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(54) Title: TARGET SEQUENCES AND METHODS TO IDENTIFY THE SAME, USEFUL IN TREATMENT OF NEURODEGENERATIVE DISEASES

(57) Abstract: The present invention relates to methods and assays for identifying agents capable of inhibiting the mutant huntingtin protein, inhibiting or reducing cell death, in particular cell death associated with polyglutamine-induced protein aggregation, which inhibition is useful in the prevention, amelioration and / or treatment of neurodegenerative diseases, and Huntington's disease more generally. In particular, the present invention provides methods and assays for identifying agents for use in the prevention and / or treatment of Huntingtons disease. The invention provides polypeptide and nucleic acid TARGETs and siRNA sequences based on these TARGETS.



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TARGET SEQUENCES AND METHODS TO IDENTIFY THE SAME, USEFUL IN TREATMENT OF
NEURODEGENERATIVE DISEASES

FIELD OF THE INVENTION

[001] The present invention relates to methods for identifying agents capable of modulating the expression or activity of proteins involved in the processes leading to Huntington's Disease (HD) pathology. Inhibition of these processes is useful in the prevention and / or treatment of Huntington's Disease and other diseases involving neurodegeneration. In particular, the present invention provides methods for identifying agents for use in the prevention and / or treatment of HD.

BACKGROUND OF THE INVENTION

[002] Huntington's Disease (HD) is an autosomal-dominant genetic neurodegenerative disease, characterized by neuropathology in the striatum and cortex. HD gives rise to progressive, selective (localized) neural cell death associated with choreic movements and dementia. No treatment exists for HD, and this disease leads to premature death in a decade from onset of clinical signs. For reviews on HD, we refer to (Bates, 2005; Tobin and Signer, 2000; Vonsattel *et al.*, 1985; Zoghbi and Orr, 2000).

[003] Neuropathological analysis of the brains of HD patients clearly evidences the regions of the brain involved in the neurodegenerative processes (Vonsattel *et al.*, 1985). The striatum (caudate nucleus) and cortex are most severely affected, explaining the motor and cognitive deficits observed during the disease process.

[004] HD is associated with increases in the length of a CAG triplet repeat present in a gene called 'huntingtin' or HD, located on chromosome 4p16.3. The Huntington's Disease Collaborative Research Group (The Huntington's Disease Collaborative Research Group, 1993) found that a 'new' gene, designated IT15 (important transcript 15) and later called huntingtin, which was isolated using cloned trapped exons from the target area, contains a polymorphic trinucleotide repeat that is expanded and unstable on HD chromosomes. A (CAG)*n* repeat longer than the normal range was observed on HD chromosomes from all 75 disease families examined. The families came from a variety of ethnic backgrounds and demonstrated a variety of 4p16.3 haplotypes. The (CAG)*n* repeat appeared to be located within the coding sequence of a predicted protein of about 348 kD that is widely expressed but unrelated to any known gene. Thus it turned out that the HD mutation involves an unstable DNA segment similar to those previously observed in several disorders, including the fragile X syndrome, Kennedy syndrome, and myotonic dystrophy. The fact that the phenotype of HD is completely dominant suggests that the disorder results from a gain-of-function mutation in which either the mRNA product or the protein product of the disease allele has some new property or is expressed inappropriately.

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[005] DiFiglia *et al.* (DiFiglia *et al.*, 1997) contributed to the understanding of the mechanism of neurodegeneration in HD. They demonstrated that an amino-terminal fragment of mutant huntingtin localizes to neuronal intranuclear inclusions (NIIs) and dystrophic neurites (DNs) in the HD cortex and striatum, which are affected in HD, and that polyglutamine length influences the extent of huntingtin accumulation in these structures. Ubiquitin, which is thought to be involved in labeling proteins for disposal by intracellular proteolysis, was also found in NIIs and DN, suggesting (DiFiglia *et al.*, 1997) that abnormal huntingtin is targeted for proteolysis but is resistant to removal. The aggregation of mutant huntingtin may be part of the pathogenic mechanism in HD.

[006] Saudou *et al.* (Saudou *et al.*, 1998) investigated the mechanisms by which mutant huntingtin induces neurodegeneration by use of a cellular model that recapitulates features of neurodegeneration seen in Huntington disease. When transfected into cultured striatal neurons, mutant huntingtin induced neurodegeneration by an apoptotic mechanism. Antiapoptotic compounds or neurotrophic factors protected neurons against mutant huntingtin. Blocking nuclear localization of mutant huntingtin suppressed its ability to form intranuclear inclusions and to induce neurodegeneration. However, the presence of inclusions did not correlate with huntingtin-induced death. The exposure of mutant huntingtin-transfected striatal neurons to conditions that suppress the formation of inclusions resulted in an increase in mutant huntingtin-induced death. These findings suggested that mutant huntingtin acts within the nucleus to induce neurodegeneration. Altogether, intranuclear inclusions may reflect a cellular mechanism to protect against huntingtin-induced cell death.

[007] A method to reduce the levels of the cell death in neurons in the striatum and cortex observed in HD is likely to confer clinical benefit to HD patients.

[008] A remarkable threshold exists, where polyglutamine stretches of 35 repeats or more in the HD gene cause HD, whereas stretches of polyglutamine fewer than 35 do not cause disease. A robust correlation between the threshold for disease and the propensity of the huntingtin protein to aggregate *in vitro*, suggests that aggregation is related to pathogenesis (Davies *et al.*, 1997; Scherzinger *et al.*, 1999).

[009] Protein aggregation follows a series of intermediate steps including an abnormal conformation of the protein, a globular intermediate, protofibrils, fibers and microscopic inclusions (Ross and Poirier, 2004). It is commonly believed that one or more of these molecular species confers toxicity in HD.

[0010] A method to reduce the expression levels of the toxic intermediates of the mutant HD protein would likely confer clinical benefit to HD patients.

Reported Developments

[0011] Neural and stem cell transplantation is a potential treatment for neurodegenerative diseases, e.g., transplantation of specific committed neuroblasts (fetal neurons) to the adult brain. Encouraged by animal studies, a clinical trial of human fetal striatal tissue transplantation for the treatment of Huntington disease was

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initially undertaken at the University of South Florida. In this series, one patient died 18 months after transplantation from causes unrelated to surgery.

[0012] The fact that activation of mechanisms mediating cell death may be involved in neurologic diseases makes apoptosis and caspases attractive therapeutic targets. Clinical trials of an inhibitor of apoptosis (minocycline) for HD are in progress.

[0013] A variety of growth factors had been shown to induce cell proliferation and neurogenesis, which could counter-act cell loss in HD (Strand *et al.*, 2007).

[0014] Inhibition of polyglutamine-induced protein aggregation could provide treatment options for polyglutamine diseases such as HD. Tanaka *et al.* (Tanaka *et al.*, 2004) showed through in vitro screening studies that various disaccharides can inhibit polyglutamine-mediated protein aggregation. They also found that various disaccharides reduced polyglutamine aggregates and increased survival in a cellular model of HD. Oral administration of trehalose, the most effective of these disaccharides, decreased polyglutamine aggregates in cerebrum and liver, improved motor dysfunction, and extended life span in a transgenic mouse model of HD. Tanaka *et al.* (Tanaka *et al.*, 2004) suggested that these beneficial effects are the result of trehalose binding to expanded polyglutamines and stabilizing the partially unfolded polyglutamine-containing protein. Lack of toxicity and high solubility, coupled with efficacy upon oral administration, made trehalose promising as a therapeutic drug or lead component for the treatment of polyglutamine diseases. The saccharide-polyglutamine interaction identified by Tanaka *et al.* (Tanaka *et al.*, 2004) thus provided a possible new therapeutic strategy for polyglutamine diseases.

[0015] Ravikumar *et al.* (Ravikumar *et al.*, 2004) presented data that provided proof of principle for the potential of inducing autophagy to treat HD. They showed that mammalian target of rapamycin (mTOR) is sequestered in polyglutamine aggregates in cell models, transgenic mice, and human brains. Such sequestration impairs the kinase activity of mTOR and induces autophagy, a key clearance pathway for mutant huntingtin fragments. This protects against polyglutamine toxicity.

[0016] There still exists a need in the art for compounds and agents for amelioration of symptoms, prevention and treatment of Huntington's Disease and other diseases associated with or exacerbated by neuronal cell death, including diseases where the cell death is linked to protein aggregation.

SUMMARY OF THE INVENTION

[0017] The present invention is based on the discovery that agents which inhibit the expression and / or activity of the TARGETS disclosed herein are able to modulate survival of neuronal cells to expression of mutant (expanded) huntingtin protein in neuronal cells. The present invention therefore provides TARGETS which are involved in the pathway involved in HD pathogenesis, methods for screening for agents capable of modulating the expression and / or activity of TARGETS and uses of these agents in the prevention and / or

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treatment of neurodegenerative diseases such as HD. The present invention provides TARGETS which are involved in or otherwise associated with neuronal cell death in neurodegenerative diseases.

[0018] The present invention relates to a method for identifying compounds that are able to modulate the expression or activity of the mutant huntingtin protein in neuronal cells, comprising contacting a compound with a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 47, 49, 51-60, 62-67, 69, 71, 75-82 and 85-90 (hereinafter "TARGETS") and fragments thereof, under conditions that allow said polypeptide to bind to said compound, and measuring a compound-polypeptide property related to huntingtin expression or activity. In a specific embodiment the compound-polypeptide property measured is huntingtin protein expression levels. In a specific embodiment, the property measured is cell death. More generally, the method relates to identifying compounds which modulate cell death and particularly neuronal cell death.

[0019] Aspects of the present method include the *in vitro* assay of compounds using polypeptide of a TARGET, or fragments thereof, such fragments including the amino acid sequences described by SEQ ID NO: 46, 47, 49, 51-60, 62-67, 69, 71, 75-82 and 85-90 and cellular assays wherein TARGET inhibition is followed by observing indicators of efficacy including, for example, TARGET expression levels, TARGET enzymatic activity and/or huntingtin protein levels.

[0020] The present invention also relates to

- (1) expression inhibitory agents comprising a polynucleotide selected from the group of an antisense polynucleotide, a ribozyme, and a small interfering RNA (siRNA), wherein said polynucleotide comprises a nucleic acid sequence complementary to, or engineered from, a naturally occurring polynucleotide sequence encoding a TARGET polypeptide said polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO: 1, 2, 4, 6-15, 17-22, 24, 26, 30-37, 40-45 and
- (2) pharmaceutical compositions comprising said agent(s), useful in the treatment, or prevention, of neurodegenerative diseases such as Huntington's disease.

[0021] Another aspect of the invention is a method of treatment, or prevention, or alleviation of a condition related to neurodegeneration, in a subject suffering or susceptible thereto, by administering a pharmaceutical composition comprising an effective TARGET-expression inhibiting amount of a expression-inhibitory agent or an effective TARGET activity inhibiting amount of a activity-inhibitory agent.

[0022] Another aspect of this invention relates to the use of agents which inhibit a TARGET as disclosed herein in a therapeutic method, a pharmaceutical composition, and the manufacture of such composition, useful for the treatment of a disease involving neurodegeneration. In particular, the present method relates to the use of the agents which inhibit a TARGET in the treatment of a disease characterized by neuronal cell death, and in particular, a disease characterized by abnormal aggregations of huntingtin protein. The agents are useful for

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amelioration or treatment of neurodegenerative conditions, particularly wherein it is desired to reduce or control protein aggregation, in particular huntingtin aggregation. Suitable neurodegenerative conditions include, but are not limited to, Alzheimer's Disease, Parkinson's Disease, Amyotrophic Lateral Sclerosis, Progressive Supranuclear Palsy, Frontotemporal Dementia and Spinocerebellar Ataxia. In particular the disease is Huntington's disease. Other objects and advantages will become apparent from a consideration of the ensuing description taken in conjunction with the following illustrative drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

- [0023] FIGURE 1: Example of a plate in the Ad-siRNA huntingtin cell death assay.
[0024] FIGURE 2: Primary screening data of 11584 Ad-siRNAs in the huntingtin cell death assay.

DETAILED DESCRIPTION

[0025] The following terms are intended to have the meanings presented therewith below and are useful in understanding the description and intended scope of the present invention.

[0026] The term 'agent' means any molecule, including polypeptides, polynucleotides, chemical compounds and small molecules. In particular the term agent includes compounds such as test compounds or drug candidate compounds.

[0027] The term 'agonist' refers to a ligand that stimulates the receptor the ligand binds to in the broadest sense.

[0028] As used herein, the term 'antagonist' is used to describe a compound that does not provoke a biological response itself upon binding to a receptor, but blocks or dampens agonist-mediated responses, or prevents or reduces agonist binding and, thereby, agonist-mediated responses.

[0029] The term 'assay' means any process used to measure a specific property of an agent, including a compound. A 'screening assay' means a process used to characterize or select compounds based upon their activity from a collection of compounds.

[0030] The term 'binding affinity' is a property that describes how strongly two or more compounds associate with each other in a non-covalent relationship. Binding affinities can be characterized qualitatively, (such as 'strong', 'weak', 'high', or 'low') or quantitatively (such as measuring the K_D).

[0031] The term 'carrier' means a non-toxic material used in the formulation of pharmaceutical compositions to provide a medium, bulk and/or useable form to a pharmaceutical composition. A carrier may comprise one or more of such materials such as an excipient, stabilizer, or an aqueous pH buffered solution.

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Examples of physiologically acceptable carriers include aqueous or solid buffer ingredients including phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN®, polyethylene glycol (PEG), and PLURONICS®.

[0032] The term ‘complex’ means the entity created when two or more compounds bind to, contact, or associate with each other.

[0033] The term ‘compound’ is used herein in the context of a ‘test compound’ or a ‘drug candidate compound’ described in connection with the assays of the present invention. As such, these compounds comprise organic or inorganic compounds, derived synthetically or from natural sources. The compounds include inorganic or organic compounds such as polynucleotides (e.g. siRNA or cDNA), lipids or hormone analogs. Other biopolymeric organic test compounds include peptides comprising from about 2 to about 40 amino acids and larger polypeptides comprising from about 40 to about 500 amino acids, including polypeptide ligands, enzymes, receptors, channels, antibodies or antibody conjugates.

[0034] The term ‘condition’ or ‘disease’ means the overt presentation of symptoms (i.e., illness) or the manifestation of abnormal clinical indicators (for example, biochemical indicators). Alternatively, the term ‘disease’ refers to a genetic or environmental risk of or propensity for developing such symptoms or abnormal clinical indicators.

[0035] The term ‘contact’ or ‘contacting’ means bringing at least two moieties together, whether in an *in vitro* system or an *in vivo* system.

[0036] The term ‘derivatives of a polypeptide’ relates to those peptides, oligopeptides, polypeptides, proteins and enzymes that comprise a stretch of contiguous amino acid residues of the polypeptide and that retain a biological activity of the protein, for example, polypeptides that have amino acid mutations compared to the amino acid sequence of a naturally-occurring form of the polypeptide. A derivative may further comprise additional naturally occurring, altered, glycosylated, acylated or non-naturally occurring amino acid residues compared to the amino acid sequence of a naturally occurring form of the polypeptide. It may also contain one or more non-amino acid substituents, or heterologous amino acid substituents, compared to the amino acid sequence of a naturally occurring form of the polypeptide, for example a reporter molecule or other ligand, covalently or non-covalently bound to the amino acid sequence.

[0037] The term ‘derivatives of a polynucleotide’ relates to DNA-molecules, RNA-molecules, and oligonucleotides that comprise a stretch of nucleic acid residues of the polynucleotide, for example, polynucleotides that may have nucleic acid mutations as compared to the nucleic acid sequence of a naturally

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occurring form of the polynucleotide. A derivative may further comprise nucleic acids with modified backbones such as PNA, polysiloxane, and 2'-O-(2-methoxy) ethyl-phosphorothioate, non-naturally occurring nucleic acid residues, or one or more nucleic acid substituents, such as methyl-, thio-, sulphate, benzoyl-, phenyl-, amino-, propyl-, chloro-, and methanocarbanucleosides, or a reporter molecule to facilitate its detection.

[0038] The term 'endogenous' shall mean a material that a mammal naturally produces. Endogenous in reference to the term 'enzyme', 'protease', 'kinase', or G-Protein Coupled Receptor ('GPCR') shall mean that which is naturally produced by a mammal (for example, and not limitation, a human). In contrast, the term non-endogenous in this context shall mean that which is not naturally produced by a mammal (for example, and not limitation, a human). Both terms can be utilized to describe both *in vivo* and *in vitro* systems. For example, and without limitation, in a screening approach, the endogenous or non-endogenous TARGET may be in reference to an *in vitro* screening system. As a further example and not limitation, where the genome of a mammal has been manipulated to include a non-endogenous TARGET, screening of a candidate compound by means of an *in vivo* system is viable.

[0039] The term 'expressible nucleic acid' means a nucleic acid coding for a proteinaceous molecule, an RNA molecule, or a DNA molecule.

[0040] The term 'expression' comprises both endogenous expression and non-endogenous expression, including overexpression by transduction.

[0041] The term 'expression inhibitory agent' means a polynucleotide designed to interfere selectively with the transcription, translation and/or expression of a specific polypeptide or protein normally expressed within a cell. More particularly, 'expression inhibitory agent' comprises a DNA or RNA molecule that contains a nucleotide sequence identical to or complementary to at least about 15-30, particularly at least 17, sequential nucleotides within the polyribonucleotide sequence coding for a specific polypeptide or protein. Exemplary expression inhibitory molecules include ribozymes, double stranded siRNA molecules, self-complementary single-stranded siRNA molecules, genetic antisense constructs, and synthetic RNA antisense molecules with modified stabilized backbones.

