# TSP-1, TSP-2, IL-17BR AND HB-EGF ASSOCIATED WITH STEM CELL ACTIVITIES AND APPLICATIONS THEREOF

## Technical Field

One or more embodiments of the present invention relate to thrombospondin 1 (TSP-1), TSP-2, interleukin 17B receptor (IL-17BR), and heparin-binding epidermal growth factor-like growth factor (HB-EGF) associated with stem cell activity, for example, activity of a mesenchymal stem cell (MSC), and use thereof.

## Background Art

Cartilage is a kind of dense and thick connective tissue, and is composed of chondrocytes distributed in a stiff yet flexible gel-like matrix. Cartilage does not contain blood vessels, and the nutrients are supplied by diffusion via the matrix. Cartilage is classified into three types: hyaline cartilage (for example, cartilage of the nose, trachea and bronchiole and articular cartilage), elastic cartilage (for example, cartilage of the external ear, part of the Eustachian tube, and part of laryngeal cartilage), and fibrocartilage (for example, meniscus and endplate cartilage). The main purpose of cartilage is to provide a framework upon which bone deposition can begin and provide a smooth surface allowing free joint movement between bones. In addition, the cartilage provides a strong yet flexible support.

There are various therapies for treating a cartilage injury or cartilage failure. Osteoarthritis is degenerative arthritis that is, in general, relatively mild at first, but aggravates with time and wear. In terms of medical treatment, medicines such as an anti-inflammatory agent (for example, diclofenac, ibuprofen, or naproxen), a COX-2 selective inhibitor, hydrocortisone, glucosamine, and chondroitin sulfate are known to relieve pain due to cartilage loss.

Thrombospondin-2 (TSP-2) is a secretory, extracellular matrix glycoprotein that exhibits strong anti-angiogenic activity (Bornstein et al., 2000, Matrix Biology 19: 557-568).

Thrombospondin-1 (TSP-1) is a multimeric glycoprotein composed of identical monomers. The monomer has a molecular weight of about 185 KDa in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions. The predominant multimer is a trimer having a molecular weight of about

450 KDa on non-reducing gels, and the molecular weights by sedimentation equilibrium are similar, at 135 kDa for monomers and 420 kDa for trimers. The predicted molecular weight from a sequence of amino acid residues in the monomer is 127,524 Da, which does not include contributions from glycosylation and β-hydroxylation. TSP-1 is known to be involved in cell adhesion, proliferation, and chemotaxis. It has also been reported that TSP-1 may be involved in the progression of malignant tumors.

Interleukin-17B receptor (IL-17BR) is a protein in humans that is encoded by the IL17BR gene. IL-17BR is a cytokine receptor that specifically binds to IL17B and IL17E, but does not bind to IL17 and IL17C.

Heparin-binding epidermal growth factor-like growth factor (HB-EGF) exerts its biological activities by binding to an erb class of EGF receptors (EGFR). HB-EGF binds heparin with high affinity. HB-EGF binds to EGFR to modulate the biological effects of the growth factor on target cells, including cellular migration and proliferation. HB-EGF is mitogenic for fibroblasts, smooth muscle cells, and epithelial cells. HB-EGF is a heat-sensitive, cationic protein, with a molecular weight of apporoximately 22,000 Da. HB-EGF is known to treat symptoms associated with intestinal ischemia, for example, intestinal cell necrosis and enterocolitis. In addition, HB-EGF is known to inhibit liver diseases and liver cell death and facilitate liver regeneration in mammals.

In spite of these disclosures, association of chondrogenic differentiation of stem cells with TSP-1, TSP-2, IL-17BR, and HB-EGF has still not been proven.

## Detailed description of the invention

### Technical problem

One aspect of the present invention provides thrombospondin 1 (TSP-1), TSP-2, interleukin 17B receptor (IL-17BR) and heparin-binding epidermal growth factor-like growth factor (HB-EGF) associated with stem cell activity or a stem cell expressing the same.

Another aspect of the present invention provides a method of using TSP-1, TSP-2, IL-17BR and HB-EGF associated with stem cell activity or a stem cell expressing the same.

Technical solution

According to an embodiment of the present invention, there is provided a compostion for stimulating a cell to differentiate into a chondrocyte, the composition including at least one selected from the group consisting of thrombospondin 2 (TSP-2) and a cell expressing TSP-2.

TSP-2 is a secretory, extracellular matrix glycoprotein that exhibits strong anti-angiogenic activity (Bornstein et al., 2000, Matrix Biology 19: 557-568). TSP-2 is a disulfide-linked homotrimer glycoprotein, and, in humans, is encoded by the THBS2 gene. TSP-2 may have an amino acid sequence disclosed in RefSeq NP_003238 (human) (SEQ ID NO: 1) or NP_035711 (mouse) (SEQ ID NO: 2) or a sequence derived therefrom.

