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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
29 August 2013 (29.08.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/124816 A2

(51) International Patent Classification:
C12N 5/0793 (2010.01)

(21) International Application Number:
PCT/IB2013/051429

(22) International Filing Date:
21 February 2013 (21.02.2013)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/601,596 22 February 2012 (22.02.2012) US

(71) Applicant: **BRAINSTEM BIOTEC LTD.** [IL/IL]; 14 Weizmann Street, 64239 Tel-Aviv (IL).

(72) Inventors: **BRODIE, Chaya**; 25236 Southwood Drive, Southfield, Michigan 48075 (US). **SLAVIN, Shimon**; 34A Shlush Street, 65149 Tel-Aviv (IL).

(74) Agents: **G. E. EHRLICH (1995) LTD.** et al.; 11 Menachem Begin Road, 5268104 Ramat Gan (IL).

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

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

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

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

WO 2013/124816 A2

(54) Title: GENERATION OF NEURAL STEM CELLS AND MOTOR NEURONS

(57) Abstract: A method of generating neural stem cells or motor neurons is disclosed, the method comprising up-regulating a level of at least one exogenous mi RNA and/or down-regulating at least one mi RNA using an agent which hybridizes to the mi RNA in mesenchymal stem cells (MSCs). Isolated cell populations and uses thereof are also disclosed.

GENERATION OF NEURAL STEM CELLS AND MOTOR NEURONS

RELATED APPLICATION

This application claims the benefit of priority of U.S. Provisional Patent Application No. 61/601,596 filed February 22, 2012, the contents of which are incorporated herein by reference in their entirety.

FIELD AND BACKGROUND OF THE INVENTION

The present invention, in some embodiments thereof, relates to methods of ex vivo differentiating mesenchymal stem cells towards neural stem cells and motor neurons using microRNAs (miRNAs).

Mesenchymal stem cells (MSCs) are a heterogeneous population of stromal cells that can be isolated from multiple species, residing in most connective tissues including bone marrow, adipose, placenta, umbilical cord and perivascular tissues. MSCs can also be isolated from the placenta and cord's Wharton's jelly.

The concentration of MSCs in all tissues, including bone marrow and adipose tissue is very low but their number can be expanded in vitro. Typically, expansion of MSCs using up to 15 passages does not result in mutations indicating genetic stability. MSC can differentiate into cells of the mesenchymal lineage, such as bone, cartilage and fat but, under certain conditions, have been reported to acquire the phenotype of cells of the endodermal and neuroectodermal lineage, suggesting some potential for "transdifferentiation".

Within the bone marrow compartment, these cells are tightly intermingled with and support hematopoiesis and the survival of hematopoietic stem cells in acquiescent state (7). In addition, after expansion in culture, MSCs retain their ability to modulate innate and adaptive immunity (8). Furthermore, MSCs migrate actively to sites of inflammation and protect damaged tissues, including the CNS, properties that supported their use as new immunosuppressive or rather immunoregulatory or anti-inflammatory agents for the treatment of inflammatory and immune-mediated diseases including autoimmune disorders (9). These features of MSCs merited their use to control life-threatening graft-versus-host-disease (GVHD) following allogeneic bone marrow transplantation, thus controlling one of the most serious complications of allogeneic

bone marrow transplantation, helping to lower transplant-related toxicity and mortality associated with multi-system organ injury (10).

Several studies have shown that MSCs following exposure to different factors *in vitro,* change their phenotype and demonstrate neuronal and glial markers [Kopen, G.C., et al., Proc Natl Acad USA. 96(19):10711-6, 1999; Sanchez-Ramos, et al. Exp Neurol. 164(2):247-56. 2000; Woodbury, D., J Neurosci Res. 61(4):364-70,2000; Woodbury, D., et al., J Neurosci Res. 69(6):908-17, 2002; Black, I.B., Woodbury, D. Blood Cells Mol Dis. 27(3):632-6, 2001; Kohyama, J., et al. Differentiation. 68(4-5):235-44, 2001; Levy, Y.S. J Mol Neurosci. 21(2):121-32, 2003].

Accordingly, MSCs (both ex-vivo differentiated and non-differentiated) have been proposed as candidates for cell replacement therapy for the treatment of various neurological disorders including multiple sclerosis, Parkinson's disease, ALS, Alzheimer's disease, spinal cord injury and stroke.

Motor neurons in the spinal cord innervate skeletal muscles, and originate from neuroepithelial cells in a restricted area of the developing spinal cord (neural tube). During embryonic development, motor neurons extend their processes (nerves) to the periphery to innervate skeletal muscles that are adjacent to the spinal cord. In an adult human body, however, motor neuron's axons are projected large distances away from the cell bodies in the spinal cord to reach their target muscles. Because of this, motor neurons have a higher metabolic rate compared to smaller neurons, and this renders them more susceptible to genetic, epigenetic, and environmental changes. Motor neurons can not renew themselves and therefore their loss or degeneration are generally associated with fatal neurological conditions including paralysis and disorders such as pediatric spinal muscular atrophy (SMA) and adult onset amyotrophic lateral sclerosis (ALS).

Roy et al., 2005 [*Exp Neurol.* 2005;196:224–234]; Zhang et al., 2006 [*Stem Cells.* 2006;24:434–442]; Bohl et al., 2008 [*Stem Cells.* 2008;26:2564–2575]; and Dimos et al., 2008 [*Science.* 2008;321:1218–1221] the contents of which are incorporated by reference teach genetic modification of different stem cells to induce differentiation into motor neurons.

SUMMARY OF THE INVENTION

According to an aspect of some embodiments of the present invention there is provided a method of predisposing mesenchymal stem cells to differentiate into neural stem cells, the method comprising up-regulating a level of at least one exogenous miRNA selected from the group consisting of miR-1275, miR-891a, miR-154, miR-1202, miR-572, miR-935a, miR302b, miR-371, miR-134, miR-219, miR-155, miR-32, miR-33, miR-126, miR-127, miR-132, let-7c, miR-665, miR-4258, miR-361-3p, miR-374a-star, miR-892b, miR-361-5p, miR-181a, miR-16, miR-636, miR-4284, miR-1208, miR-1274b, miR-30c-2-star, miR-501-3p, hsa-miR-92a, miR-378b, miR-1287, miR-425-star, miR-324-5p, miR-3178, miR-219-1-3p, miR-197, miR-181b, miR-500-star, miR-106b, miR-502-3p, miR-30c, miR-1275, miR-422a, miR-93, miR-181d, miR-1307, miR-1301, miR-99a, miR-505-star, miR-1202, miR-12, miR-532-5p, miR-195, miR-532-3p, miR-106a, miR-17, miR-1271, miR-769-3p, miR-15b, miR-324-3p, miR-20a, miR-501-5p, miR-330-3p, miR-874, miR-500, miR-25, miR-769-5p, miR-125b-2-star, miR-130b, miR-504, miR-181a-2-star, miR-885-3p, miR-1246, miR-92b, miR-362-5p, miR-572, miR-4270, miR-378c, miR-93-star, miR-149, miR-363, miR-9, miR-18a, miR-346, miR-497, miR-378, miR-1231, miR-139-5p, miR-3180-3p, miR-935 and miR-20b in the mesenchymal stem cells (MSCs), thereby predisposing the MSCs to differentiate into the neural stem cells.

According to an aspect of some embodiments of the present invention there is provided a method of predisposing MSCs to differentiate into neural stem cells, the method comprising down-regulating an expression of at least one miRNA selected from the group consisting of miR-4317, miR-153, miR-4288, miR-409-5p, miR-193a-5p, miR-10b, miR-142-3p, miR-131a, miR-125b, miR-181a, miR-145, miR-143, miR-214, miR-199a-3p, miR-199a-5p, miR-199b-3p, miR-138, miR-31, miR-21, miR-193a-5p, miR-224-star, miR-196a, miR-487b, miR-409-5p, miR-193b-star, miR-379, miR-21-star, miR-27a-star, miR-27a, miR-4317, miR-193b, miR-27b, miR-22, 574-3p, miR-4288, miR-23a, miR-221-star, miR-2113, let-7i, miR-24, miR-23b, miR-299-3p, miR-518c-star, miR-221, miR-431-star, miR-523, miR-4313, miR-559, miR-614, miR-653, miR-2278, miR-768-5p, miR-154-star, miR-302a-star, miR-3199 and miR-3137 in the mesenchymal stem cells by up-regulating a level of at least one polynucleotide agent

that hybridizes and inhibits a function of the at least one miRNA thereby predisposing the MSCs to differentiate into the neural stem cells.

According to an aspect of some embodiments of the present invention there is provided a method of predisposing MSCs to differentiate into neural stem cells, the method comprising up-regulating a level of exogenous miR-124 in the mesenchymal stem cells (MSCs) and down-regulating a level of miR-let-7 in the MSCs, thereby predisposing the MSCs to differentiate into the neural stem cells.

According to an aspect of some embodiments of the present invention there is provided a method of predisposing MSCs to differentiate into neural stem cells, the method comprising contacting the mesenchymal stem cells (MSCs) with an agent that down-regulates an amount and/or activity of Related to testis-specific, vespid and pathogenesis protein 1 (RTVP-1), thereby predisposing MSCs to differentiate into the neural stem cells.

According to an aspect of some embodiments of the present invention there is provided a method of predisposing neural stem cells to differentiate into motor neurons, the method comprising up-regulating a level of at least one exogenous miRNA selected from the group consisting of miR-368, miR-302b, miR-365-3p, miR-365-5p, miR-Let-7a, miR-Let-7b, miR-218, miR-134, miR-124, miR-125a, miR-9, miR-154, miR-20a and miR-130a in neural stem cells (NSCs), thereby predisposing NSCs to differentiate into the motor neurons.

According to an aspect of some embodiments of the present invention there is provided a method of predisposing MSCs to differentiate into motor neurons, the method comprising up-regulating a level of at least one exogenous miRNA selected from the group consisting of miR-648, miR-368, miR-365, miR-500, miR-491, miR-218, miR-155, miR-192, let-7b, miR-16, miR-210, miR-197, miR-21,miR-373, ,miR-27a, miR-122, miR-17, miR-494, miR-449, miR-503, miR-30a, miR-196a, miR-122, miR-7, miR-151-5p, miR-16, miR-22, miR-31, miR-424, miR-1, miR-29c, miR-942, miR-100, miR-520, miR-663a, miR-562, miR-449a, miR-449b-5p, miR-520b, miR-451, miR-532-59, miR-605, miR-504, miR-503, miR-155, miR-34a, miR-16, miR-7b, miR-103, miR-124, miR-1385p, miR-16, miR-330, miR-520, miR-608, miR-708, miR-107, miR-137, miR-132, miR-145, miR-204, miR-125b, miR-224, miR-30a, miR-375, miR-101, miR-106b, miR-128, miR-129-5p, miR-153, miR-203, miR-214, miR-338-3p,

miR-346, miR-98, miR-107, miR-141, miR-217, miR-424, miR-449, miR-7, miR-9, miR-93, miR-99a, miR-100, miR-1228, miR-183, miR-185, miR-190, miR-522, miR-650, miR-675, miR-342-3p, miR-31 in the mesenchymal stem cells (MSCs), thereby predisposing MSCs to differentiate into the motor neurons.

According to an aspect of some embodiments of the present invention there is provided a method of predisposing NSCs to differentiate into motor neurons, the method comprising down-regulating an expression of at least one miRNA selected from the group consisting of miR-372, miR-373, miR-141, miR-199a, miR-32, miR-33, miR-221 and miR-223 by up-regulating a level of at least one polynucleotide agent that hybridizes and inhibits a function of the at least one miRNA in the NSCs thereby predisposing NSCs to differentiate into the motor neurons.

According to an aspect of some embodiments of the present invention there is provided a method of predisposing MSCs to differentiate into motor neurons, the method comprising down-regulating an expression of at least one miRNA selected from the group consisting of miR-372, miR-373, miR-942, miR-2113, miR-199a-3p, miR-199a-5p, miR-372, miR-373, miR-942, miR-2113, miR-301a-3p, miR-302c, miR-30b-5p, miR-30c, miR-326, miR-328, miR-331-3p, miR-340, miR-345, miR-361-5p, miR-363, miR-365a-3p, miR-371a-3p, miR-373-3p, miR-374a, miR-423-3p, miR-449b-5p, miR-451a, miR-494, miR-504, miR-515-3p, miR-516a-3p, miR-519e, miR-520a-3p, miR-520c-3p, miR-520g, miR-532-5p, miR-559, miR-562, miR-572, miR-590-5p, miR-605, miR-608, miR-626, miR-639, miR-654-3p, miR-657, miR-661, miR-708-5p, miR-942, miR-96, miR-99arno and miR-194 by up-regulating a level of at least one polynucleotide agent that hybridizes and inhibits a function of the at least one miRNA in the MSCs thereby predisposing MSCs to differentiate into the motor neurons.

According to an aspect of some embodiments of the present invention there is provided a genetically modified isolated population of cells which comprise at least one exogenous miRNA selected from the group consisting of miR302b, miR-371, miR-134, miR-219, miR-154, miR-155, miR-32, miR-33, miR-126, miR-127, miR-132 and miR-137 and/or which comprise at least one polynucleotide agent that hybridizes and inhibits a function of at least one miRNA selected from the group consisting of miR-10b, miR-142-3p, miR-131a, miR-125b, miR-153 and miR-181a, wherein the cells have a neural stem cell phenotype.

According to an aspect of some embodiments of the present invention there is provided a genetically modified isolated population of cells which comprise at least one exogenous miRNA selected from the group consisting of miR-368, miR-302b, miR-365-3p, miR-365-5p, miR-Let-7a, miR-Let-7b, miR-218, miR-134, miR-124, miR-125a, miR-9, miR-154, miR-20a, miR-130a and/or which comprise at least one polynucleotide agent that hybridizes and inhibits a function of at least one miRNA selected from the group consisting of miR-372, miR-373, miR-141, miR-199a, miR-32, miR-33, miR-221 and miR-223, wherein the cells have a motor neuron phenotype.

According to an aspect of some embodiments of the present invention there is provided a method of treating a brain disease or disorder in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the isolated population of cells of claim 33, thereby treating the brain disease or disorder.

According to an aspect of some embodiments of the present invention there is provided a pharmaceutical composition comprising the isolated population of cells described herein and a pharmaceutically acceptable carrier.

According to an aspect of some embodiments of the present invention there is provided a method of selecting a miRNA which may be regulated for the treatment of a motor neuron disease comprising:

(a) differentiating a population of neural stem cells towards a motor neuron phenotype; and

(b) analyzing a change in expression of a miRNA in the population of MSCs prior to and following the differentiating of the MSCs towards a motor neuron phenotype, wherein a change of expression of a miRNA above or below a predetermined level is indicative that the miRNA may be regulated for the treatment of the motor neuron disease.

According to an aspect of some embodiments of the present invention there is provided a method of treating a motor neuron disease in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the isolated population of cells of claim 35, thereby treating the brain disease or disorder.

According to an aspect of some embodiments of the present invention there is provided a genetically modified isolated population of cells which comprise at least one exogenous miRNA selected from the group consisting of miR-1275, miR-891a, miR-154, miR-1202, miR-572 and miR-935a and/or which comprise at least one polynucleotide agent that hybridizes and inhibits a function of at least one miRNA selected from the group consisting of miR-4317, miR-153, miR-4288, miR-409-5p, miR-193a-5p, wherein said cells have a neural stem cell phenotype.

According to an aspect of some embodiments of the present invention there is provided a genetically modified isolated population of cells which comprise at least one exogenous miRNA selected from the group consisting of miR-648, miR-368, miR-365, miR-500 and miR-491 and/or which comprise at least one polynucleotide agent that hybridizes and inhibits a function of at least one miRNA selected from the group consisting of miR-372, miR-373, miR-942, miR-2113, miR-199a-3p and miR-199a-5p, wherein said cells have a motor neuron phenotype.

According to some embodiments of the invention, the at least one exogenous miRNA is selected from the group consisting of miR-1275, miR-891a, miR-154, miR-1202, miR-572 and miR-935a.

According to some embodiments of the invention, the at least one exogenous miRNA is selected from the group consisting of miR-20b, miR-925, miR-891 and miR-378.

According to some embodiments of the invention, the at least one miRNA is selected from the group consisting of miR-4317, miR-153, miR-4288, miR-409-5p, and miR-193a-5p.

According to some embodiments of the invention, the at least one miRNA is selected from the group consisting of miR-138, miR-214, miR-199a and miR-199b.

According to some embodiments of the invention, the at least one miRNA is miR-138, the method further comprises:

(i) down-regulating an expression of miR-891 using a polynucleotide agent that hybridizes and inhibits the function of miR-891;

(ii) up-regulating a level of exogenous miR20b; or

(iii) up-regulating a level of exogenous miR378.

According to some embodiments of the invention, the miRNA is selected from the group consisting of miR-648, miR-368, miR-365, miR-500 and miR-491.

According to some embodiments of the invention, the miRNA is selected from the group consisting of miR-372, miR-373, miR-942, miR-2113, miR-199a-3p and miR-199a-5p.

According to some embodiments of the invention, the at least one miRNA comprises each of miR Let-7a, miR-124, miR-368 and miR-154.

According to some embodiments of the invention, the at least one miRNA comprises each of miR-125a, miR-9 and miR-130a.

According to some embodiments of the invention, the at least one miRNA comprises each of miR-218, miR-134 and miR-20a.

According to some embodiments of the invention, the method further comprises down-regulating each of miR-141, miR-32, miR-33, miR-221, miR-223 and miR-373.

According to some embodiments of the invention, the NSCs are generated by ex vivo differentiating MSCs.

According to some embodiments of the invention, the ex vivo differentiating is affected according to any of the methods described herein.

According to some embodiments of the invention, the MSCs are isolated from a tissue selected from the group consisting of bone marrow, adipose tissue, placenta, cord blood and umbilical cord.

According to some embodiments of the invention, the MSCs are autologous to the subject.

According to some embodiments of the invention, the MSCs are non-autologous to the subject.

According to some embodiments of the invention, the MSCs are semi-allogeneic to the subject.

According to some embodiments of the invention, the up-regulating comprises introducing into the MSCs the at least one miRNA.

According to some embodiments of the invention, the up-regulating is affected by transfecting the MSCs with an expression vector which comprises a polynucleotide sequence which encodes a pre-miRNA of the at least one miRNA.

According to some embodiments of the invention, the up-regulating is affected by transfecting the MSCs with an expression vector which comprises a polynucleotide sequence which encodes the at least one miRNA.

According to some embodiments of the invention, the method further comprises analyzing an expression of at least one marker selected from the group consisting of nestin and Sox2 following the generating.

According to some embodiments of the invention, the method further comprises analyzing an expression of at least one marker selected from the group consisting of islet1, HB9 and the neuronal markers neurofilament and β3 tubulin following the generating.

According to some embodiments of the invention, the method is effected in vivo.

According to some embodiments of the invention, the method is effected ex vivo.

According to some embodiments of the invention, at least 50 % of the population of cells express at least one marker selected from the group consisting of nestin and Sox2.

According to some embodiments of the invention, the at least 50 % of the population of cells express at least one marker selected from the group consisting of islet1, HB9 and the neuronal markers neurofilament and β3 tubulin.

According to some embodiments of the invention, the isolated population of cells is for use in treating a brain disease or disorder.

According to some embodiments of the invention, the isolated population of cells is for brain disease or disorder is a neurodegenerative disorder.

According to some embodiments of the invention, the neurodegenerative disorder is selected from the group consisting of multiple sclerosis, Parkinson's, epilepsy, amyotrophic lateral sclerosis (ALS), stroke, Rett Syndrome, autoimmune encephalomyelitis, spinal cord injury, cerebral palsy, stroke, Alzheimer's disease and Huntingdon's disease.

According to some embodiments of the invention, the isolated population is for use in treating a motor neuron disease.

According to some embodiments of the invention, the motor neuron disease is selected from the group consisting of amyotrophic lateral sclerosis (ALS), primary lateral sclerosis (PLS), pseudobulbar palsy and progressive bulbar palsy.

According to some embodiments of the invention, the nerve disease or disorder is a neurodegenerative disorder.

According to some embodiments of the invention, the neurodegenerative disorder is selected from the group consisting of multiple sclerosis, Parkinson's, epilepsy, amyotrophic lateral sclerosis (ALS), stroke, Rett Syndrome, autoimmune encephalomyelitis, spinal cord injury, cerebral palsy, stroke, Alzheimer's disease and Huntingdon's disease.

Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings and images. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.

In the drawings:

FIGs. 1A-B are photographs and graphs illustrating that mesenchymal stem cells (MSCs) may be induced to differentiate to neural stem cell (NSC) -like cells and express NSC markers. MSCs were plated in neurosphere medium on bacteria dishes as described in Methods. The MSC-derived spheroids were characterized by immunofluorescence (Figure 1A) and real-time PCR (Figure 1B).

FIG. 2 is a bar graph illustrating exemplary miRNAs associated with stem cell signature and self renewal that were up-regulated during NSC differentiation.

FIG. 3 is a bar graph illustrating exemplary miRNAs associated with hematopoiesis that were up-regulated during NSC differentiation.

FIGs. 4A-D are bar graphs illustrating exemplary miRNAs associated with a neuronal signature and self renewal that were up-regulated (Figures 4A-C) or down-regulated (Figure 4D) during NSC differentiation.

FIGs. 4E-F are photographs illustrating bone marrow MSCs transfected with antagomiR-138 and miR-891 using a nestin promoter reporter assay.

FIGs. 5A-D are graphs and photographs illustrating that RTVP-1 plays a role in differentiation of MSCs towards NSCs. RTVP-1 is expressed in high levels in BM-MSCs, similar to some glioma cells that are considered as the cells that expressed the highest levels of this protein, as determined by Western blot analysis (A). A diagram showing the mesenchymal lineage differentiation of MSCs (B). Silencing of RTVP-1 in BM-MSCs using siRNA duplexes decreases the osteogenic differentiation of these cells (C). Silencing of RTVP-1 in BM-MSCs decreases the expression of the different mesenchymal markers (D).

FIG. 5E is a bar graph illustrating the expression of RTVP-1 in MSCs and MSCs differentiated to NSCs.

FIG. 5F is a bar graph illustrating the effect of silencing of RTVP-1 on nestin expression in MSCs.

FIGs. 6A-D are photographs and graphs illustrating the effect of transfection of Olig2 and differentiation medium on placenta-derived MSCs. After 12 days in culture the cells were analyzed for the expression of motor neuron progenitor (Figure 6C) and motor neuron markers (Figure 6D) using real time PCR. Figure 6A illustrates undifferentiated MSCs. Figure 6B illustrates differentiated MSCs.

FIGs. 7A-B are graphs and photographs illustrating that NSCs may be induced to differentiate into motor neuron cells. The human neural progenitor cells (Lonza) were grown as spheroids and then plated on laminin and treated with the different factors as described in the methods. Following 12-14 days, the cells were analyzed for morphological appearance and for the different markers using real time PCR.

FIG. 8 is a bar graph illustrating exemplary miRNAs associated with stem cell signature and self renewal that were up-regulated during motor neuron differentiation.

FIG. 9 is a bar graph illustrating exemplary miRNAs associated with hematopoiesis that were up-regulated during motor neuron differentiation.

FIG. 10 is a bar graph illustrating exemplary miRNAs associated with a neuronal signature and self renewal that were up-regulated during motor neuron differentiation.

DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

The present invention, in some embodiments thereof, relates to methods of ex vivo differentiating mesenchymal stem cells towards neural progenitor cells and motor neurons using microRNAs.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not necessarily limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways.

Neural stem cells (NSCs) have been isolated from embryonic and fetal mammalian and human brains and propagated in vitro in a variety of culture systems (Doetsch et al., 1999, Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. Cell 97:703-16, Johansson et al., 1999, Cell 96:25-34, Svendsen et al., 1998, J Neurosci Methods 85:141-52). A system for proliferating human neural stem cells (hNSCs) in serum-free culture medium containing human bFGF and human EGF also has been reported (Kim et al., 2002, Proc Natl Acad Sci USA 99: 4020-4025, Qu et al., 2001, NeuroReport 12: 1127-1132). Further, transplantation of hNSCs into experimental animals has been described (Qu et al., 2001, Id.; Qu et al., 2005, 35th Annual Meeting in Washington, D.C., November, 2005).

However, challenges existed in the art of stem cell therapies using stem cells derived from embryonic/fetal tissue sources. Stem cell therapies using embryonic sources face challenges such as ethical issues, technical difficulties in cell isolation, and the need for long-term immunosuppressant administration to transplant recipients; the limitations of using fetal tissue sources have been set forth above. These challenges have hindered the applicability of hNSCs for human use.

Bone marrow (BM) contains stem cells involved not only in hematopoiesis but also for production of a variety of nonhematopoietic tissues. A subset of stromal cells in bone marrow, mesenchymal stem cells (MSCs), is capable of self-renewing and producing multiple mesenchymal cell lineages, including bone, cartilage, fat tendons, and other connective tissues (Majumdar et al., 1998, J Cell Physiol. 176:57-66, Pereira et al., 1995, Proc Natl Acad Sci USA. 92: 4857-61, Pittenger et al., 1999, Science 284:143-7). Bone marrow mesenchymal stem cells normally are not committed to the neural lineage in differentiation. Although adult stem cells continue to possess some degrees of multipotency, cell types produced from adult stem cells are thought to be limited by their tissue-specific character. To overcome this barrier, it is necessary to alter the cell lineage of these adult stem cells.

Whilst reducing the present invention to practice, the present inventors have found that out of a vast number of potential micro RNAs (miRNAs), only particular miRNAs may be regulated in order to induce neural stem cell differentiation of mesenchymal stem cells (MSCs) and propose that such differentiated MSCs may be used to treat patients with brain diseases or disorders.

