METHODS OF USING SMALL RNA FROM BODILY FLUIDS FOR DIAGNOSIS AND MONITORING OF NEURODEGENERATIVE DISEASES

Described are methods for detection of neuronal pathologies using quantitative analysis in bodily fluids of synapse and/or neurite small RNAs and application of these methods to early diagnosis and monitoring of neurodegenerative diseases and other neurological disorders.
The present invention is directed to methods for noninvasive or minimally invasive detection of pathological changes in brain or other neurons by quantifying neuron and/or synapse small RNA, particularly miRNA, in bodily fluids and application of these methods to early diagnosis and monitoring of neurodegenerative diseases and other neurological disorders.

BACKGROUND OF THE INVENTION

Neurodegenerative diseases comprise a large group of pathologies caused by metabolic changes in brain cells, loss of synapses and other compartments of neurons, and finally neuronal death. For review see Neu- rodegenerative diseases: From Molecular Concepts to Therapeutic Targets. Editors: R. von Bernhardi, N.C. Inestrosa, Nova Publishers, 2008. This group of diseases includes Mild Cognitive Impairment (MCI), Alzheimer’s disease (AD), Lewy Body dementia, Parkinson’s disease (PD), Huntington’s disease (HD), frontotemporal dementia (FTD), vascular dementia, HIV Associated Neurocognitive Disorders (HAND), multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), prion diseases, different ataxias, and others. Due to increased lifespan, neurodegenerative diseases have become very common in developed countries. There are about 6 million people living with AD in the US only, 70-80 million people are in the risk group and $148 billion is spent in the US for AD patient treatment and care. Drug development and successful treatment of AD and other neurodegenerative diseases are significantly complicated by the absence of effective methods for their early diagnosis and monitoring. Development of effective diagnostic methods is further complicated by the strong brain potential to compensate for the dysfunction and loss of neurons over a long period of time. This results in late clinical manifestation of disease symptoms when treatment cannot be very successful due to serious morphologic changes in the brain including the massive loss of neurons. Thus, diagnostic methods based on detection of early events in the disease development are particularly desirable.


Currently, diagnosis of AD and other forms of dementia is based on analysis of the patient’s cognitive function. As mentioned above, due to effective compensatory mechanisms in the brain, the decrease of cognitive function is usually registered when a disease is in its later stages and fewer treatments are available. New imaging techniques, which are becoming increasingly popular (e.g., positron emission tomography (PET), computed tomography (CT), magnetic resonance imaging (MRI), multiphoton imaging, magnetoencephalography (MEG), electroencephalography (EEG) etc.), are helpful, however, they are currently not sufficiently sensitive and specific for detecting early stages of a disease before major morphological changes occur (Mucke, Nature, 2009, 461:895-897; Mistur et al., J. Clin. Neurol., 2009, 5:153-166; Miller, Science, 2009, 326:386-389; Perrin et al., Nature, 2009, 461:916-922).

The existing diagnostic molecular tests for AD and other forms of dementia can be divided into two groups. The first group is based on analysis of single nucleotide polymorphisms (SNP), which is helpful for predicting a higher risk of a disease but not for diagnostics (Bettens et al., Hum Mol Genet. 2010, 19(R1):R4-R11). The second group uses analysis of proteins involved in AD pathogenesis or brain-specific proteins, like neural thread protein (NTP), in bodily fluids (Schipper, Alzheimer’s & Dementia. 2007, 3:325-332). However, these tests are not sufficiently sensitive and specific. Recently published data have demonstrated high sensitivity of AD detection by measuring concentrations of three protein biomarkers (beta-amyloid protein 1-42, total tau protein, and phosphorylated tau181P protein) in the cerebrospinal fluid (CSF) (Meyer et al., Arch Neurol. 2010, 67:949-956). The high invasiveness of the CSF collection procedure makes such tests impractical and challenging for everyday clinical use.

Metabolic changes occurring in neurodegenera-


[0008] MicroRNAs (miRNAs) are a class of non-coding RNAs whose final product is an approximately 22 nt functional RNA molecule. They play important roles in the regulation of target genes by binding to complementary regions of messenger transcripts to repress their translation or regulate degradation (Griffiths-Jones et al. Nature Nucleic Acids Research, 2006, 34, Database issue: D140-D144). Frequently, one miRNA can target multiple mRNAs and one mRNA can be regulated by multiple miRNAs targeting different regions of the 3' UTR. Once bound to an mRNA, miRNA can modulate gene expression and protein production by affecting, e.g., mRNA translation and stability (e.g., Baek et al., Nature 455(7209):64 (2008); Selbach et al., Nature 455(7209):58 (2008); Ambros, 2004, Nature, 431, 350-355; Bartel, 2004, Cell, 116, 281-297; Cullen, 2004, Virus Research., 102, 3-9; He et al., 2004, Nat. Rev. Genet., 5, 522-531; and Ying et al., 2004, Gene, 342, 25-28). There are other classes of less characterized small RNAs (reviewed in Kim, Mol. Cells, 2005, 19: 1-15).

[0009] Many of miRNAs are specific to or over-expressed in certain organs / tissues / cells. See, e.g., Hua et al., BMC Genomics 2009, 10:214; Liang et al., BMC Genomics. 2007, 8:166; Landgraf et al., Cell. 2007, 129:1401-1414; Lee et al., RNA. 2008, 14:35-42.


[0012] Due to their small size, miRNAs can cross the blood-brain, placental and kidney barriers. Analysis of cell/tissue-specific miRNAs in bodily fluids was proposed for detection of in vivo cell death (U.S. Patent Pub. No 20090081640; Laterza et al., Clin Chem. 2009, 55:1977-1983).

[0013] Cognitive function testing and brain imaging, which are currently used as main methods for diagnosis of neurodegenerative diseases such as AD, allow only detection of later stages of disease and are not sufficiently specific. There is still a great need in the art to develop methods for early diagnosis of neurodegenerative diseases and other neurological disorders in mammals prior to occurrence of major morphological changes and massive neuronal cell death.

SUMMARY OF THE INVENTION

[0014] The present invention addresses these and other needs by providing a novel highly sensitive and non-invasive or minimally invasive diagnostic and monitoring methods based on quantification in bodily fluids of synapse and/or neurite small RNAs. The methods of the present invention allow diagnosis and monitoring of neurodegenerative diseases and other neurological disorders prior to occurrence of major morphological changes and massive neuronal cell death and thus have numerous clinical implications. For example, the use of the methods of the present invention can lead to enhanced effectiveness of currently available treatments for neurodegenerative diseases and other neurological disorders as such treatments could be administered at a significantly earlier stage of the disease. The use of the methods of the present invention can also allow development of new effective therapeutic and/or preventive treatments and can decrease costs and increase efficiency of clinical trials associated with such development.

[0015] In the first object, the present invention provides a method for diagnosing a neuronal pathology in a subject, which comprises:
In another aspect, the invention provides a method for diagnosing a neuronal pathology in a subject, which comprises:

a. determining the level of a synapse and/or neurite small RNA in a bodily fluid sample from the subject;

b. determining the level of a neuronal body small RNA (e.g., miR-181a or miR-491-5p) in a bodily fluid sample from the subject;

c. determining the ratio of the levels of the small RNAs determined in steps (a) and (b);

d. comparing the ratio of the levels of the small RNAs determined in step (c) with a corresponding control ratio, and

e. (i) identifying the subject as being afflicted with the neuronal pathology when the ratio of the levels of the small RNAs determined in step (c) is higher than the corresponding control ratio or (ii) identifying the subject as not being afflicted with the neuronal pathology when the ratio of the levels of the small RNAs determined in step (c) is not higher than the corresponding control ratio.

In another related aspect, the invention provides a method for monitoring development of a neuronal pathology in a subject, which comprises:

a. determining the level of at least one synapse or neurite small RNA in a bodily fluid sample from the subject;

b. comparing the level of the small RNA in the bodily fluid sample from the subject with a control level of the small RNA, and

c. (i) identifying the subject as being afflicted with AD when the level of the small RNA in the bodily fluid sample from the subject is increased as compared to the control or (ii) identifying the subject as not being afflicted with AD when the level of the small RNA in the bodily fluid sample from the subject is not increased as compared to the control.