[0042] The term 'fragment of a polynucleotide' relates to oligonucleotides that comprise a stretch of contiguous nucleic acid residues that exhibit substantially a similar, but not necessarily identical, activity as the complete sequence. In a particular aspect, 'fragment' may refer to a oligonucleotide comprising a nucleic acid sequence of at least 5 nucleic acid residues (preferably, at least 10 nucleic acid residues, at least 15 nucleic acid residues, at least 20 nucleic acid residues, at least 25 nucleic acid residues, at least 40 nucleic acid residues, at least 50 nucleic acid residues, at least 60 nucleic residues, at least 70 nucleic acid residues, at least 80 nucleic acid residues, at least 90 nucleic acid residues, at least 100 nucleic acid residues, at least 125 nucleic acid

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residues, at least 150 nucleic acid residues, at least 175 nucleic acid residues, at least 200 nucleic acid residues, or at least 250 nucleic acid residues) of the nucleic acid sequence of said complete sequence.

[0043] The term 'fragment of a polypeptide' relates to peptides, oligopeptides, polypeptides, proteins, monomers, subunits and enzymes that comprise a stretch of contiguous amino acid residues, and exhibit substantially a similar, but not necessarily identical, functional or expression activity as the complete sequence. In a particular aspect, 'fragment' may refer to a peptide or polypeptide comprising an amino acid sequence of at least 5 amino acid residues (preferably, at least 10 amino acid residues, at least 15 amino acid residues, at least 20 amino acid residues, at least 25 amino acid residues, at least 40 amino acid residues, at least 50 amino acid residues, at least 60 amino acid residues, at least 70 amino acid residues, at least 80 amino acid residues, at least 90 amino acid residues, at least 100 amino acid residues, at least 125 amino acid residues, at least 150 amino acid residues, at least 175 amino acid residues, at least 200 amino acid residues, or at least 250 amino acid residues) of the amino acid sequence of said complete sequence.

[0044] The term 'hybridization' means any process by which a strand of nucleic acid binds with a complementary strand through base pairing. The term 'hybridization complex' refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary bases. A hybridization complex may be formed in solution (for example, C_{0t} or R_{0t} analysis) or formed between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (for example, paper, membranes, filters, chips, pins or glass slides, or any other appropriate substrate to which cells or their nucleic acids have been fixed). The term "stringent conditions" refers to conditions that permit hybridization between polynucleotides and the claimed polynucleotides. Stringent conditions can be defined by salt concentration, the concentration of organic solvent, for example, formamide, temperature, and other conditions well known in the art. In particular, reducing the concentration of salt, increasing the concentration of formamide, or raising the hybridization temperature can increase stringency. The term 'standard hybridization conditions' refers to salt and temperature conditions substantially equivalent to 5 x SSC and 65°C for both hybridization and wash. However, one skilled in the art will appreciate that such 'standard hybridization conditions' are dependent on particular conditions including the concentration of sodium and magnesium in the buffer, nucleotide sequence length and concentration, percent mismatch, percent formamide, and the like. Also important in the determination of "standard hybridization conditions" is whether the two sequences hybridizing are RNA-RNA, DNA-DNA or RNA-DNA. Such standard hybridization conditions are easily determined by one skilled in the art according to well known formulae, wherein hybridization is typically 10-20°C below the predicted or determined T_m with washes of higher stringency, if desired.

[0045] The term 'inhibit' or 'inhibiting', in relationship to the term 'response' means that a response is decreased or prevented in the presence of a compound as opposed to in the absence of the compound.

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[0046] The term 'inhibition' refers to the reduction, down regulation of a process or the elimination of a stimulus for a process, which results in the absence or minimization of the expression of a protein or polypeptide.

[0047] The term 'induction' refers to the inducing, up-regulation, or stimulation of a process, which results in the expression of a protein or polypeptide.

[0048] The term 'ligand' means an endogenous, naturally occurring molecule specific for an endogenous, naturally occurring receptor.

[0049] The term 'pharmaceutically acceptable salts' refers to the non-toxic, inorganic and organic acid addition salts, and base addition salts, of compounds which inhibit the expression or activity of TARGETS as disclosed herein. These salts can be prepared *in situ* during the final isolation and purification of compounds useful in the present invention.

[0050] The term 'polypeptide' relates to proteins (such as TARGETS), proteinaceous molecules, fragments of proteins, monomers or portions of polymeric proteins, peptides, oligopeptides and enzymes (such as kinases, proteases, GPCR's etc.).

[0051] The term 'polynucleotide' means a polynucleic acid, in single or double stranded form, and in the sense or antisense orientation, complementary polynucleic acids that hybridize to a particular polynucleic acid under stringent conditions, and polynucleotides that are homologous in at least about 60 percent of its base pairs, and more particularly 70 percent of its base pairs are in common, most particularly 90 per cent, and in a special embodiment 100 percent of its base pairs. The polynucleotides include polyribonucleic acids, polydeoxyribonucleic acids, and synthetic analogues thereof. It also includes nucleic acids with modified backbones such as peptide nucleic acid (PNA), polysiloxane, and 2'-O-(2-methoxy)ethylphosphorothioate. The polynucleotides are described by sequences that vary in length, that range from about 10 to about 5000 bases, particularly about 100 to about 4000 bases, more particularly about 250 to about 2500 bases. One polynucleotide embodiment comprises from about 10 to about 30 bases in length. A special embodiment of polynucleotide is the polyribonucleotide of from about 17 to about 22 nucleotides, more commonly described as small interfering RNAs (siRNAs - double stranded siRNA molecules or self-complementary single-stranded siRNA molecules (shRNA)). Another special embodiment are nucleic acids with modified backbones such as peptide nucleic acid (PNA), polysiloxane, and 2'-O-(2-methoxy)ethylphosphorothioate, or including non-naturally occurring nucleic acid residues, or one or more nucleic acid substituents, such as methyl-, thio-, sulphate, benzoyl-, phenyl-, amino-, propyl-, chloro-, and methanocarbanucleosides, or a reporter molecule to facilitate its detection. Polynucleotides herein are selected to be 'substantially' complementary to different strands of a particular target DNA sequence. This means that the polynucleotides must be sufficiently complementary to hybridize with their respective strands. Therefore, the polynucleotide sequence need not reflect the exact sequence of the target sequence. For example, a non-complementary nucleotide fragment may

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be attached to the 5' end of the polynucleotide, with the remainder of the polynucleotide sequence being complementary to the strand. Alternatively, non-complementary bases or longer sequences can be interspersed into the polynucleotide, provided that the polynucleotide sequence has sufficient complementarity with the sequence of the strand to hybridize therewith under stringent conditions or to form the template for the synthesis of an extension product.

[0052] The term 'preventing' or 'prevention' refers to a reduction in risk of acquiring or developing a disease or disorder (i.e., causing at least one of the clinical symptoms of the disease not to develop) in a subject that may be exposed to a disease-causing agent, or predisposed to the disease in advance of disease onset.

[0053] The term 'prophylaxis' is related to and encompassed in the term 'prevention', and refers to a measure or procedure the purpose of which is to prevent, rather than to treat or cure a disease. Non-limiting examples of prophylactic measures may include the administration of vaccines; the administration of low molecular weight heparin to hospital patients at risk for thrombosis due, for example, to immobilization; and the administration of an anti-malarial agent such as chloroquine, in advance of a visit to a geographical region where malaria is endemic or the risk of contracting malaria is high.

[0054] The term 'solvate' means a physical association of a compound useful in this invention with one or more solvent molecules. This physical association includes hydrogen bonding. In certain instances the solvate will be capable of isolation, for example when one or more solvent molecules are incorporated in the crystal lattice of the crystalline solid. "Solvate" encompasses both solution-phase and isolable solvates. Representative solvates include hydrates, ethanولات and methanولات.

[0055] The term 'subject' includes humans and other mammals.

[0056] The term 'TARGET' or 'TARGETS' means the protein(s) identified in accordance with the assays described herein and determined to be involved in the modulation of a Huntington Disease phenotype.

[0057] 'Therapeutically effective amount' or 'effective amount' means that amount of a compound or agent that will elicit the biological or medical response of a subject that is being sought by a medical doctor or other clinician.

[0058] The term 'treating' means an intervention performed with the intention of preventing the development or altering the pathology of, and thereby ameliorating a disorder, disease or condition, including one or more symptoms of such disorder or condition. Accordingly, 'treating' refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treating include those already with the disorder as well as those in which the disorder is to be prevented. The related term 'treatment,' as used herein, refers to the act of treating a disorder, symptom, disease or condition, as the term 'treating' is defined above.

[0059] The term 'treating' or 'treatment' of any disease or disorder refers, in one embodiment, to ameliorating the disease or disorder (i.e., arresting the disease or reducing the manifestation, extent or severity of at least one of the clinical symptoms thereof). In another embodiment 'treating' or 'treatment' refers to ameliorating at

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least one physical parameter, which may not be discernible by the subject. In yet another embodiment, 'treating' or 'treatment' refers to modulating the disease or disorder, either physically, (e.g., stabilization of a discernible symptom), physiologically, (e.g., stabilization of a physical parameter), or both. In a further embodiment, 'treating' or 'treatment' relates to slowing the progression of the disease.

[0060] The term "vectors" also relates to plasmids as well as to viral vectors, such as recombinant viruses, or the nucleic acid encoding the recombinant virus.

[0061] The term "vertebrate cells" means cells derived from animals having vertera structure, including fish, avian, reptilian, amphibian, marsupial, and mammalian species. Preferred cells are derived from mammalian species, and most preferred cells are human cells. Mammalian cells include feline, canine, bovine, equine, caprine, ovine, porcine murine, such as mice and rats, and rabbits.

[0062] The term 'TARGET' or 'TARGETS' means the protein(s) identified in accordance with the assays described herein and determined to be involved in the modulation of mast cell activation . The term TARGET or TARGETS includes and contemplates alternative species forms, isoforms, and variants, such as splice variants, allelic variants, alternate in frame exons, and alternative or premature termination or start sites, including known or recognized isoforms or variants thereof such as indicated in Table 1.

[0063] The term 'neurodegenerative condition' or 'neurodegenerative disease' refers to a disorder caused by the deterioration of neurons. The exact location and type of neurons that are lost may vary between conditions. It is changes in these cells which cause them to function abnormally, eventually bringing about their death. Neurodegenerative diseases include, without limitation, Huntington's disease and other polyglutamine diseases, Alzheimer's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis, Progressive Supranuclear Palsy, Frontotemporal Dementia and Vascular Dementia.

[0064] The term 'polyglutamine disease' refers to a family of dominantly inherited neurodegenerative conditions that are caused by CAG triplet repeat expansions within genes. CAG encodes the amino acid glutamine, and the affected proteins have enlarged tracts of this amino acid. This family includes (without limitation) Huntington's disease, Spinal and bulbar muscular atrophy (SBMA), - Dentatorubral-pallidoluyisian atrophy (DRPLA), Spinocerebellar ataxia 1 (SCA1), Spinocerebellar ataxia 2 (SCA2), Spinocerebellar ataxia 3 (SCA3), Spinocerebellar ataxia 7 (SCA7) and Spinocerebellar ataxia 17 (SCA17).

TARGETS

[0065] Applicants invention is relevant to the treatment, prevention and alleviation of neurodegeneration, neural cell death, including for such diseases as Huntington's disease and other polyglutamine diseases, Alzheimer's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis, Progressive Supranuclear Palsy, Frontotemporal Dementia and Vascular Dementia. Applicant's invention further and particularly relates to

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inhibition of cell death. Applicant's invention is in part based on the TARGETs relationship to cell survival and cell death. The TARGETs are relevant, in particular, to neurodegeneration and HD.

[0066] The present invention provides methods for assaying for drug candidate compounds that modulate cell death, comprising contacting the compound with a cell expressing a cell death mediating polypeptide, such as a mutant form of huntingtin or other aggregating polypeptide whose presence or expression results in or mediates cell death, and determining the relative amount or degree of cell death in the presence and/or absence of the compound. Such methods may also be used to identify target proteins that act to modulate cell death, alternatively they may be used to identify compounds that modulate the expression or activity of target proteins. Exemplary such methods can be designed and determined by the skilled artisan. Particular such exemplary methods are provided herein.

[0067] The present invention is based on the inventor's discovery that the TARGET polypeptides and their encoding nucleic acids, identified as a result of screens described below in the Examples, are factors in neuronal cell death. A reduced activity or expression of the TARGET polypeptides and/or their encoding polynucleotides is causative, correlative or associated with reduced or inhibited cell death. Alternatively, a reduced activity or expression of the TARGET polypeptides and/or their encoding polynucleotides is causative, correlative or associated with enhanced or increased cell death.

[0068] In a particular embodiment of the invention, the TARGET polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID: 46, 47, 49, 51-60, 62-67, 69, 71, 75-82 and 85-90 as listed in Table 1.

[0069] **Table 1**

Target Gene Symbol	GenBank Nucleic Acid Acc #:	SEQ ID NO: DNA	GenBank Protein Acc #	SEQ ID NO: Protein	NAME	Class
ABCF1	NM_001090	1	NP_001081	46	Homo sapiens ATP-binding cassette, sub-family F (GCN20), member 1 (ABCF1), transcript variant 2, mRNA	Transporter
ACADM	NM_000016	2	NP_000007	47	Homo sapiens acyl-Coenzyme A dehydrogenase, C-4 to C-12 straight chain (ACADM), nuclear gene encoding mitochondrial protein, mRNA.	Enzyme
ADH5	NM_000671	3	NP_000662	48	Homo sapiens alcohol	Enzyme

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Target Gene Symbol	GenBank Nucleic Acid Acc #:	SEQ ID NO: DNA	GenBank Protein Acc #	SEQ ID NO: Protein	NAME	Class
					dehydrogenase 5 (class III), chi polypeptide (ADH5), mRNA.	
DUSP7	NM_001947	4	NP_001938	49	Homo sapiens dual specificity phosphatase 7 (DUSP7), mRNA	Phosphatase
ATP1A3	NM_152296	5	NP_689509	50	Homo sapiens ATPase, Na ⁺ /K ⁺ transporting, alpha 3 polypeptide (ATP1A3), mRNA.	Ion Channel
B4GALT7	NM_007255	6	NP_009186	51	Homo sapiens xylosylprotein beta 1,4-galactosyltransferase, polypeptide 7 (galactosyltransferase I) (B4GALT7), mRNA.	Enzyme
CSNK1G1	NM_022048	7	NP_071431	52	Homo sapiens casein kinase 1, gamma 1 (CSNK1G1), transcript variant 2, mRNA.	Kinase
CTSL1	NM_145918	8	NP_666023	53	Homo sapiens cathepsin L (CTSL), transcript variant 2, mRNA.	Protease
DAPK2	NM_014326	9	NP_055141	54	Homo sapiens death-associated protein kinase 2 (DAPK2), mRNA	Kinase
DHCR24	NM_014762	10	NP_055577	55	Homo sapiens 24-dehydrocholesterol reductase (DHCR24), mRNA.	Enzyme
DMPK	NM_004409	11	NP_004400	56	Homo sapiens dystrophin myotonic-protein kinase (DMPK), mRNA.	Kinase
DUSP5	NM_004419	12	NP_004410	57	Homo sapiens dual specificity phosphatase 5 (DUSP5), mRNA.	Phosphatase
FGF17	NM_003867	13	NP_003858	58	Homo sapiens fibroblast growth factor 17 (FGF17), mRNA.	Secreted
C10orf59	NM_018363	14	NP_060833	59	Homo sapiens chromosome 10 open reading frame 59 (C10orf59), mRNA.	Enzyme

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Target Gene Symbol	GenBank Nucleic Acid Acc #:	SEQ ID NO: DNA	GenBank Protein Acc #	SEQ ID NO: Protein	NAME	Class
FZD5	NM_003468	15	NP_003459	60	Homo sapiens frizzled homolog 5 (Drosophila) (FZD5), mRNA	GPCR
GAK	NM_005255	16	NP_005246	61	Homo sapiens cyclin G associated kinase (GAK), mRNA.	Kinase
HSD17B8	NM_014234	17	NP_055049	62	Homo sapiens hydroxysteroid (17-beta) dehydrogenase 8 (HSD17B8), mRNA	Enzyme
KCNA1	NM_133329	18	NP_579875	63	Homo sapiens potassium voltage-gated channel, subfamily G, member 3 (KCNG3), transcript variant 1, mRNA.	Ion Channel
WDR81	NM_152348	19	NP_689561	64	Homo sapiens WD repeat domain 81 (WDR81), mRNA.	Enzyme
DUSP18	NM_152511	20	NP_689724	65	Homo sapiens dual specificity phosphatase 18 (DUSP18), mRNA.	Phosphatase
KCTD8	NM_198353	21	NP_938167	66	Homo sapiens potassium channel tetramerisation domain containing 8 (KCTD8), mRNA.	Ion Channel
CYB5R1	NM_016243	22	NP_057327	67	Homo sapiens cytochrome b5 reductase 1 (CYB5R1), mRNA.	Enzyme
LPL	NM_000237	23	NP_000228	68	Homo sapiens lipoprotein lipase (LPL), mRNA.	Enzyme
MTMR2	NM_016156	24	NP_057240	69	Homo sapiens myotubularin related protein 2 (MTMR2), transcript variant 1, mRNA.	Phosphatase
NDUFS2	NM_004550	25	NP_004541	70	Homo sapiens NADH dehydrogenase (ubiquinone) Fe-S protein 2, 49kDa (NADH-coenzyme Q reductase) (NDUFS2),	Enzyme

