COMPOSITION COMPRISING MESENCHYMAL STEM CELLS OR CULTURE SOLUTION OF MESENCHYMAL STEM CELLS FOR THE PREVENTION OR TREATMENT OF NEURAL DISEASES

Technical Field

The present invention relates to a composition including mesenchymal stem cells (MSCs), a culture solution of MSCs, proteins contained in a culture solution of MSCs, or a signal transduction system-stimulating factor inducing expression of the proteins for the prevention or treatment of Alzheimer's disease, related to damage of neurites.

The present invention relates to a composition including mesenchymal stem cells (MSCs), a culture solution of MSCs, proteins contained in a culture solution of MSCs, or a signal transduction system-stimulating factor inducing expression of the proteins, for the prevention or treatment of a disease related to damage of neurites.

Background Art

Alzheimer's disease, which is a brain disorder that destroys brain cells by a destructive accumulation of amyloid-beta protein and generally outbreaks with aging, is a serious disease resulting in speech impediment and recognition disorder. Alzheimer's disease proceeds in stages and gradually destroys memory, reasoning, judgment, language, and the ability to carry out even simple tasks. Eventually, loss of emotional control may cause degradation of human life. Currently, Alzheimer's disease cannot be completely cured, but drugs relieving symptoms are clinically applied. However, effects of these drugs on patients are limited. Around half of Alzheimer's disease patients fail to be cured from initial drug treatment. Even if the initial drug treatment is successful, only a slight alleviation of symptoms is experienced. Thus, there is a need to develop a novel treatment for satisfying medical demands, and the development of a treatment for Alzheimer's disease will have large economical and social effects. It is known that as Alzheimer's disease proceeds, the cerebral cortex and hippocampus are destroyed and cannot be restored, and thus there is no treatmen therefor.

Research on Alzheimer's disease has been driven by a focus on two proteins, tau and amyloid precursor protein (APP) (Stuart M. and Mark P. M, Nature Medicine,

12(4), 392-393, 2006). Brains of affected individuals accumulate aberrant forms of both of these proteins. Tau becomes hyperphosphorylated and APP is cleaved by secretase to produce amyloid-beta (Aβ) protein which aggregates in the brain in plaque form. In general, the number of synapses is reduced and neurites are damaged in brain regions in which plaque is accumulated. This indicates that the amyloid-beta damages synapses and neurites (Mark P.M, Nature, 430, 631-639, 2004).

Research on pathogenetic mechanism has been actively conducted for the treatment of Alzheimer's disease. In particular, research on an inhibitor of beta-secretase and/or gamma-secretase producing amyloid-beta protein, a protease degrading accumulated amyloid-beta protein, and an inhibitor of acetylcholine esterase degrading acetylcholine have been intensively performed. Furthermore, research on a treatment for Alzheimer's disease using an inflammation inhibitor has been conducted since Alzheimer's disease is an aging-related chronic inflammatory disease.

The amount of amyloid-beta in the brain is determined by the balance between reactions for production and removal of the amyloid-beta. Accordingly, if the amyloid-beta removal is reduced, the amount of amyloid-beta is increased. Deficiency of neprilysin (NEP), which is an enzyme with activity for degrading amyloid-beta, results in accelerating extracellular accumulation of amyloid (Kanae Iijima-Ando, etc., J. Biol. Chem., 283(27), 19066-19076, 2008).

Abnormal neurites projected from a cell body of a neuron is related to neural diseases. Examples of the neural diseases are Alzheimer's disease, Parkinson's disease, depression, epilepsy, multiple sclerosis, and mania. In particular, epilepsy occurs due to death of neuron and gliosis of human hippocampus. Neurites are cleaved by the death of neuron. Multiple sclerosis is a chronic autoimmune disease occurring in the brain due to abnormalities of Nogo A, a neurite outgrowth inhibiting protein. Depression is a brain disorder caused by abnormalities of M6a, a neurite outgrowth-related protein. Alleviation of symptoms of mania has been reported in mice by activating a signal transduction pathway stimulating neurite outgrowth.

Mesenchymal stem cells (MSCs) are multipotent stem cells differentiating into mesodermal lineage cells such as osteocytes, chondrocytes, adipocytes, and myocytes or ectodermal lineage cells such as neurons. It has recently been reported that MSCs have a potential to differentiate into neuroglia in the brain, and thus attempts to

differentiate MSCs into neurons have been made (Korean Patent Publication No. 10-2004-0016785, February 25, 2004).

Among the MSCs, a bone marrow-derived MSC can be obtained from a patient. If the MSC is autologously transplanted, there is no immune rejection response, and thus can be clinically applied to patients. However, since bone marrow-derived MSC collection requires various stages of complicated medical treatments, bone marrow donation is time-consuming, psychologically and physically painful and expensive. However, since an umbilical cord blood-derived MSC is simply obtained from an umbilical cord, and the umbilical cord blood preservation industry is being actively developed, and donors are easily found due to the umbilical cord blood infrastructure, MSCs are easily obtained. Furthermore, MSCs obtained from allogeneic cord blood do not exhibit an immune response after transplantation, thereby exhibiting immunological stability.

All the cited references are incorporated herein by reference in their entireties.

Detailed Description of the Invention

Technical Problem

For the treatment of neural diseases using stem cells, differentiation of stem cells into neurons needs to be performed in advance, or stem cells need to be administered with materials differentiating the stem cells into neurons according to the conventional methods.

One or more embodiments of the present invention include a cellular treatment method for a neural disease without differentiating stem cells into neurons.

One or more embodiments of the present invention include a composition for preventing and treating a neural disease comprising MSCs.

One or more embodiments of the present invention include a method of preventing of neurocytoxicity caused by amyloid-beta, preventing phosphorylation of tau protein in neurons, preventing neurite damage, and inducing expression of neprilysin in neurons or microglial cells.

One or more embodiments of the present invention include a kit for preventing neurocytoxicity caused by amyloid-beta, preventing phosphorylation of tau protein in neurons, preventing neurite damage, and inducing expression of neprilysin in neurons or

microglial cells

Technical Solution

Inventors of the present invention have found that neurocytoxicity caused by amyloid-beta, phosphorylation of tau protein in neurons, and damage of neurites are prevented, and expression of neprilysin is induced in neurons or microglial cells when neurons or microglial cells treated with or without amyloid-beta are co-cultured with MSCs, a culture solution of MSCs, or proteins contained in the culture solution of MSCs.

Advantageous Effects

Neurocytoxicity caused by amyloid-beta is prevented, phosphorylation of tau protein in neurons is prevented, expression of neprilysin is induced in neurons or microglial cells, and damage of neurites is prevented when neurons or microglial cells are co-cultured with MSCs, a culture solution of MSCs, proteins contained in the culture solution of MSCs, and/or a signal transduction system-stimulating factor inducing expression of the proteins.

A composition including MSCs, a culture solution of MSCs, proteins contained in the culture solution of MSCs, or a signal transduction system-stimulating factor inducing expression of the proteins according to the present invention may be used as an effective cellular treatment composition for the prevention and treatment of neural diseases.

In addition, there are provided a method of and a kit for preventing neurocytoxicity caused by amyloid-beta, preventing phosphorylation of tau protein in neurons, preventing damage of neurites, and inducing expression of Neprilysin in neurons using MSCs, a culture solution of MSCs, proteins contained in the culture solution of MSCs, and/or a signal transduction system-stimulating factor inducing expression of the proteins.

Brief Description of the Drawings

The above and other features and advantages of the present invention will become more apparent by describing in detail exemplary embodiments thereof with reference to the attached drawings in which:

FIG. 1 illustrates optical microscopic images of live neurons untreated and treated with amyloid-beta for 24 hours;

FIG. 2 shows a co-culture system for co-culturing neurons treated with amyloid-beta with human UCB-derived MSCs;

FIG. 3 illustrates results of fluorescent staining to explain effects of co-culturing neurons with human UCB-derived MSCs on death of neuron caused by amyloid-beta (Aβ42);

FIG. 4 is a graph illustrating the percentage of dead neurons to explain effects of co-culturing neurons with human UCB-derived MSCs on death of neuron caused by Aβ42;

FIG. 5 illustrates results of fluorescent staining to explain effects of co-culturing neurons with human bone marrow-derived MSCs on death of neuron caused by Aβ42;

FIG. 6 illustrates neurons fluorescent stained using an anti-phosphor-tau antibody;

FIG. 7 illustrates neurons treated with Aβ42, co-cultured with MSCs, and stained using immunofluorescent staining ;

FIG. 8 illustrates expression of neprilysin in neurons treated with Aβ42 and co-cultured with bone marrow-derived MSCs or UCB-derived MSCs;

FIG. 9 illustrates expression of neprilysin in neurons and microglial cells when neurons and microglial cells treated with Aβ42 are co-cultured with MSCs;

FIG. 10 is a graph illustrating the percentage of dead neurons treated with Aβ42 and co-cultured with proteins secreted from MSCs;

FIG. 11 is a graph illustrating the length of neurites of neurons cultured with Aβ42 and proteins secreted from MSCs;

FIG. 12 shows the results of RT-PCR using the total RNA isolated from UCB-MSC as a template after co-culturing microglial cells with UCB-MSC;

FIG. 13 shows the results of western blotting indicating the increase in the expression of NEP when neurons and microglial cells are cultured in the presence of IL-4;

FIG. 14 shows images of Aβ protein plaque in a brain tissue including hippocampus and cerebral cortex stained using a Thio-S staining;

FIG. 15 is a graph illustrating the total area of Aβ plaque in the images of FIG. 14;

FIG. 16 shows the results of immunoblotting indicating the change of Aβ protein produced in the brain of a mouse used for an experiment;

FIG. 17 shows the degree of expression of NEP in a brain tissue of a normal mouse and a mouse transformed to have Alzheimer's disease including hippocampus and cerebral cortex;

FIG. 18 is a graph illustrating band intensity of NEP of FIG. 17 measured using Quantity One software (Bio-RAD);

FIG. 19 shows the degree of expression of NEP in a brain tissue of a mouse into which MSCs and IL-4 are administered and including hippocampus and cerebral cortex; and

FIG. 20 shows the expression of NEP in microglial cells of a mouse into which UCB-derived MSCs and IL-4 are administered.

Best mode for carrying out the Invention

According to embodiments of the present invention, damage of neurons caused by amyloid-beta may be prevented or repaired when the neurons are co-cultured with mesenchymal stem cells (MSCs), which are not differentiated into neurons, without direct contact between the neurons and the MSCs. In addition, the inventors of the present invention have found that damage of neurons by amyloid-beta may be prevented or repaired when co-cultured with a culture solution of MSCs or a specific protein contained in the culture solution.

When neurons treated with 10 μM of amyloid-beta42 (Aβ42) for 24 hours (Ct+Aβ shown in FIGS. 1 and 3) are compared with untreated neurons (Ct shown in FIG. 3), most neurons treated with Aβ42 die. However, if the damaged neural cells are co-cultured with umbilical cord blood (UCB)-derived MSCs, death of neuron is prevented and cell maturation is increased (Ct+Aβ+MSC of FIG. 3 and FIG. 4). The effects of the UCB-derived MSCs on the prevention of death of neuron caused by amyloid-beta may also be observed in bone marrow-derived MSCs (Cortex/Aβ/BM-MSC of FIG. 5). When cerebral cortex-derived neurons and MSCs are co-cultured in the same culture medium in the presence of Aβ42 for 24 hours, the same result as shown in Ct+Aβ+MSC of FIG. 3 is obtained. This indicates that damaged neurons by Aβ42 may be reparied and the damge of neurons by Aβ42 may be prevented if the neurons are co-cultured with MSCs.

In addition, phosphorylation of tau protein, which is rapidly phosphorylated by Aβ42, is prevented by co-culturing the tau protein with human UCB-derived MSCs (FIG. 6).

As a result of observing neurons using antibodies angainst Tubulin β III and MAP2, i.e., markers of neurons, neurites are damaged and cleaved and the shape of the neurons is condensed in neurons treated with Aβ42 due to toxicity. However, when the neurons are co-cultured with the UCB-derived MSCs, the neurites are maintained in the neurons and differentiation and maturation of the neurons are accelerated (FIG. 7).

