgtctgca atgttctaat gcttcaaaac atgtacgtaa 1020

gccttgttta tctggaaatc atttctttct gcttatatca tttataaata gaaaatgttc 1080

tgtaataact taaaatagtt ccacatacat aatgctttta gtgtcataat acttactact 1140

ggtctatatt taccaacatt tatcacattt tacaaaatga agtagaagaa aaaaaagaca 1200

acgactttat ggccctggaa ttccagtaat ggtgaccaac atgttttaaa ttccagtaaa 1260

ggttatggtt acatttcaa 1279

<210> 4
<211> 1478
<212> DNA
<213> 智人

<400> 4
atcgcgagat caggaaggtg gccgagtgtg tcgccgcggc catcaggcac ttctccttcc 60

tgcccttgta tgaagaagga tgtgtttgct tccccttgtg ccatgattgt aaatttcctg 120

[0010]

aggcctcctc agccctgcag aactggggtt atagccatgt gactgatctt cgtccaagaa 180

tatgtaaaga aaaagtgttg agttggcttt tagggctaga gcaatgtatc ttaggctcac 240

ttaaggaagc tgtagagatg agcccaagga gggaaaccag aagagccccc caggctcacc 300

agttgtttgt tggctccCta caaacatgtc attcaagtgg ctaatcttac aacagcacaa 360

attcatctaa ccagaaagag aagaggaggc tccaaaggca cttgactact gagcatcacc 420

ctggacgtgt acaagtctgc gtccttattg ttttcttcat tgggccgaac tttctggtcc 480

tcatccaaca gctcttctat cacgtgttcg aaagtgtcag ccaatgatgt caagcctctt 540

gaacctgcct tgggcccatt cacgctctcc agagtcccat gggtccgcac acctggagat 600

actctattat agcaaagaag aaagataatt tcattgagcc atcctgtttt acaggaaatt 660

ctgcaagtgg caacgtgggt ccattccgtg tgtgtcacta gagctggcgc aagcccatgg 720

ccatggtgag gcagcgtttc cactggaact aatctgatac ctgcaccagc tcttgcaact 780

gtgcagtgtt cccactgcaa actacggatg gggtaaaaga ctgctcacct cctatttctc 840

atctaatctc acacactctg tttgatgagg ctatggagaa acaggtcttc tcatacacta 900

aaggtgggag tacaaacaat tcaagccctg tgcaggacaa ttaggcaata cctatcaaaa 960

ttatacatga tttttcctgc tgacccagca attccacttc tgggaataat tgacagatat 1020

aggtgcatat gtacaaaatg atggaaagct ctctggtata tattagtaag tgataaaaca 1080

aggtgtaaaa tagtgtatat atggctacta ccttttgttt taaaaatggg ggaaaatggt 1140

ggagcttgcg gtgagccgag atcgtgccac tgcactccag cctgggcgac agagcgagac 1200

tccgtctcaa aaaaaaaaca gggtggggtg gggggaaat aatagtacat actcatattt 1260

acctgtatct atataaaaca cactatcaag gattcacaag aaactaatac aaatgatcac 1320

cttatagatg gtatgtattg ggggatactg aggtgagcag ggtataagtg gggcaagact 1380

tttcagtgta aacttctttt aaattttatt ttgatttttg aataatgtaa attaactgtc 1440

[0011]

aaataattaa attaaaaata accaatttat taacaaaa 1478

<210> 5
<211> 1437
<212> DNA
<213> 智人

<400> 5
atcgcgagat caggaaggtg gccgagtgtg tcgccgcggc catcaggcac ttctccttcc 60

tgcccttgta tgaagaagga tgtgtttgct tccccttgtg ccatgattgt aaatttcctg 120

aggcctcctc agccctgcag aactggctag agcaatgtat cttaggctca cttaaggaag 180

ctgtagagat gagcccaagg agggaaacca gaagagcccc ccaggctcac cagttgtttg 240

ttggctccct acaaacatgt cattcaagtg gctaatctta caacagcaca aattcatcta 300

accagaaaga gaagaggagg ctccaaaggc acttgactac tgagcatcac cctggacgtg 360

tacaagtctg cgtccttatt gttttcttca ttgggccgaa ctttctggtc ctcatccaac 420

agctcttcta tcacgtgttc gaaagtgtca gccaatgatg tcaagcctct tgaacctgcc 480

ttgggcccat tcacgctctc cagagtccca tgggtccgca cacctggaga tactctatta 540

tagcaaagaa gaaagataat ttcattgagc catcctgttt tacaggaaat tctgcaagtg 600

gcaacgtggg tccattccgt gtgtgtcact agagctggcg caagcccatg gccatggtga 660

ggcagcgttt ccactggaac taatctgata cctgcaccag ctcttgcaac tgtgcagtgt 720

tcccactgca aactacggat ggggattttc cctcctggtg agtcaaaatg aacaagaaat 780

accccaggac ctcccttccc tccttggcca ttaatgagat gaaggcaatt aactcacata 840

gtataaatga atcatttgag gtgatgactg catttaggc aaatgatgac tttcttggtt 900

ccattggttt gcaagtaaaa gttacacaca ttgaaaagac actgaaacag atttcctaaa 960

tgcttcattt tctggatgca ccaatgttga cctactatac atgttaaatg gttttaaaat 1020

atcaccttaa aataaaggaa acttccagct actaactcag ctctgaatgg gctatgaaag 1080

[0012]

gctccaaagg tatgtgaaaa attactgtta ttttgcttta aaaaatgtga tgtctaagag 1140

tgtctgcaat gttctaatgc ttcaaaacat gtacgtaagc cttgtttatc tggaaatcat 1200

ttctttctgc ttatatcatt tataaataga aaatgttctg taataactta aaatagttcc 1260

acatacataa tgcttttagt gtcataatac ttactactgg tctatattta ccaacattta 1320

tcacatttta caaaatgaag tagaagaaaa aaaagacaac gactttatgg ccctggaatt 1380

ccagtaatgg tgaccaacat gttttaaatt ccagtaaagg ttatggttac atttcaa 1437

<210> 6
<211> 2322
<212> DNA
<213> 智人

<400> 6
atcgcgagat caggaaggtg gccgagtgtg tcgccgcggc catcaggcac ttctccttcc 60

tgcccttgta tgaagaagga tgtgtttgct tccccttgtg ccatgattgt aaatttcctg 120

aggcctcctc agccctgcag aactggggtt atagccatgt gactgatctt cgtccaagaa 180

tatgtaaaga aaaagtgttg agttggcttt tagggctaga gcaatgtatc ttaggctcac 240

ttaaggaagc tgtagagatg agcccaagga gggaaaccag aagagccccc caggctcacc 300

agttgtttgt tggctcccta caaacatgtc attcaagtgg ctaatcttac aacagcacaa 360

attcatctaa ccagaaagag aagaggaggc tccaaaggca cttgactact gagcatcacc 420

ctggacgtgt acaagtctgc gtccttattg ttttcttcat tgggccgaac tttctggtcc 480

tcatccaaca gctcttctat cacgtgttcg aaagtgtcag ccaatgatgt caagcctctt 540

gaacctgcct tgggcccatt cacgctctcc agagtcccat gggtccgcac acctggagat 600

actctattat agcaaagaag aaagataatt tcattgagcc atcctgtttt acagtattga 660

attattacca caaggtacca accatatatg catacttaat agggtatttt gtcaaaacta 720

[0013]

