COMPOSITION FOR THE DIAGNOSIS, PREVENTION OR TREATMENT OF DISEASES RELATED TO CELLS EXPRESSING IL-8 OR GRO-ALPHA, COMPRISING UCB-MSCS

TECHNICAL FIELD

The present invention relates to a gene therapy composition for transferring a therapeutical gene, a marker gene, or a product thereof to a cell that expresses interleukin-8(IL-8) or GRO-α and induces tropism of umbilical cord blood-derived mesenchymal stem cells or mesenchymal stem cells isolated from umbilical cord blood and/or the mesenchymal stem cells expanded from said mesenchymal stem cells (UCB-MSCs), wherein the gene therapy composition includes UCB-MSCs.

The present invention also relates to treating disease related to a cell expressing IL-8 or GRO-α, or brain tumors in gene therapy, using the composition includes UCB-MSCs.

The present invention also relates to a composition or kit for diagnosing brain tumors, preventing brain tumors, treating brain tumors, or monitoring brain tumor treatment progression, using UCB-MSCs.

BACKGROUND ART

It is known that stem cells migrate toward sites of pathology. Recently, it was found that bone marrow-derived mesenchymal stem cells (BM-MSCs) have a tropism for tumors and migrate toward tumor sites. Such BM-MSCs that can migrate to sites of specific tumors may prove to be a useful tool in gene therapy. For example, BM-MSCs having a tropism for tumors can be used as vehicles for transferring a therapeutic suicide gene to tumor sites [see Ponte A.L. et al., *Stem Cells*, 25, 1737-1745 (2005); Kahler C.M. et al., *Respir Res* 8, 50 (2007)]. Despite this interesting phenomenon, the molecular mechanisms regulating MSCs trafficking to tumor are unclear.

Growing evidence over several years indicates that induction of BM-MSCs migration seems to be stimulated by several soluble factors. Recently, monocyte chemoattractant protein-1 (MCP-1) secreted from breast cancer cells has been shown to stimulate BM-MSCs migration [see Dwyer R.M. et al. *Clin Cancer Res* 13, 5020-5027 (2007)]. Furthermore, chemokine ligand2 (CCL2) and chemokine ligand10 (CCL-10) can induce the migration of neural progenitor cells to sites damaged within the middle

cerebral artery occlusion (MCAo) stroke model [see *J Neurosci Res* 85, 2120-2125 (2007)]. An insulin-like growth factor-1 (IGF-1) markedly increased the rat BM-MSCs migratory response [see Li Y. et al. *Biochem Biophys Res Commun* 356, 780-784 (2007)]. Therefore, identifing the soluble factors that affect migration events of MSCs is important for understanding how MSCs migrate toward tumors or damaged tissues.

Genes introduced to BM-MSCs are over-expressed *in vivo* and show bioactivity. For example, BM-MSCs to which a human hAng1 gene is introduced stimulate generation of blood vessels in an infarction site of an acute myocardial infarction model animal [see Sun L. et al., *Biochemical Biophysical Research Communication* 357(2007) 779-784], BM-MSCs overexpressing Akt surprisingly treat myocardial infarction and improve functions of heart [see Nicolas N.et al., *Molecular Therapy* 14(6), 840-850, 2006], Bcl-2 gene-modified BM-MSCs prevent apoptosis and improve functions of heart [see *Stem Cells* 25, 2118-2127 (2007)], and BM-MSCs overexpressing endothelial nitric oxide synthase recovers the damage of right ventricular caused by pulmonary hypertension [see Sachiko et al., *Circulation*, 114[suppl I]:I-181~I-185]. These results indicate that MSCs to which genes are introduced can be used as a tool in gene therapy.

Meanwhile, in general, cells of the central nervous system are well regulated, wherein the central nervous system consists of a brain and a spinal cord. However, when this regulation collapses, cells are continuously divided and tumors are formed. Tumors can be categorized as benign tumors or malignant tumors. The central nervous system has neurons, and glia cells that support and protect the neurons. Tumors generated in glia cells are known as glioma. Glioma accounts for 50% of primary brain tumors and accounts for 15% of primary spinal cord tumors. In addition, brain tumors include neural tumors, blood vessel tumors, and gland tumors. There is also a secondary brain tumor caused by other tumors developed in other sites of the body. The secondary brain tumor is the most common type of brain tumor

Treating brain tumors are difficult due to the sites of the tumours. Brain tumors can be treated by physical surgery or chemotherapy. For physical surgery, when tumor sites are completely removed, complications are likely to occur. For chemotherapy, a high-concentration anticancer drug needs to be injected due to a brain-blood barrier, and thus, it seriously damages other organs. Recently, gene therapy has been used to treat brain tumors. In gene therapy, a gene is introduced for suppressing growth of cancer cells by using a virus vector. Since the virus vector does not have a selective migration capability toward a target cancer site, the virus vector is surface-modified to obtain such

capability. However, there is a limit to migrate a sufficient amount of virus vectors to the target cancer site.

Research results on a homing effect, which is a phenomenon in which stem cells migrate toward a disease site, have been disclosed, indicating that stem cells can be useful delivery media for treating brain tumors. However, mechanisms regulating stem cells trafficking to tumors are unclear. It is known that neural stem cells have a tropism for a type of brain tumor, that is, malignant glioma. Based on this theory, research into a method of transferring genes to a brain tumor site by using neural stem cells that function as a vehicle is being conducted (see Yip S et al., *The Cancer J* 9(3), 189-204, 2003; Kim SK et al., *Clin Cancer Res* 12(18), 5550-5556, 2006). Yip et al. found that brain tumors can be treated with neural stem cells carrying an immune regulatory gene, an apophosis promoting gene, a pro-drug converting enzyme, an oncolytic virus, etc. Brown et al. identified that brain tumors can be effectively treated by injecting a cytosine deaminase gene-containing vector into the brain, wherein the cytosine deaminase gene changes 5-fluorocytosine (5-FC) into 5-fluorouracil (5-FU), wherein 5-FU is an anticancer drug and 5-FC is a prodrug of 5-FU (see Brown AB et al., *Human Gene Ther.* 14(18), 1777-1785, 2003). Ehtesham et al. reported that growth of brain tumors was decreased by injecting neural stem cells treated for delivering interleukin-12 or a tumor necrosis factor-related apoptosis-inducing ligand (*Cancer Res* 62, 5657-5663, 2002; *Cancer Res* 62, 7170-7174, 2002). However, using neural stem cell in clinical experiments causes ethical problems related to how neural stem cells are taken, and immunological rejection caused by allogenic transplantation. Accordingly, there is a need to find other types of stem cells that do not cause these problems and can be easily obtained.

Akira et al. disclosed that BM-MSCs have a tropism for brain tumors (see *Cancer Res* 65(8), 3307-3316, 2005). BM-MSCs can be taken from patients. When BM-MSCs are injected through autologous transplantation, immunological rejection does not occur, which is an advantage for a clinical use. In a study, human BM-MSCs were injected into nude mice having skulls transplanted with human glioma cell lines through a carotid artery. As a result, the human BM-MSCs were found only in glioma and not in a normal part of the brain adjacent to glioma. In addition, even when human BM-MSCs were transplanted into a skull, human BM-MSCs migrated toward glioma. When human BM-MSCs were infected with an adenovirus vector containing cDNA of an IFN-beta gene and then the resultant vector was injected into a glioma-transplanted skull

of a nude mouse through a carotid artery, the lifetime of the nude mouse was increased. WO07/037653A1 discloses a composition for treating cancer, comprising BM-MSCs expressing a cytosine deaminase gene. In this case, however, since BM-MSCs are taken through a plurality of complex processes, subjects from which the BM-MSCs are taken suffer from mental and physical stress for a long period of time. Accordingly, there is a need to find other types of stem cells.

Unlike bone marrow, umbilical cord blood (UCB) having many MSCs can be easily taken from umbilical cords which are discarded in delivery processes. Also, the UCB storage industry is well established and thus, it is easy to find donors. Even when MSCs taken from other human-induced UCB are used, immunological rejection does not occur after transplantation. Accordingly, high immunological stability can be obtained. Therefore, it is very important to identify whether a disease such as a brain tumor can be treated based on tropisom of UCB MSCs. However, such attempts for identifying availability of UCB-MSCs have not yet been made. All the references cited in the present specification are incorporated by reference in their entity. Also, all the information disclosed in the present specification is used only to help understanding of the background of the present inventive concept and cannot be prior art.

DETAILED DESCRIPTION OF THE INVENTION

TECHNICAL PROBLEM

Target gene therapy recently developed uses specific migration characteristics of mesenchymal stem cells (MSCs), in which therapeutical genes are introduced to MSCs and then the resultant MSCs are migrated to a disease site and the disease is treated. To develop gene therapy using tropism of MSCs, a molecular mechanism regulating the migration of MSCs toward tumors should be completely understood. However, the molecular mechanism has not yet been determined. Accordingly, the present inventive concept is to find out the molecular mechanism of UCB-MSCs migrating toward a tumor cell and uses the molecular mechanism in gene therapy.

Although research into whether brain tumors can be treated with neural stem cells or bone marrow-derived mesenchymal stem cells (BM-MSCs) are being conducted, collecting neural stem cells and BM-MSCs may lead to ethical problems, immunological rejection, and mental and physical stress of a subject from which BM-MSCs are taken. Accordingly, the present inventive concept also provides a stem cell that can be taken without these problems described above and have better tropism for brain tumors.

TECHNICAL SOLUTION

The inventors of the present invention have conducted research in order to solve these problems, and found that mesenchymal stem cells isolated from umbilical cord blood and/or the mesenchymal stem cells expanded from said mesenchymal stem cells (UCB-MSCs) have a tropism for brain tumor cells and that UCB-MSCs have a stronger migration capability than mesenchymal stem cells isolated from bone marrow and/or the mesenchymal stem cells expanded from said mesenchymal stem cells (BM-MSCs). The inventors have provided a therapeutical application for treating brain tumor using UCB-MSCs.

Also, the inventors of the present invention found the tropism of UCB-MSCs is affected by interleukin-8 (IL-8) or GRO-α. Based on this finding, the inventors of the present invention provide a method of delivering a therapeutical gene or a product thereof to a cell that expresses IL-8 or GRO-α and induces the tropism of UCB-MSCs, and a therapeutical application thereof.

ADVANTAGEOUS EFFECTS

Umbilical cord blood derived mesenchymal stem cells (UCB-MSCs) in the composition according to the present invention has a selective tropism for cells that express IL-8 or GRO-α and thus induce the tropism of UCB-MSCs or brain tumor cells. The tropism capability of the UCB-MSCs is better than that of other stem cells and thus, therapeutical genes or a product thereof can be more effectively delivered than when other conventional stem cells are used. Accordingly, a pharmaceutical composition or a kit comprising UCB-MSCs according to the present invention can be used to diagnose, prevent, and treat diseases related to cells expressing interleukin(IL)-8 or GRO-α or brain tumors.

MODE OF INVENTIONS

The inventors of the present invention have studied stem cells having an effective tropism for tumors, and surprisingly found that umbilical cord blood-derived mesenchymal stem cells (UCB-MSCs) have a strong tropism for brain tumors, specifically a stronger tropism for brain tumors than bone marrow-derived mesenchymal stem cells (BM-MSCs), which has never been known before. Also, the inventors found that interleukin (IL)-8 or GRO-α relates to the tropism of UCB-MSCs.