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Target Gene Symbol	GenBank Nucleic Acid Acc #:	SEQ ID NO: DNA	GenBank Protein Acc #	SEQ ID NO: Protein	NAME	Class
					mRNA.	
NEK7	NM_133494	26	NP_598001	71	Homo sapiens NIMA (never in mitosis gene a)-related kinase 7 (NEK7), mRNA.	Kinase
P4HB	NM_000918	27	NP_000909	72	Homo sapiens procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), beta polypeptide (protein disulfide isomerase-associated 1) (P4HB), mRNA.	Enzyme
PDE8B	NM_003719	28	NP_003710	73	Homo sapiens phosphodiesterase 8B (PDE8B), transcript variant 1, mRNA.	PDE
PIK3R3	NM_003629	29	NP_003620	74	Homo sapiens phosphoinositide-3-kinase, regulatory subunit 3 (p55, gamma) (PIK3R3), mRNA.	Kinase
PPIG	NM_004792	30	NP_004783	75	Homo sapiens peptidyl-prolyl isomerase G (cyclophilin G) (PPIG), mRNA.	Enzyme
PRMT3	NM_005788	31	NP_005779	76	Homo sapiens HMT1 hnRNP methyltransferase-like 3 (S. cerevisiae) (HRMT1L3), mRNA.	Enzyme
RHOBTB1	NM_198225	32	NP_937868	77	Homo sapiens Rho-related BTB domain containing 1 (RHOBTB1), transcript variant 2, mRNA.	Enzyme
RPS6KB1	NM_003161	33	NP_003152	78	Homo sapiens ribosomal protein S6 kinase, 70kDa, polypeptide 1 (RPS6KB1), mRNA.	Kinase
RPS6KC1	NM_058253	34	NP_490654	79	Homo sapiens ribosomal protein S6 kinase, 52kD, polypeptide 1	Kinase

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Target Gene Symbol	GenBank Nucleic Acid Acc #:	SEQ ID NO: DNA	GenBank Protein Acc #	SEQ ID NO: Protein	NAME	Class
					(RPS6KC1), mRNA.	
DHRS3	NM_004753	35	NP_004744	80	Homo sapiens dehydrogenase/reductase (SDR family) member 3 (DHRS3), mRNA.	Enzyme
SLC20A2	NM_006749	36	NP_006740	81	Homo sapiens solute carrier family 20 (phosphate transporter), member 2 (SLC20A2), mRNA.	Transporter
SLCO1A2	NM_022148	37	NP_071431	82	Homo sapiens cytokine receptor-like factor 2 (CRLF2), transcript variant 1, mRNA.	Transporter
SLC9A1	NM_003047	38	NP_003038	83	Homo sapiens solute carrier family 9 (sodium/hydrogen exchanger), member 1 (antiporter, Na ⁺ /H ⁺ , amiloride sensitive) (SLC9A1), mRNA.	Ion Channel
SMARCA1	NM_139035	39	NP_620604	84	Homo sapiens SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 1 (SMARCA1), transcript variant 2, mRNA.	Enzyme
SPTLC2	NM_004863	40	NP_004854	85	Homo sapiens serine palmitoyltransferase, long chain base subunit 2 (SPTLC2), mRNA.	Enzyme
SRPK2	NM_003138	41	NP_003129	86	Homo sapiens SFRS protein kinase 2 (SRPK2), mRNA.	Kinase
ST3GAL6	NM_006100	42	NP_006091	87	Homo sapiens ST3 beta-galactoside alpha-2,3-sialyltransferase 6 (ST3GAL6), mRNA.	Enzyme
UCK1	NM_031432	43	NP_113620	88	Homo sapiens uridine-cytidine kinase 1 (UCK1), mRNA.	Kinase
UCKL1	NM_017859	44	NP_060329	89	Homo sapiens uridine-	Kinase

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Target Gene Symbol	GenBank Nucleic Acid Acc #:	SEQ ID NO: DNA	GenBank Protein Acc #	SEQ ID NO: Protein	NAME	Class
					cytidine kinase 1-like 1 (UCKL1), mRNA.	
YAP1	NM_006106	45	NP_006097	90	Homo sapiens Yes-associated protein 1, 65kDa (YAP1), mRNA.	Not classified

[0070] A particular embodiment of the invention comprises the transporter TARGETs identified as SEQ ID NOs: 46, 81 and 82. A particular embodiment of the invention comprises the TARGET identified as SEQ ID NO: 90. A further particular embodiment of the invention comprises the enzyme TARGETs identified as SEQ ID NOs: 47, 51, 55, 59, 62, 64, 67, 75, 76, 77, 80, 85 and 87. A further particular embodiment of the invention comprises the protease TARGET identified as SEQ ID NO: 53. A further particular embodiment of the invention comprises the kinase TARGETs identified as SEQ ID NOs: 52, 54, 56, 71, 78, 79, 86, 88 and 89. A further particular embodiment of the invention comprises the GPCR TARGETs identified as SEQ ID NO: 60. A further particular embodiment of the invention comprises the ion channel TARGETs identified as SEQ ID NOs: 63 and 66. A further particular embodiment of the invention comprises the secreted TARGETs identified as SEQ ID NO: 58. A further particular embodiment of the invention comprises the phosphatase TARGETs identified as SEQ ID NOs: 49, 57, 65 and 69.

[0071] Confirming the validity of the screens used herein and the TARGETs, certain TARGET polypeptides, SEQ ID NOs: 48, 50, 61, 68, 70, 72, 73, 74, 83 and 84, have been identified as huntingtin interacting proteins using yeast two-hybrid screening or affinity pull down (Kaltenbach, L.S. *et al* (2007) PLoS Genet 3(5):689-708). Specific inhibition of these particular TARGET polypeptides and/or inhibition of cell death thereby has not been described or demonstrated.

[0072] In one aspect, the present invention relates to a method for assaying for drug candidate compounds that inhibit cell death, comprising contacting the compound with a polypeptide comprising an amino acid sequence of SEQ ID NO: 46, 47, 49, 51-60, 62-67, 69, 71, 75-82 and 85-90, or a fragment thereof, under conditions that allow said polypeptide to bind to the compound, and detecting the formation of a complex between the polypeptide and the compound. One particular means of measuring the complex formation is to determine the binding affinity of said compound to said polypeptide.

[0073] More particularly, the invention relates to a method for identifying an agent that modulates cell death, the method comprising:

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- (a) contacting a population of mammalian cells with one or more compound that exhibits binding affinity for a TARGET polypeptide, or fragment thereof, and
- (b) measuring a compound-polypeptide property related to cell death.

[0074] In a further aspect, the present invention relates to a method for assaying for drug candidate compounds that inhibit cell death, comprising contacting the compound with a polypeptide comprising an amino acid sequence of SEQ ID NO: 46, 47, 49, 51-60, 62-67, 69, 71, 75-82 and 85-90, or a fragment thereof, under conditions that allow said compound to modulate the activity or expression of the polypeptide, and determining the activity or expression of the polypeptide. One particular means of measuring the activity or expression of the polypeptide is to determine the amount of said polypeptide using a polypeptide binding agent, such as an antibody, or to determine the activity of said polypeptide in a biological or biochemical measure, for instance the amount of phosphorylation of a target of a kinase polypeptide. A further means of measuring the activity or expression of the polypeptide is to determine the amount or extent of cell death or cell death mediators.

[0075] The compound-polypeptide property referred to above is related to the expression and/or activity of the TARGET, and is a measurable phenomenon chosen by the person of ordinary skill in the art. The measurable property may be, for example, the binding affinity for a peptide domain of the polypeptide TARGET or the enzyme activity of the polypeptide TARGET or the level of any one of a number of biochemical markers including markers for cell death.

[0076] Depending on the choice of the skilled artisan, the present assay method may be designed to function as a series of measurements, each of which is designed to determine whether the drug candidate compound is indeed acting on the polypeptide to thereby modulate neuronal cell death, and particularly the Huntington Disease phenotype. For example, an assay designed to determine the binding affinity of a compound to the polypeptide, or fragment thereof, may be necessary, but may be one exemplary assay or one assay among additional and more particular or specific assays to ascertain whether the test compound would be useful for modulating neuronal cell death, including particularly the Huntington Disease phenotype, when administered to a subject.

[0077] Suitable controls should always be in place to insure against false positive readings. In a particular embodiment of the present invention the screening method comprises the additional step of comparing the compound to a suitable control. In one embodiment, the control may be a cell or a sample that has not been in contact with the test compound. In an alternative embodiment, the control may be a cell that does not express the TARGET; for example in one aspect of such an embodiment the test cell may naturally express the TARGET and the control cell may have been contacted with an agent, e.g. an siRNA, which inhibits or prevents expression of the TARGET. Alternatively, in another aspect of such an embodiment, the cell in its native state does not express the TARGET and the test cell has been engineered so as to express the TARGET,

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so that in this embodiment, the control could be the untransformed native cell. The control may also or alternatively utilize a known mediator of cell death. Whilst exemplary controls are described herein, this should not be taken as limiting; it is within the scope of a person of skill in the art to select appropriate controls for the experimental conditions being used.

[0078] The order of taking these measurements is not believed to be critical to the practice of the present invention, which may be practiced in any order. For example, one may first perform a screening assay of a set of compounds for which no information is known respecting the compounds' binding affinity for the polypeptide. Alternatively, one may screen a set of compounds identified as having binding affinity for a polypeptide domain, or a class of compounds identified as being an inhibitor of the polypeptide. However, for the present assay to be meaningful to the ultimate use of the drug candidate compounds, a measurement of modulation of neuronal cell death, and particularly of the Huntington Disease phenotype, is preferred. The means by which to measure, assess, or determine neuronal cell death, or activation of a cell death pathway, may be selected or determined by the skilled artisan. Validation studies including controls and measurements of binding affinity to the polypeptides or modulation of activity or expression of the polypeptides of the invention are nonetheless useful in identifying a compound useful in any therapeutic or diagnostic application.

[0079] Analogous approaches based on art-recognized methods and assays may be applicable with respect to the TARGETS and compounds in any of various disease(s) characterized by neurodegeneration and / or neural cell death. An assay or assays may be designed to confirm that the test compound, having binding affinity for the TARGET, inhibits neurodegeneration and / or neural cell death.

[0080] The present assay method may be practiced *in vitro*, using one or more of the TARGET proteins, or fragments thereof, including monomers, portions or subunits of polymeric proteins, peptides, oligopeptides and enzymatically active portions thereof.

[0081] The binding affinity of a compound with the polypeptide TARGET can be measured by methods known in the art, such as using surface plasmon resonance biosensors (Biacore®), by saturation binding analysis with a labeled compound (for example, Scatchard and Lindmo analysis), by differential UV spectrophotometer, fluorescence polarization assay, Fluorometric Imaging Plate Reader (FLIPR®) system, Fluorescence resonance energy transfer, and Bioluminescence resonance energy transfer. The binding affinity of compounds can also be expressed in dissociation constant (Kd) or as IC₅₀ or EC₅₀. The IC₅₀ represents the concentration of a compound that is required for 50% inhibition of binding of another ligand to the polypeptide. The EC₅₀ represents the concentration required for obtaining 50% of the maximum effect in any assay that measures TARGET function. The dissociation constant, Kd, is a measure of how well a ligand binds to the polypeptide, it is equivalent to the ligand concentration required to saturate exactly half of the binding-sites on the polypeptide. Compounds with a high affinity binding have low Kd, IC₅₀ and EC₅₀ values, for example, in

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the range of 100 nM to 1 pM; a moderate- to low-affinity binding relates to high K_d , IC_{50} and EC_{50} values, for example in the micromolar range.

[0082] The present assay method may also be practiced in a cellular assay. A host cell expressing the TARGET, or fragment(s) thereof, can be a cell with endogenous expression or a cell modified to express or over-expressing the TARGET, for example, by transduction. When the endogenous expression of the polypeptide is not sufficient to determine a baseline that can easily be measured, one may use host cells that over-express TARGET. Over-expression has the advantage that the level of the TARGET substrate end-products is higher than the activity level by endogenous expression. Accordingly, measuring such levels using presently available techniques is easier. Alternatively, a non-endogenous form of TARGET may be expressed or overexpressed in a cell and utilized in screening.

[0083] The assay method may be based on the particular expression or activity of the TARGET polypeptide, including but not limited to an enzyme activity. Thus, assays for the enzyme TARGETs identified as SEQ ID NOs: 47, 48, 51, 55, 59, 62, 64, 67, 68, 70, 72, 75, 76, 77, 80, 84, 85 and 87 may be based on enzymatic activity or enzyme expression. Assays for the protease TARGET identified as SEQ ID NOs: 53 may be based on protease activity or expression. Assays for the kinase TARGETs identified as SEQ ID NOs: 52, 54, 56, 61, 71, 74, 78, 79, 86, 88 and 89 may be based on kinase activity or expression, including but not limited to phosphorylation of a kinase target. Assays for the phosphatase TARGETs identified as SEQ ID NOs: 49, 57, 65 may be based on phosphatase activity or expression, including but not limited to dephosphorylation of a phosphatase target. Assays for the GPCR TARGETs identified as SEQ ID NO: 60 may be based on GPCR activity or expression, including downstream mediators or activators. Assays for the phosphodiesterase (PDE) TARGET identified as SEQ ID NO: 73 may be based on PDE activity or expression. Assays for the secreted TARGETs identified as SEQ ID NOs: 58 may utilize activity or expression in soluble culture media or secreted activity. Assays for ion channel TARGETs identified as SEQ ID NOs: 50, 63, 66 and 83 may use techniques well known to those of skill in the art including classical patch clamping, high-throughput fluorescence based or tracer based assays which measure the ability of a compound to open or close an ion channel thereby changing the concentration of fluorescent dyes or tracers across a membrane or within a cell. The measurable phenomenon, activity or property may be selected or chosen by the skilled artisan. The person of ordinary skill in the art may select from any of a number of assay formats, systems or design one using his knowledge and expertise in the art.

[0084] The present inventors have identified certain target proteins and their encoding nucleic acids by screening recombinant adenoviruses mediating the expression of a library of shRNAs, referred to herein as 'Ad-siRNAs'. This type of library is a screen in which siRNA molecules are transduced into cells by recombinant adenoviruses, which siRNA molecules inhibit or repress the expression of a specific gene as well as expression and activity of the corresponding gene product in a cell. Each siRNA in a viral vector

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corresponds to a specific natural gene. By identifying a siRNA or shRNA that regulates cell death, for example as described in the examples herein, a direct correlation can be drawn between the specific gene expression and the pathway for regulating cell death and / or neurodegeneration. The TARGET genes identified using the knock-down library (the protein expression products thereof herein referred to as “TARGET” polypeptides) are then used in the present inventive method for identifying compounds that can be used to in the treatment of diseases associated with the abnormal protein aggregation. The knock down (KD) target sequences, identified in the Ad-siRNA screens more particularly described herein, include those set out below in Table 2 SEQ ID NOs: 91-135) and shRNA compounds comprising the sequences listed in Table 2 have been shown herein to inhibit the expression and/or activity of these TARGET genes and the examples herein confirm the role of the TARGETS in the pathway modulating the cell death in neurodegenerative conditions.

[0085]

Table 2

Exemplary KD target sequences useful in the practice of the present expression-inhibitory agent invention

HIT REF	GeneSymbol	19-mer	SEQ ID NO
1	ABCF1	AATCGACCCACACAGAAGTTC	91
2	ACADM	AACCAGACCTGTAGTAGCTGC	92
3	ADH5	AAGGGCCAAAGAGTTTGGAGC	93
4	DUSP7	ACAGAGTACTCTGAGCACTGC	94
5	ATP1A3	AAGCAGGCAGCTGACATGATC	95
6	B4GALT7	AACATCATGTTGGACTGTGAC	96
7	CSNK1G1	AATCACGTGCTCCACAGCTTC	97
8	CTSL1	AAGTGGAAGGCGATGCACAAC	98
9	DAPK2	AAATTGTGAACTACGAGCCCC	99
10	DHCR24	ACAGGCATCGAGTCATCATCC	100
11	DMPK	AAGATCATGAACAAGTGGGAC	101
12	DUSP5	AAACCAGTGGTAAATGTCAGC	102
13	FGF17	ACGGAGATCGTGCTGGAGAAC	103
14	C10orf59	ACATTCACAGGTACCAAGTGC	104
15	FZD5	AAGCTCATGATCCGCATCGGC	105
16	GAK	AAGATCTTCTACCAGACGTGC	106
17	HSD17B8	ACATGGGATCCGCTGTAAGTC	107
18	KCNA1	ACGAGTACTTCTTCGACCGGC	108

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HIT REF	GeneSymbol	19-mer	SEQ ID NO
19	WDR81	AACAAGATTGGCGTCTGCTCC	109
20	DUSP18	AACTCACGTCTCTGTGACTTC	110
21	KCTD8	AAGTACACGTCCCGCTTCTAC	111
22	CYB5R1	ACGACTGCTAGACAAGACGAC	112
23	LPL	AATGTATGAGAGTTGGGTGCC	113
24	MTMR2	ACTTTGTGATACATACCCTGC	114
25	NDUFS2	AAGTTGTATACTGAGGGCTAC	115
26	NEK7	AATGGATGCCAAAGCACGTGC	116
27	P4HB	ACTTCCAACAGTGACGTGTTC	117
28	PDE8B	ACCAGTGATCTTGTGGAGGC	118
29	PIK3R3	AAATGGATCCTCCAGCTCTTC	119
30	PPIG	AAGAACACCACCAGGAAGATC	120
31	PRMT3	AAGAATTGCCACAACAGGGTC	121
32	RHOBTB1	ACAACCAGGAATACTTCGAGC	122
33	RPS6KB1	AACTCAATTTGCCTCCCTACC	123
34	RPS6KC1	AACACTATGCACAGGAGGATC	124
35	DHRS3	AAGCATACTTCCACAGGCTGC	125
36	SLC20A2	AACAGTTACACCTGCTACACC	126
37	SLCO1A2	AAGAGTATTTGCTGGCATTCC	127
38	SLC9A1	AAGAGATCCACACACAGTTCC	128
39	SMARCA1	AACTACGCAGTGGATGCCTAC	129
40	SPTLC2	ACCAGGTATTTTCAGGAGACGC	130
41	SRPK2	AATCCAACATCAAGGCCTCC	131
42	ST3GAL6	AAACTGCAGAGTTGTGATCTC	132
43	UCK1	AACCTGATCGTGCAGCACATC	133
44	UCKL1	AAGCAAGCGTACCATCTACAC	134
45	YAP1	CTTAACAGTGGCACCTATCAC	135

[0086] Table 1 lists the TARGETS identified using applicants' knock-down library in the cell death assay described below, including the class of polypeptides identified. TARGETS have been identified in polypeptide

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classes including kinase, protease, enzyme, ion channel, GPCR, phosphodiesterase and phosphatase, for instance.