The composition may further include a carrier that may be pharmaceutically acceptable. For example, the carrier may be selected from the group consisting of a medium, a buffer, and a biocompatible polymer. The biocompatible polymer may be selected from commonly used polymers that may support cells and/or maintain cell activity in a two- or three-dimensional structure. For example, the biocompatible polymer may include at least one polymer selected from the group consisting of hyaluronic acid, hydroxy apatite, chitosan, collagen, and fibrin.

The composition may be used to treat or prevent injury, degeneration, loss or defect of cartilage. The injury, degeneration, loss or defect of cartilage may include arthritis or joint deformity. The arthritis may be rheumatic arthritis or degenerative arthritis. For example, the injury, degeneration, loss or defect of cartilage may be caused by at least one selected from the group consisting of degenerative arthritis due to aging; early degenerative arthritis due to joint overload, including obesity; external injuries due to sports, falling, accidents and the like; degenerative arthritis secondarily developed by not appropriately treating a cartilage injury due to external injuries; and joint deformity due to ligament injury, muscle weakness around joints, dislocation of joints, formation of joint mice and bone growth retardation. In addition, the cell to be differentiated into a chondrocyte may be a cell derived from at least one of tissues exposed by a cartilage injury, cartilage degeneration, cartilage loss, or a cartilage defect, for example, tissues such as synovial fluid, periosteum, bone, and bone marrow.

The composition may include TSP-2 in an amount ranging from about 30 pg to about 300 mg. For example, the composition may include TSP-2 in an amount

ranging from about 10 ng to about 300 mg, from about 100 ng to about 300 mg, from about 1 $\mu g$ to about 300 mg, from about 10 $\mu g$ to about 300 mg, or from about 10 $\mu g$ to about 300 mg.

In addition, the composition may include a cell producing TSP-2 in a concentration ranging from about $1x10^4$ cells/ml to about $1x10^6$ cells/ml, from about $5x10^4$ cells/ml to about $1x10^6$ cells/ml, from about $2.5x10^5$ cells/ml to about $1x10^6$ cells/ml, or from about $5x10^5$ cells/ml to about $1x10^6$ cells/ml.

The composition may facilitate the chondrogenic differentiation in vitro or in vivo. In the case of facilitation of the chondrogenic differentiation in vivo, a subject in which the chondrogenic differentiation occurs may be a mammal.

The cell may be a stem cell. The stem cell may be selected from the group consisting of an induced pluripotent stem cell (iPS cell), an embryonic stem cell, and an adult stem cell. The adult stem cell may be selected from the group consisting of a mesenchymal stem cell (MSC), an adipose-derived stem cell, an endothelial stem cell, and a hematopoietic stem cell. The MSC may be derived from a mammal, for example, a human. The MSC may include at least one selected from the group consisting of a bone marrow-derived mesenchymal stem cell (BM-MSC), an umbilical cord blood-derived mesenchymal stem cell (UCB-MSC), an adipose-derived mesenchymal stem cell (AD-MSC), an embryonic yolk sac-derived MSC, a placenta-derived MSC, a skin-derived MSC, a peripheral blood-derived MSC, a muscle-derived MSC, a liver-derived MSC, a nervous tissue-derived MSC, a periosteum-derived MSC, a umbilical cord-derived MSC, a fetal membrane-derived MSC, a synovium-derived MSC, an amniotic membrane-derived MSC, a meniscus-derived MSC, an anterior cruciate ligament-derived MSC, an articular chondrocytes-derived MSC, and an MSC separated and/or cultured from other tissues including MSCs.

The cell may be a cell that produces and extracellularly secretes the TSP-2. That is, the cell itself secretes TSP-2, and interacts with the TSP-2 to be differentiated into a chondrocyte. In addition, the cell may be a cell contacting TSP-2 that is produced by other cells to be secreted or externally administered. For example, the cell contacting TSP-2 may be a cell existing in tissue with a cartilage injury, cartilage degeneration, cartilage loss, or a cartilage defect. The cell existing in tissue with the cartilage injury, cartilage degeneration, cartilage loss, or cartilage defect may be a cell existing in tissue exposed due to the cartilage injury, cartilage degeneration,

cartilage loss, or cartilage defect. The tissue exposed may vary depending on a degree of the cartilage injury, cartilage degeneration, cartilage loss, or cartilage defect. The tissue may be selected from the group consisting of synovial fluid, periosteum, bone, and bone marrow. The tissue may be a tissue with arthritis or joint deformity. The arthritis may be rheumatic arthritis or degenerative arthritis. The cell contacting TSP-2 may be a cell derived from at least one of the tissues with degenerative arthritis due to aging; early degenerative arthritis due to joint overload, including obesity; external injuries due to sports, falling, accidents and the like; degenerative arthritis secondarily developed by not appropriately treating a cartilage injury due to external injuries; and joint deformity due to ligament injury, muscle weakness around joints, dislocation of joints, formation of joint mice and bone growth retardation.