In another related aspect, the invention provides a method for diagnosing mild cognitive impairment (MCI) in a subject, which comprises:

a. determining the level of at least one synapse or neurite small RNA in a bodily fluid sample from the subject;

b. comparing the level of the small RNA in the bodily fluid sample from the subject with a control level of the small RNA, and

c. (i) identifying the subject as being afflicted with MCI when the level of the small RNA in the bodily fluid sample from the subject is increased as compared to the control or (ii) identifying the subject as not being afflicted with MCI when the level of the small RNA in the bodily fluid sample from the subject is not increased as compared to the control.

In any of the above diagnostic methods, the control level of the small RNA can be, for example, (i) the level of said small RNA in a similarly processed bodily fluid sample from an age-matched control subject, (ii) the level of said small RNA in a similarly processed bodily fluid sample from the same subject obtained in the past, or (iii) a predetermined standard.

Any of the above diagnostic methods can further comprise normalizing the level of the small RNA in the bodily fluid sample from the subject and in the control to the level of a small RNA which is not expressed in brain (e.g., miR-10b or miR-141).
ues to develop at the same rate if the level of the small RNA is not changed in the later obtained bodily fluid sample(s) as compared to the earlier obtained sample(s), and (iii) determining that the development of the neuronal pathology in the subject is slowed down if the level of the small RNA is decreased in the later obtained bodily fluid sample(s) as compared to the earlier obtained sample(s).

[0023] In an additional aspect, the invention provides a method for monitoring the effectiveness of a treatment of a neuronal pathology in a subject, which comprises:

a. determining the level of at least one synapse or neurite small RNA in a bodily fluid sample from the subject obtained prior to initiation of the treatment;

b. determining the level of the small RNA in one or more bodily fluid sample(s) from the subject obtained in the course of or following the treatment (e.g., within 1 week-12 months intervals), and

c. comparing the level of the small RNA determined in steps (a) and (b), and optionally between different samples in step (b).

[0024] Such method can further comprise (d) (i) determining that the treatment is effective if the level of the small RNA has decreased in the course of or following the treatment or (ii) determining that the treatment is not effective if the level of the small RNA has not decreased in the course of or following the treatment.

[0025] Non-limiting examples of neuronal pathologies which can be diagnosed and monitored using any of the above methods include neurodegenerative diseases (such as, e.g., Alzheimer’s disease (AD), Parkinson’s disease (PD), Lewy Body dementia, Huntingdon’s disease (HD), frontotemporal dementia (FTD), vascular dementia, HIV Associated Neurocognitive Disorders (HAND), mild cognitive impairment (MCI), mixed dementia, Creutzfeldt-Jakob Disease (CJD), normal pressure hydrocephalus, Wernicke-Korsakoff syndrome, multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), prion diseases, and different ataxias) and neuronal pathologies associated with an encephalopathy or neuropathy.

[0026] In any of the above methods, a neuronal pathology can be diagnosed and monitored prior to massive neuronal cell death characteristic of said pathology.

[0027] In one embodiment, the small RNA used in any of the methods of the invention is present in synapses. In another embodiment, the small RNA used in any of the methods of the invention is present in spines. In yet another embodiment, the small RNA used in any of the methods of the invention is present in axons. In a further embodiment, the small RNA used in any of the methods of the invention is present in dendrites.

[0028] In one embodiment, the small RNA used in any of the methods of the invention is miRNA. Non-limiting examples of synapse and/or neurite miRNAs useful in any of the methods of the invention include miR-7, miR-9, miR-9*, miR-25, miR-26a, miR-26b, miR-98, miR-124, miR-125b, miR-128, miR-132, miR-134, miR-138, miR-146, miR-182, miR-183, miR-200b, miR-200c, miR-213, miR-292-5p, miR-297, miR-322, miR-323-3p, miR-325, miR-337, miR-339, miR-345, miR-350, miR-351, miR-370, miR-425, miR-429, miR-433-5p, miR-446, miR-467, and miR-874. In one specific embodiment, the miRNA is selected from the group consisting of miR-7, miR-125b, miR-128, miR-132, miR-323-3p, miR-370, and miR-874. In one embodiment, any of the methods of the invention comprise determining the level of two or more synapse and/or neurite small RNAs.

[0029] Non-limiting examples of bodily fluid samples useful in any of the methods of the invention include blood plasma, serum, urine, and saliva.

[0030] Non-limiting examples of methods for determining the level of small RNAs useful in any of the methods of the invention include hybridization, RT-PCR, and sequencing.

[0031] In one embodiment, prior to step (a) in any of the above methods, the small RNA is purified from the bodily fluid sample.

[0032] Any of the above methods can further comprise the step of reducing or eliminating degradation of the small RNA.

BRIEF DESCRIPTION OF THE DRAWINGS

[0034] Figures 1A-G are graphs showing comparisons of miRNA concentrations in plasma of AD patients and age-matched controls. All concentrations were normalized per ubiquitous miR-16 and presented in relative units (ordinate axis). miR-7 (A), miR-125b (B), miR-128 (C), miR-132 (D), and miR-323-3p (E) are neurite and/or synapse miRNA; miR-181a (F) and miR-491-5p (G) are neuronal body miRNA.

Figures 2A-D are graphs showing comparison of miRNA concentrations in plasma of MCI patients and age-matched controls. All concentrations were normalized per spiked miRNA and presented in relative units (ordinate axis). miR-7 (A) and miR-874 (B) are neurite and/or synapse miRNA; miR-181a (C) and miR-491-5p (D) are neuronal body miRNA.

Figures 3A-B are graphs showing comparison of miRNA concentrations in plasma of MCI patients and age-matched controls. All concentrations were normalized per miR-141 and presented in relative units (ordinate axis). miR-128 (A) is neurite and synapse miRNA; miR-539 (B) is neuronal body miRNA.

Figures 4A-D are graphs showing comparison of miRNA concentrations in plasma of MCI and AD patients and age-matched controls. Concentrations of neurite and/or synapse miRNA miR-128 (A), miR-
DETAILED DESCRIPTION OF THE INVENTION

[0035] The present invention is based on the inventors' realization that since neurite (axon and/or dendrite and/or spine) destruction and synapse loss as well as some metabolic events precede neuronal death in the course of development of neurodegenerative diseases, methods based on detection of those phenomena could be used for earlier disease diagnosis than the ones based on detecting cell death.

[0036] The instant invention is further based on the inventors' discovery that levels of synapse and/or neurite miRNAs increase in bodily fluids of patients with Mild Cognitive Impairment (MCI) and/or Alzheimer's disease (AD) compared to respective age-matched controls reflecting excessive destruction of neurites and/or loss of synapses.

[0037] Within the meaning of the present invention, the term "synapse and/or neurite small RNA" refers to small RNA (e.g., miRNA or BC200 RNA) which (i) is "neuron-enriched", i.e., present in increased amounts (e.g., at least 5-times higher concentrations) in neurons, as compared to cell types that can be a source of significant amounts of small RNA in a bodily fluid being tested and (ii) is present in a synapse and/or neurite (i.e., axon and/or dendrite and/or spine). To be useful in the diagnostic methods of the present invention, such synapse and/or neurite small RNA should be detectable in bodily fluids as a result of its release from neurons (e.g., due to neurite/synapse destruction or neuronal death).

[0038] The present invention provides a novel highly sensitive and noninvasive or minimally invasive method for diagnosing a neuronal pathology (e.g., a neuronal pathology associated with a neurodegenerative disease or another neurological disorder) in a subject, said method comprising determining the level in a bodily fluid sample from the subject (e.g., blood plasma or serum, urine, saliva, or other bodily fluids) of one or more synapse and/or neurite small RNA (e.g., miRNA or BC200 RNA). Specifically, this method comprises (a) determining the level of at least one synapse and/or neurite small RNA in a bodily fluid sample from the subject; (b) comparing the level of the small RNA in the bodily fluid sample with a control level of the small RNA (e.g., a similarly processed bodily fluid sample from a control subject [e.g., an age-matched control or the same subject in the past (e.g., 1, 3, 6, 12, 24, 36, or 48 months earlier)] or a predetermined standard), and (c) identifying the subject as being affected with the neuronal pathology when the level of the small RNA in the bodily fluid sample from the subject is increased as compared to the control or (ii) identifying the subject as not being affected with the neuronal pathology when the level of the small RNA in the bodily fluid sample from the subject is not increased as compared to the control.