tgcatgaagg tcatttgttt gagatgtcag aacattttcc cgtgagaaga tctcattggg 780

cattgaaaca gaaccacatg ctcttcagac cagcaaccgc gactaccaaa tactcctctg 840

tcaactctac ttgagtaaga acgctttcaa ttaaggccta agtgtcaaca tgcctttaaa 900

aaaaatcgtg gtgacacaaa atctttcttt ttagcaccca acagaatccc ttcaaagcct 960

cgtggtctga caccctatgc tacgtgactt gtgacccatc catttgtcat gttcttcggg 1020

aatgtggcta aggggctaag atgtgacttg aaaagaaagg tagaacaaga tcatctcaaa 1080

tttattatca aggaatagtt cagaaaacga cttcagacca cagagacagc agaacagatg 1140

gtccggcatg gatagagcat cagacactca cagactgtgc caacaagagc catcgagtca 1200

aaacagccaa aggaaggagg gtcatggaat gggttctctc acaccaaact gatgcccaga 1260

ggccctcagc atgaataaca aaggcaacca gacccacaag ccatactgag tggatacaaa 1320

acctatacct aggctgacat cccaaatgtg tgtggcaagt tagatgatga tggcacaaaa 1380

gacagaacac cttgcttctg gccattgtca gctcttggaa gagagcacac ttttagagga 1440

gcagctgcaa ggagcctgag aacaaaactg gaaatgtctg ttatgaaagc cttcacagga 1500

aattctgcaa gtggcaacgt gggtccattc cgtgtgtgtc actagagctg gcgcaagccc 1560

atggccatgg tgaggcagcg tttccactgg aactaatctg atacctgcac cagctcttgc 1620

aactgtgcag tgttcccact gcaaactacg gatggggtaa aagactgctc acctcctatt 1680

tctcatctaa tctcacacac tctgtttgat gaggctatgg agaaacaggt cttctcatac 1740

actaaaggtg ggagtacaaa caattcaagc cctgtgcagg acaattaggc aatacctatc 1800

aaaattatac atgatttttc ctgctgaccc agcaattcca cttctgggaa taattgacag 1860

atataggtgc atatgtacaa aatgatggaa agctctctgg tatatattag taagtgataa 1920

aacaaggtgt aaaatagtgt atatatggct actacctttt gttttaaaaa tgggggaaaa 1980

tggtggagct tgcggtgagc cgagatcgtg ccactgcact ccagcctggg cgacagagcg 2040

[0014]

agactccgtc tcaaaaaaaa aacagggtgg ggtgggggggg aaataatagt acatactcat 2100

atttacctgt atctatataa aacacactat caaggattca caagaaacta atacaaatga 2160

tcaccttata gatggtatgt attgggggat actgaggtga gcagggtata agtggggcaa 2220

gacttttcag tgtaaacttc ttttaaattt tattttgatt tttgaataat gtaaattaac 2280

tgtcaaataa ttaaattaaa aataaccaat ttattaacaa aa 2322

<210> 7
<211> 2036
<212> DNA
<213> 智人

<400> 7
atcgcgagat caggaaggtg gccgagtgtg tcgccgcggc catcaggcac ttctccttcc 60

tgcccttgta tgaagaagga tgtgtttgct tccccttgtg ccatgattgt aaatttcctg 120

aggcctcctc agccctgcag aactggctag agcaatgtat cttaggctca cttaaggaag 180

ctgtagagat gagcccaagg agggaaacca gaagagcccc ccaggctcac cagttgtttg 240

ttggctccct acaaacatgt cattcaagtg gctaatctta caacagcaca aattcatcta 300

accagaaaga gaagaggagg ctccaaaggc acttgactac tgagcatcac cctggacgtg 360

tacaagtctg cgtccttatt gttttcttca ttgggccgaa ctttctggtc ctcatccaac 420

agctcttcta tcacgtgttc gaaagtgtca gccaatgatg tcaagcctct tgaacctgcc 480

ttgggcccat tcacgctctc cagagtccca tgggtccgca cacctggaga tactctatta 540

tagcaaagaa gaaagataat ttcattgagc catcctgttt tacagcaccc aacagaatcc 600

cttcaaagcc tcgtggtctg acaccctatg ctacgtgact tgtgacccat ccatttgtca 660

tgttcttcgg gaatgtggct aaggggctaa gatgtgactt gaaaagaaag gtagaacaag 720

atcatctcaa atttattatc aaggaatagt tcagaaaacg acttcagacc acagagacag 780

cagaacagat ggtccggcat ggatagagca tcagacactc acagactgtg ccaacaagag 840

[0015]

ccatcgagtc aaaacagcca aaggaaggag ggtcatggaa tgggttctct cacaccaaac 900

tgatgcccag aggccctcag catgaataac aaaggcaacc agacccacaa gccatactga 960

gtggatacaa aacctatacc taggctgaca tcccaaatgt gtgtggcaag ttagatgatg 1020

atggcacaaa agacagaaca ccttgcttct ggccattgtc agctcttgga agagagcaca 1080

cttttagagg agcagctgca aggagcctga gaacaaaact ggaaatgtct gttatgaaag 1140

ccttcacagg aaattctgca agtggcaacg tgggtccatt ccgtgtgtgt cactagagct 1200

ggcgcaagcc catggccatg gtgaggcagc gtttccactg gaactaatct gatacctgca 1260

ccagctcttg caactgtgca gtgttcccac tgcaaactac ggatgggaga ggataaagaa 1320

cttcaatctt taaaaaagag aggattttcc ctcctggtga gtcaaaatga acaagaaata 1380

ccccaggacc tcccttccct ccttggccat taatgagatg aaggcaatta actcacatag 1440

tataaatgaa tcatttgagg tgatgactgc attttaggca aatgatgact ttcttggttc 1500

cattggtttg caagtaaaag ttacacacat tgaaaagaca ctgaaacaga tttcctaaat 1560

gcttcatttt ctggatgcac caatgttgac ctactataca tgttaaatgg ttttaaaata 1620

tcaccttaaa ataaaggaaa cttccagcta ctaactcagc tctgaatggg ctatgaaagg 1680

ctccaaaggt atgtgaaaaa ttactgttat tttgctttaa aaaatgtgat gtctaagagt 1740

gtctgcaatg ttctaatgct tcaaaacatg tacgtaagcc ttgtttatct ggaaatcatt 1800

tctttctgct tatatcattt ataaatagaa aatgttctgt aataacttaa aatagttcca 1860

catacataat gcttttagtg tcataatact tactactggt ctatatttac caacatttat 1920

cacattttac aaaatgaagt agaagaaaaa aaagacaacg actttatggc cctggaattc 1980

cagtaatggt gaccaacatg ttttaaattc cagtaaaggt tatggttaca tttcaa 2036

<210> 8

<211> 2364

[0016]

<212> DNA
<213> 智人

<400> 8
gtcatcgctg tctggaacag cgatgactcg atcgcgagat caggaaggtg gccgagtgtg 60

tcgccgcggc catcaggcac ttctccttcc tgcccttgta tgaagaagga tgtgtttgct 120

tcccccttgtg ccatgattgt aaatttcctg aggcctcctc agccctgcag aactggggtt 180

atagccatgt gactgatctt cgtccaagaa tatgtaaaga aaaagtgttg agttggcttt 240

tagggctaga gcaatgtatc ttaggctcac ttaaggaagc tgtagagatg agcccaagga 300

gggaaaccag aagagccccc caggctcacc agttgtttgt tggctcccta caaacatgtc 360

attcaagtgg ctaatcttac aacagcacaa attcatctaa ccagaaagag aagaggaggc 420

tccaaaggca cttgactact gagcatcacc ctggacgtgt acaagtctgc gtccttattg 480

ttttcttcat tgggccgaac tttctggtcc tcatccaaca gctcttctat cacgtgttcg 540

aaagtgtcag ccaatgatgt caagcctctt gaacctgcct tgggcccatt cacgctctcc 600

agagtcccat gggtccgcac acctggagat actctattat agcaaagaag aaagataatt 660

tcattgagcc atcctgtttt acagtattga attattacca caaggtacca accatatatg 720

catacttaat agggtatttt gtcaaaacta tgcatgaagg tcatttgttt gagatgtcag 780

aacattttcc cgtgagaaga tctcattggg cattgaaaca gaaccacatg ctcttcagac 840

cagcaaccgc gactaccaaa tactcctctg tcaactctac ttgagtaaga acgctttcaa 900

ttaaggccta agtgtcaaca tgcctttaaa aaaaatcgtg gtgacacaaa atctttcttt 960

ttagcaccca acagaatccc ttcaaagcct cgtggtctga caccctatgc tacgtgactt 1020

gtgacccatc catttgtcat gttcttcggg aatgtggcta aggggctaag atgtgacttg 1080

aaaagaaagg tagaacaaga tcatctcaaa tttattatca aggaatagtt cagaaaacga 1140

cttcagacca cagagacagc agaacagatg gtccggcatg gatagagcat cagacactca 1200

[0017]