The “cell producing TSP-2” may be a cell that naturally produces TSP-2, or a cell induced to produce TSP-2. The composition may further include an inducer that induces a cell to produce TSP-2.

The cell producing TSP-2 may be a stem cell. The stem cell may be selected from the group consisting of an iPS cell, an embryonic stem cell, and an adult stem cell. The adult stem cell may be selected from the group consisting of an MSC, an adipose-derived stem cell, an endothelial stem cell, and a hematopoietic stem cell. The MSC may be derived from a mammal, for example, a human. The MSC may include at least one selected from the group consisting of a BM-MSC, an UCB-MSC, an AD-MSC, an embryonic yolk sac-derived MSC, a placenta-derived MSC, a skin-derived SMSC, a peripheral blood-derived MSC, a muscle-derived MSC, a liver-derived MSC, a nervous tissue-derived MSC, a periosteum-derived MSC, a umbilical cord-derived MSC, a fetal membrane-derived MSC, a synovium-derived MSC, an amniotic membrane-derived MSC, a meniscus-derived MSC, an anterior cruciate ligament-derived MSC, an articular chondrocytes-derived MSC, and an MSC separated and/or cultured from other tissues including MSCs.

The cell producing TSP-2 and the cell to differentiate into a chondrocyte may be identical or different from each other. That is, the cell producing TSP-2 may act by paracrine or autocrine mechanisms. The cell to differentiate into a chondrocyte may be a cell that produces TSP-2 and extracellularly secretes TSP-2. That is, the cell itself secretes TSP-2, and interacts with the secreted TSP-2, thereby differentiating into a chondrocyte. In addition, the cell may be a cell contacting

TSP-2 that is produced by other cells to be secreted or externally administered. For example, the cell contacting TSP-2 may be a cell existing in tissue with a cartilage injury, cartilage degeneration, cartilage loss, or a cartilage defect. The cell existing in tissue with the cartilage injury, cartilage degeneration, cartilage loss, or cartilage defect may be a cell existing in the tissue itself and tissue exposed due to the cartilage injury, cartilage degeneration, cartilage loss, or cartilage defect. The tissue exposed may vary depending on a degree of the cartilage injury, cartilage degeneration, cartilage loss, or cartilage defect. For example, the tissue may be selected from the group consisting of synovial fluid, periosteum, bone, and bone marrow. The tissue may be a tissue with arthritis or joint deformity. The arthritis may be rheumatic arthritis or degenerative arthritis. The cell contacting TSP-2 may be a cell derived from at least one of the tissues with degenerative arthritis due to aging; early degenerative arthritis due to joint overload, including obesity; external injuries due to sports, falling, accidents and the like; degenerative arthritis secondarily developed by not appropriately treating a cartilage injury due to external injuries; and joint deformity due to ligament injury, muscle weakness around joints, dislocation of joints, formation of joint mice and bone growth retardation.

The cell producing TSP-2 may be a cell expressing TSP-2 to an amount higher than a set value. The set value may be an amount expressed by a reference cell. The reference cell may be known to have a chondrogenic differentiation capability. Such a differentiation capability may be known by the fact that the reference cell is cultured in an in vitro differentiation medium to induce chondorgenic differentiation. In addition, the reference cell administered to a subject may be identified to have a differentiation capability in vivo. The reference cell may be selected from the group consisting of a BM-MSC, a fibroblast, and an UCB-MSC. The UCB-MSC may be selected from the group consisting of a C5 UCB-MSC, a C6 UCB-MSC, and a C7 UCB-MSC.

The set value may be at least an amount expressed from an MSC that differentiates into a chondrocyte in a maintenance medium or an induction medium and has low activity. The MSC differentiating into a chondrocyte and with low activity may be a C5, C6 or C7 UCB-MSC.

The set value may be 72 pg/$10^5$ cells/ml or greater when the cell producing TSP-2 is cultured in a maintenance medium for 1 day. On the other hand, when the cell producing TSP-2 is pellet cultured in an induction medium for 7 days, the set

value may be 550 pg/$10^5$ cells/ml or greater.

The set value may be a value of TSP-2 expressed in a medium selected from the group consisting of a α-minimum essential medium (MEM-α) medium, a MSC maintenance medium (for example, a MEM-α medium containing 10% fetal bovine serum (FBS) and 50μg/ml of gentamicin), and a chondrogenic differentiation medium of a MSC (for example, a medium containing a high glucose Dulbecco's modified Eagle's medium (DMEM) (containing 4500 mg/l of glucose), 50 μg/ml of ascorbate, 0.1 μM dexamethasone, 40 μg/ml of L-proline, 100 μg/ml of pyruvate, 10 ng/ml of TGF-β3, 500 ng/ml of bone morphogenetic protein 6 (BMP-6), 50mg/ml ITS+, and 50 μg/ml of gentamicin).

The TSP-2 may be expressed in a cell lysate and/or a culture supernatant. The concentration of the TSP-2 may be measured on mRNA level or protein level.