[0087] Specific methods to determine the activity of a kinase, such as the TARGETs represented by SEQ ID NOs: 52, 54, 56, 61, 71, 74, 78, 79, 86, 88 and 89, by measuring the phosphorylation of a substrate by the kinase, which measurements are performed in the presence or absence of a compound, are well known in the art.

[0088] Ion channels are membrane protein complexes and their function is to facilitate the diffusion of ions across biological membranes. Membranes, or phospholipid bilayers, build a hydrophobic, low dielectric barrier to hydrophilic and charged molecules. Ion channels provide a high conducting, hydrophilic pathway across the hydrophobic interior of the membrane. The activity of an ion channel can be measured using classical patch clamping. High-throughput fluorescence-based or tracer-based assays are also widely available to measure ion channel activity. These fluorescent-based assays screen compounds on the basis of their ability to either open or close an ion channel thereby changing the concentration of specific fluorescent dyes across a membrane. In the case of the tracer-based assay, the changes in concentration of the tracer within and outside the cell are measured by radioactivity measurement or gas absorption spectrometry.

[0089] Specific methods to determine the inhibition by the compound by measuring the cleavage of the substrate by the polypeptide, which is a protease, are well known in the art. The TARGET represented by SEQ ID NO: 53 is a protease. Classically, substrates are used in which a fluorescent group is linked to a quencher through a peptide sequence that is a substrate that can be cleaved by the target protease. Cleavage of the linker separates the fluorescent group and quencher, giving rise to an increase in fluorescence.

[0090] G-protein coupled receptors (GPCR) are capable of activating an effector protein, resulting in changes in second messenger levels in the cell. The TARGET represented by SEQ ID NO: 60 is a GPCR. The activity of a GPCR can be measured by measuring the activity level of such second messengers. Two important and useful second messengers in the cell are cyclic AMP (cAMP) and Ca^{2+} . The activity levels can be measured by methods known to persons skilled in the art, either directly by ELISA or radioactive technologies or by using substrates that generate a fluorescent or luminescent signal when contacted with Ca^{2+} or indirectly by reporter gene analysis. The activity level of the one or more secondary messengers may typically be determined with a reporter gene controlled by a promoter, wherein the promoter is responsive to the second messenger. Promoters known and used in the art for such purposes are the cyclic-AMP responsive promoter that is responsive for the cyclic-AMP levels in the cell, and the NF-AT responsive promoter that is sensitive to cytoplasmic Ca^{2+} -levels in the cell. The reporter gene typically has a gene product that is easily detectable. The reporter gene can either be stably infected or transiently transfected in the host cell. Useful reporter genes are alkaline phosphatase, enhanced green fluorescent protein, destabilized green fluorescent protein, luciferase and β -galactosidase.

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[0091] It should be understood that the cells expressing the polypeptides, may be cells naturally expressing the polypeptides, or the cells may be may be transfected to express the polypeptides, as described above. Also, the cells may be transduced to overexpress the polypeptide, or may be transfected to express a non-endogenous form of the polypeptide, which can be differentially assayed or assessed. In one particular embodiment the methods of the present invention further comprise the step of contacting the population of cells with an agonist of the polypeptide. This is useful in methods wherein the expression of the polypeptide in a certain chosen population of cells is too low for a proper detection of its activity. By using an agonist the polypeptide may be triggered, enabling a proper read-out if the compound inhibits the polypeptide

[0092] The population of cells may be exposed to the compound or the mixture of compounds through different means, for instance by direct incubation in the medium, or by nucleic acid transfer into the cells. Such transfer may be achieved by a wide variety of means, for instance by direct transfection of naked isolated DNA, or RNA, or by means of delivery systems, such as recombinant vectors. Other delivery means such as liposomes, or other lipid-based vectors may also be used. Particularly, the nucleic acid compound is delivered by means of a (recombinant) vector such as a recombinant virus.

[0093] For high-throughput purposes, libraries of compounds may be used such as antibody fragment libraries, peptide phage display libraries, peptide libraries (for example, LOPAPTM, Sigma Aldrich), lipid libraries (BioMol), synthetic compound libraries (for example, LOPACTM, Sigma Aldrich) or natural compound libraries (Specs, TimTec).

[0094] Particular drug candidate compounds are low molecular weight compounds. Low molecular weight compounds, for example with a molecular weight of 500 Dalton or less, are likely to have good absorption and permeation in biological systems and are consequently more likely to be successful drug candidates than compounds with a molecular weight above 500 Dalton (Lipinski *et al.*, 2001)). Peptides comprise another particular class of drug candidate compounds. Peptides may be excellent drug candidates and there are multiple examples of commercially valuable peptides such as fertility hormones and platelet aggregation inhibitors. Natural compounds are another particular class of drug candidate compound. Such compounds are found in and extracted from natural sources, and which may thereafter be synthesized. The lipids are another particular class of drug candidate compound.

[0095] Another particular class of drug candidate compounds is an antibody. The present invention also provides antibodies directed against a TARGET. These antibodies may be endogenously produced to bind to the TARGET within the cell, or added to the tissue to bind to TARGET polypeptide present outside the cell. These antibodies may be monoclonal antibodies or polyclonal antibodies. The present invention includes chimeric, single chain, and humanized antibodies, as well as Fab fragments and the products of a Fab expression library, and Fv fragments and the products of an Fv expression library. In another embodiment, the

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compound may be a nanobody, the smallest functional fragment of naturally occurring single-domain antibodies (Cortez-Retamozo *et al.* 2004).

[0096] In certain embodiments, polyclonal antibodies may be used in the practice of the invention. The skilled artisan knows methods of preparing polyclonal antibodies. Polyclonal antibodies can be raised in a mammal, for example, by one or more injections of an immunizing agent and, if desired, an adjuvant. Typically, the immunizing agent and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections. Antibodies may also be generated against the intact TARGET protein or polypeptide, or against a fragment, derivatives including conjugates, or other epitope of the TARGET protein or polypeptide, such as the TARGET embedded in a cellular membrane, or a library of antibody variable regions, such as a phage display library.

[0097] It may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. Examples of adjuvants that may be employed include Freund's complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate). One skilled in the art without undue experimentation may select the immunization protocol.

[0098] In some embodiments, the antibodies may be monoclonal antibodies. Monoclonal antibodies may be prepared using methods known in the art. The monoclonal antibodies of the present invention may be "humanized" to prevent the host from mounting an immune response to the antibodies. A "humanized antibody" is one in which the complementarity determining regions (CDRs) and/or other portions of the light and/or heavy variable domain framework are derived from a non-human immunoglobulin, but the remaining portions of the molecule are derived from one or more human immunoglobulins. Humanized antibodies also include antibodies characterized by a humanized heavy chain associated with a donor or acceptor unmodified light chain or a chimeric light chain, or vice versa. The humanization of antibodies may be accomplished by methods known in the art (see, for example, Mark and Padlan, (1994) "Chapter 4. Humanization of Monoclonal Antibodies", The Handbook of Experimental Pharmacology Vol. 113, Springer-Verlag, New York). Transgenic animals may be used to express humanized antibodies.

[0099] Human antibodies can also be produced using various techniques known in the art, including phage display libraries (Hoogenboom and Winter, (1991) *J. Mol. Biol.* 227:381-8; Marks *et al.* (1991). *J. Mol. Biol.* 222:581-97). The techniques of Cole *et al.* and Boerner *et al.* are also available for the preparation of human monoclonal antibodies (Cole, *et al.* (1985) *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77; Boerner, *et al.* (1991). *J. Immunol.*, 147(1):86-95).

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[0100] Techniques known in the art for the production of single chain antibodies can be adapted to produce single chain antibodies to the TARGET polypeptides and proteins of the present invention. The antibodies may be monovalent antibodies. Methods for preparing monovalent antibodies are well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain cross-linking. Alternatively, the relevant cysteine residues are substituted with another amino acid residue or are deleted so as to prevent cross-linking.

[0101] Bispecific antibodies are monoclonal, particularly human or humanized, antibodies that have binding specificities for at least two different antigens and particularly for a cell-surface protein or receptor or receptor subunit. In the present case, one of the binding specificities is for one domain of the TARGET, while the other one is for another domain of the same or different TARGET.

[0102] Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, (1983) Nature 305:537-9). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. Affinity chromatography steps usually accomplish the purification of the correct molecule. Similar procedures are disclosed in Traunecker, *et al.* (1991) EMBO J. 10:3655-9.

[0103] In a further embodiment the present invention relates to a method for identifying a compound that modulates cell death comprising:

- a) contacting a compound with a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 47, 49, 51-60, 62-67, 69, 71, 75-82 and 85-90;
- b) determining the binding affinity of the compound to the polypeptide;
- c) contacting a population of mammalian cells expressing said polypeptide with the compound that exhibits a binding affinity of at least 10 micromolar; and
- d) identifying the compound that modulates the expression of mutant huntingtin protein.

[0104] The present invention further relates to a method for identifying a compound that modulates cell death, comprising:

- a) contacting a compound with a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 47, 49, 51-60, 62-67, 69, 71, 75-82 and 85-90;
- b) determining the ability of the compound inhibit the expression or activity of the polypeptide;
- c) contacting a population of mammalian cells expressing said polypeptide with the compound that significantly inhibits the expression or activity of the polypeptide ; and
- d) identifying the compound that modulates the expression of the mutant huntingtin protein.

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e) identifying the compound that modulates the phenotypic effect of the expression of the mutant huntingtin protein, in particular cell death caused by mutant huntingtin.

[0105] In particular aspects of the invention, , the ability of the compound to modulate cell death may be measured by methods well known to those of skill in the art, including (without limitation) using propidium iodide exclusion or annexin-V staining to quantify the number of dead cells.

[0106] According to another particular embodiment, the assay method uses a drug candidate compound identified as having a binding affinity for a TARGET, and/or has already been identified as having down-regulating activity such as antagonist activity vis-à-vis one or more TARGET.

[0107] Candidate compound or agents may be validated or rescreened in the huntingtin cell death assay. Other assays for confirming activity in ameliorating, preventing or treating HD or other neurodegenerative diseases include neural cell death assays, assays for apoptosis, and animal models for HD or neurodegenerative diseases such as R6/2 (Mangiarini *et al.*, 1996) and YAC128 (Slow *et al.*, 2003)

[0108] The present invention further relates to a method for modulating the Huntington Disease phenotype comprising contacting mammalian cells with an expression inhibitory agent comprising a polyribonucleotide sequence that complements at least about 15 to 30, particularly at least 17 to 30, most particularly at least 17 to 25 contiguous nucleotides of the nucleotide sequence selected from the group consisting of SEQ ID NO: 1, 2, 4, 6-15, 17-22, 24, 26, 30-37, 40-45.

[0109] Another aspect of the present invention relates to a method for modulating the Huntington Disease phenotype, comprising by contacting mammalian cells with an expression-inhibiting agent that inhibits the translation in the cell of a polyribonucleotide encoding a TARGET polypeptide. A particular embodiment relates to a composition comprising a polynucleotide including at least one antisense strand that functions to pair the agent with the TARGET mRNA, and thereby down-regulate or block the expression of TARGET polypeptide. The inhibitory agent particularly comprises antisense polynucleotide, a ribozyme, and a small interfering RNA (siRNA), wherein said agent comprises a nucleic acid sequence complementary to, or engineered from, a naturally-occurring polynucleotide sequence selected from the group consisting of SEQ ID NO: 1, 2, 4, 6-15, 17-22, 24, 26, 30-37, 40-45.

[0110] A special embodiment of the present invention relates to a method wherein the expression-inhibiting agent is selected from the group consisting of antisense RNA, antisense oligodeoxynucleotide (ODN), a ribozyme that cleaves the polyribonucleotide coding for SEQ ID NO: 46, 47, 49, 51-60, 62-67, 69, 71, 75-82 and 85-90, a small interfering RNA (siRNA, particularly shRNA,) that is sufficiently homologous to a portion of the polyribonucleotide corresponding to SEQ ID NO: 1, 2, 4, 6-15, 17-22, 24, 26, 30-37, 40-45, such that the antisense RNA, ODN, ribozyme, particularly siRNA, particularly shRNA, interferes with the translation of the TARGET polyribonucleotide to the TARGET polypeptide.

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[0111] In one embodiment, the TARGET is a transporter, therefore the ribozyme may cleave a polynucleotide coding for SEQ ID NO: 46, 81 or 82 or the siRNA or shRNA is homologous to a portion of the polyribonucleotide corresponding to SEQ ID NO: 1, 36 or 37, exemplary oligonucleotide sequences include SEQ ID NO: 91, 126 and 127. In a further embodiment, the TARGET is an enzyme, therefore the ribozyme may cleave a polynucleotide coding for SEQ ID NO: 47, 51, 55, 59, 62, 64, 67, 75, 76, 77, 80, 85 or 87 or the siRNA or shRNA is homologous to a portion of the polyribonucleotide corresponding to SEQ ID NO: 2, 6, 10, 14, 17, 19, 22, 30, 31, 32, 35, 40 or 42, exemplary oligonucleotide sequences include SEQ ID NO: 92, 96, 100, 104, 107, 109, 112, 120, 121, 122, 125, 130 and 132. In a further embodiment, the TARGET is a protease, therefore the ribozyme may cleave a polynucleotide coding for SEQ ID NO: 53 or the siRNA or shRNA is homologous to a portion of the polyribonucleotide corresponding to SEQ ID NO: 8, exemplary oligonucleotide sequences include SEQ ID NO: 98. In a further embodiment, the TARGET is a kinase, therefore the ribozyme may cleave a polynucleotide coding for SEQ ID NO: 52, 54, 56, 71, 78, 79, 86, 88 or 89 or the siRNA or shRNA is homologous to a portion of the polyribonucleotide corresponding to SEQ ID NO: 7, 9, 11, 26, 33, 34, 41, 43 or 44, exemplary oligonucleotide sequences include SEQ ID NO: 97, 99, 101, 116, 123, 124, 131, 133 and 134. In a further embodiment, the TARGET is a GPCR, therefore the ribozyme may cleave a polynucleotide coding for SEQ ID NO: 60 or the siRNA or shRNA is homologous to a portion of the polyribonucleotide corresponding to SEQ ID NO: 15, exemplary oligonucleotide sequences include SEQ ID NO: 105. In a further embodiment, the TARGET is an ion channel, therefore the ribozyme may cleave a polynucleotide coding for SEQ ID NO: 63 or 66 or the siRNA or shRNA is homologous to a portion of the polyribonucleotide corresponding to SEQ ID NO: 18 or 21, exemplary oligonucleotide sequences include SEQ ID NO: 108 and 111. In a further embodiment, the TARGET is a secreted protein, therefore the ribozyme may cleave a polynucleotide coding for SEQ ID NO: 58 or the siRNA or shRNA is homologous to a portion of the polyribonucleotide corresponding to SEQ ID NO: 13, exemplary oligonucleotide sequences include SEQ ID NO: 103.

[0112] Another embodiment of the present invention relates to a method wherein the expression-inhibiting agent is a nucleic acid expressing the antisense RNA, antisense oligodeoxynucleotide (ODN), a ribozyme that cleaves the polyribonucleotide corresponding to SEQ ID 46, 47, 49, 51-60, 62-67, 69, 71, 75-82 and 85-90, a small interfering RNA (siRNA, particularly shRNA,) that is sufficiently complementary to a portion of the polyribonucleotide corresponding to SEQ ID NO: 1, 2, 4, 6-15, 17-22, 24, 26, 30-37, 40-45, such that the antisense RNA, ODN, ribozyme, particularly siRNA, particularly shRNA, interferes with the translation of the TARGET polyribonucleotide to the TARGET polypeptide. Particularly the expression-inhibiting agent is an antisense RNA, ribozyme, antisense oligodeoxynucleotide, or siRNA, particularly shRNA, comprising a polyribonucleotide sequence that complements at least about 17 to about 30 contiguous nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID NO: 1, 2, 4, 6-15, 17-22, 24, 26, 30-37, 40-

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45. More particularly, the expression-inhibiting agent is an antisense RNA, ribozyme, antisense oligodeoxynucleotide, or siRNA, particularly shRNA, comprising a polyribonucleotide sequence that complements at least 15 to about 30, particularly at least 17 to about 30, most particularly at least 17 to about 25 contiguous nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID NO: 1, 2, 4, 6-15, 17-22, 24, 26, 30-37, 40-45. A special embodiment comprises a polyribonucleotide sequence that complements a polynucleotide sequence selected from the group consisting of SEQ ID NO: 91, 92, 94, 96-105, 107-112, 114, 116, 120-127 and 130-135.

[0113] The down regulation of gene expression using antisense nucleic acids can be achieved at the translational or transcriptional level. Antisense nucleic acids of the invention are particularly nucleic acid fragments capable of specifically hybridizing with all or part of a nucleic acid encoding a TARGET polypeptide or the corresponding messenger RNA. In addition, antisense nucleic acids may be designed which decrease expression of the nucleic acid sequence capable of encoding a TARGET polypeptide by inhibiting splicing of its primary transcript. Any length of antisense sequence is suitable for practice of the invention so long as it is capable of down-regulating or blocking expression of a nucleic acid coding for a TARGET. Particularly, the antisense sequence is at least about 15-30, and particularly at least 17 nucleotides in length. The preparation and use of antisense nucleic acids, DNA encoding antisense RNAs and the use of oligo and genetic antisense is known in the art.

[0114] One embodiment of expression-inhibitory agent is a nucleic acid that is antisense to a nucleic acid comprising SEQ ID NO: 1, 2, 4, 6-15, 17-22, 24, 26, 30-37, 40-45, for example, an antisense nucleic acid (for example, DNA) may be introduced into cells *in vitro*, or administered to a subject *in vivo*, as gene therapy to inhibit cellular expression of nucleic acids comprising SEQ ID NO: 1, 2, 4, 6-15, 17-22, 24, 26, 30-37, 40-45. Antisense oligonucleotides may comprise a sequence containing from about 15 to about 100 nucleotides, more particularly from 15 to 30 nucleotides, and most particularly, from about 17 to about 25 nucleotides. Antisense nucleic acids may be prepared from about 15 to about 30 contiguous nucleotides selected from the sequences of SEQ ID NO: 1, 2, 4, 6-15, 17-22, 24, 26, 30-37, 40-45, expressed in the opposite orientation.