The inventors co-cultured UCB-MSCs and representative tumor cell lines to identify characteristics of the tropism of UCB-MSCs and cytokine related to those tumor cell lines. Specifically, UCB-MSCs, and one selected from human brain tumor cell lines, such as U-87 MG, LN18, U138, or U251 cells; human rectal cancer cell line such as LS-174T; human B lymphocyte such as NC37; mouse's fibroblast (NIH3T3); a gastric cancer cell line such as KATO III; a lung cancer cell line such as A549, and a liver cancer cell line such as PLC/PRF5 were co-cultured in a transwell chamber to measure mobility of UCB-MSCs. As a result, it was found that UCB-MSCs have a strong tropism for U-87 MG, LN18, U138, and U251 cells which are brain tumor cells (see FIGS. 3 and 4). UCB-MSCs also had tropism for a conditioned media that did not include U-87 MG cells and was obtained by culturing U-87 MG (see FIG. 4).

Also, the tropism for a brain tumor cell line of UCB-MSCs was compared with that of BM-MSCs that is currently used as a source of stem cells. As a result, it was found that UCB-MSCs have a stronger tropism for a brain tumor cell line than BM-MSCs (FIG. 5). Also, the chemotactic index of UCB-MSCs was largest with respect to the brain tumor cell line among various cancer cell lines (FIG. 6). Such a high chemotactic index with respect to the brain tumor cell is an additional advantage of UCB-MSCs, in addition to the fact that UCB-MSCs can be obtained more easily and have more immunological stability than BM-MSCs, proving that UCB-MSCs are a very suitable medium for gene therapy of a brain tumor because a therapeutical gene can be efficiently transferred to the inside or neighboring portions of the brain tumor.

The tropism of UCB-MSCs in the transwell chamber may be derived by cytokines that are derived to be secreted in the co-culture of two cells. Thus, two cells were co-cultured in a transwell chamber to prepare a medium and then, the medium was analyzed using the cytokine array. As a result, it was identified that high levels of cytokines, such as IL-8 or GRO-α, were secreted in a medium in which UCB-MSCs and U87 MG had been co-cultured (see FIG. 7). Accordingly, it is highly likely that these cytokines may derive a tropism of the UCB-MSCs..

The inventors of the present invention cultured UCB-MSCs alone, cultured U-87 MG alone, and co-cultured UCB-MSCs and U-87 MG, and then analyzed IL-8 mRNA levels of these cells using RT-PCR. As a result, UCB-MSCs did not express IL-8 in either the presence or absence of U-87 MG cells. However, U-87 MG expressed IL-8 constitutively in both the presence and absence of UCB-MSCs (see FIG. 8). Treatment of UCB-MSCs with IL-8 significantly enhanced its migration when compared to untreated

cells (see (A) of FIG. 9). However, when UCB-MSCs were pre-incubated with anti-CXCR1 antibodies that are antibodies with respect to an IL-8 receptor and recombinant IL-8 was applied to UCB-MSCs, IL-8 mediated migration of UCB-MSCs were reduced in a dose-dependent manner by anti-CXCR1 treatment ((B) of FIG. 9). Anti-CXCR2 treatment also showed the same effect. Similarly, GRO-α treatment also enhanced UCB-MSCs migration when compared to untreated UCB-MSCs ((C) of FIG. 9). In contrast, there were no significant differences in UCB-MSCs migration in cultures treated with MCP-1 ((D) of FIG. 9). This data indicates that IL-8 and GRO-α participate in UCB-MSCs migration toward U-87 MG cells.

The co-relationship between the concentration of IL-8 secreted by each cancer cell and UCB-MSCs migration was measured. As a result, U-87 MG, which migrated the highest concentration of UCB-MSCs, showed the highest IL-8 production (FIG. 10A). The inventor also measured IL-8 secretion level in various glioma cells. All tested glioma cell lines which were target cell of UCB-MSC tropism also showed high secretion level of IL-8 ((B) of FIG. 10). This data suggested that UCB-MSCs had a strong migration attraction toward IL-8 secreting cells. In order to identify this, IL-8 was artificially overexpressed in A549 that is a low level of IL-8 expressing human lung cancer cells and then, A549 overexpressing IL-8 and UCB-MSCs were co-cultured. As a result, it was found that although the UCB-MSCs had a poor migration attraction toward A549, UCB-MSCs had a strong migration attraction toward A549 overexpressing IL-8. Therefore, IL-8 could be a strong inducer of UCB-MSCs (see FIG. 10C).

The inventors of the present invention compared migration characteristics of BM-MSCs and UCB-MSCs with respect to U-87 MG cells or IL-8. As a result, it was found that UCB-MSCs migrate more strongly toward U-87 MG cells or IL-8 than BM-MSCs. UCB-MSCs migration is enhanced dramatically in response to IL-8 treatment, but BM-MSCs migration is weak in response to IL-8 treatment (see FIG. 11).

Expression levels of CXC chemokine receptor 1 (CXCR1) and CXC chemokine receptor 2 (CXCR2) in UCB-MSCs and BM-MSCs were compared by measuring mRNA and protein (see FIG. 12). RT-PCR analysis using total RNA isolated from UCB-MSCs and BM-MSCs reveal that the PCR product of both CXCR1 and CXCR2 has a higher intensity in UCB-MSCs when compared to BM-MSCs. In regard of protein expression of CXCR1 and CXCR2, CXCR1 and CXCR2 are highly expressed both in UCB-MSCs and BM-MSCs. Since IL-8 has a high affinity to CXCR1 and CXCR2, increased

UCB-MSCs migration toward U-87 MG may be due to up-regulated expression of CXCR1 and CXCR2.

The inventors of the present invention performed an experiment of introducing a gene coding green fluorescent protein (GFP) into UCB-MSCs. As a result, it was found that GFP was successfully introduced and expressed (see FIG.13). Also, it was found that UCB-MSCs overexpressing the gene coding GFP also have a tropism for U87 MG (see FIG. 14). Such results show that UCB-MSCs to which a gene or a product thereof is introduced can be transferred to cells secreting IL-8 or GRO-α.

Based on the results described above, the present invention relates to a method of transferring a gene or product thereof to a cell expressing IL-8 or GRO-α by using UCB-MSCs. The present invention also relates to UCB-MSCs containing a therapeutical composition for transferring a therapeutical or marker gene or a product thereof to a cell that expresses IL-8 or GRO-α and drives tropism of UCB-MSCs. The present invention also relates to a therapeutical pharmaceutical composition, a kit, a use for preventing or treating brain tumors, and a treatment method of brain tumors, using UCB-MSCs. The present invention also relates to a composition, a kit for diagnosing brain tumors or monitoring the progression of brain tumor treatment and a diagnosing method of brain tumor or monitoring method of the progression of brain tumor treatment, using UCB-MSCs.

Specifically, the present inventive concept relates to:

[1] a pharmaceutical composition for preventing or treating brain tumors, wherein the pharmaceutical composition comprises a UCB-MSC;

[2] a pharmaceutical composition for preventing or treating brain tumors, wherein the pharmaceutical composition comprises a UCB-MSC, wherein the UCB-MSC functions as a carrier for gene therapy for brain tumors;

[3] a pharmaceutical composition for preventing or treating brain tumors, wherein the pharmaceutical composition comprises a UCB-MSC, wherein an anti-tumor gene is introduced to the UCB-MSC;

[4] a pharmaceutical composition for preventing or treating brain tumors, wherein the pharmaceutical composition comprises a UCB-MSC, wherein an anti-tumor gene is introduced to the UCB-MSC, wherein the anti-tumor gene is selected from a tumor suppressor gene, an apoptosis-inducing factor gene, a cell cycle regulatory gene, and an angiogenesis inhibitor gene;

[5] a pharmaceutical composition for preventing or treating brain tumors, wherein the pharmaceutical composition comprises a UCB-MSC, wherein an anti-tumor gene is introduced to the UCB-MSC, wherein the anti-tumor gene is selected from a tumor suppressor gene, an apoptosis inducing factor gene, a cell cycle regulatory gene, and an angiogenesis inhibitor gene, wherein the tumor suppressor gene may be selected from the group consisting of a gene of phosphatase and tensin homolog (PTEN), a gene of Maspin, a gene of RUNX3, a gene of Caveolin-1, a gene of nm23, a gene of Rb protein, a gene of Brush-1, a gene of inhibitor of tumor growth (ING-4), a gene of survivin, a gene of X chromosome linked inhibitor apoptosis protein (XIAP), a gene of neural apoptosis inhibitory protein (NAIP), and genes of proteins regulating these genes;

[6] a pharmaceutical composition for preventing or treating brain tumors, wherein the pharmaceutical composition comprises a UCB-MSC, wherein an anti-tumor gene is introduced to the UCB-MSC, wherein the anti-tumor gene is selected from a tumor suppressor gene, an apoptosis-inducing factor gene, a cell cycle regulatory gene, and an angiogenesis inhibitor gene, wherein the apoptosis-inducing factor gene may be selected from the group consisting of a gene of cytokine, a gene of interleukin, a gene of a tumor necrosis factor (TNF), a gene of interferon(INF-α, INF-β, INF-γ), a gene of a colony stimulating factor (CSFs), a gene of p53, a gene of Apaf-1, a gene of TRAIL, a gene of Caspase, a gene of Bax, a gene of Bad, a gene of FADD, a gene of JNK, a gene of p38 kinase, and genes of proteins regulating these genes;

[7] a pharmaceutical composition for preventing or treating brain tumors, wherein the pharmaceutical composition comprises a UCB-MSC, wherein an anti-tumor gene is introduced to the UCB-MSC, wherein the anti-tumor gene is selected from a tumor suppressor gene, an apoptosis-inducing factor gene, a cell cycle regulatory gene, and an angiogenesis inhibitor gene, wherein the cell cycle regulatory gene may be selected from the group consisting of a gene of cdc2, a gene of Cyclin(Cyclin A, Cyclin D, Cyclin E), a gene of cdc25C, a gene of p21WAF, a gene of p16INK4, a gene of CDK(CDK1, CDK2, CDK4, CDK6), a gene of Rb protein, a gene of E2F, an antisense or SiRNA thereof, and genes of proteins regulating these genes;

[8] a pharmaceutical composition for preventing or treating brain tumors, wherein the pharmaceutical composition comprises a UCB-MSC, wherein an anti-tumor gene is introduced to the UCB-MSC, wherein the anti-tumor gene is selected from a tumor suppressor gene, an apoptosis-inducing factor gene, a cell cycle regulatory gene, and an angiogenesis inhibitor gene, wherein the angiogenesis inhibitor gene may be selected

from the group consisting of a gene of thrombospondin-1, a gene of endostatin, a gene of tumstatin, a gene of canstatin, a gene of vastatin, a gene of restin, a gene of a vascular endothelial growth inhibitor, a gene of maspin, a gene of angiopoietins, a gene of 16-kd prolactin fragment, and a gene of endorepellin;

[9] a pharmaceutical composition for preventing or treating brain tumors, wherein the pharmaceutical composition comprises a UCB-MSC, wherein a prodrug converting enzyme gene is introduced to the UCB-MSC;

[10] a pharmaceutical composition for preventing or treating brain tumors, wherein the pharmaceutical composition comprises a UCB-MSC, wherein a prodrug converting enzyme gene is introduced to the UCB-MSC, wherein the prodrug converting enzyme gene is selected from cytosine deaminase and CYP2B1 gene;

[11] a pharmaceutical composition for preventing or treating brain tumors, wherein the pharmaceutical composition comprises a UCB-MSC, wherein an antisense or SiRNA of a gene related to a brain tumor is introduced to the UCB-MSC;