The composition may stimulate activity of a cell to differentiate into a chondrocyte. The cell may be identical or different from the cell expressing TSP-2.

The cell may be a cell that produces TSP-2 and extracellularly secretes the TSP-2. That is, the cell itself secretes TSP-2, and interacts with the TSP-2 to be differentiated into a chondrocyte. In addition, the cell may be a cell contacting TSP-2 that is produced by other cells to be secreted or externally administered. For example, the cell contacting TSP-2 may be a cell existing in tissues with cartilage injury, cartilage degeneration, cartilage loss, or cartilage defect. The cell existing in tissue with a cartilage injury, cartilage degeneration, cartilage loss, or a cartilage defect may be a cell existing in the tissue itself or tissue exposed due to the cartilage injury, cartilage degeneration, cartilage loss, or cartilage defect. The tissue exposed may vary depending on a degree of the cartilage injury, cartilage degeneration, cartilage loss, or cartilage defect. The tissue may be selected from the group consisting of synovial fluid, periosteum, bone, and bone marrow. The tissue may be a tissue with arthritis or joint deformity. The arthritis may be rheumatic arthritis or degenerative arthritis. The cell contacting TSP-2 may be a cell derived from at least one of the tissues with degenerative arthritis due to aging; early degenerative arthritis due to joint overload including obesity; external injuries due to sports, falling, accidents and the like; degenerative arthritis secondarily developed by not appropriately treating a cartilage injury due to external injuries; and joint deformity due to ligament injury, muscle weakness around joints, dislocation of joints, formation of joint mice and bone growth retardation. For example, the cell may be located in

the vicinity of the cell expressing TSP-2.

According to another embodiment of the present invention, there is provided a method of differentiating a cell into a chondrocyte in a subject, the method including administering a composition including at least one selected from the group consisting of TSP-2 and cells expressing TSP-2 to an amount effective enough to differentiate a cell into a chondrocyte.

The amount effective enough to differentiate a cell into a chondrocyte may be a sufficient amount at a constant ratio allowing chondrogenic differentiation of a cell. The amount may easily be selected by those of ordinary skill in the art according to the selected cell and a cell expressing TSP-2. For example, the amount may be an amount allowing at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% of a total stem cell to differentiate into a chondrocyte within 1 to 7 days. A detailed description of the cell expressing TSP-2 and the cell to differentiate into a chondrocyte has already been provided. The subject may be selected from the group consisting of mammals, for example, a human, a mouse, and a rabbit.

According to another embodiment of the present invention, there is provided a method of identifying a capability of a stem cell to differentiate a cell into a chondrocyte, the method including: culturing a stem cell in a medium; measuring the concentration of at least one selected from the group consisting of TSP-1, TSP-2, interleukin 17B receptor (IL-17BR), and heparin-binding epidermal growth factor-like growth factor (HB-EGF) from the culture; and identifying a chondrogenic differentiation and/or induction capability of the cultured stem cell based on the measured concentration.

The method will now be described in detail. The method includes culturing a stem cell in a medium. The culturing of the stem cell in a medium is known in the art, and thus media and conditions may be appropriately selected by one of ordinary skill in the art depending on selected stem cells.

The stem cell may be selected from the group consisting of an iPS cell, an embryonic stem cell, and an adult stem cell. The adult stem cell may be selected from the group consisting of an MSC, an adipose-derived stem cell, an endothelial stem cell, and a hematopoietic stem cell. The MSC may be derived from a mammal, for example, a human. The MSC may include at least one selected from the group consisting of a BM-MSC, an UCB-MSC, an adipose-derived MSC, an embryonic yolk sac-derived SMC, a placenta-derived MSC, a skin-derived MSC, a peripheral

blood-derived MSC, a muscle-derived MSC, a liver-derived MSC, a nervous tissue-derived MSC, a periosteum-derived MSC, a umbilical cord-derived MSC, a fetal membrane-derived MSC, a synovium-derived MSC, an amniotic membrane-derived MSC, a meniscus-derived MSC, an anterior cruciate ligament-derived MSC, an articular chondrocytes-derived MSC, and an MSC separated and/or cultured from other tissues including MSCs.

For example, the stem cell may an MSC, and the medium may be an MSC maintenance medium or a chondrogenic differentiation medium of an MSC. The medium may be selected from the group consisting of a MEM-α medium, a MSC maintenance medium (for example, a MEM-α medium containing 10% FBS and 50 μg/ml of gentamicin), and a chondrogenic differentiation medium of an MSC (for example, a medium containing a high glucose DMEM, 50 μg/ml of ascorbate, 0.1 μM dexamethasone, 40 μg/ml of L-proline, 100 μg/ml of pyruvate, 10 ng/ml of TGF-β3, 500 ng/ml of BMP-6, 50mg/ml ITS+, and 50 μg/ml of gentamicin). The culturing process may be performed using a method that is commonly used in an MSC culture.