cagactgtgc caacaagagc catcgagtca aaacagccaa aggaaggagg gtcatggaat 1260

gggttctctc acaccaaact gatgcccaga ggccctcagc atgaataaca aaggcaacca 1320

gacccacaag ccatactgag tggatacaaa acctatacct aggctgacat cccaaatgtg 1380

tgtggcaagt tagatgatga tggcacaaaa gacagaacac cttgcttctg gccattgtca 1440

gctcttggaa gagagcacac ttttagagga gcagctgcaa ggagcctgag aacaaaactg 1500

gaaatgtctg ttatgaaagc cttcacagga aattctgcaa gtggcaacgt gggtccattc 1560

cgtgtgtgtc actagagctg gcgcaagccc atggccatgg tgaggcagcg tttccactgg 1620

aactaatctg atacctgcac cagctcttgc aactgtgcag tgttcccact gcaaactacg 1680

gatggggtaa aagactgctc acctcctatt tctcatctaa tctcacacac tctgtttgat 1740

gaggctatgg agaaacaggt cttctcatac actaaaggtg ggagtacaaa caattcaagc 1800

cctgtgcagg acaattaggc aatacctatc aaaattatac atgatttttc ctgctgaccc 1860

agcaattcca cttctgggaa taattgacag atataggtgc atatgtacaa aatgatggaa 1920

agctctctgg tatatattag taagtgataa aacaaggtgt aaaatagtgt atatatggct 1980

actacctttt gttttaaaaa tgggggaaaa tggtggagct tgcggtgagc cgagatcgtg 2040

ccactgcact ccagcctggg cgacagagcg agactccgtc tcaaaaaaaa aacagggtgg 2100

ggtgggggggg aaataatagt acatactcat atttacctgt atctatataa aacacactat 2160

caaggattca caagaaacta atacaaatga tcaccttata gatggtatgt attgggggat 2220

actgaggtga gcagggtata agtggggcaa gacttttcag tgtaaacttc ttttaaattt 2280

tattttgatt tttgaataat gtaaattaac tgtcaaataa ttaaattaaa aataaccaat 2340

ttattaacaa aaaaaaaaaa aaaa 2364

<210> 9
<211> 3136
<212> DNA

[0018]

<213> 智人

<400> 9

ctgcataaag atttcttcac agggtccttt aaaactgtct atttctaaga ggtcccttcc 60

actacaggta aaaggaaatt ccctctaccc tagggcccct caggatccct tcctctctca 120

cacgtttctt cgactgctcc tgatttaagc attcagctgg ccacgcaacg caagaagcaa 180

taagaacaca aaaaccctac cctgttcctc ctctatccgt ggcctttgcc accacctcca 240

caacctagtt cagattcctc ttcttttctc aagggaacgt ctaaagctct caagtccgtt 300

ttggcagggc gattttgtaa gtctgaaaca tttcagtgtg tctctcgatc tcaggcagct 360

caaaagaaaa gatctgctgg ctgcgtgaag gtgcattaga aacctgctgc taccttgcag 420

cctgggctga gcatatgctc cggaacttgc ttctcttcca acatcctgca cctcagggtt 480

gcacgctctg gttcccaaac ccccggccgc tggcttatgc aaatcactta ggtacatgca 540

aaagtatccc ttctcccgga gcgccattgg cccggggagg tctcgagctc attactatgc 600

agagaggaga gccgccattg gccaagagga ggaccagagg ggcgtgtttc tcgggcaaat 660

tggatctcct aaattggatg acctgggctg aaagacaact taaagacccc cagaaaactc 720

tggttttata gataagaaat ctgaggctcg agagagagtg tgttctgccc aacatcatca 780

cggaacagct cctgggctcc tggctcctaa tctgatcgcg agatcaggaa ggtggccgag 840

tgtgtcgccg cggccatcag gcacttctcc ttcctgccct tgtatgaaga aggatgtgtt 900

tgcttccccc tgtgccatga ttgtaaattt cctgaggcct cctcagcccc gcagaactgg 960

ggttatagcc atgtgactga tcttcgtcca agaatatgta aagaaaaagt gttgagttgg 1020

cttttagggc tagagcaatg tatcttaggc tcacttaagg aagctgtaga gatgagccca 1080

aggagggaaa ccagaagagc cccccaggct caccagttgt ttgttggctc cctacaaaca 1140

tgtcattcaa gtggctaatc ttacaacagc acaaattcat ctaaccagaa agagaagagg 1200

aggctccaaa ggcacttgac tactgagcat caccctggac gtgtacaagt ctgcgtcctt 1260

[0019]

attgttttct tcattgggcc gaactttctg gtcctcatcc aacagctctt ctatcacgtg 1320

ttcgaaagtg tcagccaatg atgtcaagcc tcttgaacct gccttgggcc cattcacgct 1380

ctccagagtc ccatgggtcc gcacacctgg agatactcta ttatagcaaa gaagaaagat 1440

aatttcattg agccatcctg ttttacagta ttgaattatt accacaaggt accaaccata 1500

tatgcatact taatagggta ttttgtcaaa actatgcatg aaggtcattt gtttgagatg 1560

tcagaacatt ttcccgtgag aagatctcat tgggcattga aacagaacca catgctcttc 1620

agaccagcaa ccgcgactac caaatactcc tctgtcaact ctacttgagt aagaacgctt 1680

tcaattaagg cctaagtgtc aacatgcctt taaaaaaaat cgtggtgaca caaaatcttt 1740

ctttttagca cccaacagaa tcccttcaaa gcctcgtggt ctgacaccct atgctacgtg 1800

acttgtgacc catccatttg tcatgttctt cgggaatgtg gctaaggggc taagatgtga 1860

cttgaaaaga aaggtagaac aagatcatct caaatttatt atcaaggaat agttcagaaa 1920

acgacttcag accacagaga cagcagaaca gatggtccgg catggataga gcatcagaca 1980

ctcacagact gtgccaacaa gagccatcga gtcaaaacag ccaaaggaag gagggtcatg 2040

gaatgggttc tctcacacca aactgatgcc cagaggccct cagcatgaat aacaaaggca 2100

accagaccca caagccatac tgagtggata caaaacctat acctaggctg acatcccaaa 2160

tgtgtgtggc aagttagatg atgatggcac aaaagacaga acaccttgct tctggccatt 2220

gtcagctctt ggaagagagc acacttttag aggagcagct gcaaggagcc tgagaacaaa 2280

actggaaatg tctgttatga aagccttcac aggaaattct gcaagtggca acgtgggtcc 2340

attccgtgtg tgtcactaga gctggcgcaa gcccatggcc atggtgaggc agcgtttcca 2400

ctggaactaa tctgatacct gcaccagctc ttgcaactgt gcagtgttcc cactgcaaac 2460

tacggatggg gtaaaagact gctcacctcc tatttctcat ctaatctcac acactctgtt 2520

tgatgaggct atggagaaac aggtcttctc atacactaaa ggtgggagta caaacaattc 2580

[0020]

aagccctgtg caggacaatt aggcaatacc tatcaaaatt atacatgatt tttcctgctg 2640

acccagcaat tccacttctg ggaataattg acagatatag gtgcatatgt acaaaatgat 2700

ggaaagctct ctggtatata ttagtaagtg ataaaacaag gtgtaaaata gtgtatatat 2760

ggctactacc ttttgtttta aaaatggggg aaaatggtgg agcttgcggt gagccgagat 2820

cgtgccactg cactccagcc tgggcgacag agcgagactc cgtctcaaaa aaaaaacagg 2880

gtggggtggg ggggaaataa tagtacatac tcatatttac ctgtatctat ataaaacaca 2940

ctatcaagga ttcacaagaa actaatacaa atgatcacct tatagatggt atgtattggg 3000

ggatactgag gtgagcaggg tataagtggg gcaagacttt tcagtgtaaa cttcttttaa 3060

attttatttt gatttttgaa taatgtaaat taactgtcaa ataattaaat taaaaataac 3120

caatttatta acaaaa 3136

<210> 10
<211> 906
<212> DNA
<213> 小鼠

<400> 10
cgctgtctca atataatcta tacaacataa atccactatc ttccccttttt aatggtcagt 60

gtacatacac aggaagtgtc tatccttatg aatcgccagc caattctctt tttgctatcc 120

atagtaaggg cccgaacata cgattgggta gttcggcatt gcgagttcca gtgccttttg 180

tctatgcccc tgcagccttc cttggtgtaa cccatgggat tacacttggt ctcgtagaaa 240

tactgcttca gttggccttt ggataccggg actttctcta ggactgtgac cgtcccgcca 300

gacatgtcca ctgcagtctt tttatctgcc gctgtgaccc actcgctaat actgtcacac 360

acgctcagct ccccacggcg ggcagggtcg gagtggcgcc gaaccctcat agacatgttt 420

gcggcatcca ggtaatttttt gtattcctcc agcagaaaga gtagaggagg ctccaaaggc 480

[0021]