As a result of observing expression of neprilysin (NEP), known as protein degrading and removing Aβ42, the expression of NEP is reduced in neurons treated with Aβ42. However, when the neurons are co-cultured with UCB-derived MSCs, the expression of NEP is increased in the protein level and mRNA level (FIG. 8A). FIG. 8B illustrates stained neurons using an anti-NEP antibody. If the neurons are treated with Aβ42, the portion stained in red is considerably reduced, thereby indicating that the expression of NEP is reduced in the neurons. However, if the neurons are co-cultured with MSCs, the expression of the NEP is increased. These results are also observed in experiments using bone marrow-derived MSCs as well as UCB-derived MSCs (FIG. 8C). Thus, when a neural cell treated with or without Aβ42 is co-cultured with MSCs, the expression level of NEP in the neural cell is increased in mRNA and protein level. The MSCs includes UCB-MSCs and BM-MSCs.

Furthermore, it is also identified that UCB-derived MSCs induce the expression of NEP not only in the neurons (neurons) but also in microglial cells, which are known as macrophage of the brain and remove toxic substances accumulated in the brain, for example, Aβ of Alzheimer's disease (FIG. 9).

Since the effects described above are obtained by co-culturing the MSCs and the neurons without direct contact therebetween, it is considered that substances secreted from the MSCs cause the effects. Proteins that are not expressed or rarely expressed when MSCs are singly clutured, but increasingly expressed in the MSCs when the neurons and the MSCs are co-cultured are analyzed. As a result, it is identified total 14 proteins are related to the prevention of toxicity caused by Aβ42 and differentiation and maturation of the neurons. The 14 proteins are activin A, platelet factor 4 (PF4), decorin, galectin 3, growth differentiation factor 15 (GDF15), glypican 3, membrane-type

frizzled-related protein (MFRP), intercellular adhesion molecule 5 (ICAM5), insulin-like growth factor binding protein 7 (IGFBP7), platelet-derived growth factor-AA (PDGF-AA), secreted protein acidic and rich in cysteine (SPARCL1), thrombospondin-1, wnt-1 induced secreted protein 1 (WISP1), and progranulin. When the neurons treated with Aβ42 and each of the proteins instead of the MSCs, the death of neuron is considerably reduced, and the length of neurites is significantly increased when compared to the neurons treated only with Aβ42 (FIGS. 10 and 11). In this regard, the 14 proteins described above will be described in more detail.

Activin A that is known as inhibin βA (INHBA) is a homodimer protein. It is known that INHBA is coded by an INHBA gene in humans. INHBA may have an amino acid sequence of NCBI Accession No.: NP_002183 (SEQ ID NO: 1).

Platelet factor 4 (PF4) that is known as chemokine (C-X-C motif) ligand 4 (CXCL4) is a small cytokine belonging to a CXC chemokine family. The gene for human PF4 is located on human chromosome 4. PF4 may have an amino acid sequence of NCBI Accession No.: NP_002610 (SEQ ID NO: 2).

Decorin is a proteoglycan having an average molecular weight of about 90 to about 140 kDa. Decorin belongs to a small leucine-rich proteoglycan (SLRP) family and includes a protein core having leucine repeats with glycosaminoglycan (GAG) consisting of chondroitin sulfate (CS) or dermatan sulfate (DS). Decorin may have an amino acid sequence of NCBI Accession No.: NP_001911 (SEQ ID NO: 3).

Galectin 3 that is known as LGAL3 (lectin, galactoside-binding, soluble 3) is a lectin binding to beta-galactoside. For example, galectin 3 may have an amino acid sequence of NCBI Accession No.: NP_919308 (SEQ ID NO: 4).

Growth differentiation factor 15 (GDF15) that is known as macrophage inhibitory cytokine 1 (MIC1) is a protein belonging to a transforming growth factor beta superfamily and controlling an inflammatory pathway in wounds and a cell death pathway in a diseases process. For example, GDF15 may have an amino acid sequence of NCBI Accession No.: NP_004855 (SEQ ID NO: 5).

Glypican 3 that is known as GPC3 is a protein belongs to a glypican family. For example, glypican 3 may have an amino acid sequence of NCBI Accession No.: NP_004475 (SEQ ID NO: 6). Glypican belongs to a heparan sulfate proteoglycan family and is attached to the surface of cells through a covalent bond with

glycosylphosphatidylinositol (GPI).

Membrane frizzled-related protein (MFRP), for example, may have an amino acid sequence of NCBI Accession No.: NP_113621 (SEQ ID NO: 7).

Intercellular adhesion molecule 5 (ICAM5) that is known as telencephalin belongs to an ICAM family. ICAM is a type I transmembrane glycoprotein, contains 2 to 9 immunoglobulin pseudo C2 type domains, and binds to leukocyte adhesion lymphocyte function-associated antigen 1 (LFA-1) protein. For example, ICAM5 may have an amino acid sequence of NCBI Accession No.: NP_003250 (SEQ ID NO: 8).

Insulin-like growth factor binding protein 7 (IGFBP7) belongs to an IGFBP family specifically binding to insulin-like growth factor (IGF). IGFBP7 is also known as IGF-binding protein-related protein 1 (IGFBP-rp1). For example, IGFBP7 may have an amino acid sequence of NCBI Accession No.: NP_001544 (SEQ ID NO: 9).

Platelet-derived growth factor AA (PDGF-AA) belongs to PDGF. PDGF-AA is a homodimer glycoprotein including PDGF alpha polypeptide that is known as two PDGFA. PDGF is a protein controlling the growth and differentiation of cells. PDGF is also related to angiogenesis. For example, PDGFA may have an amino acid sequence of NCBI Accession No.: XP_001126441 (SEQ ID NO: 10).

For example, secreted protein acidic and rich in cysteines-like 1 (SPARCL1) may have an amino acid sequence of NCBI accession No.: NP_004675 (SEQ ID NO: 11).

Thrombospondin 1 (TSP1) is a homotrimeric protein bound through a disulfide. Thrombospondin 1 is an adhesive glycoprotein that mediates cell-to-cell and cell-to-maxtrix interactions. Thrombospondin 1 can bind to fibrinogen, fibronectin, laminin, and type V collagen. For example, Thrombospondin 1 may have an amino acid sequence of NCBI Accession No.: NP_003237 (SEQ ID NO: 12).

WNT1 inducible signalling pathway protein 1 (WISP1) that is known as CCN4 belongs to a WISP protein sub-family and a connective tissue growth factor (CTGF) family. WNT1 is a cysteine-rich, glycosylated signalling proteins that mediate a variety of developmental process. A CTGF family members are characterized by four conserved cysteine-rich domains: an IGF binding domain, a vWF type C module, a thrombospondin domain and a C-terminal cystine knot-like domain. For example, WISP1 may have an amino acid sequence of NCBI Accession No.: NP_003873 (SEQ ID NO: 13).

Progranulin (PGN) is a precursor of granulin. Progranulin is a single precursor protein having 7.5 repeats of highly preserved 12-cysteine granulin/epithelin motif, and granulin (GRN) is cleaved from the progranulin and belongs to a secreted and glycosylated peptide family. Progranulin is also known as a proepithelin and a PC cell-derived growth factor. For example, progranulin may have an amino acid sequence of NCBI Accession No.: NP_001012497 (SEQ ID NO: 14).

If microglial cells and neurons are cultured in the presence of Interleukin-4 (IL-4), it was identified that the expression of neprilysin (NEP) is increased in the microglial cells and neurons. In addition, it was identified that amyloid plaque was reduced if UCB-derived MSCs (UCB-MSC) or IL-4 are administered to a mouse having Alzheimer's disease. It was also identified that the expression of NEP is increased in brain tissues including hippocampus and/or cerebral cortex if UCB-MSC or IL-4 are administered to a mouse having Alzheimer's disease. It was also identified that the expression of NEP is increased in microglial cell in brain tissues if UCB-MSC or IL-4 are administered to a mouse having Alzheimer's disease.

Interleukin-4 (IL-4) is a cytokine inducing differentiation of a naïve helper T cell (Th0 cell) into a Th2 cell. Th2 cell activated by IL-4 further produces IL-4. IL-4 may have an amino acid sequence of NCBI Accession Nos.: NP_000580 (SEQ ID NO: 30) or NP_067258.

The 14 proteins may include not only human-derived proteins but also mammal-derived proteins. For example, the mammal includes a rodent and the rodent may include for example, a mouse or a rat.

Even though the possibility of treating of neurodegenerative disorders, such as Alzheimer's disease, has been raised with recent research on tissue regenerative medicines using stem cells, currently available stem cell technology is not sufficiently developed to be applied to a wide range of memory loss in the brain such as Alzheimer's disease. However, the inventors of the present invention have found that MSCs reduce neurocytoxicity caused by amyloid-beta, and accelerate differentiation and proliferation of neural stem cells in the brain. Thus, the possibility of developing a cellular preparation for the treatment of Alzheimer's disease and other neural diseases is raised. In addition, it has been found that several proteins secreted from MSCs have therapeutic effects on neural diseases such as Alzheimer's disease, and thus the potential for the

prevention and treatment of neural diseases is increased.

The present invention provides a pharmaceutical composition for the prevention or treatment of a neural disease, including mesenchymal stem cells (MSCs), a culture solution of the MSCs, proteins contained in the culture solution of MSCs and/or a signal transduction system-stimulating factor inducing expression of the proteins. The neural disease may be a disease caused by a damaged neurite. The neural disease may be Alzheimer's disease, Parkinson's disease, depression, epilepsy, multiple sclerosis, mania, or any combination thereof.

A pre-dementia syndrome exhibiting mild cognitive impairment may be diagnosed using a neuropsychological test. It has been reported that about 12% of patients with mild cognitive impairment progress to Alzheimer's disease per year. Surprisingly, about 80% of patients with mild cognitive impairment progress to Alzheimer's disease after 6 years without any treatment. Thus, when a pharmaceutical composition according to the present invention is administered to patients with mild cognitive impairment, the progress to Alzheimer's disease may be prevented or delayed.

The present invention also provides a method and a kit for preventing neurocytoxicity caused by treatment with amyloid-beta in neurons, preventing phosphorylation of tau protein in neurons, preventing neurite damage, and inducing expression of neprilysin in neurons using MSCs, a culture solution of MSCs, proteins contained in the culture solution of MSCs, or a signal transduction system-stimulating factor inducing expression of the proteins in vitro or in vivo. The kit may further include ingredients required for culturing the neurons.

The pharmaceutical composition including MSCs, a culture solution of MSCs, proteins contained in the culture solution of MSCs, or a signal transduction system-stimulating factor inducing expression of the proteins according to the present invention may be administered with other effective ingredients having effects on the prevention or treatment of Alzheimer's disease, Parkinson's disease, depression, epilepsy, multiple sclerosis, mania, etc.

The pharmaceutical composition may further include pharmaceutically acceptable additives in addition to effective ingredients, and may be formulated in a unit dosage formulation suitable for administering to a patient using any known method in the pharmaceutical field. For this purpose, a formulation for parenteral administration such

as injection formulation or topical administration formulation may be used. For example, a formulation for parenteral administration such as injection formulation of a sterile solution or suspension, if required, using water or other pharmaceutically acceptable solvents, may be used. For example, a unit dosage formulation may be prepared using a pharmaceutically acceptable carrier or medium, e.g., sterile water, saline, vegetable oil, an emulsifier, a suspension, a surfactant, a stabilizer, an excipient, a vehicle, a preservative, and a binder.

The pharmaceutical formulation may be administered parenterally using any known method in the art. The parenteral administration may include a topical administration and a systematic administration. The topical administration may be performed by directly administering the pharmaceutical formulation into an injury region or peripheral regions of the injury region, for example, brain or spinal cord, peripheral regions thereof, or opposite regions thereof. The systematic administration may be performed by administering the pharmaceutical formulation into spinal fluid, vein or artery. The spinal fluid includes cerebrospinal fluid. The arterty may be a region supplying blood to the injury region. In addition, the administration may be performed according to a method disclosed in (Douglas Kondziolka, Pittsburgh, Neurology, vol. 55, pp. 565-569, 2000). Specifically, a skull of a subject is incised to make a hole having a diameter of 1 cm and a suspension of MSCs in Hank's balanced salt solution (HBSS) is injected into the hole by employing a long-needle syringe and a stereotactic frame used to inject the suspension into a right position.

A dose of the MSCs may range from $1x10^4$ to $1x10^7$ cells/kg (body weight) per day, for example, from $5x10^5$ to $5x10^6$ cells/kg (body weight) per day, which can be administered in a single dose or in divided doses. However, it should be understood that the amount of the MSCs, for example, UCB-derived MSCs, actually administered to a patient should be determined in light of various relevant factors including type of diseases, severity of diseases, chosen route of administration, and body weight, age, and gender of an individual patient.

The present invention also provides a method of preventing or treating a neural disease of an individual, the method including administering a pharmaceutical composition comprising at least one selected from the group consisting of mesenchymal stem cells (MSCs) and a culture solution of the MSCs to the individual.