[0115] The skilled artisan can readily utilize any of several strategies to facilitate and simplify the selection process for antisense nucleic acids and oligonucleotides effective in inhibition of TARGET and/or Huntington Disease phenotype modulation. Predictions of the binding energy or calculation of thermodynamic indices between an oligonucleotide and a complementary sequence in an mRNA molecule may be utilized (Chiang et al. (1991) J. Biol. Chem. 266:18162-18171; Stull *et al.* (1992) Nucl. Acids Res. 20:3501-3508). Antisense oligonucleotides may be selected on the basis of secondary structure (Wickstrom *et al* (1991) in Prospects for Antisense Nucleic Acid Therapy of Cancer and AIDS, Wickstrom, ed., Wiley-Liss, Inc., New York, pp. 7-24; Lima *et al.* (1992) Biochem. 31:12055-12061). Schmidt and Thompson (U.S. Patent 6,416,951) describe a method for identifying a functional antisense agent comprising hybridizing an RNA with an oligonucleotide

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and measuring in real time the kinetics of hybridization by hybridizing in the presence of an intercalation dye or incorporating a label and measuring the spectroscopic properties of the dye or the label's signal in the presence of unlabelled oligonucleotide. In addition, any of a variety of computer programs may be utilized which predict suitable antisense oligonucleotide sequences or antisense targets utilizing various criteria recognized by the skilled artisan, including for example the absence of self-complementarity, the absence hairpin loops, the absence of stable homodimer and duplex formation (stability being assessed by predicted energy in kcal/mol). Examples of such computer programs are readily available and known to the skilled artisan and include the OLIGO 4 or OLIGO 6 program (Molecular Biology Insights, Inc., Cascade, CO) and the Oligo Tech program (Oligo Therapeutics Inc., Wilsonville, OR). In addition, antisense oligonucleotides suitable in the present invention may be identified by screening an oligonucleotide library, or a library of nucleic acid molecules, under hybridization conditions and selecting for those which hybridize to the target RNA or nucleic acid (see for example U.S. Patent 6,500,615). Mishra and Toulme have also developed a selection procedure based on selective amplification of oligonucleotides that bind target (Mishra *et al* (1994) Life Sciences 317:977-982). Oligonucleotides may also be selected by their ability to mediate cleavage of target RNA by RNase H, by selection and characterization of the cleavage fragments (Ho *et al* (1996) Nucl Acids Res 24:1901-1907; Ho *et al* (1998) Nature Biotechnology 16:59-630). Generation and targeting of oligonucleotides to GGGA motifs of RNA molecules has also been described (U.S. Patent 6,277,981).

[0116] The antisense nucleic acids are particularly oligonucleotides and may consist entirely of deoxyribonucleotides, modified deoxyribonucleotides, or some combination of both. The antisense nucleic acids can be synthetic oligonucleotides. The oligonucleotides may be chemically modified, if desired, to improve stability and/or selectivity. Specific examples of some particular oligonucleotides envisioned for this invention include those containing modified backbones, for example, phosphorothioates, phosphotriesters, methyl phosphonates, short chain alkyl or cycloalkyl intersugar linkages or short chain heteroatomic or heterocyclic intersugar linkages. Since oligonucleotides are susceptible to degradation by intracellular nucleases, the modifications can include, for example, the use of a sulfur group to replace the free oxygen of the phosphodiester bond. This modification is called a phosphorothioate linkage. Phosphorothioate antisense oligonucleotides are water soluble, polyanionic, and resistant to endogenous nucleases. In addition, when a phosphorothioate antisense oligonucleotide hybridizes to its TARGET site, the RNA-DNA duplex activates the endogenous enzyme ribonuclease (RNase) H, which cleaves the mRNA component of the hybrid molecule. Oligonucleotides may also contain one or more substituted sugar moieties. Particular oligonucleotides comprise one of the following at the 2' position: OH, SH, SCH₃, F, OCN, 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. Similar

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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.

[0117] In addition, antisense oligonucleotides with phosphoramidite and polyamide (peptide) linkages can be synthesized. These molecules should be very resistant to nuclease degradation. Furthermore, chemical groups can be added to the 2' carbon of the sugar moiety and the 5 carbon (C-5) of pyrimidines to enhance stability and facilitate the binding of the antisense oligonucleotide to its TARGET site. Modifications may include 2'-deoxy, O-pentoxo, O-propoxo, O-methoxy, fluoro, methoxyethoxy phosphorothioates, modified bases, as well as other modifications known to those of skill in the art.

[0118] Another type of expression-inhibitory agent that reduces the levels of TARGETS is the ribozyme. Ribozymes are catalytic RNA molecules (RNA enzymes) that have separate catalytic and substrate binding domains. The substrate binding sequence combines by nucleotide complementarity and, possibly, non-hydrogen bond interactions with its TARGET sequence. The catalytic portion cleaves the TARGET RNA at a specific site. The substrate domain of a ribozyme can be engineered to direct it to a specified mRNA sequence. The ribozyme recognizes and then binds a TARGET mRNA through complementary base pairing. Once it is bound to the correct TARGET site, the ribozyme acts enzymatically to cut the TARGET mRNA. Cleavage of the mRNA by a ribozyme destroys its ability to direct synthesis of the corresponding polypeptide. Once the ribozyme has cleaved its TARGET sequence, it is released and can repeatedly bind and cleave at other mRNAs.

[0119] Ribozyme forms include a hammerhead motif, a hairpin motif, a hepatitis delta virus, group I intron or RNaseP RNA (in association with an RNA guide sequence) motif or Neurospora VS RNA motif. Ribozymes possessing a hammerhead or hairpin structure are readily prepared since these catalytic RNA molecules can be expressed within cells from eukaryotic promoters (Chen, et al. (1992) *Nucleic Acids Res.* 20:4581-9). A ribozyme of the present invention can be expressed in eukaryotic cells from the appropriate DNA vector. If desired, the activity of the ribozyme may be augmented by its release from the primary transcript by a second ribozyme (Ventura, et al. (1993) *Nucleic Acids Res.* 21:3249-55).

[0120] Ribozymes may be chemically synthesized by combining an oligodeoxyribonucleotide with a ribozyme catalytic domain (20 nucleotides) flanked by sequences that hybridize to the TARGET mRNA after transcription. The oligodeoxyribonucleotide is amplified by using the substrate binding sequences as primers. The amplification product is cloned into a eukaryotic expression vector.

[0121] Ribozymes are expressed from transcription units inserted into DNA, RNA, or viral vectors. Transcription of the ribozyme sequences are driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type will depend on nearby gene regulatory sequences. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Gao and Huang, (1993) *Nucleic*

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Acids Res. 21:2867-72). It has been demonstrated that ribozymes expressed from these promoters can function in mammalian cells (Kashani-Sabet, *et al.* (1992) Antisense Res. Dev. 2:3-15).

[0122] A particular inhibitory agent is a small interfering RNA (siRNA, particularly small hairpin RNA, "shRNA"). siRNA, particularly shRNA, mediate the post-transcriptional process of gene silencing by double stranded RNA (dsRNA) that is homologous in sequence to the silenced RNA. siRNA according to the present invention comprises a sense strand of 15-30, particularly 17-30, most particularly 17-25 nucleotides complementary or homologous to a contiguous 17-25 nucleotide sequence selected from the group of sequences described in SEQ ID NO: 1, 2, 4, 6-15, 17-22, 24, 26, 30-37 and 40-45, particularly from the group of sequences described in SEQ ID No: 91, 92, 94, 96-105, 107-112, 114, 116, 120-127 and 130-135, and an antisense strand of 15-30, particularly 17-30, most particularly 17-25 nucleotides complementary to the sense strand. The most particular siRNA comprises sense and anti-sense strands that are 100 per cent complementary to each other and the TARGET polynucleotide sequence. Particularly the siRNA further comprises a loop region linking the sense and the antisense strand.

[0123] A self-complementing single stranded shRNA molecule polynucleotide according to the present invention comprises a sense portion and an antisense portion connected by a loop region linker. Particularly, the loop region sequence is 4-30 nucleotides long, more particularly 5-15 nucleotides long and most particularly 8 or 12 nucleotides long. In a most particular embodiment the linker sequence is UUGCUAUA or GUUUGCUAUAAC (SEQ ID NO: 136). Self-complementary single stranded siRNAs form hairpin loops and are more stable than ordinary dsRNA. In addition, they are more easily produced from vectors.

[0124] Analogous to antisense RNA, the siRNA can be modified to confirm resistance to nucleolytic degradation, or to enhance activity, or to enhance cellular distribution, or to enhance cellular uptake, such modifications may consist of modified internucleoside linkages, modified nucleic acid bases, modified sugars and/or chemical linkage the siRNA to one or more moieties or conjugates. The nucleotide sequences are selected according to siRNA designing rules that give an improved reduction of the TARGET sequences compared to nucleotide sequences that do not comply with these siRNA designing rules (For a discussion of these rules and examples of the preparation of siRNA, WO 2004/094636 and US 2003/0198627, are hereby incorporated by reference).

[0125] The present invention also relates to compositions, and methods using said compositions, comprising a DNA expression vector capable of expressing a polynucleotide capable of modulating a Huntington Disease phenotype and described hereinabove as an expression inhibition agent.

[0126] A special aspect of these compositions and methods relates to the down-regulation or blocking of the expression of a TARGET polypeptide by the induced expression of a polynucleotide encoding an intracellular binding protein that is capable of selectively interacting with the TARGET polypeptide. An intracellular binding protein includes any protein capable of selectively interacting, or binding, with the polypeptide in the

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cell in which it is expressed and neutralizing the function of the polypeptide. Particularly, the intracellular binding protein is a neutralizing antibody or a fragment of a neutralizing antibody having binding affinity to an epitope of the TARGET polypeptide of SEQ ID NO: 46, 47, 49, 51-60, 62-67, 69, 71, 75-82 and 85-90. More particularly, the intracellular binding protein is a single chain antibody.

[0127] A special embodiment of this composition comprises the expression-inhibiting agent selected from the group consisting of antisense RNA, antisense oligodeoxynucleotide (ODN), a ribozyme that cleaves the polyribonucleotide coding for SEQ ID NO: 46, 47, 49, 51-60, 62-67, 69, 71, 75-82 and 85-90, and a small interfering RNA (siRNA) that is sufficiently homologous to a portion of the polyribonucleotide corresponding to SEQ ID NO: 1, 2, 4, 6-15, 17-22, 24, 26, 30-37 and 40-45, such that the siRNA interferes with the translation of the TARGET polyribonucleotide to the TARGET polypeptide.

[0128] The polynucleotide expressing the expression-inhibiting agent, or a polynucleotide expressing the TARGET polypeptide in cells, is particularly included within a vector. The polynucleic acid is operably linked to signals enabling expression of the nucleic acid sequence and is introduced into a cell utilizing, particularly, recombinant vector constructs, which will express the nucleic acid or antisense nucleic acid once the vector is introduced into the cell. A variety of viral-based systems are available, including adenoviral, retroviral, adeno-associated viral, lentiviral, herpes simplex viral or a sendai viral vector systems. All may be used to introduce and express polynucleotide sequence for the expression-inhibiting agents in TARGET cells.

[0129] Particularly, the viral vectors used in the methods of the present invention are replication defective. Such replication defective vectors will usually pack at least one region that is necessary for the replication of the virus in the infected cell. These regions can either be eliminated (in whole or in part), or be rendered non-functional by any technique known to a person skilled in the art. These techniques include the total removal, substitution, partial deletion or addition of one or more bases to an essential (for replication) region. Such techniques may be performed in vitro (on the isolated DNA) or in situ, using the techniques of genetic manipulation or by treatment with mutagenic agents. Particularly, the replication defective virus retains the sequences of its genome, which are necessary for encapsidating, the viral particles.

[0130] In a particular embodiment, the viral element is derived from an adenovirus. Particularly, the vehicle includes an adenoviral vector packaged into an adenoviral capsid, or a functional part, derivative, and/or analogue thereof. Adenovirus biology is also comparatively well known on the molecular level. Many tools for adenoviral vectors have been and continue to be developed, thus making an adenoviral capsid a particular vehicle for incorporating in a library of the invention. An adenovirus is capable of infecting a wide variety of cells. However, different adenoviral serotypes have different preferences for cells. To combine and widen the TARGET cell population that an adenoviral capsid of the invention can enter in a particular embodiment, the vehicle includes adenoviral fiber proteins from at least two adenoviruses. Particular adenoviral fiber protein

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sequences are serotype 17, 45 and 51. Techniques of construction and expression of these chimeric vectors are disclosed in US 2003/0180258 and US 2004/0071660, hereby incorporated by reference.

[0131] In a particular embodiment, the nucleic acid derived from an adenovirus includes the nucleic acid encoding an adenoviral late protein or a functional part, derivative, and/or analogue thereof. An adenoviral late protein, for instance an adenoviral fiber protein, may be favorably used to TARGET the vehicle to a certain cell or to induce enhanced delivery of the vehicle to the cell. Particularly, the nucleic acid derived from an adenovirus encodes for essentially all adenoviral late proteins, enabling the formation of entire adenoviral capsids or functional parts, analogues, and/or derivatives thereof. Particularly, the nucleic acid derived from an adenovirus includes the nucleic acid encoding adenovirus E2A or a functional part, derivative, and/or analogue thereof. Particularly, the nucleic acid derived from an adenovirus includes the nucleic acid encoding at least one E4-region protein or a functional part, derivative, and/or analogue thereof, which facilitates, at least in part, replication of an adenoviral derived nucleic acid in a cell. The adenoviral vectors used in the examples of this application are exemplary of the vectors useful in the present method of treatment invention.

[0132] Certain embodiments of the present invention use retroviral vector systems. Retroviruses are integrating viruses that infect dividing cells, and their construction is known in the art. Retroviral vectors can be constructed from different types of retrovirus, such as, MoMuLV ("murine Moloney leukemia virus" MSV ("murine Moloney sarcoma virus"), HaSV ("Harvey sarcoma virus"); SNV ("spleen necrosis virus"); RSV ("Rous sarcoma virus") and Friend virus. Lentiviral vector systems may also be used in the practice of the present invention. Retroviral systems and herpes virus system may be particular vehicles for transfection of neuronal cells.

[0133] In other embodiments of the present invention, adeno-associated viruses ("AAV") are utilized. The AAV viruses are DNA viruses of relatively small size that integrate, in a stable and site-specific manner, into the genome of the infected cells. They are able to infect a wide spectrum of cells without inducing any effects on cellular growth, morphology or differentiation, and they do not appear to be involved in human pathologies.

[0134] In the vector construction, the polynucleotide agents of the present invention may be linked to one or more regulatory regions. Selection of the appropriate regulatory region or regions is a routine matter, within the level of ordinary skill in the art. Regulatory regions include promoters, and may include enhancers, suppressors, etc.

[0135] Promoters that may be used in the expression vectors of the present invention include both constitutive promoters and regulated (inducible) promoters. The promoters may be prokaryotic or eukaryotic depending on the host. Among the prokaryotic (including bacteriophage) promoters useful for practice of this invention are lac, lacZ, T3, T7, lambda P.sub.r, P.sub.l, and trp promoters. Among the eukaryotic (including viral) promoters useful for practice of this invention are ubiquitous promoters (for example, HPRT, vimentin, actin, tubulin), intermediate filament promoters (for example, desmin, neurofilaments, keratin, GFAP), therapeutic

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gene promoters (for example, MDR type, CFTR, factor VIII), tissue-specific promoters (for example, actin promoter in smooth muscle cells, or Flt and Flk promoters active in endothelial cells), including animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift, *et al.* (1984) *Cell* 38:639-46; Ornitz, *et al.* (1986) *Cold Spring Harbor Symp. Quant. Biol.* 50:399-409; MacDonald, (1987) *Hepatology* 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, (1985) *Nature* 315:115-22), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl, *et al.* (1984) *Cell* 38:647-58; Adames, *et al.* (1985) *Nature* 318:533-8; Alexander, *et al.* (1987) *Mol. Cell. Biol.* 7:1436-44), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder, *et al.* (1986) *Cell* 45:485-95), albumin gene control region which is active in liver (Pinkert, *et al.* (1987) *Genes and Devel.* 1:268-76), alpha-fetoprotein gene control region which is active in liver (Krumlauf, *et al.* (1985) *Mol. Cell. Biol.*, 5:1639-48; Hammer, *et al.* (1987) *Science* 235:53-8), alpha 1-antitrypsin gene control region which is active in the liver (Kelsey, *et al.* (1987) *Genes and Devel.*, 1: 161-71), beta-globin gene control region which is active in myeloid cells (Mogram, *et al.* (1985) *Nature* 315:338-40; Kollias, *et al.* (1986) *Cell* 46:89-94), myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead, *et al.* (1987) *Cell* 48:703-12), myosin light chain-2 gene control region which is active in skeletal muscle (Sani, (1985) *Nature* 314:283-6), and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason, *et al.* (1986) *Science* 234:1372-8).

[0136] Other promoters which may be used in the practice of the invention include promoters which are preferentially activated in dividing cells, promoters which respond to a stimulus (for example, steroid hormone receptor, retinoic acid receptor), tetracycline-regulated transcriptional modulators, cytomegalovirus immediate-early, retroviral LTR, metallothionein, SV-40, E1a, and MLP promoters.