Further, the present inventors identified particular combinations of miRNAs whose regulation was found to synergistically increase the differentiation towards NSCs, as measured by nestin and SOX-2 expression.

Whilst further reducing the present invention to practice the present inventors uncovered that upon manipulation of the miRNA expression of NSCs, cells expressing motor neurons markers may be generated.

Thus, the present inventors showed that upreguation of at least one of miR-368, miR-302b, miR-365-3p, miR-365-5p, miR-Let-7a, miR-Let-7b, miR-218, miR-134, miR-124, miR-125a, miR-9, miR-154, miR-20a, miR-130a in neural stem cells (NSCs), induced a motor neuron phenotype, whilst down-regulation of at least one of miR-372, miR-373, miR-141, miR-199a, miR-32, miR-33, miR-221 and miR-223 in NSCs also induced a motor neuron phenotype.

Further, the present inventors identified particular combinations of miRNAs whose regulation was found to synergistically increase the differentiation towards motor neurons, as measured by expression of motor neuron markers including islet1, HB9 and the neuronal markers neurofilament and β3 tubulin.

Thus, according to one aspect of the present invention there is provided a method of predisposing mesenchymal stem cells to differentiate into neural stem cells, the method comprising up-regulating a level of at least one exogenous miRNA selected from the group consisting of miR302b, miR-371, miR-134, miR-219, miR-154, miR-155, miR-32, miR-33, miR-126, miR-127, miR-132, miR-137, miR-572, miR-935a, miR-891a, miR-1202, miR-1275, let-7c, miR-665, miR-4258, miR-361-3p, miR-374a-star, miR-892b, miR-361-5p, miR-181a, miR-16, miR-636, miR-4284, miR-1208, miR-1274b, miR-30c-2-star, miR-501-3p, hsa-miR-92a, miR-378b, miR-1287, miR-425-star, miR-324-5p, miR-3178, miR-219-1-3p, miR-197, miR-181b, miR-500-star, miR-106b, miR-502-3p, miR-30c, miR-1275, miR-422a, miR-93, miR-181d, miR-1307, miR-1301, miR-99a, miR-505-star, miR-1202, miR-12, miR-532-5p, miR-195, miR-532-3p, miR-106a, miR-17, miR-1271, miR-769-3p, miR-15b, miR-324-3p, miR-20a, miR-501-5p, miR-330-3p, miR-874, miR-500, miR-25, miR-769-5p, miR-125b-2-star, miR-130b, miR-504, miR-181a-2-star, miR-885-3p, miR-1246, miR-92b, miR-362-5p, miR-572, miR-4270, miR-378c, miR-93-star, miR-149, miR-363, miR-9, miR-18a, miR-891a, miR-346, miR-124, miR-497, miR-378, miR-1231, miR-139-5p, miR-3180-3p, miR-9-star, miR-935 and miR-20b in mesenchymal stem cells (MSCs), thereby predisposing mesenchymal stem cells to differentiate into the neural stem cells.

As used herein, the phrase "predisposing MSCs to differentiate into neural stem cells (NSCs)" refers to causing the MSCs to differentiate along the NSC lineage. The generated cells may be fully differentiated into NSCs, or partially differentiated into NSCs.

The phrase "at least one" as used in the specification refers to one, two, three four, five six, seven, eight, nine, ten or more miRNAs. Examples of particular combinations of miRNAs are provided herein below.

Mesenchymal stem cells give rise to one or more mesenchymal tissues (e.g., adipose, osseous, cartilaginous, elastic and fibrous connective tissues, myoblasts) as well as to tissues other than those originating in the embryonic mesoderm (e.g., neural cells) depending upon various influences from bioactive factors such as cytokines. Although such cells can be isolated from embryonic yolk sac, placenta, umbilical cord, fetal and adolescent skin, blood and other tissues, their abundance in the easily

accessible fat tissue and BM far exceeds their abundance in other tissues and as such isolation from BM and fat tissue is presently preferred.

Methods of isolating, purifying and expanding mesenchymal stem cells (MSCs) are known in the arts and include, for example, those disclosed by Caplan and Haynesworth in U.S. Pat. No. 5,486,359 and Jones E.A. et al., 2002, Isolation and characterization of bone marrow multipotential mesenchymal progenitor cells, Arthritis Rheum. 46(12): 3349-60.

Mesenchymal stem cells may be isolated from various tissues including but not limited to bone marrow, peripheral blood, blood, chorionic and amniotic placenta (e.g. fetal side of the placenta), cord blood, umbilical cord, amniotic fluid, placenta and from adipose tissue.

A method of isolating mesenchymal stem cells from peripheral blood is described by Kassis et al [Bone Marrow Transplant. 2006 May; 37(10):967-76]. A method of isolating mesenchymal stem cells from placental tissue is described by Zhang et al [Chinese Medical Journal, 2004, 117 (6):882-887]. Methods of isolating and culturing adipose tissue, placental and cord blood mesenchymal stem cells are described by Kern et al [Stem Cells, 2006; 24:1294-1301].

According to a preferred embodiment of this aspect of the present invention, the mesenchymal stem cells are human.

According to another embodiment of this aspect of the present invention, the mesenchymal stem cells are isolated from placenta and umbilical cord of newborn humans.

Bone marrow can be isolated from the iliac crest of an individual by aspiration. Low-density BM mononuclear cells (BMMNC) may be separated by a FICOL-PAQUE density gradient or by elimination of red blood cells using Hetastarch (hydroxyethyl starch). Preferably, mesenchymal stem cell cultures are generated by diluting BM aspirates (usually 20 ml) with equal volumes of Hank's balanced salt solution (HBSS; GIBCO Laboratories, Grand Island, NY, USA) and layering the diluted cells over about 10 ml of a Ficoll column (Ficoll-Paque; Pharmacia, Piscataway, NJ, USA). Following 30 minutes of centrifugation at 2,500 x g, the mononuclear cell layer is removed from the interface and suspended in HBSS. Cells are then centrifuged at 1,500 x g for 15 minutes and resuspended in a complete medium (MEM, α medium without

deoxyribonucleotides or ribonucleotides; GIBCO); 20 % fetal calf serum (FCS) derived from a lot selected for rapid growth of MSCs (Atlanta Biologicals, Norcross, GA); 100 units/ml penicillin (GIBCO), 100 μg/ml streptomycin (GIBCO); and 2 mM L-glutamine (GIBCO). Resuspended cells are plated in about 25 ml of medium in a 10 cm culture dish (Corning Glass Works, Corning, NY) and incubated at 37 °C with 5 % humidified CO_2. Following 24 hours in culture, non-adherent cells are discarded, and the adherent cells are thoroughly washed twice with phosphate buffered saline (PBS). The medium is replaced with a fresh complete medium every 3 or 4 days for about 14 days. Adherent cells are then harvested with 0.25 % trypsin and 1 mM EDTA (Trypsin/EDTA, GIBCO) for 5 min at 37 °C, re-plated in a 6-cm plate and cultured for another 14 days. Cells are then trypsinized and counted using a cell counting device such as for example, a hemocytometer (Hausser Scientific, Horsham, PA). Cultured cells are recovered by centrifugation and resuspended with 5 % DMSO and 30 % FCS at a concentration of 1 to 2 X 10^6 cells per ml. Aliquots of about 1 ml each are slowly frozen and stored in liquid nitrogen.

Adipose tissue-derived MSCs can be obtained by liposuction and mononuclear cells can be isolated manually by removal of the fat and fat cells, or using the Celution System (Cytori Therapeutics) following the same procedure as described above for preparation of MSCs.

According to one embodiment the populations are plated on polystyrene plastic surfaces (e.g. in a flask) and mesenchymal stem cells are isolated by removing non-adherent cells. Alternatively mesenchymal stem cell may be isolated by FACS using mesenchymal stem cell markers.

Preferably the MSCs are at least 50 % purified, more preferably at least 75 % purified and even more preferably at least 90 % purified.

To expand the mesenchymal stem cell fraction, frozen cells are thawed at 37 °C, diluted with a complete medium and recovered by centrifugation to remove the DMSO. Cells are resuspended in a complete medium and plated at a concentration of about 5,000 cells/cm^2. Following 24 hours in culture, non-adherent cells are removed and the adherent cells are harvested using Trypsin/EDTA, dissociated by passage through a narrowed Pasteur pipette, and preferably re-plated at a density of about 1.5 to about 3.0 cells/cm^2. Under these conditions, MSC cultures can grow for about 50 population

doublings and be expanded for about 2000 fold [Colter DC., et al. Rapid expansion of recycling stem cells in cultures of plastic-adherent cells from human bone marrow. Proc Natl Acad Sci USA. 97: 3213-3218, 2000].

MSC cultures utilized by some embodiments of the invention preferably include three groups of cells which are defined by their morphological features: small and agranular cells (referred to as RS-1, herein below), small and granular cells (referred to as RS-2, herein below) and large and moderately granular cells (referred to as mature MSCs, herein below). The presence and concentration of such cells in culture can be assayed by identifying a presence or absence of various cell surface markers, by using, for example, immunofluorescence, *in situ* hybridization, and activity assays.

When MSCs are cultured under the culturing conditions of some embodiments of the invention they exhibit negative staining for the hematopoietic stem cell markers CD34, CD11B, CD43 and CD45. A small fraction of cells (less than 10 %) are dimly positive for CD31 and/or CD38 markers. In addition, mature MSCs are dimly positive for the hematopoietic stem cell marker, CD117 (c-Kit), moderately positive for the osteogenic MSCs marker, Stro-1 [Simmons, P. J. & Torok-Storb, B. (1991). Blood 78, 5562] and positive for the thymocytes and peripheral T lymphocytes marker, CD90 (Thy-1). On the other hand, the RS-1 cells are negative for the CD117 and Stro1 markers and are dimly positive for the CD90 marker, and the RS-2 cells are negative for all of these markers.

The mesenchymal stem cells of the present invention may be of autologous, syngeneic or allogeneic related (matched siblings or haploidentical family members) or unrelated fully mismatched source, as further described herein below.

Culturing of the mesenchymal stem cells can be performed in any media that supports neural stem cell differentiation (or at least does not prevent neural stem cell differentiation) such as those described in U.S. Pat. No. 6,528,245 and by Sanchez-Ramos et al. (2000); Woodburry et al. (2000); Woodburry et al. (J. Neurisci. Res. 96:908-917, 2001); Black and Woodbury (Blood Cells Mol. Dis. 27:632-635, 2001); Deng et al. (2001), Kohyama et al. (2001), Reyes and Verfatile (Ann. N.Y. Acad. Sci. 938:231-235, 2001) and Jiang et al. (Nature 418:47-49, 2002).

The differentiating media may be G5, neurobasal medium, DMEM or DMEM/F12, OptiMEM™ or any other medium that supports neuronal growth.

As mentioned, the mesenchymal stem cells are contacted (either ex vivo or in vivo) with at least one of the following miRNAs in order to induce differentiation into neural stem cells - miR302b, miR-371, miR-134, miR-219, miR-154, miR-155, miR-32, miR-33, miR-126, miR-127, miR-132, miR-137, miR-572, miR-935a, miR-891a, miR-1202, miR-1275, let-7c, miR-665, miR-4258, miR-361-3p, miR-374a-star, miR-892b miR-361-5p, miR-181a, miR-16, miR-636, miR-4284, miR-1208, miR-1274b, miR-30c-2-star, miR-501-3p, hsa-miR-92a, miR-378b, miR-1287, miR-425-star, miR-324-5p, miR-3178, miR-219-1-3p, miR-197, miR-181b, miR-500-star, miR-106b, miR-502-3p, miR-30c, miR-1275, miR-422a, miR-93, miR-181d, miR-1307, miR-1301, miR-99a, miR-505-star, miR-1202, miR-12, miR-532-5p, miR-195, miR-532-3p, miR-106a, miR-17, miR-1271, miR-769-3p, miR-15b, miR-324-3p, miR-20a, miR-501-5p, miR-330-3p, miR-874, miR-500, miR-25, miR-769-5p, miR-125b-2-star, miR-130b, miR-504, miR-181a-2-star, miR-885-3p, miR-1246, miR-92b, miR-362-5p, miR-572, miR-4270, miR-378c, miR-93-star, miR-149, miR-363, miR-18a, miR-891a, miR-346, miR-497, miR-378, miR-1231, miR-139-5p, miR-3180-3p, miR-935 and miR-20b.

According to a particular embodiment, the miRNA is selected from the group consisting of miR302b, miR-371, miR-134, miR-219, miR-154, miR-155, miR-32, miR-33, miR-126, miR-127, miR-132.

According to another embodiment, the miRNA is selected from the group consisting of miR-20b, miR-925, miR-891 and miR-378.

The present invention also contemplate differentiation of mesenchymal stem cells towards a neural stem cell phenotype by down-regulation of particular miRNAs – namely miR-10b, miR-142-3p, miR-131a, miR-125b, miR-153 and miR-181a.

The present inventors contemplates down-regulation of additional miRNAs for the differentiation of MSCs towards a neural stem cell phenotype. These miRNAs include miR-409-5p, miR-193a-5p, miR-4317, miR-4288, miR-145, miR-143, miR-214, miR-199a-3p, miR-199a-5p, miR-199b-3p, miR-138, miR-31, miR-21, miR-193a-5p, miR-224-star, miR-196a, miR-487b, miR-409-5p, miR-193b-star, miR-379, miR-21-star, miR-27a-star, miR-27a, miR-4317, miR-193b, miR-27b, miR-22, 574-3p, miR-4288, miR-23a, miR-221-star, miR-2113, let-7i, miR-24, miR-23b, miR-299-3p, miR-518c-star, miR-221, miR-431-star, miR-523, miR-4313, miR-559, miR-614, miR-653, miR-2278, miR-768-5p, miR-154-star, miR-302a-star, miR-3199 and miR-3137.

According to a particular embodiment, the miRNA which is to be downregulated is selected from the group consisting of miR-138, miR-214, miR-199a and miR-199b.

Down-regulating such miRNAs can be affected using a polynucleotide which is hybridizable in cells under physiological conditions to the miRNA.

According to a particular embodiment, the cell population is generated by up-regulating an expression of miR-124 in mesenchymal stem cells (MSCs) whilst simultaneously down-regulating an expression of miR-let-7 in the population of MSCs.

According to a particular embodiment, the cell population is generated by down-regulating an expression of miR-891 in mesenchymal stem cells (MSCs) whilst simultaneously down-regulating an expression of miR-138 in the population of MSCs.

According to a particular embodiment, the cell population is generated by up-regulating an expression of miR-20b in mesenchymal stem cells (MSCs) whilst simultaneously down-regulating an expression of miR-138 in the population of MSCs.

According to a particular embodiment, the cell population is generated by up-regulating an expression of miR-378 in mesenchymal stem cells (MSCs) whilst simultaneously down-regulating an expression of miR-138 in the population of MSCs.

As used herein, the term "hybridizable" refers to capable of hybridizing, *i.e.*, forming a double strand molecule such as RNA:RNA, RNA:DNA and/or DNA:DNA molecules. "Physiological conditions" refer to the conditions present in cells, tissue or a whole organism or body. Preferably, the physiological conditions used by the present invention include a temperature between 34-40 °C, more preferably, a temperature between 35-38 °C, more preferably, a temperature between 36 and 37.5 °C, most preferably, a temperature between 37 to 37.5 °C; salt concentrations (e.g., sodium chloride NaCl) between 0.8-1 %, more preferably, about 0.9 %; and/or pH values in the range of 6.5-8, more preferably, 6.5-7.5, most preferably, pH of 7-7.5.

As mentioned, the present inventors have also uncovered that upon manipulation of particular miRNAs in neural stem cells, cells expressing motor neurons markers may be generated.

Thus according to another aspect of the present invention there is provided a method of predisposing neural stem cells to differentiate into motor neurons comprising up-regulating a level of at least one exogenous miRNA selected from the group consisting of miR-368, miR-302b, miR-365-3p, miR-365-5p, miR-Let-7a, miR-Let-7b,

miR-218, miR-134, miR-124, miR-125a, miR-9, miR-154, miR-20a, miR-130a in neural stem cells (NSCs).

The neural stem cells of this aspect of the present invention may be non-committed neural stem cells that are not committed to any particular type of neural cell such as but not limited to neuronal and glial cell types. Preferably these cells have a potential to commit to a neural fate. Alternatively, the neural stem cells may be committed to a particular neural cell type, such as a motor neuron, but do not express/secrete markers of terminal differentiation – e.g. do not secrete neurotransmitters.

According to a particular embodiment, the neural stem cells express at least one of nestin and/ or SOX-2. Additional markers include SOX1, SOX3, PSA-NCAM and MUSASHI-1. Methods of confirming expression of the markers are provided herein below. Formation of ''neural rosettes'' is another morphologic marker of neural stem cell formation.

According to one embodiment, the neural stem cells have been generated by ex vivo differentiation of mesenchymal stem cells or embryonic stem cells (or induced embryonic stem cells).

Mesenchymal stem cells have been described herein above. Numerous methods are known in the art for differentiating MSCs towards a neural stem cell fate including genetic modification and/or culturing in a medium which promotes differentiation towards that fate. The medium typically comprises growth factors and/or cytokines including, but not limited to epidermal growth factor (EGF), basic fibroblast growth factor (bFGF). Typically, the differentiation is affected in serum free medium, or serum replacements.

According to a particular embodiment, NSCs are generated by genetically modifying the MSCs to express an exogenous miRNA, as described herein above.

The phrase "embryonic stem cells" refers to embryonic cells which are capable of differentiating into cells of all three embryonic germ layers *(i.e.*, endoderm, ectoderm and mesoderm), or remaining in an undifferentiated state. The phrase "embryonic stem cells" may comprise cells which are obtained from the embryonic tissue formed after gestation (e.g., blastocyst) before implantation of the embryo (*i.e.*, a pre-implantation blastocyst), extended blastocyst cells (EBCs) which are obtained from a post-

implantation/pre-gastrulation stage blastocyst (see WO2006/040763) and embryonic germ (EG) cells which are obtained from the genital tissue of a fetus any time during gestation, preferably before 10 weeks of gestation.

Induced pluripotent stem cells (iPS; embryonic-like stem cells), are cells obtained by de-differentiation of adult somatic cells which are endowed with pluripotency (*i.e.*, being capable of differentiating into the three embryonic germ cell layers, *i.e.,* endoderm, ectoderm and mesoderm). According to some embodiments of the invention, such cells are obtained from a differentiated tissue (e.g., a somatic tissue such as skin) and undergo de-differentiation by genetic manipulation which re-program the cell to acquire embryonic stem cells characteristics. According to some embodiments of the invention, the induced pluripotent stem cells are formed by inducing the expression of Oct-4, Sox2, Kfl4 and c-Myc in a somatic stem cell.

The embryonic stem cells of some embodiments of the invention can be obtained using well-known cell-culture methods. For example, human embryonic stem cells can be isolated from human blastocysts. Human blastocysts are typically obtained from human *in vivo* pre-implantation embryos or from *in vitro* fertilized (IVF) embryos. Alternatively, a single cell human embryo can be expanded to the blastocyst stage. For the isolation of human ES cells the zona pellucida is removed from the blastocyst and the inner cell mass (ICM) is isolated by immunosurgery, in which the trophectoderm cells are lysed and removed from the intact ICM by gentle pipetting. The ICM is then plated in a tissue culture flask containing the appropriate medium which enables its outgrowth. Following 9 to 15 days, the ICM derived outgrowth is dissociated into clumps either by a mechanical dissociation or by an enzymatic degradation and the cells are then re-plated on a fresh tissue culture medium. Colonies demonstrating undifferentiated morphology are individually selected by micropipette, mechanically dissociated into clumps, and re-plated. Resulting ES cells are then routinely split every 4-7 days. For further details on methods of preparation human ES cells see Thomson et al., [U.S. Pat. No. 5,843,780; Science 282: 1145, 1998; Curr. Top. Dev. Biol. 38: 133, 1998; Proc. Natl. Acad. Sci. USA 92: 7844, 1995]; Bongso et al., [Hum Reprod 4: 706, 1989]; and Gardner et al., [Fertil. Steril. 69: 84, 1998].

It will be appreciated that commercially available stem cells can also be used with this aspect of some embodiments of the invention. Human ES cells can be

purchased from the NIH human embryonic stem cells registry (www.escr.nih.gov). Non-limiting examples of commercially available embryonic stem cell lines are BG01, BG02, BG03, BG04, CY12, CY30, CY92, CY10, TE03 and TE32.

In addition, ES cells can be obtained from other species as well, including mouse (Mills and Bradley, 2001), golden hamster [Doetschman et al., 1988, Dev Biol. 127: 224-7], rat [Iannaccone et al., 1994, Dev Biol. 163: 288-92] rabbit [Giles et al. 1993, Mol Reprod Dev. 36: 130-8; Graves & Moreadith, 1993, Mol Reprod Dev. 1993, 36: 424-33], several domestic animal species [Notarianni et al., 1991, J Reprod Fertil Suppl. 43: 255-60; Wheeler 1994, Reprod Fertil Dev. 6: 563-8; Mitalipova et al., 2001, Cloning. 3: 59-67] and non-human primate species (Rhesus monkey and marmoset) [Thomson et al., 1995, Proc Natl Acad Sci U S A. 92: 7844-8; Thomson et al., 1996, Biol Reprod. 55: 254-9].

Induced pluripotent stem cells (iPS) (embryonic-like stem cells) can be generated from somatic cells by genetic manipulation of somatic cells, e.g., by retroviral transduction of somatic cells such as fibroblasts, hepatocytes, gastric epithelial cells with transcription factors such as Oct-3/4, Sox2, c-Myc, and KLF4 [Yamanaka S, Cell Stem Cell. 2007, 1(1):39-49; Aoi T, et al., Generation of Pluripotent Stem Cells from Adult Mouse Liver and Stomach Cells. Science. 2008 Feb 14. (Epub ahead of print); IH Park, Zhao R, West JA, et al. Reprogramming of human somatic cells to pluripotency with defined factors. Nature 2008;451:141-146; K Takahashi, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007;131:861-872]. Other embryonic-like stem cells can be generated by nuclear transfer to oocytes, fusion with embryonic stem cells or nuclear transfer into zygotes if the recipient cells are arrested in mitosis.

Methods of generating neural stem cells from ESCs or iPS cells are known in the art and include for example those which induce differentiation via embryoid bodies and those which induce differentiation via adherent culture. Particular protocols for differentiating ESCs towards a neuronal fate are reviewed in Dhara et al., Journal of Cellular Biochemistry 105:633–640 (2008), the contents of which are incorporated herein by reference. It will be appreciated that many other methods are known for differentiating ESC, iPSCs and MSCs towards neuronal stem cells and the present application contemplates use of all these methods.

The neuronal stem cells of the present invention may be of autologous, syngeneic or allogeneic related (matched siblings or haploidentical family members) or unrelated fully mismatched source.

Culturing of neuronal stem cells can be performed in any media that supports neural stem cell differentiation, examples of which are described herein above.

As mentioned, the neuronal stem cells are contacted (either ex vivo or in vivo) with at least one of the following miRNAs in order to induce differentiation towards the motor neuron lineage - miR-368, miR-302b, miR-365-3p, miR-365-5p, miR-Let-7a miR-Let-7b, miR-218, miR-134, miR-124, miR-125a, miR-9, miR-154, miR-20a and miR-130a.

The present invention also contemplates differentiation of neuronal stem cells towards motor neuron phenotype by down-regulation of particular miRNAs – namely miR-372, miR-373, miR-141, miR-199a, miR-32, miR-33, miR-221 and miR-223.

Down-regulating such miRNAs can be affected using a polynucleotide which is hybridizable in cells under physiological conditions to the miRNA molecule.

According to a particular embodiment, the cell population is generated by up-regulating an expression of each of miR Let-7a, miR-124, miR-368 and miR-154 in the neural stem cells.

According to a particular embodiment, the cell population is generated by up-regulating an expression of each of miR-125a, miR-9 and miR-130a in the neural stem cells.

According to still another embodiment, the cell population is generated by up-regulating an expression of each of each of miR-218, miR-134 and miR-20a.

The present inventors further contemplate down-regulating each of miR-141, miR-32, miR-33, miR-221, miR-223 and miR-373 in addition to any of the methods described herein above to enhance the differentiation towards the motor neuron phenotype.

Mesenchymal stem cells were differentiated into motor neurons by overexpressing Olig2 and HB9. The present inventors performed a miRNA array analysis on the differentiated and non-differentiated cells and found a number of miRNAs that were overexpressed in a statistically significant manner (more than 3 fold) and a number of miRNAs that were downregulated in a statistically significant manner

(more than 3 fold). The present inventors contemplate that the miRNAs whose expression was increased in the differentiated cells may be candidates for overexpression in order to generate motor neurons from MSCs. The present inventors contemplate that the miRNAs whose expression was decreased in the differentiated cells are candidates for downregulation in order to generate motor neurons from MSCs.