The administration used in the method may be a topical administration or a systematic administration. The pharmaceutical composition may be administered by an amount effective for preventing or treating the disease. It would be obvious to one of ordinary skill in the art that the effective amount may vary according to the conditions of the disease.

The pharmaceutical composition used in the method is the same as that described above. In the method, the MSCs contained in the pharmaceutical composition may be collected from not only autologous cells but also allogeneic cells from others and animals for medical experiments. Cells preserved in a frozen form may also be used. This therapeutic method is not limited to humans. In general, MSCs may also be applied to mammals as well as humans.

In the method, the neural disease may be a disease caused by at least one selected from the group consisting of amyloid-beta, hyperphosphorylation of tau protein, hypoexpression of neprilysin, and damage to neurites. The neural disease may be Alzheimer's disease, Parkinson's disease, depression, epilepsy, multiple sclerosis, or mania.

The amyloid-beta (Aβ) used herein indicates a major element of amyloid plaque found in the brain of a patient having Alzheimer's disease. The amyloid-beta (Aβ) may be a peptide including an amino acid derived from the C-terminal of amyloid precursor protein (APP) that is a transmembrane glycoprotein. The Aβ may be produced from APP by a continuous operation of β- secretase and γ-secretase. For example, the Aβ may include 39 to 43 amino acids, for example 40 to 42 amino acids. The Aβ may include 672-713 residues (Aβ42) or 672-711 residues (Aβ40) of an amino acid sequence of NCBI Accession No.: NP_000475 (SEQ ID NO: 19) which is human amyloid-beta A4 protein isoform precursor (APP). The amyloid-beta (Aβ) may be derived from a mammal. For example, the Aβ may be derived from a human or a mouse.

The "tau protein" used in this specification is a microtubule-associated protein found in neurons of a central nervous system. The tau protein interacts with tubulin to stabilize microtubule and promotes tubulin assembly of the microtubule. It is known that a cerebral tissue includes 6 different tau isoforms. It is known that hyperphosphorylation of tau protein is related to the outbreak of Alzheimer's disease. Tau protein is microtubule-associated protein having high solubility. In humans, tau

protein is mainly found in neurons rather than non-neuron cells. One of the functions of tau protein is to control stabilization of axonal microtubule. For example, tau protein may be microtubule-associated protein tau isoform 2 having an amino acid sequence of NCBI Accession No.: NP_005901 (SEQ ID NO: 20). The tau protein may be derived from a mammal. For example, the tau protein may be derived from a human or a mouse.

Neprilysin is a zinc-dependent metalloprotease enzyme decomposing a large number of small secreted peptides. Neprilysin decomposes amyloid-beta that causes Alzheimer's disease if amyloid-beta is abnormally misfolded and aggregated in neural tissues. For example, neprilysin may have an amino acid sequence of NCBI Accession No.: NP_000893 (SEQ ID NO: 21). The neprilysin may be derived from a mammal. For example, the neprilysin may be derived from a human or a mouse.

The present invention also provides a method of reducing amyloid plaque in neural tissues, the method including culturing the neural tissues in the presence of at least one selected from the group consisting of mesenchymal stem cells (MSCs) and a culture solution of the MSCs.

In the method, the neural tissues such as neurons may be cultured in vitro or in vivo. The in vitro culture may be performed in a culture medium for MSCs and/or neural tissues such neurons which is known in the art. The MSCs and neural tissues such as neurons may be cultured with or without direct contact therebetween. For example, the MSCs and neural tissues such as neurons may be cultured by being separated from each other by a membrane with pores. The membrane may have a pore size and configuration sufficiently large for biologically active materials in the culture medium for the MSCs to pass through the pore but for cells not to pass therethrough. The biologically active materials may be proteins, sugars and nucleic acids. The membrane may be disposed such that the MSCs are cultured on the membrane and the neural tissues such as neurons are cultured below the membrane so that the biologically active materials pass through the membrane to the below of the membrane by the gravity.

The in vivo culture may further include administering at least one selected from the group consisting of MSCs and a culture solution of the MSCs into an individual. The administration may be a topical administration or a systematic administration. An effective amount for reducing the amount of plaque may be administered. It would be

obvious to one of ordinary skill in the art that the effective amount may vary according to the conditions of the disease. The individual may be any animal in need of reducing amyloid plaque in it's neural tissues. The animal may include a mammal. The mammal may include a human, a mouse or a rat.

The reducing of amyloid plaque in the neural tissues may be reducing the amount of amyloid plaque in the neural tissues compared to that of amyloid plaque when the neural tissues such as neurons are cultured in the absence of the MSCs and a culture solution of the MSCs.

The term "amyloid plaque" used in this specification may be an insoluble fibrous protein aggregates including amyloid beta. The amyloid plaque may be present within a cell, on the cell membrane and/or in a space between cells.

The tem "neural tissues" used herein, include central nerve system, for example, brain tissues. The brain tissues include cerebral tissues and hippocampus. The cerebral tissues include cerebral cortex. The neural tissues include neural cells as well as the neural tissues per se. The neural cells include neuronal cells and/or microglial cells. The culturing the neural tissues includes culturing the neural cells such as neuronal cell and/or microglial cells in vivo or in vitro.

The present invention also provides a method of reducing the degree of phosphorylation of tau protein in neurons, the method including culturing the neurons in the presence of at least one selected from the group consisting of mesenchymal stem cells (MSCs) and a culture solution of the MSCs.

The culturing is described above with reference to the method of reducing amyloid plaque.

The reducing of phosphorylation of tau protein in the neurons may be reducing the amount of phosphorylation of tau protein compared to that of phosphorylation of tau protein when the neurons are cultured in the absence of the MSCs and a culture solution of the MSCs.

The present invention also provides a method of increasing expression of neprilysin in neurons or microglial cells, the method including culturing the neurons or microglial cells in the presence of at least one selected from the group consisting of mesenchymal stem cells (MSCs) and a culture solution of the MSCs.

The culturing is described above with reference to the method of reducing amyloid

plaque in the neural tissues. The increasing of neprilysin expression in the neurons or microglial cells may be increasing neprilysin expression in the neurons or microglial cells compared to neprilysin expression in the neurons or microglial cells when the neurons or microglial cells are cultured in the absence of the MSCs and a culture solution of the MSCs.

The present invention also provides a method of increasing growth of neurites of neurons, the method including culturing the neurons in the presence of at least one selected from the group consisting of mesenchymal stem cells (MSCs) and a culture solution of the MSCs.

The culturing is described above with reference to the method of reducing amyloid plaque in the neural tissues. The neurons may be normal neurons or neurons having damaged neurites, for example, by Aβ. The increasing of neurites growth of the neurons may be increasing of neurites growth of the neurons compared to neurites growth of the neurons when the neurons are cultured in the absence of the MSCs and a culture solution of the MSCs.

The present invention also provides a method of preventing or treating a neural disease of an individual, the method including administering a pharmaceutical composition including at least one selected from the group consisting of activin A, PF4, decorin, galectin 3, GDF15, glypican 3, MFRP, ICAM5, IGFBP7, PDGF-AA, SPARCL1, thrombospondin-1, WISP1, progranulin, IL-4, a factor inducing expression thereof, and any combination thereof.

The administration used in the method may be a topical administration or a systematic administration. An effective amount for preventing or treating the neural disease may be administered. It would be obvious to one of ordinary skill in the art that the effective amount may vary according to the conditions of the disease.

The pharmaceutical composition used in the method is the same as that described above.

In the method, the neural disease may be a disease caused by at least one selected from the group consisting of amyloid-beta, hyperphosphorylation of tau protein, hypoexpression of neprilysin, and damage to neurites. The neural disease may be Alzheimer's disease, Parkinson's disease, depression, epilepsy, multiple sclerosis, or mania.

The present invention also provides a method of reducing amyloid plaque in neural tissues, the method including culturing the neural tissues in the presence of at least one selected from the group consisting of activin A, PF4, decorin, galectin 3, GDF15, glypican 3, MFRP, ICAM5, IGFBP7, PDGF-AA, SPARCL1, thrombospondin-1, WISP1, progranulin, IL-4, a factor inducing expression thereof, and any combination thereof.

In the method, the neural tissues such as neurons may be cultured in vitro or in vivo. The in vivo culture may further include administering at least one selected from the group consisting of activin A, PF4, decorin, galectin 3, GDF15, glypican 3, MFRP, ICAM5, IGFBP7, PDGF-AA, SPARCL1, thrombospondin-1, WISP1, progranulin, IL-4, a factor inducing expression thereof, and any combination thereof to the individual. The administration may be a topical administration or a systematic administration. An effective amount for reducing the amount of the plaque may be administered. It would be obvious to one of ordinary skill in the art that the effective amount may vary according to the conditions of the disease. For example, each one selected from the group consisting of activin A, PF4, decorin, galectin 3, GDF15, glypican 3, MFRP, ICAM5, IGFBP7, PDGF-AA, SPARCL1, thrombospondin-1, WISP1, progranulin, IL-4, a factor inducing expression thereof, and any combination thereof may be administered in amount from about 1ng/kg body weight to about 100mg/kg body weight, for example, about 10ng/kg body weight to about 50mg/kg body weight. The administered formulation may further include additives such as water, a culture medium, a buffer, or an excipient. The individual may be any animal in need of reducing amyloid plaque in it's neural tissues. The animal may include a mammal. The mammal may include a human, a mouse or a rat.

The amyloid plaque may be reduced in the presence of at least one selected from the group consisting of activin A, PF4, decorin, galectin 3, GDF15, glypican 3, MFRP, ICAM5, IGFBP7, PDGF-AA, SPARCL1, thrombospondin-1, WISP1, progranulin, IL-4, a factor inducing expression thereof, and any combination thereof when compared to in the absence thereof.

The present invention also provides a method of reducing the degree of phosphorylation of tau protein in neurons, the method including culturing the neurons in the presence of at least one selected from the group consisting of activin A, PF4, decorin,

galectin 3, GDF15, glypican 3, MFRP, ICAM5, IGFBP7, PDGF-AA, SPARCL1, thrombospondin-1, WISP1, progranulin, IL-4, a factor inducing expression thereof, and any combination thereof.

The culturing is described above with reference to the method of reducing amyloid plaque in the neural tissues. The degree of phosphorylation of tau protein in neurons may be reduced in the presence of at least one selected from the group consisting of activin A, PF4, decorin, galectin 3, GDF15, glypican 3, MFRP, ICAM5, IGFBP7, PDGF-AA, SPARCL1, thrombospondin-1, WISP1, progranulin, IL-4, a factor inducing expression thereof, and any combination thereof when compared to in the absence thereof.

The present invention also provides a method of increasing expression of neprilysin of neurons or microglial cells, the method including culturing the neurons or microglial cells in the presence of at least one selected from the group consisting of activin A, PF4, decorin, galectin 3, GDF15, glypican 3, MFRP, ICAM5, IGFBP7, PDGF-AA, SPARCL1, thrombospondin-1, WISP1, progranulin, IL-4, a factor inducing expression thereof, and any combination thereof.

The culturing is described above with reference to the method of reducing amyloid plaque in the neural tissues. The expression of neprilysin of neurons or microglial cells may be increased in the presence of at least one selected from the group consisting of activin A, PF4, decorin, galectin 3, GDF15, glypican 3, MFRP, ICAM5, IGFBP7, PDGF-AA, SPARCL1, thrombospondin-1, WISP1, progranulin, IL-4, a factor inducing expression thereof, and any combination thereof when compared to in the absence thereof.

The present invention also provides a method of increasing growth of neurites of neurons, the method including culturing the neurons in the presence of at least one selected from the group consisting of activin A, PF4, decorin, galectin 3, GDF15, glypican 3, MFRP, ICAM5, IGFBP7, PDGF-AA, SPARCL1, thrombospondin-1, WISP1, progranulin, IL-4, a factor inducing expression thereof, and any combination thereof.

The culturing is described above with reference to the method of reducing amyloid plaque in the neural tissues. The neurons may be normal neurons or neurons having damaged neurites, for example, by Aβ. The growth of neurites of neurons may be increased in the presence of at least one selected from the group consisting of activin A,

PF4, decorin, galectin 3, GDF15, glypican 3, MFRP, ICAM5, IGFBP7, PDGF-AA, SPARCL1, thrombospondin-1, WISP1, progranulin, IL-4, a factor inducing expression thereof, and any combination thereof when compared to in the absence thereof.