acttgactgc tgagcatcac ccgggaagtg tacaagtccg cgtccttatg gttttcttcg 540

ttgggccgaa ccttctggtc ctcatccagc agctcttcga tgacgtgctc aaaagtgtca 600

gccagtgatg tcgtcgtcag acctctcgaa cctgccctgg gcccattcac gctctccaga 660

gtcccatggg tccgcacacc tgggtaggcc aagttgcctt gtccgtggac gtttacttct 720

ttcatgggcg ccgccttcat gcaaccgaag tatgaaataa ccatagtaag gaaaaggatg 780

gtcatcactc ttctcacctg gtggaactgt gggaaggaag cagagacaga cacagaacag 840

gttagaactt ctttctcggg gacagcatgt ggcccatctg cttcaataat ttaatttaaa 900

aaaaaa 906

<210> 11
<211> 992
<212> DNA
<213> 小鼠

<400> 11
cgctgtctca atataatcta tacaacataa atccactatc ttccccttt aatggtcagt 60

gtacatacac aggaagtgtc tatccttatg aatcgccagc caattctctt tttgctatcc 120

atagtaaggg cccgaacata cgattgggta gttcggcatt gcgagttcca gtgccttttg 180

tctatgcccc tgcagccttc cttggtgtaa cccatgggat tacacttggt ctcgtagaaa 240

tactgcttca gttggccttt ggataccggg actttctcta ggactgtgac cgtcccgcca 300

gacatgtcca ctgcagtctt tttatctgcc gctgtgaccc actcgctaat actgtcacac 360

acgctcagct ccccacggcg ggcagggtcg gagtggcgcc gaaccctcat agacatgttt 420

gcggcatcca ggtaatttt gtattcctcc agcagaaaga gtagaggagg ctccaaaggc 480

acttgactgc tgagcatcac ccgggaagtg tacaagtccg cgtccttatg gttttcttcg 540

ttgggccgaa ccttctggtc ctcatccagc agctcttcga tgacgtgctc aaaagtgtca 600

gccagtgatg tcgtcgtcag acctctcgaa cctgccctgg gcccattcac gctctccaga 660

[0022]

gtcccatggg tccgcacacc tgggtaggcc aagttgcctt gtccgtggac gtttacttct 720

ttcatgggcg ccgccttcat gcaaccgaag tatgaaataa ccatagtaag gaaaaggatg 780

gtcatcactc ttctcacctg gtggaactgt gggaaggaag cagagacaga cacagaacag 840

gatggttggc tccagcctca ttccttctgt cccagcttct gaagttctgg gaatacaaca 900

tgtaccatca tgccaagctc tacttttga aatcatggct cctgatatgt tgggcagccc 960

tgttgtgctt tgaagataaa atgctccaca aa 992

<210> 12
<211> 43
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 12
ararcrarar arcrararcr urgrgrurgr argrcrcrur grg 43

<210> 13
<211> 44
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 13
rurgrargrc rcrurararg rarurarcra rururgrcru rcru 44

<210> 14
<211> 44
<212> DNA
<213> 人工序列

<220>

[0023]

<223> 反义寡核苷酸

<400> 14
rgrurgrcru rgrururgru rarargraru rurargrcrc rarc 44

<210> 15
<211> 44
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 15
rararurgra rcrarurgru rururgrura rgrgrgrarg rcrc 44

<210> 16
<211> 23
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 16
cmcmagmgmu gmumgmcgmg mac 23

<210> 17
<211> 23
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 17
cmcmaumgmg gmamcmucmu mgg 23

<210> 18
<211> 23

[0024]

<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 18
amgmagmcmg umgmamaumg mgg 23

<210> 19
<211> 23
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 19
cmcmcamamg gmcmamggmu muc 23

<210> 20
<211> 23
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 20
amamgamumg cmumumgamc mau 23

<210> 21
<211> 23
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 21
cmamuumgmg cmumgmacma mcu 23

[0025]

<210> 22
<211> 23
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 22
umumcgmama cmamcmgumg mau 23

<210> 23
<211> 23
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 23
amgmaamgma gmcmumgumu mgg 23

<210> 24
<211> 23
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 24
amumgamgmg amcmcmagma maa 23

<210> 25
<211> 23
<212> DNA
<213> 人工序列

<220>

[0026]

<223> 反义寡核苷酸

<400> 25
gmumucmgmg cmcmcmaamu mga 23

<210> 26
<211> 23
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 26
amgmaamama cmamamuama mgg 23

<210> 27
<211> 23
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 27
amcmgcmamg amcmumugmu mac 23

<210> 28
<211> 23
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 28
amcmgumcmc amgmgmgumg mau 23

<210> 29
<211> 23

[0027]

<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 29
gmcmucmamg umamgmucma mag 23

<210> 30
<211> 23
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 30
umgmccmumu umgmgmagmc mcu 23

<210> 31
<211> 23
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 31
cmcmucmumu cmumcmuumu mcu 23

<210> 32
<211> 16
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 32
cccggtatcc aaaggc 16

[0028]

<210> 33
<211> 16
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 33
gtattagcga gtgggt 16

<210> 34
<211> 16
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 34
gtctatgagg gttcgg 16

<210> 35
<211> 16
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 35
cctcctctac tctttc 16

<210> 36
<211> 16
<212> DNA
<213> 人工序列

<220>

[0029]

<223> 反义寡核苷酸

<400> 36
ggcaggttcg agaggt 16

<210> 37
<211> 16
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 37
ttccttccca cagttc 16

<210> 38
<211> 16
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 38
cggttgcatg aaggcg 16

<210> 39
<211> 16
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 39
tggctggcga ttcata 16

<210> 40
<211> 16

[0030]

<210> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 40
caacatatca ggagcc 16

<210> 41
<211> 16
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 41
tgtattccca gaactt 16

<210> 42
<211> 56
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 42
rurarurgrg rururararu rurcrarura rcrururcrg rgrururgrc rarurg 56

<210> 43
<211> 56
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 43
rargrararg rurarararc rgrurcrcra rcrgrgrarc rarargrgrc rararc 56

[0031]

<210> 44
<211> 56
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 44
rarurururc rurarcrgra rgrarcrcra rargrurgru rarararcrc rcraru 56

<210> 45
<211> 56
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 45
rurarargrg rarcrgrcrg rgrarcruru rgrurarcra rcrururcrc rgrgrg 56

<210> 46
<211> 56
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 46
rargrarara rgrarararg rururcrura rarcrcrurg rururcrurg rurgru 56

<210> 47
<211> 16
<212> DNA
<213> 人工序列

<220>

[0032]

<223> 反义寡核苷酸

<400> 47
gatttcagag ccgcag 16

<210> 48
<211> 16
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 48
gacacatcca tcccag 16

<210> 49
<211> 16
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 49
cctcgtcatg tctgtg 16

<210> 50
<211> 15
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 50
cttgaattgt ttgta 15

<210> 51
<211> 15

[0033]

<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 51
agttgcaaga gttgg 15

<210> 52
<211> 15
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 52
atctgttctg ctgtc 15

<210> 53
<211> 15
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 53
catattcttg gacga 15

<210> 54
<211> 15
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 54
tgtgctgttg taaga 15

[0034]

<210> 55
<211> 15
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸

<400> 55
tgacagagga gtatt 15

<210> 56
<211> 38
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸 SEQ ID NO: 11 的反向互补序列

<400> 56
rgrgrcrurc rarcrcrarg rururgruru rurgruru 38

<210> 57
<211> 38
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸 SEQ ID NO: 12 的反向互补序列

<400> 57
rgrcrararu rgrurarurc rururargrg rcrurcra 38

<210> 58
<211> 38
<212> DNA
<213> 人工序列

<220>

[0035]

<223> 反义寡核苷酸 SEQ ID NO: 13 的反向互补序列

<400> 58
rgrcrurara rurcrurura rcrararcra rgrcrarc 38

<210> 59
<211> 38
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸 SEQ ID NO: 14 的反向互补序列

<400> 59
rurcrercru rarcrarara rcrarurgru rcraruru 38

<210> 60
<211> 50
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸 SEQ ID NO: 41 的反向互补序列

<400> 60
rgrcrararc rcrgrararg rurarurgra rararurara rcrcrarura 50

<210> 61
<211> 50
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸 SEQ ID NO: 42 的反向互补序列

<400> 61
rgrcrcruru rgrurcrcrg rurgrgrarc rgrururura rcrururcru 50

<210> 62
<211> 50

[0036]

<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸 SEQ ID NO: 43 的反向互补序列

<400> 62
rgrgraruru rarcrarcru rurgrgrurc rurcrgrura rgrarararu 50

<210> 63
<211> 50
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸 SEQ ID NO: 44 的反向互补序列