In the culturing of the stem cell in a medium, only the stem cell may be cultured without including other cells, or other cells, in addition to the stem cell may be cultured together. The other cells may be cells that produce TSP-2 and extracellularly secretes the TSP-2. That is, the cells themselves secrete TSP-2, and interact with the TSP-2, thereby differentiating into a chondrocyte. In addition, the cells may be cells contacting TSP-2 that is produced by other cells to be secreted or externally administered. For example, the cells contacting TSP-2 may be cells existing in tissues with cartilage injury, cartilage degeneration, cartilage loss, or cartilage defect. The cell existing in tissue with a cartilage injury, cartilage degeneration, cartilage loss, or a cartilage defect may be the tissue itself or a cell existing in tissue exposed due to the cartilage injury, cartilage degeneration, cartilage loss, or cartilage defect. The tissue exposed may vary depending on a degree of the cartilage injury, cartilage degeneration, cartilage loss, or cartilage defect. The tissue may be selected from the group consisting of synovial fluid, periosteum, bone, and bone marrow. The tissue may be a tissue with arthritis or joint deformity. The arthritis may be rheumatic arthritis or degenerative arthritis. The cell contacting TSP-2 may be a cell derived from at least one of the tissues with degenerative arthritis due to aging; early degenerative arthritis due to joint overload including obesity; external injuries due to sports, falling, accidents and the like; degenerative arthritis secondarily developed by

not appropriately treating a cartilage injury due to external injuries; and joint deformity due to ligament injury, muscle weakness around joints, dislocation of joints, formation of joint mice and bone growth retardation. For example, the cell may be located in the vicinity of the cell expressing TSP-2.

The method includes measuring the concentration of at least one selected from the group consisting of TSP-1, TSP-2, IL-17BR, and HB-EGF from the culture. The concentration of at least one selected from the group consisting of TSP-1, TSP-2, IL-17BR, and HB-EGF may be measured from a cell lysate or a culture supernatant. The concentration of at least one selected from the group consisting of TSP-1, TSP-2, IL-17BR, and HB-EGF may be measured on an mRNA level or a protein level. The measurement on an mRNA or protein level is well-known in the art. For example, a quantitative polymerase chain reaction (PCR) or enzyme-linked immunosorbent assay (ELISA) may be used.

TSP-1 is a multimeric glycoprotein composed of identical monomers. The monomer has a molecular weight of about 185 kDa in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions. A predominant multimer is a trimer having a molecular weight of about 450 kDa on non-reducing gels, and molecular weights by sedimentation equilibrium are similar, at 135 kDa for monomers and 420 kDa for trimers. The predicted molecular weight from the sequence of amino acid residues in the monomer is 127,524 Da, which does not include contributions from glycosylation and β-hydroxylation. TSP-1 is known to be involved in cell adhesion, proliferation, and chemotaxis. It has also been reported that TSP-1 may be involved in the progression of malignant tumors. TSP-1 may have an amino acid sequence disclosed in RefSeq NP_003237 (human) (SEQ ID NO: 3) or NP_035710 (mouse) (SEQ ID NO: 4) or a sequence derived therefrom.

IL-17BR is a protein that in humans is encoded by the IL17RB gene. IL-17BR is a cytokine receptor that specifically binds to IL17B and IL17E, but does not bind to IL17 and IL17C. IL-17BR may have an amino acid sequence disclosed in RefSeq NP_758434 (human) (SEQ ID NO: 5) or NP_062529 (mouse) (SEQ ID NO: 6) or a sequence derived therefrom.

Heparin-binding epidermal growth factor-like growth factor (HB-EGF) exerts its biological activities by binding to an erb class of EGF receptors (EGFR). HB-EGF binds heparin with high affinity. HB-EGF binds to EGFR to modulate the biologic effects of the growth factor on target cells, including cellular migration and

proliferation. HB-EGF may have an amino acid sequence disclosed in RefSeq NP_001936 (human) (SEQ ID NO: 7) or NP_034545 (mouse) (SEQ ID NO: 8) or a sequence derived therefrom.

The method may include identifying a chondrogenic differentiation capability of the cultured stem cell based on the measured concentration.

The identifying process may include comparing the concentration of at least one selected from the group consisting of TSP-1, TSP-2, IL-17BR, and HB-EGF with the concentration obtained from a reference cell as a control, which is identified to have a chondrogenic differentiation capability.

In the identifying process, when the measured concentration is higher than the concentration obtained from the reference cell, it may be confirmed that the stem cell has a high capability of differentiating into a chondrocyte. On the other hand, when the measured concentration is lower than or the same as the concentration obtained from the reference cell, it may be determined that the stem cell has a low capability of differentiating into a chondrocyte.