[12] a pharmaceutical composition for preventing or treating brain tumors, wherein the pharmaceutical composition comprises a UCB-MSC, wherein an antisense or SiRNA of a gene related to a brain tumor is introduced to the UCB-MSC, wherein the gene related to a brain tumor may be selected from the group consisting of a gene of Ras family, a gene of c-myc, a gene of abl, a gene of erbB-1, a gene of EGF-R, a gene of Bax, a gene of Apaf-1 interacting protein (APIP), a gene of Wnt-1-induced secreted protein 1 (WISP-1), a gene of Wnt, a gene of Raf-1, a gene of Src, a gene of Akt, a gene of Erk-1,2 and a gene of BcL-2;

[13] a pharmaceutical composition for preventing or treating brain tumors, wherein the pharmaceutical composition comprises a UCB-MSC, wherein an oncolytic virus is introduced to the UCB-MSC;

[14] a pharmaceutical composition for preventing or treating brain tumors, wherein the pharmaceutical composition comprises a UCB-MSC, wherein an oncolytic virus is introduced to the UCB-MSC, wherein the oncolytic virus is selected from Herpes simplex virus and Reovirus type 3;

[15] any one of the pharmaceutical compositions for preventing or treating brain tumors described above, wherein the brain tumor is selected from the group consisting of Astrocytoma, Pilocytic astrocytoma, Low-grade Astrocytoma, Anaplastic Astrocytoma, Glioblastoma Multiforme, Brain Stem Glioma, Ependymoma, Subependymoma, Ganglioneuroma, Mixed Glioma, Oligodendroglioma, Optic Nerve Glioma, Acoustic

Neuroma, Chordoma, CNS Lymphoma, Craniopharyngioma, Hemangioblastoma, Medulloblastoma, Meningioma, Pineal Tumors, Pituitary Tumors, Primitive Neuroectodermal Tumors, Rhabdoid Tumors, Schwannoma, Cysts, Neurofibromatosis, Pseudotumor Cerebri and Tuberous Sclerosis;

[16] a composition for diagnosing brain tumors or monitoring brain tumor treatment progression, wherein the composition includes a UCB-MSC;

[17] a composition for diagnosing brain tumors or monitoring brain tumor treatment progression, wherein the composition includes a UCB-MSC, wherein the UCB-MSC is labeled with a detectable marker;

[18] a composition for diagnosing brain tumors or monitoring brain tumor treatment progression, wherein the composition includes a UCB-MSC, wherein the UCB-MSC is labeled with a detectable marker, wherein the detectable marker is selected from luciferase-containing enzyme-based fluorescent detector and Tat peptide-derivatized magnetic nanoparticles;

[19] any one of the compositions for diagnosing brain tumors or monitoring brain tumor treatment progression, wherein the brain tumor is selected from the group consisting of Astrocytoma, Pilocytic astrocytoma, Low-grade Astrocytoma, Anaplastic Astrocytoma, Glioblastoma Multiforme, Brain Stem Glioma, Ependymoma, Subependymoma, Ganglioneuroma, Mixed Glioma, Oligodendroglioma, Optic Nerve Glioma, Acoustic Neuroma, Chordoma, CNS Lymphoma, Craniopharyngioma, Hemangioblastoma, Medulloblastoma, Meningioma, Pineal Tumors, Pituitary Tumors, Primitive Neuroectodermal Tumors, Rhabdoid Tumors, Schwannoma, Cysts, Neurofibromatosis, Pseudotumor Cerebri, and Tuberous Sclerosis;

[20] a kit for treating brain tumors, including: an expression vector having a prodrug converting enzyme gene; an UCB-MSC; and an prodrug of anticancer drug;

[21] a kit for treating brain tumors, including: an expression vector having a prodrug converting enzyme gene; an UCB-MSC; and an prodrug of anticancer drug, wherein the prodrug converting enzyme gene is selected from a cytosine deaminase gene and a CYP2B1 gene;

[22] a kit for treating brain tumors, including: an expression vector having a prodrug converting enzyme gene; an UCB-MSC; and an prodrug of anticancer drug, wherein the prodrug converting enzyme gene is selected from a cytosine deaminase gene and a CYP2B1 gene, wherein the UCB-MSC is transfected with the expression vector having a prodrug converting enzyme gene;

[23] any one of the kits described above, wherein the brain tumor is selected from the group consisting of Astrocytoma, Pilocytic astrocytoma, Low-grade Astrocytoma, Anaplastic Astrocytoma, Glioblastoma Multiforme, Brain Stem Glioma, Ependymoma, Subependymoma, Ganglioneuroma, Mixed Glioma, Oligodendroglioma, Optic Nerve Glioma, Acoustic Neuroma, Chordoma, CNS Lymphoma, Craniopharyngioma, Hemangioblastoma, Medulloblastoma, Meningioma, Pineal Tumors, Pituitary Tumors, Primitive Neuroectodermal Tumors, Rhabdoid Tumors, Schwannoma, Cysts, Neurofibromatosis, Pseudotumor Cerebri and Tuberous Sclerosis;

[24] a gene therapy composition for transferring a therapeutical gene or product thereof to a cell, wherein the gene therapy composition includes a UCB-MSC, wherein the cell expresses IL-8 or GRO-α and induces tropism of the UCB-MSC;

[25] a gene therapy composition for transferring a therapeutical gene or product thereof to a cell, wherein the gene therapy composition includes a UCB-MSC, wherein the cell expresses IL-8 or GRO-α and induces tropism of the UCB-MSC, wherein the UCB-MSC function as a carrier for gene therapy;

[26] a gene therapy composition for transferring a therapeutical gene or product thereof to a cell, wherein the gene therapy composition includes a UCB-MSC, wherein the cell expresses IL-8 or GRO-α and induces tropism of the UCB-MSC, wherein an anti-tumor gene is introduced to the UCB-MSC;

[27] a gene therapy composition for transferring a therapeutical gene or product thereof to a cell, wherein the gene therapy composition includes a UCB-MSC, wherein the cell expresses IL-8 or GRO-α and induces a tropism of the UCB-MSC, wherein an anti-tumor gene is introduced to the UCB-MSC, wherein the anti-tumor gene is selected from the group consisting of a tumor suppressor gene, an apoptosis-inducing factor gene, a cell cycle regulatory gene, and an angiogenesis inhibitor gene;

[28] a gene-treating composition for transferring a therapeutical gene or product thereof to a cell, wherein the gene therapy composition includes a UCB-MSC, wherein the cell expresses IL-8 or GRO-α and induces a tropism of the UCB-MSC, wherein an anti-tumor gene is introduced to the UCB-MSC, wherein the anti-tumor gene is selected from the group consisting of a tumor suppressor gene, an apoptosis-inducing factor gene, a cell cycle regulatory gene, and an angiogenesis inhibitor gene, wherein the tumor suppressor gene may be selected from the group consisting of a gene of phosphatase and tensin homolog (PTEN), a gene of Maspin, a gene of RUNX3, a gene of Caveolin-1, a gene of nm23, a gene of Rb protein, a gene of Brush-1, a gene of inhibitor of tumor

growth (ING-4), a gene of survivin, a gene of X chromosome linked inhibitor apoptosis protein (XIAP), a gene of neural apoptosis inhibitory protein (NAIP), and genes of proteins regulating these genes;

[29] a gene therapy omposition for transferring a therapeutical gene or product thereof to a cell, wherein the gene therapy composition includes a UCB-MSC, wherein the cell expresses IL-8 or GRO-α and induces a tropism of the UCB-MSC, wherein an anti-tumor gene is introduced to the UCB-MSC, wherein the anti-tumor gene is selected from the group consisting of a tumor suppressor gene, an apoptosis-inducing factor gene, a cell cycle regulatory gene, and an angiogenesis inhibitor gene, wherein the apoptosis-inducing factor gene may be selected from the group consisting of a gene of cytokine, a gene of interleukin, a gene of a tumor necrosis factor (TNF), a gene of interferon(INF-α, INF-β, INF-γ), a gene of a colony stimulating factor (CSFs), a gene of p53, a gene of Apaf-1, a gene of TRAIL, a gene of Caspase, a gene of Bax, a gene of Bad, a gene of FADD, a gene of JNK, a gene of p38 kinase, and genes of proteins regulating these genes;

[30] a gene therapy composition for transferring a therapeutical gene or product thereof to a cell, wherein the gene therapy composition includes a UCB-MSC, wherein the cell expresses IL-8 or GRO-α and induces tropism of the UCB-MSC, wherein an anti-tumor gene is introduced to the UCB-MSC, wherein the anti-tumor gene is selected from the group consisting of a tumor suppressor gene, an apoptosis-inducing factor gene, a cell cycle regulatory gene, and an antiogenesis inhibitor gene, wherein the cell cycle regulatory gene may be selected from the group consisting of a gene of cdc2, a gene of Cyclin(Cyclin A, Cyclin D, Cyclin E), a gene of cdc25C, a gene of p21WAF, a gene of p16INK4, a gene of CDK(CDK1, CDK2, CDK4, CDK6), a gene of Rb protein, a gene of E2F, an antisense or SiRNA thereof, and genes of proteins regulating these genes;

[31] a gene therapy composition for transferring a therapeutical gene or product thereof to a cell, wherein the gene therapy composition includes a UCB-MSC, wherein the cell expresses IL-8 or GRO-α and induces a tropism of the UCB-MSC, wherein an anti-tumor gene is introduced to the UCB-MSC, wherein the anti-tumor gene is selected from the group consisting of a tumor suppressor gene, an apoptosis-inducing factor gene, a cell cycle regulatory gene, and an antiogenesis inhibitor gene, wherein the angiogenesis inhibitor gene may be selected from the group consisting of a gene of thrombospondin-1, a gene of endostatin, a gene of tumstatin, a gene of canstatin, a gene of vastatin, a gene of restin, a gene of a vascular endothelial growth inhibitor, a gene of

maspin, a gene of angiopoietins, a gene of 16-kd prolactin fragment, and a gene of endorepellin;

[32] a gene therapy composition for transferring a therapeutical gene or product thereof to a cell, wherein the gene therapy composition includes a UCB-MSC, wherein the cell expresses IL-8 or GRO-α and induces a tropism of the UCB-MSC, wherein a prodrug converting enzyme gene is introduced to the UCB-MSC;

[33] a gene therapy composition for transferring a therapeutical gene or product thereof to a cell, wherein the gene therapy composition includes a UCB-MSC, wherein the cell expresses IL-8 or GRO-α and induces a tropism of the UCB-MSC, wherein a prodrug converting enzyme gene is introduced to the UCB-MSC, wherein the prodrug converting enzyme gene is selected from the group consisting of cytosine deaminase gene and a CYP2B1 gene;

[34] a gene therapy composition for transferring a therapeutical gene or product thereof to a cell, wherein the gene therapy composition includes a UCB-MSC, wherein the cell expresses IL-8 or GRO-α and induces a tropism of the UCB-MSC, wherein an antisense or SiRNA of a gene related to a tumor is introduced to the UCB-MSC;

[35] a gene therapy composition for transferring a therapeutical gene or product thereof to a cell, wherein the gene therapy composition includes a UCB-MSC, wherein the cell expresses IL-8 or GRO-α and induces a tropism of the UCB-MSC, wherein an antisense or SiRNA of a gene related to a tumor is introduced to the UCB-MSC, wherein the gene related to a tumor may be selected from the group consisting of a gene of Ras family, a gene of c-myc, a gene of abl, a gene of erbB-1, a gene of EGF-R, a gene of Bax, a gene of an Apaf-1 interacting protein (APIP), a gene of Wnt-1-induced secreted protein 1 (WISP-1), a gene of Wnt, a gene of Raf-1, a gene of Src, a gene of Akt, a gene of Erk-1,2 and a gene of BcL-2;