Thus, according to still another aspect of the present invention there is provided a method of predisposing MSCs to differentiate into motor neurons, the method comprising up-regulating a level of at least one exogenous miRNA selected from the group consisting of miR-368, miR-365, miR-500, miR-648, miR-491, miR-218, miR-155, miR-192, let-7b, miR-16, miR-210, miR-197, miR-21, miR-373, miR-27a, miR-122, miR-17, miR-494, miR-449, miR-503, miR-30a, miR-196a, miR-122, miR-7, miR-151-5p, miR-16, miR-22, miR-31, miR-424, miR-1, miR-29c, miR-942, miR-100, miR-520, miR-663a, miR-562, miR-449a, miR-449b-5p, miR-520b, miR-451, miR-532-59, miR-605, miR-504, miR-503, miR-155, miR-34a, miR-16, miR-7b, miR-103, miR-124, miR-1385p, miR-16, miR-330, miR-520, miR-608, miR-708, miR-107, miR-137, miR-132, miR-145, miR-204, miR-125b, miR-224, miR-30a, miR-375, miR-101, miR-106b, miR-128, miR-129-5p, miR-153, miR-203, miR-214, miR-338-3p, miR-346, miR-98, miR-107, miR-141, miR-217, miR-424, miR-449, miR-7, miR-9, miR-93, miR-99a, miR-100, miR-1228, miR-183, miR-185, miR-190, miR-522, miR-650, miR-675, miR-342-3p, miR-31in the mesenchymal stem cells (MSCs).

According to yet another aspect of the present invention there is provided a method of predisposing MSCs to differentiate into motor neurons, the method comprising down-regulating an expression of at least one miRNA selected from the group consisting of miR-199a, miR-372, miR-373, miR-942, miR-2113, miR-301a-3p, miR-302c, miR-30b-5p, miR-30c, miR-326, miR-328, miR-331-3p, miR-340, miR-345, miR-361-5p, miR-363, miR-365a-3p, miR-371a-3p, miR-373-3p, miR-374a, miR-423-3p, miR-449b-5p, miR-451a, miR-494, miR-504, miR-515-3p, miR-516a-3p, miR-519e, miR-520a-3p, miR-520c-3p, miR-520g, miR-532-5p, miR-559, miR-562, miR-572, miR-590-5p, miR-605, miR-608, miR-626, miR-639, miR-654-3p, miR-657, miR-661, miR-708-5p, miR-942, miR-96, miR-99arno and miR-194 by up-regulating a level of at least one polynucleotide agent that hybridizes and inhibits a function of said at

least one miRNA in the MSCs thereby predisposing MSCs to differentiate into the motor neurons.

The term "microRNA", "miRNA", and "miR" are synonymous and refer to a collection of non-coding single-stranded RNA molecules of about 19-28 nucleotides in length, which regulate gene expression. miRNAs are found in a wide range of organisms and have been shown to play a role in development, homeostasis, and disease etiology.

Below is a brief description of the mechanism of miRNA activity.

Genes coding for miRNAs are transcribed leading to production of an miRNA precursor known as the pri-miRNA. The pri-miRNA is typically part of a polycistronic RNA comprising multiple pri-miRNAs. The pri-miRNA may form a hairpin with a stem and loop. The stem may comprise mismatched bases.

The hairpin structure of the pri-miRNA is recognized by Drosha, which is an RNase III endonuclease. Drosha typically recognizes terminal loops in the pri-miRNA and cleaves approximately two helical turns into the stem to produce a 60–70 nt precursor known as the pre-miRNA. Drosha cleaves the pri-miRNA with a staggered cut typical of RNase III endonucleases yielding a pre-miRNA stem loop with a 5' phosphate and ~2 nucleotide 3' overhang. It is estimated that approximately one helical turn of stem (~10 nucleotides) extending beyond the Drosha cleavage site is essential for efficient processing. The pre-miRNA is then actively transported from the nucleus to the cytoplasm by Ran-GTP and the export receptor exportin-5.

The double-stranded stem of the pre-miRNA is then recognized by Dicer, which is also an RNase III endonuclease. Dicer may also recognize the 5' phosphate and 3' overhang at the base of the stem loop. Dicer then cleaves off the terminal loop two helical turns away from the base of the stem loop leaving an additional 5' phosphate and ~2 nucleotide 3' overhang. The resulting siRNA-like duplex, which may comprise mismatches, comprises the mature miRNA and a similar-sized fragment known as the miRNA*. The miRNA and miRNA* may be derived from opposing arms of the pri-miRNA and pre-miRNA. miRNA* sequences may be found in libraries of cloned miRNAs but typically at lower frequency than the miRNAs.

Although initially present as a double-stranded species with miRNA*, the miRNA eventually become incorporated as a single-stranded RNA into a

ribonucleoprotein complex known as the RNA-induced silencing complex (RISC). Various proteins can form the RISC, which can lead to variability in specificity for miRNA/miRNA* duplexes, binding site of the target gene, activity of miRNA (repress or activate), and which strand of the miRNA/miRNA* duplex is loaded in to the RISC.

When the miRNA strand of the miRNA:miRNA* duplex is loaded into the RISC, the miRNA* is removed and degraded. The strand of the miRNA:miRNA* duplex that is loaded into the RISC is the strand whose 5' end is less tightly paired. In cases where both ends of the miRNA:miRNA* have roughly equivalent 5' pairing, both miRNA and miRNA* may have gene silencing activity.

The RISC identifies target nucleic acids based on high levels of complementarity between the miRNA and the mRNA, especially by nucleotides 2-7 of the miRNA.

A number of studies have looked at the base-pairing requirement between miRNA and its mRNA target for achieving efficient inhibition of translation (reviewed by Bartel 2004, Cell 116-281). In mammalian cells, the first 8 nucleotides of the miRNA may be important (Doench & Sharp 2004 GenesDev 2004-504). However, other parts of the microRNA may also participate in mRNA binding. Moreover, sufficient base pairing at the 3' can compensate for insufficient pairing at the 5' (Brennecke et al, 2005 PLoS 3-e85). Computation studies, analyzing miRNA binding on whole genomes have suggested a specific role for bases 2-7 at the 5' of the miRNA in target binding but the role of the first nucleotide, found usually to be "A" was also recognized (Lewis et at 2005 Cell 120-15). Similarly, nucleotides 1-7 or 2-8 were used to identify and validate targets by Krek et al (2005, Nat Genet 37-495).

The target sites in the mRNA may be in the 5' UTR, the 3' UTR or in the coding region. Interestingly, multiple miRNAs may regulate the same mRNA target by recognizing the same or multiple sites. The presence of multiple miRNA binding sites in most genetically identified targets may indicate that the cooperative action of multiple RISCs provides the most efficient translational inhibition.

miRNAs may direct the RISC to down-regulate gene expression by either of two mechanisms: mRNA cleavage or translational repression. The miRNA may specify cleavage of the mRNA if the mRNA has a certain degree of complementarity to the miRNA. When a miRNA guides cleavage, the cut is typically between the nucleotides

pairing to residues 10 and 11 of the miRNA. Alternatively, the miRNA may repress translation if the miRNA does not have the requisite degree of complementarity to the miRNA. Translational repression may be more prevalent in animals since animals may have a lower degree of complementarity between the miRNA and binding site.

It should be noted that there may be variability in the 5' and 3' ends of any pair of miRNA and miRNA*. This variability may be due to variability in the enzymatic processing of Drosha and Dicer with respect to the site of cleavage. Variability at the 5' and 3' ends of miRNA and miRNA* may also be due to mismatches in the stem structures of the pri-miRNA and pre-miRNA. The mismatches of the stem strands may lead to a population of different hairpin structures. Variability in the stem structures may also lead to variability in the products of cleavage by Drosha and Dicer.

The term "microRNA mimic" refers to synthetic non-coding RNAs that are capable of entering the RNAi pathway and regulating gene expression. miRNA mimics imitate the function of endogenous microRNAs (miRNAs) and can be designed as mature, double stranded molecules or mimic precursors (e.g., or pre-miRNAs). miRNA mimics can be comprised of modified or unmodified RNA, DNA, RNA-DNA hybrids, or alternative nucleic acid chemistries (e.g., LNAs or 2'-O,4'-C-ethylene-bridged nucleic acids (ENA)). Other modifications are described herein below. For mature, double stranded miRNA mimics, the length of the duplex region can vary between 13-33, 18-24 or 21-23 nucleotides. The miRNA may also comprise a total of at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39 or 40 nucleotides. The sequence of the miRNA may be the first 13-33 nucleotides of the pre-miRNA. The sequence of the miRNA may also be the last 13-33 nucleotides of the pre-miRNA. The sequence of the miRNA may comprise any of the sequences described herein, or variants thereof.

It will be appreciated from the description provided herein above, that contacting mesenchymal stem cells may be affected in a number of ways:

1. Transiently transfecting the mesenchymal stem cells with the mature miRNA (or modified form thereof, as described herein below). The miRNAs designed according to the teachings of the present invention can be generated according to any oligonucleotide synthesis method known in the art, including both enzymatic syntheses and solid-phase syntheses. Equipment and reagents for executing solid-phase synthesis

are commercially available from, for example, Applied Biosystems. Any other means for such synthesis may also be employed; the actual synthesis of the oligonucleotides is well within the capabilities of one skilled in the art and can be accomplished via established methodologies as detailed in, for example: Sambrook, J. and Russell, D. W. (2001), "Molecular Cloning: A Laboratory Manual"; Ausubel, R. M. et al., eds. (1994, 1989), "Current Protocols in Molecular Biology," Volumes I-III, John Wiley & Sons, Baltimore, Maryland; Perbal, B. (1988), "A Practical Guide to Molecular Cloning," John Wiley & Sons, New York; and Gait, M. J., ed. (1984), "Oligonucleotide Synthesis"; utilizing solid-phase chemistry, e.g. cyanoethyl phosphoramidite followed by deprotection, desalting, and purification by, for example, an automated trityl-on method or HPLC.

2. Stably, or transiently transfecting the mesenchymal stem cells with an expression vector which encodes the mature miRNA or with miRNA mimic.

3. Stably, or transiently transfecting the mesenchymal stem cells with an expression vector which encodes the pre-miRNA. The pre-miRNA sequence may comprise from 45-90, 60-80 or 60-70 nucleotides. The sequence of the pre-miRNA may comprise a miRNA and a miRNA* as set forth herein. The sequence of the pre-miRNA may also be that of a pri-miRNA excluding from 0-160 nucleotides from the 5' and 3' ends of the pri-miRNA. The sequence of the pre-miRNA may comprise the sequence of the miRNA, or variants thereof.

4. Stably, or transiently transfecting the mesenchymal stem cells with an expression vector which encodes the pri-miRNA. The pri-miRNA sequence may comprise from 45-30,000, 50-25,000, 100-20,000, 1,000-1,500 or 80-100 nucleotides. The sequence of the pri-miRNA may comprise a pre-miRNA, miRNA and miRNA*, as set forth herein, and variants thereof. Preparation of miRNAs mimics can be affected by chemical synthesis methods or by recombinant methods.

miRNA antagonists may be introduced into cells using transfection protocols known in the art using either siRNAs or expression vectors such as Anatgomirs.

As mentioned herein above, the polynucleotides which down-regulate the miRNAs described herein above may be provided as modified polynucleotides using various methods known in the art.

For example, the oligonucleotides (e.g. miRNAs) or polynucleotides of the present invention may comprise heterocylic nucleosides consisting of purines and the pyrimidines bases, bonded in a 3'-to-5' phosphodiester linkage.

Preferably used oligonucleotides or polynucleotides are those modified either in backbone, internucleoside linkages, or bases, as is broadly described herein under.

Specific examples of preferred oligonucleotides or polynucleotides useful according to this aspect of the present invention include oligonucleotides or polynucleotides containing modified backbones or non-natural internucleoside linkages. Oligonucleotides or polynucleotides having modified backbones include those that retain a phosphorus atom in the backbone, as disclosed in U.S. Pat. Nos.: 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; and 5,625,050.

Preferred modified oligonucleotide or polynucleotide backbones include, for example: phosphorothioates; chiral phosphorothioates; phosphorodithioates; phosphotriesters; aminoalkyl phosphotriesters; methyl and other alkyl phosphonates, including 3'-alkylene phosphonates and chiral phosphonates; phosphinates; phosphoramidates, including 3'-amino phosphoramidate and aminoalkylphosphoramidates; thionophosphoramidates; thionoalkylphosphonates; thionoalkylphosphotriesters; and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogues of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts, and free acid forms of the above modifications can also be used.

Alternatively, modified oligonucleotide or polynucleotide backbones that do not include a phosphorus atom therein have backbones that are formed by short-chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short-chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in

part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide, and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene-containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts, as disclosed in U.S. Pat. Nos.: 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439.

Other oligonucleotides or polynucleotides which may be used according to the present invention are those modified in both sugar and the internucleoside linkage, *i.e.*, the backbone of the nucleotide units is replaced with novel groups. The base units are maintained for complementation with the appropriate polynucleotide target. An example of such an oligonucleotide mimetic includes a peptide nucleic acid (PNA). A PNA oligonucleotide refers to an oligonucleotide where the sugar-backbone is replaced with an amide-containing backbone, in particular an aminoethylglycine backbone. The bases are retained and are bound directly or indirectly to aza-nitrogen atoms of the amide portion of the backbone. United States patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Pat. Nos. 5,539,082; 5,714,331; and 5,719,262; each of which is herein incorporated by reference. Other backbone modifications which may be used in the present invention are disclosed in U.S. Pat. No. 6,303,374.

Oligonucleotides or polynucleotides of the present invention may also include base modifications or substitutions. As used herein, "unmodified" or "natural" bases include the purine bases adenine (A) and guanine (G) and the pyrimidine bases thymine (T), cytosine (C), and uracil (U). "Modified" bases include but are not limited to other synthetic and natural bases, such as: 5-methylcytosine (5-me-C); 5-hydroxymethyl cytosine; xanthine; hypoxanthine; 2-aminoadenine; 6-methyl and other alkyl derivatives of adenine and guanine; 2-propyl and other alkyl derivatives of adenine and guanine; 2-thiouracil, 2-thiothymine, and 2-thiocytosine; 5-halouracil and cytosine; 5-propynyl uracil and cytosine; 6-azo uracil, cytosine, and thymine; 5-uracil (pseudouracil); 4-

thiouracil; 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl, and other 8-substituted adenines and guanines; 5-halo, particularly 5-bromo, 5-trifluoromethyl, and other 5-substituted uracils and cytosines; 7-methylguanine and 7-methyladenine; 8-azaguanine and 8-azaadenine; 7-deazaguanine and 7-deazaadenine; and 3-deazaguanine and 3-deazaadenine. Additional modified bases include those disclosed in: U.S. Pat. No. 3,687,808; Kroschwitz, J. I., ed. (1990),"The Concise Encyclopedia Of Polymer Science And Engineering," pages 858-859, John Wiley & Sons; Englisch et al. (1991), "Angewandte Chemie," International Edition, *30*, 613; and Sanghvi, Y. S., "Antisense Research and Applications," Chapter 15, pages 289-302, S. T. Crooke and B. Lebleu, eds., CRC Press, 1993. Such modified bases are particularly useful for increasing the binding affinity of the oligomeric compounds of the invention. These include 5-substituted pyrimidines, 6-azapyrimidines, and N-2, N-6, and O-6-substituted purines, including 2-aminopropyladenine, 5-propynyluracil, and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2ºC (Sanghvi, Y. S. et al. (1993), "Antisense Research and Applications," pages 276-278, CRC Press, Boca Raton), and are presently preferred base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

To express miRNAs or polynucleotide agents which regulate miRNAs in mesencyhymal stem cells or neural stem cells, a polynucleotide sequence encoding the miRNA (or pre-miRNA, or pri-miRNA, or polynucleotide which down-regulates the miRNAs) is preferably ligated into a nucleic acid construct suitable for mesenchymal stem cell (or neural stem cell) expression. Such a nucleic acid construct includes a promoter sequence for directing transcription of the polynucleotide sequence in the cell in a constitutive or inducible manner.

It will be appreciated that the nucleic acid construct of some embodiments of the invention can also utilize miRNA homologues which exhibit the desired activity (*e.g.* motor neuron or neural stem cell differentiating ability). Such homologues can be, for example, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identical to any of the sequences described herein above, as determined using the BestFit software of the Wisconsin sequence analysis

package, utilizing the Smith and Waterman algorithm, where gap weight equals 50, length weight equals 3, average match equals 10 and average mismatch equals -9.

In addition, the homologues can be, for example, at least 60 %, at least 61 %, at least 62 %, at least 63 %, at least 64 %, at least 65 %, at least 66 %, at least 67 %, at least 68 %, at least 69 %, at least 70 %, at least 71 %, at least 72 %, at least 73 %, at least 74 %, at least 75 %, at least 76 %, at least 77 %, at least 78 %, at least 79 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identical to the sequences described herein above, as determined using the BestFit software of the Wisconsin sequence analysis package, utilizing the Smith and Waterman algorithm, where gap weight equals 50, length weight equals 3, average match equals 10 and average mismatch equals -9.

Constitutive promoters suitable for use with some embodiments of the invention are promoter sequences which are active under most environmental conditions and most types of cells such as the cytomegalovirus (CMV) and Rous sarcoma virus (RSV). Inducible promoters suitable for use with some embodiments of the invention include for example tetracycline-inducible promoter (Zabala M, et al., Cancer Res. 2004, 64(8): 2799-804).

Eukaryotic promoters typically contain two types of recognition sequences, the TATA box and upstream promoter elements. The TATA box, located 25-30 base pairs upstream of the transcription initiation site, is thought to be involved in directing RNA polymerase to begin RNA synthesis. The other upstream promoter elements determine the rate at which transcription is initiated.

Preferably, the promoter utilized by the nucleic acid construct of some embodiments of the invention is active in the specific cell population transformed – i.e. mesenchymal stem cells or neural stem cells.

Enhancer elements can stimulate transcription up to 1,000 fold from linked homologous or heterologous promoters. Enhancers are active when placed downstream or upstream from the transcription initiation site. Many enhancer elements derived from viruses have a broad host range and are active in a variety of tissues. For example, the SV40 early gene enhancer is suitable for many cell types. Other enhancer/promoter

combinations that are suitable for some embodiments of the invention include those derived from polyoma virus, human or murine cytomegalovirus (CMV), the long term repeat from various retroviruses such as murine leukemia virus, murine or Rous sarcoma virus and HIV. See, Enhancers and Eukaryotic Expression, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. 1983, which is incorporated herein by reference.

In the construction of the expression vector, the promoter is preferably positioned approximately the same distance from the heterologous transcription start site as it is from the transcription start site in its natural setting. As is known in the art, however, some variation in this distance can be accommodated without loss of promoter function.

In addition to the elements already described, the expression vector of some embodiments of the invention may typically contain other specialized elements intended to increase the level of expression of cloned nucleic acids or to facilitate the identification of cells that carry the recombinant DNA. For example, a number of animal viruses contain DNA sequences that promote the extra chromosomal replication of the viral genome in permissive cell types. Plasmids bearing these viral replicons are replicated episomally as long as the appropriate factors are provided by genes either carried on the plasmid or with the genome of the host cell.

The vector may or may not include a eukaryotic replicon. If a eukaryotic replicon is present, then the vector is amplifiable in eukaryotic cells using the appropriate selectable marker. If the vector does not comprise a eukaryotic replicon, no episomal amplification is possible. Instead, the recombinant DNA integrates into the genome of the engineered cell, where the promoter directs expression of the desired nucleic acid.

Examples for mammalian expression vectors include, but are not limited to, pcDNA3, pcDNA3.1(+/-), pGL3, pZeoSV2(+/-), pSecTag2, pDisplay, pEF/myc/cyto, pCMV/myc/cyto, pCR3.1, pSinRep5, DH26S, DHBB, pNMT1, pNMT41, pNMT81, which are available from Invitrogen, pCI which is available from Promega, pMbac, pPbac, pBK-RSV and pBK-CMV which are available from Strategene, pTRES which is available from Clontech, and their derivatives. Other expression vectors are available from SBI or Sigma.

Expression vectors containing regulatory elements from eukaryotic viruses such as retroviruses can be also used. SV40 vectors include pSVT7 and pMT2. Vectors derived from bovine papilloma virus include pBV-1MTHA, and vectors derived from Epstein Bar virus include pHEBO, and p2O5. Other exemplary vectors include pMSG, pAV009/A$^+$, pMTO10/A$^+$, pMAMneo-5, baculovirus pDSVE, and any other vector allowing expression of proteins under the direction of the SV-40 early promoter, SV-40 later promoter, metallothionein promoter, murine mammary tumor virus promoter, Rous sarcoma virus promoter, polyhedrin promoter, or other promoters shown effective for expression in eukaryotic cells.

As described above, viruses are very specialized infectious agents that have evolved, in many cases, to elude host defense mechanisms. Typically, viruses infect and propagate in specific cell types. The targeting specificity of viral vectors utilizes its natural specificity to specifically target predetermined cell types and thereby introduce a recombinant gene into the infected cell. Thus, the type of vector used by some embodiments of the invention will depend on the cell type transformed. The ability to select suitable vectors according to the cell type transformed is well within the capabilities of the ordinary skilled artisan and as such no general description of selection consideration is provided herein. For example, bone marrow cells can be targeted using the human T cell leukemia virus type I (HTLV-I) and kidney cells may be targeted using the heterologous promoter present in the baculovirus Autographa californica nucleopolyhedrovirus (AcMNPV) as described in Liang CY et al., 2004 (Arch Virol. 149: 51-60).

According to one embodiment, a lentiviral vector is used to transfect the mesenchymal stem cells or neural stem cells.

Various methods can be used to introduce the expression vector of some embodiments of the invention into mesenchymal stem cells. Such methods are generally described in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Harbor Laboratory, New York (1989, 1992), in Ausubel et al., Current Protocols in Molecular Biology, John Wiley and Sons, Baltimore, Md. (1989), Chang et al., Somatic Gene Therapy, CRC Press, Ann Arbor, Mich. (1995), Vega et al., Gene Targeting, CRC Press, Ann Arbor Mich. (1995), Vectors: A Survey of Molecular Cloning Vectors and Their Uses, Butterworths, Boston Mass. (1988) and Gilboa et at.

[Biotechniques 4 (6): 504-512, 1986] and include, for example, stable or transient transfection, lipofection, electroporation and infection with recombinant viral vectors. In addition, see U.S. Pat. Nos. 5,464,764 and 5,487,992 for positive-negative selection methods.

Introduction of nucleic acids by viral infection offers several advantages over other methods such as lipofection and electroporation, since higher transfection efficiency can be obtained due to the infectious nature of viruses.

Other vectors can be used that are non-viral, such as cationic lipids, polylysine, and dendrimers.

The miRNAs, miRNA mimics and pre-miRs can be transfected into cells also using nanoparticles such as gold nanoparticles and by ferric oxide magnetic NP – see for example Ghosh et al., Biomaterials. 2013 Jan;34(3):807-16; Crew E, et al., Anal Chem. 2012 Jan 3;84(1):26-9.

Other modes of transfection that do not involved integration include the use of minicircle DNA vectors or the use of PiggyBac transposon that allows the transfection of genes that can be later removed from the genome.

As mentioned hereinabove, a variety of prokaryotic or eukaryotic cells can be used as host-expression systems to express the miRNAs or polynucleotide agent capable of down-regulating the miRNA of some embodiments of the invention. These include, but are not limited to, microorganisms, such as bacteria transformed with a recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vector containing the coding sequence; yeast transformed with recombinant yeast expression vectors containing the coding sequence; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors, such as Ti plasmid, containing the coding sequence. Mammalian expression systems can also be used to express the miRNAs of some embodiments of the invention.

Examples of bacterial constructs include the pET series of E. coli expression vectors [Studier et al. (1990) Methods in Enzymol. 185:60-89).

In yeast, a number of vectors containing constitutive or inducible promoters can be used, as disclosed in U.S. Pat. Application No: 5,932,447. Alternatively, vectors can

be used which promote integration of foreign DNA sequences into the yeast chromosome.

The conditions used for contacting the mesenchymal stem cells or neural stem cells are selected for a time period/concentration of cells/concentration of miRNA/ratio between cells and miRNA which enable the miRNA (or inhibitors thereof) to induce differentiation thereof. The present invention further contemplates incubation of the stem cells with a differentiation factor which promotes differentiation towards a motor neuron or neural stem cell lineage. The incubation with such differentiation factors may be affected prior to, concomitant with or following the contacting with the miRNA. Examples of such agents are provided in the Examples section herein below.

Alternatively, or additionally, the mesenchymal stem cells may be genetically modified so as to express such differentiation factors, using expression constructs such as those described herein above.

During or following the differentiation step the stem cells may be monitored for their differentiation state. Cell differentiation can be determined upon examination of cell or tissue-specific markers which are known to be indicative of differentiation.

For example, the neural stem cells may express at least one of nestin and SOX-2. Additional markers include SOX1, SOX3, PSA-NCAM and MUSASHI-1.

Below is a list of markers that may be used to confirm differentiation into motor neurons: ChAT (choline acetyltransferase), Chox10, En1, Even-skipped (Eve) transcription factor, Evx1/2, Fibroblast growth factor-1 (FGF1 or acidic FGF), HB9, Isl1 (Islet-1), Isl2, Islet1/2, Lim3, p75(NTR) (p75 neurotrophin receptor), REG2, Sim1, SMI32 (SMI-32) and Zfh1.

Tissue/cell specific markers can be detected using immunological techniques well known in the art [Thomson JA et al., (1998). Science 282: 1145-7]. Examples include, but are not limited to, flow cytometry for membrane-bound markers, immunohistochemistry for extracellular and intracellular markers and enzymatic immunoassay, for secreted molecular markers.

It will be appreciated that the cells obtained according to the methods described herein may be enriched for a particular cell type – e.g. progenitor cell type or mature cell type. Thus for example, the time of differentiation may be selected to obtain an earlier progenitor type (e.g. one that expresses at least one of the following markers

nestin, olig2 and Sox2) or a later mature cell type (e.g. one that expresses at least one of the following markers ChAT, islet1, HB9 and β3 tubulin).

Further enrichment of a particular cell type may be affected using cell sorting techniques such as FACS and magnetic sorting.