The identifying process may include, when an expression amount of TSP-2 is larger than 72 pg/ml/1.0x$10^5$ cells when the stem cell is monolayer cultured in a maintenance medium for 1 day, or when an expression amount of TSP-2 is larger than 550 pg/ml/1.0x$10^5$ cells when the stem cell is pellet cultured in a maintenance medium for 7 days, determining that the stem cell has a high capability of differentiating into a chondrocyte. The maintenance medium of the stem cell may be a medium containing MEM-α, 10% FBS, and 50 μg/ml of gentamicin, and a chondrogenic induction medium of the stem cell may be a medium containing a high glucose DMEM, 50 μg/ml of ascorbate, 0.1 μM dexamethasone, 40 μg/ml of L-proline, 100 μg/ml of pyruvate, 10 ng/ml of TGF-β3, 500 ng/ml of BMP-6, 50mg/ml ITS+, and 50 μg/ml of gentamicin.

The method may further include comparing the measured concentration of at least one selected from the group consisting of TSP-1, TSP-2, IL-17BR, and HB-EGF with the concentration of at least one selected from the group consisting of TSP-1, TSP-2, IL-17BR, and HB-EGF obtained from a reference cell as a control, which is identified to have a low capability of differentiating into a chondrocyte.

In addition, in the comparing method, when the measured concentration of at least one selected from the group consisting of TSP-1, TSP-2, IL-17BR, and HB-EGF is at least 10%, at least 20% or at least 30% higher than the concentration of at least

one selected from the group consisting of TSP-1, TSP-2, IL-17BR, and HB-EGF obtained from the reference cell, it may be determined that the stem cell has a high capability of differentiating into a chondrocyte. The reference cell may be selected from the group consisting of a BM-MSC, a fibroblast, and an UCB-MSC. The UCB-MSC may be selected from the group consisting of a C5 UCB-MSC, a C6 UCB-MSC, and a C7 UCB-MSC.

According to another embodiment of the present invention, there is provided a method of differentiating a cell into a chondrocyte, the method including differentiating a cell which is determined to have a high capability of differentiating into a chondrocyte according to the method described above, into a chondrocyte.

The differentiating process may be performed in vitro or in vivo. The method may include culturing a cell determined to have a high capability of differentiating into a chondrocyte, for example, an MSC, in a chondrogenic differentiation medium of a cell to differentiate the cell, for example, the MSC, into a chondrocyte in vitro. In the culturing process, the cell may be cultured with a biocompatible polymer.

The biocompatible polymer may be selected from commonly used polymers that may support cells and/or maintain cell activity in a two- or three-dimensional structure. For example, the biocompatible polymer may include at least one polymer selected from the group consisting of hyaluronic acid, hydroxyapatite, chitosan, fibrin, and collagen.

The method may further include administering the cell, for example, an MSC to a subject in need of chondrogenic differentiation. The subject may be a subject with cartilage injury, cartilage degeneration, cartilage loss, or cartilage defect. For example, the subject may be a subject with arthritis or joint deformity. The arthritis may be rheumatic arthritis or degenerative arthritis. The subject may have at least one of the tissues with degenerative arthritis due to aging; early degenerative arthritis due to joint overload including obesity; external injuries due to sports, falling, accidents and the like; degenerative arthritis secondarily developed by not appropriately treating a cartilage injury due to external injuries; and joint deformity due to ligament injury, muscle weakness around joints, dislocation of joints, formation of joint mice and bone growth retardation. The external injury includes a fracture. The administering process may be performed by intravenous injection or muscular injection, or may be locally performed on lesion sites. The cell, for example, a MSC may be administered with a carrier. The carrier may be a medium, a buffer, or a

biocompatible polymer. The biocompatible polymer may be selected from commonly used polymers that may support cells and/or maintain cell activity in a two- or three-dimensional structure. The biocompatible polymer may include at least one polymer selected from the group consisting of hyaluronic acid, hydroxyapatite, chitosan, fibrin, and collagen. The cell may be a stem cell. The stem cell may include at least one selected from the group consisting of an iPS cell, an embryonic stem cell, and an adult stem cell. The adult stem cell may be selected from the group consisting of an MSC, an adipose-derived stem cell, an endothelial stem cell, and a hematopoietic stem cell. The MSC may be derived from a mammal, for example, a human. The MSC may include at least one selected from the group consisting of a BM-MSC, an UCB-MSC, an adipose-derived MSC, an embryonic yolk sac-derived MSC, a placenta-derived MSC, a skin-derived MSC, a peripheral blood-derived MSC, a muscle-derived MSC, a liver-derived MSC, a nervous tissue-derived MSC, a periosteum-derived MSC, a umbilical cord-derived MSC, a fetal membrane-derived MSC, a synovium-derived MSC, an amniotic membrane-derived MSC, a meniscus-derived MSC, an anterior cruciate ligament-derived MSC, an articular chondrocytes-derived MSC, and an MSC separated and/or cultured from other tissues including MSCs.

According to another embodiment of the present invention, there is provided a method of identifying a sample including a cell capable of differentiating into a chondrocyte, the method including culturing a cell-containing sample in a medium; and measuring the concentration of at least one selected from the group consisting of TSP-1, TSP-2, IL-17BR, and HB-EGF from the culture.