[36] a gene therapy composition for transferring a therapeutical gene or product thereof to a cell, wherein the gene-treating composition includes a UCB-MSC, wherein the cell expresses IL-8 or GRO-α and induces a tropism of the UCB-MSC, wherein an oncolytic virus is introduced to the UCB-MSC;

[37] a gene therapy composition for transferring a therapeutical gene or product thereof to a cell, wherein the gene therapy composition includes a UCB-MSC, wherein the cell expresses IL-8 or GRO-α and induces a tropism of the UCB-MSC, wherein a oncolytic virus is introduced to the UCB-MSC, wherein the oncolytic virus is selected from the group consisting of Herpes simplex virus and Reovirus type 3;

[38] a composition for diagnosing a disease occuring in a site including a cell or monitoring treatment progression of the disease, wherein the composition includes a UCB-MSC, wherein the cell expresses IL-8 or GRO-α and induces a tropism of the UCB-MSC;

[39] a composition for diagnosing a disease occurring in a site including a cell or monitoring treatment progression of the disease, wherein the composition includes a UCB-MSC, wherein the cell expresses IL-8 or GRO-α and induces a tropism of the UCB-MSC, wherein the UCB-MSC is labeled with a detectable marker; and

[40] a composition for diagnosing a disease occurring in a site including a cell or monitoring treatment progression of the disease, wherein the composition includes a UCB-MSC, wherein the cell expresses IL-8 or GRO-α and induces a tropism of the UCB-MSC, wherein the UCB-MSC is labeled with a detectable marker, wherein the detectable marker may be selected from the group consisting of a luciferase-containing enzyme based fluorescent detector and Tat peptide-derivatized magnetic nanoparticles.

Hereinafter, the present invention will be described in detail.

In the present specification, the term "umbilical blood" refers to blood taken from an umbilical vein connecting a placenta to a fetus in all mammals including humans.

In the present specification, the term "umbilical cord blood derived mesenchymal stem cell (UCB-MSC)" refers to a MSC that is isolated from umbilical blood of mammals, preferably humans, and cultured.

In the present specification, the term "treatment" refers to: preventing disease or disorder development in an animal, preferably a mammal, and more preferably humans which are not yet diagnosed with but susceptible to a disease; suppressing disease progression; and alleviating disease.

In the present specification, the term "brain tumor" refers to a malignant or benign tumor developing in the brain and the spinal cord and all kinds of tumors developing in a glial cell and a non-glial cell. In this regard, the brain tumor may be a primary brain tumor or a secondary brain tumor.

Meanwhile, terms which are not defined in the present specification may have meanings which are conventionally defined in the art.

To isolate a monocyte including a MSC from an UCB, any known method such as the method disclosed in Korean Registered Patent No. 489248 filed by the applicant of the present application and registered can be used. For example, the isolating method may be a ficoll-Hypaque density gradient method, but is not limited thereto. Specifically,

UCB taken from an umbilical vein after delivery and before the placenta is separated is centrifuged with a ficoll-hypaque gradient to obtain a monocyte, and then the monocyte is washed several times to remove impurities therefrom. The resultant monocyte can be directly used for isolation or culture of MSCs, or cryo preserved for a long period of time.

A MSC can be isolated from UCB and cultured using any known method (see Pittinger MF et al. *Science*, 284: 143-7, 1999; and Lazarus HM et al. *Bone Marrow Transplant*, 16: 557-64, 1995), such as a method disclosed in Korean Publicated Patent No. 2003-0069115.

First, the isolated UCB is centrifuged with, for example, a ficoll-Hypaque gradient to separate monocytes including a hematopoietic cell and a MSC, and then, the monocyte is washed several times to remove impurities therefrom. Then, monocytes are seeded in an appropriate concentration in a culture dish to grow cells in a form of a single layer. These cells were identified with a phase-contrast microscope. In the phase-contrast microscopic image, a colony of cells having a homogeneous spindle shape are MSCs. Then, when cells are cultured and grow, cells are sub-cultured and then multiplied until the number of the cells reaches a desired number.

UCB-MSCs included in the composition according to the present inventon can be cryopreserved using known methods (see Campos et al., Cryobiology 35:921-924, 1995). A medium for the cryoporeserving process may include 10~20% fetal bovine serum (FBS) and 10% dimethylsulfoxide (DMSO). The cells are suspended in the medium until the concentration of cells is about 1×10^6 to 5×10^6 cells per 1 mL of medium.

The cell suspension is divided and each part is loaded onto a glass or plastic ample for cryopreservation, and then, the sample is sealed and loaded into a temperature-controlled programed freezer. Cells may be frozen using a freezing program providing a temperature change of -1℃/minute so that damage to cells can be reduced when frozen cells are thawed. When the temperature of the sample reaches -90℃ or lower, the sample is moved to a liquid nitrogen storage tank having a temperature of -150℃ or lower.

When the frozen cells are thawed, the sample is quickly moved from the liquid nitrogen storage tank to a water bath having a controlled temperature of 37℃. The thawed content in the ample is immediately moved to a culture dish containing a culture medium in a stabilized condition.

According to the present invention, a culture medium for isolating and culturing a MSC may be a cell culture medium containing 10% to 30% of FBS. The cell culture medium may be any cell culture medium that is conventionally used in the art. Examples of the cell culture medium include a DMEM medium, an MEM medium, a α-MEM medium, a McCoys 5A medium, an eagle's basal medium, a CMRL medium, a Glasgow minimum nessarily medium, a (Ham's) F-12 medium, an iscove's modified Dulbecco's medium (IMDM), a (Liebovitz') L-15 medium, and a RPMI 1640 medium. For example, the cell culture medium may be the DMEM medium. During culturing, cells may be suspended so that the concentration of cells is about 5×10^3 to 2×10^4 cells per 1 mL of the medium

Also, the cell culture medium may further include one or more additives, if required. The additives may include at least one material selected from the group consisting of: a serum of fetal calf, horse or human; a penicillin G for preventing microorganism contamination; antibiotics such as streptomycin sulfate or gentamycin; antifungal agents such as amphotericin B or nystatin; and mixtures of at least two materials selected from the forgoing.

According to the present invention, UCB-MSCs may be genetically engineered so as to transfer a therapeutic drug for substantially inhibiting growth of brain tumor cells. Also, according to the present invention, UCB-MSCs may be genetically engineered so as to transfer a therapeutical gene or product thereof to cells secreting IL-8 or GRO-α. In this regard, the term "inhibiting" refers to inhibiting cell proliferation and growth, in addition necrosis and apoptosis. The therapeutical gene may be, for example, an anti-tumor gene, a gene of an enzyme converting a prodrug into a drug, an antisense or SiRNA of a gene related to a tumor, or oncolytic virus (see Yip S et al., *The Cancer J.* 9(3), 189-204, 2003). The anti-tumor gene may be a tumor suppressor gene, an apoptosis-inducing factor gene, a cell cycle regulatory gene, or an angiogenesis inhibitor gene. Specifically, the tumor suppressor gene may be selected from the group consisting of a gene of phosphatase and tensin homolog (PTEN), a gene of Maspin, a gene of RUNX3, a gene of Caveolin-1, a gene of nm23, a gene of Rb protein, a gene of Brush-1, a gene of inhibitor of tumor growth (ING-4), a gene of urvivin, a gene of X chromosome linked inhibitor apoptosis protein (XIAP), a gene of neural apoptosis inhibitory protein (NAIP), and genes of proteins regulating these genes. The tumor suppressor gene is not limited to those genes described above. The apoptosis inducing factor gene may be selected from the group consisting of a gene of cytokine, a

gene of interleukin, a gene of a tumor necrosis factor (TNF), a gene of interferon(INF-α, INF-β, INF-γ), a gene of a colony stimulating factor (CSFs), a gene of p53, a gene of Apaf-1, a gene of TNF-related apoptosis-inducing ligand(TRAIL), a gene of Caspase, a gene of Bax, a gene of Bad, a gene of FADD, a gene of JNK, a gene of p38 kinase, and genes of proteins regulating these genes. The apoptosis inducing factor gene is not limited to those genes described above. The cell cycle regulatory gene may be selected from the group consisting of a gene of cdc2, a gene of Cyclin(Cyclin A, Cyclin D, Cyclin E), a gene of cdc25C, a gene of p21WAF, a gene of p16INK4, a gene of CDK(CDK1, CDK2, CDK4, CDK6), a gene of Rb protein, a gene of E2F. The cell cycle regulatory gene is not limited to those genes described above. The angiogenesis inhibitor gene may be selected from the group consisting of a gene of thrombospondin-1, a gene of endostatin, a gene of tumstatin, a gene of canstatin, a gene of vastatin, a gene of restin, a gene of a vascular endothelial growth inhibitor, a gene of maspin, a gene of angiopoietins, a gene of 16-kd prolactin fragment, and a gene of Endorepellin. The angiogenesis inhibitor gene is not limited to those genes described above. The gene of an enzyme converting a prodrug into a drug may be cytosine deaminase converting 5-FC into 5-FU that is an anticancer drug, or a CYP2B1 gene participating in bio-activating cyclophosphamide and ifosfamide which are anticancer drugs, wherein 5-FC is a prodrug of 5-FU. The gene of an enzyme converting a prodrug into a drug is not limited to these materials described above. The antisense or SiRNA of a gene related to a tumor may be, but is not limited to, an antisense or SiRNA of a gene of Ras family, a gene of c-myc, a gene of abl, a gene of erbB-1, a gene of EGF-R, a gene of Bax, a gene of an Apaf-1 interacting protein (APIP), a gene of Wnt-1-induced secreted protein 1 (WISP-1), a gene of Wnt, a gene of Raf-1, a gene of Src, a gene of Akt, a gene of Erk-1,2 and a gene of BcL-2.

The oncolytic virus that can be carried by UCB-MSCs may be a Herpes simplex virus or a Reovirus type 3, but is not limited thereto.

UCB-MSCs to which a desired gene is introduced in an expressionable state can be appropriately manufactured using techniques that are known in the art. For example, a vector including a to-be-introduced gene is prepared (see Dehari H et al., *Cancer Gene Ther.*, 10, 75-85, 2003; WO07/037653) and then the vector can be transduced in ex vivo into primary culture MSCs. In this regard, examples of the vector include adenovirus vector, retrovirus vector, adeno-related virus vector, herpes simplex virus vector, SV40 vector, poliomavirus vector, papillomavirus vector, picarnovirus vector, vacciniavirus

vector, and lentivirus vector. For example, a method developed by Tsuda H et al. can be used (*Mol Ther* 2003, 7, 354-365). Specifically, one day before being infected with an adenovirus gene, MSCs ($5x10^5$ cells) may be inoculated into a culture dish. Then the MSCs and a solution including an adenovirus vector to which the adenovirus gene is introduced are incubated at 37℃ in a 5% CO_2 incubator for 1 hour, so that the MSCs are infected with the adenovirus gene. Then the infected MSCs are washed with a phosphoric acid buffer solution and then a conventional medium is applied thereto. Alternatively, without use of a virus vector, a desired gene can be introduced into UCB-MSCs by using a naked DNA and any method selected from a calcium-phosphate method, a cationic liposome method, and an electrophoration method. Alternatively, a fusion protein gene of protein transduction domain (PTD) and anti-tumor protein can be introduced to UCB-MSCs using the fusion protein gene-including vector (see Wu SP et al. *Biochem Biophys Res Commun.* 346(1), 1-6, 2006).