<400> 63
rgrgrararg rurgrurarc rarargrurc rcrgrcrgru rcrcrurura 50

<210> 64
<211> 50
<212> DNA
<213> 人工序列

<220>
<223> 反义寡核苷酸 SEQ ID NO: 45 的反向互补序列

<400> 64
rcrargrara rcrargrgru rurargrara rcrurururc rurururcru 50

<210> 65
<211> 25
<212> DNA
<213> 人工序列

<220>
<223> 分析序列

<400> 65
gcacacctgg agatactcta ttata 25

[0037]

<210> 66
<211> 25
<212> DNA
<213> 人工序列

<220>
<223> 分析序列

<400> 66
cctgcagaat ggcctggaat tacaa 25

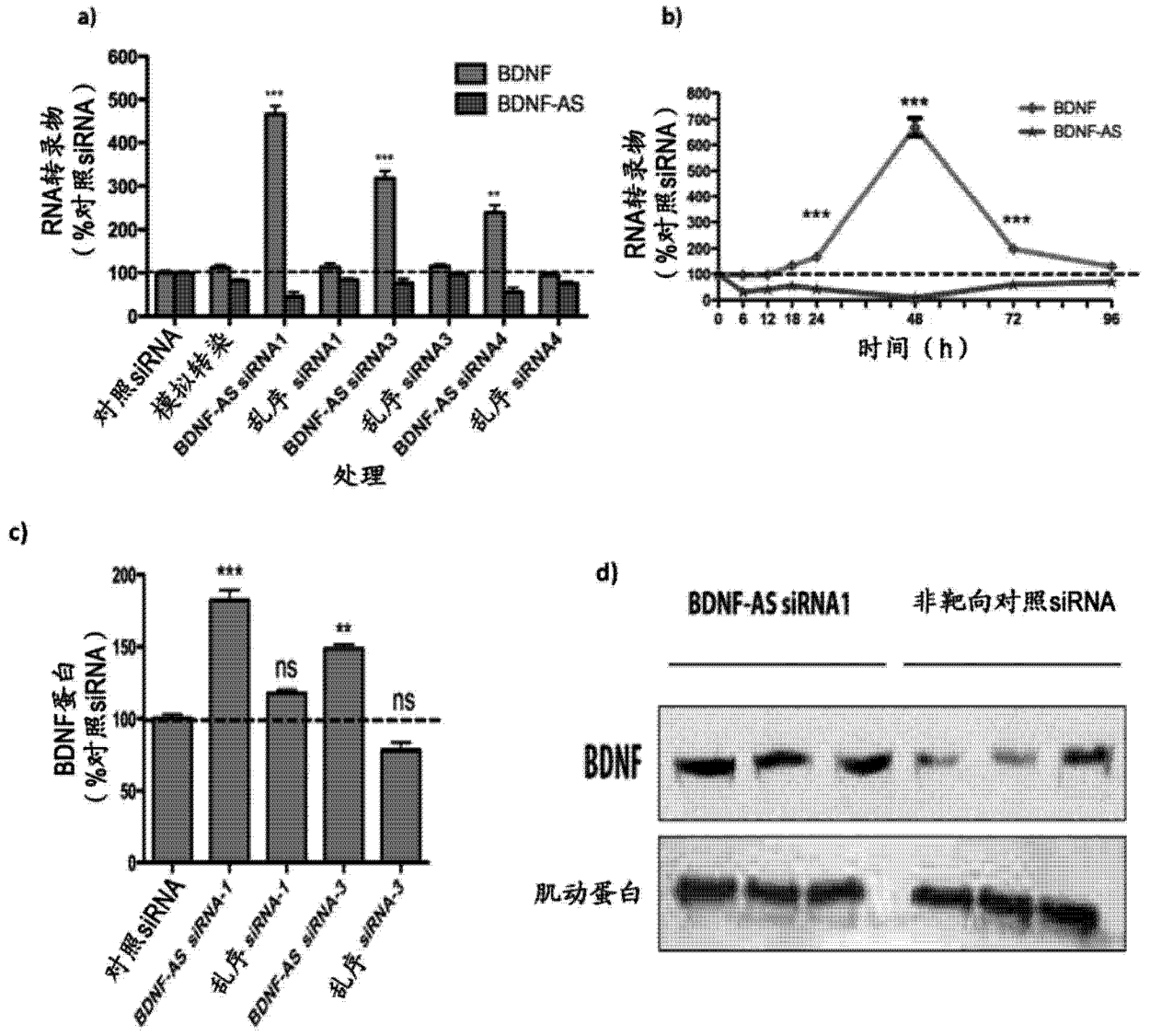

a)
RNA转录物
（%对照siRNA）
BDNF
BDNF-AS
对照siRNA
模拟转染
BDNF-AS siRNA1
乱序 siRNA1
BDNF-AS siRNA3
乱序 siRNA3
BDNF-AS siRNA4
乱序 siRNA4
处理
b)
RNA转录物
（%对照siRNA）
BDNF
BDNF-AS
时间（h）
c)
BDNF蛋白
（%对照siRNA）
对照siRNA
BDNF-AS siRNA-1
乱序 siRNA-1
BDNF-AS siRNA-3
乱序 siRNA-3
ns
d)
BDNF-AS siRNA1
非靶向对照siRNA
BDNF
肌动蛋白

图 1

e)

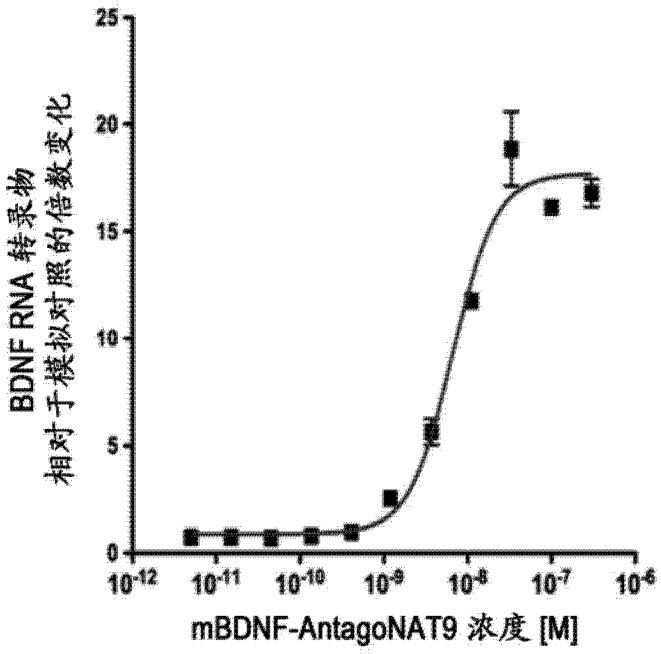

BDNF RNA 转录物
相对于模拟对照的倍数变化
25
20
15
10
5
0
10-12
10-11
10-10
10-9
10-8
10-7
10-6
mBDNF-AntagoNAT9 浓度 [M]