[0039] The diagnostic method of the invention makes possible early diagnosis of neurodegenerative diseases and other neurological disorders, e.g., prior to occurrence of major morphological changes and/or massive neuronal cell death associated with such diseases and disorders.

[0040] Furthermore, analysis of synapse and/or neurite small RNAs significantly enhances the sensitivity of the small RNA detection as compared to detecting neuronal body small RNAs which are not present or depleted in synapses and neurites, because the amount of synapses and neurites in the brain is 10^3 times higher than the amount of neurons. This approach also provides detailed and comprehensive information for monitoring disease development and treatment effectiveness, since various specific events in neurons (e.g., changes in miRNA profile, their secretion, neurite degradation, synapse loss, and finally neuronal death) can be detected and quantitated.

[0041] Differences in levels of synapse and/or neurite small RNAs in bodily fluids of subjects having neurodegenerative diseases or other neurological disorders as compared to normal subjects detectable by the method of the present invention may be due to (i) disease-associated destruction of neurites and/or synapses, (ii) disease-associated changes in expression or metabolism of these small RNAs, (iii) disease-associated changes in transport and intracellular distribution of these small RNAs, (iv) disease-associated changes in secretion of these small RNAs (Rabinowits et al. Clin Lung Cancer, 2009, 10:42-46), as well as other causes.

[0042] In a separate embodiment, the invention provides a related diagnostic method for diagnosing a neuronal pathology which comprises (a) determining the level of a synapse and/or neurite small RNA in a bodily fluid sample from the subject; (b) determining the level of a neuronal body small RNA in a bodily fluid sample from the subject; (c) determining the ratio of the levels of the small RNAs determined in steps (a) and (b); (d) comparing the ratio of the levels of the small RNAs determined in step (c) with a corresponding control ratio, and (e) identifying the subject as being affected with the neuronal pathology when the ratio of the levels of the small RNAs determined in step (c) is higher than the corresponding control ratio or (ii) identifying the subject as not being affected with the neuronal pathology when ratio of the levels of the small RNAs determined in step (c) is not higher than the corresponding control ratio.

[0043] Within the meaning of the present invention, the term "neuronal body small RNA" refers to small RNA (e.g., miRNA) which (i) is "neuron-enriched", i.e., present in increased amounts (e.g., at least 5-times higher concentrations) in neurons, as compared to cell types that can be a source of significant amounts of small RNA in a bodily fluid being tested and (ii) is absent from or present in significantly lower concentrations in neurites or synapses than in neuronal cell bodies.
[0044] In another related embodiment, the present invention provides a method for monitoring development of a neuronal pathology (e.g., a neuronal pathology associated with a neurodegenerative disease or another neurological disorder) by periodically (e.g., every 1, 3, 6, 12, 24, 36, 48 months) obtaining samples of a bodily fluid from a subject under observation and determining changes in the level of one or more synapse and/or neurite small RNA (e.g., miRNA or BC200 RNA) in the bodily fluid. Specifically, this method comprises (a) determining the level of at least one synapse and/or neurite small RNA in two or more bodily fluid samples from the subject, wherein the samples have been obtained at spaced apart time points, and (b) comparing the levels of the small RNA between the earlier obtained and later obtained bodily fluid sample(s). If the level of the small RNA is increased in the later obtained bodily fluid sample(s) as compared to the earlier obtained sample(s), this is indicative of acceleration of development of the neuronal pathology in the subject. If the level of the small RNA is not changed in the later obtained bodily fluid sample(s) as compared to the earlier obtained sample(s), this is indicative of that the neuronal pathology in the subject continues to develop at the same rate. If the level of the small RNA is decreased in the later obtained bodily fluid sample(s) as compared to the earlier obtained sample(s), this is indicative of slow down in development of the neuronal pathology in the subject.

[0045] In another related embodiment, the invention provides a method for monitoring the effectiveness of a treatment of a neuronal pathology (e.g., a neuronal pathology associated with a neurodegenerative disease or another neurological disorder) in a subject by determining changes in the level of one or more synapse and/or neurite small RNA in bodily fluid samples from the subject, wherein said samples have been obtained prior to initiation of the treatment and at different time points (e.g., every 1 week, 2 weeks, 1 month, 3 months, 6 months, 12 months, 24 months, 36 months, 48 months) in the course of or following the treatment. Specifically, this method comprises (a) determining the level of at least one synapse and/or neurite small RNA in a bodily fluid sample from the subject obtained prior to initiation of the treatment; (b) determining the level of the small RNA in one or more bodily fluid sample(s) from the subject obtained in the course of or following the treatment, and (c) comparing the level of the small RNA determined in steps (a) and (b), and optionally between different samples in step (b). If the level of the small RNA has decreased in the course of or following the treatment, this is indicative that the treatment is effective. If the level of the small RNA has not decreased in the course of or following the treatment, this is indicative that the treatment is not effective. This method can also involve comparison with placebo treated patients or other relevant controls.

[0046] The diagnostic and monitoring methods of the invention are useful for detecting and monitoring any stage of development of a neuronal pathology (e.g., a neuronal pathology associated with a neurodegenerative disease or another neurological disorder) and provide the advantage of a simple and minimally invasive (or non-invasive) assay. As noted above, unlike methods known in the art, the methods of the invention allow for diagnosis and monitoring of neuronal pathologies prior to occurrence of major morphological changes and/or massive neuronal cell death associated with such pathologies.

[0047] The methods of the present invention can be used to diagnose and monitor various neuronal pathologies including, without limitation, neurodegenerative diseases (e.g., Alzheimer’s disease (AD), Parkinson’s disease (PD), Lewy Body dementia, Huntington’s disease (HD), frontotemporal dementia (FTD), vascular dementia, HIV Associated Neurocognitive Disorders (HAND), mild cognitive impairment (MCI), mixed dementia, Creutzfeldt-Jakob Disease (CJD), normal pressure hydrocephalus, Wernicke-Korsakoff syndrome, multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), prion diseases, different ataxias, etc.), various encephalopathies (e.g. viral encephalopathies such as AIDS dementia) and neuropathies (e.g., glaucoma [optical neuropathy], spinal muscular atrophy, etc.). In a separate embodiment of the present invention, a spectrum of various small RNAs (e.g., various miRNAs) can be analyzed for differential diagnosis of various neurodegenerative diseases with similar clinical symptoms, for example, different forms of dementia.

[0048] Neurite and/or synapse small RNAs useful in the methods of the present invention include, without limitation, miRNAs such as miR-7; miR-9; miR-9*; miR-25; miR-26a; miR-26b; miR-98; miR-124; miR-125b; miR-128; miR-132; miR-134; miR-138; miR-146; miR-182; miR-183; miR-200b; miR-200c; miR-213; miR-292-5p; miR-297; miR-322; miR-323-3p; miR-325; miR-337; miR-339; miR-345; miR-350; miR-351; miR-370; miR-425; miR-429; miR-433-5p; miR-446; miR-467; miR-874 (see Schratt et al., Nature 439:283-289, 2006; Lugli et al., J Neurochem. 106:650-661, 2008; Bicker and Schratt, J Cell Mol Med. 12:1466-1476, 2008; Smalheiser, Lugli, Neurouromolecular Med. 11:133-140, 2009; Rajasethupathy, Neuron, 63:714-716, 2009; Kye, RNA, 13:1224-1234, 2007; Yu, et al., Exp Cell Res. 314:2618-2633, 2008; Cougat et al., J Neurosci. 28:13793-13804, 2008; Kawahara, Brain Nerve, 60:1437-1444, 2008), and other small RNAs such as BC200 RNA (Brain Cytosplasm RNA 200-nucleotides; Dahm et al., Seminars in Cell & Dev. Biol. 18: 216-223, 2007; Mus et al., Proc. Natl. Acad. Sci. U S A., 104:10679-10684, 2007). Additional small RNAs useful in the methods of the invention can be identified, for example, based on their enrichment in neurons (and in certain regions of the brain depending on a disease) and intracellular localization in axons and/or dendrites and/or spines and/or synapses. If urine samples are selected for conducting diagnostic methods of the invention, preferred small RNAs for detection would be those small RNAs which are not significantly expressed in cells of...
the urinary system. Similarly, if blood samples (e.g., serum or plasma) are used for conducting diagnostic methods of the invention, preferred small RNAs for detection would be those small RNAs which are not expressed or are present at very low levels in blood cells.