The "mesenchymal stem cell (MSC)" used herein may be a MSC isolated from at least one selected from a group consisting of a mammalian, e.g. human, embryonic yolk sac, placenta, umbilical cord, umbilical cord blood, skin, peripheral blood, bone marrow, adipose tissue, muscle, liver, neural tissue, periosteum, fetal membrane, synovial membrane, synovial fluid, amniotic membrane, meniscus, anterior cruciate ligament, articular chondrocytes, decidous teeth, pericyte, trabecular bone, infra patellar fat pad, spleen, thymus, and other tissues including MSCs or expanded by culturing the isolated MSC.

As used herein, the "umbilical cord blood" refers to the blood taken from the umbilical cord vein which links the placenta of mammals including humans with a newborn baby thereof. The "umbilical cord blood-derived MSC" as used herein refers to a MSC which is isolated from the umbilical cord blood of mammals, for example, humans or a MSC expanded by culturing the isolated UCB-MSC.

The "treating" used herein refers to: preventing the manifestation of a not-yet-diagnosed disease or disorder in animals, for example, mammals including humans, which are prone to acquiring such diseases or disorders; inhibiting the development a disease; or relieving a disease.

Terminology that is not defined herein have meanings commonly used in the art.

Any known method, for example, a method disclosed in Korean Patent No. 489248 may be used to isolate mononuclear cells including MSCs from umbilical cord blood. For example, a Ficoll-Hypaque density gradient method may be used, but the method is not limited thereto. Specifically, umbilical cord blood collected from the umbilical vein after childbirth and before the placenta is removed is centrifuged using a Ficoll-Hypaque gradient to obtain mononuclear cells. The mononuclear cells were washed several times to remove impurities. The isolated mononuclear cells may be subjected to isolation and cultivation of MSCs or to be frozen for long-term safekeeping at a very low temperature until use.

Any known method may be used for MSC isolation from the umbilical cord blood and cultivation of the MSC (Korean patent Publication No. 2003-0069115, and Pittinger

MF, *Science*, 284: 143-7, 1999; and Lazarus HM, etc. *Bone Marrow Transplant*, 16: 557-64, 1995).

First, collected umbilical cord blood is centrifuged using a Ficoll-Hypaque gradient to isolate mononuclear cells including hematopoietic stem cells and MSCs, and the mononuclear cells are washed several times to remove impurities. The mononuclear cells are cultured in a culture dish with an appropriate density. Then, the mononuclear cells are proliferated to form a monolayer. Among the mononuclear cells, MSCs proliferate in a homogenous and spindle-shaped long colony of cells when observed using a phase contrast microscope. The grown cells are repeatedly sub-cultured to obtain a desired number of cells.

Cells contained in the composition according to the present invention may be preserved in a frozen form using known methods. (Campos, etc., Cryobiology 35:921-924, 1995). A culture medium used for the frozen form may include 10% dimethylsulfoxide (DMSO) and one of 10 to 20% fetal bovine serum (FBS), human peripheral blood, or plasma or serum of umbilical cord blood. The cells may be suspended such that about 1×10^6 to 5×10^6 cells exist in 1 mL of the medium.

The cell suspension is distributed into glass or plastic ampoules for deep freezing, and then the ampoules may be sealed and put in a deep freezer kept at a programmed temperature. In this regard, for example, a freeze-program that controls the freezing rate at -1℃/min is used so that cell damage during thawing is minimized. When the temperature of the ampoules reaches less than -90℃, it may be transferred into a liquid nitrogen tank and maintained at less than -150℃.

To thaw the cells, the ampoules have to be quickly transferred from the liquid nitrogen tank into a 37℃ water bath. The thawed cells in the ampoules are quickly placed in a culture vessel containing a culture medium under an aseptic condition.

In the present invention, the medium used in the isolation and cultivation of the MSCs may be any medium for general cell culture well-known in the art containing 10 to 30% FBS, human peripheral blood, or plasma or serum of umbilical cord blood. For example, the culture medium may be Dulbecco's modified eagle medium (DMEM), minimum essential medium (MEM), α-MEM, McCoys 5A medium, Eagle's basal medium, Connaught Medical Research Laboratory (CMRL) medium, Glasgow minimum essential medium, Ham's F-12 medium, Iscove's modified Dulbecco's medium (IMDM), Liebovitz'

L-15 medium, or Roswell Park Memorial Institute (RPMI) 1640 medium, for example, DMEM. The cells may be suspended at the concentration of 5×10^3 to 2×10^4 cells per 1ml of the medium.

Furthermore, the cell culture medium of the present invention may further include one or more auxiliary components. The auxiliary compnents may be fetal bovine serum, horse serum or human serum; and antibiotics such as Penicillin G, streptomycin sulfate, and gentamycin; antifungal agents such as amphotericin B and nystatin; and a mixture thereof to prevent microorganism contamination.

Umbilical cord blood-derived cells do not express histocompatibility antigen HLA-DR (class II) which is the major cause of rejection after tissue or organ transplantation (Le Blanc, K C, *Exp Hematol,*31:890-896, 2003; and Tse W T et al., *Transplantation,* 75:389-397, 2003). Since these cells can minimize the immune response after transplantation, for example, rejection of transplanted tissue or organs, autologous as well as allogeneic umbilical cord blood can be used. Frozen cells may also be used.

The culture solution of MSCs may be a culture solution used for culturing mammalian cells, for example, human bone marrow-derived MSCs, UCB-derived MSCs, adipose tissue-derived stem cells, embryonic yolk sac-derived MSCs, placenta-derived MSCs, skin-derived MSCs, peripheral blood-derived MSCs, muscle-derived MSCs, liver-derived MSCs, neural tissue-derived MSCs, periosteum-derived MSCs, umbilical cord-derived MSCs, fetal membrane-derived MSCs, synovial membrane-derived MSCs, synovial fluid-derived MSCs, amniotic membrane-derived MSCs, meniscus-derived MSCs, anterior cruciate ligament-derived MSCs, articular chondrocytes-derived MSCs, decidous teeth-derived MSCs, pericyte-derived MSCs, trabecular bone-derived MSCs, infra patellar fat pad-derived MSCs, spleen-derived MSCs, thymus-derived MSCs, and MSCs isolated from other tissues including MSCs, and/or culturd MSCs.

The culture medium may be for example, a cell culture medium containing FBS, or plasma or serum of human peripheral blood or umbilical cord blood. The cell culture medium may include, for example, DMEM, MEM, α-MEM, McCoys 5A medium, Eagle's basal medium, CMRL medium, Glasgow minimum essential medium, Ham's F-12 medium, Iscove's modified Dulbecco's medium (IMDM), Liebovitz' L-15 medium, and RPMI 1640 medium, but is not limited thereto.

The culture solution of MSCs according to the present invention may include at least one selected from the group consisting of activin A, PF4, decorin, galectin3, GDF15, glypican3, MFRP, ICAM5, IGFBP, PDGF-AA, SPARCL1, thrombospondin1, WISP1, and progranulin, IL-4, or a factor inducing at least one of the proteins.

The pharmaceutical composition according to the present invention may include at least one protein selected from the group consisting of activin A, PF4, decorin, galectin3, GDF15, glypican3, MFRP, ICAM5, IGFBP, PDGF-AA, SPARCL1, thrombospondin1, WISP1, and progranulin, IL-4, or a factor inducing at least one of the proteins as an active ingredient.

The factor inducing at least one of the proteins may be a signal transduction system-stimulating factor and any known factor. The factor may be the following examples, but is not limited thereto. The factor inducing galectin 3 may include at least one selected from the group consisting of phorbol 12-myristate 13-acetate (PMA) and a modified lipoprotein. The PMA or the lipoprotein is known to induce galectin 3 via protein kinase C (PKC), mitogen-activated protein kinase 1,2 (MAPK-1,2) and p38 kinase. The factor inducing PDGF-AA may include at least one selected from the group consisting of avian erythroblastosis virus E26 (v ets) oncogene homolog 1 (Ets-1) and lysophosphatidylcholine. Lysophosphatidylcholine is known to induce PDGF-AA via MAPK-1,2.

All cited references may be incorporated herein by reference in their entireties.

The present invention will be described in further detail with reference to the following examples. These examples are for illustrative purposes only and are not intended to limit the scope of the present invention.

Examples

Example 1: Isolation and cultivation of neural stem cells

Neural stem cells used herein were isolated as follows. Neural stem cells were isolated from the cerebral cortex and hippocampus of an embryonic day 14 (E14) Sprague-Dawley rat (Orient Bion Inc., Korea). First, the abdomen of a pregnant rat was incised, and the embryo was isolated using a scissors and forceps. The embryo was washed with a Hank's balanced salt solution (HBSS) for dissection and placed in a dish containing ice-cold HBSS. The cerebral cortex and hippocampus were isolated from the E14 embryo using needles and forceps under a microscope. The isolated cerebral

cortex was pipetted 10 to 20 times into single cells in a serum-free culture solution using pipettes. The single cells were treated with poly-L-ornithine (15 μg/ml, Sigma, St. Louis, MO) at 37℃ for 16 hours and smeared on a cover slip coated with fibronectin (1 μg/ml, Sigma) for at least 2 hours. The single cells were cultured in a serum-free Neurobasal™ culture medium (GIBCO) supplemented with 20 ng/ml of basic fibroblast growth factor (bFGF) and B-27 serum-free supplement for about 2 to 4 days until about 70% of the bottom surface of the culture dish was covered with the single cells (70 to 80% confluence). The bFGF was removed and differentiation of the neuron cells was induced for 4 to 6 days. During the differentiation, the cells were incubated in a 5% CO_2 incubator at 37℃, while the culture medium and the B27 supplement were changed every other day and the bFGF was added thereto everyday. The differentiated neurons were used in the following examples.

Example 2: Isolation and amplification of UCB-derived MSCs

An umbilical cord blood (UCB) sample was collected from the umbilical vein right after childbirth with the mother's approval. Specifically, the umbilical vein was pricked with a 16-gauge needle connected to an UCB collection bag containing 44 mL of a citrate phosphate dextrose anticoagulant-1 (CPDA-1) anticoagulant (Green Cross Corp., Korea) such that the UCB was collected in the collection bag by gravity. The UCB thus obtained was handled within 48 hours after collection, and the viability of the monocytes was more than 90%. The collected UCB was centrifuged using a Ficoll-Hypaque gradient (density: 1.077 g/mL, Sigma) to obtain mononuclear cells and the mononuclear cells were washed several times to remove impurities. The cells were suspended in a minimal essential medium (α-MEM, Gibco BRL) supplemented with 10% to 20% of FBS (HyClone). The cells were introduced into the minimal essential medium supplemented with 10% to 20% of FBS to an optimized concentration, and cultured in a 5% CO_2 incubator at 37℃, while changing the culture medium twice a week. When the cultured cells formed a monolayer, and MSCs amplified in a spindle shape were identified using a phase contrast microscope, sub-cultures of the cells were repeated so as to sufficiently amplify the MSCs. The UCB-derived MSCs were cultured in α-MEM supplemented with 10 to 20% of FBS.

Example 3: Toxicity of amyloid-beta protein

In order to prepare ideal conditions for an outbreak of Alzheimer's disease, the

neurons differentiated as described in Example 1 were cultured in a serum-free Neurobasal™ culture medium without bFGF and B27 and including 10 μM of amyloid-beta protein fragment 1-42 (Aβ42, sigma, A9810) that is known to cause Alzheimer's disease. After 3 to 4 days of differentiation of the neural stem cells, morphological characteristics of the neural stem cells were observed using a microscope. If the differentiation into neurons was identified, the cells were treated with Aβ for 24 hours.

FIG. 1 illustrates optical microscopic images of live neurons untreated and treated with amyloid-beta for 24 hours to measure morphological changes of the neurons. As the concentration of the amyloid-beta increased, the number of dead neurons increased. In FIG. 1, the control shows neurons cultured in a serum-free Neurobasal™ culture medium without amyloid-beta, the Aβ-1μM, Aβ-5μM, and Aβ-10μM respectively show neurons cultured in culture media respectively including 1μM, 5μM, and 10μM of amyloid-beta for 24 hours.

Example 4: Effects of co-culture of human UCB-derived MSCs and neurons treated with amyloid-beta on death of neuron

When neurons treated with amyloid-beta were co-cultured with human UCB-derived MSCs, neurons damaged by toxic substances such as amyloid-beta were observed.