图 1 续

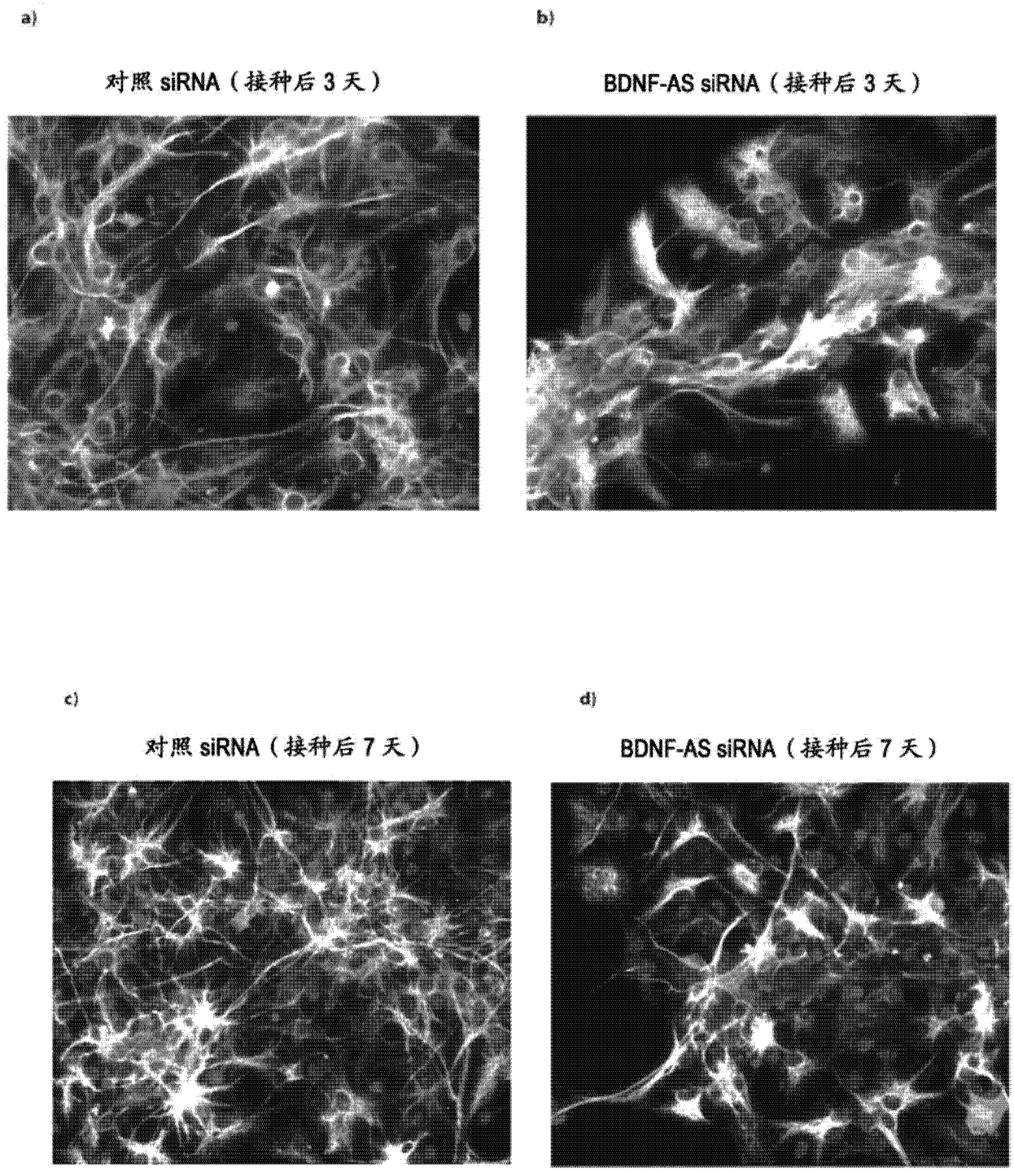

a)
对照 siRNA（接种后 3 天）
b)
BDNF-AS siRNA（接种后 3 天）
c)
对照 siRNA（接种后 7 天）
d)
BDNF-AS siRNA（接种后 7 天）

图 2

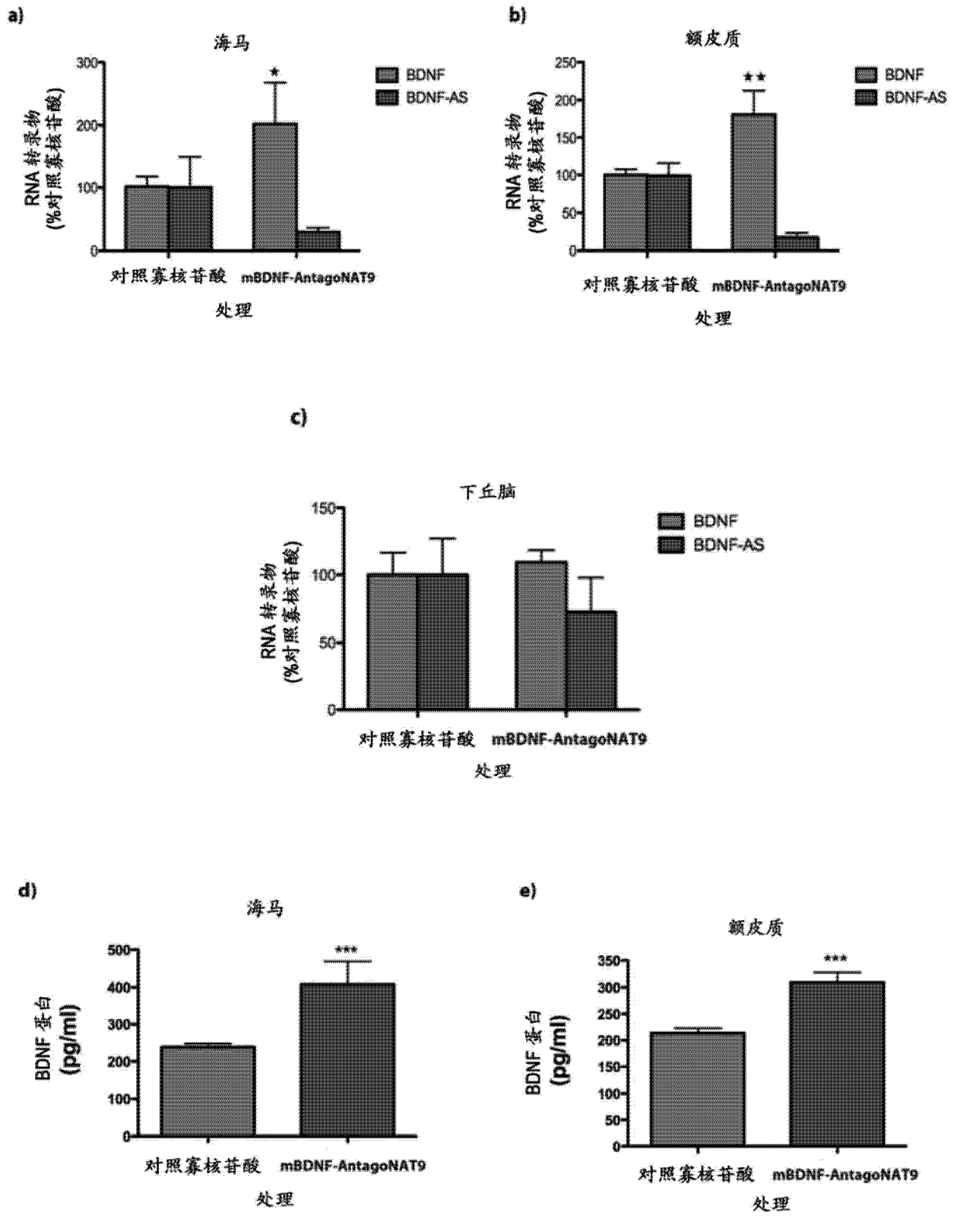

a)
海马
RNA 转录物
(%对照寡核苷酸)
300
200
100
0
*
BDNF
BDNF-AS
对照寡核苷酸
mBDNF-AntagoNAT9
处理
b)
额皮质
RNA 转录物
(%对照寡核苷酸)
250
200
150
100
50
0
**
BDNF
BDNF-AS
对照寡核苷酸
mBDNF-AntagoNAT9
处理
c)
下丘脑
RNA 转录物
(%对照寡核苷酸)
150
100
50
0
BDNF
BDNF-AS
对照寡核苷酸
mBDNF-AntagoNAT9
处理
d)
海马
BDNF 蛋白
(pg/ml)
500
400
300
200
100
0
***
对照寡核苷酸
mBDNF-AntagoNAT9
处理
e)
额皮质
BDNF 蛋白
(pg/ml)
350
300
250
200
150
100
50
0
***
对照寡核苷酸
mBDNF-AntagoNAT9
处理