**[0049]** The methods of the instant invention are based on measurement of levels of certain small RNAs in bodily fluids. The use of bodily fluids that can be obtained by non-invasive or minimally invasive techniques (e.g., as opposed to detection in the brain or CSF) allows for a cheap and minimally invasive or noninvasive diagnostic procedure. Preferred bodily fluids for use in the methods of the invention are blood plasma, serum, urine, and saliva. However, any other bodily fluid can also be used.

**[0050]** Examples of useful methods for measuring small RNA level in bodily fluids include hybridization with selective probes (e.g., using Northern blotting, bead-based flow-cytometry, oligonucleotide microchip [microarray], or solution hybridization assays such as Ambion mirVana mirna Detection Kit), polymerase chain reaction (PCR)-based detection (e.g., stem-loop reverse transcription-polymerase chain reaction [RT-PCR], quantitative RT-PCR based array method [qPCR-array]), or direct sequencing by one of the next generation sequencing technologies (e.g., Helicos small RNA sequencing, mirNA BeadArray [Illumina], Roche 454 (FLX-Titanium), and ABI SOLiD). For review of additional applicable techniques see, e.g., Chen et al., BMC Genomics, 2009, 10:407; Kong et al., J Cell Physiol. 2009; 218:22-25.

**[0051]** In some embodiments, small RNAs are purified prior to quantification. Small RNAs (e.g., miRNAs) can be isolated and purified from bodily fluids by various methods, including the use of commercial kits (e.g., miRNeasy kit [Qiagen], MirVana RNA isolation kit [Ambion/ABI], miRACLE [Agilent], High Pure miRNA isolation kit [Roche], and miRNA Purification kit [Norgen Biotek Corp.]), Trizol extraction (see Example 1, below), concentration and purification on anion-exchangers, magnetic beads covered by RNA-binding substances, or adsorption of certain miRNA on complementary oligonucleotides.

**[0052]** In some embodiments, small RNA degradation in bodily fluid samples and/or during small RNA purification is reduced or eliminated. Useful methods for reducing or eliminating small RNA degradation, include, without limitation, adding RNase inhibitors (e.g., RNasin Plus [Promega], SUPERase-In [ABI], etc.), use of guanidine chloride, guanidine isothiocyanate, N-lauroylsarcosine, sodium dodecyl sulphate (SDS), or a combination thereof. Also, when working with urine samples, lower risk of RNA degradation can be achieved when the sample has been held in the bladder for a shorter time (e.g., less than 4 hours). Reducing small RNA degradation in bodily fluid samples is particularly important when sample storage and transportation is required prior to small RNA quantification.

**[0053]** To account for possible losses of a given small RNA during purification, potential RT-PCR inhibition, small RNA contaminants derived from dying or damaged blood or urine cells during sample isolation and treatment, variations in kidney filtration, etc., various methods of experimental data normalization can be employed. For example, the following normalization methods can be used in the present invention:

a) Concentration of a target small RNA can be normalized to one of ubiquitous miRNAs (e.g., miR-16), small nuclear RNAs (snRNAs), miRNAs which are not expressed in neurons (e.g., miR-122a, miR-10b, miR-141), U6 small nuclear RNA (U6 RNA), or neuron body miRNAs (e.g., miR-137, miR-181a, miR-491-5p, miR-298, miR-339 [Kye, RNA, 13:1224-1234, 2007], and others).

b) Synthetic small RNA (e.g., miRNA) oligonucleotides can be synthesized and used as controls for losses during purification and RT-PCR inhibition (by adding them to bodily fluid samples before RNA purification).

c) To account for variations in kidney filtration (when working with urine samples), small RNA concentration in urine can be normalized on creatinine and/or albumin level.

**Definitions**

**[0054]** The term "neuronal cell body" refers to the portion of a nerve cell that contains the nucleus surrounded by the cytoplasm and the plasma membrane but does not incorporate the dendrites or axons.

**[0055]** The term "neurite" as used herein refers to any projection from the cell body of a neuron. This projection can be an axon, a dendrite, or a spine.

**[0056]** The term "axon" refers to a long, slender projection of a neuron that conducts electrical impulses away from the neuron’s cell body or soma. Axons are distinguished from dendrites by several features, including shape (dendrites often taper while axons usually maintain a constant radius), length (dendrites are restricted to a small region around the cell body while axons can be much longer), and function (dendrites usually receive signals while axons usually transmit them). Axons and dendrites make contact with other cells (usually other neurons but sometimes muscle or gland cells) at junctions called synapses.

**[0057]** The term "dendrite" refers to a branched projection of a neuron that acts to conduct the electrochemical stimulation received from other neural cells to the cell body of the neuron from which the dendrites project.

**[0058]** The terms "spine" or "dendritic spine" refer to a small membranous protrusion from a neuron’s dendrite that typically receives input from a single synapse of an axon. Dendritic spines serve as a storage site for synaptic strength and help transmit electrical signals to the neuronal cell body. Most spines have a bulbous head (the
spine head), and a thin neck that connects the head of the spine to the shaft of the dendrite. The dendrites of a single neuron can contain hundreds to thousands of spines. In addition to spines providing an anatomical substrate for memory storage and synaptic transmission, they may also serve to increase the number of possible contacts between neurons.

The term "synapse" refers to specialized junctions, through which neurons signal to each other and to non-neuronal cells such as those in muscles or glands. A typical neuron gives rise to several thousand synapses. Most synapses connect axons to dendrites, but there are also other types of connections, including axon-to-cell-body, axon-to-axon, and dendrite-to-dendrite. In the brain, each neuron forms synapses with many others, and, likewise, each receives synaptic inputs from many others. As a result, the output of a neuron may depend on the input of many others, each of which may have a different degree of influence, depending on the strength of its synapse with that neuron. There are two major types of synapses, chemical synapses and electrical synapses. In electrical synapses, cells approach within about 3.5 nm of each other, rather than the 20 to 40 nm distance that separates cells at chemical synapses. In chemical synapses, the postsynaptic potential is caused by the opening of ion channels by chemical transmitters, while in electrical synapses it is caused by direct electrical coupling between both neurons. Electrical synapses are therefore faster than chemical synapses.

Within the meaning of the present invention, the term "synapse and/or neurite small RNA" refers to small RNA (e.g., miRNA or BC200 RNA) which (i) is "neuron-enriched", i.e., is present in increased amounts (e.g., at least 5-times higher concentrations) in neurons, as compared to cell types that can be a source of significant amounts of small RNA in a bodily fluid being tested and (ii) is present in a synapse and/or neurite (i.e., axon and/or dendrite and/or spine). To be useful in the diagnostic methods of the present invention, such synapse and/or neurite small RNA should be detectable in bodily fluids as a result of its release from neurons (e.g., due to neurite/synapse destruction or neuronal death).

The term "neuronal body small RNA" as used herein refers to small RNA (e.g., miRNA) which (i) is "neuron-enriched", i.e., is present in increased amounts (e.g., at least 5-times higher concentrations) in neurons, as compared to cell types that can be a source of significant amounts of small RNA in a bodily fluid being tested and (ii) is present in a synapse and/or neurite (i.e., axon and/or dendrite and/or spine). To be useful in the diagnostic methods of the present invention, such synapse and/or neurite small RNA should be detectable in bodily fluids as a result of its release from neurons (e.g., due to neurite/synapse destruction or neuronal death).