In addition, cell differentiation can be also followed by specific reporters that are tagged with GFP or RFP and exhibit increased fluorescence upon differentiation.

By determining the targets of the miRNAs of the present invention that are proposed for up-regulation, it will be appreciated that the scope of the present invention may be broadened to include down-regulation of the targets by means other than contacting with miRNA. Correspondingly, by determining the targets of the miRNAs of the present invention that are proposed for down-regulation, it will be appreciated that the scope of the present invention may be broadened to include up-regulation of the targets.

For example, the present inventors have shown that one of the targets of miR-137 is Related to testis-specific, vespid and pathogenesis protein 1 (RTVP-1) Thus the present invention contemplates that differentiation towards the neural stem cell lineage may be affected by down-regulation of this protein.

Thus, according to another aspect of the invention, there is provided a method of generating neural stem cells, the method comprising contacting mesenchymal stem cells (MSCs) with an agent that down-regulates an amount and/or activity of Related to testis-specific, vespid and pathogenesis protein 1 (RTVP-1), thereby generating the neural stem cells.

Related to testis-specific, vespid and pathogenesis protein 1 (RTVP-1) was cloned from human GBM cell lines by two groups and was termed glioma pathogenesis-related protein- GLIPR1 or RTVP-1 [Rich T, et al., Gene 1996; 180: 125-30], incorporated herein by reference. RTVP-1 contains a putative signal peptide, a trans membrane domain and a SCP domain, with a yet unknown function which is also found in other RTVP-1 homologs including TPX-1, the venom allergen antigen 5 and group 1 of the plant pathogenesis-related proteins (PR-1).

Down-regulation of RTVP-1 (or any of the other miRNA targets of the present invention) can be obtained at the genomic and/or the transcript level using a variety of molecules which interfere with transcription and/or translation (e.g., RNA silencing

agents, Ribozyme, DNAzyme and antisense), or on the protein level using e.g., antagonists, enzymes that cleave the polypeptide and the like.

Following is a list of agents capable of down-regulating expression level and/or activity of RTVP-1.

One example of an agent capable of down-regulating RTVP-1 is an antibody or antibody fragment capable of specifically binding thereto. Preferably, the antibody is capable of being internalized by the cell and entering the nucleus.

The term "antibody" as used in this invention includes intact molecules as well as functional fragments thereof, such as Fab, F(ab')2, and Fv that are capable of binding to macrophages. These functional antibody fragments are defined as follows: (1) Fab, the fragment which contains a monovalent antigen-binding fragment of an antibody molecule, can be produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and a portion of one heavy chain; (2) Fab', the fragment of an antibody molecule that can be obtained by treating whole antibody with pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain; two Fab' fragments are obtained per antibody molecule; (3) (Fab')2, the fragment of the antibody that can be obtained by treating whole antibody with the enzyme pepsin without subsequent reduction; F(ab')2 is a dimer of two Fab' fragments held together by two disulfide bonds; (4) Fv, defined as a genetically engineered fragment containing the variable region of the light chain and the variable region of the heavy chain expressed as two chains; and (5) Single chain antibody ("SCA"), a genetically engineered molecule containing the variable region of the light chain and the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule.

Down-regulation of RTVP-1 can be also achieved by RNA silencing. As used herein, the phrase "RNA silencing" refers to a group of regulatory mechanisms [e.g. RNA interference (RNAi), transcriptional gene silencing (TGS), post-transcriptional gene silencing (PTGS), quelling, co-suppression, and translational repression] mediated by RNA molecules which result in the inhibition or "silencing" of the expression of a corresponding protein-coding gene. RNA silencing has been observed in many types of organisms, including plants, animals, and fungi.

As used herein, the term "RNA silencing agent" refers to an RNA which is capable of inhibiting or "silencing" the expression of a target gene. In certain embodiments, the RNA silencing agent is capable of preventing complete processing (e.g., the full translation and/or expression) of an mRNA molecule through a post-transcriptional silencing mechanism. RNA silencing agents include non-coding RNA molecules, for example RNA duplexes comprising paired strands, as well as precursor RNAs from which such small non-coding RNAs can be generated. In one embodiment, the RNA silencing agent is capable of inducing RNA interference. In another embodiment, the RNA silencing agent is capable of mediating translational repression.

RNA interference refers to the process of sequence-specific post-transcriptional gene silencing in animals mediated by short interfering RNAs (siRNAs). The corresponding process in plants is commonly referred to as post-transcriptional gene silencing or RNA silencing and is also referred to as quelling in fungi. The process of post-transcriptional gene silencing is thought to be an evolutionarily-conserved cellular defense mechanism used to prevent the expression of foreign genes and is commonly shared by diverse flora and phyla. Such protection from foreign gene expression may have evolved in response to the production of double-stranded RNAs (dsRNAs) derived from viral infection or from the random integration of transposon elements into a host genome via a cellular response that specifically destroys homologous single-stranded RNA or viral genomic RNA.

The presence of long dsRNAs in cells stimulates the activity of a ribonuclease III enzyme referred to as dicer. Dicer is involved in the processing of the dsRNA into short pieces of dsRNA known as short interfering RNAs (siRNAs). Short interfering RNAs derived from dicer activity are typically about 21 to about 23 nucleotides in length and comprise about 19 base pair duplexes. The RNAi response also features an endonuclease complex, commonly referred to as an RNA-induced silencing complex (RISC), which mediates cleavage of single-stranded RNA having sequence complementary to the antisense strand of the siRNA duplex. Cleavage of the target RNA takes place in the middle of the region complementary to the antisense strand of the siRNA duplex.

Accordingly, the present invention contemplates use of dsRNA to down-regulate protein expression from the mRNA.

According to one embodiment, the dsRNA is greater than 30 bp. The use of long dsRNAs (i.e. dsRNA greater than 30 bp) has been very limited owing to the belief that these longer regions of double stranded RNA will result in the induction of the interferon and PKR response. However, the use of long dsRNAs can provide numerous advantages in that the cell can select the optimal silencing sequence alleviating the need to test numerous siRNAs; long dsRNAs will allow for silencing libraries to have less complexity than would be necessary for siRNAs; and, perhaps most importantly, long dsRNA could prevent viral escape mutations when used as therapeutics.

Various studies demonstrate that long dsRNAs can be used to silence gene expression without inducing the stress response or causing significant off-target effects - see for example [Strat et al., Nucleic Acids Research, 2006, Vol. 34, No. 13 3803–3810; Bhargava A et al. Brain Res. Protoc. 2004;13:115–125; Diallo M., et al., Oligonucleotides. 2003;13:381–392; Paddison P.J., et al., Proc. Natl Acad. Sci. USA. 2002;99:1443–1448; Tran N., et al., FEBS Lett. 2004;573:127–134].

In particular, the present invention also contemplates introduction of long dsRNA (over 30 base transcripts) for gene silencing in cells where the interferon pathway is not activated (e.g. embryonic cells and oocytes) see for example Billy et al., PNAS 2001, Vol 98, pages 14428-14433. and Diallo et al, Oligonucleotides, October 1, 2003, 13(5): 381-392. doi:10.1089/154545703322617069.

The present invention also contemplates introduction of long dsRNA specifically designed not to induce the interferon and PKR pathways for down-regulating gene expression. For example, Shinagwa and Ishii [*Genes & Dev.* 17 (11): 1340-1345, 2003] have developed a vector, named pDECAP, to express long double-strand RNA from an RNA polymerase II (Pol II) promoter. Because the transcripts from pDECAP lack both the 5'-cap structure and the 3'-poly(A) tail that facilitate ds-RNA export to the cytoplasm, long ds-RNA from pDECAP does not induce the interferon response.

Another method of evading the interferon and PKR pathways in mammalian systems is by introduction of small inhibitory RNAs (siRNAs) either via transfection or endogenous expression.

The term "siRNA" refers to small inhibitory RNA duplexes (generally between 18-30 basepairs) that induce the RNA interference (RNAi) pathway. Typically, siRNAs

are chemically synthesized as 21mers with a central 19 bp duplex region and symmetric 2-base 3'-overhangs on the termini, although it has been recently described that chemically synthesized RNA duplexes of 25-30 base length can have as much as a 100-fold increase in potency compared with 21mers at the same location. The observed increased potency obtained using longer RNAs in triggering RNAi is theorized to result from providing Dicer with a substrate (27mer) instead of a product (21mer) and that this improves the rate or efficiency of entry of the siRNA duplex into RISC.

It has been found that position of the 3'-overhang influences potency of an siRNA and asymmetric duplexes having a 3'-overhang on the antisense strand are generally more potent than those with the 3'-overhang on the sense strand (Rose et al., 2005). This can be attributed to asymmetrical strand loading into RISC, as the opposite efficacy patterns are observed when targeting the antisense transcript.

The strands of a double-stranded interfering RNA (e.g., an siRNA) may be connected to form a hairpin or stem-loop structure (e.g., an shRNA). Thus, as mentioned the RNA silencing agent of the present invention may also be a short hairpin RNA (shRNA).

The term "shRNA", as used herein, refers to an RNA agent having a stem-loop structure, comprising a first and second region of complementary sequence, the degree of complementarity and orientation of the regions being sufficient such that base pairing occurs between the regions, the first and second regions being joined by a loop region, the loop resulting from a lack of base pairing between nucleotides (or nucleotide analogs) within the loop region. The number of nucleotides in the loop is a number between and including 3 to 23, or 5 to 15, or 7 to 13, or 4 to 9, or 9 to 11. Some of the nucleotides in the loop can be involved in base-pair interactions with other nucleotides in the loop. Examples of oligonucleotide sequences that can be used to form the loop include 5'-UUCAAGAGA-3'; (Brummelkamp, T. R. et al. (2002) Science 296: 550) and 5'-UUUGUGUAG-3' (Castanotto, D. et al. (2002) RNA 8:1454). It will be recognized by one of skill in the art that the resulting single chain oligonucleotide forms a stem-loop or hairpin structure comprising a double-stranded region capable of interacting with the RNAi machinery.

According to another embodiment the RNA silencing agent may be a miRNA, as further described herein above.

Synthesis of RNA silencing agents suitable for use with the present invention can be affected as follows. First, the miRNA target mRNA sequence (e.g. CTGF sequence) is scanned downstream of the AUG start codon for AA dinucleotide sequences. Occurrence of each AA and the 3' adjacent 19 nucleotides is recorded as potential siRNA target sites. Preferably, siRNA target sites are selected from the open reading frame, as untranslated regions (UTRs) are richer in regulatory protein binding sites. UTR-binding proteins and/or translation initiation complexes may interfere with binding of the siRNA endonuclease complex [Tuschl ChemBiochem. 2:239-245]. It will be appreciated though, that siRNAs directed at untranslated regions may also be effective, as demonstrated for GAPDH wherein siRNA directed at the 5' UTR mediated about 90 % decrease in cellular GAPDH mRNA and completely abolished protein level (www.ambion.com/techlib/tn/91/912.html).

Second, potential target sites are compared to an appropriate genomic database (e.g., human, mouse, rat etc.) using any sequence alignment software, such as the BLAST software available from the NCBI server (www.ncbi.nlm.nih.gov/BLAST/). Putative target sites which exhibit significant homology to other coding sequences are filtered out.

Qualifying target sequences are selected as template for siRNA synthesis. Preferred sequences are those including low G/C content as these have proven to be more effective in mediating gene silencing as compared to those with G/C content higher than 55 %. Several target sites are preferably selected along the length of the target gene for evaluation. For better evaluation of the selected siRNAs, a negative control is preferably used in conjunction. Negative control siRNA preferably include the same nucleotide composition as the siRNAs but lack significant homology to the genome. Thus, a scrambled nucleotide sequence of the siRNA is preferably used, provided it does not display any significant homology to any other gene.

The RNA silencing agents of the present invention may comprise nucleic acid analogs that may have at least one different linkage, e.g., phosphoramidate, phosphorothioate, phosphorodithioate, or O-methylphosphoroamidite linkages and peptide nucleic acid backbones and linkages. Other analog nucleic acids include those with positive backbones; non-ionic backbones, and non-ribose backbones, including those described in U.S. Pat. Nos. 5,235,033 and 5,034,506, which are incorporated by

reference. Nucleic acids containing one or more non-naturally occurring or modified nucleotides are also included within one definition of nucleic acids. The modified nucleotide analog may be located for example at the 5'-end and/or the 3'-end of the nucleic acid molecule. Representative examples of nucleotide analogs may be selected from sugar- or backbone-modified ribonucleotides. It should be noted, however, that also nucleobase-modified ribonucleotides, i.e. ribonucleotides, containing a non-naturally occurring nucleobase instead of a naturally occurring nucleobase such as uridines or cytidines modified at the 5-position, e.g. 5-(2-amino) propyl uridine, 5-bromo uridine; adenosines and guanosines modified at the 8-position, e.g. 8-bromo guanosine; deaza nucleotides, e.g. 7-deaza-adenosine; O- and N-alkylated nucleotides, e.g. N6-methyl adenosine are suitable. The 2'-OH-group may be replaced by a group selected from H, OR, R, halo, SH, SR, NH_2, NHR, NR_2 or CN, wherein R is C_1-C_6 alkyl, alkenyl or alkynyl and halo is F, Cl, Br or I. Modified nucleotides also include nucleotides conjugated with cholesterol through, e.g., a hydroxyprolinol linkage as described in Krutzfeldt et al., Nature 438:685-689 (2005), Soutschek et al., Nature 432:173-178 (2004), and U.S. Patent Publication No. 20050107325, which are incorporated herein by reference. Additional modified nucleotides and nucleic acids are described in U.S. Patent Publication No. 20050182005, which is incorporated herein by reference. Modifications of the ribose-phosphate backbone may be done for a variety of reasons, e.g., to increase the stability and half-life of such molecules in physiological environments, to enhance diffusion across cell membranes, or as probes on a biochip. The backbone modification may also enhance resistance to degradation, such as in the harsh endocytic environment of cells. The backbone modification may also reduce nucleic acid clearance by hepatocytes, such as in the liver and kidney. Mixtures of naturally occurring nucleic acids and analogs may be made; alternatively, mixtures of different nucleic acid analogs, and mixtures of naturally occurring nucleic acids and analogs may be made.

In some embodiments, the RNA silencing agent provided herein can be functionally associated with a cell-penetrating peptide." As used herein, a "cell-penetrating peptide" is a peptide that comprises a short (about 12-30 residues) amino acid sequence or functional motif that confers the energy-independent (i.e., non-endocytotic) translocation properties associated with transport of the membrane-

permeable complex across the plasma and/or nuclear membranes of a cell. The cell-penetrating peptide used in the membrane-permeable complex of the present invention preferably comprises at least one non-functional cysteine residue, which is either free or derivatized to form a disulfide link with a double-stranded ribonucleic acid that has been modified for such linkage. Representative amino acid motifs conferring such properties are listed in U.S. Pat. No. 6,348,185, the contents of which are expressly incorporated herein by reference. The cell-penetrating peptides of the present invention preferably include, but are not limited to, penetratin, transportan, pIsl, TAT(48-60), pVEC, MTS, and MAP.

Another agent capable of down-regulating RTVP-1 is a DNAzyme molecule capable of specifically cleaving an mRNA transcript or DNA sequence of CTGF. DNAzymes are single-stranded polynucleotides which are capable of cleaving both single and double stranded target sequences (Breaker, R.R. and Joyce, G. Chemistry and Biology 1995;2:655; Santoro, S.W. & Joyce, G.F. Proc. Natl, Acad. Sci. USA 1997;943:4262) A general model (the "10-23" model) for the DNAzyme has been proposed. "10-23" DNAzymes have a catalytic domain of 15 deoxyribonucleotides, flanked by two substrate-recognition domains of seven to nine deoxyribonucleotides each. This type of DNAzyme can effectively cleave its substrate RNA at purine:pyrimidine junctions (Santoro, S.W. & Joyce, G.F. Proc. Natl, Acad. Sci. USA 199; for rev of DNAzymes see Khachigian, LM [Curr Opin Mol Ther 4:119-21 (2002)].

Examples of construction and amplification of synthetic, engineered DNAzymes recognizing single and double-stranded target cleavage sites have been disclosed in U.S. Pat. No. 6,326,174 to Joyce et al.

Down-regulation of RTVP-1 can also be obtained by using an antisense polynucleotide capable of specifically hybridizing with an mRNA transcript encoding RTVP-1.

Design of antisense molecules which can be used to efficiently down-regulate RTVP-1should take into consideration two aspects important to the antisense approach. The first aspect is delivery of the oligonucleotide into the cytoplasm of the appropriate cells, while the second aspect is design of an oligonucleotide which specifically binds the designated mRNA within cells in a way which inhibits translation thereof.

The prior art teaches of a number of delivery strategies which can be used to efficiently deliver oligonucleotides into a wide variety of cell types [see, for example, Luft J Mol Med 76: 75-6 (1998); Kronenwett et al. Blood 91: 852-62 (1998); Rajur et al. Bioconjug Chem 8: 935-40 (1997); Lavigne et al. Biochem Biophys Res Commun 237: 566-71 (1997) and Aoki et al. (1997) Biochem Biophys Res Commun 231: 540-5 (1997)].

In addition, algorithms for identifying those sequences with the highest predicted binding affinity for their target mRNA based on a thermodynamic cycle that accounts for the energetics of structural alterations in both the target mRNA and the oligonucleotide are also available [see, for example, Walton et al. Biotechnol Bioeng 65: 1-9 (1999)].

Such algorithms have been successfully used to implement an antisense approach in cells. For example, the algorithm developed by Walton et al. enabled scientists to successfully design antisense oligonucleotides for rabbit beta-globin (RBG) and mouse tumor necrosis factor-alpha (TNF alpha) transcripts. The same research group has more recently reported that the antisense activity of rationally selected oligonucleotides against three model target mRNAs (human lactate dehydrogenase A and B and rat gp130) in cell culture as evaluated by a kinetic PCR technique proved effective in almost all cases, including tests against three different targets in two cell types with phosphodiester and phosphorothioate oligonucleotide chemistries.

In addition, several approaches for designing and predicting efficiency of specific oligonucleotides using an in vitro system were also published (Matveeva et al., Nature Biotechnology 16: 1374 - 1375 (1998)].

Another agent capable of down-regulating RTVP-1is a ribozyme molecule capable of specifically cleaving an mRNA transcript encoding RTVP-1. Ribozymes are being increasingly used for the sequence-specific inhibition of gene expression by the cleavage of mRNAs encoding proteins of interest [Welch et al., Curr Opin Biotechnol. 9:486-96 (1998)]. The possibility of designing ribozymes to cleave any specific target RNA has rendered them valuable tools in both basic research and therapeutic applications.

An additional method of regulating the expression of a RTVP-1gene in cells is via triplex forming oligonuclotides (TFOs). Recent studies have shown that TFOs can

be designed which can recognize and bind to polypurine/polypirimidine regions in double-stranded helical DNA in a sequence-specific manner. These recognition rules are outlined by Maher III, L. J., et al., Science,1989;245:725-730; Moser, H. E., et al., Science,1987;238:645-630; Beal, P. A., et al, Science,1992;251:1360-1363; Cooney, M., et al., Science,1988;241:456-459; and Hogan, M. E., et al., EP Publication 375408. Modification of the oligonuclotides, such as the introduction of intercalators and backbone substitutions, and optimization of binding conditions (pH and cation concentration) have aided in overcoming inherent obstacles to TFO activity such as charge repulsion and instability, and it was recently shown that synthetic oligonucleotides can be targeted to specific sequences (for a recent review see Seidman and Glazer, J Clin Invest 2003;112:487-94).

In general, the triplex-forming oligonucleotide has the sequence correspondence:

oligo	3'--A	G	G	T
duplex	5'--A	G	C	T
duplex	3'--T	C	G	A

However, it has been shown that the A-AT and G-GC triplets have the greatest triple helical stability (Reither and Jeltsch, BMC Biochem, 2002, Sept12, Epub). The same authors have demonstrated that TFOs designed according to the A-AT and G-GC rule do not form non-specific triplexes, indicating that the triplex formation is indeed sequence specific.

Triplex-forming oligonucleotides preferably are at least 15, more preferably 25, still more preferably 30 or more nucleotides in length, up to 50 or 100 bp.

Transfection of cells (for example, via cationic liposomes) with TFOs, and formation of the triple helical structure with the target DNA induces steric and functional changes, blocking transcription initiation and elongation, allowing the introduction of desired sequence changes in the endogenous DNA and resulting in the specific down-regulation of gene expression. Examples of such suppression of gene expression in cells treated with TFOs include knockout of episomal supFG1 and endogenous HPRT genes in mammalian cells (Vasquez et al., Nucl Acids Res. 1999;27:1176-81, and Puri, et al, J Biol Chem, 2001;276:28991-98), and the sequence- and target specific down-regulation of expression of the Ets2 transcription factor, important in prostate cancer etiology (Carbone, et al, Nucl Acid Res. 2003;31:833-43),

and the pro-inflammatory ICAM-1 gene (Besch et al, J Biol Chem, 2002;277:32473-79). In addition, Vuyisich and Beal have recently shown that sequence specific TFOs can bind to dsRNA, inhibiting activity of dsRNA-dependent enzymes such as RNA-dependent kinases (Vuyisich and Beal, Nuc. Acids Res 2000;28:2369-74).

Additionally, TFOs designed according to the abovementioned principles can induce directed mutagenesis capable of effecting DNA repair, thus providing both down-regulation and up-regulation of expression of endogenous genes (Seidman and Glazer, J Clin Invest 2003;112:487-94). Detailed description of the design, synthesis and administration of effective TFOs can be found in U.S. Patent Application Nos. 2003 017068 and 2003 0096980 to Froehler et al, and 2002 0128218 and 2002 0123476 to Emanuele et al, and U.S. Pat. No. 5,721,138 to Lawn.

The conditions used for contacting the mesenchymal stem cells are selected for a time period/concentration of cells/concentration of RTVP-1 down-regulatory agent/ratio between cells and RTVP-1down-regulatory agent which enable the RTVP-1 down-regulatory agent to induce differentiation thereof.

Isolated cell populations obtained according to the methods describe herein are typically non-homogeneous, although homogeneous cell populations are also contemplated.

According to a particular embodiment, the cell populations are genetically modified to express an exogenous miRNA or a polynucleotide agent capable of down-regulating the miRNA.

The term "isolated" as used herein refers to a population of cells that has been removed from its in-vivo location (e.g. bone marrow, neural tissue). Preferably the isolated cell population is substantially free from other substances (e.g., other cells) that are present in its in-vivo location.

Cell populations may be selected such that more than about 50 % (alternatively more than about 60 %, more than about 70 %, more than about 80 %, more than about 90 % or even more than about 95 %) of the cells express at least one, at least two, at least three, at least four, at least five of the markers for motor neurons or at least one, at least two, at least three, at least four, at least five of the markers for neural stem cells.

Isolation of particular subpopulations of cells may be affected using techniques known in the art including fluorescent activated cell sorting and/or magnetic separation of cells.

The cells of the populations of this aspect of the present invention may comprise structural motor neuron or neural stem cell phenotypes including a cell size, a cell shape, an organelle size and an organelle number. These structural phenotypes may be analyzed using microscopic techniques (e.g. scanning electro microscopy). Antibodies or dyes may be used to highlight distinguishing features in order to aid in the analysis.

The cells and cell populations of the present invention may be useful for a variety of therapeutic purposes. Representative examples of CNS diseases or disorders that can be beneficially treated with the cells described herein include, but are not limited to, a pain disorder, a motion disorder, a dissociative disorder, a mood disorder, an affective disorder, a neurodegenerative disease or disorder, psychiatric disorders and a convulsive disorder.

More specific examples of such conditions include, but are not limited to, Parkinson's, ALS, Multiple Sclerosis, Huntingdon's disease, autoimmune encephalomyelitis, spinal cord injury, cerebral palsy, diabetic neuropathy, glaucatomus neuropathy, macular degeneration, action tremors and tardive dyskinesia, panic, anxiety, depression, alcoholism, insomnia, manic behavior, schizophrenia, autism-spectrum disorder, manic-depressive disorders, Alzheimer's and epilepsy.

The use of differentiated MSCs may be also indicated for treatment of traumatic lesions of the nervous system including spinal cord injury and also for treatment of stroke caused by bleeding or thrombosis or embolism because of the need to induce neurogenesis and provide survival factors to minimize insult to damaged neurons.

The motor neuron like cells of the present invention may be useful for motor neuron diseases including, but not limited to amyotrophic lateral sclerosis (ALS), primary lateral sclerosis (PLS), pseudobulbar palsy and progressive bulbar palsy.

In any of the methods described herein the cells may be obtained from an autologous, semi-allogeneic or non-autologous (i.e., allogeneic or xenogeneic) human donor or embryo or cord/placenta. For example, cells may be isolated from a human cadaver or a donor subject.

The term semi-allogeneic refers to donor cells which are partially-mismatched to recipient cells at a major histocompatibility complex (MHC) class I or class II locus.

The cells of the present invention can be administered to the treated individual using a variety of transplantation approaches, the nature of which depends on the site of implantation.

The term or phrase "transplantation", "cell replacement" or "grafting" are used interchangeably herein and refer to the introduction of the cells of the present invention to target tissue. As mentioned, the cells can be derived from the recipient or from an allogeneic, semi-allogeneic or xenogeneic donor.