图 3

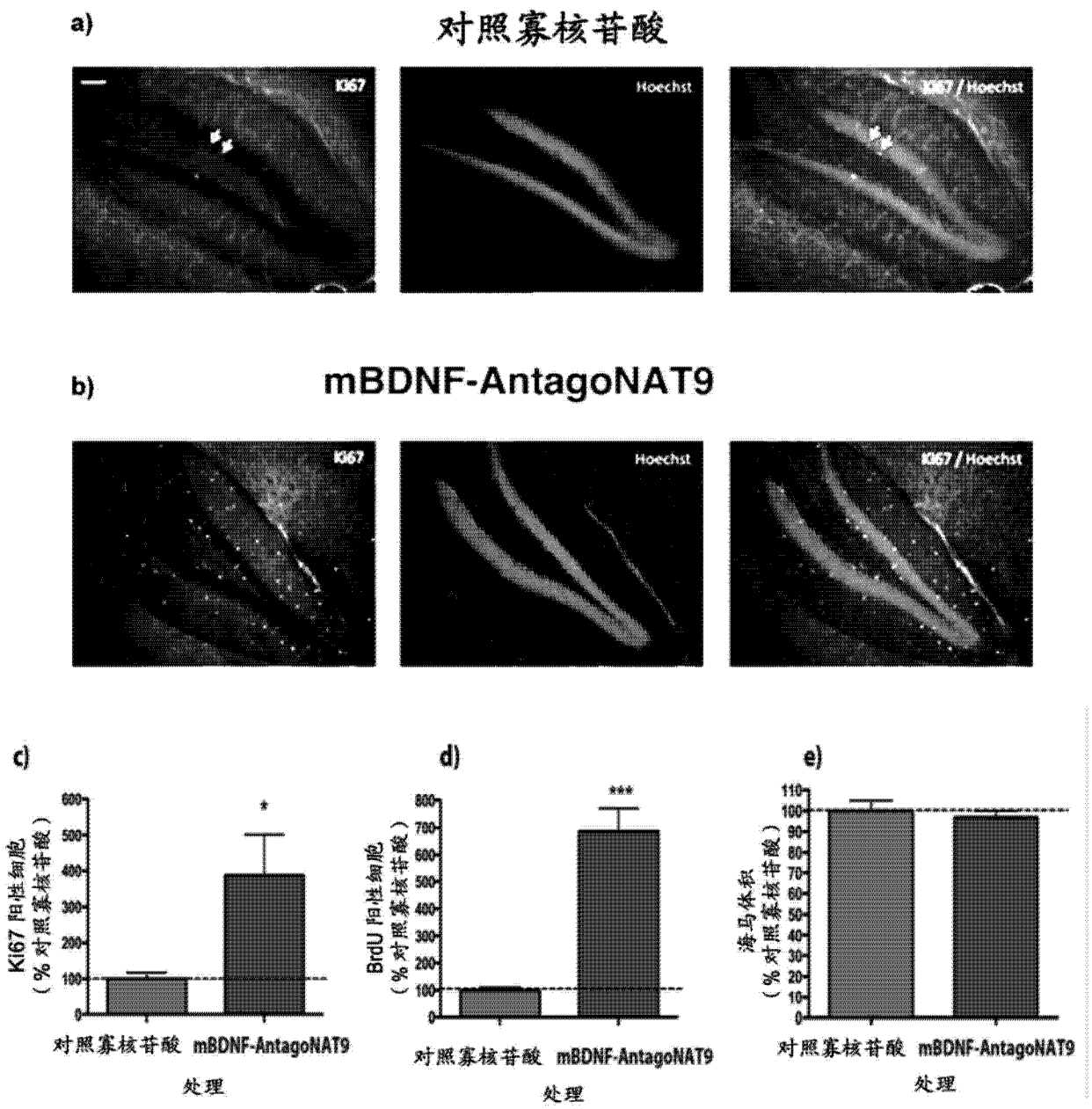

a)
对照寡核苷酸
Ki67
Hoechst
Ki67 / Hoechst
b)
mBDNF-AntagoNAT9
Ki67
Hoechst
Ki67 / Hoechst
c)
Ki67 阳性细胞
（% 对照寡核苷酸）
*
对照寡核苷酸 mBDNF-AntagoNAT9
处理
d)
BrdU 阳性细胞
（% 对照寡核苷酸）
***
对照寡核苷酸 mBDNF-AntagoNAT9
处理
e)
海马体积
（% 对照寡核苷酸）
对照寡核苷酸 mBDNF-AntagoNAT9
处理

图 4

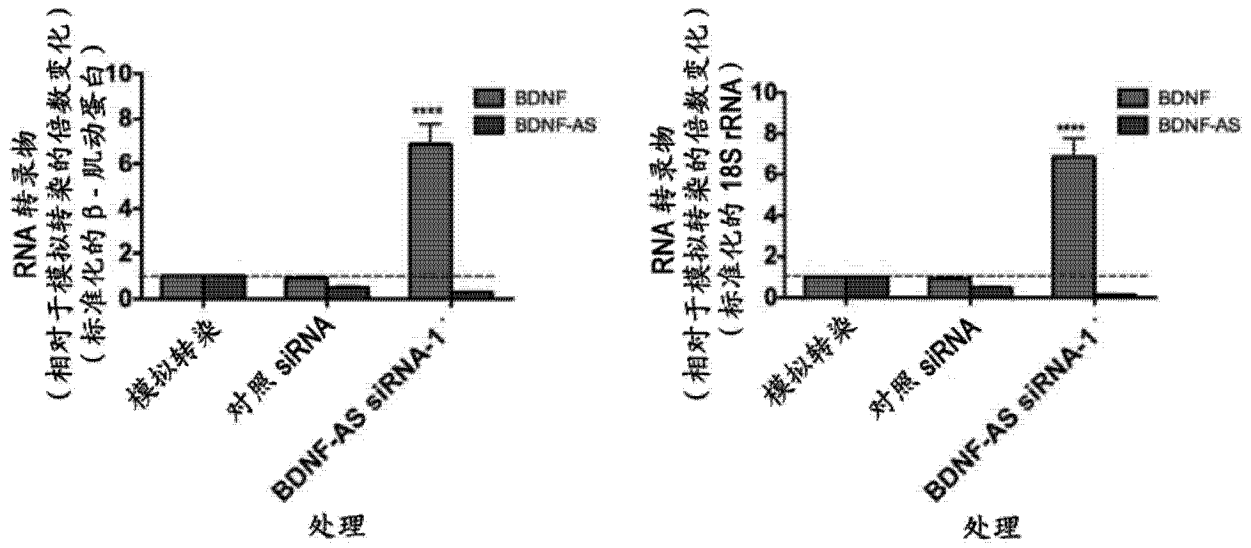

RNA 转录物
（相对于模拟转染的倍数变化）
（标准化的 β - 肌动蛋白）
BDNF
BDNF-AS
模拟转染
对照 siRNA
BDNF-AS siRNA-1
处理
RNA 转录物
（相对于模拟转染的倍数变化）
（标准化的 18S rRNA）
BDNF
BDNF-AS
模拟转染
对照 siRNA
BDNF-AS siRNA-1
处理

图 5

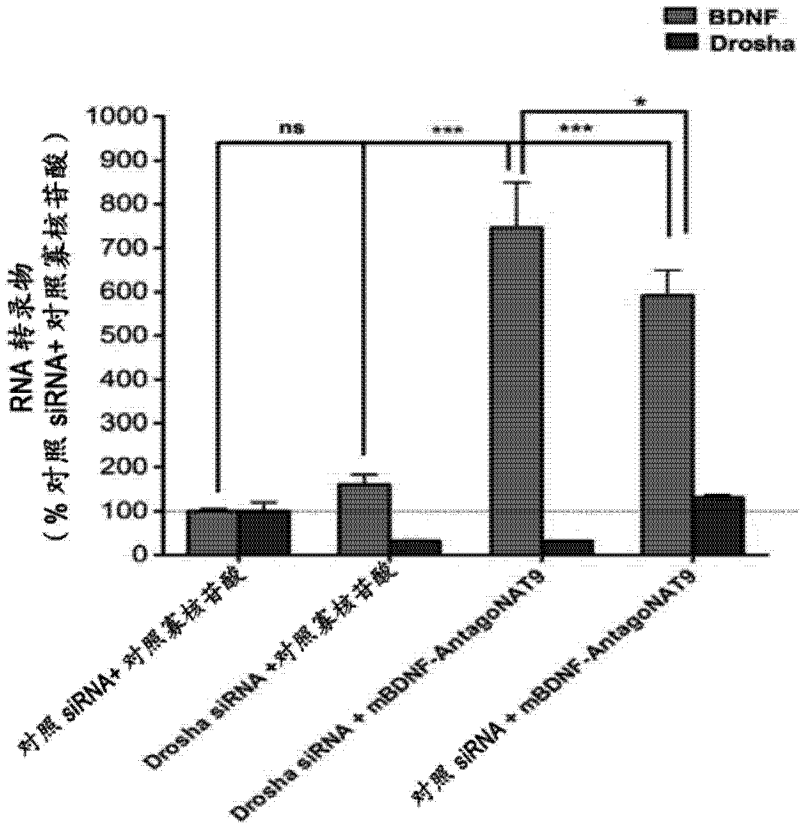

BDNF
Drosha
RNA 转录物
（% 对照 siRNA+ 对照寡核苷酸）
ns
***
***
*
对照 siRNA+ 对照寡核苷酸
Drosha siRNA +对照寡核苷酸
Drosha siRNA + mBDNF-AntagoNAT9
对照 siRNA + mBDNF-AntagoNAT9