In particular, E14 embryo cerebral cortex stem cells and hippocampus stem cells were isolated, and the isolated stem cells were proliferated and differentiated into neurons in the same manner as described in Example 1, and then treated with 10 μM of amyloid-beta as in Example 3. After 12 hours of the amyloid-beta treatment, the neurons treated with the amyloid-beta were co-cultured with human UCB-derived MSCs in the presence of the amyloid-beta for 12 hours, so that the cells were cultured for 24 hours in total in the presence of the amyloid-beta. The co-culture was performed in a co-culture system as shown in FIG. 2. FIG. 2 shows a co-culture system for co-culturing neurons treated with amyloid-beta with human UCB-derived MSCs. Referring to FIG. 2, a co-culture system 100 includes an upper chamber 10 and a lower chamber 40, wherein the bottom of the upper chamber 10 includes a microporous membrane 30 having a pore size of about 1 μm. Human UCB-derived MSCs 20 were cultured in the upper chamber 10, and neurons 50 differentiated from cerebral cortex

stem cells or hippocampus stem cells were cultured in the lower chamber 40. The upper chamber 10 and the lower chamber 40 may be separated from each other, and the lower surface of the bottom of the upper chamber 10 is spaced apart from the upper surface of the bottom of the lower chamber 40 by about 1 mm. The co-culture was performed by respectively culturing cells in the lower chamber 40 and the upper chamber 10, and adding the upper chamber 10 to the culture medium of the lower chamber 40.

Cerebral cortex and hippocampus-derived neurons untreated, cerebral cortex and hippocampus-derived neurons treated with amyloid-beta, and cerebral cortex and hippocampus-derived neurons untreated with amyloid-beta and co-cultured with MSCs were also cultured and observed. Damaged cerebral cortex and hippocampus-derived neurons and human UCB-derived MSCs were co-cultured for 24 hours after the amyloid-beta treatment, and then the degree of the damage of the neurons was observed using a microscope. The cultivation was performed using serum-free Neurobasal™ culture media (GIBCO) without bFGF and B27.

In order to quantitively measure death of neuron caused by treatment with amyloid-beta, live and dead cells were measured using a fluorescent staining analysis. Cytoxicity was analyzed using a LIVE/DEAD™ viability/cytotoxicity assy kit for animal cells (Sigma, L3224). The kit includes calcein AM and ethidium homodimer, wherein the calcein AM is used to identify live cells, and the ethidium homodimer is used to identify dead cells. The calcein AM is a non-fluorescent cell permeable dye and converted into a green fluorescent calcein in a live cell by hydrolysis of acetoxymethyl ester by esterase in the cell. The ethidium homodimer cannot permeate a membrane of a live cell but permeates a damaged cell membrane and binds to nucleic acids of the cell to emit red fluorescence.

Cerebral cortex and hippocampus-derived neurons were cultured in a culture medium containng Aβ42 in a lower chamber 40 of the co-culture system 100 to directly treating the Aβ42 to the neurons. Dead cells were stained in red and live cells were stained in green by a live/dead staining. As a result, when cells treated with 10 μM Aβ42 for 24 hours (Ct+Aβ of FIG. 3) were compared with untreated cells (Ct of FIG. 3), green fluorescence was significantly reduced and a wide range of red fluorescence was observed by the treatment with Aβ42, thereby indicating that most neurons were dead by

the treatment with Aβ42. However, if the damaged neural stem cells were co-cultured with UCB-derived MSCs in the co-culture system 100 shown in FIG. 2, death of the neurons was prevented and maturation of neuron was increased (Ct+Aβ+MSC of FIG. 3). This indicates that if neurons damaged by Aβ42 are co-cultured with UCB-MSCs, the damaged cells may be restored. In FIG. 3, Ct+Aβ+MSC shows cerebral cortex-derived neurons cultured in a serum-free Neurobasal™ culture medium including 10μM of Aβ42 for 12 hours and then co-cultured with UCB-derived MSCs in the presence of 10μM of Aβ42 for 12 hours. In addition, when cerebral cortex-derived neurons were cultured in a serum-free Neurobasal™ culture medium in the presence of 10 μM of Aβ42 in the lower chamber 40 and UCB-derived MSCs were simultaneously cultured in the same culture medium in the upper chamber 10 for 24 hours, the results were the same shown in the Ct+Aβ+MSC of FIG. 3. Thus, if neurons were co-cultured with UCB-MSCs, the neurons damaged by Aβ42 may be restored and the damage by Aβ42 may be prevented.

In FIG. 3, Ct shows cerebral cortex-derived neurons cultured in a serum-free Neurobasal™ culture medium without Aβ42 for 24 hours, Ct+Aβ shows cerebral cortex-derived neurons cultured in a serum-free Neurobasal™ culture medium including 10 μM of Aβ42 for 24 hours, Ct+Aβ+MSC shows cerebral cortex-derived neurons cultured in a serum-free Neurobasal™ culture medium including 10 μM of Aβ42 for 12 hours and then co-cultured with UCB-derived MSCs in the presence of 10μM of Aβ42 for 12 hours, and Ct+MSC shows cerebral cortex-derived neurons cultured in a serum-free Neurobasal™ culture medium without Aβ42 for 12 hours and then co-cultured with UCB-derived MSCs for 12 hours.

FIG. 4 is a graph illustrating the percentage of dead neurons based on the results of FIG. 3. In FIG. 4, cortex shows the results of the control in which cerebral cortex-derived neurons were cultured in a culture medium without Aβ42, Cortex+Aβ shows the results of culturing cerebral cortex-derived neurons in a culture medium including 10 μM of Aβ42 for 24 hours, Cortex+Aβ+MSC shows the results of culturing cerebral cortex-derived neurons in a culture medium including 10 μM of Aβ42 for 12 hours and then co-culturing the cerebral cortex-derived neurons with human UCB-derived MSCs in the presence of 10μM of Aβ42 for 12 hours, and Cortex+MSC shows the results of culturing cerebral cortex-derived neurons in a culture medium

without Aβ42 for 12 hours and then co-culturing the cerebral cortex-derived neurons with human UCB-derived MSCs for 12 hours.

Example 5: Effects of co-culture of human bone marrow-derived MSCs and neurons treated with amyloid-beta on death of neuron

Experiments were performed in the same manner as in Example 4 using bone marrow-derived MSCs (BM-MSC) collected from donated bone marrow. When neurons treated with Aβ were co-cultured with bone marrow-derived MSCs, death of neuron was prevented as in Examples 4 (Ct/Aβ/BM-MSC of FIG. 5).

FIG. 5 illustrates results of fluorescent staining to explain effects of co-culturing neurons with human bone marrow-derived MSCs on death of neuron caused by Aβ42. In FIG. 5, Ct shows the results of the control in which cerebral cortex-derived neurons were cultured in a culture medium without Aβ, Ct+Aβ shows the results of culturing cerebral cortex-derived neurons in a culture medium including 10 μM of Aβ for 24 hours, Ct/Aβ/BM-MSC shows the results of culturing cerebral cortex-derived neurons in a culture medium including 10 μM of Aβ for 12 hours and then co-culturing the cerebral cortex-derived neurons with human bone marrow-derived MSCs in the presence of 10 μM of Aβ for 12 hours, and Ct+BM-MSC shows the results of culturing cerebral cortex-derived neurons in a culture medium without Aβ for 12 hours and then co-culturing the cerebral cortex-derived neurons with human bone marrow-derived MSCs for 12 hours.

Example 6: Effects of co-culture of human UCB-derived MSCs and neurons treated with amyloid-beta on phosphorylation of tau protein

FIG. 6 illustrates neurons stained using an anti-phosphor-tau antibody that is an antibody binding to phosphorylated tau by Aβ42, wherein tau is known as a protein inducing death of neuron. The anti-phosphor-tau antibody were conjugated with a red fluorescent Cy3 to visualize the binding of the anti-phosphor-tau antibody and the phosphor-tau.

The first row of FIG. 6 shows neurons stained with Cy3-conjugated anti-phosphor-tau antibody, and the second row of FIG. 6 shows neurons stained with 4',6-diamidino-2-phenylindole (DAPI). In the first row of FIG. 6, Ct shows the results of the control in which cerebral cortex-derived neurons were cultured in a culture medium without Aβ, Aβ42 shows the results of culturing cerebral cortex-derived neurons in a

culture medium including 10 μM of Aβ for 24 hours, Aβ42/MSC shows the results of culturing cerebral cortex-derived neurons in a culture medium including 10 μM of Aβ for 12 hours and then co-culturing the cerebral cortex-derived neurons with human UCB-derived MSCs in the presence of 10μM of Aβ42 for 12 hours, and MSC shows the results of culturing cerebral cortex-derived neurons in a culture medium without Aβ for 12 hours and then co-culturing the cerebral cortex-derived neurons with human UCB-derived MSCs for 12 hours. As shown in the first row of FIG. 6, tau protein was rapidly phosphorylated in the neurons but dephosphorylated by the co-culturing with the human UCB-derived MSCs (see Aβ42 and Aβ42/MSC of FIG. 6).

As shown in the second row of FIG. 6, DAPI staining shows that cerebral cortex-derived neurons that are not stained by the anti-phosphor-tau antibody in the first row of FIG. 6 are maintained. DAPI staining was performed using VECTASHIELD™ (VECTOR LABORATORIES), and a DAPI-containing mounting medium was added to a slide glass on which cells are deposited right before observing the cells using a microscope.

Example 7: Analysis of differentiated neurons using immunofluorescent staining when neurons treated with amyloid-beta are co-cultured with human UCB-derived MSCs

Neurons derived from the cerebral cortex and hippocampus were stained using antibodies specifically binding to microtubule-associated protein (MAP2) and Tubulin β III which are known as markers of differentiation of neurons.

An immunofluorescent staining was performed as follows. Neurons were fixed to wells of a 12-well plate using 4% paraformaldehyde for 20 minutes at room temperature, and washed four times with 0.1% BSA/PBS for 5 minutes each. Then, non-specific reaction was prevented by adding a solution containing 10% normal goat serum (NGS), 0.3% Triton X-100, and 0.1% BSA/PBS thereto and conducting reaction at room temperature for 30 to 45 minutes. A solution including a primary antibody, 10% NGS, and 0.1% BSA/PBS was added to the wells and reaction was conducted at 4℃ overnight. The resultant was washed three times with 0.1% BSA/PBS for 5 minutes each. A secondary antibody and a 0.1% BSA/PBS solution including a reagent binding to the secondary antibody was added thereto, and reaction was conducted for 4 minutes, and then the resultant was washed four times with 0.1% BSA/PBS for 5 minutes each.

The primary antibody was prepared by diluting monoclonal anti-Tubulin β III antibody produced in mouse (Sigma) and rabbit anti-microtubule associated protein (MAP) 2 polyclonal antibody (Chemicon) in a buffer solution respectively at 1:500 and 1:200. The secondary antibody was prepared by respectively diluting biotinylated anti-mouse antibody and biotinylated anti-rabbit antibody, (Vector) in a buffer solution at 1:200. The reagent binding to the secondary antibody was prepared by diluting dichlorotriazinyl fluorescein (DTAF, Jackson immuno Research) in a buffer solution at 1:200.

In the neurons (cerebral and hippocampus-derived neurons) treated with Aβ42, neurites were cleaved and the shape of neurons was condensed due to toxicity. On the other hand, in neurons co-cultured with UCB-derived MSCs, neurites were maintained and maturation of the neurons were accelerated (FIGS. 7A, 7B, and 7C).

FIG. 7 illustrates neurons treated with Aβ42, co-cultured with UCB-derived MSCs, and stained using immunofluorescent staining using anti-Tubulin β III and anti-MAP2 and western blotting.

FIG. 7A shows cerebral cortex-derived neurons, FIG. 7B shows hippocampus-derived neurons. MAP2 and Tubulin β III respectively show the results of the stained immunofluorescent staining using anti-MAP2 and anti-Tubulin β III. Control shows the results of the control in which cerebral cortex-derived neurons or hippocampus-derived neurons were cultured in a serum-free Neurobasal™ culture medium without Aβ for 24 hours, Aβ42 shows the results of culturing cerebral cortex-derived neurons or hippocampus-derived neurons in a culture medium including 10 μM of Aβ for 24 hours, Aβ42/MSC shows the results of culturing cerebral cortex-derived neurons or hippocampus-derived neurons in a serum-free Neurobasal™ culture medium including 10 μM of Aβ for 12 hours and then co-culturing the cerebral cortex-derived neurons or hippocampus-derived neurons with human UCB-derived MSCs in the presence of 10μM of Aβ42 for 12 hours, and MSC shows the results of culturing cerebral cortex-derived neurons or hippocampus-derived neurons in a culture medium without Aβ for 12 hours and then co-culturing the cerebral cortex-derived neurons or hippocampus-derived neurons with human UCB-derived MSCs for 12 hours.