The cells can be injected systemically into the circulation, administered intrathecally or grafted into the central nervous system, the spinal cord or into the ventricular cavities or subdurally onto the surface of a host brain. Conditions for successful transplantation include: (i) viability of the implant; (ii) retention of the graft at the site of transplantation; and (iii) minimum amount of pathological reaction at the site of transplantation. Methods for transplanting various nerve tissues, for example embryonic brain tissue, into host brains have been described in: "Neural grafting in the mammalian CNS", Bjorklund and Stenevi, eds. (1985); Freed et al., 2001; Olanow et al., 2003). These procedures include intraparenchymal transplantation, i.e. within the host brain (as compared to outside the brain or extraparenchymal transplantation) achieved by injection or deposition of tissue within the brain parenchyma at the time of transplantation.

Intraparenchymal transplantation can be performed using two approaches: (i) injection of cells into the host brain parenchyma or (ii) preparing a cavity by surgical means to expose the host brain parenchyma and then depositing the graft into the cavity. Both methods provide parenchymal deposition between the graft and host brain tissue at the time of grafting, and both facilitate anatomical integration between the graft and host brain tissue. This is of importance if it is required that the graft becomes an integral part of the host brain and survives for the life of the host.

Alternatively, the graft may be placed in a ventricle, e.g. a cerebral ventricle or subdurally, i.e. on the surface of the host brain where it is separated from the host brain parenchyma by the intervening pia mater or arachnoid and pia mater. Grafting to the ventricle may be accomplished by injection of the donor cells or by growing the cells in

a substrate such as 3% collagen to form a plug of solid tissue which may then be implanted into the ventricle to prevent dislocation of the graft. For subdural grafting, the cells may be injected around the surface of the brain after making a slit in the dura. Injections into selected regions of the host brain may be made by drilling a hole and piercing the dura to permit the needle of a microsyringe to be inserted. The microsyringe is preferably mounted in a stereotaxic frame and three dimensional stereotaxic coordinates are selected for placing the needle into the desired location of the brain or spinal cord. The cells may also be introduced into the putamen, nucleus basalis, hippocampus cortex, striatum, substantia nigra or caudate regions of the brain, as well as the spinal cord.

The cells may also be transplanted to a healthy region of the tissue. In some cases the exact location of the damaged tissue area may be unknown and the cells may be inadvertently transplanted to a healthy region. In other cases, it may be preferable to administer the cells to a healthy region, thereby avoiding any further damage to that region. Whatever the case, following transplantation, the cells preferably migrate to the damaged area.

For transplanting, the cell suspension is drawn up into the syringe and administered to anesthetized transplantation recipients. Multiple injections may be made using this procedure.

The cellular suspension procedure thus permits grafting of the cells to any predetermined site in the brain or spinal cord, is relatively non-traumatic, allows multiple grafting simultaneously in several different sites or the same site using the same cell suspension, and permits mixtures of cells from different anatomical regions. Multiple grafts may consist of a mixture of cell types, and/or a mixture of transgenes inserted into the cells. Preferably from approximately 10^4 to approximately 10^9 cells are introduced per graft. Cells can be administered concomitantly to different locations such as combined administration intrathecally and intravenously to maximize the chance of targeting into affected areas.

For transplantation into cavities, which may be preferred for spinal cord grafting, tissue is removed from regions close to the external surface of the central nerve system (CNS) to form a transplantation cavity, for example as described by Stenevi et al. (Brain Res. 114:1-20., 1976), by removing bone overlying the brain and stopping

bleeding with a material such a gelfoam. Suction may be used to create the cavity. The graft is then placed in the cavity. More than one transplant may be placed in the same cavity using injection of cells or solid tissue implants. Preferably, the site of implantation is dictated by the CNS disorder being treated. Demyelinated MS lesions are distributed across multiple locations throughout the CNS, such that effective treatment of MS may rely more on the migratory ability of the cells to the appropriate target sites.

Intranasal administration of the cells is also contemplated.

MSCs typically down regulate MHC class 2 and are therefore less immunogenic. Embryonal or newborn cells obtained from the cord blood, cord's Warton's gelly or placenta are further less likely to be strongly immunogenic and therefore less likely to be rejected, especially since such cells are immunosuppressive and immunoregulatory to start with.

Notwithstanding, since non-autologous cells may induce an immune reaction when administered to the body several approaches have been developed to reduce the likelihood of rejection of non-autologous cells. Furthermore, since diseases such as multiple sclerosis are inflammatory based diseases, the problem of immune reaction is exacerbated. These include either administration of cells to privileged sites, or alternatively, suppressing the recipient's immune system, providing anti-inflammatory treatment which may be indicated to control autoimmune disorders to start with and/or encapsulating the non-autologous/semi-autologous cells in immunoisolating, semipermeable membranes before transplantation.

As mentioned herein above, the present inventors also propose use of newborn mesenchymal stem cells to limit the immune reaction.

The following experiments may be performed to confirm the potential use of newborn's MSCs isolated from the cord / placenta for treatment of neurological disorders:

1) Differentiated MSCs (to various neural cells or neural progenitor cells) may serve as stimulators in one way mixed lymphocyte culture with allogeneic T cells and proliferative responses in comparison with T cells responding against allogeneic lymphocytes isolated from the same donor may be evaluated by ^{3}H-Thymidine uptake to document hyporesponsiveness.

2) Differentiated MSCs may be added/co-cultured to one way mixed lymphocyte cultures and to cell cultures with T cell mitogens (phytohemmaglutinin and concanavalin A) to confirm the immunosuppressive effects on proliferative responses mediated by T cells.

3) Cord and placenta cells cultured from Brown Norway rats (unmodified and differentiated), may be enriched for MSCs and these cells may be infused into Lewis rats with induced experimental autoimmune encephalomyelitis (EAE). Alternatively, cord and placenta cells cultured from BALB/c mice, (BALB/cxC57BL/6)F1 or xenogeneic cells from Brown Norway rats (unmodified and differentiated), may be enriched for MSCs and these cells may be infused into C57BL/6 or SJL/j recipients with induced experimental autoimmune encephalomyelitis (EAE). The clinical effects against paralysis may be investigated to evaluate the therapeutic effects of xenogeneic, fully MHC mismatched or haploidentically mismatched MSCs. Such experiments may provide the basis for treatment of patients with a genetic disorder or genetically proned disorder with family member's haploidentical MSCs.

4) BALB/c MSCs cultured from cord and placenta may be transfused with pre-miR labeled with GFP or RFP, which will allow the inventors to follow the migration and persistence of these cells in the brain of C57BL/6 recipients with induced EAE. The clinical effects of labeled MHC mismatched differentiated MSCs may be evaluated by monitoring signs of disease, paralysis and histopathology. The migration and localization of such cells may be also monitored by using fluorescent cells from genetically transduced GFP "green" or Red2 "red" donors.

As mentioned, the present invention also contemplates encapsulation techniques to minimize an immune response.

Encapsulation techniques are generally classified as microencapsulation, involving small spherical vehicles and macroencapsulation, involving larger flat-sheet and hollow-fiber membranes (Uludag, H. et al. Technology of mammalian cell encapsulation. Adv Drug Deliv Rev. 2000; 42: 29-64).

Methods of preparing microcapsules are known in the arts and include for example those disclosed by Lu M Z, et al., Cell encapsulation with alginate and alpha-

phenoxycinnamylidene-acetylated poly(allylamine). Biotechnol Bioeng. 2000, 70: 479-83, Chang T M and Prakash S. Procedures for microencapsulation of enzymes, cells and genetically engineered microorganisms. Mol. Biotechnol. 2001, 17: 249-60, and Lu M Z, et al., A novel cell encapsulation method using photosensitive poly(allylamine alpha-cyanocinnamylideneacetate). J. Microencapsul. 2000, 17: 245-51.

For example, microcapsules are prepared by complexing modified collagen with a ter-polymer shell of 2-hydroxyethyl methylacrylate (HEMA), methacrylic acid (MAA) and methyl methacrylate (MMA), resulting in a capsule thickness of 2-5 .mu.m. Such microcapsules can be further encapsulated with additional 2-5 .mu.m ter-polymer shells in order to impart a negatively charged smooth surface and to minimize plasma protein absorption (Chia, S. M. et al. Multi-layered microcapsules for cell encapsulation Biomaterials. 2002 23: 849-56).

Other microcapsules are based on alginate, a marine polysaccharide (Sambanis, A. Encapsulated islets in diabetes treatment. Diabetes Technol. Ther. 2003, 5: 665-8) or its derivatives. For example, microcapsules can be prepared by the polyelectrolyte complexation between the polyanions sodium alginate and sodium cellulose sulphate with the polycation poly(methylene-co-guanidine) hydrochloride in the presence of calcium chloride.

It will be appreciated that cell encapsulation is improved when smaller capsules are used. Thus, the quality control, mechanical stability, diffusion properties, and in vitro activities of encapsulated cells improved when the capsule size was reduced from 1 mm to 400 .mu.m (Canaple L. et al., Improving cell encapsulation through size control. J Biomater Sci Polym Ed. 2002; 13:783-96). Moreover, nanoporous biocapsules with well-controlled pore size as small as 7 nm, tailored surface chemistries and precise microarchitectures were found to successfully immunoisolate microenvironments for cells (Williams D. Small is beautiful: microparticle and nanoparticle technology in medical devices. Med Device Technol. 1999, 10: 6-9; Desai, T. A. Microfabrication technology for pancreatic cell encapsulation. Expert Opin Biol Ther. 2002, 2: 633-46).

Examples of immunosuppressive agents include, but are not limited to, methotrexate, cyclophosphamide, cyclosporine, cyclosporin A, chloroquine, hydroxychloroquine, sulfasalazine (sulphasalazopyrine), gold salts, D-penicillamine,

leflunomide, azathioprine, anakinra, infliximab (REMICADE™), etanercept, TNF alpha blockers, a biological agent that targets an inflammatory cytokine, and Non-Steroidal Anti-Inflammatory Drug (NSAIDs). Examples of NSAIDs include, but are not limited to acetyl salicylic acid, choline magnesium salicylate, diflunisal, magnesium salicylate, salsalate, sodium salicylate, diclofenac, etodolac, fenoprofen, flurbiprofen, indomethacin, ketoprofen, ketorolac, meclofenamate, naproxen, nabumetone, phenylbutazone, piroxicam, sulindac, tolmetin, acetaminophen, ibuprofen, Cox-2 inhibitors and tramadol.

In any of the methods described herein, the cells can be administered either per se or, preferably as a part of a pharmaceutical composition that further comprises a pharmaceutically acceptable carrier.

As used herein a "pharmaceutical composition" refers to a preparation of one or more of the cell compositions described herein, with other chemical components such as pharmaceutically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of the cells to a subject.

Hereinafter, the term "pharmaceutically acceptable carrier" refers to a carrier or a diluent that does not cause significant irritation to a subject and does not abrogate the biological activity and properties of the administered compound. Examples, without limitations, of carriers are propylene glycol, saline, emulsions and mixtures of organic solvents with water.

Herein the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of a compound. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, Pa., latest edition, which is incorporated herein by reference.

Suitable routes of administration include direct administration into the circulation (intravenously or intra-arterial), into the spinal fluid or into the tissue or organ of interest. Thus, for example the cells may be administered directly into the brain.

For any preparation used in the methods of the invention, the therapeutically effective amount or dose can be estimated initially from in vitro and cell culture assays. Preferably, a dose is formulated in an animal model to achieve a desired concentration or titer. Such information can be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of the active ingredients described herein can be determined by standard pharmaceutical procedures in vitro, in cell cultures or experimental animals. For example, animal models of demyelinating diseases include shiverer (shi/shi, MBP deleted) mouse, MD rats (PLP deficiency), Jimpy mouse (PLP mutation), dog shaking pup (PLP mutation), twitcher mouse (galactosylceramidase defect, as in human Krabbe disease), trembler mouse (PMP-22 deficiency). Virus induced demyelination model comprise use if Theiler's virus and mouse hepatitis virus. Autoimmune EAE is a possible model for multiple sclerosis.

The data obtained from these in vitro and cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition, (see e.g., Fingl, et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p. 1). For example, a multiple sclerosis patient can be monitored symptomatically for improved motor functions indicating positive response to treatment.

For injection, the active ingredients of the pharmaceutical composition may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological salt buffer.

Dosage amount and interval may be adjusted individually to levels of the active ingredient which are sufficient to effectively treat the brain disease/disorder. Dosages necessary to achieve the desired effect will depend on individual characteristics and route of administration. Detection assays can be used to determine plasma concentrations.

Depending on the severity and responsiveness of the condition to be treated, dosing can be of a single or a plurality of administrations, with course of treatment lasting from several days to several weeks or diminution of the disease state is achieved.

The amount of a composition to be administered will, of course, be dependent on the individual being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc. The dosage and timing of administration will be responsive to a careful and continuous monitoring of the individual changing condition. For example, a treated multiple sclerosis patient will be administered with an amount of cells which is sufficient to alleviate the symptoms of the disease, based on the monitoring indications.

The cells of the present invention may be co-administered with therapeutic agents useful in treating neurodegenerative disorders, such as gangliosides; antibiotics, neurotransmitters, neurohormones, toxins, neurite promoting molecules; and antimetabolites and precursors of neurotransmitter molecules such as L-DOPA.

As used herein the term "about" refers to +/-10%.

Throughout this application, various embodiments of this invention may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

As used herein, the term “treating” includes abrogating, substantially inhibiting, slowing or reversing the progression of a condition, substantially ameliorating clinical or aesthetical symptoms of a condition or substantially preventing the appearance of clinical or aesthetical symptoms of a condition.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the following examples.

As used herein the term “about” refers to ± 10 %.

The terms "comprises", "comprising", "includes", "including", “having” and their conjugates mean "including but not limited to".

The term “consisting of” means “including and limited to”.

The term "consisting essentially of" means that the composition, method or structure may include additional ingredients, steps and/or parts, but only if the additional ingredients, steps and/or parts do not materially alter the basic and novel characteristics of the claimed composition, method or structure.

As used herein, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures thereof.

Throughout this application, various embodiments of this invention may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well

as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases "ranging/ranges between" a first indicate number and a second indicate number and "ranging/ranges from" a first indicate number "to" a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals there between.

As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

As used herein, the term "treating" includes abrogating, substantially inhibiting, slowing or reversing the progression of a condition, substantially ameliorating clinical or aesthetical symptoms of a condition or substantially preventing the appearance of clinical or aesthetical symptoms of a condition.

It is noted that for each miR described herein the corresponding sequence (mature and pre) is provided in the sequence listing which should be regarded as part of the specification.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions illustrate some embodiments of the invention in a non limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Culture of Animal Cells - A Manual of Basic Technique" by Freshney, Wiley-Liss, N. Y. (1994), Third Edition; "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); “Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by

reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

EXAMPLE 1

Differentiation of Mesenchymal stem cells (MSCs) to Neural stem cells (NSCs)

METHODS

Mesenchymal stem cells (MSCs) from either bone marrow, adipose, placenta or umbilical cord were plated in high density in bacterial dishes in serum free medium supplemented with 10 mg/ml EGF and bFGF for 10 days. The cells started to aggregates and after 4-5 days were disaggregated mechanically to promote their detachment from the plates. The cells were then maintained for two weeks after which they were analyzed for the expression of NSC markers and for their ability to generate neurons, astrocytes and oligodendrocytes when plated on laminin in low-serum (5 %) medium.

The cells were then subjected to miRNA microarray as described.

RESULTS

As illustrated in Figures 1A-B, the mesenchymal stem cells expressed neuronal markers following neural stem cell differentiation.

EXAMPLE 2

Changes in miRNA expression during NSC differentiation

MATERIALS AND METHODS

miRNAs have been shown to play a role in the differentiation of various neural cells and neural stem cells. To analyze the expression and function of specific miRNAs in MSC-derived NSCs, the MSCs were differentiated towards NSCs as described in Example 1 and miRNA array analysis was performed to the control and differentiated cells. A qRT-PCR microarray was run that contained 96 miRNAs, all of which were related to stem cells and that were divided into subgroups based on their known association with stem cells, neural-related, hematopoietic and organ-related miRNAs.

For analyzing the differential expression of specific miRNA in control and differentiated MSCs, the Stem cell microRNA qPCR array was employed with quantiMiR from SBI company (catalog # RA620A-1), according to the user protocol, the contents of which are incorporated herein by reference. For the qPCR, the Applied Biosystems Power SYBR master mix (cat# 4367659) was used.

The system allows for the ability to quantitate fold differences of 95 separate microRNAs between 2 separate experimental RNA samples. The array plate also includes the U6 transcript as a normalization signal. All 95 microRNAs chosen for the array have published implications with regard to potential roles in stem cell self-renewal, hematopoiesis, neuronal development and differentiated tissue identification. The array plate also includes the U6 RNA as a normalization signal.

Total RNA was isolated from 10^5-10^6 cells of control and differentiated MSCs using miRneasy total RNA isolation kit from Qiagen (catalog # 217004) that isolate RNA fraction with sizes < 200 bp.

500 ng of total RNA was processed according to "SBI Stem Cell MicroRNA qPCR Array with QuantiMir™" (Cat. # RA620A-1) user protocol. For the qPCR, the Applied Biosystems Power SYBR master mix (cat# 4367659) was used.

For validation, sybr-green qPCR of the specific miRNA of interest was performed on the same RNA samples processed according to QIAGEN miScript System handbook (cat # 218061 & 218073)

Hu hsa-miR MicroRNA Profiling Kit (System Biosciences) "SBI Stem Cell MicroRNA qPCR Array with QuantiMir™" (Cat. # RA620A-1) which detects the expression of 96 miRNAs, was used to profile the miRNAs in unmodified BM-MSC compared with MSCs differentiated to astrocytes. 500 ng of total RNA was tagged with poly(A) to its 3' end by poly A polymerase, and reverse-transcribed with oligo-dT adaptors by QuantiMir RT technology. Expression levels of the miRNAs were measured by quantitative PCR using SYBR green reagent and VIIA7, Real-Time PCR System (Applied Biosystems). All miRNAs could be measured with miRNA specific forward primers and a universal reverse primer (SBI). Expression level of the miRNAs was normalized to U6 snRNA, using the comparative CT method for relative

quantification as calculated with the following equation: $2^{-[(\text{CT astrocyte diff miRNA-CT astrocyte endogenous control})-(\text{CT DMEM miRNA-CT DMEM endogenous control})]}$.

In addition, an Affymetrix miRNA 3.0 array was used to compare BM-MSCs and human NSCs and identify differentially expressed miRNAs.

RESULTS

As presented in Figures 2, 3 and 4A, there were significant changes in the expression of specific miRNA of each group between the control MSCs and the differentiated ones.

The results of the Affymetrix miRNA 3.0 array analysis are detailed in Table 1 herein below.

Table 1

MSCs/NSCs	Up regulated		MSCs/NSCs	Down regulated		
miRNA	Fold change		miRNA	Fold change	miRNA	Fold change
hsa-miR-145_st	1379.78		hsa-let-7c_st	-1.53698	hsa-miR-324-3p_st	-7.34456
hsa-miR-143_st	752.7381		hsa-miR-665_st	-1.58884	hsa-miR-20a_st	-7.83858
hsa-miR-214_st	552.6854		hsa-miR-4258_st	-1.61841	hsa-miR-501-5p_st	-8.36351
hsa-miR-199a-3p_st	511.1263		hsa-miR-361-3p_st	-1.63684	hsa-miR-330-3p_st	-8.71869
hsa-miR-199a-5p_st	362.5667		hsa-miR-374a-star_st	-1.76218	hsa-miR-874_st	-9.13392
hsa-miR-199b-3p_st	347.4311		hsa-miR-892b_st	-1.85672	hsa-miR-500_st	-9.68441
hsa-miR-138_st	229.2463		hsa-miR-361-5p_st	-1.90874	hsa-miR-25_st	-9.86881
hsa-miR-31_st	190.5331		hsa-miR-181a_st	-1.93941	hsa-miR-769-5p_st	-10.1382
hsa-miR-21_st	59.83459		hsa-miR-16_st	-2.19583	hsa-miR-125b-2-star_st	-10.3325
hsa-miR-193a-5p_st	23.8986		hsa-miR-636_st	-2.27398	hsa-miR-130b_st	-16.7436
hsa-miR-224-star_st	21.60842		hsa-miR-4284_st	-2.79417	hsa-miR-504_st	-16.9435
hsa-miR-196a_st	21.38142		hsa-miR-1208_st	-3.00768	hsa-miR-181a-2-star_st	-17.7877
hsa-miR-487b_st	19.18475		hsa-miR-1274b_st	-3.01855	hsa-miR-885-3p_st	-20.1501
hsa-miR-409-5p_st	17.45522		hsa-miR-30c-2-star_st	-3.46182	hsa-miR-1246_st	-21.0971

hsa-miR-193b-star_st	10.34438		hsa-miR-501-3p_st	-3.49025	hsa-miR-92b_st	-22.8735
hsa-miR-379_st	9.571106		hsa-miR-92a_st	-3.7152	hsa-miR-362-5p_st	-23.3686
hsa-miR-21-star_st	8.401508		hsa-miR-378b_st	-3.72739	hsa-miR-572_st	-23.3743
hsa-miR-27a-star_st	7.080883		hsa-miR-1287_st	-3.87466	hsa-miR-4270_st	-24.4173
hsa-miR-27a_st	6.122331		hsa-miR-425-star_st	-4.0524	hsa-miR-378c_st	-26.6758
hsa-miR-4317_st	5.715753		hsa-miR-324-5p_st	-4.37339	hsa-miR-93-star_st	-28.4948
hsa-miR-193b_st	4.920511		hsa-miR-3178_st	-4.40631	hsa-miR-149_st	-28.7369
hsa-miR-27b_st	4.889609		hsa-miR-219-1-3p_st	-4.52146	hsa-miR-363_st	-28.9968
hsa-miR-22_st	4.798265		hsa-miR-197_st	-4.609	hsa-miR-9_st	-31.2283
hsa-miR-574-3p_st	3.402782		hsa-miR-181b_st	-4.61406	hsa-miR-18a_st	-32.3908
hsa-miR-4288_st	3.375774		hsa-miR-500-star_st	-4.72807	hsa-miR-891a_st	-33.1912
hsa-miR-23a_st	3.34163		hsa-miR-106b_st	-4.96582	hsa-miR-346_st	-38.7283
hsa-miR-221-star_st	3.09015		hsa-miR-502-3p_st	-4.97984	hsa-miR-124_st	-50.7583
hsa-miR-2113_st	3.030064		hsa-miR-30c_st	-5.17107	hsa-miR-497_st	-72.2314
hsa-let-7i_st	2.551577		hsa-miR-1275_st	-5.29365	hsa-miR-378_st	-73.6306
hsa-miR-24_st	2.300083		hsa-miR-422a_st	-5.54416	hsa-miR-1231_st	-82.7066
hsa-miR-23b_st	2.217338		hsa-miR-93_st	-5.6233	hsa-miR-139-5p_st	-92.6078
hsa-miR-299-3p_st	2.201907		hsa-miR-181d_st	-5.74741	hsa-miR-3180-3p_st	-94.3695
hsa-miR-518c-star_st	2.197822		hsa-miR-1307_st	-5.82664	hsa-miR-9-star_st	-114.107
hsa-miR-221_st	2.186328		hsa-miR-1301_st	-5.84397	hsa-miR-935_st	-140.688
hsa-miR-431-star_st	2.177192		hsa-miR-99a_st	-5.88481	hsa-miR-20b_st	-156.762
hsa-miR-523_st	2.116276		hsa-miR-505-star_st	-5.9383		
hsa-miR-4313_st	1.937531		hsa-miR-1202_st	-5.94177		
hsa-miR-559_st	1.916531		hsa-miR-128_st	-6.05212		
hsa-miR-614_st	1.894046		hsa-miR-532-5p_st	-6.11976		
hsa-miR-	1.803374		hsa-miR-	-6.5161		

653_st			195_st			
hsa-miR-2278_st	1.675887		hsa-miR-532-3p_st	-6.66014		
v11_hsa-miR-768-5p_st	1.647103		hsa-miR-106a_st	-6.91155		
hsa-miR-154-star_st	1.608659		hsa-miR-17_st	-6.91565		
hsa-miR-302a-star_st	1.598961		hsa-miR-1271_st	-7.05548		
hsa-miR-3199_st	1.580479		hsa-miR-769-3p_st	-7.1367		
hsa-miR-3137_st	1.476948		hsa-miR-15b_st	-7.31636		

Using a nestin promoter based reporter assay, the present inventors confirmed that overexpression of miR-20b, miR-935, miR-891 and miR-378 also induced differentiation of the MSCs into NSCs (Figure 4B).

Similarly, silencing of miR-138, miR-214, miR-199a and miR-199b decreased the mesenchymal phenotypes of all the MSCs and induced their NSC differentiation (Figure 4C).

Co-transfection of the MSCs with combination of miR-20b or miR-378 with antagomiR-138 further increased the differentiation of the MSCs to nestin positive cells (Figure 4D).

As presented in Figures 4E-F, overexpression of antagomiR-138 and miR-891 mimic induced a significant increase in the generation of nestin positive cells in the transfected MSCs as demonstrated by the increased fluorescence intensity of cells transduced with the nestin-GFP reporter.

EXAMPLE 3

miRNAs that play a role in the differentiation of MSCs to NSCs

The present inventors further examined the role of the specific miRNAs that were found to be altered in the miR microarray on the differentiation of the MSCs to NSCs. These experiments were performed by transfecting MSCs with either specific or combination of mature miRNA mimics or miRNA inhibitors and then their ability to generate neurospheres and express the markers nestin and Sox2 was examined.

RESULTS

It was found that the inhibition of let-7 together with expression of miR-124 increased NSC differentiation.

In addition, it was found that up-regulation of the following miRNAs: miR302b, miR-371, miR-134, miR-219, miR-154, miR-155, miR-32, miR-33, miR-126 and miR-127 and down-regulation of the following miRs- miR-10b, miR-142-3p, miR-131a, miR-125b, miR-153 and miR-181a either alone or in various combinations induced differentiation of the MSCs to NSCs albeit to different degrees.