In the culturing process, the cell may be a stem cell. The stem cell may be a UCB-MSC. The cell capable of differentiating into a chondrocyte may be the stem cell, for example, the UCB-MSC. In addition, the cell capable of differentiating into a chondrocyte may be a stem cell, for example, a UCB-MSC and other cells cultured with the stem cell. The other cells may be other types of stem cells. The other cells may be cells that produce TSP-2 and extracellularly secretes the TSP-2. That is, the cells themselves secrete TSP-2, and interact with the TSP-2 to be differentiated into a chondrocyte. In addition, the cells may be cells contacting TSP-2 that is produced by other cells to be secreted or externally administered. For example, the cells contacting TSP-2 may be cells existing in tissues with a cartilage injury, cartilage degeneration, cartilage loss, or a cartilage defect. The cell existing in tissue with the

cartilage injury, cartilage degeneration, cartilage loss, or cartilage defect may be a cell existing in the tissue itself and tissue exposed due to the cartilage injury, cartilage degeneration, cartilage loss, or cartilage defect. The tissue exposed may vary depending on a degree of the cartilage injury, cartilage degeneration, cartilage loss, or cartilage defect. For example, the tissue may be selected from the group consisting of synovial fluid, periosteum, bone, and bone marrow. The tissue may be a tissue with arthritis or joint deformity. The arthritis may be rheumatic arthritis or degenerative arthritis. The cell contacting TSP-2 may be a cell derived from at least one of the tissues with degenerative arthritis due to aging; early degenerative arthritis due to joint overload including obesity; external injuries due to sports, falling, accidents and the like; degenerative arthritis secondarily developed by not appropriately treating a cartilage injury due to external injuries; and joint deformity due to ligament injury, muscle weakness around joints, dislocation of joints, formation of joint mice and bone growth retardation. For example, the cell contacting TSP-2 may be a cell located in the vicinity of the cell expressing TSP-2. The medium may be a cell maintenance medium or a chondrogenic induction medium of a cell.

According to another embodiment of the present invention, there is provided a composition for decreasing chondrocyte death, the composition including a heparin binding EGF-like growth factor (HB-EGF) and a stem cell expressing a HB-EGF.

The HB-EGF may exert its biological activities by binding to an erb class of EGF receptors (EGFR). HB-EGF binds heparin with high affinity. HB-EGF binds to EGFR to modulate the biologic effects of the growth factor on target cells, including cellular migration and proliferation. HB-EGF may have an amino acid sequence disclosed in RefSeq NP_001936 (human) (SEQ ID NO: 7) or NP_034545 (mouse) (SEQ ID NO: 8) or a sequence derived therefrom.

The composition may include a stem cell expressing a HB-EGF. The stem cell may be an UCB-MSC. The composition may include a carrier. A detailed description of the carrier has already been provided.

According to another embodiment of the present invention, there is provided a method of decreasing chondrocyte death of a subject, the method including administering a composition for decreasing chondrocyte death to a subject, the composition including a HB-EGF in an amount enough to decrease chondrocyte death and a stem cell expressing a HB-EGF.

The amount enough to decrease chondrocyte death refers to an amount

enough to decrease chondrocyte death more than a control. For example, the amount enough to decrease chondrocyte death refers to an amount enough to decrease chondrocyte death at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% greater than a control. A detailed description of the composition has already been provided. The subject may be a subject with a cartilage injury, cartilage degeneration, cartilage loss, or a cartilage defect. The injury, degeneration, loss, or defect of cartilage may include arthritis, osteoporosis, a fracture, or joint deformity. The arthritis may be rheumatic arthritis or degenerative arthritis. The injury, degeneration, loss, or defect of cartilage may be derived from at least one selected from the group consisting of degenerative arthritis due to aging; early degenerative arthritis due to joint overload including obesity; external injuries due to sports, falling, accidents and the like; degenerative arthritis secondarily developed by not appropriately treating a cartilage injury due to external injuries; and joint deformity due to ligament injury, muscle weakness around joints, dislocation of joints, formation of joint mice and bone growth retardation. In addition, the chondrocyte may be a cell derived from at least one of the tissues exposed due to the injury, degeneration, loss, or defect of cartilage, for example, tissues such as synovial fluid, periosteum, bone, and bone marrow. The administering process may be performed by intravenous injection or muscular injection, or may be locally performed on lesion sites. The subject may be a mammal. The mammal may include a human, a cow, a pig, a dog, and a mouse.

The composition may further include a carrier. The carrier may be a medium, a buffer, or a biocompatible polymer. The biocompatible polymer may be selected from commonly used polymers that may support cells and/or maintain cell activity in a two- or three-dimensional structure. The biocompatible polymer may include at least one polymer selected from the group consisting of hyaluronic acid, hydroxyapatite, chitosan, fibrin, and collagen. The method may be performed in vitro or in vivo.