The vectors may further include a gene marker for an additional historical examination. The gene marker may be, for example, a gene for coding a chromogenic or fluorescent protein, such as lacZ or a green fluorescent protein (GFP), but is not limited thereto (see Yip Set al., *The Cancer J.* 9(3), 189-204, 2003).

Brain tumors that can be diagnosed, prevented, and treated using the pharmaceutical composition according to the present invention may be primary brain tumors or secondary brain tumors. Examples of brain tumors that can be diagnosed, prevented, and treated using the composition according to the present invention include Astrocytoma, Pilocytic astrocytoma, Low-grade Astrocytoma, Anaplastic Astrocytoma, Glioblastoma Multiforme, Brain Stem Glioma, Ependymoma, Subependymoma, Ganglioneuroma, Mixed Glioma, Oligodendroglioma, Optic Nerve Glioma, Acoustic Neuroma, Chordoma, CNS Lymphoma, Craniopharyngioma, Hemangioblastoma, Medulloblastoma, Meningioma, Pineal Tumors, Pituitary Tumors, Primitive Neuroectodermal Tumors, Rhabdoid Tumors, Schwannoma, Cysts, Neurofibromatosis, Pseudotumor Cerebri, and uberous Sclerosis. However, brain tumors that can be diagnosed, prevented, and treated using the composition according to the present invention are not limited to these tumors described above.

The pharmaceutical composition according to the present invention may be administered together with any known anticancer drug.

Since UCB-MSCs do not express HLA-DR (Major Histocompatibility Complex class II) that is a major cause for immunological rejection when a tissue or organ is

transplanted (see Le Blanc, KC, *Exp Hematol,* 31:890-896, 2003; and Tse WT et al., *Transplantation*, 75:389-397, 2003), immunological reactions, such as rejection, which are major problems of transplantation do not occur or can be minimized. Accordingly, UCB-MSCs included in the pharmaceutical composition according to the present invention can be taken from, in addition to a self-derived UCB, another subject-derived UCB. According to the present invention, UCB-MSCs can be used after being cryopreserved.

The UCB-MSCs-containing pharmaceutical composition for gene therapy or for preventing or treating diseases according to the present invention may further include pharmaceutically acceptable additives, in addition to the effective component. The UCB-MSCs-containing pharmaceutical composition may be formed into a suitable formulation that can be administered to a body. The suitable formulation may be a non-oral administration formulation, such as an injectable formulation or a locally administrable formulation. For example, a sterilized solution or suspension that includes water or a pharmaceutically acceptable solvent can be non-orally administered in an injectable form. Specifically, water or the pharmaceutically acceptable solvent is appropriately combined with a pharmaceutically acceptable carrier or medium, thereby forming an injectable formulation in a conventionally acceptable unit dosage. Examples of the pharmaceutically acceptable carrier or medium may include sterile water, saline, vegetable oils, emulsifier, suspension, surfactant, stabilizer, excipient, vehicle, an antiseptic substance, a binder, etc.

The injectable formulation described above can be non-orally administered, specifically directly administered to a site of disease, by using a conventional method. Alternatively, the injectable formulation described above can be administered through a cerebrospinal fluid, a vein, or an artery supplying blood to the site of disease, preferably directly administered to neighboring portions of the site of disease in the brain or the spinal cord or the opposite portions thereto. For example, the injectable formulation described can be administered using a clinical method developed by Douglas Kondziolka in Pittsburgh in 1998. That is, a skull of a subject to be administered is cut to a diameter of about 1 cm, the size of a pea, and then a MSC solution mixed with a Hank's balanced salt solution (HBSS) is injected thereto. In this regard, the injecting the MSC solution is performed using an injector including a long needle and a stereotactic frame for accuratly injecting the MSC solution into the brain.

The daily dose of UCB-MSCs may be $1x10^4$ to $1x10^7$ cells/kg body weight, preferably $5x10^5$ to $5x10^6$ cells/kg body weight. The daily dose may be administered at once or be divided for several treatments. However, according to the present invention, the administration dose of UCB-MSCs may vary according to the kinds of diseases to be treated, the severity of disease to be treated, administration routes, and the weight, age, and gender of a patient. Accordingly, the administration dose described above does not limit the scope of the present invention.

The present invention also provides a method of treating a disease related to a cell that produces IL-8 or GRO-α and induces a tropism of UCB-MSCs, or a brain tumor, wherein the method includes administering a therapeutically effective amount of the pharmaceutical composition according to the present invention to a patient. In this regard, UCB-MSCs included in the pharmaceutical composition may be taken from UCB of the patient itself or other humans or animals used for medical purposes. In this regard, UCB-MSCs may be used after cyropreservation. Such a method using UCB-MSCs is not limited to humans, and can be applied to other mammals.

The present invention also provides a method of diagnosing a disease related to a cell that produces IL-8 or GRO-α and induces a tropism of UCB-MSCs, or a brain tumor, and a kit used to perform the method, wherein the method uses UCB-MSCs. In this method, UCB-MSCs may be labeled with a detectable marker. The UCB-MSCs labeled with the detectable marker can be visualized by a state-of-the-art technique, and can be tracked in real time in a live animal. The detectable marker may be a luciferase-containing enzyme based fluorescent detector or Tat peptide-derivatized magnetic nanoparticles (Yip S et al., *The Cancer J.* 9(3), 189-204, 2003). If a stem cell expressing luciferase is used, the administered stem cell migrating to the site of disease can be tracked by identifying bioluminescence in real time and thus, diseases can be diagnosed and the site of disease can be identified (see Weissleder R et al., *Nat Med* 9, 123-128, 2003). Tat peptide-derivatized magnetic nanoparticles are connected to UCB-MSCs using a method developed by Lewin et al. (see *Nat Biotech* 18, 410-414, 2000) and then administered in vivo. The administered UCB-MSCs can be tracked by magnetic resonance imaging (MRI). Accordingly, UCB-MSCs labeled with a marker can be administered in vivo and then, by identifying a location where UCB-MSCs gather, the tumor and the site of the tumor can be identified. In addition, UCB-MSCs labeled with the marker can be administered during the brain tumor treatment or after the brain tumor treatment to identify the location and size of the distribution of the administered

UCB-MSCs. That is, the progress of the brain tumor treatment can also be monitored. Accordingly, the present invention also provides a method of monitoring progression of the brain tumor treatment and a kit used to perform the method, wherein the method uses UCB-MSCs. The monitoring method and the kit used to perform the method can also be applied to a disease related to a cell that produces IL-8 or GRO-α and induces a tropism of UCB-MSCs.

It is noted that all the prior art referred to in the present specification is incorporated by reference in its entirety.

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 inventive concept.

Examples

U-87 MG, A549, KATO III, PLC/PRF5, LN18, U138 and U251 cell lines were purchased from American Type Culture Collection (ATCC) and used in the present experiments. A549, KATOIII and PLC/PRF5 cells were cultured in RPMI containing 10-20% (v/v) FBS (HyClone, Logan, UT, US) and Gentamicin at 37℃ in a 5% CO_2 incubator. U-87 MG CELLS, LN18, U138 and U251 cells were grown in an Eagle's minimum essential medium (MEM) containing 10-20% (v/v) FBS. Bone marrow-derived mesenchymal stem cell (BM-MSCs) was purchased from LONZA. BM-MSCs and established UCB-MSCs were cultured in α-MEM media containing 10-20% FBS.

<Example 1> Preparation of umbilical cord blood-derived mesenchymal stem cells (UCB-MSCS)

UCB samples were collected from the umbilical vein of deliveries, with informed maternal consent. Specifically, a 16-gauge needle of a UCB collection bag containing 44 mL of CPDA-1 anticoagulant (Greencross Co., Yongin, Kyungki-do, Korea) was inserted into the umbilical vein and UCB was collected by gravity. In all cases, UCB harvests were processed within 48 hours of collection, with viability of 90% or more.

<Example 2> Isolation and expansion of UCB-MSCs

UCB-MSCs prepared according to Example 1 were centrifuged with a Ficoll-Hypaque gradient (produced by Sigma Co., density: 1.077 g/mL), and then washed several times to remove impurities. 10 to 20% FBS (HyClone Co.)-containing a basic medium (α-MEM, Gibco BRL Co.) was added to the resultant product to suspend UCB-MSCs. The UCB-MSCs were portioned at a suitable concentration into each of 10 to 20% FBS- containing a basic media, and then cultivated at 37℃ in a 5% CO_2 incubator while the medium was altered twice in a week (FIG. 1). When the cultured cells formed a single layer, MSCs expanding in a spindle shape were identified with a phase-contrast microscope. Then, sub-cultivation was repeatedly performed until the MSCs expanded sufficiently.

<Example 3> Preparation of UCB-MSCs labeled with PKH-26

UCB-MSCs cultivated according to Example 2 were dyed with PKH-26 (Sigma Co.) using a method disclosed in a reference [Barreda DA et al., *Developmental and Comparative Immunology*, 24:395-406, 2000]. First, UCB-MSCs were separated from the cell culture dish by using Trypsin and then, 2x 10^7 cells were washed with an FBS-free medium. The washed cells were collected using a centrifuge and then suspended in 1 mL of Diluent C in a kit provided by a manufacturer. Then the resultant cell suspension solution(2x) was mixed with 1ml of the PKH fluorescent dye solution(2x) and then the mixture was reacted at 25℃ for 5 minutes. To terminate the labeling reaction, a medium containing an equal volume of fetal bovine serum (FBS) was added to the reaction product and then left to sit for 1 minute. Cells labeled with PKH26 were collected by centrifuging and then, washed with a 10 to 20% FBS-containing medium three times and used in experiments.

<Example 4> Co-culture of MSCs and other cell lines in transwell chamber

Human UCB-MSCs (hUCB-MSCs) were dyed with PKH-26 (produced by Sigma). The dyed hUCB-MSCs and other tumor cell lines were co-cultured under a culture condition in a transwell chamber (FALCON) (MSC: cancer cell lines=1:5). For a control group, cancer cell lines-free MSCs were cultured in the same condition as described above. The transwell chamber used in the co-culturing, as illustrated in FIG. 2, included a lower compartment and an upper compartment, wherein the lower compartment is separated from the upper compartment by a microporous membrane (8 μm size.) In the upper compartment, PKH26-labeled hUCB-MSCs were cultured; and in lower

compartment, each of human brain tumor cell line U87-MG, human rectal cancer cell line LS174-T, human B lymphocyte NC-37, and mouse's fibroblast NIH3T3 was cultured. After co-culturing for one day, two days, and three days each, the migration of PKH26-labled UCB-MSCs was identified using a phase-contrast microscope (×100) and PKH26-labled UCB-MSCs migrated was counted (FIG. 3). The same experiment was performed using KATO III, A549, PLC/PRF5, LN18, U138, and U251 which are tumor cell lines and the migration of PKH26-labeled UCB-MSCs was identified. The same experiment was performed in a condition in which a medium conditioned by U-87 MG cells was placed in the lower compartment (FIG. 4).