In addition to the miRNAs that were described in the miRNA array, it was also found that transfection of the MSCs with miR-132 and miR-137 also increased the NSC differentiation.

EXAMPLE 4

Additional factors that promote the differentiation of MSCs to NSCs

Related to testis-specific, vespid and pathogenesis protein 1 (RTVP-1) was cloned from human GBM cell lines by two groups and was termed glioma pathogenesis-related protein- GLIPR1 or RTVP-1 [3]. RTVP-1 contains a putative signal peptide, a trans membrane domain and a SCP domain, with a yet unknown function which is also found in other RTVP-1 homologs including TPX-1 [4], the venom allergen antigen 5 [5] and group 1 of the plant pathogenesis-related proteins (PR-1). It has recently been reported that RTVP-1 acts as a tumor promoter in gliomas. Thus, the expression of RTVP-1 correlates with the degree of malignancy of astrocytic tumors and over-expression of RTVP-1 increases cell proliferation, invasion, migration and anchorage independent growth. Moreover, silencing of RTVP-1 induces apoptosis in glioma cell lines and primary glioma cultures [6]. Interestingly, RTVP-1 acts as a tumor suppressor in prostate cancer cells and adenovirus mediated delivery of RTVP-1 has therapeutic effects in a mouse prostate cancer model [7-9].

RESULTS

Expression of RTVP-1 in MSCs is very high, as determined by Western blot (Figure 5A). Moreover, silencing of RTVP-1 in MSCs abrogated their ability to

differentiate to mesenchymal lineage cells and decreased the expression of neural stem cell and neural markers (Figures 5C-D).

Further, silencing of RTVP-1 in MSCs increased the expression of both nestin and Sox 2 and some levels of beta 3 tubulin (data not shown).

Interestingly, it was found that RTVP-1 is a novel target of miR-137, suggesting that the positive effect of miR-137 on the NSC differentiation of MSCs may be mediated by RTVP-1.

To further examine the role of RTVP-1, its expression was examined in MSCs, NSCs and in MSCs that were differentiated into NSCs.

Human NSCs did not express RTVP-1 at all (data not shown) and the expression of RTVP-1 in MSCs was significantly higher than that of MSCs differentiated to NSCs irrespective of the source of MSCs that were examined (Figure 5E).

The effect of RTVP-1 overexpression in human NSCs was examined. It was found that these cells acquired mesenchymal phenotypes and especially were predisposed to differentiate into adipocytes (data not shown).

Silencing of RTVP-1 in the different MSCs examined increased the expression of nestin in these cells (Figure 5F).

To further analyze the effect of RTVP-1 on mesenchymal transformation, gene array analysis was performed on BM-MSCs in which the expression of RTVP-1 was silenced. Silencing of RTVP-1 decreased the expression of ALDH1A3 by 3.2 fold, VAV3 by 15 fold, CD200 by 5 fold and the stemness markers Oct4, Nanog and Sox2 by 2.3, 3.4 and 4.2, respectively. Collectively these results indicate that RTVP-1 decreases the proliferation and stemness signature of these cells.

In contrast, RTVP-1 increased the expression of certain genes such as nestin (3.4 fold), NKX2.2 (4.7 fold) and calcium channel, voltage dependent (3 fold).

Together, these results implicate RTVP-1 as a major mesenchymal regulator and demonstrate that silencing of RTVP-1 induces differentiation of MSCs to cells with neural phenotypes.

EXAMPLE 5

Differentiation of neural progenitor cells to motor neurons

MATERIALS AND METHODS

Plates were coated with 20 μg/ml laminin overnight and were then washed twice with PBS. The NPC were plated in the confluency of 50 % and after 24 hr were incubated with priming medium: NM medium with heparin (use 10 μg/mL) and bFGF (100 μg/mL) for 5 days. After day 5 the medium was changed to the differentiation medium: F12 with 1 mL of B27 in 50 mL F12 (or 2%), retinoic acid (RA, 1 μM), and SHH (200 ng/mL). The RA was added every other day. After 5 days GDNF and BDNF were added to the medium (10 ng/mL).

RESULTS

In the developing spinal cord, there is sequential generation of motor neurons (MNs) and oligodendrocytes (OLPs). There are common progenitors called pMN that first generate MN and then oligodendrocytes. The basic helix-loop-helix (bHLH) transcription factor Olig2, is expressed in the pMN domain and it's one of the important transcription factors that play a role in the development of both cell types. Over-expression of Olig2 in MSCs that were grow in NM medium supplemented with 200 ng/ml recombinant SHH, 20 ng/ml of each, GDNF, BDNF, CNTF and NT-3 and 1 mM retinoic acid induced the expression of two specific markers of motor neurons Hb9 and Islet1 (Figures 6A-D).

EXAMPLE 6

Involvement of miRNAs in the differentiation of NPCs to Motor neurons

MATERIALS AND METHODS

To identify specific miRNAs involved with motor neuron differentiation, the present inventors differentiated two types of neural stem/progenitor cells into motor neurons at different stages of development using the protocol described in Example 5. The characterization of the cells as motor neurons was characterized by the expression of the specific markers, islet1, HB9 and the neuronal markers neurofilament and β3 tubulin.

To analyze the expression and function of specific miRNAs in motor neurons the neural progenitor cell system described herein above was used. miRNA array

analysis was performed on the control and differentiated cells. A qRT-PCR microarray that contained 96 miRNAs, all of which were related to stem cells and that were divided into subgroups based on their known association with stem cells, neural-related, hematopoietic and organ-related miRNAs, as described in Example 2.

RESULTS

As illustrated in Figures 7A-B, neural stem cells may be induced to differentiate into motor neurons.

As presented in Figures 8-10, there were significant changes in the expression of specific miRNA of each group between the control MSCs and the differentiated MSCs.

qRT-PCR studies were performed to validate the differences in the miRNA expression that were observed between the control and differentiated cells.

Similar to the results that were obtained with the microarray data, the qRT-PCR results demonstrated a decrease in miRs, 372, 373, 141, 199a, 32, 33, 221 and 223.

In contrast a significant increase was observed in all the miRNAs that increased in the array and specifically the following miRNAs: miR-368, 302b, 365-3p, 365-5p, Let-7a, Let-7b, 218, 134, 124, 125a, 9, 154, 20a, 130a.

The present inventors further examined the role of the specific miRNAs in the differentiation of MSCs to motor neurons. It was found that the combination of Let-7a and miR-124, 368 and miR-154 increased the expression of Hb9 and Islet-1. Similarly, transfection with combinations of miR-125a, 9, 130a and 218, 134 and 20a together and in combination with miRNA inhibitors of miR-141, 32, 33, 221, 223 and miR373 also induced differentiation of MSCs to either motor neuron progenitors or to immature motor neurons.

EXAMPLE 7

Sequences

Table 2

Name	Sequence of mature miRNA	Sequence of premiRNA
hsa-let-7a	seq id no: 1	seq id no: 73
		seq id no: 74
		seq id no: 75
hsa-let-7b	seq id no: 2	seq id no: 76

hsa-let-7c	seq id no: 3	seq id no: 77
hsa-let-7d	seq id no: 4	seq id no: 78
hsa-let-7e	seq id no: 5	seq id no: 79
hsa-let-7f	seq id no: 6	seq id no: 80
hsa-let-7g	seq id no: 7	seq id no: 81
hsa-let-7i	seq id no: 8	seq id no: 82
hsa-mir-106a	seq id no: 9	seq id no: 83
hsa-mir-106b	seq id no: 10	seq id no: 84
hsa-mir-1294	seq id no: 11	seq id no: 85
hsa-mir-1297	seq id no: 12	seq id no: 86
hsa-mir-143	seq id no: 13	seq id no: 87
hsa-mir-144	seq id no: 14	seq id no: 88
hsa-mir-145	seq id no: 15	seq id no: 89
hsa-mir-17	seq id no: 16	seq id no: 90
miR-181a	seq id no: 17	seq id no: 91
miR-181a	seq id no: 18	seq id no: 92
miR-181b	seq id no: 19	seq id no: 93
miR-181b	seq id no: 20	seq id no: 94
miR-181c	seq id no: 21	seq id no: 95
hsa-mir-181d	seq id no: 22	seq id no: 96
hsa-mir-199a-3p	seq id no: 23	seq id no: 97
hsa-mir-199b-3p	seq id no: 24	seq id no: 98
hsa-mir-202	seq id no: 25	seq id no: 99
hsa-mir-20a	seq id no: 26	seq id no: 100
hsa-mir-20b	seq id no: 27	seq id no: 101
hsa-mir-2113	seq id no: 28	seq id no: 102
hsa-mir-25	seq id no: 29	seq id no: 103
hsa-mir-26a	seq id no: 30	seq id no: 104
	seq id no: 31	seq id no: 105
hsa-mir-26b	seq id no: 32	seq id no: 106
hsa-mir-29a	seq id no: 33	seq id no: 107
hsa-mir-29b	seq id no: 34	seq id no: 108
		seq id no: 109
hsa-mir-29c	seq id no: 35	seq id no: 110
hsa-mir-3129-5p	seq id no: 36	seq id no: 111
hsa-mir-3177-5p	seq id no: 37	seq id no: 112
hsa-mir-32	seq id no: 38	seq id no: 113
hsa-mir-326	seq id no: 39	seq id no: 114
hsa-mir-330-5p	seq id no: 40	seq id no: 115
hsa-mir-363	seq id no: 41	seq id no: 116

hsa-mir-3659	seq id no: 42	seq id no: 117
hsa-mir-3662	seq id no: 43	seq id no: 118
hsa-mir-367	seq id no: 44	seq id no: 119
hsa-mir-372	seq id no: 45	seq id no: 120
hsa-mir-373	seq id no: 46	seq id no: 121
hsa-mir-3927	seq id no: 47	seq id no: 122
hsa-mir-4262	seq id no: 48	seq id no: 123
hsa-mir-4279	seq id no: 49	seq id no: 124
hsa-mir-4458	seq id no: 50	seq id no: 125
hsa-mir-4465	seq id no: 51	seq id no: 126
hsa-mir-4500	seq id no: 52	seq id no: 127
hsa-mir-4658	seq id no: 53	seq id no: 128
hsa-mir-4724-3p	seq id no: 54	seq id no: 129
hsa-mir-4742-3p	seq id no: 55	seq id no: 130
hsa-mir-4770	seq id no: 56	seq id no: 131
hsa-mir-519d	seq id no: 57	seq id no: 132
hsa-mir-520a-3p	seq id no: 58	seq id no: 133
hsa-mir-520b	seq id no: 59	seq id no: 134
hsa-mir-520c-3p	seq id no: 60	seq id no: 135
hsa-mir-520d-3p	seq id no: 61	seq id no: 136
hsa-mir-520d-5p	seq id no: 62	seq id no: 137
hsa-mir-520e	seq id no: 63	seq id no: 138
hsa-mir-524-5p	seq id no: 64	seq id no: 139
hsa-mir-642b	seq id no: 65	seq id no: 140
hsa-mir-656	seq id no: 66	seq id no: 141
hsa-mir-767-5p	seq id no: 67	seq id no: 142
hsa-mir-92a	seq id no: 68	seq id no: 143
	seq id no: 69	seq id no: 144
hsa-mir-92b	seq id no: 70	seq id no: 145
hsa-mir-93	seq id no: 71	seq id no: 146
hsa-mir-98	seq id no: 72	seq id no: 147

Table 3

Name	Sequence of mature	Sequence of premiRNA
hsa-mir-410	seq id no: 148	seq id no: 156
hsa-mir-3163	seq id no: 149	seq id no: 157
hsa-mir-148a	seq id no: 150	seq id no: 158
hsa-mir-148b	seq id no: 151	seq id no: 159
hsa-mir-152	seq id no: 152	seq id no: 160

hsa-mir-3121-3p	seq id no: 153	seq id no: 161
hsa-mir-495	seq id no: 154	seq id no: 162
hsa-mir-4680-3p	seq id no: 155	seq id no: 163

Table 4

Name	Sequence of mature	PMIR id	Sequence of premiRNA
miR-92ap	seq id no: 164	MI0000093	seq id no: 269
	seq id no: 165	MI0000094	seq id no: 270
miR-21	seq id no: 166	MI0000077	seq id no: 271
miR-26a 5P	seq id no: 167	MI0000083	seq id no: 272
	seq id no: 168	MI0000750	seq id no: 273
miR-18a	seq id no: 169	MI0000072	seq id no: 274
miR-124	seq id no: 170	MI0000445	seq id no: 275
	seq id no: 171	MI0000443	seq id no: 276
	seq id no: 172	MI0000444	seq id no: 277
miR-99a	seq id no: 173	MI0000101	seq id no: 278
miR-30c	seq id no: 174	MI0000736	seq id no: 279
		MI0000254	seq id no: 280
miR-301a 3P	seq id no: 175	MI0000745	seq id no: 281
miR-145-50	seq id no: 176	MI0000461	seq id no: 282
miR-143-3p	seq id no: 177	MI0000459	seq id no: 283
miR-373 3P	seq id no: 178	MI0000781	seq id no: 284
miR-20b	seq id no: 179	MI0001519	seq id no: 285
miR-29c 3P	seq id no: 180	MI0000735	seq id no: 286
miR-29b 3P	seq id no: 181	MI0000105	seq id no: 287
		MI0000107	seq id no: 288
miR-143			
let-7g	seq id no: 182	MI0000433	seq id no: 289
let-7a	seq id no: 183	MI0000060	seq id no: 290
		MI0000061	seq id no: 291
		MI0000062	seq id no: 292
let-7b	seq id no: 184	MI0000063	seq id no: 293
miR-98	seq id no: 185	MI0000100	seq id no: 294
miR-30a*	seq id no: 186	MI0000088	seq id no: 295
miR-17	seq id no: 187	MI0000071	seq id no: 296
miR-1-1	seq id no: 188	MI0000651	seq id no: 297
miR-1-2	seq id no: 189	MI0000437	seq id no: 298
miR-192	seq id no: 190	MI0000234	seq id no: 299
miR-155	seq id no: 191	MI0000681	seq id no: 300
miR-516-ap a1-5p--	seq id no: 192	MI0003180	seq id no: 301
a2-3p--	seq id no: 193	MI0003181	seq id no: 302
miR-31	seq id no: 194	MI0000089	seq id no: 303
miR-181a	seq id no: 195	MI0000289	seq id no: 304
	seq id no: 196	MI0000269	seq id no: 305
miR-181b	seq id no: 197	MI0000270	seq id no: 306

	seq id no: 198	MI0000683	seq id no: 307
miR-181c	seq id no: 199	MI0000271	seq id no: 308
miR-34-c	seq id no: 200	MI0000743	seq id no: 309
miR-34b*	seq id no: 201	MI0000742	seq id no: 310
miR-103a	seq id no: 202	MI0000109	seq id no: 311
	seq id no: 203	MI0000108	seq id no: 312
miR-210	seq id no: 204	MI0000286	seq id no: 313
miR-16	seq id no: 205	MI0000070	seq id no: 314
	seq id no: 206	MI0000115	seq id no: 315
miR-30a	seq id no: 207	MI0000088	seq id no: 316
miR-31	seq id no: 208	MI0000089	seq id no: 317
miR-222	seq id no: 209	MI0000299	seq id no: 318
miR-17	seq id no: 210	MI0000071	seq id no: 319
miR-17*	seq id no: 211	MI0000071	seq id no: 320
miR-200b	seq id no: 212	MI0000342	seq id no: 321
miR-200c	seq id no: 213	MI0000650	seq id no: 322
miR-128	seq id no: 214	MI0000447	seq id no: 323
		MI0000727	seq id no: 324
miR-503	seq id no: 215	MI0003188	seq id no: 325
miR-424	seq id no: 216	MI0001446	seq id no: 326
miR-195	seq id no: 217	MI0000489	seq id no: 327
miR-1256	seq id no: 218	MI0006390	seq id no: 328
miR-203a	seq id no: 219	MI0000283	seq id no: 329
miR-199 ??			
hsa-miR-199a-3p_st	seq id no: 220	MI0000242	seq id no: 330
hsa-miR-199a-5p_st	seq id no: 221	MI0000242	seq id no: 331
hsa-miR-199b-3p_st	seq id no: 222	MI0000282	seq id no: 332
miR-93	seq id no: 223	MI0000095	seq id no: 333
miR-98	seq id no: 224	MI0000100	seq id no: 334
miR-125-a	seq id no: 225	MI0000469	seq id no: 335
miR-133a	seq id no: 226	MI0000450	seq id no: 336
		MI0000451	seq id no: 337
miR-133b	seq id no: 227	MI0000822	seq id no: 338
miR-126	seq id no: 228	MI0000471	seq id no: 339
miR-194	seq id no: 229	MI0000488	seq id no: 340
		MI0000732	seq id no: 341
miR-346	seq id no: 230	MI0000826	seq id no: 342
miR-15b	seq id no: 231	MI0000438	seq id no: 343
miR-338-3p	seq id no: 232	MI0000814	seq id no: 344
miR-373			
miR-205	seq id no: 233	MI0000285	seq id no: 345
miR-210			
miR-125			
miR-1226	seq id no: 234	MI0006313	seq id no: 346
miR-708	seq id no: 235	MI0005543	seq id no: 347
miR-449	seq id no: 236	MI0001648	seq id no: 348
miR-422	seq id no: 237	MI0001444	seq id no: 349

miR-340	seq id no: 238	MI0000802	seq id no: 350
miR-605	seq id no: 239	MI0003618	seq id no: 351
miR-522	seq id no: 240	MI0003177	seq id no: 352
miR-663	seq id no: 241	MI0003672	seq id no: 353
miR-130a	seq id no: 242	MI0000448	seq id no: 354
miR-130b	seq id no: 243	MI0000748	seq id no: 355
miR-942	seq id no: 244	MI0005767	seq id no: 356
miR-572	seq id no: 245	MI0003579	seq id no: 357
miR-520			
miR-639	seq id no: 246	MI0003654	seq id no: 358
miR-654	seq id no: 247	MI0003676	seq id no: 359
miR-519			
miR-204		seq id no: 248	MI0000284
miR-224	seq id no: 249	MI0000301	seq id no: 360
miR-616	seq id no: 250	MI0003629	seq id no: 361
miR-122	seq id no: 251	MI0000442	seq id no: 362
miR-299 3p-	seq id no: 252	MI0000744	seq id no: 363
5p-	seq id no: 253		seq id no: 364
miR-100	seq id no: 254	MI0000102	
miR-138	seq id no: 255	MI0000476	seq id no: 365
miR-140	seq id no: 256	MI0000456	seq id no: 366
miR-375	seq id no: 257	MI0000783	seq id no: 367
miR-217	seq id no: 258	MI0000293	seq id no: 368
miR-302			seq id no: 369
miR-372	seq id no: 259	MI0000780	
miR-96	seq id no: 260	MI0000098	seq id no: 370
miR-127-3p	seq id no: 261	MI0000472	seq id no: 371
miR-449			seq id no: 372
miR-135b	seq id no: 262	MI0000810	
miR-101	seq id no: 263	MI0000103	seq id no: 373
		MI0000739	seq id no: 374
miR-326	seq id no: 264	MI0000808	seq id no: 375
miR-3245p-	seq id no: 265	MI0000813	seq id no: 376
3p-	seq id no: 266	MI0000813	seq id no: 377
miR-335	seq id no: 267	MI0000816	seq id no: 378
miR-141	seq id no: 268	MI0000457	seq id no: 379

Table 5

Name	Sequence of mature miRNA	Sequence of premiRNA
miR-1275	seq id no: 381	seq id no: 414
miR-891a	seq id no: 382	seq id no: 415
miR-154	seq id no: 383	seq id no: 416

miR-1202	seq id no: 384	seq id no: 417
miR-572	seq id no: 385	seq id no: 418
miR-935a	seq id no: 386	seq id no: 419
miR-4317	seq id no: 387	seq id no: 420
miR-153	seq id no: 388	seq id no: 421
		seq id no: 422
miR-4288	seq id no: 389	seq id no: 423
miR-409-5p	seq id no: 390	seq id no: 424
miR-193a-5p	seq id no: 391	seq id no: 425
miR-648	seq id no: 392	seq id no: 426
miR-368		
miR-365	seq id no: 393	seq id no: 427
miR-500	seq id no: 394	seq id no: 428
miR-491	seq id no: 395	seq id no: 429
hsa-miR-199a-3p_st	seq id no: 396	seq id no: 430
	seq id no: 397	seq id no: 431
hsa-miR-199a-5p_st	seq id no: 398	seq id no: 432
	seq id no: 399	seq id no: 433
miR-2113	seq id no: 400	seq id no: 434
miR-372	seq id no: 401	seq id no: 435
miR-373	seq id no: 402	seq id no: 436
miR-942	seq id no: 403	seq id no: 437
miR-1293	seq id no: 404	seq id no: 438
miR-18	seq id no: 405	seq id no: 439
miR-1182	seq id no: 406	seq id no: 440
miR-1185	seq id no: 407	seq id no: 441
		seq id no: 442
miR-1276	seq id no: 408	seq id no: 443

miR-193b	seq id no: 409	seq id no: 444
miR-1238	seq id no: 410	seq id no: 445
miR-889	seq id no: 411	seq id no: 446
miR-370	seq id no: 412	seq id no: 447
miR-548-d1	seq id no: 413	seq id no: 448

Table 6

mir designation	**seq id no:**
hsa-miR-302b	seq id no: 449
hsa-miR-371	seq id no: 450
hsa-miR-134	seq id no: 451
hsa-miR-219	seq id no: 452
hsa-miR-154	seq id no: 453
hsa-miR-155	seq id no: 454
hsa-miR-32	seq id no: 455
hsa-miR-33	seq id no: 456
hsa-miR-126	seq id no: 457
hsa-miR-127	seq id no: 458
hsa-miR-132	seq id no: 459
hsa-miR-137	seq id no: 460
hsa-miR-10b	seq id no: 461
hsa-miR-142-3p	seq id no: 462
hsa-miR-131a	
hsa-miR-125b	seq id no: 463
hsa-miR-153	seq id no: 464
hsa-miR-181a	seq id no: 465
hsa-miR-123	
hsa-miR-let-7a	seq id no: 466
hsa-miR-let-7b	seq id no: 467
hsa-miR-368	seq id no: 468
hsa-miR-365-3p	
hsa-miR-365-5p	
hsa-miR-218	seq id no: 469
hsa-miR-124	seq id no: 470
hsa-miR-125a	seq id no: 471
hsa-miR-9	seq id no: 472
hsa-miR-20a	seq id no: 473
hsa-miR-130a	seq id no: 474
hsa-miR-372	seq id no: 475
hsa-miR-373	seq id no: 476
hsa-miR-141	seq id no: 477
hsa-miR-199a	seq id no: 478
hsa-miR-221	seq id no: 479
hsa-miR-223	seq id no: 480

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting.

WHAT IS CLAIMED IS:

1. A method of predisposing mesenchymal stem cells to differentiate into neural stem cells, the method comprising up-regulating a level of at least one exogenous miRNA selected from the group consisting of miR-1275, miR-891a, miR-154, miR-1202, miR-572, miR-935a, miR302b, miR-371, miR-134, miR-219, miR-155, miR-32, miR-33, miR-126, miR-127, miR-132, let-7c, miR-665, miR-4258, miR-361-3p, miR-374a-star, miR-892b, miR-361-5p, miR-181a, miR-16, miR-636, miR-4284, miR-1208, miR-1274b, miR-30c-2-star, miR-501-3p, hsa-miR-92a, miR-378b, miR-1287, miR-425-star, miR-324-5p, miR-3178, miR-219-1-3p, miR-197, miR-181b, miR-500-star, miR-106b, miR-502-3p, miR-30c, miR-1275, miR-422a, miR-93, miR-181d, miR-1307, miR-1301, miR-99a, miR-505-star, miR-1202, miR-12, miR-532-5p, miR-195, miR-532-3p, miR-106a, miR-17, miR-1271, miR-769-3p, miR-15b, miR-324-3p, miR-20a, miR-501-5p, miR-330-3p, miR-874, miR-500, miR-25, miR-769-5p, miR-125b-2-star, miR-130b, miR-504, miR-181a-2-star, miR-885-3p, miR-1246, miR-92b, miR-362-5p, miR-572, miR-4270, miR-378c, miR-93-star, miR-149, miR-363, miR-9, miR-18a, miR-346, miR-497, miR-378, miR-1231, miR-139-5p, miR-3180-3p, miR-935 and miR-20b in the mesenchymal stem cells (MSCs), thereby predisposing the MSCs to differentiate into the neural stem cells.

2. The method of claim 1, wherein said at least one exogenous miRNA is selected from the group consisting of miR-1275, miR-891a, miR-154, miR-1202, miR-572 and miR-935a.

3. The method of claim 1, wherein said at least one exogenous miRNA is selected from the group consisting of miR-20b, miR-925, miR-891 and miR-378.

4. A method of predisposing MSCs to differentiate into neural stem cells, the method comprising down-regulating an expression of at least one miRNA selected from the group consisting of miR-4317, miR-153, miR-4288, miR-409-5p, miR-193a-5p, miR-10b, miR-142-3p, miR-131a, miR-125b, miR-181a, miR-145, miR-143, miR-214, miR-199a-3p, miR-199a-5p, miR-199b-3p, miR-138, miR-31, miR-21, miR-193a-5p,

miR-224-star, miR-196a, miR-487b, miR-409-5p, miR-193b-star, miR-379, miR-21-star, miR-27a-star, miR-27a, miR-4317, miR-193b, miR-27b, miR-22, 574-3p, miR-4288, miR-23a, miR-221-star, miR-2113, let-7i, miR-24, miR-23b, miR-299-3p, miR-518c-star, miR-221, miR-431-star, miR-523, miR-4313, miR-559, miR-614, miR-653, miR-2278, miR-768-5p, miR-154-star, miR-302a-star, miR-3199 and miR-3137 in the mesenchymal stem cells by up-regulating a level of at least one polynucleotide agent that hybridizes and inhibits a function of said at least one miRNA thereby predisposing the MSCs to differentiate into the neural stem cells.