FIG. 7C shows the results of co-culturing cerebral cortex-derived neurons treated with Aβ42 with UCB-derived MSCs and performing western blotting the co-cultured neurons using anti-MAP2 antibody. First, membranes of neurons were crushed using

an ultra-sonicator in a Lysis buffer containing sodium dodecyl sulfate (SDS) to extract protein. The extracted protein was electrophoresed using a SDS-polyacrylamide gel to separate the protein according to the size. When the electrophoresis was terminated, the protein was transferred to a nitrocellulose membrane using electrical properties of the protein and reacted with the anti-MAP2 antibody (Millipore chem) diluted in PBS containing 3% skimmed milk. Then, an anti-rabbit antibody (Vector) conjugated to streptavidin-conjugated dichlorotriazinyl fluorescein (DTAF, Jackson immuno Research) was added thereto, and the resultant was treated with a substrate of enhanced chemiluminescence (ECL) solution, and then the resultant was developted using an X-ray film. In FIG. 7C, Control, Aβ, Aβ+MSC and MSC are the same as described above. In FIG. 7C, 200 indicates the molecular weight marker of 200 kDa.

Example 8: Induction of expression of neprilysin by human UCB-derived MSCs in neurons and microglial cells

Neprilysin (NEP) is known as a protein degrading Aβ42 in vivo with insulin degrading enzyme (IDE). In addition, it has been reported that knockout of NEP caused symptoms of Alzheimer's disease in mice. Neurons prepared in Examples 4 to 7 were collected and lysed to extract protein. The protein was separated using electrophoresis in a SDS-PAGE, and expression of the protein was measured by western blotting the separated protein using anti-neprilysin antibody. In addition, mRNA expression of NEP was measured using an NEP-specific primer by RT-PCR. In addition, the cultured cells were stained with anti-NEP antibody.

First, neurons were fixed to wells of a 12-well plate using 4% paraformaldehyde for 20 minutes at room temperature, and washed four times with 0.1% BSA/PBS for 5 minutes each. Then, non-specific reactions were prevented by adding a solution containing 10% normal goat serum (NGS), 0.3% Triton X-100, and 0.1% BSA/PBS thereto at room temperature for 30 to 45 minutes. A 10% NGS containing a primary antibody and 0.1% BSA/PBS were added to the wells and reaction was conducted at 4℃ overnight. The resultant was washed three times with 0.1% BSA/PBS for 5 minutes each. A secondary antibody and 0.1% BSA/PBS solution containing a reagent binding to the secondary antibody were added thereto, and reaction was conducted at room temperature for 40 minutes, and the resultant was washed four times with 0.1%BSA/PBS for 5 minutes each. Monoclonal anti-NEP antibody produced in mouse

(Sigma) diluted in a buffer solution at 1:500 was used as the primary antibody. Biotinylated anti-mouse antibody (Vector) diluted in a buffer solution at 1:200 was used as the secondary antibody. Streptavidin-conjugated dichlorotriazinyl fluorescein (DTAF, Jackson Immuno Research) diluted in a buffer solution at 1:200 was used as the reagent binding to the secondary antibody.

FIG. 8 illustrates expression of neprilysin in rat neurons treated with Aβ42 and co-cultured with human bone marrow-derived MSCs or human UCB-derived MSCs.

In FIG. 8A, the top shows a western blotting analysis of cultured rat cerebral cortex-derived neurons. Neuron shows the results of the control in which rat cerebral cortex-derived neurons were cultured in a serum-free Neurobasal™ culture medium without Aβ for 24 hours, Neuron+Aβ shows the results of culturing rat cerebral cortex-derived neurons in a culture medium including 10 μM of Aβ for 24 hours, the Neuron+Aβ+MSC shows the results of culturing rat cerebral cortex-derived neurons in a serum-free Neurobasal™ culture medium including 10 μM of Aβ for 12 hours and then co-culturing the rat cerebral cortex-derived neurons with human UCB-derived MSCs in the presence of 10μM of Aβ for 12 hours, and Neuron+MSC shows the results of culturing rat cerebral cortex-derived neurons in a culture medium without Aβ for 12 hours and then co-culturing the rat cerebral cortex-derived neurons with human UCB-derived MSCs for 12 hours.

In FIG. 8A, the bottom shows a RT-PCR result using mRNA isolated from the cultured rat neurons as a template. PCR primers specific for NEP genes of a rat (SEQ ID NOS: 15 and 16) and PCR primers specific for β-actin genes (SEQ ID NOS: 17 and 18) were used. As a result of RT-PCR, amplified NEP gene (422bp) and amplified β-actin gene (300bp) were produced. Neuron, Neuron+Aβ, Neuron+Aβ+MSC, and Neuron+MSC are described above.

As shown in FIG. 8A, if rat neurons were treated with Aβ42, the expression of NEP was reduced. If the rat neurons treated with Aβ42 were co-cultured with human UCB-derived MSCs, the expression of NEP was increased in the protein and mRNA level. This indicates that human MSCs stimulate rat neurons to increase production of NEP and remove toxic Aβ42 protein.

In FIG. 8B, Ct, Aβ, Aβ+MSC, and MSC respectively correspond to Neuron, Neuron+Aβ, Neuron+Aβ+MSC, and Neuron+MSC.

The cells were stained according to the following process. First, neurons were fixed to wells of a 12-well plate using 4% paraformaldehyde for 20 minutes at room temperature, and washed four times with 0.1% BSA/PBS for 5 minutes each. Then, non-specific reactions were prevented by adding a solution containing 10% normal goat serum (NGS), 0.3% Triton X-100, and 0.1% BSA/PBS thereto at room temperature for 30 to 45 minutes. A 10% NGS containing a primary antibody and 0.1% BSA/PBS were added to the wells and reaction was conducted at 4℃ overnight. The resultant was washed three times with 0.1% BSA/PBS for 5 minutes each. A secondary antibody and a 0.1% BSA/PBS solution containing a reagent binding to the secondary antibody was added thereto, and reaction was conducted at room temperature for 40 minutes, and the resultant was washed four times with 0.1%BSA/PBS for 5 minutes each. Monoclonal anti-NEP antibody produced in mouse (Sigma) diluted in a buffer solution at 1:500 was used as the primary antibody. Biotinylated anti-mouse antibody (Vector) diluted in a buffer solution at 1:200 was used as the secondary antibody. Streptavidin-conjugated dichlorotriazinyl fluorescein (DTAF, Jackson immuno Research) diluted in a buffer solution at 1:200 was used as the reagent binding to the secondary antibody.

As shown in FIG. 8B, if the neurons were treated with Aβ42, the portion stained in red was considerably reduced, thereby indicating that the expression of NEP is reducing in the neurons. However, if the neurons were co-cultured with MSCs, the expression of the NEP was restored.

FIG. 8C shows the results of RT-PCR indicating that the expression of NEP in rat neurons was increased using bone marrow-derived MSCs (BM-MSCs).

The RT-PCR of NEP and β-actin were performed in the same condition using the same primers described with reference to FIG. 8A. In FIG. 8C, Lane 1 shows the results of the control in which rat cerebral cortex-derived neurons were cultured in a serum-free Neurobasal™ culture medium without Aβ for 24 hours, Lanes 2 and 3 show the results of culturing rat cerebral cortex-derived neurons in a culture medium without including Aβ for 12 hours, and then co-culturing the rat cerebral cortex-derived neurons with human bone marrow-derived MSCs (BM-MSC1 and BM-MSC2) for 12 hours. In this regard, BM-MSC1 and BM-MSC2 represents cells obtained from different donors. The results shown in FIG. 8C exhibit an increase of NEP expression in rat cerebral cortex-derived neurons when rat cerebral cortex-derived neurons are co-cultured with

human BM-MSC at mRNA level. Further, according to the western blotting analysis and immunoblotting analysis, it was confirmed that when rat cerebral cortex-derived neurons are co-cultured with human BM-MSC, the NEP expression in the neurons are increased at a protein level.

The brain includes not only neurons but also microglial cells which are known as macrophage of the brain and remove toxic substances accumulated in the brain. The microglial cells remove Aβ in Alzheimer's disease. According to a recent report, a reduction in the expression of NEP in the microglial cells accelerates the progress of Alzheimer's disease. Thus, restoration of expression of NEP by human UCB cells was identified in neurons and microglial cells using an immunofluorescent staining (FIG. 9). FIG. 9 illustrates expression of neprilysin in neurons and microglial cells when neurons treated with Aβ42 are co-cultured with MSCs.

The first row of FIG. 9 shows cerebral cortex-derived neurons cultured in a serum-free Neurobasal™ culture medium including 10 μM of Aβ for 12 hours, then co-cultured with human UCB-derived MSCs in the presence of 10μM of Aβ42 for 12 hours, and double stained using an antibody specifically binding to each of the markers of neurons MAP2 and NEP. The staining was performed in the same manner as in FIG. 8B, except that a rabbit anti-MAP2 antibody was used as a primary antibody, a biotinylated anti-rabbit antibody was used as a secondary antibody binding to the primary antibody, and streptavidin-conjugated dichlorotriazinyl fluorescein (DTAF, Jackson immuno Research) was used as a reagent binding to the secondary antibody for MAP2, and a monoclonal anti-NEP antibody produced in mouse (Sigma) was used as a primary antibody, a biotinylated anti-mouse antibody (Vector) was used as a secondary antibody, and streptavidin-conjugated dichlorotriazinyl fluorescein (DTAF, Jackson immuno Research) was used as a reagent binding to the secondary antibody for NEP. In the first row of FIG. 9, MAP2 and NEP show the neurons stained respectively using the anti-MAP2 antibody and the anti-NEP antibody, and MAP2+NEP shows an overlap image of the neurons stained respectively using the anti-MAP2 antibody and the anti-NEP antibody. DAPI shows the results stained using DAPI in the same manner as in the second row of FIG. 6.

Since both MAP2 and NEP show stained cells as shown in the first row of FIG. 9, it was identified that both of MAP2 and NEP are expressed in the neurons. In addition,

as a result of the image overlap (MAP2+NEP), MAP2 and NEP are found in the same area, and thus it was identified that both of MAP2 and NEP are expressed. The neurons were stained by DAPI, and thus it was identified that the neurons are maintained in normal conditions.

The second row of FIG. 9 shows the results of the same experiments shown in the first row of FIG. 9, except that microglial cells were used instead of neurons and CD40 and NEP, as markers of microglial cells, were used, as markers of microglial cells instead of MAP2 and NEP. The staining of CD40 was performed using a goat anti-CD40 antibody as a primary antibody for CD40, biotin-conjugated anti-goat antibody as a secondary antibody binding to the primary antibody, and streptavidin-conjugated dichlorotriazinyl fluorescein (DTAF, Jackson immuno Research) diluted in a buffer solution at 1:200 as a reagent binding to the secondary antibody.

Since both CD40 and NEP show stained cells as shown in the second row of FIG. 9, it was identified that both of CD40 and NEP are expressed in the microglial cells. In addition, as a result of the image overlap (CD40+NEP), CD40 and NEP are found in the same area, and thus it was identified that both of MAP2 and NEP are expressed in the microglial cells. The microglial cells were stained by DAPI, and thus it was identified that the microglial cells are maintained in normal conditions.

According to the results of the first and second rows of FIG. 9, if the neurons and the microglial cells are co-cultured with UCB-derived MSCs, the expression of NEP was induced in the neurons and the microglial cells treated with Aβ.

Example 9: Identification of protein secreted by MSCs and preventing toxicity of Aβ42 and verification of effects of the protein

As a result of Examples 4 to 8, it was identified that toxicity of Aβ42 was inhibited in the neurons, if the neurons treated with Aβ42 were co-cultured with MSCs without direct contact therebetween. It can be predicted that the toxicity of Aβ42 can be inhibited by the interaction between substances secreted from the MSCs and the neurons.

In Example 9, substances that are secreted from the MSCs and inhibit toxicity of Aβ42 are detected and identified.

(1) Detecting MSC-derived substances inhibiting toxicity of Aβ42

First, cells were cultured in various conditions.

Culture group 1: Cerebral cortex-derived neurons were cultured in a serum-free Neurobasal™ culture medium without Aβ for 24 hours.

Culture group 2: Cerebral cortex-derived neurons were cultured in a serum-free Neurobasal™ culture medium including 10 μM of Aβ for 24 hours.

Culture group 3: Cerebral cortex-derived neurons were cultured in a serum-free Neurobasal™ culture medium including 10 μM of Aβ for 12 hours and then co-cultured with human UCB-derived MSCs in the presence of 10μM of Aβ for 12 hours.

Culture group 4: Human UCB-derived MSCs were cultured in a serum-free Neurobasal™ culture medium including 10 μM of Aβ for 24 hours.