图 6

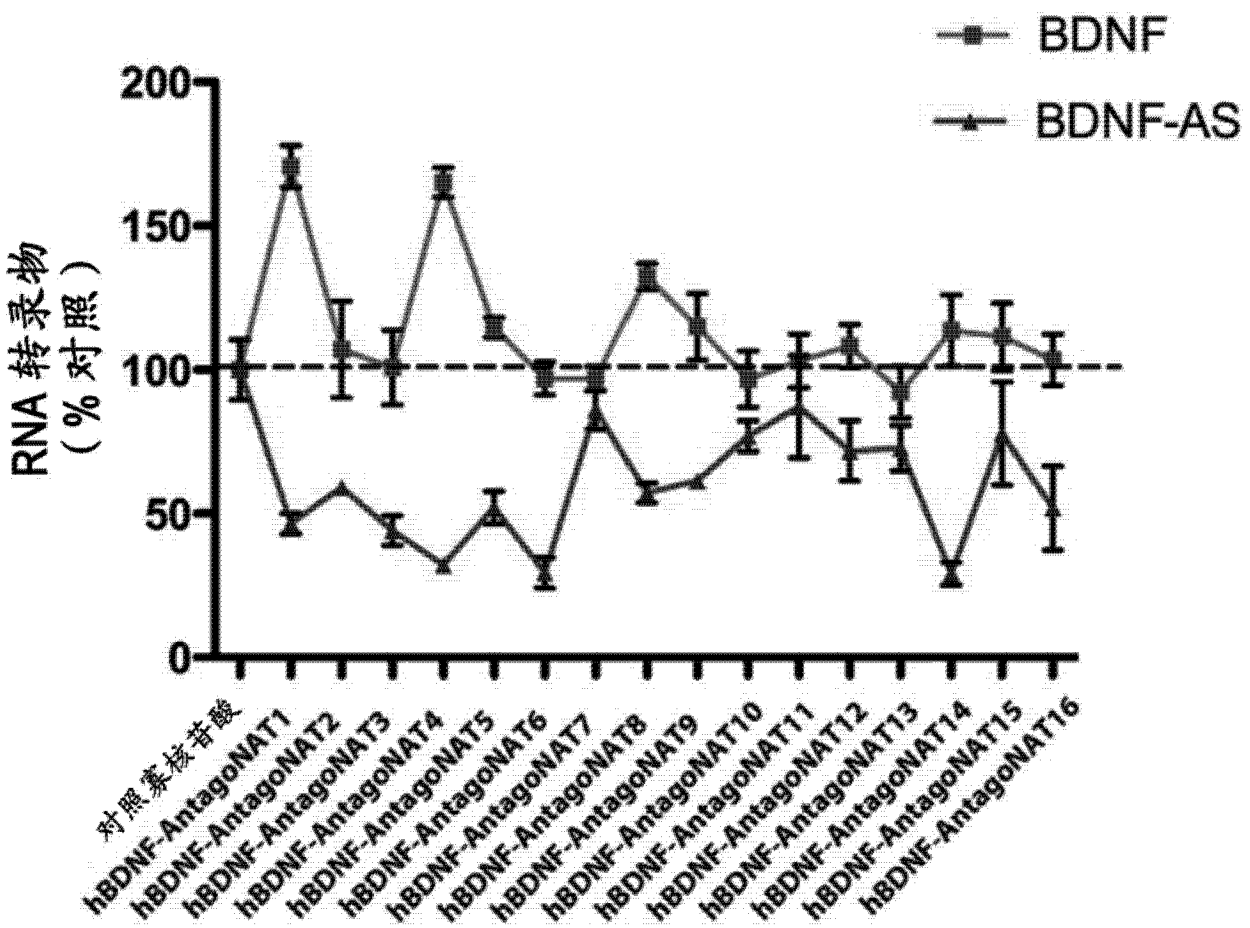

BDNF
BDNF-AS
RNA 转录物
（% 对照）
200
150
100
50
0
对照寡核苷酸
hBDNF-AntagoNAT1
hBDNF-AntagoNAT2
hBDNF-AntagoNAT3
hBDNF-AntagoNAT4
hBDNF-AntagoNAT5
hBDNF-AntagoNAT6
hBDNF-AntagoNAT7
hBDNF-AntagoNAT8
hBDNF-AntagoNAT9
hBDNF-AntagoNAT10
hBDNF-AntagoNAT11
hBDNF-AntagoNAT12
hBDNF-AntagoNAT13
hBDNF-AntagoNAT14
hBDNF-AntagoNAT15
hBDNF-AntagoNAT16

图 7

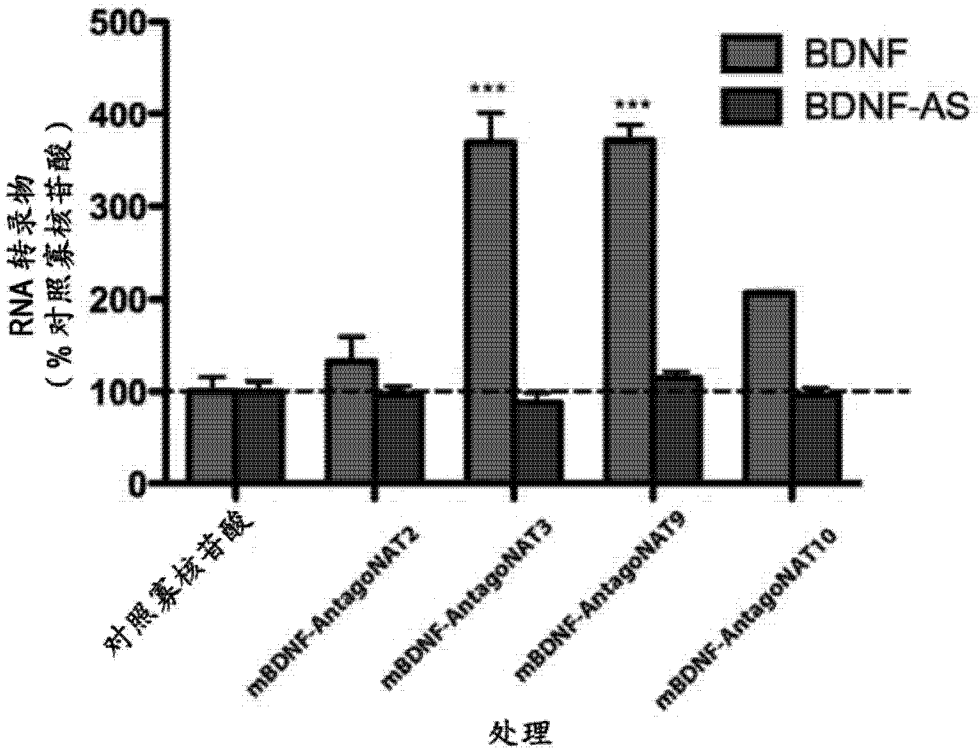

500
400
300
200
100
0
RNA 转录物
（% 对照寡核苷酸）
BDNF
BDNF-AS
***
***
对照寡核苷酸
mBDNF-AntagoNAT2
mBDNF-AntagoNAT3
mBDNF-AntagoNAT9
mBDNF-AntagoNAT10
处理

图 8

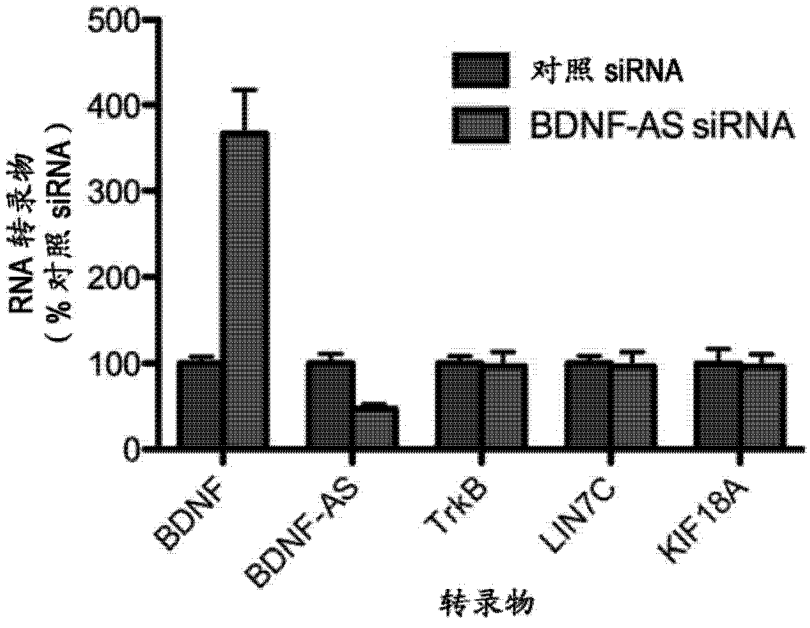

500
400
300
200
100
0
RNA 转录物
（% 对照 siRNA）
对照 siRNA
BDNF-AS siRNA
BDNF
BDNF-AS
TrkB
LIN7C
KIF18A
转录物

图 9