The terms "microRNA" or "miRNA" as used herein refer to a class of small approximately 22 nt long non-coding mature RNA molecules. They play important roles in the regulation of target genes by binding to complementary regions of messenger transcripts (mRNA) to repress their translation or regulate degradation (Griffiths-Jones Nucleic Acids Research, 2006, 34, Database issue: D140-D144). Frequently, one miRNA can target multiple mRNAs and one mRNA can be regulated by multiple miRNAs targeting different regions of the 3' UTR. Once bound to an mRNA, miRNA can modulate gene expression and protein production by affecting, e.g., mRNA translation and stability (Baek et al., Nature 455(7209):64 (2008); Selbach et al., Nature 455(7209):58 (2008); Ambros, 2004, Nature, 431, 350-355; Bartel, 2004, Cell, 116, 281-297; Cullen, 2004, Virus Research., 102, 3-9; He et al., 2004, Nat. Rev. Genet., 5, 522-531; and Ying et al., 2004, Gene, 342, 25-28). Examples of neurite and/or synapse miRNAs useful in the methods of the present invention include, without limitation, miR-7, miR-9, miR-7*, miR-9*, miR-25, miR-26a, miR-26b, miR-98, miR-124, miR-125b, miR-128, miR-132, miR-134, miR-138, miR-146, miR-182, miR-183, miR-200b, miR-200c, miR-213, miR-292-5p, miR-297, miR-322, miR-323-3p, miR-325, miR-337, miR-339, miR-345, miR-350, miR-351, miR-370, miR-425, miR-429, miR-433-5p, miR-446, miR-467 and miR-874. Information on most currently known miRNAs can be found in the miRNA database miRBase (available at the world wide web at mirbase.org). See also Burside et al., BMC Genomics 9:185 (2008); Williams et al., BMC Genomics 8:172 (2007); Landgraf et al., Cell 129:1401 (2007).

The term "miRNA array" refers to a multiplex technology used in molecular biology and in medicine. It consists of an arrayed series of multiple (e.g., thousands)
microscopic spots of oligonucleotides, each containing a specific sequence (probe) complementary to a particular target miRNA. After probe-target hybridization under high-stringency conditions the resulting hybrids are usually detected and quantified by quantifying fluorophore-, silver-, or chemiluminescence-labeled targets to determine relative abundance of miRNA. In the methods of the present invention, both custom-made and commercially available miRNA arrays can be used. Examples of useful commercially available miRNA arrays (based on various methods of target labeling, hybrid detection and analysis) include arrays produced by Agilent, Illumina, Invitrogen, Febit, and LC Sciences.

[0068] The term "next generation sequencing technologies" broadly refers to sequencing methods which generate multiple sequencing reactions in parallel. This allows vastly increased throughput and yield of data. Non-limiting examples of commonly used next generation sequencing platforms include Helicos small RNA sequencing, miRNA BeadArray (Illumina), Roche 454 (FLX-Titanium), and ABI SOLID.

[0069] An "individual" or "subject" or "animal", as used herein, refers to humans, veterinary animals (e.g., cats, dogs, cows, horses, sheep, pigs, etc.) and experimental animal models of neurodegenerative diseases or other neuronal pathologies (see Examples, below). In a preferred embodiment, the subject is a human.

[0070] The term "urinary tract" refers to the organs and ducts, which participate in the secretion and elimination of urine from the body.

[0071] The term "purified" as used herein refers to material that has been isolated under conditions that reduce or eliminate the presence of unrelated materials, i.e., contaminants, including native materials from which the material is obtained. For example, RNA purification includes elimination of proteins, lipids, salts and other unrelated compounds present in bodily fluids. Besides, for some methods of analysis a purified miRNA is preferably substantially free of other RNA oligonucleotides contained in bodily fluid samples (e.g., RNA and mRNA fragments, ubiquitous miRNAs, which are expressed at high levels in almost all tissues [e.g., miR-16, etc.]). As used herein, the term "substantially free" is used operationally, in the context of analytical testing of the material. Preferably, purified material substantially free of contaminants is at least 50% pure; more preferably, at least 90% pure, and still more preferably at least 99% pure. Purity can be evaluated by chromatography, gel electrophoresis, composition analysis, biological assay, and other methods known in the art.

[0072] As used herein, the term "similarly processed" refers to samples (e.g., bodily fluid samples or purified RNAs) which have been obtained using the same protocol.

[0073] The term "a control level" as used herein encompasses predetermined standards (e.g., a published value in a reference) as well as levels determined experimentally in similarly processed samples from control subjects (e.g., age-matched healthy subjects, placebo treated patients, etc.).

[0074] The term "about" or "approximately" means within a statistically meaningful range of a value. Such a range can be within an order of magnitude, preferably within 50%, more preferably within 20%, still more preferably within 10%, and even more preferably within 5% of a given value or range. The allowable variation encompassed by the term "about" or "approximately" depends on the particular system under study, and can be readily appreciated by one of ordinary skill in the art.


EXAMPLES

[0076] The present invention is also described and demonstrated by way of the following examples. However, the use of these and other examples anywhere in the specification is illustrative only and in no way limits the scope and meaning of the invention or of any exemplified
Example 1: Comparison of different methods used for miRNA purification from serum or plasma.

[0077] Since most of the commercially available kits for miRNA isolation have been developed for miRNA purification from cells and tissues various kits are compared with in-house modifications to adjust them for miRNA isolation from serum or plasma. Commercial kits include the miRNeasy kit (Qiagen), the MirVana RNA isolation kit (Ambion/ABI), miRACLE (Agilent), High Pure miRNA isolation kit (Roche), and miRNA Purification kit (Norgen Biotek Corp.). Besides, the in-house techniques based on the use of Trizol (Invitrogen) are developed. In some experiments, miRNA is pre-adsorbed on anion-exchanger, such as Q-Sepharose, or on magnetic beads covered with a RNA-binding material (Q-Sepharose GE Healthcare), PEI-polyethyleneimine, or other. After Trizol deproteinization, RNA is precipitated with isopropyl alcohol or additionally purified on silica columns. In some experiments, purified RNA is treated with RNase-free DNAses (Qiagen, ABI, Invitrogen or other). miRNA preparations obtained by different methods are compared using RT PCR. The quality of miRNA preparations is also evaluated by measurement of the RT PCR inhibition (see Example 3, below).

[0078] miRNA was purified from plasma and serum samples obtained from the same 5 healthy donors. $10^7$ copies of Arabidopsis thaliana miR-159a (ath-miR-159a) were spiked per 1 ml plasma or serum after addition of guanidine-containing solution for evaluation of miRNA yield. Two techniques, one based on MirVana Paris kit (Ambion/ABI), and another based on Trizol (Invitrogen) deproteinization, and subsequent purification on silica columns, were compared. After RNA purification concentrations of spiked miRNA and human endogenous miR-9, miR-16, and miR-134 in final prep were measured by RT-PCR. MirVana Paris kit was more effective in miRNA isolation then the Trizol-based technique and was selected for future experiments. Although all analyzed miRNA were detectable in serum and plasma and both sample types are suitable for miRNA testing, the final PCR Ct values were about 2 cycles lower for plasma, and the latter was used in subsequent experiments. Based on the quantitative measurement of spiked ath-miR-159a, average yield of miRNA isolated from plasma with MirVana kit was 71.4%.

Example 2: Selection of miRNA for testing.

[0079] Tested miRNAs were initially selected based on literature data on their enrichment in brain compartments and presence in neurites (i.e., axons and/or dendrites and/or spines) and/or synapses (Hua et al., BMC Genomics. 2009, 10:214; Liang et al., BMC Genomics. 2007, 8:166; Landgraf et al., Cell. 2007, 129:1401-1414; Lee et al., RNA. 2008, 14:35-42; Schratt et al., Nature, 439:283-289, 2006; Lugli et al., J Neurochem. 106:650-661, 2008; Bicker and Schratt, J Cell Mol Med., 12:1466-1476, 2008; Smalheiser and Lugli, Neuronomolecular Med. 11:133-140, 2009; Rajasekharapathy, Neuron, 63:714-716, 2009; Kye, RNA 13:1224-1234, 2007; Yu et al., Exp Cell Res. 314:2618-2633, 2008; Cougot, et al., J Neurosci. 28:13793-13804, 2008; Kawahara, Brain Nerve. 60:1437-1444, 2008; Schratt G. Rev Neurosci. 2009;10:842-849) as well as on their involvement in neurite- and synapse-associated processes (The miR-Ontology Data Base: available at the world wide web at ferrolab.dmi.unict.it/mirol/). For normalization, in addition to spiked miRNA, ubiquitous miRNA, such as miR-16, and miRNA expressed in numerous tissues but not in brain, such as miR-10b and miR-141, were used.