Culture groups 5 and 6: Human UCB-derived MSCs were cultured in a serum-free Neurobasal™ culture medium for 24 hours.

Then, the culture media of Culture groups 1 to 6 were collected, and cytokine and protein were assayed and compared with each other to detect cytokine or protein that are not expressed or rarely expressed when stem cells are only cultured but increasingly expressed when the stem cells and the neurons are co-cultured. The cytokine assay was performed using RayBio™ Human Cytokine Antibody Array I G series (RayBiotech, Inc), and the protein assay was performed using RayBio™ Human Cytokine Antibody Array I G series/Biotin Label Based Antibody Array I G series (RayBiotech, Inc). 54,504 proteins may be assayed using the two arrays.

By comparing data of the assays, protein that is not expressed or rarely expressed when stem cells are only cultured but increasingly expressed when the stem cells and the neurons are co-cultured was selected. As a result, the following 14 proteins were identified:

Activin A, platelet factor 4 (PF4), decorin, galectin 3, growth differentiation factor 15 (GDF15), glypican 3, membrane-type frizzled-related protein (MFRP), intercellular adhesion molecule 5 (ICAM5), insulin-like growth factor binding protein 7 (IGFBP7), platelet-derived growth factor-AA (PDGF-AA), secreted protein acidic and rich in cysteine (SPARCL1), thrombospondin-1 (TSP1), wnt-1 induced secreted protein 1 (WISP1), and progranulin (PGN).

It was estimated that the 14 proteins inhibit toxicity of neuron treated with Aβ and promote differentiation and maturation of the neurons.

(2) Identifying activity of detected 14 proteins

Recombinant proteins of the detected 14 proteins were purchased from (R&D SYSTEMS). Then, cerebral cortex-derived neurons were treated with Aβ and cultured in a serum-free Neurobasal™ culture medium respectively containing 25 ng/ml of activin A, 25 ng/ml of PF4, 3 ng/ml of galectin 3, 100 ng/ml of decorin, 50 ng/ml of GDF15, 50 ng/ml of glypican 3, 50 ng/ml of MFRP, 50 ng/ml of ICAM5, 30 ng/ml of IGFBP7, 50 ng/ml of PDGF-AA, 50 ng/ml of SPARCL1, 50 ng/ml of TSP1, 50 ng/ml of WISP1 and 50 ng/ml of progranulin, for 24 hours. Then, the death of neuron was measured by fluorescent staining using a LIVE/DEAD™ viability/cytotoxicity assay kit (Sigma, L3224). The degree of cell death caused by Aβ was calculated based on the numbers of dead cells and live cells. The cell death was calculated using a ratio of the number of dead cells to the total number of cells.

FIG. 10 is a graph illustrating the percentage of dead neurons treated with Aβ42 and co-cultured with proteins secreted from MSCs. In FIG. 10, Cortex shows cerebral cortex-derived neurons cultured in a serum-free Neurobasal™ culture medium without Aβ42 for 24 hours, Cortex+Aβ shows cerebral cortex-derived neurons cultured in a serum-free Neurobasal™ culture medium including 10 μM of Aβ42 for 24 hours, Cortex+Aβ+MSC shows cerebral cortex-derived neurons cultured in a serum-free Neurobasal™ culture medium including 10 μM of Aβ42 for 12 hours and then co-cultured with UCB-derived MSCs in the presence of 10μM of Aβ42 for 12 hours, and Cortex+MSC shows cerebral cortex-derived neurons cultured in a serum-free Neurobasal™ culture medium without Aβ42 for 12 hours and then co-cultured with UCB-derived MSCs for 12 hours. Aβ shows cerebral cortex-derived neurons cultured in a serum-free Neurobasal™ culture medium including Aβ42 and each of the 14 proteins having a concentration described above or 24 hours (In FIG. 10, $p<0.03$ and $p<0.01$ respectively indicate that error ranges of t-tests are respectively less than 3% and 1%).

As shown in FIG. 10, each of the 14 proteins inhibited the death of neuron caused by Aβ42. The degree of inhibiting the cell death decreases in the order of Cortex+Aβ+MSC, galectin 3, WISP1, and MFRP. This indicates that the co-culture with the MSCs, i.e., the combination of the 14 proteins has the greatest effect on inhibiting toxicity of Aβ.

In order to measure effects of protein on maturation of the neurons, the length of

neurites in the cultured cells was measured. The neurons were cultured in the same conditions described with reference to FIG. 10. 100 cells were randomly selected from each culture group, and the length of neurites was measured using i-solution software (iMTechnology).

FIG. 11 is a graph illustrating the length of neurites of neurons cultured with Aβ42 and proteins secreted from MSCs. In FIG. 11, the culture groups are the same as those in FIG. 10, and the length of neurites are an average length. As shown in FIG. 11, each of the 14 proteins or a combination of the 14 proteins significantly increased the length of neurites compared to the neurons treated with Aβ42.

Example 10: Identification of cytokine secreted from MSCs and inducing expression of neprilysin in microglial cells

The co-culture system 100 described in Example 4 was used herein. Microglial cells (BV2) were cultured in the lower chamber 40, and UCB-derived MSCs (UCB-MSC) were cultured in the upper chamber 10. BV2 cells are immortalized cells prepared by infecting microglial cells of a mouse with v-raf/v-myc recombinant retrovirus and express traits of activated microglial cells. The co-culture was performed by culturing BV2 cells in a DMEM supplemented with 5% FBS in the lower chamber 40, adding UCB-derived MSCs cultured in a α-MEM supplemented with 5% FBS to the upper chamber 10, and replacing the culture medium with a serum-free DMEM. The cells were co-cultured in a serum-free DMEM for 24 hours. Then, the MSCs were collected from the upper chamber 10, and total RNA was obtained using a trizol reagent, and then RT-PCR was performed using the total RNA as a template. Primers that amplify genes of IL-4 (SEQ ID NOS: 22 and 23), IL-6 (SEQ ID NOS: 24 and 25), IL-8 (SEQ ID NOS: 26 and 27) and monocyte chemoattractant protein-1 (MCP-1, SEQ ID NOS: 28 and 29) were used. As a control group, β-actin was amplified using primers (SEQ ID NOS: 17 and 18). In the control group, UCB-derived MSCs (UCB-MSC) cultured in the same conditions described above, except that the UCB-derived MSCs were not co-cultured with microglial cells (BV2), were used.

FIG. 12 shows the results of RT-PCR using the total RNA isolated from UCB-MSC after co-culturing microglial cells with UCB-MSC as a template. As shown in FIG. 12, if the microglial cells and UCB-MSC are co-cultured, the expression of IL-4, IL-6, IL-8, and MCP-1 in UCB-MSC increased.

Microglial cells, BV2 cells, neurons, and SH-SY5Y cells (ATCC) were cultured respectively in the presence of IL-4, IL-6, IL-8 and MCP-1, and then BV2 cells and SH-SY5Y cells were collected. The collected cells were lysed and proteins were separated from the lysates according to the size, and the resultant was western blotted using an anti-NEP antibody. As a result, the expression of NEP increased with time in BV2 cells and SHY-5Y cells cultured in the presence of IL-4 when compared to in the absence of IL-4. The SH-SY5Y cells are thrice-cloned neurobastoma derived from SK-N-SH. The SH-SY5Y cells represent neuronal cells.

FIG. 13 shows the results of western blotting indicating the increase in the expression of NEP when neurons and microglial cells are cultured in the presence of IL-4. FIG. 13A shows the results of western blotting of microglial cells (BV2 cells) cultured in DMEM including 10ng/ml of IL-4 for 24 hours. FIG. 13B shows the results of western blotting of neurons (SH-SY5Y cells) cultured in α-MEM including 10ng/ml of IL-4 for 24 hours.

Example 11: Reduction of amyloid protein plaque by administering UCB-derived MSCs into hippocampus and cortex of a mouse transformed to have Alzheimer's disease (thioflavin-S staining and immuno-blotting)

In order to improve effects of the treatment, PBS, $1x10^4$ of UCB-derived MSCs in PBS, and 200㎍/kg (weight) of IL-4 (Peprotech) in PBS were administered into hippocampus of a 10 month-old mouse transformed to have Alzheimer's disease using a stereotactic frame. After 10 days, the mouse was killed, and brain tissue weres collected from hippocampus and cerebral cortex thereof. The obtained brain tissues were cut into slices and stained using thiosulfate (Sigma) to identify the amyloid-beta protein plaque. In order to identify the plaque, the brain tissue was reacted with a thioflavin solution (Sigma) dissolved in 50% ethanol for 5 minutes. After the reaction, the slices of the brain tissue was washed with 50% ethanol and water for 5 minutes. This slices were observed using a fluorescent microscope to identigy amyloid protein plaque in the brain tissue.

FIG. 14 shows images of amyloid-beta protein plaque in a brain tissue including hippocampus and cerebral cortex stained using a Thio-S staining. As shown in FIG. 14, the amyloid-beta protein plaque was significantly reduced in the culture groups into which UCB-derived MSCs and IL-4 were administered. In FIG. 14, PBS, MSC, and IL-4

respectively show the culture groups into which PBS, UCB-derived MSCs, and IL-4 were administered.

FIG. 15 is a graph illustrating the total area of amyloid-beta plaque in the images of FIG. 14. The area was measured using a Metamorpho software (Molecular devices). As shown in FIG. 15, the amyloid-beta plaque was significantly reduced in the culture groups into which MSCs and IL-4 were administered when compared to the control group.

FIG. 16 shows the results of immunoblotting indicating the change of amyloid-beta protein produced in the brain of a mouse used for an experiment. The graph of FIG. 16 was obtained according to the following process. First, protein was extracted from a brain tissue of a mouse including hippocampus and cerebral cortex and treated in the conditions described above using a sonicator (Branson). Then, the extract was separated according to the size using electrophoresis. The separated protein was transferred to a nitrocellulose membrane by a potential difference and an immuno-blotting was performed using an antibody capable of specifically detecting Aβ42. The proteins were stained using coomassie blue (bottom part). As shown in FIG. 16, the amount of Aβ42 protein was significantly reduced in the culture groups into which MSCs and IL-4 were administered when compared to the culture group into which PBS was administered. In FIG. 16, Litter indicates a littermate of a transformed mouse, and APP/PS1 mice indicates a mouse transformed to have Alzhemer's disease. In addition, PBS, MSC and IL-4 respectively show the culture groups into which PBS, MSC and IL-4 were administered.

Example 12: Effect of UCB-derived MSCs and IL-4 on expression of NEP

(1) Expression of NEP in brain tissue of normal animal and animal transformed to have Alzheimer's disease

Brain tissues of normal mice and mice transformed to have Alzheimer's diseases respectively raised for 6, 9, 12 and 18 months were obtained, and protein was extracted in the same manner as in Example 11 and separated using electrophoresis. The separated protein was transferred to a nitrocellulose membrane and reacted with anti-NEP antiboty (R&D systems) to analyze the expression of NEP.

FIG. 17 shows the degree of expression of NEP in a brain tissue of a normal mouse and a mouse transformed to have Alzheimer's disease including hippocampus

and cerebral cortex. As shown in FIG. 17, the expression of NEP was reduced in the brain tissue of the mouse transformed to have Alzheimer's disease. In FIG. 17, Litter and APP/PS1 mice are the same as those described with reference to FIG. 16. In additon, Lanes 6, 9, 12, and 18 respectively show the culture group cultured for 6, 9, 12, and 18 months (M: month).

FIG. 18 is a graph illustrating band intensity of NEP of FIG. 17 measured using Quantity One software (Bio-RAD). The band intensity is a relative intensity. As shown in FIG. 18, the expression of NEP was reduced in the brain tissue of the mouse transformed to have Alzheimer's disease comapred to that of the normal mouse.

(2) Effect of UCB-derived MSCs and IL-4 on expression of NEP

PBS, $1x10^4$ of UCB-derived MSCs in PBS, and 200μg/kg (weight) of IL-4 in PBS (Peprotech) were administered into hippocampus of a 10 month-old mouse transformed to have Alzheimer's disease. After 10 days, the mouse was killed, and brain tissue including hippocampus and cerebral cortex was collected. Proteins were extracted from each brain tissue and separated using electrophotosis to analyze the amount of expressed NEP using an immuno-blotting.

FIG. 19 shows the degree of expression of NEP in a brain tissue of a mouse into which MSCs and IL-4 are administered and including hippocampus and cerebral cortex. Coomassie blue was used for staining (bottom part). As shown in FIG. 19, the expression of NEP was reduced in the culture group into which PBS was administered when compared to the normal mouse as shown in operation (1) described above, and the expression of NEP in the culture group into which UCB-derived MSCs and IL-4 were administered was similar to that of the normal mouse.