5. The method of claim 4, wherein said at least one miRNA is selected from the group consisting of miR-4317, miR-153, miR-4288, miR-409-5p and miR-193a-5p.

6. The method of claim 4, wherein said at least one miRNA is selected from the group consisting of miR-138, miR-214, miR-199a and miR-199b.

7. The method of claim 6, wherein when said at least one miRNA is miR-138, the method further comprises:

(i) down-regulating an expression of miR-891 using a polynucleotide agent that hybridizes and inhibits the function of miR-891;

(ii) up-regulating a level of exogenous miR20b; or

(iii) up-regulating a level of exogenous miR378.

8. A method of predisposing MSCs to differentiate into neural stem cells, the method comprising up-regulating a level of exogenous miR-124 in the mesenchymal stem cells (MSCs) and down-regulating a level of miR-let-7 in said MSCs, thereby predisposing the MSCs to differentiate into the neural stem cells.

9. A method of predisposing MSCs to differentiate into neural stem cells, the method comprising contacting the mesenchymal stem cells (MSCs) with an agent that down-regulates an amount and/or activity of Related to testis-specific, vespid and pathogenesis protein 1 (RTVP-1), thereby predisposing MSCs to differentiate into the neural stem cells.

10. A method of predisposing neural stem cells to differentiate into motor neurons, the method comprising up-regulating a level of at least one exogenous miRNA selected from the group consisting of miR-368, miR-302b, miR-365-3p, miR-365-5p, miR-Let-7a, miR-Let-7b, miR-218, miR-134, miR-124, miR-125a, miR-9, miR-154, miR-20a and miR-130a in neural stem cells (NSCs), thereby predisposing NSCs to differentiate into the motor neurons.

11. A method of predisposing MSCs to differentiate into motor neurons, the method comprising up-regulating a level of at least one exogenous miRNA selected from the group consisting of miR-648, miR-368, miR-365, miR-500, miR-491, miR-218, miR-155, miR-192, let-7b, miR-16, miR-210, miR-197, miR-21,miR-373, ,miR-27a, miR-122, miR-17, miR-494, miR-449, miR-503, miR-30a, miR-196a, miR-122, miR-7, miR-151-5p, miR-16, miR-22, miR-31, miR-424, miR-1, miR-29c, miR-942, miR-100, miR-520, miR-663a, miR-562, miR-449a, miR-449b-5p, miR-520b, miR-451, miR-532-59, miR-605, miR-504, miR-503, miR-155, miR-34a, miR-16, miR-7b, miR-103, miR-124, miR-1385p, miR-16, miR-330, miR-520, miR-608, miR-708, miR-107, miR-137, miR-132, miR-145, miR-204, miR-125b, miR-224, miR-30a, miR-375, miR-101, miR-106b, miR-128, miR-129-5p, miR-153, miR-203, miR-214, miR-338-3p, miR-346, miR-98, miR-107, miR-141, miR-217, miR-424, miR-449, miR-7, miR-9, miR-93, miR-99a, miR-100, miR-1228, miR-183, miR-185, miR-190, miR-522, miR-650, miR-675, miR-342-3p and miR-31 in the mesenchymal stem cells (MSCs), thereby predisposing MSCs to differentiate into the motor neurons.

12. The method of claim 11, wherein said miRNA is selected from the group consisting of miR-648, miR-368, miR-365, miR-500 and miR-491.

13. A method of predisposing NSCs to differentiate into motor neurons, the method comprising down-regulating an expression of at least one miRNA selected from the group consisting of miR-372, miR-373, miR-141, miR-199a, miR-32, miR-33, miR-221 and miR-223 by up-regulating a level of at least one polynucleotide agent that

hybridizes and inhibits a function of said at least one miRNA in the NSCs thereby predisposing NSCs to differentiate into the motor neurons.

14. A method of predisposing MSCs to differentiate into motor neurons, the method comprising down-regulating an expression of at least one miRNA selected from the group consisting of miR-372, miR-373, miR-942, miR-2113, miR-199a-3p, miR-199a-5p, miR-372, miR-373, miR-942, miR-2113, miR-301a-3p, miR-302c, miR-30b-5p, miR-30c, miR-326, miR-328, miR-331-3p, miR-340, miR-345, miR-361-5p, miR-363, miR-365a-3p, miR-371a-3p, miR-373-3p, miR-374a, miR-423-3p, miR-449b-5p, miR-451a, miR-494, miR-504, miR-515-3p, miR-516a-3p, miR-519e, miR-520a-3p, miR-520c-3p, miR-520g, miR-532-5p, miR-559, miR-562, miR-572, miR-590-5p, miR-605, miR-608, miR-626, miR-639, miR-654-3p, miR-657, miR-661, miR-708-5p, miR-942, miR-96, miR-99arno and miR-194 by up-regulating a level of at least one polynucleotide agent that hybridizes and inhibits a function of said at least one miRNA in the MSCs thereby predisposing MSCs to differentiate into the motor neurons.

15. The method of claim 14, wherein said miRNA is selected from the group consisting of miR-372, miR-373, miR-942, miR-2113, miR-199a-3p and miR-199a-5p.

16. The method of claim 10, wherein said at least one miRNA comprises each of miR Let-7a, miR-124, miR-368 and miR-154.

17. The method of claim 10, wherein said at least one miRNA comprises each of miR-125a, miR-9 and miR-130a.

18. The method of claim 10, wherein said at least one miRNA comprises each of miR-218, miR-134 and miR-20a.

19. The method of claims 16, 17 or 18 further comprising down-regulating each of miR-141, miR-32, miR-33, miR-221, miR-223 and miR-373.

20. The method of claims 10 or 13, wherein said NSCs are generated by ex vivo differentiating MSCs.

21. The method of claim 20, wherein said ex vivo differentiating is affected according to any of the methods of claims 1-9.

22. The method of any of claims 1, 4, 8, 9 and 20, wherein said MSCs are isolated from a tissue selected from the group consisting of bone marrow, adipose tissue, placenta, cord blood and umbilical cord.

23. The method of any of claims 1, 4, 8, 9 and 20, wherein said MSCs are autologous to said subject.

24. The method of any of claims 1, 4, 8, 9 and 20, wherein said MSCs are non-autologous to said subject.

25. The method of any of claims 1, 4, 8, 9 and 20, wherein said MSCs are semi-allogeneic to said subject.

26. The method of any of claims 1, 8 and 10, wherein said up-regulating comprises introducing into said MSCs said at least one miRNA.

27. The method of claims 1, 8 and 10 wherein said up-regulating is affected by transfecting said MSCs with an expression vector which comprises a polynucleotide sequence which encodes a pre-miRNA of said at least one miRNA.

28. The method of claims 1, 8 and 10 wherein said up-regulating is effected by transfecting said MSCs with an expression vector which comprises a polynucleotide sequence which encodes said at least one miRNA.

29. The method of any of claims 1-9 further comprising analyzing an expression of at least one marker selected from the group consisting of nestin and Sox2 following said generating.

30. The method of any of claims 10 or 13 further comprising analyzing an expression of at least one marker selected from the group consisting of islet1, HB9 and the neuronal markers neurofilament and β3 tubulin following said generating.

31. The method of claims 1, 4, 8, 9, 10, 13 and 19 being effected in vivo.

32. The method of claims 1, 4, 8, 9, 10, 13 and 19 being effected ex vivo.

33. A genetically modified isolated population of cells which comprise at least one exogenous miRNA selected from the group consisting of miR302b, miR-371, miR-134, miR-219, miR-154, miR-155, miR-32, miR-33, miR-126, miR-127, miR-132 and miR-137 and/or which comprise at least one polynucleotide agent that hybridizes and inhibits a function of at least one miRNA selected from the group consisting of miR-10b, miR-142-3p, miR-131a, miR-125b, miR-153 and miR-181a, wherein said cells have a neural stem cell phenotype.

34. The isolated population of cells of claim 33, wherein at least 50 % of the population of cells express at least one marker selected from the group consisting of nestin and Sox2.

35. A genetically modified isolated population of cells which comprise at least one exogenous miRNA selected from the group consisting of miR-368, miR-302b, miR-365-3p, miR-365-5p, miR-Let-7a, miR-Let-7b, miR-218, miR-134, miR-124, miR-125a, miR-9, miR-154, miR-20a, miR-130a and/or which comprise at least one polynucleotide agent that hybridizes and inhibits a function of at least one miRNA selected from the group consisting of miR-372, miR-373, miR-141, miR-199a, miR-32, miR-33, miR-221 and miR-223, wherein said cells have a motor neuron phenotype.

36. The isolated population of cells of claim 35, wherein at least 50 % of the population of cells express at least one marker selected from the group consisting of islet1, HB9 and the neuronal markers neurofilament and β3 tubulin.

37. The isolated population of cells of claim 33 for use in treating a brain disease or disorder.

38. The isolated population of cells of claim 37, wherein said brain disease or disorder is a neurodegenerative disorder.

39. The isolated population of cells of claim 38, wherein said neurodegenerative disorder is selected from the group consisting of multiple sclerosis, Parkinson's, epilepsy, amyotrophic lateral sclerosis (ALS), stroke, Rett Syndrome, autoimmune encephalomyelitis, spinal cord injury, cerebral palsy, stroke, Alzheimer's disease and Huntingdon's disease.

40. The isolated population of cells of claim 35, for use in treating a motor neuron disease.

41. The isolated population of cells of claim 40, wherein said motor neuron disease is selected from the group consisting of amyotrophic lateral sclerosis (ALS), primary lateral sclerosis (PLS), pseudobulbar palsy and progressive bulbar palsy.

42. A method of treating a brain disease or disorder in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the isolated population of cells of claim 33, thereby treating the brain disease or disorder.

43. The method of claim 42, wherein the nerve disease or disorder is a neurodegenerative disorder.

44. The method of claim 43, wherein said neurodegenerative disorder is selected from the group consisting of multiple sclerosis, Parkinson's, epilepsy, amyotrophic lateral sclerosis (ALS), stroke, Rett Syndrome, autoimmune encephalomyelitis, spinal cord injury, cerebral palsy, stroke, Alzheimer's disease and Huntingdon's disease.

45. A method of treating a motor neuron disease in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the isolated population of cells of claim 35, thereby treating the brain disease or disorder.

46. A pharmaceutical composition comprising the isolated population of cells of claim 33 and a pharmaceutically acceptable carrier.

47. A pharmaceutical composition comprising the isolated population of cells of claim 35 and a pharmaceutically acceptable carrier.

48. A method of selecting a miRNA which may be regulated for the treatment of a motor neuron disease comprising:

(a) differentiating a population of neural stem cells towards a motor neuron phenotype; and

(b) analyzing a change in expression of a miRNA in said population of MSCs prior to and following said differentiating of said MSCs towards a motor neuron phenotype, wherein a change of expression of a miRNA above or below a predetermined level is indicative that said miRNA may be regulated for the treatment of the motor neuron disease.

49. A genetically modified isolated population of cells which comprise at least one exogenous miRNA selected from the group consisting of miR-1275, miR-891a, miR-154, miR-1202, miR-572 and miR-935a and/or which comprise at least one polynucleotide agent that hybridizes and inhibits a function of at least one miRNA

selected from the group consisting of miR-4317, miR-153, miR-4288, miR-409-5p, miR-193a-5p, wherein said cells have a neural stem cell phenotype.

50. A genetically modified isolated population of cells which comprise at least one exogenous miRNA selected from the group consisting of miR-648, miR-368, miR-365, miR-500 and miR-491 and/or which comprise at least one polynucleotide agent that hybridizes and inhibits a function of at least one miRNA selected from the group consisting of miR-372, miR-373, miR-942, miR-2113, miR-199a-3p and miR-199a-5p, wherein said cells have a motor neuron phenotype.

FIG. 1A

MSCs can form neural-like precursor cells

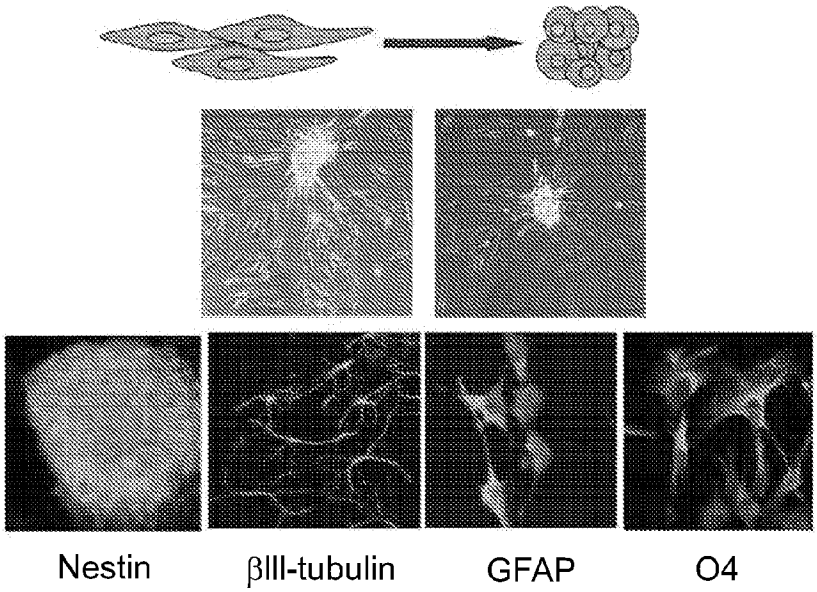


FIG. 1B

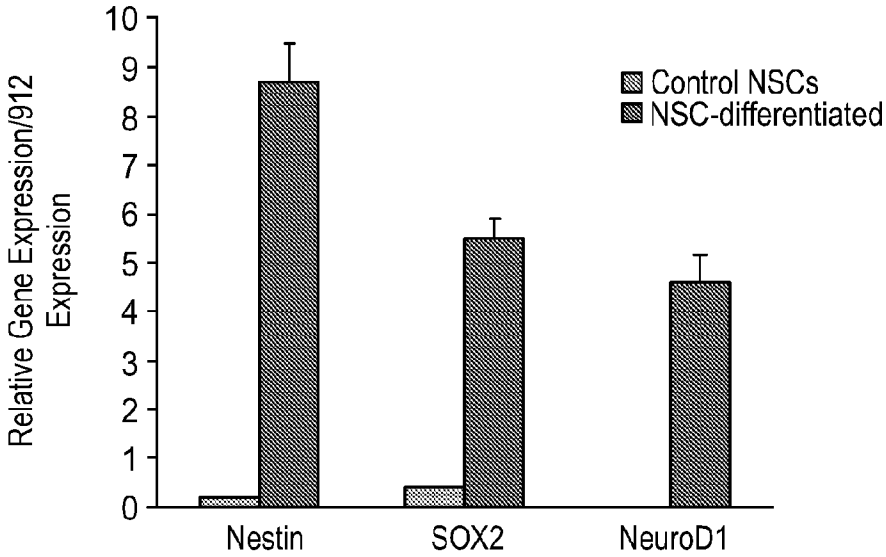


FIG. 2

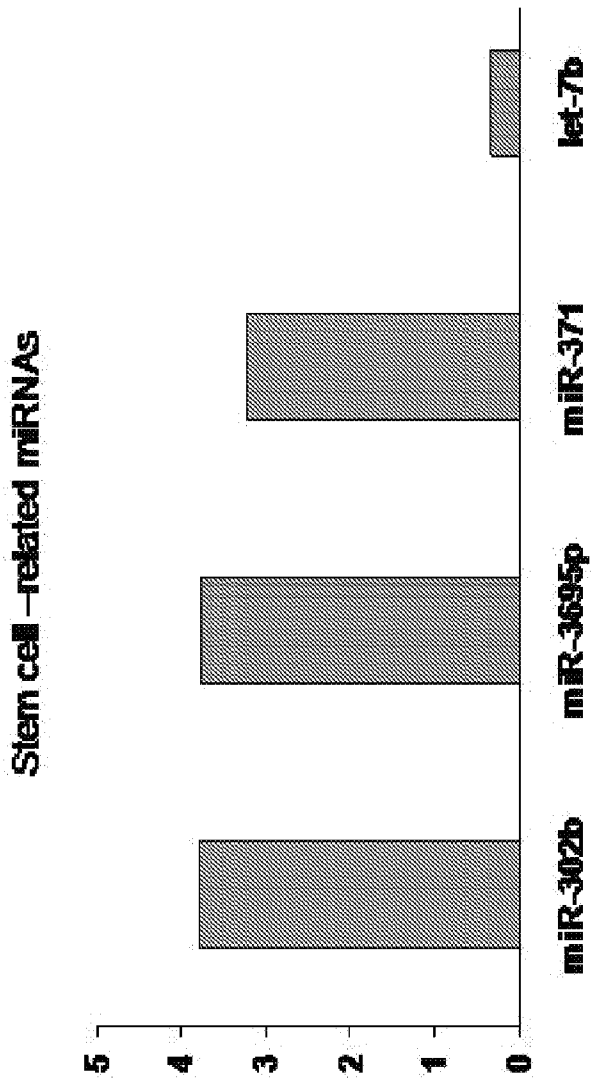

Stem cell –related miRNAs
5
4
3
2
1
0
miR-302b
miR-369-5p
miR-371
let-7b

FIG. 3

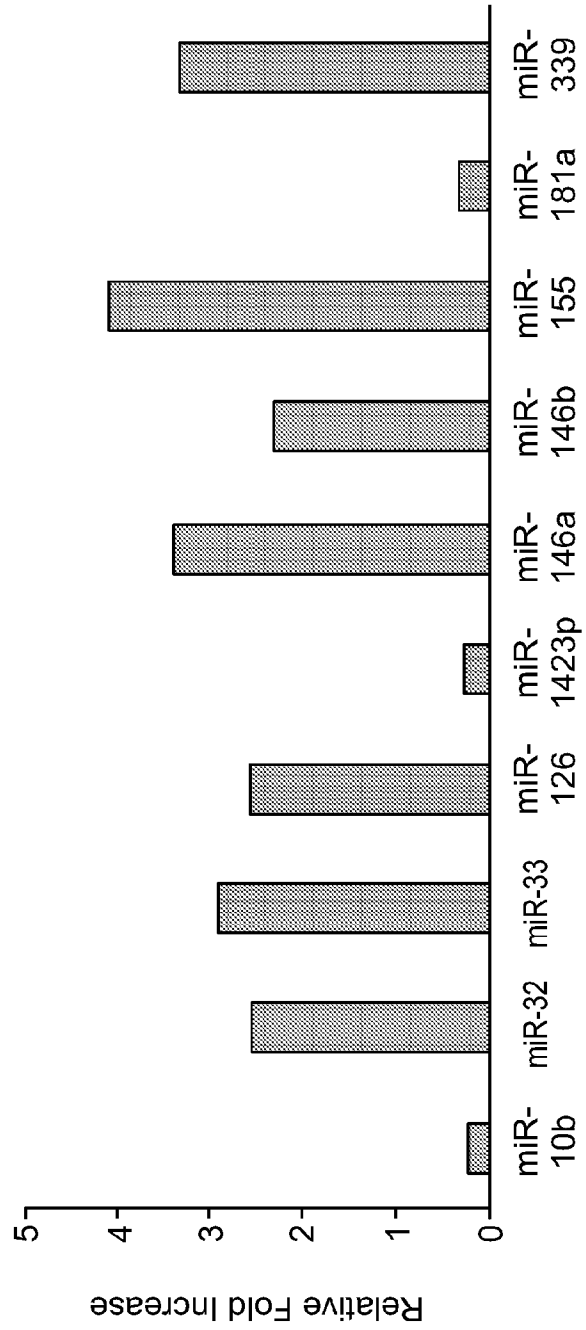

Hematopoietic-related miRNAs
Relative Fold Increase
0
1
2
3
4
5
miR-10b
miR-32
miR-33
miR-126
miR-1423p
miR-146a
miR-146b
miR-155
miR-181a
miR-339

FIG. 4A

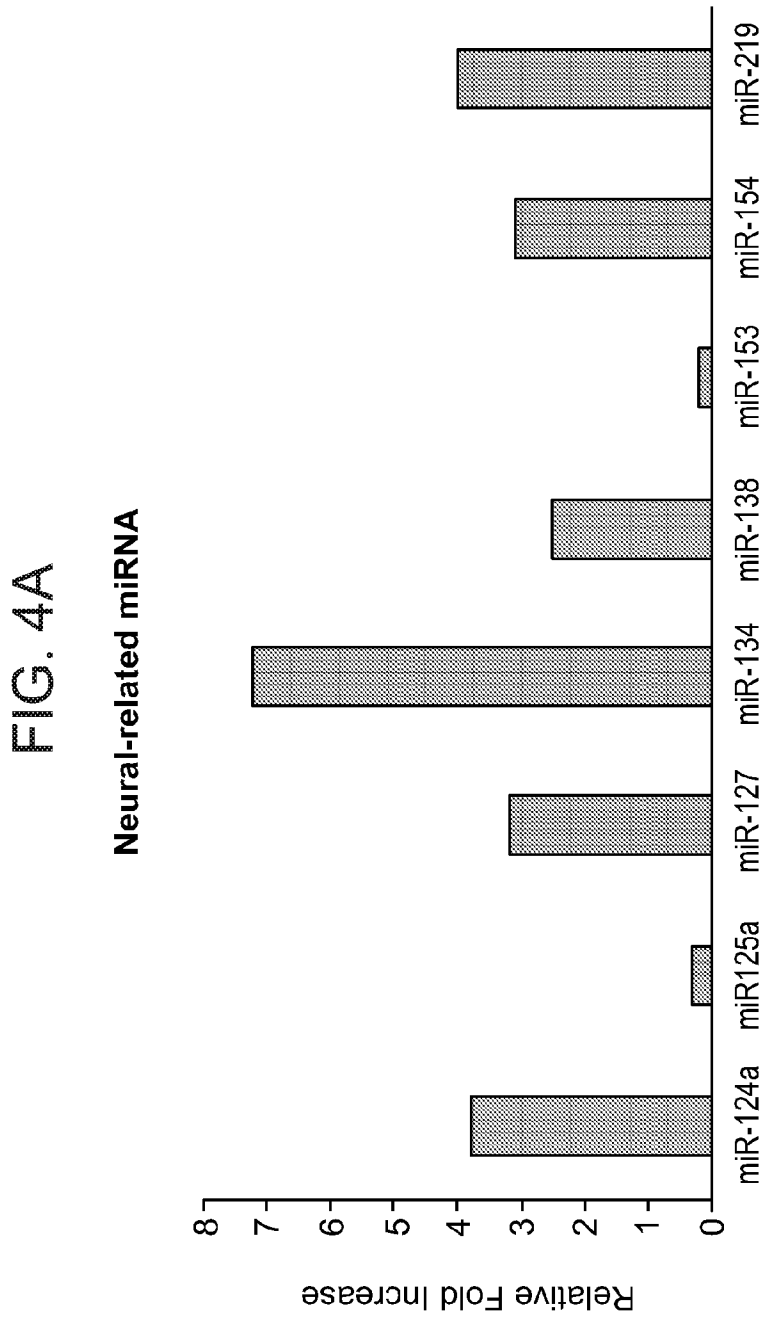

Neural-related miRNA
Relative Fold Increase
0
1
2
3
4
5
6
7
8
miR-124a
miR125a
miR-127
miR-134
miR-138
miR-153
miR-154
miR-219