Example 3: Detection of an increase in levels of synapse and/or neurite miRNA in plasma of AD patients.

[0080] Plasma samples were obtained from patients diagnosed with developed AD by cognitive test and brain imaging. Profiles of several neuron-enriched miRNAs from plasma of these patients were analyzed using RT-PCR with primers and probes for each individual miRNA. The amount of RNA equivalent to 30 µL plasma was taken in each PCR reaction, and 1/15 of RT product was taken into final PCR. Thus, the amount of miRNA equivalent to 2 µL plasma was detected. The results were normalized per various miRNA, usually per ubiquitous miR-16, converted into Relative Quantity (RQ) of miRNA according the ABI protocol (2-ΔCt), and compared with miRNA profiles from age-matched controls. In addition, data obtained with neurite and/or synapse miRNA were compared with data obtained with neuronal body miRNA.

[0081] As shown in Figures 1A-G, the data obtained clearly demonstrate that concentrations of many neuron-enriched miRNAs increase in plasma of AD patients. However, this effect is much more prominent for neurite and/or synapse miRNAs (miR-7 (A), miR-125b (B), miR-128 (C), miR-132 (D), and miR-323-3p (E)) than for neuronal body miRNAs (miR-181a (F) and miR-491-5p (G)).

[0082] Other techniques can be used for measuring miRNA concentration in bodily fluids with some precautions. For example, application of next generation sequencing technologies to quantitative analysis of miRNAs and other small RNAs in bodily fluids is complicated by two factors. First, fragments of ribosomal RNA (rRNA) and to a lesser degree messenger RNA (mRNA) comprise major part of small oligonucleotides present in bod-
Plasma samples were obtained from patients diagnosed with MCI and AD. Profiles of neuron-enriched miRNA from plasma of these patients were analyzed using RT-PCR with primers and probes for each individual miRNA (ABI). The amount of RNA equivalent to 30 μL plasma were taken in each PCR reaction, and 1/15 of RT product was taken into final PCR. Thus, the amount of miRNA equivalent to 2 μL plasma was detected. Then the concentrations of neurite and/or synapse miRNAs were normalized per miRNA, located mainly in neuronal body, according the ABI protocol (2^ΔCt), and compared with respective numbers from age-matched controls.

Example 4: Demonstration that the increase in levels of some neurite and/or synapse miRNAs (i.e., miR-128 (A), miR-132 (B), miR-370 (C), and miR-125b (D)) from Control to MCI to AD. These data suggest that periodic screening of elderly people can help with early diagnostics and monitoring of MCI and AD.

Example 5: Comparison of neuron-enriched miRNA levels in plasma of Control, MCI and AD patients.

Example 6: Detection of neurite destruction and synapse loss (in the absence of massive neuronal cell death) in animal models of early and mild AD by analysis of neurite and/or synapse miRNAs in blood.

Various transgenic mice models are currently available that overexpress Familial Alzheimer’s disease (FAD) mutant forms of human APP. Most currently studied models show cognitive deficits and age-related disruption of synaptic markers and amyloid plaque deposition, but few strains show evidence of significant cell death (Janus et al. 2000; Ashe 2001; Chapman et al. 2001; Richardson & Burns 2002). Examples of such transgenic mice are (i) PDAPP mice overexpressing hAPP V717F; (ii) Tg2576 mice overexpressing hAPP 695 mutated with both K670N and M671L (Hsiao et al., 1996), (iii) TgAPP/Ld/2 mice overexpressing hAPP V642L; (iv) mice overexpressing hAPP V717I; (v) human APP transgenic mice with mutation of Asp-664, which prevents caspase cleavage and accumulation of cytotoxic peptide APP-C31 with partial reversal of Alzheimer’s-like pathology (Galvan et al. Proc Natl Acad Sci U S A. 2006;103:7130-7135). Also useful is a double mutant transgenic mouse model expressing APP minigenes that encode FAD-linked APP mutants and an early-onset fa-
milial AD (FAD)-linked human presenilin 1 (PS1) variant (A246E) and a chimeric mouse/human APP harboring mutations linked to Swedish FAD kindreds (APPswe) (see U.S. Patent No. 5,912,410; Borchelt et al., Neuron 1997, 19:939-945; Holcomb et al., 1998). These mice develop numerous amyloid deposits much earlier than age-matched mice expressing APPswe and wild-type human PS1. Expression of APP minigene results in FAD-linked APP mutants and, in particular, co-expression of the mutant human PS1 A246E and APPswe elevates levels of Aβ in the brain, and these mice develop numerous diffuse Aβ deposits and plaques in the hippocampus and cortex (Calhoun et al., Proc. Natl. Acad. Sci. USA 1999; 96:14088-14093). Similarly to humans suffering from AD, these and other transgenic animal models are characterized by various cognitive defects such as loss of neurons, learning deficits, problems in object recognition memory, and problems with alternation-spatial reference and working memory (Chen et al., Nature 2000; 408:975-979).

[0091] To detect neurite destruction and synapse loss (in the absence of massive neuronal cell death), neurite and/or synapse miRNAs are isolated from the blood serum/plasma of AD model transgenic mice and analyzed by RT-PCR, and data obtained are compared with brain histopathology.

The following items are herein disclosed.

Item 1. A method for diagnosing a neuronal pathology in a subject, which comprises:

a. determining the level of at least one synapse or neurite small RNA in a bodily fluid sample from the subject;

b. comparing the level of the small RNA in the bodily fluid sample from the subject with a control level of the small RNA, and

c. (i) identifying the subject as being afflicted with the neuronal pathology when the level of the small RNA in the bodily fluid sample from the subject is increased as compared to the control or (ii) identifying the subject as not being afflicted with the neuronal pathology when the level of the small RNA in the bodily fluid sample from the subject is not increased as compared to the control.

Item 2. A method for diagnosing a neuronal pathology in a subject, which comprises:

a. determining the level of a synapse or neurite small RNA in a bodily fluid sample from the subject;

b. determining the level of a neuronal body small RNA in a bodily fluid sample from the subject;

c. determining the ratio of the levels of the small RNAs determined in steps (a) and (b);

d. comparing the ratio of the levels of the small RNAs determined in step (c) with a corresponding control ratio, and

e. (i) identifying the subject as being afflicted with the neuronal pathology when the ratio of the levels of the small RNAs determined in step (c) is higher than the corresponding control ratio or (ii) identifying the subject as not being afflicted with the neuronal pathology when the ratio of the levels of the small RNAs determined in step (c) is not higher than the corresponding control ratio.

Item 3. The method of item 1 or 2, wherein the neuronal pathology is associated with a neurodegenerative disease.

Item 4. The method of item 3, wherein the neurodegenerative disease is a member selected from the group consisting of Alzheimer’s disease (AD), Parkinson’s disease (PD), Lewy Body dementia, Huntington’s disease (HD), frontotemporal dementia (FTD), vascular dementia, HIV Associated Neurocognitive Disorders (HAND), mild cognitive impairment (MCI), mixed dementia, Creutzfeldt-Jakob Disease (CJD), normal pressure hydrocephalus, Wernicke-Korsakoff syndrome, multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), prion diseases, and different ataxias.

Item 5. The method of item 1 or 2, wherein the neuronal pathology is associated with an encephalopathy or neuropathy.

Item 6. The method of item 1 or 2, wherein the neuronal pathology is diagnosed prior to massive neuronal cell death characteristic of said pathology.

Item 7. A method for diagnosing Alzheimer’s disease (AD) in a subject, which comprises:

a. determining the level of at least one synapse or neurite small RNA in a bodily fluid sample from the subject;

b. comparing the level of the small RNA in the bodily fluid sample from the subject with a control level of the small RNA, and

c. (i) identifying the subject as being afflicted with AD when the level of the small RNA in the bodily fluid sample from the subject is increased as compared to the control or (ii) identifying the subject as not being afflicted with AD when the level of the small RNA in the bodily fluid sample from
the subject is not increased as compared to the control.

Item 8. The method of item 7, wherein AD is diagnosed prior to massive neuronal cell death characteristic of AD.