[0137] Additional vector systems include the non-viral systems that facilitate introduction of polynucleotide agents into a patient, for example, a DNA vector encoding a desired sequence can be introduced in vivo by lipofection. Synthetic cationic lipids designed to limit the difficulties encountered with liposome-mediated transfection can be used to prepare liposomes for in vivo transfection of a gene encoding a marker (Felgner, *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:7413-7); see Mackey, *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:8027-31; Ulmer, *et al.* (1993) *Science* 259:1745-8). The use of cationic lipids may promote encapsulation of negatively charged nucleic acids, and also promote fusion with negatively charged cell membranes (Felgner and Ringold, (1989) *Nature* 337:387-8). Particularly useful lipid compounds and compositions for transfer of nucleic acids are described in WO 95/18863 and WO 96/17823, and in U.S. Pat. No. 5,459,127. The use of lipofection to introduce exogenous genes into the specific organs in vivo has certain practical advantages and directing transfection to particular cell types would be particularly advantageous in a tissue with cellular heterogeneity, for example, pancreas, liver, kidney, and the brain. Lipids may be chemically coupled to other

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molecules for the purpose of targeting. Targeted peptides, for example, hormones or neurotransmitters, and proteins, for example, antibodies, or non-peptide molecules could be coupled to liposomes chemically. Other molecules are also useful for facilitating transfection of a nucleic acid in vivo, for example, a cationic oligopeptide (for example, WO 95/21931), peptides derived from DNA binding proteins (for example, WO 96/25508), or a cationic polymer (for example, WO 95/21931).

[0138] It is also possible to introduce a DNA vector in vivo as a naked DNA plasmid (see U.S. Pat. Nos. 5,693,622; 5,589,466; and 5,580,859). Naked DNA vectors for therapeutic purposes can be introduced into the desired host cells by methods known in the art, for example, transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, use of a gene gun, or use of a DNA vector transporter (see, for example, Wilson, *et al.* (1992) J. Biol. Chem. 267:963-7; Wu and Wu, (1988) J. Biol. Chem. 263:14621-4; Hartmut, *et al.* Canadian Patent Application No. 2,012,311, filed Mar. 15, 1990; Williams, *et al.* (1991). Proc. Natl. Acad. Sci. USA 88:2726-30). Receptor-mediated DNA delivery approaches can also be used (Curiel, *et al.* (1992) Hum. Gene Ther. 3:147-54; Wu and Wu, (1987) J. Biol. Chem. 262:4429-32).

[0139] A biologically compatible composition is a composition, that may be solid, liquid, gel, or other form, in which the compound, polynucleotide, vector, or antibody of the invention is maintained in an active form, for example, in a form able to effect a biological activity. For example, a compound of the invention would have inverse agonist or antagonist activity on the TARGET; a nucleic acid would be able to replicate, translate a message, or hybridize to a complementary mRNA of a TARGET; a vector would be able to transfect a TARGET cell and express the antisense, antibody, ribozyme or siRNA as described hereinabove; an antibody would bind a TARGET polypeptide domain.

[0140] A particular biologically compatible composition is an aqueous solution that is buffered using, for example, Tris, phosphate, or HEPES buffer, containing salt ions. Usually the concentration of salt ions will be similar to physiological levels. Biologically compatible solutions may include stabilizing agents and preservatives. In a more particular embodiment, the biocompatible composition is a pharmaceutically acceptable composition. Such compositions can be formulated for administration by topical, oral, parenteral, intranasal, subcutaneous, and intraocular, routes. Parenteral administration is meant to include intravenous injection, intramuscular injection, intraarterial injection or infusion techniques. The composition may be administered parenterally in dosage unit formulations containing standard, well-known non-toxic physiologically acceptable carriers, adjuvants and vehicles as desired.

[0141] A particular embodiment of the present composition invention is a modulation of the Huntington Disease phenotype inhibiting pharmaceutical composition comprising a therapeutically effective amount of an expression-inhibiting agent as described hereinabove, in admixture with a pharmaceutically acceptable carrier. Another particular embodiment is a pharmaceutical composition for the treatment or prevention of a condition

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involving bone resorption, or a susceptibility to the condition, comprising an effective cell death inhibiting amount of a TARGET antagonist or inverse agonist, its pharmaceutically acceptable salts, hydrates, solvates, or prodrugs thereof in admixture with a pharmaceutically acceptable carrier.

[0142] Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient. Pharmaceutical compositions for oral use can be prepared by combining active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethyl-cellulose; gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate. Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinyl-pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

[0143] Pharmaceutical preparations that can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

[0144] Particular sterile injectable preparations can be a solution or suspension in a non-toxic parenterally acceptable solvent or diluent. Examples of pharmaceutically acceptable carriers are saline, buffered saline, isotonic saline (for example, monosodium or disodium phosphate, sodium, potassium; calcium or magnesium chloride, or mixtures of such salts), Ringer's solution, dextrose, water, sterile water, glycerol, ethanol, and combinations thereof 1,3-butanediol and sterile fixed oils are conveniently employed as solvents or suspending media. Any bland fixed oil can be employed including synthetic mono- or di-glycerides. Fatty acids such as oleic acid also find use in the preparation of injectables.

[0145] The compounds or compositions of the invention may be combined for administration with or embedded in polymeric carrier(s), biodegradable or biomimetic matrices or in a scaffold. The carrier, matrix or scaffold may be of any material that will allow composition to be incorporated and expressed and will be

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compatible with the addition of cells or in the presence of cells. Particularly, the carrier matrix or scaffold is predominantly non-immunogenic and is biodegradable. Examples of biodegradable materials include, but are not limited to, polyglycolic acid (PGA), polylactic acid (PLA), hyaluronic acid, catgut suture material, gelatin, cellulose, nitrocellulose, collagen, albumin, fibrin, alginate, cotton, or other naturally-occurring biodegradable materials. It may be preferable to sterilize the matrix or scaffold material prior to administration or implantation, e.g., by treatment with ethylene oxide or by gamma irradiation or irradiation with an electron beam. In addition, a number of other materials may be used to form the scaffold or framework structure, including but not limited to: nylon (polyamides), dacron (polyesters), polystyrene, polypropylene, polyacrylates, polyvinyl compounds (e.g., polyvinylchloride), polycarbonate (PVC), polytetrafluorethylene (PTFE, teflon), thermax (TPX), polymers of hydroxy acids such as polylactic acid (PLA), polyglycolic acid (PGA), and polylactic acid-glycolic acid (PLGA), polyorthoesters, polyanhydrides, polyphosphazenes, and a variety of polyhydroxyalkanoates, and combinations thereof. Matrices suitable include a polymeric mesh or sponge and a polymeric hydrogel. In the particular embodiment, the matrix is biodegradable over a time period of less than a year, more particularly less than six months, most particularly over two to ten weeks. The polymer composition, as well as method of manufacture, can be used to determine the rate of degradation. For example, mixing increasing amounts of polylactic acid with polyglycolic acid decreases the degradation time. Meshes of polyglycolic acid that can be used can be obtained commercially, for instance, from surgical supply companies (e.g., Ethicon, N.J.). In general, these polymers are at least partially soluble in aqueous solutions, such as water, buffered salt solutions, or aqueous alcohol solutions, that have charged side groups, or a monovalent ionic salt thereof.

[0146] The composition medium can also be a hydrogel, which is prepared from any biocompatible or non-cytotoxic homo- or hetero-polymer, such as a hydrophilic polyacrylic acid polymer that can act as a drug absorbing sponge. Certain of them, such as, in particular, those obtained from ethylene and/or propylene oxide are commercially available. A hydrogel can be deposited directly onto the surface of the tissue to be treated, for example during surgical intervention.

[0147] Embodiments of pharmaceutical compositions of the present invention comprise a replication defective recombinant viral vector encoding the agent of the present invention and a transfection enhancer, such as poloxamer. An example of a poloxamer is Poloxamer 407, which is commercially available (BASF, Parsippany, N.J.) and is a non-toxic, biocompatible polyol. A poloxamer impregnated with recombinant viruses may be deposited directly on the surface of the tissue to be treated, for example during a surgical intervention. Poloxamer possesses essentially the same advantages as hydrogel while having a lower viscosity.

[0148] The active agents may also be entrapped in microcapsules prepared, for example, by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres,

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microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences (1980) 16th edition, Osol, A. Ed.

[0149] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semi-permeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, for example, films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOTTM. (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

[0150] As defined above, therapeutically effective dose means that amount of protein, polynucleotide, peptide, or its antibodies, agonists or antagonists, which ameliorate the symptoms or condition. Therapeutic efficacy and toxicity of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, for example, ED₅₀ (the dose therapeutically effective in 50% of the population) and LD₅₀ (the dose lethal to 50% of the population). The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD₅₀/ED₅₀. Pharmaceutical compositions that exhibit large therapeutic indices are particular. The data obtained from cell culture assays and animal studies are used in formulating a range of dosage for human use. The dosage of such compounds lies particularly within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

[0151] For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models, usually mice, rabbits, dogs, or pigs. The animal model is also used to achieve a desirable concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans. The exact dosage is chosen by the individual physician in view of the patient to be treated. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Additional factors which may be taken into account include the

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severity of the disease state, age, weight and gender of the patient; diet, desired duration of treatment, method of administration, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long acting pharmaceutical compositions might be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular formulation.

[0152] The pharmaceutical compositions according to this invention may be administered to a subject by a variety of methods. They may be added directly to targeted tissues, complexed with cationic lipids, packaged within liposomes, or delivered to targeted cells by other methods known in the art. Localized administration to the desired tissues may be done by direct injection, transdermal absorption, catheter, infusion pump or stent. The DNA, DNA/vehicle complexes, or the recombinant virus particles are locally administered to the site of treatment. Alternative routes of delivery include, but are not limited to, intravenous injection, intramuscular injection, subcutaneous injection, aerosol inhalation, oral (tablet or pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal delivery. Examples of ribozyme delivery and administration are provided in Sullivan *et al.* WO 94/02595.

[0153] Antibodies according to the invention may be delivered as a bolus only, infused over time or both administered as a bolus and infused over time. Those skilled in the art may employ different formulations for polynucleotides than for proteins. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

[0154] As discussed hereinabove, recombinant viruses may be used to introduce DNA encoding polynucleotide agents useful in the present invention. Recombinant viruses according to the invention are generally formulated and administered in the form of doses of between about 10^4 and about 10^{14} pfu. In the case of AAVs and adenoviruses, doses of from about 10^6 to about 10^{11} pfu are particularly used. The term pfu ("plaque-forming unit") corresponds to the infective power of a suspension of virions and is determined by infecting an appropriate cell culture and measuring the number of plaques formed. The techniques for determining the pfu titre of a viral solution are well documented in the prior art.

[0155] Administration of the expression-inhibiting agent of the present invention to the subject patient includes both self-administration and administration by another person. The patient may be in need of treatment for an existing disease or medical condition, or may desire prophylactic treatment to prevent or reduce the risk for diseases and medical conditions affected by a disturbance in bone metabolism. The expression-inhibiting agent of the present invention may be delivered to the subject patient orally, transdermally, via inhalation, injection, nasally, rectally or via a sustained release formulation.

[0156] The polypeptides and polynucleotides useful in the practice of the present invention described herein may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. To perform the methods it is feasible to immobilize either the TARGET polypeptide or the compound to facilitate

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separation of complexes from uncomplexed forms of the polypeptide, as well as to accommodate automation of the assay. Interaction (for example, binding of) of the TARGET polypeptide with a compound can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates, test tubes, and microcentrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows the polypeptide to be bound to a matrix. For example, the TARGET polypeptide can be "His" tagged, and subsequently adsorbed onto Ni-NTA microtitre plates, or ProtA fusions with the TARGET polypeptides can be adsorbed to IgG, which are then combined with the cell lysates (*for example*, (35)^S-labelled) and the candidate compound, and the mixture incubated under conditions favorable for complex formation (for example, at physiological conditions for salt and pH). Following incubation, the plates are washed to remove any unbound label, and the matrix is immobilized. The amount of radioactivity can be determined directly, or in the supernatant after dissociation of the complexes. Alternatively, the complexes can be dissociated from the matrix, separated by SDS-PAGE, and the level of the protein binding to the TARGET protein quantified from the gel using standard electrophoretic techniques.

[0157] Other techniques for immobilizing protein on matrices can also be used in the method of identifying compounds. For example, either the TARGET or the compound can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated TARGET protein molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well known in the art (for example, biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with the TARGETS but which do not interfere with binding of the TARGET to the compound can be derivatized to the wells of the plate, and the TARGET can be trapped in the wells by antibody conjugation. As described above, preparations of a labeled candidate compound are incubated in the wells of the plate presenting the TARGETS, and the amount of complex trapped in the well can be quantitated.

[0158] The invention is further illustrated in the following figures and examples.

Examples

[0159] As described in the introduction, both cell death caused by expression of mutant huntingtin and the abnormal conformation of the expanded huntingtin protein are phenotypes that serve as an entry-point for development of a drug that prevents or stops the neurodegeneration observed in HD and similar neurodegenerative diseases. The following assays, when used in combination with arrayed adenoviral shRNA (small hairpin RNA) or adenoviral cDNA expression libraries (the production and use of which are described in WO99/64582), compounds or compound libraries are useful for the discovery of factors that modulate neuronal cell death and/or the survival of neurons in neurodegenerative diseases.

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[0160] **Example 1** describes the design and setup of a high-throughput screening method for the identification of regulators or modulators of mutant huntingtin-induced cell death and is referred to herein as the “cell death assay”.

[0161] **Example 2** describes the screening and its results of 11584 “Ad-siRNA’s” in the cell death assay.

[0162] **Example 3** describes the rescreen of the primary hits using independent repropagation material.

[0163] **Example 4** describes gene expression analysis of the TARGETs.

[0164] **Example 5** describes further “on target analysis” which may be used to further validate a hit.

[0165] **Example 6** describes a cell based assay which may be used for further confirmation of the hits.

**Example 1. Design and setup of a high-throughput screening method for the identification of regulators
mutant huntingtin-induced cell death**

[0166] The cell death assay that has been developed for the screening of the SilenceSelect[®] collections has following distinctive features:

- 1) The assay is run on SH-SY5Y neuroblastoma cells differentiated towards a neuronal phenotype (Biedler *et al.*, 1973), but could be used for any other source of primary neuronal cells or cell lines.
- 2) The assay has been optimized for the use with arrayed adenoviral collections for functional genomics purposes.
- 3) The assay can also be used adapted for use to screen compounds or compound collections.
- 4) The assay can be run in high throughput mode.
- 5) The assay can also be adapted to screen other RNA or DNA collections for functional genomics purposes, for example but without limitation dominant negative (DN), cDNA or RNAi collections.

[0167] The protocol of the cell death assay is described below. This protocol is the result of the testing of various read-outs and various protocols:

[0168] Retinoic acid differentiated SH-SY5Y neuroblastoma cells expressing huntingtin containing an expanded polyglutamine repeat are a preferred cell model due to the human origin and neuronal-like phenotype and genotype of these cells. Targets identified in human model systems are commonly considered to have a lower attrition during clinical assessment as compared to targets identified in models from different species. SH-SY5Y neuroblastoma cells (ATCC # CRL-2266) were cultured on tissue culture grade plastic in high-glucose Dulbecco's modified Eagle medium containing 10% FCS, supplemented with 100 U / ml penicillin, 100 µg / ml streptomycin and 10 mM Hepes Buffer. For high-throughput screening, cells were cultured in clear 96-well plates at 5,000 cells per well, at 37°C, 5 % CO₂ in a humidified chamber.

[0169] Expression of huntingtin constructs containing an expanded polyglutamine repeat is the preferred method to measure the toxicity induced by expanded huntingtin. To efficiently express the expanded huntingtin

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in SH-SY5Y cells the polyglutamine repeat containing human huntingtin fragment cDNA is synthesized and cloned in adenoviral adapter plasmids. dE1/dE2A (deleted for adenoviral genes E1 and E2A) adenoviruses are generated from these adapter plasmids by co-transfection of the helper plasmid pWEAd5AflIII-rITR.dE2A in PerC6.E2A packaging cells, as described in WO99/64582.

[0170] Cells were cultured overnight and refreshed with medium containing 10 μ M all-trans retinoic acid (tRA). 4 hours after medium refreshment the cells were transduced with 2 μ l of our proprietary SilenceSelect[®] libraries. After 72 hours, the cells were refreshed with medium containing 10 μ M tRA and adenoviral constructs containing expanded huntingtin with a green-fluorescent protein tag (HD-Q121-N171-GFP) at 1000 virus particles per cell (VPU).

[0171] Four days after huntingtin knock-in transduction (HD-Q121-N171-GFP), a cell-death and nuclear stain were applied to a final concentration of 2 μ g / mL propidium iodide and 20 μ g / mL Hoechst-33342 respectively. Propidium iodide is a membrane impermeable DNA stain which is excluded from viable cells and is commonly used for identifying dead cells in a population (Macklis and Madison, 1990). The cell membrane loses its integrity in the process of cell-death whereby it becomes permeable to stains like propidium iodide. Hoechst-33342 is a membrane permeable DNA stain that is commonly used for the identification of nuclei in both live and dead cells. Stains were incubated at room-temperature for 30 minutes and measured on a high-content imager (GE-Healthcare; InCell-1000) using a 10x objective. Acquisition was performed for Hoechst-33342 (500 ms at wavelength 360 excitation – 460 emission), for GFP-tagged expanded huntingtin (200 ms at wavelength 475 excitation – 535 emission) and propidium iodide (200 ms at wavelength 535 excitation – 620 emission).

[0172] Image analysis was performed using Developer software (GE-Healthcare; version 1.6 build 725), specifically measuring cell-death of expanded huntingtin transduced cells based on GFP-signal and propidium iodide. The total number of cells was determined on the basis of the Hoechst-33342 staining of all nuclei. Segmentation was performed with an object identifier to measure local differences in intensity using kernel size 9 and sensitivity 50. The number of expanded huntingtin transduced cells was assessed on the basis of the GFP-signal tagged to the expanded huntingtin. Segmentation was achieved with an object identifier at kernel size 31 and sensitivity 50. The number of cells that were permeable to propidium iodide is assessed with an object identifier with kernel size 19 and sensitivity 1. Nuclear condensation was based on the Hoechst-33342 stain using an object identifier at kernel size 3 and sensitivity 1. The number of expanded huntingtin transduced cells was determined on the basis of the overlap between the defined nuclei and the GFP-identifier of the expanded huntingtin transduced cells. The number of propidium iodide positive cells was resolved on the basis of the overlap between the propidium iodide identifier and the defined nuclei. The number of cells with condensed nuclei was established on the basis of the overlap between the defined nuclei and the nuclear condensation

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identifier. The percentage of cell-death was consecutively calculated on the basis of the number of propidium iodide plus the number of nuclear condensating cells specifically for the expanded huntingtin defined cells.