That is, PKH26-labeled UCB-MSCs were co-cultured with various tumor cell lines in a transwell chamber and then, PKH-labeled mesenchymal stem cells that have migrated into the lower compartment were counted. As a result, UCB-MSCs had a strong tropism for U87-MG, LN18, U138, and U251 that are brain tumor cell lines, and a weak tropism for other tumor cells (FIGS. 3 and 4). In B of FIG. 3, the left image shows the case of the control group in which a human cell line-free medium was added instead of tumor cells, and the right image shows the case in which UCB-MSCs were co-cultured with U87-MGcell. Referring to FIG. 3, the migration of many PKH26-labeled UCB-MSCs was identified. When the human cell line-free medium was used instead of tumor cells and cultured, the migration of PKH26-labeled UCB-MSCs was negligible (see the left image of B of FIG. 3). However, PKH26-labeled UCB-MSCs had a tropism for a conditioned medium that did not contain U-87 MG cells but U-87 MG cells had been cultured therein (see D of FIG. 4). Such results show that the conditioned medium contained soluble factors that function to attract UCB-MSCs toward U-87 MG cells.

<Example 5 > Comparison of tropism of BM-MSCs for U87-MG and tropism of UCB-MSCs for U87-MG

A tropism of BM-MSCs for U87-MG was compared to a tropism of UCB-MSCs for U87-MG, using a transwell chamber. U87-MG cancer cells or medium were placed in a lower compartment, and BM-MSCs and UCB-MSCs each were placed in an upper compartment. In all cases, the culturing was performed for two days. As a result, UCB-MSCs were found to have a stronger tropism for U87-MG than BM-MSCs (FIG. 5).

<Example 6> Comparison of migrations of MSC

UCB-MSCs donated from four donors in the upper compartment and each of A549 that is lung cancer cell, HeLa that is cervical cancer cell, and U87-MG that is brain cancer-glioma cell in the lower compartment were co-cultured in a transwell chamber. Then, chemotactic indices of of UCB-MSCs in each case were compared (FIG. 6).

A549, HeLa, and U87-MG cells were purchased from American type culture collection (ATCC). Each of A549 and U87 MG cells was cultured in 10-20% bovine serum-containing RPMI1640, and HeLa was cultured in DMEM. In each case, the chemotactic index was calculated by dividing the number of UCB-MSCs migrated toward U87 MG by the number of UCB-MSCs migrated in the control experiment. A tropism of UCB-MSCs for those cancer cells was analyzed. As a result, it was found that UCB-MSCs had the stronggest tropism for U87 MG that is a brain tumor cell line.

<Example 7> Cytokine Array

MSCs and each of three kinds of human cells, including U87-MG tumor cell, were co-cultured, and the cultured medium was collected. The cytokine profile from the cultured medium was examined by using a cytokine antibody array.

A film to which antibodies of various cytokine were attached was taken from a kit for a cytokine array purchased from R&D System Co. and was reacted with a blocking solution for one hour. Separately, amounts of the media prepared by co-culturing UCB-MSCs and each of three kinds of human cells, including U87-MG tumor cell were adjusted to 1.5 mL or less, and each medium was mixed with the mixed antibodies of cyokines contained in the kit and an antigen-antibody reaction was induced for one hour. The medium in which the supplied cytokine antibodies were combined with secreted cytokines was reacted with the film that was subjected to the blocking for 4℃ for 12 hours. After the reaction, the film was placed in a washing solution and washed, and then washed with tertiary distilled water. Then the film was dried at room temperature. After repeating the washing and drying process two or three times, the film was reacted in a solution containg streptavidin-HRP for 30 minutes. Then the film was washed with a washing solution three times, reacted with a chromattic reagent, and then exposed to an X-ray film in the dark room.

U87-MG inducing strong tropism of UCB-MSCs secreted growth-related oncogene (GRO-alpha), IL-8, MCP-1, G-CSF, GM-DSF, IL-6, IL-1β, a migration inhibitory factor (MIF), and Serpin E1. Specifically the amounts of GRO-alpha and IL-8 were higher than those of MCP-1, G-CSF, GM-DSF, IL-6, IL-1β, a MIF, and Serpin E1.

FIG. 7 shows results obtained by analyzing cell lysates and cell culture supernatants through a cytokine array. Array results and information about changed spots compared to the control group are shown in FIG. 1. In Table 1, cytokines in parentheses are cytokines that were derived by the co-culturing with tumor cells. It is highly likely that these cytokines may induce a tropism of UCB-MSCs.

【Table 1】

	Cell Lysate	Supernatants
NC37	MIF+++	MIF+++
	sICAM-1++	Serpin E1+++
	MIP-1a++	MIP-1a++
	IL-16++	MIP-1b++
		IL-6+
		IL-8+
LS174-T	MIF+++	MIF+++
	IL-1ra++	SerpinE1+++
	GROa+	IL-6+
		IL-8+
U87	sICAM-1+++	(GROa+++)
	IL-6+++	IL-6+++
	MIF+++	(IL-8+++)
	SerpinE1+++	Serpin E1+++
	IL-1ra++	(G-CSF++)
	IL-8++	MIF++
	G-CSF++	(MCP-1+)
	IL-1a+	
	IL-1b+	

Conditioned media collected from UCB-MSCs cultures only, U-87 MG cell cultures only, and the co-culture of both cells were prepared. Each conditioned medium was incubated on an array membrane, and then visualized by an ECL reagent. Then the visualized conditioned media were compared to each other (see FIG. 8). FIG. 8A shows cytokine antibody array analysis results from the medium prepared by culturing UCS-MSCs only and a control medium. FIG. 8B shows cytokine antibody array analysis results from the medium prepared by culturing the U-87 MG only, and the medium prepared by co-culturing UCB-MSCs and U-87 MG.

FIG. 8C shows results identified by isolating mRNA from UCB-MSCs cultured with and without U-87 MG (left) or from U-87 MG cultured with and without UCB-MSCs (right). RT-PCR was performed with IL-8 specific primers and GAPDH was used as a control. When the level of IL-8 mRNA was measured by RT-PCR, it was found that U-87 MG expresses IL-8 in both cases which US-87 MG cultured with and without UCB-MSCs.

<Example 8> Effect of cytokine with respect to UCB-MSCs migration

The effect of IL-8, GRO-α, MCP-1(RND Systems, MN, USA) with respect to migration of UCB-MSCs was measured using traswell migration assay. PHK-26-labelled UCB-MSCs were placed in the upper compartment and no cells were placed in the lower compartment. UCB-MSCs were cultured in an IL-8 free medium or in a medium containing different concentrations of recombinant humane IL-8 for 24 hours. As a result, it can be seen that UCB-MSCs migrated more when treated with IL-8 than when they were not treated with IL-8 (FIG. 9A).

IL-8 receptor on UCB-MSCs can be effectively blocked by anti-human CXC chemokine receptor 1 (CXCR1) antibodies. After pre-incubation of UCB-MSCs with anti-CXCR1 antibodies, recombinant IL-8 was again applied to UCB-MSCs. IL-8 mediated migration of UCB-MSCs was reduced, in a dose-dependant manner, by anti-CXCR1 treatment (FIG. 9B). Anti-CXCR2 treatment also showed the same effect.

Similarly, GRO-α treatment also enhanced UCB-MSCs migration when compared to untreated cells (FIG. 9C). GRO-α also belongs to the CC subfamily and can interact with the CXCR2 receptor [see Wuyts A. et al. *Eur J Biochem* 255, 67-73 (1998)].

In contrast, significant differences were not found in UCB-MSCs migration in cultures treated with MCP-1 (FIG. 9 D). These data strongly indicate that IL-8 and GRO-α participate in migration of UCB-MSCs towards U-87 MG cells.

<Example 9> UCB-MSCs migrates toward A549 cell overexpressing IL-8

The relationship between the concentration of IL-8 secreted from several cancer cells and UCB-MSCs migration toward each cancer cell was identified. FIG. 10A shows ELISA results indicating concentrations of IL-8 secreted in media in which U87 MG (brain tumor), KATOIII (gastric cancer), A549 (lung cancer), and PLC/PRF5 (liver cancer) cells were cultured. In this regard, the concentration of IL-8 was measured per $1x10^5$ cells. Among the cancer cell lines assayed, U-87 MG showed the highest IL-8 production. This data suggested that UCB-MSCs had a strong migration attraction toward IL-8 producing cells. In addition, other human brain tumor cells, that is, LN18, U138, and U251 cells, also showed the similar concentration level of IL-8 as that of U-87 MG (see FIG. 10B). Therefore, it can be seen that UCB-MSCs has a tropism for IL-8 secreted by brain tumor cells.

In order to know whether addition of IL-8 to cells expressing a lower level of IL-8 induces the migration of UCB-MSCs, IL-8 was overexpressed in A549 that is a human lung cancer cell. FIG. 10C shows migration results of UCB-MSCs after IL-8 gene was introduced to A549 cells that secrete a low level of IL-8 by using a lipofectamine reagent and then IL-8 was overexpressed. Referring to FIG. 10C, more UCB-MSCs migrated in A549 cells having overexpreesed IL-8 than in A549 cells. Accordingly, it can be seen that IL-8 could be a strong inducer of UCB-MSCs migration. FIG. 10D shows the concentration of IL-8 secreted into a medium in a condition of C measured by ELISA.

<Example 10> Comparison of reaction of BM-MSCs and UCB-MSCs with respect to IL-8

Since it is known that BM-MSCs migrate toward U-87 MG cells *in vitro* and *in vivo*, migration characteristics of BM-MSCs and UCB-MSCs with respect to U-87 MG cells were compared to those with respect to IL-8 (FIG. 11). A of FIG. 11 shows tropism characteristics of BM-MSCs and UCB-MSCs migrated toward the lower compartment B, that is, toward U87 MG. B of FIG. 11 shows tropism characteristics of BM-MSCs and UCB-MSCs migrated toward the lower compartment B when they are treated with IL-8 for 14 hours. Referring to FIG. 11, more UCB-MSCs migrated than BM-MSCs. Therefore, it can be seen that UCB-MSCs strongly corresponded to IL-8 and migrated more, but BM-MSCs relatively weakly corresponded to IL-8.

<Example 11> Expression levels of the IL-8 receptor CXCR1 and CXCR2 in UCB-MSCs

Expression levels of CXCR1 and CXCR2 which are IL-8 receptors in UCB-MSCs and BM-MSCs were compared by measuring mRNA and protein in each of CXCR1 and CXCR2. FIG. 12 A shows results of RT-PCR which was performed with CXCR1 and CXCR2 primers after mRNA was isolated in each of CXCR1 and CXCR2. GAPDH was included into each sample to assess the quantity of the isolated RNA. FIG. 12 B shows results of expression levels of CXCR1 and CXCR2 obtained by measuring each mRNA band intensity of gel obtained from FIG. 12A using a densitometer (* and **, $p<0.001$; n=4). As a result of RT-PCR analysis for all the RNA isolated from UCB-MSCs and BM-MSCs, the band density of the PCR product of CXCR1 and CXCR2 was higher in UCB-MSCs than in BM-MSCs. FIG. 12 C shows analysis results obtained by immuostaining UCB-MSCs and BM-MSCs with anti-CXCR1 and CXCR2 antibodies to identify expression levels of CXCR1 and CXCR2 (x400). Referring to C of FIG. 12, UCB-MSCs and BM-MSCs showed high expression levels of CXCR1 and CXCR2. Since IL-8 has high affinity to CXCR1 and CXCR2, increased UCB-MSCs migration toward U-87 MG may be due to up-regulated expression of CXCR1 and CXCR2. FIG. 12 D shows analysis results obtained by immuostaining UCB-MSCs and BM-MSCs with a secondary antibody only without anti-CXCR1 and CXCR2 antibodies to identify antigen specificity of the anti-CXCR1 and CXCR2 antibodies.