FIG. 4B

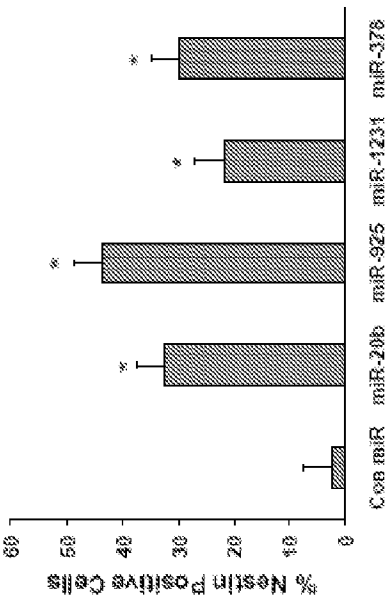


FIG. 4C

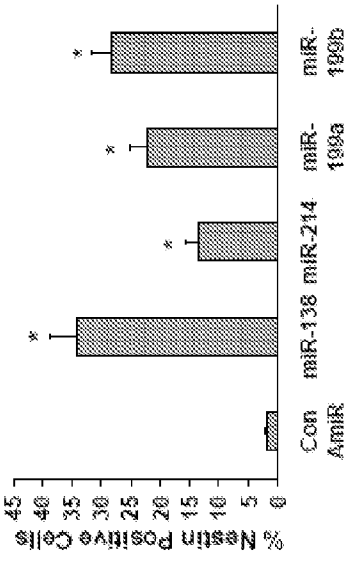


FIG. 4D

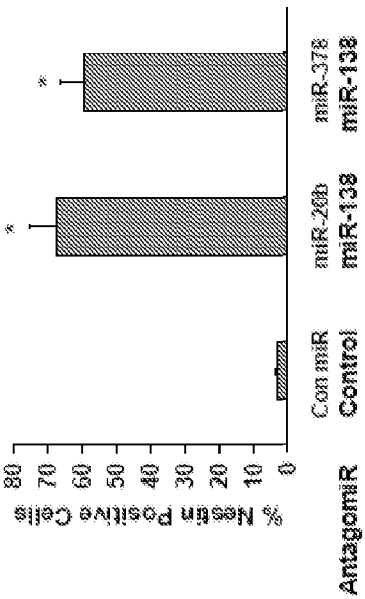


FIG. 4F

MSC-derived NSCs

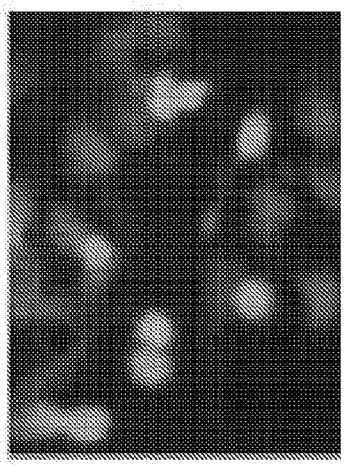


FIG. 4E

MSCs

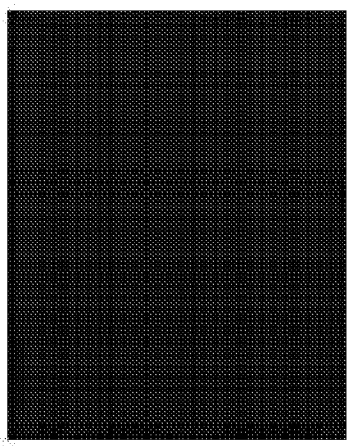


FIG. 5A

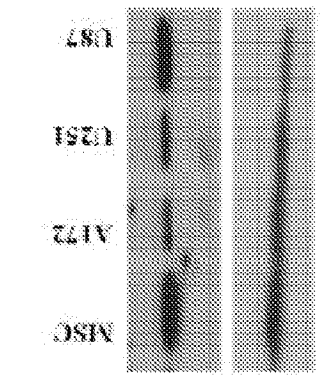


FIG. 5B

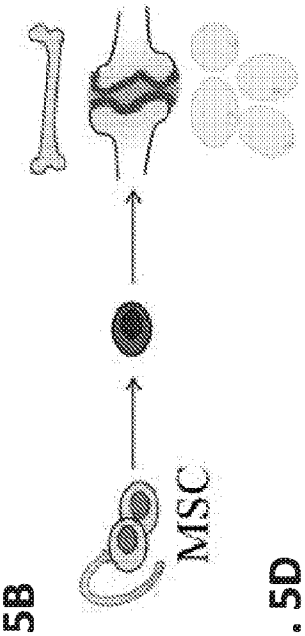


FIG. 5C

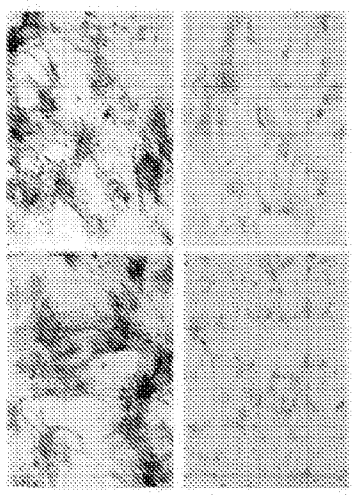


FIG. 5D

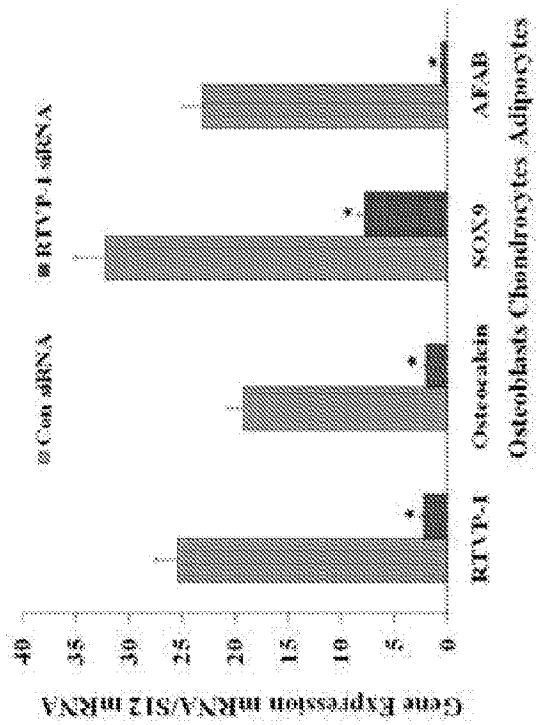


FIG. 5E

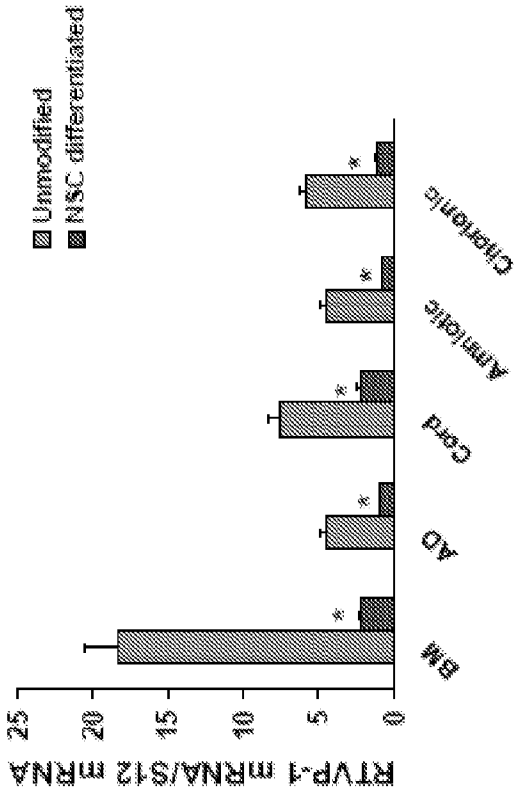

RTVP-1 mRNA/S12 mRNA
25
20
15
10
5
0
Unmodified
NSC differentiated
BM
AD
Cord
Amniotic
Chorionic
*

FIG. 5F

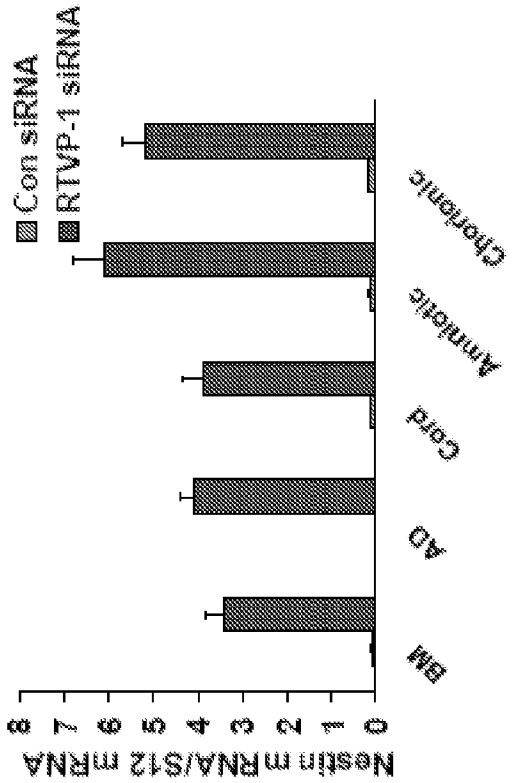

Nestin mRNA/S12 mRNA
8
7
6
5
4
3
2
1
0
Con siRNA
RTVP-1 siRNA
BM
AD
Cord
Amniotic
Chorionic

FIG. 6A

Control

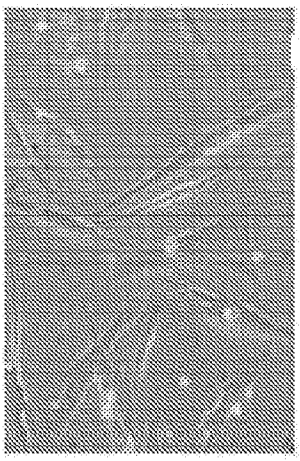


FIG. 6B

Treatment

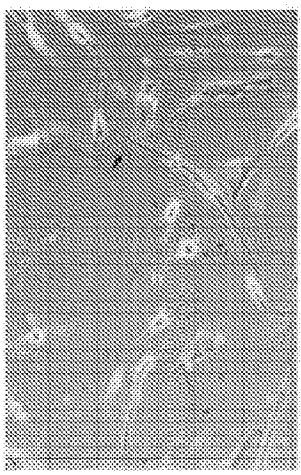


FIG. 6C

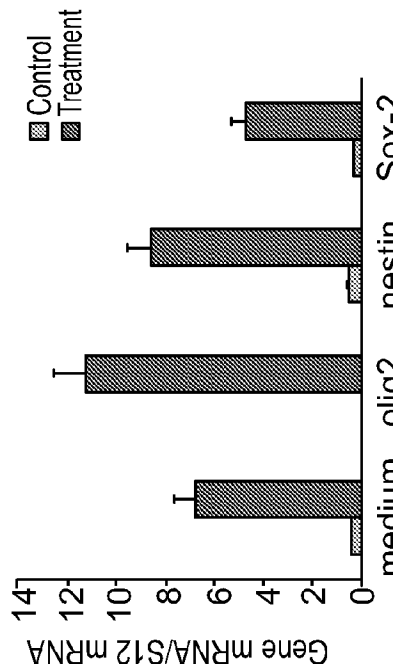


FIG. 6D

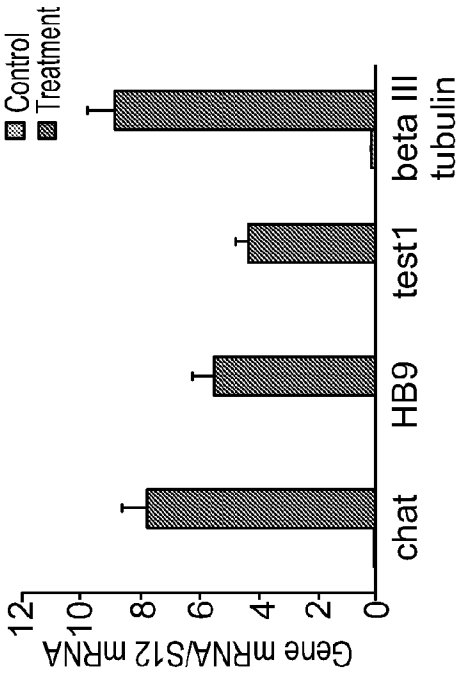


FIG. 7A

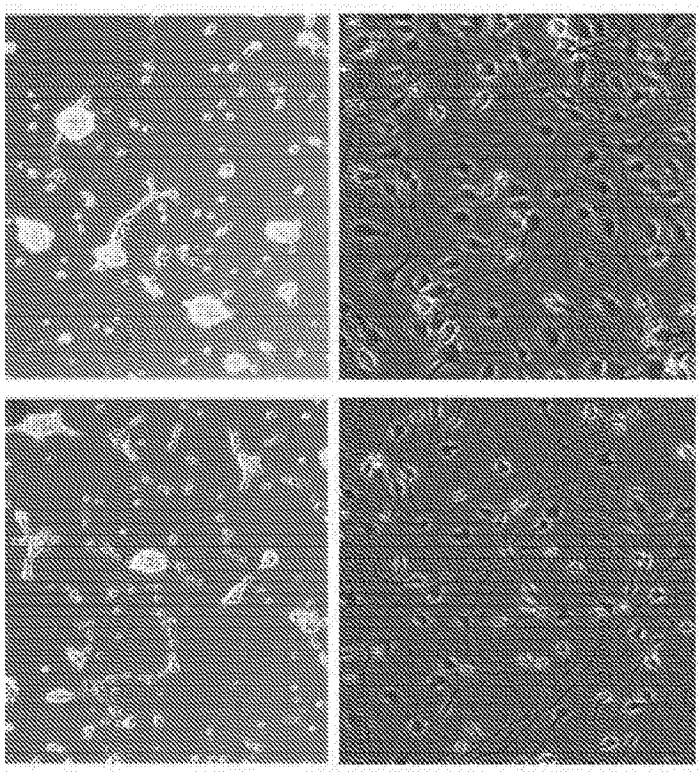


FIG. 7B

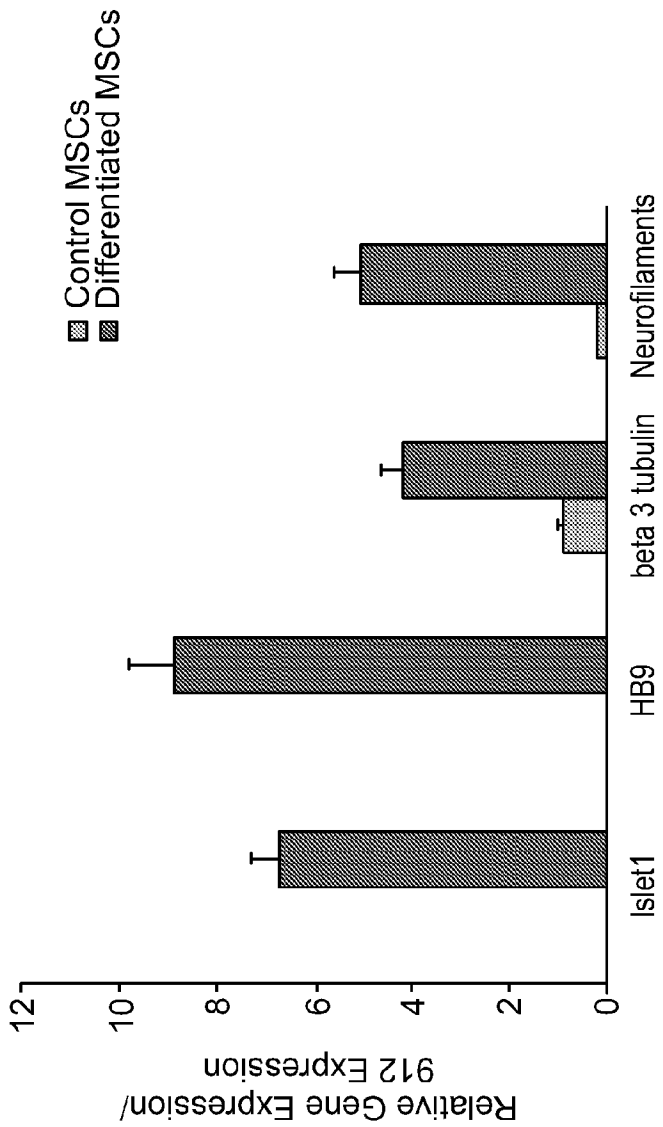

Control MSCs
Differentiated MSCs
Relative Gene Expression/
912 Expression
0
2
4
6
8
10
12
Islet1
HB9
beta 3 tubulin
Neurofilaments

FIG. 8

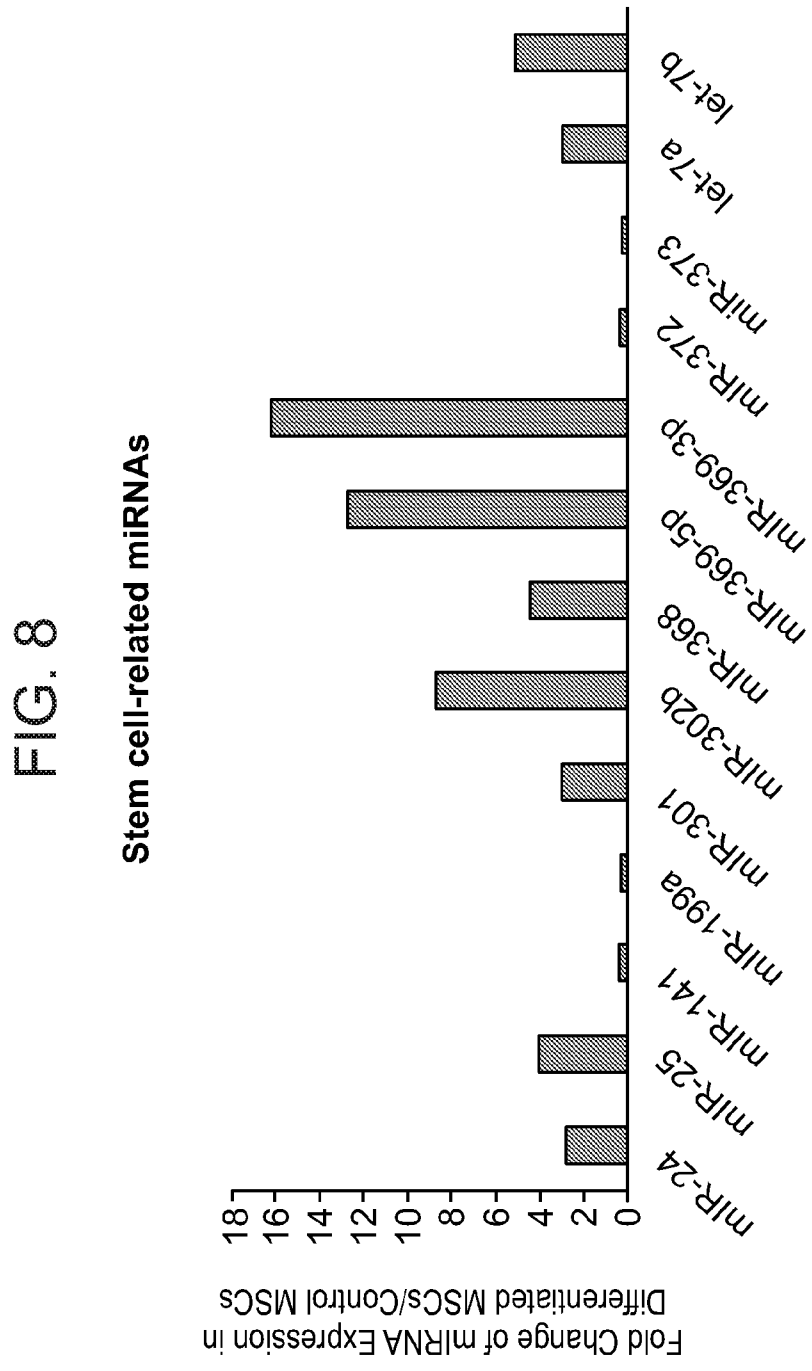

Stem cell-related miRNAs
Fold Change of miRNA Expression in Differentiated MSCs/Control MSCs
0
2
4
6
8
10
12
14
16
18
miR-24
miR-25
miR-141
miR-199a
miR-301
miR-302b
miR-368
miR-369-5p
miR-369-3p
miR-372
miR-373
let-7a
let-7b

FIG. 9

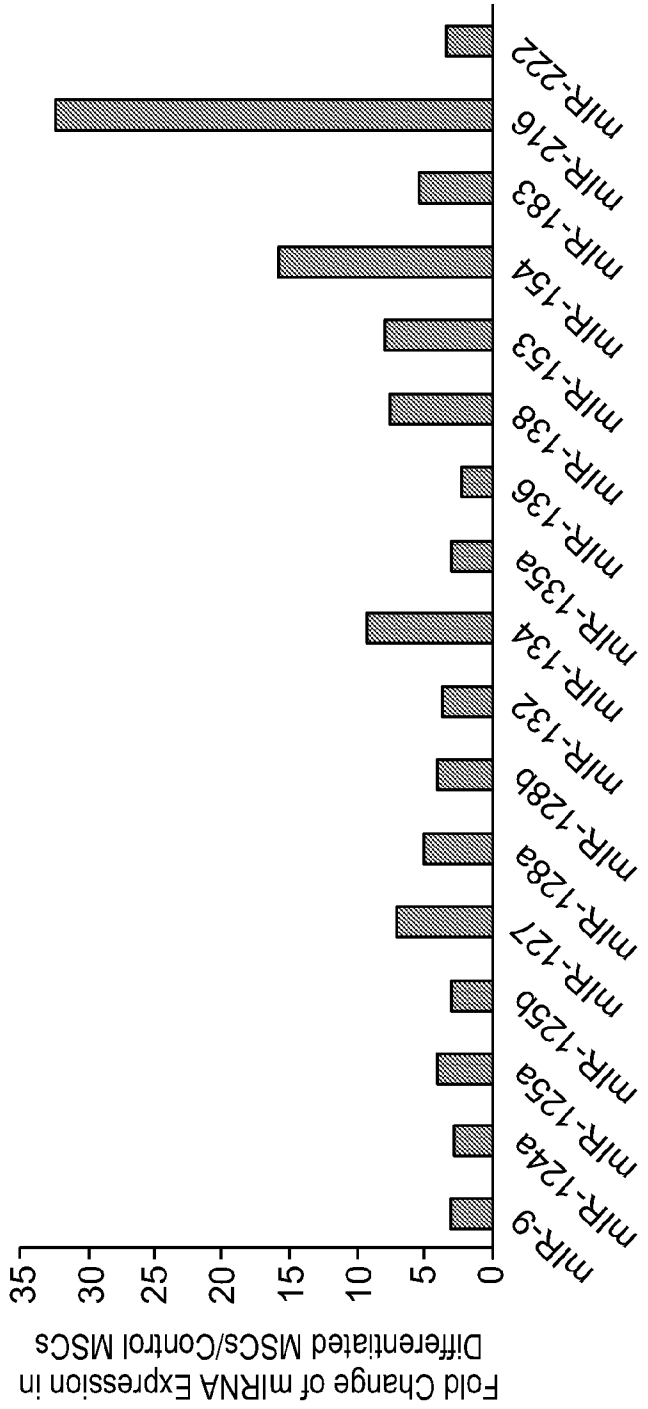
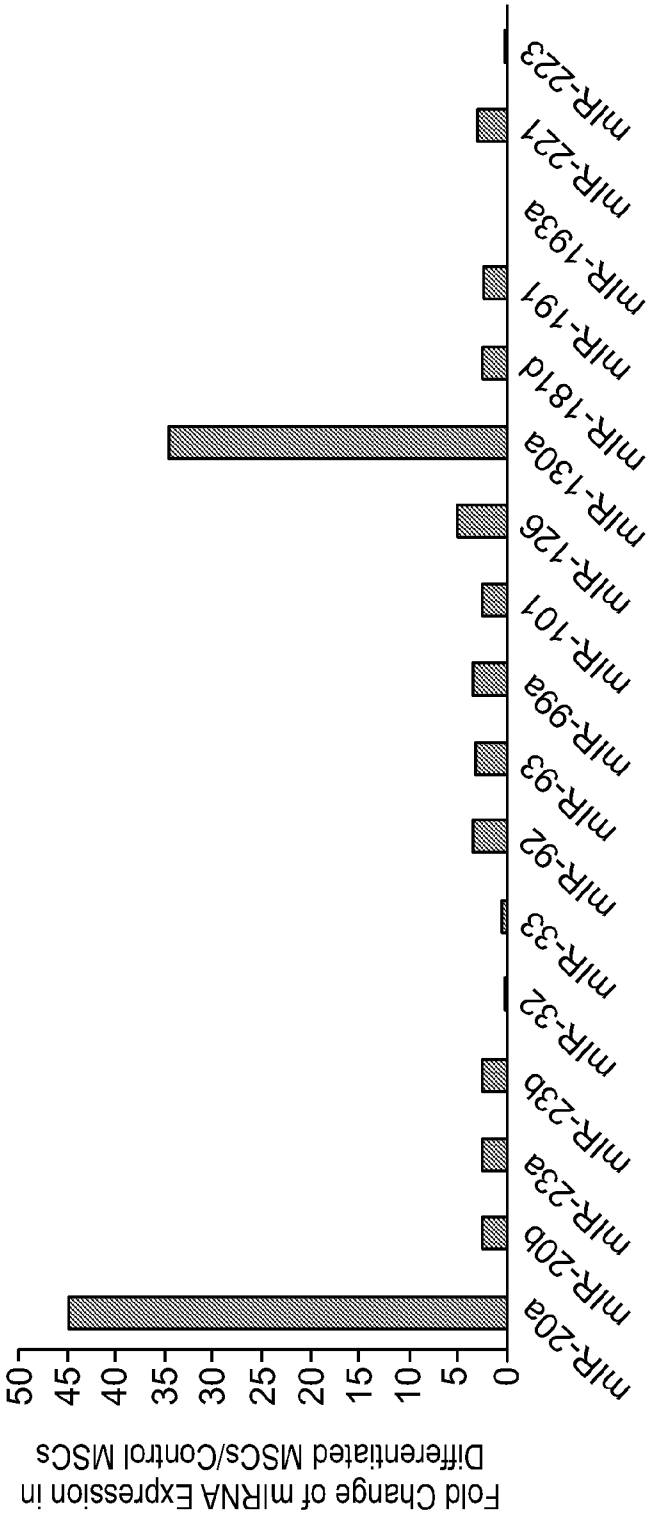

Neuronal-related miRNAs
Fold Change of miRNA Expression in Differentiated MSCs/Control MSCs
0
5
10
15
20
25
30
35
miR-9
miR-124a
miR-125a
miR-125b
miR-127
miR-128a
miR-128b
miR-132
miR-134
miR-135a
miR-136
miR-138
miR-153
miR-154
miR-183
miR-216
miR-222

FIG. 10


Hematopoietic-related miRNAs
Fold Change of mIRNA Expression in Differentiated MSCs/Control MSCs
50
45
40
35
30
25
20
15
10
5
0
miR-20a
miR-20b
miR-23a
miR-23b
miR-32
miR-33
miR-92
miR-93
miR-99a
miR-101
miR-126
miR-130a
miR-181d
miR-191
miR-193a
miR-221
miR-223