[0173] From the expanded huntingtin defined cells the average GFP-intensity was measured within the identifier. The number of large inclusions was based on the GFP-signal using an intensity identifier with a minimal threshold of 3000. The number of inclusion forming cells was defined by the overlap of the inclusion identifier with the huntingtin transduced cells.

Example 2. Screening of 11584 “Ad-siRNA’s” in the cell death assay.

[0174] The cell death assay, the development of which is described in Example 1, has been screened against an arrayed collection of 11584 different recombinant adenoviruses mediating the expression of shRNAs in retinoic acid-differentiated neuroblastoma cells. These shRNAs cause a reduction in expression levels of genes that contain homologous sequences by a mechanism known as RNA interference (RNAi), whereas the expression of the cDNAs cause over-expression of the respective gene. The 11584 Ad-siRNA’s contained in the arrayed collection target 5119 different transcripts. On average, every transcript is targeted by 2 to 3 independent Ad-siRNA’s.

[0175] Every Ad-siRNA plate contains control viruses that are produced under the same conditions as the SilenceSelect[®] adenoviral collection. The viruses include three sets of negative control viruses (N₁ (Ad5-empty_KD)), N₂ (Ad5-Luc_v13_KD), N₃ (Ad5-mmSrc_v2_KD)), together with positive control viruses (P₁ (Ad5-HD_v5_KD)), P₂ (Ad5-HSPCB_v15_KD), P₃ (Ad5-FRAP1_v2_KD), P₄ (Ad5-HDAC6_v1_KD)), P₅ (Ad5-TP53_v2_KD)). Every well of a virus plate contains 150 µL of virus crude lysate. A representative example of the performance of a plate tested with the screening protocol described above is shown in Figure 1. In this figure, the calculated cell death ratio (the number of dead GFP-positive cells divided by the number of GFP-positive cells) detected upon performing the assay for every recombinant adenovirus on the plate is shown. When the value for the cell death level exceeds the cutoff value (defined as 1.5 fold the standard deviation over the sample), an Ad-siRNA virus is marked as a hit (either suppressing cell death at values smaller than -1.5, or increasing cell death at values greater than 1.5).

[0176] The complete SilenceSelect[®] collection (11584 Ad-siRNA’s targeting 5119 transcripts, contained in 130 96-well plates) was screened in the cell death assay according to the protocol described above. Every virus was used in biological duplicate measurements. Threshold settings for the screen were set at average of all data points per plate plus or minus 1.5 times standard deviation over all data points per plate. A total of 550 Ad-siRNA hits was isolated that scored below the threshold of -1.5-fold st dev from the mean of the sample viruses. A total of 680 Ad-siRNA hits was isolated that scored above the threshold of 1.5-fold stdev from the mean of the sample viruses.

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[0177] In, Figure 2, all datapoints obtained in the screening of the SilenceSelect® collection in the cell death assay are shown.

Example 3: Rescreen of the primary hits using independent repropagation material

[0178] To confirm the results of the identified Ad-siRNA in the cell death assay the following approach may be taken: the Ad-siRNA hits are repropagated using PerC6.E2A cells (CruceCell, Leiden, The Netherlands) in a 96-well plate format, followed by retesting in the cell death assay protocol as described above. Crude lysate samples of the identified Ad-siRNA hits are selected from the SilenceSelect® collection and rearranged in 96-well plates together with the negative (N₁ to N₃) and positive controls (P₁ to P₃). Vials containing crude lysate Ad-siRNA samples are labeled with a barcode (Screenmates™, Matrix technologies) to perform quality checks on the rearranged plates. To propagate the rearranged hit viruses, 40,000 PerC6.E2A cells are seeded in 200 µL of DMEM containing 10% FBS into each well of a 96-well plate and incubated overnight at 39°C in a humidified incubator at 10% CO₂ (PERC6 medium). Subsequently, 2 µL of crude lysate from the hit Ad-siRNA's rearranged in the 96-well plates as indicated above is added to the PerC6.E2A cells using a 96 well pipettor. The plates may then be incubated at 34°C in a humidified incubator at 10% CO₂ for 5 to 10 days. After this period, the repropagation plates are frozen at -80°C, provided that complete CPE (cytopathic effect) could be seen. The propagated Ad-siRNAs are rescreened in the cell death assay.

[0179] Data analysis for the cell death repressor rescreen is performed as follows. For every plate the average and standard deviation is calculated for the negative controls and may be used to set a "cutoff value" that indicates the fold-difference between the sample and the average of all negatives in terms of standard deviation of all negatives. Threshold settings for the cell death repressor rescreen were set at -4 fold standard deviation of the negative controls from the mean of the negative controls. At this cut-off, 485 Ad-siRNAs are again positive in the cell death assay.

[0180] The activators of cell death were rescreened both in the original set-up using a GFP-fused huntingtin fragment to induce cell death, and in the presence of the GFP protein lacking a polyglutamine containing huntingtin fragment. This allows the identification of Ad-siRNAs that activate cell death specifically in the presence of the expanded poly-glutamine protein. For each Ad-siRNA, both a cutoff value (fold standard deviation of the negative controls from the mean of the negative controls) and a polyglutamine-dependence (ratio of induction of cell death for polyglutamine-GFP versus GFP transduction) is calculated. Threshold settings for the cell death activator rescreen were for Ad-siRNAs either a cutoff of greater than 2 or a polyglutamine dependence of greater than 2. 97 of the 680 primary Ad-siRNA hits were confirmed in this way.

[0181] A quality control of target Ad- was performed as follows: Target Ad-siRNAs are propagated using derivatives of PER.C6® cells (CruceCell, Leiden, The Netherlands) in 96-well plates, followed by sequencing the siRNAs encoded by the target Ad-siRNA viruses. PERC6.E2A cells are seeded in 96 well plates at a density of

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40,000 cells/well in 180 μ L PERC6.E2A medium. Cells are then incubated overnight at 39°C in a 10% CO₂ humidified incubator. One day later, cells are infected with 1 μ L of crude cell lysate from SilenceSelect[®] stocks containing target Ad-siRNAs. Cells are incubated further at 34°C, 10% CO₂ until appearance of cytopathic effect (as revealed by the swelling and rounding up of the cells, typically 7 days post infection). The supernatant is collected, and the virus crude lysate is treated with proteinase K by adding 4 μ L Lysis buffer (4x Expand High Fidelity buffer with MgCl₂ (Roche Molecular Biochemicals, Cat. No 1332465) supplemented with 1 mg/mL proteinase K (Roche Molecular Biochemicals, Cat No 745 723) and 0.45% Tween-20 (Roche Molecular Biochemicals, Cat No 1335465) to 12 μ L crude lysate in sterile PCR tubes. These tubes are incubated at 55°C for 2 hours followed by a 15 minutes inactivation step at 95°C. For the PCR reaction, 1 μ L lysate is added to a PCR master mix composed of 5 μ L 10x Expand High Fidelity buffer with MgCl₂, 0.5 μ L of dNTP mix (10 mM for each dNTP), 1 μ L of "Forward primer" (10 mM stock, sequence: 5' CCG TTT ACG TGG AGA CTC GCC 3') (SEQ. ID NO: 137), 1 μ L of "Reverse Primer" (10 mM stock, sequence: 5' CCC CCA CCT TAT ATA TAT TCT TTC C) (SEQ. ID NO: 138), 0.2 μ L of Expand High Fidelity DNA polymerase (3.5 U/ μ L, Roche Molecular Biochemicals) and 41.3 μ L of H₂O. PCR is performed in a PE Biosystems GeneAmp PCR system 9700 as follows: the PCR mixture (50 μ L in total) is incubated at 95°C for 5 minutes; each cycle runs at 95°C for 15 sec., 55°C for 30 sec., 68°C for 4 minutes, and is repeated for 35 cycles. A final incubation at 68°C is performed for 7 minutes. For sequencing analysis, the siRNA constructs expressed by the target adenoviruses are amplified by PCR using primers complementary to vector sequences flanking the SapI site of the pIPspAdapt6-U6 plasmid. The sequence of the PCR fragments is determined and compared with the expected sequence. All sequences are found to be identical to the expected sequence.

[0182]

Table 3

[0183] Summary of the data obtained for the rescreen for all huntingtin cell death hits. The activity of each hit is presented in fold standard deviation in cell death of the 96-well plate from the average in cell death of the 96-well plate. In the primary screen, standard deviation and average were calculated on the library viruses. In the re-screen, standard deviation and average were calculated on the negative control viruses.

HIT REF	SYMBOL	primary screen		re-screen	
		RUN A	RUN B	RUN A	RUN B
		score	score	score	score
1	ABCF1	-1.71	-1.52	-9.48	-7.31
2	ACADM	-1.68	-1.77	-11.36	-7.19
3	ADH5	-0.62	-3.94	-8.48	-7.58
4	DUSP7	-2.26	-2.42	-4.95	-5.48
5	ATP1A3	-1.73	-2.02	-5.18	-6.11

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HIT REF	SYMBOL	primary screen		re-screen	
		RUN A	RUN B	RUN A	RUN B
		score	score	score	score
6	B4GALT7	-1.53	-1.7	-8.28	-6.7
7	CSNK1G1	-2.19	-2.3	-13.05	-9.28
8	CTSL1	-1.92	-2.11	-6.88	-5.63
9	DAPK2	-2.11	-2	-6.27	-7.38
10	DHCR24	-2.02	-1.95	-12.07	-8.63
11	DMPK	-1.51	-1.63	-13.14	-8.77
12	DUSP5	-1.63	-1.86	-11.43	-7.98
13	FGF17	-1.6	-1.83	-6.3	-8.31
14	C10orf59	-1.59	-1.92	-6.31	-5.37
15	FZD5	-1.75	-1.51	-8.38	-9.42
16	GAK	-1.92	-2.2	-6.42	-5.34
17	HSD17B8	-1.9	-1.93	-10.22	-7.61
18	KCNA1	-1.69	-2.38	-5.41	-6.69
19	WDR81	-1.54	-1.71	-7.56	-5.48
20	DUSP18	-1.96	-1.66	-10.87	-7.61
21	KCTD8	-1.84	-1.88	-14.04	-9.12
22	CYB5R1	2.01	1.1	6.32	6.11
23	LPL	-1.96	-1.99	-8.7	-9.34
24	MTMR2	-1.68	-1.63	-6.24	-7.25
25	NDUFS2	-1.61	-1.67	-11.35	-10.36
26	NEK7	-2.45	-2.25	-6.73	-5.26
27	P4HB	-1.59	-1.65	-5.49	-7.72
28	PDE8B	-2.02	-1.94	-6.23	-9.9
29	PIK3R3	-1.63	-1.69	-7.68	-8.56
30	PPIG	-1.72	-2.22	-11.61	-8.52
31	PRMT3	-1.92	-1.86	-11.68	-8.8
32	RHOBTB1	-1.64	-1.89	-6.08	-5.01
33	RPS6KB1	-1.92	-2.01	-8.85	-9.6
34	RPS6KC1	-1.57	-1.63	-7.9	-9.22
35	DHRS3	-1.56	-1.61	-11.21	-7.42
36	SLC20A2	-1.82	-2.22	-9.04	-6.28
37	SLCO1A2	-1.87	-2.25	-8.38	-11.12
38	SLC9A1	-2.49	-2.61	-8.31	-8.7
39	SMARCA1	-3.33	-3.22	-7.09	-8.78
40	SPTLC2	-1.61	-1.56	-12.06	-8.02
41	SRPK2	-1.74	-1.93	-7.24	-7.91
42	ST3GAL6	-1.89	-1.93	-7.5	-6.4
43	UCK1	-2.25	-1.9	-11.15	-7.36
44	UCKL1	-1.99	-2.02	-8.31	-9.31

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HIT REF	SYMBOL	primary screen		re-screen	
		RUN A	RUN B	RUN A	RUN B
		score	score	score	score
45	YAP1	-1.97	-2	-5.9	-5.44

Example 4: Gene expression analysis

[0184] To validate these targets as actively expressed in the human brain, particularly the striatum and cortex, areas which are affected in HD (Vonsattel *et al.*, 1985), the gene expression in the human brain of the transcripts represented by the hit viruses may be measured by either one of two methods.

4.1

[0185] A publicly (Hodges *et al.*, 2006) available microarray data-set is analyzed (NCBI Gene Expression Omnibus entry GSE3790). The arrays with good quality RNA are used (Table 4).

Table 4 Microarrays analyzed

Sample	No. of arrays
Caudate Nucleus - control	26
Caudate Nucleus – Vonsattel grade 1&2	32
Cortex Brodman Area 9 - control	12
Cortex Brodman Area 9 - Vonsattel grade 4	4

[0186] The hybridization levels are reported as p-values (statistical significance that the gene is expressed, the cut-off for significance was $p=0.05$). Genes expressed on more than 50 % of the arrays are ranked as expressed genes. The median p-value of expression across the striatum and cortex is presented in Table 5. Furthermore, a ratio between the $-\log$ of the median p-values from the striatum of HD patients with Vonsattel grade 1 or 2 and from the striatum of control subjects is used to indicate disease-specific expression.

4.2

[0187] For genes not analyzed in this (Hodges *et al.*, 2006) data-set, RNA may be isolated from fresh frozen brain tissue from control subjects and from HD patients, both from the striatum and from the cortex. The gene expression may be analyzed using Real-time TaqMan analysis of gene expression mRNA expression data (quantitative RT-PCR).

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[0188] Total RNA from these samples is isolated using the Qiagen RNeasy kit and the quality of RNA is assessed using an Agilent 2100 Bioanalyzer Pico chip. RNAs are selected on the basis of quality (28S and 18S peaks rRNA). cDNA is prepared from the RNA and pools of cDNA are made if appropriate (Table 5).

Table 5 Clinical status of RNA samples used in TaqMan analysis.

Some cDNA samples are pooled cDNAs from 2 or 3 samples (indicated by multiple entries in the fields).

RNA sample	Clinical status	Area of the brain	Sex	Age	CAG repeat
1	control	striatum	m	48	N/A
2	control	parietal cortex	m	51	N/A
		frontal cortex	m	46	N/A
3	HD	striatum	m	55	21-43
	Vonsattel II	striatum	m	81	19-41
4	HD	frontal cortex	f	52	17-47
	Vonsattel II	frontal cortex	m	55	21-43
		frontal cortex	m	81	19-41
5	HD	striatum	f	52	16-53
	Vonsattel IV				
6	HD	frontal cortex	f	52	16-53
	Vonsattel IV				

[#N/A = not applicable – no CAG repeat]

[0189] Each sample is measured in duplicate on different plates. The gene expression is calculated in cycle thresholds (Ct) (Applied Biosystems manual). A low cycle threshold indicates high expression, a Ct of 35 or greater indicates no expression. A differential gene expression in the striatum of HD patients with Vonsattel grade 1 or 2 and from the striatum of control subjects is calculated with $2^{(\Delta Ct)}$. Targets showing a ratio greater than 1 are over-expressed in HD striatum, and therefore of increased value as a drug target.

Table 6: Results of gene expression analysis.

Target Gene Symbol	SEQ ID NO: DNA	Expression array (p value)	Expression TaqMan (Ct)	Relative expression HD (ratio –logP or $2^{\Delta Ct}$)
ABCF1	1	0.0025		1.00
ACADM	2	0.0017		1.00

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Target Gene Symbol	SEQ ID NO: DNA	Expression array (p value)	Expression TaqMan (Ct)	Relative expression HD (ratio $-\log P$ or $2^{\Delta\Delta Ct}$)
ADH5	3		30.83	4.11
DUSP7	4		24.62	1.00
ATP1A3	5	0.0081		0.80
B4GALT7	6	0.0452		1.05
CSNK1G1	7	0.0395		0.93
CTSL1	8	0.0050		1.06
DAPK2	9		30.61	1.48
DHCR24	10	0.0022		0.91
DMPK	11	0.0331		0.69
DUSP5	12	0.0166		0.86
FGF17	13		27.69	1.15
C10orf59	14	0.0144		0.88
FZD5	15		28.43	4.04
GAK	16	0.0760		1.20
HSD17B8	17		30.33	1.91
KCNA1	18	0.0318		0.62
WDR81	19	0.0808		1.28
DUSP18	20	0.0435		1.15
KCTD8	21		25.36	0.73
CYB5R1	22	0.0153		1.00
LPL	23	0.0042		0.95
MTMR2	24	0.0506		0.98
NDUFS2	25	0.0124		0.88
NEK7	26		26.78	2.57
P4HB	27	0.0128		1.01
PDE8B	28	0.0025		0.95
PIK3R3	29	0.0453		0.73
PPIG	30	0.0068		1.06
PRMT3	31	0.0360		1.26
RHOBTB1	32	0.0258		1.43
RPS6KB1	33	0.0017		1.00

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Target Gene Symbol	SEQ ID NO: DNA	Expression array (p value)	Expression TaqMan (Ct)	Relative expression HD (ratio $-\log P$ or $2^{\Delta\Delta Ct}$)
RPS6KC1	34	0.0018		0.94
DHRS3	35	0.0326		1.08
SLC20A2	36	0.0548		1.13
SLC01A2	37	0.0266		1.22
SLC9A1	38		28.10	0.42
SMARCA1	39	0.0064		0.96
SPTLC2	40		26.70	1.48
SRPK2	41	0.0035		1.03
ST3GAL6	42	0.0832		1.03
UCK1	43	0.0220		0.96
UCKL1	44		27.38	1.61
YAP1	45	0.0036		1.10

Example 5: “On target analysis” using KD viruses

[0190] To strengthen the validation of a hit, it is helpful to recapitulate its effect using a completely independent siRNA targeting the same target gene through a different sequence. This analysis is called the “on target analysis”. In practice, this will be done by designing multiple new shRNA oligonucleotides against the target using a specialised algorithm previously described, and incorporating these into adenoviruses, according to WO 03/020931. After virus production, these viruses will be arrayed in 96 well plates, together with positive and negative control viruses. On average, 6 new independent Ad-siRNA’s will be produced for a set of targets. One independent repropagation of these virus plates will then be performed as described above for the rescreen in Example 3. The plates produced in this repropagation will be tested in biological duplicate in the primary screening assay at 3 MOIS according to the protocol described (Example 1). Ad-siRNA’s mediating a functional effect above the set cutoff value in at least 1 MOI will be nominated as hits scoring in the “on target analysis”. The cutoff value in these experiments will be defined as the average over the negative controls + 2 times the standard deviation over the negative controls. These hits are considered “on target”, and proceed to the next validation experiment.