Item 9. A method for diagnosing mild cognitive impairment (MCI) in a subject, which comprises:

a. determining the level of at least one synapse or neurite small RNA in a bodily fluid sample from the subject;

b. comparing the level of the small RNA in the bodily fluid sample from the subject with a control level of the small RNA, and

c. (i) identifying the subject as being afflicted with MCI when the level of the small RNA in the bodily fluid sample from the subject is increased as compared to the control or (ii) identifying the subject as not being afflicted with MCI when the level of the small RNA in the bodily fluid sample from the subject is not increased as compared to the control.

Item 10. The method of any one of items 1, 2, 7, or 9, wherein the small RNA is present in synapses.

Item 11. The method of any one of items 1, 2, 7, or 9, wherein the small RNA is present in spines.

Item 12. The method of any one of items 1, 2, 7, or 9, wherein the small RNA is present in axons.

Item 13. The method of any one of items 1, 2, 7, or 9, wherein the small RNA is present in dendrites.

Item 14. The method of any one of items 1, 2, 7, or 9, wherein the small RNA is miRNA.

Item 15. The method of item 14, wherein the miRNA is a member selected from the group consisting of miR-7, miR-9, miR-9*, miR-25, miR-26a, miR-26b, miR-98, miR-124, miR-125b, miR-128, miR-132, miR-134, miR-138, miR-146, miR-182, miR-183, miR-200b, miR-200c, miR-213, miR-292-5p, miR-297, miR-322, miR-323-3p, miR-325, miR-337, miR-339, miR-345, miR-350, miR-351, miR-370, miR-425, miR-429, miR-433-5p, miR-446, miR-467, and miR-874.

Item 16. The method of item 14, wherein the miRNA is a member selected from the group consisting of miR-7, miR-125b, miR-128, miR-132, miR-323-3p, miR-370, and miR-874.

Item 17. The method of any one of items 1, 2, 7, or 9, wherein the control level of the small RNA is the level of said small RNA in a similarly processed bodily fluid sample from an age-matched control subject.

Item 18. The method of any one of items 1, 2, 7, or 9, wherein the control level of the small RNA is the level of said small RNA in a similarly processed bodily fluid sample from the same subject obtained in the past.

Item 19. The method of any one of items 1, 2, 7, or 9, wherein the control level of the small RNA is a predetermined standard.

Item 20. The method of item 2, wherein the neuronal body small RNA is miR-181a or miR-491-5p.

Item 21. The method of any one of items 1, 2, 7, or 9 comprising determining the level of two or more synapse or neurite small RNAs.

Item 22. The method of any one of items 1, 2, 7, or 9, further comprising normalizing the level of the small RNA in the bodily fluid sample from the subject and in the control to the level of a small RNA which is not expressed in brain.

Item 23. The method of item 22, wherein the small RNA which is not expressed in brain is miR-10b or miR-141.

Item 24. The method of any one of items 1, 2, 7, or 9, wherein the bodily fluid sample is blood plasma.

Item 25. The method of any one of items 1, 2, 7, or 9, wherein the level of the small RNA is determined using a method selected from the group consisting of hybridization, RT-PCR, and sequencing.

Item 26. The method of any one of items 1, 2, 7, or 9, wherein, prior to step (a), the small RNA is purified from the bodily fluid sample.

Item 27. The method of any one of items 1, 2, 7, or 9, further comprising the step of reducing or eliminating degradation of the small RNA.

Item 28. A method for monitoring development of a neuronal pathology in a subject, which comprises:

a. determining the level of at least one synapse or neurite small RNA in two or more bodily fluid samples from the subject, wherein the samples have been obtained at spaced apart time points, and

b. comparing the levels of the small RNA between the earlier obtained and later obtained.
bodily fluid sample(s).

Item 29. The method of item 28, which further comprises (c) (i) determining that the development of the neuronal pathology in the subject is accelerated if the level of the small RNA is increased in the later obtained bodily fluid sample(s) as compared to the earlier obtained sample(s); (ii) determining that the neuronal pathology in the subject continues to develop at the same rate if the level of the small RNA is not changed in the later obtained bodily fluid sample(s) as compared to the earlier obtained sample(s), and (iii) determining that the development of the neuronal pathology in the subject is slowed down if the level of the small RNA is decreased in the later obtained bodily fluid sample(s) as compared to the earlier obtained sample(s).

Item 30. The method of item 28 or 29, wherein the bodily fluid samples have been obtained within 1-48 months intervals.

Item 31. A method for monitoring the effectiveness of a treatment of a neuronal pathology in a subject, which comprises:

a. determining the level of at least one synapse or neurite small RNA in a bodily fluid sample from the subject obtained prior to initiation of the treatment;

b. determining the level of the small RNA in one or more bodily fluid sample(s) from the subject obtained in the course of or following the treatment, and

c. comparing the level of the small RNA determined in steps (a) and (b), and optionally between different samples in step (b).

Item 32. The method of item 31, which further comprises (d) (i) determining that the treatment is effective if the level of the small RNA has decreased in the course of or following the treatment or (ii) determining that the treatment is not effective if the level of the small RNA has not decreased in the course of or following the treatment.

Item 33. The method of item 31 or 32, wherein the bodily fluid samples have been obtained within 1-12 months intervals.

Item 34. The method of any one of items 28, 29, 31, or 32, wherein, the small RNA is present in synapses.

Item 35. The method of any one of items 28, 29, 31, or 32, wherein, the small RNA is present in dendrites.

Item 36. The method of any one of items 28, 29, 31, or 32, wherein, the small RNA is present in axons.

Item 37. The method of any one of items 28, 29, 31, or 32, wherein, the small RNA is present in dendrites.

Item 38. The method of any one of items 28, 29, 31, or 32, wherein the small RNA is miRNA.

Item 39. The method of item 38, wherein the miRNA is a member selected from the group consisting of miR-7, miR-9, miR-9*, miR-25, miR-26a, miR-26b, miR-98, miR-124, miR-125b, miR-128, miR-132, miR-134, miR-138, miR-146, miR-182, miR-183, miR-200b, miR-200c, miR-213, miR-292-5p, miR-297, miR-322, miR-323-3p, miR-325, miR-337, miR-339, miR-345, miR-350, miR-351, miR-370, miR-425, miR-429, miR-433-5p, miR-446, miR-467, and miR-874.

Item 40. The method of item 38, wherein the miRNA is a member selected from the group consisting of miR-7, miR-125b, miR-128, miR-132, miR-323-3p, miR-370, and miR-874.

Item 41. The method of any one of items 28, 29, 31, or 32 comprising determining the level of two or more synapse or neurite small RNAs.

Item 42. The method of any one of items 28, 29, 31, or 32 wherein the bodily fluid sample is blood plasma.

Item 43. The method of any one of items 28, 29, 31, or 32, wherein the level of the small RNA is determined using a method selected from the group consisting of hybridization, RT-PCR, and sequencing.

Item 44. The method of any one of items 28, 29, 31, or 32, wherein, prior to step (a), the small RNA is purified from the bodily fluid sample.

Item 45. The method of any one of items 28, 29, 31, or 32, further comprising the step of reducing or eliminating degradation of the small RNA.

Item 46. The method of any one of items 28, 29, 31, or 32, wherein the neuronal pathology is associated with a neurodegenerative disease.

Item 47. The method of item 46, wherein the neurodegenerative disease is a member selected from the group consisting of Alzheimer's disease (AD), Parkinson's disease (PD), Lewy Body dementia, Huntington's disease (HD), frontotemporal dementia (FTD), vascular dementia, HIV Associated Neurocognitive Disorders (HAND), mild cognitive impairment (MCI), mixed dementia, Creutzfeldt-Jakob Dis-
ease (CJD), normal pressure hydrocephalus, Wer-
nicke-Korsakoff syndrome, multiple sclerosis (MS),
amyotrophic lateral sclerosis (ALS), prion diseases,
and different ataxias.

Item 48. The method of any one of items 28, 29, 31,
or 32, wherein the neuronal pathology is monitored
prior to massive neuronal cell death characteristic of
said pathology.