Example 13: Effect of UCB-derived MSCs and IL-4 on expression of NEP in microglial cells

In Example 8, it was identified that NEP was overexpressed in neurons and microglial cells when the neurons and microglial cells are respectively co-cultured with MSCs.

In Example 13, this effect was identified in an animal model. Brain hippocampus tissue of the culture groups into which PBS, UCB-derived MSCs, and IL-4 were administered described in Example 12 were stained in the same manner as shown in FIG. 8B. The anti-NEP antibody and the anti-CD40 antibody, as a marker of microglial

cells (Santacruz Biotechnology) were used and the results were merged. In the anti-NEP antibody staining, the secondary antibody and the reagent binding to the secondary antibody are the same as those described in Examle 8. Also, in the anti-CD40 antibody staining, the secondary antibody and the reagent binding to the secondary antibody are the same as those described in Example 8.

FIG. 20 shows the expression of NEP in microglial cells of a mouse into which UCB-derived MSCs and IL-4 are administered. As shown in FIG. 20, when UCB-derived MSCs and IL-4 are administered into an animal model, overexpression of NEP was induced in microglial cells.

While the present invention has been particularly shown and described with reference to exemplary embodiments thereof, it will be understood by those of ordinary skill in the art that various changes in form and details may be made therein without departing from the spirit and scope of the present invention as defined by the following claims.

CLAIMS

1. A pharmaceutical composition for the prevention or treatment of a neural disease, comprising at least one selected from the group consisting of mesenchymal stem cells (MSCs) and a culture solution of the MSCs.

2. The pharmaceutical composition of claim 1, wherein the mesenchymal stem cells is MSCs isolated from at least one tissue having mesenchymal stem cells selected from the group consisting of human embryonic yolk sac, placenta, umbilical cord, umbilical cord blood, skin, peripheral blood, bone marrow, adipose tissue, muscle, liver, neural tissue, periosteum, fetal membrane, synovial membrane, synovial fluid, amniotic membrane, meniscus, anterior cruciate ligament, articular chondrocytes, decidous teeth, pericyte, trabecular bone, infra patellar fat pad, spleen, and thymus, and/or MSCs expanded from the MSCs.

3. The pharmaceutical composition of claim 1, wherein the mesenchymal stem cells comprise umbilical cord blood-derived mesenchymal stem cells or bone marrow-derived mesenchymal stem cells.

4. The pharmaceutical composition of claim 1, wherein the culture solution comprises at least one selected from the group consisting of activin A, platelet factor 4 (PF4), decorin, galectin 3, growth differentiation factor 15 (GDF15), glypican 3, membrane-type frizzled-related protein (MFRP), intercellular adhesion molecule 5 (ICAM5), insulin-like growth factor binding protein 7 (IGFBP7), platelet-derived growth factor-AA (PDGF-AA), secreted protein acidic and rich in cysteine (SPARCL1), thrombospondin-1, wnt-1 induced secreted protein 1 (WISP1), progranulin, IL-4, a factor inducing expression thereof, and any combination thereof.

5. The pharmaceutical composition of claim 1, wherein the neural disease is a disease caused by at least one selected from the group consisting of formation of amyloid-beta plaque in neural tissues, phosphorylation of tau protein in neurons, damage to neurites, reduction in expression of Neprilysin in neurons, and any combination thereof.

6. The pharmaceutical composition of claim 1, wherein the neural diseases comprises at least one selected from the group consisting of Alzheimer's disease, Parkinson's disease, depression, epilepsy, multiple sclerosis, and mania.

7. A pharmaceutical composition for the prevention or treatment of a neural disease, comprising at least one selected from the group consisting of activin A, platelet factor 4 (PF4), decorin, galectin 3, growth differentiation factor 15 (GDF15), glypican 3, membrane-type frizzled-related protein (MFRP), intercellular adhesion molecule 5 (ICAM5), insulin-like growth factor binding protein 7 (IGFBP7), platelet-derived growth factor-AA (PDGF-AA), secreted protein acidic and rich in cysteine (SPARCL1), thrombospondin-1, wnt-1 induced secreted protein 1 (WISP1), progranulin, IL-4, a factor inducing expression thereof, and any combination thereof.

8. The pharmaceutical composition of claim 7, wherein the neural disease is a disease caused by at least one selected from the group consisting of formation of amyloid-beta plaque in neural tissues, phosphorylation of tau protein in neurons, damage to neurites, reduction in expression of Neprilysin in neurons, and any combination thereof.

9. The pharmaceutical composition of claim 7, wherein the neural diseases comprises at least one selected from the group consisting of Alzheimer's disease, Parkinson's disease, depression, epilepsy, multiple sclerosis, and mania.

10. A kit for preventing neurocytoxicity caused by amyloid-beta, comprising at least one selected from the group consisting of mesenchymal stem cells (MSCs) and a culture solution of the MSCs.

11. A kit for preventing phosphorylation of tau protein in neurons, comprising at least one selected from the group consisting of mesenchymal stem cells (MSCs) and a culture solution of the MSCs.

12. A kit for inducing expression of neprilysin in neurons and/or microglial cells, comprising at least one selected from the group consisting of mesenchymal stem cells (MSCs) and a culture solution of the MSCs.

13. A kit for the preventing neurocytoxicity by amyloid-beta, comprising at least one selected from the group consisting of activin A, platelet factor 4 (PF4), decorin, galectin 3, growth differentiation factor 15 (GDF15), glypican 3, membrane-type frizzled-related protein (MFRP), intercellular adhesion molecule 5 (ICAM5), insulin-like growth factor binding protein 7 (IGFBP7), platelet-derived growth factor-AA (PDGF-AA), secreted protein acidic and rich in cysteine (SPARCL1), thrombospondin-1, wnt-1 induced secreted protein 1 (WISP1), progranulin, IL-4, a factor inducing expression thereof, and any combination thereof.

14. A kit for the preventing phosphorylation of tau protein in neurons, comprising at least one selected from the group consisting of activin A, platelet factor 4 (PF4), decorin, galectin 3, growth differentiation factor 15 (GDF15), glypican 3, membrane-type frizzled-related protein (MFRP), intercellular adhesion molecule 5 (ICAM5), insulin-like growth factor binding protein 7 (IGFBP7), platelet-derived growth factor-AA (PDGF-AA), secreted protein acidic and rich in cysteine (SPARCL1), thrombospondin-1, wnt-1 induced secreted protein 1 (WISP1), progranulin, IL-4, a factor inducing expression thereof, and any combination thereof.

15. A kit for inducing expression of neprilysin in neurons and/or microglial cells, comprising at least one selected from the group consisting of activin A, platelet factor 4 (PF4), decorin, galectin 3, growth differentiation factor 15 (GDF15), glypican 3, membrane-type frizzled-related protein (MFRP), intercellular adhesion molecule 5 (ICAM5), insulin-like growth factor binding protein 7 (IGFBP7), platelet-derived growth factor-AA (PDGF-AA), secreted protein acidic and rich in cysteine (SPARCL1), thrombospondin-1, wnt-1 induced secreted protein 1 (WISP1), progranulin, IL-4, a factor inducing expression thereof, and any combination thereof.

16. A method of preventing or treating a neural disease of an individual, the method comprising administering a pharmaceutical composition comprising at least one selected from the group consisting of mesenchymal stem cells (MSCs) and a culture solution of the MSCs to the individual.

17. The method of claim 16, wherein the neural disease is a disease caused

by at least one selected from the group consisting of formation of amyloid-beta plaque in neural tissues, phosphorylation of tau protein in neurons, damage to neurites, reduction in expression of Neprilysin in neurons, and any combination thereof.

18. The method of claim 16, wherein the neural diseases comprises at least one selected from the group consisting of Alzheimer's disease, Parkinson's disease, depression, epilepsy, multiple sclerosis, and mania.

19. A method of preventing or treating a neural disease of an individual, the method comprising administering a pharmaceutical composition comprising at least one selected from the group consisting of activin A, platelet factor 4 (PF4), decorin, galectin 3, growth differentiation factor 15 (GDF15), glypican 3, membrane-type frizzled-related protein (MFRP), intercellular adhesion molecule 5 (ICAM5), insulin-like growth factor binding protein 7 (IGFBP7), platelet-derived growth factor-AA (PDGF-AA), secreted protein acidic and rich in cysteine (SPARCL1), thrombospondin-1, wnt-1 induced secreted protein 1 (WISP1), progranulin, IL-4, a factor inducing expression thereof, and any combination thereof to the individual.

20. The method of claim 19, wherein the neural disease is a disease caused by at least one selected from the group consisting of formation of amyloid-beta plaque in neural tissues, phosphorylation of tau protein in neurons, damage to neurites, reduction in expression of Neprilysin in neurons, and any combination thereof.

21. The methodof claim 19, wherein the neural diseases comprises at least one selected from the group consisting of Alzheimer's disease, Parkinson's disease, depression, epilepsy, multiple sclerosis, and mania.

22. A method of reducing amyloid plaque in neural tissues, by culturing the neural tissues in the presence of at least one selected from the group consisting of mesenchymal stem cells (MSCs) and a culture solution of the MSCs.

23. A method of reducing the degree of phosphorylation of tau protein in neurons, by culturing the neurons in the presence of at least one selected from the group consisting of mesenchymal stem cells (MSCs) and a culture solution of the MSCs.

24. A method of increasing expression of neprilysin of cells, by culturing the cells in the presence of at least one selected from the group consisting of mesenchymal stem cells (MSCs) and a culture solution of the MSCs, wherein the cells is at least one cells selected from the group consisting of neuronal cells and microglial cells.

25. A method of increasing growth of neurites of neurons, by culturing the neurons in the presence of at least one selected from the group consisting of mesenchymal stem cells (MSCs) and a culture solution of the MSCs.

26. A method of reducing amyloid plaque in neural tissues, by culturing the neural tissues in the presence of at least one selected from the group consisting of activin A, platelet factor 4 (PF4), decorin, galectin 3, growth differentiation factor 15 (GDF15), glypican 3, membrane-type frizzled-related protein (MFRP), intercellular adhesion molecule 5 (ICAM5), insulin-like growth factor binding protein 7 (IGFBP7), platelet-derived growth factor-AA (PDGF-AA), secreted protein acidic and rich in cysteine (SPARCL1), thrombospondin-1, wnt-1 induced secreted protein 1 (WISP1), progranulin, IL-4, a factor inducing expression thereof, and any combination thereof.

27. A method of reducing the degree of phosphorylation of tau protein in neurons, by culturing the neurons in the presence of at least one selected from the group consisting of activin A, platelet factor 4 (PF4), decorin, galectin 3, growth differentiation factor 15 (GDF15), glypican 3, membrane-type frizzled-related protein (MFRP), intercellular adhesion molecule 5 (ICAM5), insulin-like growth factor binding protein 7 (IGFBP7), platelet-derived growth factor-AA (PDGF-AA), secreted protein acidic and rich in cysteine (SPARCL1), thrombospondin-1, wnt-1 induced secreted protein 1 (WISP1), progranulin, IL-4, a factor inducing expression thereof, and any combination thereof.

28. A method of increasing expression of neprilysin of cells, by culturing the cells in the presence of at least one selected from the group consisting of activin A, platelet factor 4 (PF4), decorin, galectin 3, growth differentiation factor 15 (GDF15), glypican 3, membrane-type frizzled-related protein (MFRP), intercellular adhesion molecule 5 (ICAM5), insulin-like growth factor binding protein 7 (IGFBP7), platelet-derived growth factor-AA (PDGF-AA), secreted protein acidic and rich in

cysteine (SPARCL1), thrombospondin-1, wnt-1 induced secreted protein 1 (WISP1), progranulin, IL-4, a factor inducing expression thereof, and any combination thereof wherein the cells is at least one cells selected from the group consisting of neuronal cells and microglial cells.

29. A method of increasing growth of neurites of neurons, by culturing the neurons in the presence of at least one selected from the group consisting of activin A, platelet factor 4 (PF4), decorin, galectin 3, growth differentiation factor 15 (GDF15), glypican 3, membrane-type frizzled-related protein (MFRP), intercellular adhesion molecule 5 (ICAM5), insulin-like growth factor binding protein 7 (IGFBP7), platelet-derived growth factor-AA (PDGF-AA), secreted protein acidic and rich in cysteine (SPARCL1), thrombospondin-1, wnt-1 induced secreted protein 1 (WISP1), progranulin, IL-4, a factor inducing expression thereof, and any combination thereof.