<Example 12> Introduction of gene into umbilical cord blood mesenchymal stem cell

As an example of an experiment in which a gene is introduced into UCB-MSCs, a green fluorescent protein (GFP) was overexpressed using an electroporation method with a human MSC neucleofector produced by amaxa biosystem Co. and an electroporation method. $4x10^5$ cells of UCB-MSCs were cultured for two days and then placed in a mixture of 5 mg of a GFP encoding gene and 100 ml of a human MSC nucelofector for 15 minutes and the gene was introduced in an electroporator. The resultant UCB-MSCs were moved to a plate and after 24 hours, expression levels of GFP were identified with a fluorescent microscope. The GFP could be identified in a cytoplasm of each MSC (see FIG. 13).

To test a tropism of UCB-MSCs to which the GFP encoding gene is introduced, each of a GFP encoding gene and an empty gene was overexpressed in UCB-MSCs

and then, a tropism of the resultant UCB-MSCs for U-87 MG was identified. After the gene was introduced as described above, UCB-MSCs expressing GFP were placed in an upper compartment of a transwell chamber and U-87 MG was placed in a lower compartment of the transwell chamber, and then UCB-MSCs expressing GFP and U-87 MG were co-cultured for 24 hours. Among cells migrated toward the lower compartment, GFP positive cells were identified. As a result, it can be seen that UCB-MSCs overexpressing GFP due to introduction of a GFP gen had a strong tropism for U87 MG (FIG. 14).

CLAIMS

1. A pharmaceutical composition for preventing or treating brain tumors, wherein the pharmaceutical composition comprises mesenchymal stem cells isolated from umbilical cord blood and/or mesenchymal stem cells expanded from said mesenchymal stem cells, wherein the brain tumor cell expresses at least one selected from the group consisting of IL-8 and GRO-α and is not a glioma cell.

2. The pharmaceutical composition of claim 1, wherein the mesenchymal stem cells isolated from umbilical cord blood and/or the mesenchymal stem cells expanded from said mesenchymal stem cells function as a carrier for gene therapy for treating brain tumors.

3. The pharmaceutical composition of claim 1, wherein an anti-tumor gene is introduced to the mesenchymal stem cells isolated from umbilical cord blood and/or the mesenchymal stem cells expanded from said mesenchymal stem cells.

4. The pharmaceutical composition of claim 3, wherein the anti-tumor gene is selected from the group consisting of a tumor suppressor gene, an apoptosis-inducing factor gene, a cell cycle regulatory gene and an angiogenesis inhibitor gene.

5. The pharmaceutical composition of claim 4, wherein the tumor suppressor gene is selected from the group consisting of a gene of phosphatase and tensin homolog (PTEN), a gene of Maspin, a gene of RUNX3, a gene of Caveolin-1, a gene of nm23, a gene of Rb protein, a gene of Brush-1, a gene of inhibitor of tumor growth (ING-4), a gene of survivin, a gene of X chromosome linked inhibitor apoptosis protein (XIAP), a gene of neural apoptosis inhibitory protein (NAIP) and genes of proteins related to regulating said genes.

6. The pharmaceutical composition of claim 4, wherein the apoptosis inducing factor gene is selected from the group consisting of a gene of cytokine, a gene of interleukin, a gene of a tumor necrosis factor (TNF), a gene of interferon(INF-α, INF-β, INF-γ), a gene of a colony stimulating factor (CSFs), a gene of p53, a gene of Apaf-1, a gene of TRAIL, a gene of Caspase, a gene of Bax, a gene

of Bad, a gene of FADD, a gene of JNK, a gene of p38 kinase and genes of proteins related to regulating said genes.

7. The pharmaceutical composition of claim 4, wherein the cell cycle regulatory gene is selected from the group consisting of a gene of cdc2, a gene of Cyclin(Cyclin A, Cyclin D, Cyclin E), a gene of cdc25C, a gene of p21WAF, a gene of p16INK4, a gene of CDK(CDK1, CDK2, CDK4, CDK6), a gene of Rb protein, a gene of E2F, an antisense or siRNA thereof and genes of proteins related to regulating said genes.

8. The pharmaceutical composition of claim 4, wherein the angiogenesis inhibitor gene is selected from the group consisting of a gene of thrombospondin-1, a gene of endostatin, a gene of tumstatin, a gene of canstatin, a gene of vastatin, a gene of restin, a gene of a vascular endothelial growth inhibitor, a gene of maspin, a gene of angiopoietins, a gene of 16-kd prolactin fragment and a gene of endorepellin.

9. The pharmaceutical composition of claim 1, wherein a prodrug converting enzyme gene is introduced to the mesenchymal stem cells isolated from umbilical cord blood and/or the mesenchymal stem cells expanded from said mesenchymal stem cells.

10. The pharmaceutical composition of claim 9, wherein the prodrug converting enzyme gene is selected from cytosine deaminase gene and CYP2B1 gene.

11. The pharmaceutical composition of claim 1, wherein an antisense or siRNA of a gene related to brain tumor is introduced to the mesenchymal stem cells isolated from umbilical cord blood and/or the mesenchymal stem cells expanded from said mesenchymal stem cells.

12. The pharmaceutical composition of claim 11, wherein the gene related to brain tumor is selected from the group consisting of a gene of Ras family, a gene of c-myc, a gene of abl, a gene of erbB-1, a gene of EGF-R, a gene of Bax, a gene of Apaf-1 interacting protein (APIP), a gene of Wnt-1-induced secreted protein 1 (WISP-1), a gene of Wnt, a gene of Raf-1, a gene of Src, a gene of Akt, a gene of Erk-1,2 and a gene of BcL-2.

13. The pharmaceutical composition of claim 1, wherein an oncolytic virus is introduced to the mesenchymal stem cells isolated from umbilical cord blood and/or the mesenchymal stem cells expanded from said mesenchymal stem cells.

14. The pharmaceutical composition of claim 13, wherein the oncolytic virus is selected from Herpes simplex virus and Reovirus type 3.

15. The pharmaceutical composition of any one of claims 1-14, wherein the brain tumor is selected from the group consisting of Ganglioneuroma, Acoustic Neuroma, Chordoma, CNS Lymphoma, Craniopharyngioma, Hemangioblastoma, Medulloblastoma, Meningioma, Pineal Tumors, Pituitary Tumors, Primitive Neuroectodermal Tumors, Rhabdoid Tumors, Schwannoma, Cysts, Neurofibromatosis, Pseudotumor Cerebri and Tuberous Sclerosis.

16. A composition for diagnosing brain tumor or monitoring brain tumor treatment progression, wherein the composition includes the mesenchymal stem cells isolated from umbilical cord blood and/or the mesenchymal stem cells expanded from said mesenchymal stem cells, wherein the brain tumor cell expresses at least one selected from the group consisting of IL-8 and GRO-α and is not a glioma cell, wherein the mesenchymal stem cells isolated from umbilical cord blood and/or the mesenchymal stem cells expanded from said mesenchymal stem cells are labeled with a detectable marker.

17. The composition of claim 16, wherein the detectable marker is selected from luciferase-containing enzyme-based fluorescent detector and Tat peptide-derivatized magnetic nanoparticles.

18. The composition of any one of claims 16 and 17, wherein the brain tumor is selected from the group consisting of Ganglioneuroma, Acoustic Neuroma, Chordoma, CNS Lymphoma, Craniopharyngioma, Hemangioblastoma, Medulloblastoma, Meningioma, Pineal Tumors, Pituitary Tumors, Primitive Neuroectodermal Tumors, Rhabdoid Tumors, Schwannoma, Cysts, Neurofibromatosis, Pseudotumor Cerebri and Tuberous Sclerosis.

19. A kit for treating brain tumor, comprising: a prodrug of anticancer drug; an umbilical cord blood-derived mesenchymal stem cell; and an expression vector having the prodrug converting enzyme gene, wherein the umbilical cord blood-derived mesenchymal stem cell is transfected with the expression vector having a gene encoding the prodrug converting enzyme, wherein the brain tumor

cell expresses at least one selected from the group consisting of IL-8 and GRO-α and is not a glioma cell, wherein the umbilical cord blood-derived mesenchymal stem cell is mesenchymal stem cells isolated from umbilical cord blood and/or the mesenchymal stem cells expanded from said mesenchymal stem cells.

20. The kit of claim 19, wherein the gene encoding the prodrug converting enzyme is selected from a cytosine deaminase gene and a CYP2B1 gene.

21. The kit of claim 19 or 20, wherein the brain tumor is selected from the group consisting of Gangliomeuroma, Acoustic Neuroma, Chordoma, CNS Lymphoma, Craniopharyngioma, Hemangioblastoma, Medulloblastoma, Meningioma, Pineal Tumors, Pituitary Tumors, Primitive Neuroectodermal Tumors, Rhabdoid Tumors, Schwannoma, Cysts, Neurofibromatosis, Pseudotumor Cerebri and Tuberous Sclerosis.

22. A gene therapy composition for transferring a therapeutical gene or product thereof to a cell, wherein the gene therapy composition comprises the mesenchymal stem cells isolated from umbilical cord blood and/or the mesenchymal stem cells expanded from said mesenchymal stem cells, wherein the cell expresses at least one selected from the group consisting of IL-8 and GRO-α and induces tropism of the mesenchymal stem cells isolated from umbilical cord blood and/or the mesenchymal stem cells expanded from said mesenchymal stem cells and the cell is not a glioma.

23. The gene therapy composition of claim 22, wherein the mesenchymal stem cells isolated from umbilical cord blood and/or the mesenchymal stem cells expanded from said mesenchymal stem cells function as a carrier for gene therapy.

24. The gene therapy composition of claim 22, wherein an anti-tumor gene is introduced to the mesenchymal stem cells isolated from umbilical cord blood and/or the mesenchymal stem cells expanded from said mesenchymal stem cells.

25. The gene therapy composition of claim 24, wherein the anti-tumor gene is selected from the group consisting of a tumor suppressor gene, an apoptosis-inducing factor gene, a cell cycle regulatory gene and an angiogenesis inhibitor gene.

26. The gene therapy composition of claim 25, wherein the tumor suppressor gene is selected from the group consisting of a gene of phosphatase and tensin homolog (PTEN), a gene of Maspin, a gene of RUNX3, a gene of Caveolin-1, a gene of nm23, a gene of Rb protein, a gene of Brush-1, a gene of inhibitor of tumor growth (ING-4), a gene of survivin, a gene of X chromosome linked inhibitor apoptosis protein (XIAP), a gene of neural apoptosis inhibitory protein (NAIP), and genes of proteins related to regulating said genes.

27. The gene therapy composition of claim 25, wherein the apoptosis-inducing factor gene is selected from the group consisting of a gene of cytokine, a gene of interleukin, a gene of a tumor necrosis factor (TNF), a gene of interferon(INF-α, INF-β, INF-γ), a gene of a colony stimulating factor (CSFs), a gene of p53, a gene of Apaf-1, a gene of TRAIL, a gene of Caspase, a gene of Bax, a gene of Bad, a gene of FADD, a gene of JNK, a gene of p38 kinase, and genes of proteins related to regulating said genes.

28. The gene therapy composition of claim 25, wherein the cell cycle regulatory gene is selected from the group consisting of a gene of cdc2, a gene of Cyclin(Cyclin A, Cyclin D, Cyclin E), a gene of cdc25C, a gene of p21WAF, a gene of p16INK4, a gene of CDK(CDK1, CDK2, CDK4, CDK6), a gene of Rb protein, a gene of E2F, an antisense or siRNA thereof, and genes of proteins related to regulating said genes.