Claims

1. A method for detecting in a subject neurite destruc-
tion and synapse loss, associated with a neuronal
pathology, prior to neuronal cell death, which method
comprises:

a. measuring the level of a synapse or neurite
small RNA in a bodily fluid sample collected from
the subject, wherein the bodily fluid is selected
from the group consisting of blood plasma, ser-
num, urine, and saliva;

b. measuring the level of a normalizer small RNA
in the same bodily fluid sample collected from
the subject, wherein the normalizer small RNA
is a neuronal body small RNA or a small RNA
which is not expressed in brain or miRNA se-
lected from the group consisting of miR-181a,
miR-491-5p, miR-10b and miR-141;

c. calculating the ratio of the levels of the small
RNAs measured in steps (a) and (b);

d. comparing the ratio of the levels of the small
RNAs calculated in step (c) with a corresponding
control ratio, wherein the control ratio is selected
from the group consisting of (i) a predetermined
standard, (ii) the ratio of said synapse or neurite
small RNA to said normalizer small RNA in a
similarly processed bodily fluid sample from the
same subject collected in the past, and (iii) the
ratio of said synapse or neurite small RNA to
said normalizer small RNA in a similarly proc-
essed bodily fluid sample from a control subject,

and
e. (i) identifying the subject as being afflicted with
neurite destruction and synapse loss associated
with the neuronal pathology when the ratio of
the levels of the small RNAs calculated in step
(c) is higher than the corresponding control ratio
or (ii) identifying the subject as not being afflicted
with neurite destruction and synapse loss asso-
ciated with the neuronal pathology when the ra-
tio of the levels of the small RNAs calculated in
step (c) is not higher than the corresponding
control ratio.

2. A method for monitoring changes in neurite destruc-
tion and synapse loss associated with development
of a neuronal pathology in a subject, which method
comprises:

a. measuring the level of a synapse or neurite
small RNA in two or more bodily fluid samples
collected from the subject, wherein the samples
have been collected at spaced apart time points,
and wherein the bodily fluid is selected from the
group consisting of blood plasma, serum, urine,
and saliva;

b. measuring the level of a normalizer small RNA
in the same bodily fluid samples as in step (a),
wherein the normalizer small RNA is a neuronal
body small RNA or a small RNA which is not
expressed in brain or miRNA selected from the

3. A method for monitoring the effect of a treatment on
neurite destruction and synapse loss in a subject suf-
ferring from a neuronal pathology, which method
comprises:

a. measuring the level of a synapse or neurite
small RNA in a bodily fluid sample collected from
the subject prior to initiation of the treatment,
wherein the bodily fluid is selected from the
group consisting of blood serum, blood plasma,
urine, and saliva;

b. measuring the level of a normalizer small RNA
in the same bodily fluid sample as in step (a),
wherein the normalizer small RNA is a neuronal

4. A method for detecting in a subject neurite destruc-
tion and synapse loss, associated with a neuronal
pathology, prior to neuronal cell death, which method
comprises:

a. measuring the level of a synapse or neurite
small RNA in a bodily fluid sample collected from
the subject, wherein the bodily fluid is selected
from the group consisting of blood plasma, serum,
urine, and saliva;

b. measuring the level of a normalizer small RNA
in the same bodily fluid sample as in step (a),
wherein the normalizer small RNA is a neuronal
body small RNA or a small RNA which is not
expressed in brain or miRNA selected from the

5. A method for detecting in a subject neurite destruc-
tion and synapse loss, associated with a neuronal
pathology, prior to neuronal cell death, which method
comprises:

a. measuring the level of a synapse or neurite
small RNA in a bodily fluid sample collected from
the subject, wherein the bodily fluid is selected
from the group consisting of blood plasma, serum,
urine, and saliva;

b. measuring the level of a normalizer small RNA
in the same bodily fluid sample as in step (a),
wherein the normalizer small RNA is a neuronal
body small RNA or a small RNA which is not
expressed in brain or miRNA selected from the

6. A method for detecting in a subject neurite destruc-
tion and synapse loss, associated with a neuronal
pathology, prior to neuronal cell death, which method
comprises:

a. measuring the level of a synapse or neurite
small RNA in a bodily fluid sample collected from
the subject, wherein the bodily fluid is selected
from the group consisting of blood plasma, serum,
urine, and saliva;

b. measuring the level of a normalizer small RNA
in the same bodily fluid sample as in step (a),
wherein the normalizer small RNA is a neuronal
body small RNA or a small RNA which is not
expressed in brain or miRNA selected from the

7. A method for detecting in a subject neurite destruc-
tion and synapse loss, associated with a neuronal
pathology, prior to neuronal cell death, which method
comprises:

a. measuring the level of a synapse or neurite
small RNA in a bodily fluid sample collected from
the subject, wherein the bodily fluid is selected
from the group consisting of blood plasma, serum,
urine, and saliva;

b. measuring the level of a normalizer small RNA
in the same bodily fluid sample as in step (a),
wherein the normalizer small RNA is a neuronal
body small RNA or a small RNA which is not
expressed in brain or miRNA selected from the

body small RNA or a small RNA which is not expressed in brain or miRNA selected from the group consisting of miR-181a, miR-491-5p, miR-10b and miR-141;
c. calculating the ratio of the levels of the small RNAs measured in steps (a) and (b);
d. measuring the level of the same synapse or neurite small RNAs as in step (a) in one or more bodily fluid sample(s) collected from the subject in the course of or following the treatment, wherein the bodily fluid is selected from the group consisting of blood plasma, serum, urine, and saliva;
e. measuring the level of the same normalizer small RNA as in step (b) in the same bodily fluid sample(s) as in step (d);
f. calculating the ratio of the levels of the small RNAs measured in steps (d) and (e) for each bodily fluid sample;
g. comparing the ratios of the levels of the small RNAs calculated in steps (c) and (f), and optionally comparing the ratios of the levels of the small RNAs calculated in step (f) between different samples in step (d), and
h. (i) determining that the treatment is effective in decreasing neurite destruction and synapse loss if the ratio of the levels of the small RNAs calculated in step (c) is higher than the corresponding ratio(s) calculated in step (f) or (ii) determining that the treatment is not effective in decreasing neurite destruction and synapse loss if the ratio of the levels of the small RNAs calculated in step (c) is not higher than the corresponding ratio(s) calculated in step (f).

4. The method of any of claims 1 to 3, wherein the synapse or neurite small RNA is miRNA selected from the group consisting of miR-7, miR-9, miR-9*, miR-25, miR-26a, miR-26b, miR-98, miR-124, miR-125b, miR-128, miR-132, miR-134, miR-138, miR-146, miR-182, miR-183, miR-200b, miR-200c, miR-213, miR-292-5p, miR-297, miR-322, miR-323-3p, miR-325, miR-337, miR-339, miR-345, miR-350, miR-351, miR-370, miR-425, miR-429, miR-433-5p, miR-446, miR-467, and miR-874.

5. The method of any of claims 1 to 4, comprising measuring the level of two or more synapse or neurite small RNAs.

6. The method of any of claims 1 to 5, wherein the neuronal pathology is associated with a neurodegenerative disease.

7. The method of claim 6, wherein the neurodegenerative disease is selected from the group consisting of Alzheimer’s disease (AD), Parkinson’s disease (PD), Lewy Body dementia, Huntington’s disease (HD), frontotemporal dementia (FTD), vascular dementia, HIV Associated Neurocognitive Disorders (HAND), mild cognitive impairment (MCI), mixed dementia, Creutzfeldt-Jakob Disease (CJD), normal pressure hydrocephalus, Wernicke-Korsakoff syndrome, multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), prion diseases, and different ataxias and neuronal pathologies associated with an encephalopathy or neuropathy.
FIG. 1A

miR-7

Median
C: 139
AD: 1322

FIG. 1B

miR-125b

Median
C: 364
AD: 2077
**FIG. 1C**

**FIG. 1D**
**FIG. 1E**

- **miR-323-3p**
  - C
  - MCI
  - Median
    - C: 93
    - AD: 479

**FIG. 1F**

- **miR-181a**
  - C
  - AD
  - Median
    - C: 59
    - AD: 99
FIG. 4A

miR-128

C
MCI
AD

FIG. 4B

miR-132

C
MCI
AD
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The present search report has been drawn up for all claims

**Place of search:** Munich  
**Date of completion of the search:** 8 November 2016  
**Examiner:** Ripaud, Leslie
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