Example 6: Primary cell based assay confirmation

[0191] A cell model with increased clinical relevance for Huntington’s Disease will have a phenotype similar to the population of neurons most severely affected in Huntington’s Disease. Neuropathological

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analysis of the brains of HD patients clearly evidences the regions of the brain involved in the neurodegenerative processes (Vonsattel *et al.*, 1985). The striatum (caudate nucleus) and cortex are most severely affected, explaining the motor and cognitive deficits observed during the disease process. A conditionally immortalized cell line derived from the human fetal striatum will be used to replicate the assay described in Example 1. Such a cell line may be cultured under the conditions that allow active proliferation, but upon turning off the immortalization gene such as c-myc, cells will terminally differentiate to a striatal neuron phenotype. The response of such neurons to the assay described in example 1 will be more relevant to the sensitivity of the striatal neuron population in the HD patient. Hit Ad-siRNAs active in the human striatal neuron assay will represent genes with increased validation as a drug target compared to Ad-siRNAs that fail to show an effect in the human striatal neuron assay. An example of a human striatal neuron cell line is the STROC05 cell line described in Usapat application 20060067918 (Sinden et al., ReNeuron Ltd.).

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[0193] From the foregoing description, various modifications and changes in the compositions and methods of this invention will occur to those skilled in the art. All such modifications coming within the scope of the appended claims are intended to be included therein.

[0194] All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

WE CLAIM:

1. A method for identifying a compound that modulates cell death, said method comprising:
 - a) contacting a compound with a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 47, 49, 51-60, 62-67, 69, 71, 75-82 and 85-90;
 - b) determining the binding affinity of the compound to the polypeptide;
2. The method according to claim 1 which additionally comprises the steps of
 - c) contacting a population of mammalian cells expressing said polypeptide with the compound that exhibits a binding affinity of at least 10 micromolar; and
 - d) identifying the compound that modulates the expression of mutant huntingtin protein.
3. A method for identifying a compound that modulates cell death, said method comprising:
 - a) contacting a compound with a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 47, 49, 51-60, 62-67, 69, 71, 75-82 and 85-90;
 - b) determining the ability of the compound inhibit the expression or activity of the polypeptide;
4. The method according to claim 3 which additionally comprises the steps of
 - c) contacting a population of mammalian cells expressing said polypeptide with the compound that significantly inhibits the expression or activity of the polypeptide ; and
 - d) identifying the compound that modulates the expression of mutant huntingtin protein.
5. The method according to any one of claims 1-4, wherein said polypeptide is in an *in vitro* cell-free preparation.
6. The method according to any one of claims 1-4, wherein said polypeptide is present in a cell.
7. The method according to claim 6, wherein the cell is a mammalian cell
8. The method according to claim 6 or 7, wherein the cell naturally expresses said polypeptide.
9. The method according to claim 6 or 7, wherein the cell has been engineered so as to express the target.

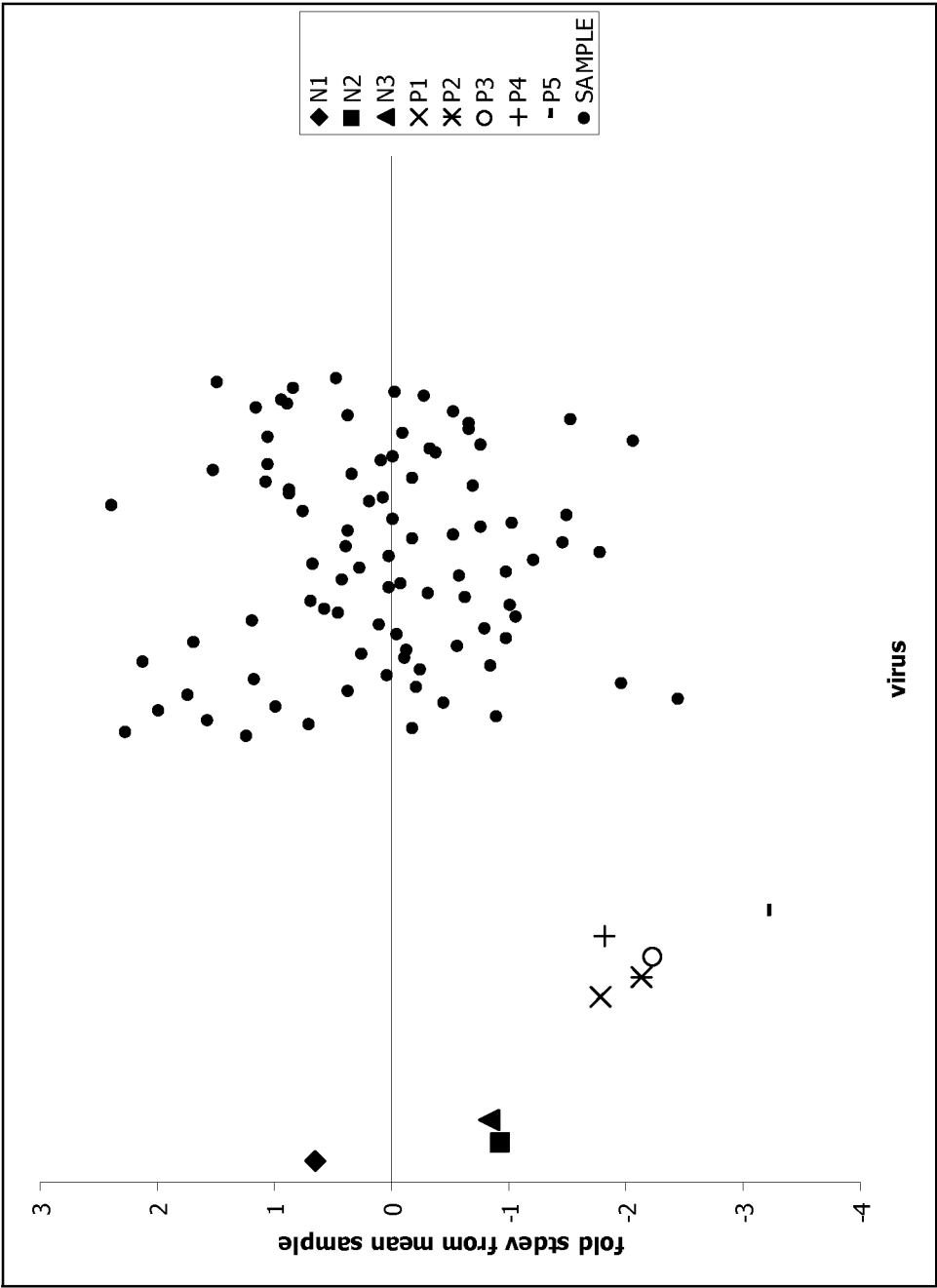
10. The method according to any one of claims 1-9, wherein said compound is selected from the group consisting of compounds of a commercially available screening library and compounds having binding affinity for a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 46, 47, 49, 51-60, 62-67, 69, 71, 75-82 and 85-90.
11. The method according to any one of claims 1-9, wherein said compound is a peptide in a phage display library or an antibody fragment library.
12. An agent effective in modulating polyglutamine-induced cell death, selected from the group consisting of an antisense polynucleotide, a ribozyme, and a small interfering RNA (siRNA), wherein said agent comprises a nucleic acid sequence complementary to, or engineered from, a naturally-occurring polynucleotide sequence of about 17 to about 30 contiguous nucleotides of a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, 2, 4, 6-15, 17-22, 24, 26, 30-37, 40-45.
13. The agent according to claim 12, wherein a vector in a mammalian cell expresses said agent.
14. The agent according to claim 12, which is effective in modulating polyglutamine-induced cell death in a polyglutamine cell death assay.
15. The agent according to claim 13, wherein said vector is an adenoviral, retroviral, adeno-associated viral, lentiviral, a herpes simplex viral or a sendai viral vector.
16. The agent according to claim 12, wherein said antisense polynucleotide and said siRNA comprise an antisense strand of 17-25 nucleotides complementary to a sense strand, wherein said sense strand is selected from 17-25 continuous nucleotides of a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, 2, 4, 6-15, 17-22, 24, 26, 30-37, 40-45.
17. The agent according to claims 16, wherein said siRNA further comprises said sense strand.
18. The agent according to claim 17, wherein said sense strand is selected from the group consisting of SEQ ID NO: 91, 92, 94, 96-105, 107-112, 114, 116, 120-127 and 130-135.
19. The agent according to claim 18, wherein said siRNA further comprises a loop region connecting said sense and said antisense strand.
20. The agent according to claim 19, wherein said loop region comprises a nucleic acid sequence selected from the group consisting of UUGCUAUA and GUUUGCUAUAAC (SEQ ID NO: 136).

21. The agent according to claim 19, wherein said agent is an antisense polynucleotide, ribozyme, or siRNA comprising a nucleic acid sequence complementary to a nucleic acid sequence selected from the group consisting of SEQ ID NO: 91, 92, 94, 96-105, 107-112, 114, 116, 120-127 and 130-135.
22. A cell death modulating pharmaceutical composition comprising a therapeutically effective amount of an agent of claim 12 in admixture with a pharmaceutically acceptable carrier.
23. A method of treating and/or preventing a disease involving neurodegeneration, comprising administering to said subject a pharmaceutical composition according to claim 22.
24. The method according to claim 23 wherein the disease is a polyglutamine disease.
25. The method according to claim 24, wherein the disease is Huntington's disease.
26. The method according to claim 23, wherein the disease is selected from Huntington's disease Alzheimer's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis, Progressive Supranuclear Palsy, Frontotemporal Dementia and Vascular Dementia.
27. Use of an agent according to claims 12-21 in the manufacture of a medicament for the treatment and/or prevention of a disease involving neurodegeneration.
28. Use according to claim 27, wherein the disease is a polyglutamine disease.
29. Use according to claim 27 or 28, wherein the disease is Huntington's disease.
30. Use according to claim 27 wherein the disease is selected from Huntington's disease Alzheimer's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis, Progressive Supranuclear Palsy, Frontotemporal Dementia and Vascular Dementia.
31. An agent according to any one of claims 12-21 for use in the treatment and/or prevention of a disease involving neurodegeneration, comprising administering to said subject a pharmaceutical composition according to claim 22.
32. The agent according to claim 31 wherein the disease is a polyglutamine disease.
33. The agent according to claim 32, wherein the disease is Huntington's disease.
34. The agent according to claim 31, wherein the disease is selected from Huntington's disease Alzheimer's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis, Progressive Supranuclear Palsy, Frontotemporal Dementia and Vascular Dementia.

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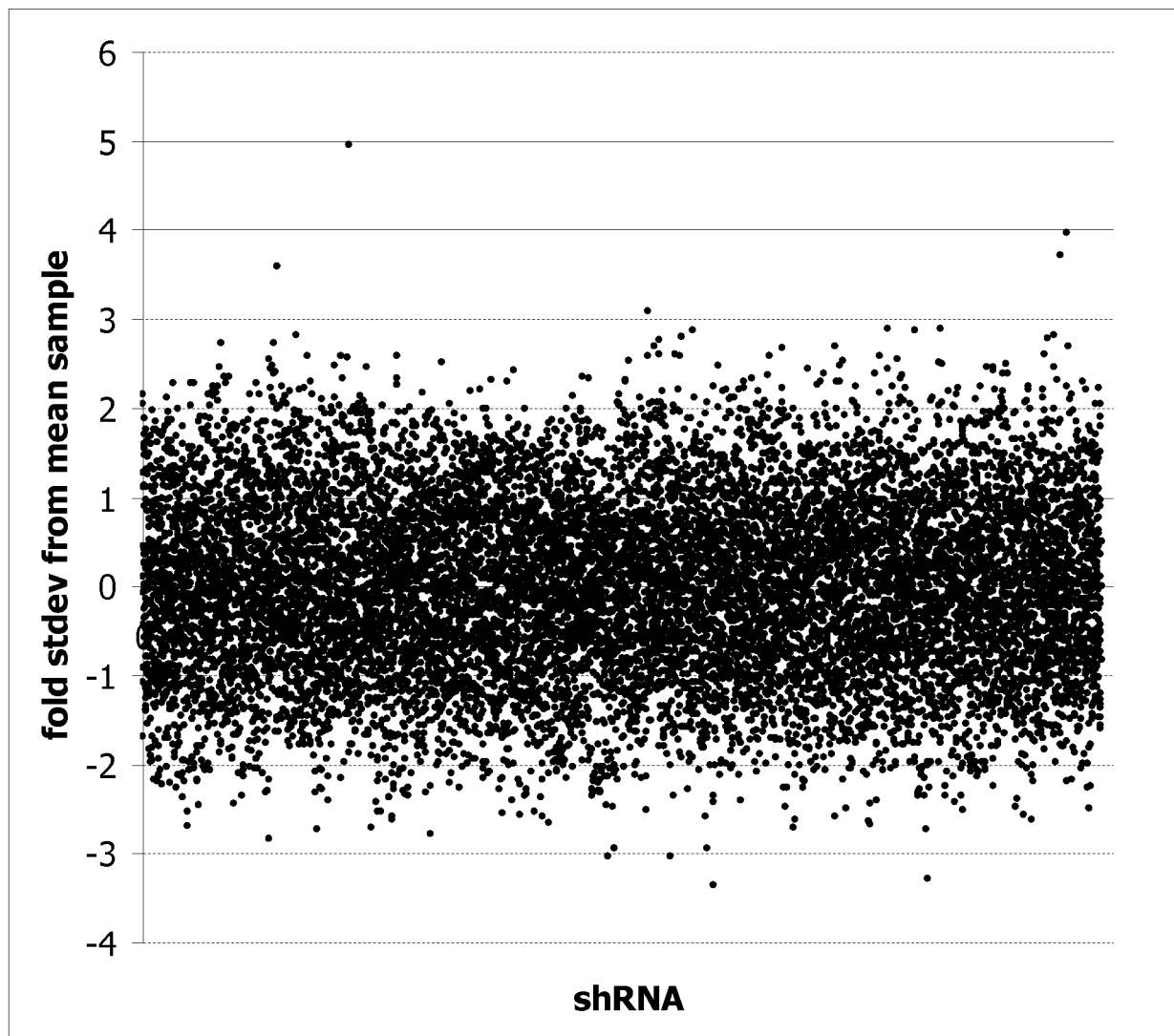
FIGURE 1



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FIGURE 2



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2009/051184

A. CLASSIFICATION OF SUBJECT MATTER

INV. G01N33/50

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N

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

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

EPO-Internal, BIOSIS, EMBASE, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KALTENBACH LINDA S ET AL: "Huntingtin interacting proteins are genetic modifiers of neurodegeneration" PLOS GENETICS, vol. 3, no. 5, May 2007 (2007-05), pages 689-708, XP002522374 ISSN: 1553-7390(print) 1553-7404(ele -----	1-11
X	WO 03/035832 A (EXELIXIS INC [US]; COSTA MICHAEL A [US]; HUNG TAK [US]; FRIEDMAN LORI) 1 May 2003 (2003-05-01) abstract; claim 1 ----- -/--	1-11

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *Z* document member of the same patent family

Date of the actual completion of the international search

29 May 2009

Date of mailing of the international search report

17/06/2009

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Authorized officer

Hinchliffe, Philippe

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2009/051184

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; July 2006 (2006-07), SEVENICH LISA ET AL: "Human cathepsin L rescues the neurodegeneration and lethality in cathepsin B/L double-deficient mice" XP002522376 Database accession no. PREV200600447807 abstract & BIOLOGICAL CHEMISTRY, vol. 387, no. 7, July 2006 (2006-07), pages 885-891, ISSN: 1431-6730 -----	1-11
X	KATUNUMA N ET AL: "Study of the functional share of lysosomal cathepsins by the development of specific inhibitors." ADVANCES IN ENZYME REGULATION 1999, vol. 39, 1999, pages 247-260, XP002522375 ISSN: 0065-2571 abstract -----	3-11
X	US 2002/193305 A1 (RUBEN STEVEN M [US] ET AL) 19 December 2002 (2002-12-19) seq.2 is identical to claimed sequence 82 paragraph [0495]; claims 1,20,22 -----	1-11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2009/051184

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-11 (part)
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: invention 1, claims 1-11 (parts)

Method of using sequence 53 (CTSL) for identifying agents that modulate cell death.

2. claims: inventions 2-35, claims 1-11 (parts)

Methods of using sequences 46,47,49, 51,52,54-60,62-67,69,71,75-82,85-90, indivisually, for identifying agents that modulate cell death.

3. claims: inventions 36+; claims 12-22,23-34 (all parts)

agents based upon sequences 1,2,4,6-15,17-22,24, 26,30-37,40-45 used for treating polyglutamine induced cell death. Each and every agent (whatever they are) must be searched individually for their effects on modulating polyglutamine induced cell death.
Methods of treatment of diseases involving neurodegeneration using sequences 1,2,4,6-15,17-22,24, 26,30-37,40-45.
Therefore there are at least 35 separate inventions when the simplest si RNAs encompassed by these claims are considered.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2009/051184

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 03035832	A	01-05-2003	AU 2002337915 A1	06-05-2003
US 2002193305	A1	19-12-2002	NONE	