29. The gene therapy composition of claim 25, wherein the angiogenesis inhibitor gene is selected from the group consisting of a gene of thrombospondin-1, a gene of endostatin, a gene of tumstatin, a gene of canstatin, a gene of vastatin, a gene of restin, a gene of a vascular endothelial growth inhibitor, a gene of maspin, a gene of angiopoietins, a gene of 16-kd prolactin fragment and a gene of endorepellin.

30. The gene therapy composition of claim 22, wherein a prodrug converting enzyme gene is introduced to the mesenchymal stem cells isolated from umbilical cord blood and/or the mesenchymal stem cells expanded from said mesenchymal stem cells.

31. The gene-therapy composition of claim 30, wherein the prodrug converting enzyme gene is selected from the group consisting of cytosine deaminase gene and a CYP2B1 gene.

32. The gene therapy composition of claim 22, wherein an antisense or siRNA of a gene related to tumor is introduced to the mesenchymal stem cells isolated from umbilical cord blood and/or the mesenchymal stem cells expanded from said mesenchymal stem cells.

33. The gene therapy composition of claim 32, wherein the gene related to tumor is selected from the group consisting of a gene of Ras family, a gene of c-myc, a gene of abl, a gene of erbB-1, a gene of EGF-R, a gene of Bax, a gene of an Apaf-1 interacting protein (APIP), a gene of Wnt-1-induced secreted protein 1 (WISP-1), a gene of Wnt, a gene of Raf-1, a gene of Src, a gene of Akt, a gene of Erk-1,2 and a gene of BcL-2.

34. The gene therapy composition of claim 22, wherein an oncolytic virus is introduced to the mesenchymal stem cells isolated from umbilical cord blood and/or the mesenchymal stem cells expanded from said mesenchymal stem cells.

35. The gene therapy composition of claim 34, wherein the oncolytic virus is selected from the group consisting of Herpes simplex virus and Reovirus type 3.

36. A composition for diagnosing a disease occurring in a site, comprising a cell or monitoring treatment procession of the disease, wherein the composition comprises the mesenchymal stem cells isolated from umbilical cord blood and/or the mesenchymal stem cells expanded from said mesenchymal stem cells, wherein the cell expresses at least one selected from the group consisting of IL-8 and GRO-α and induces tropism of the mesenchymal stem cells isolated from umbilical cord blood and/or the mesenchymal stem cells expanded from said mesenchymal stem cells and the cell is not a glioma, wherein the mesenchymal stem cells isolated from umbilical cord blood and/or the mesenchymal stem cells expanded from said mesenchymal stem cells are labeled with a detectable marker.

37. The composition of claim 36, wherein the detectable marker is selected from the group consisting of a luciferase-containing enzyme based fluorescent detector and Tat peptide-derivatized magnetic nanoparticles.

38. The gene therapy composition of any one of claims 22 to 35, wherein the cell expressing IL-8 or GRO-α is a brain tumor cell.

39. The composition of any one of claims 36 to 37, wherein the cell expressing IL-8 or GRO-α is a brain tumor cell.

DESCRIPTION OF THE DRAWINGS

FIG. 1 is a view illustrating a process of collecting mesenchymal stem cells (MSCs);

FIG. 2 is a schematic view of a transwell chamber that is used to co-culture umbilical cord blood derived mesenchymal stem cells (UCB-MSCs) and various cell lines according to the present invention;

FIG. 3 is a graph of the number of UCB-MSCs migrated toward a lower compartment of a transwell chamber when PKH-labeled UCB-MSCs placed in an upper compartment of the transwell chamber and each of U87-MG, LS174-T, NC-37, and NIH3T3 cells placed in the lower compartment of the transwell chamber are co-cultured, wherein: in (A), a left bar indicates a case in which the cell number of cell lines in the lower compartment in the transwell chamber is $1X10^5$ cells and a right bar indicates a case in which the cell number of cell lines in the lower compartment in the transwell chamber is $5X10^5$ cells, and in both cases, the number of UCB-MSCs is $1X10^5$cells and in (B), a left fluorescent microscopic image shows PKH26-labeled UCB-MSCs migrated toward the lower compartment of the transwell chamber when a human cell line-free medium only is used (control), and a right fluorescent microscopic image shows PKH26-labeled UCB-MSCs migrated toward the lower compartment of the transwell chamber when when UCB-MSCs are co-cultured with U87-MG cells;

FIG. 4 is a graph of the number of UCB-MSCs migrated toward the lower compartment of the transwell chamber when PKH-labeled UCB-MSCs placed in the upper compartment of the transwell chamber and each of U87-MG, KATO III, A549, PLC/PRF5, LN18, U138, and U251 cells placed in the lower compartment of the transwell chamber are co-cultured (A and C), wherein (B) is an fluorescent microscopic image of PKH26-labeled UCB-MSCs migrated toward the lower compartment, and (D) is a graph of the number of UCB-MSCs migrated when UCB-MSCs are co-cultured with U-87 MG cells, or with U87 MG cells-free cultured-conditioned media which is prepared by culturing U87 MG cells in media and then removing U87 MG cells from the media;

FIG. 5 is a graph for comparing tropisms of BM-MSCs and UCB-MSCs for U-87 MG cells, wherein each of PKH-labeled UCB-MSCs and PKH-labeled BM-MSCs placed in the upper compartment in the transwell chamber and U-87 MG cells placed in the lower compartment in the transwell chamber are co-cultured and the number of PKH-labeled UCB-MSCs migrated toward the lower compartment is compared with the

number of PKH-labeled BM-MSCs, wherein a left bar indicates a case in which U-87 MG cells do not exist and a right bar indicates a case in which U-87 MG cells exist (the number of U-87 MG cells is $5X10^5$, and in both cases, the number of MSCs is $1X10^5$;

FIG. 6 is a graph of chemotactic indices of UCB-MSCs when UCB-MSCs are co-cultured with cancer cell lines (A549, HeLa, and U-87 MG cells) ;

FIG. 7 shows results obtained by analyzing cell lysates and cell culture supernatants through a cytokine array after each of NC37, LS174-T, and U-87 MG cells is co-cultured with UCB-MSCs;

FIG. 8 shows analysis results obtained by collecting conditioned media in which UCB-MSCs were cultured alone, U-87 MG cells were cultured alone, and both of UCB-MSCs and U-87 MG cells were co-cultured, incubating the conditioned media on an array membrane, and then visualizing the incubated results with ECL reagents, wherein (A) shows cytokine antibody array analysis results from the conditioned media with UCB-MSCs and medium control, (B) shows analysis results when the conditioned media in which U-87 MG only is cultured is used and when the media in which a UCB-MSCs and U-87 MG are co-cultured is used, and (C) shows mRNA isolation results from UCB-MSCs (left) cultured with or without U-87 MG cells or UCB-MSCs, and from U-87 MG cells cultured with and without UCB-MSCs, wherein RP-PCT is performed with IL-8 specific primers and GAPDH is used as a control group;

FIG. 9 is a graph for identifying the effect of IL-8 and GRO-α, among the cytokines analyzed with reference to FIG. 8, on MSC migration, wherein (A) is a graph of cell migration toward the lower compartment when MSCs are treated with a recombinant IL-8 protein for 0, 1, 10, and 100 ng for 24 hours, (B) is a graph of cell migration toward the lower compartment when UCB-MSCs are pre-treated with 0.02, 0.2, and 2 μg of a CXC chemokine receptor 1 (CXCR1) antibody that is known as a receptor of IL-8 in cells and then treated with 50 ng of IL-8 to promote the MSC migration. (*, $p=0.007$; **, $p<0.001$), (C) is a graph of cell migration toward the lower compartment when UCB-MSCs are treated with GRO-α (*,$p<0.005$), and (D) is a graph of cell migration when MSCs are treated with Monocyte chemoattractant protein-1 (MCP-1);

FIG. 10 consists of (A), (B), (C), and (D), wherein (A) and (B) are graphs of the amount of IL-8 secreted in the cultured media with U-87 MG, KATO III, A549, PLC/PRF5, LN18, U138, and U251 cells, measured by ELISA, (C) is a graph of cell migration when IL-8 gene is introduced to A549 cell secreting a low level of IL-8 and overexpressed, and (D) is a graph of the amount of IL-8 secreted in the media in the condition of (C),

measured by ELISA;

FIG. 11 are graphs for comparing tropisms of UCB-MSCs and BM-MSCs for U-87 MG cells, wherein (A) is a graph for comparing tropism of UCB-MSCs and BM-MSCs moved toward the lower compartment, with respect to U-87 MG cells, and (B) is a graph for comparing tropism of UCB-MSCs and BM-MSCs when UCB-MSCs and BM-MSCs are treated with IL-8 for 14 hours;

FIG. 12 show analysis results for comparing expression levels of CXC chemokine receptor 1 and CXC chemokine receptor 2 (CXCR1 and CXCR2), which are known as IL-8 receptors, in UCB-MSCs and BM-MSCs, by measuring mRNA and protein, wherein (A) shows analysis results when mRNA is separated from each of UCB-MSCs and BM-MSCs and RT-PCR was performed with CXCR1 and CXCR2 primers in UCB-MSCs and BM-MSCs, wherein the separated RNA was quantified with reference to GAPDH reacted with each sample, (B) is a graph of expression levels of CXCR1 and CXCR2 by measuring the band intensity of mRNA of each gel obtained from (A) with a densitometer (* and **, $p<0.001$; $n=4$), (C) shows analysis results of protein expression levels of CXCR1 and CXCR2 in UCB-MSCs and BM-MSCs by performing a immunostaining process and anti- CXCR1 and CXR2 antibodies (x400), and (D) shows analysis results obtained by immuostaining UCB-MSCs and BM-MSCs with a secondary antibody only instead of anti-CXCR1 and CXCR2 antibodies to identify antigen specificity of the anti-CXCR1 and CXCR2 antibodies;

FIG. 13 is a fluorescent microscopic image of UCB-MSCs to which a gene coding green fluorescent protein (GFP) is introduced and overexpressed;

FIG. 14 shows results of an experiment in which each of the gene coding GFP and an empty gene is overexpressed in UCB-MSCs. Said UCB-MSCs are placed in a upper compartment in a transwell and U-87 MG cells are placed in a lower compartment in the transwell and cocultured for 24 hours, and then UCB-MSCs migrated toward the lower compartment are identified; and

FIG. 15 shows primer sequences used in Examples of the present invetion.

<110> MEDIPOST CO., LTD.

<120> Composition for the diagnosis, prevention or treatment of diseases related to cells expressing IL-8 or GRO-alpha, comprising UCB-MSCs

<150> KR1020070087228
<151> 2007-08-29

<150> US 60/972966
<151> 2007-09-17

<150> KR 1020080067247
<151> 2008-07-10

<150> US 61/089733
<151> 2008-08-18

<160> 8

<170> KopatentIn 1.71

<210> 1
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> primer sequence for GAPDH

<400> 1
accacagtcc atgccatcac 20

<210> 2
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> primer sequence for GAPDH

<400> 2
tccaccaccc tgttgctgta 20

<210> 3
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> primer sequence for IL-8

<400> 3
tcctgatttc tgcagctctg tg 22

<210> 4
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> primer sequence for IL-8

<400> 4
tgcttgaagt ttcactggca tc 22

<210> 5
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> primer sequence for CXCR1

<400> 5
gagccccgaa tctgacatta 20

<210> 6
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> primer sequence for CXCR1

<400> 6
gcagacactg caacacacct 20

<210> 7
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> primer sequence for CXCR2

<400> 7
attctgggca tccttcacag 20

<210> 8
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> primer sequence for CXCR2

<400> 8
tgcacttagg caggaggtct 20