According to another embodiment of the present invention, there is provided a method of increasing an expression of at least one protein selected from the group consisting of TSP-1, TSP-2, IL-17BR, and HB-EGF from a stem cell, the method including culturing a stem cell in the presence of a joint fluid of a patient with at least one ailment selected from the group consisting of a cartilage injury, cartilage degeneration, cartilage loss, a cartilage defect, and combinations thereof.

The stem cell may be a BM-MSC or an UCB-MSC. The expression may be

measured on a protein or mRNA level. The stem cell may be allogeneic or autologous with respect to the joint fluid.

The at least one ailment selected from the group consisting of the cartilage injury, cartilage degeneration, cartilage loss, cartilage defect, and combinations thereof may include arthritis and joint deformity. The arthritis may be rheumatic arthritis or degenerative arthritis. The ailment may include at least one selected from the group consisting of degenerative arthritis due to aging; early degenerative arthritis due to joint overload including obesity; external injuries due to sports, falling, accidents and the like; degenerative arthritis secondarily developed by not appropriately treating a cartilage injury due to external injuries; and joint deformity due to ligament injury, muscle weakness around joints, dislocation of joints, formation of joint mice and bone growth retardation. The method may be performed in vitro or in vivo. The joint fluid may be a cell existing in tissue ifself with the ailment described above and in tissues exposed due to a cartilage injury, cartilage degeneration, cartilage loss, or a cartilage defect. The cell existing in tissue with the cartilage injury, cartilage degeneration, cartilage loss, or the cartilage defect may be a cell existing in the tissue itself and tissue exposed due to the cartilage injury, cartilage degeneration, cartilage loss, or cartilage defect. The tissue exposed may vary depending on a degree of the cartilage injury, cartilage degeneration, cartilage loss, or cartilage defect. For example, the tissue may be selected from the group consisting of synovial fluid, periosteum, bone, and bone marrow. In addition, the joint fluid may be a joint fluid located at an arbitrary position in a subject with the ailment.

According to another embodiment of the present invention, there is provided a method of differentiating a stem cell into a lesion tissue cell, the method including culturing a stem cell in the presence of a lesion tissue.

The lesion tissue may be a joint fluid of a patient with arthritis; a synovial fluid derived from a joint cavity of a patient with arthritis; a bronchoalveolar lavage fluid (BALF) of a patient with acute respiratory distress syndrome (ARDS), bronchial asthma, lung cancer, an interstitial lung disease, or a chronic obstructive pulmonary disease (COPD); or a spinal fluid, a pleural fluid, an ascite fluid or a gastric fluid collected from a patient. The culturing process may be performed using a known method in the art related to culturing of stem cells.

The method may further include administering a stem cell differentiated into a

lesion tissue cell, obtained by the culturing process, for example, a MSC, to a subject with a lesion tissue to treat the lesion tissue. The lesion tissue and the stem cell, for example, a MSC may be allogeneic or autologous with respect to each other.

In the method, the stem cell may include at least one selected from the group consisting of an iPS cell, an embryonic stem cell, and an adult stem cell. The adult stem cell may be selected from the group consisting of an MSC, an adipose-derived stem cell, an endothelial stem cell, and a hematopoietic stem cell. The MSC may be derived from a mammal, for example, a human. The MSC may include at least one selected from the group consisting of a BM-MSC, an UCB-MSC, an adipose-derived MSC, an embryonic yolk sac-derived MSC, a placenta-derived MSC, a skin-derived MSC, a peripheral blood-derived MSC, a muscle-derived MSC, a liver-derived MSC, a nervous tissue-derived MSC, a periosteum-derived MSC, an umbilical cord-derived MSC, a fetal membrane-derived MSC, a synovium-derived MSC, an amniotic membrane-derived MSC, a meniscus-derived MSC, an anterior cruciate ligament-derived MSC, an articular chondrocytes-derived MSC, and an MSC separated and/or cultured from other tissues including MSCs. The method may be performed in vitro or in vivo.

According to another aspect of the present invention, there is provided a method of screening a material regulating stem cell activity, the method including culturing a stem cell in the presence of a lesion tissue; and measuring a product expressed from the culture.

The method includes culturing a stem cell, for example, an MSC in the presence of a lesion tissue. The lesion tissue may be a joint fluid of a patient with arthritis; a synovial fluid derived from a joint cavity of a patient with arthritis; a bronchoalveolar lavage fluid (BALF) of a patient with acute respiratory distress syndrome (ARDS), bronchial asthma, lung cancer, an interstitial lung disease, or a chronic obstructive pulmonary disease (COPD); or a spinal fluid, a pleural fluid, an ascite fluid or a gastric fluid collected from a patient. The culturing may be performed in the presence of a maintenance medium of the stem cell, for example, a MSC or a differentiation medium of the stem cell, for example, a MSC, into a tissue corresponding to a lesion tissue. The lesion tissue may be included in the culture in an amount ranging from 5 to 30%, for example, from 10 to 20% based on a cell suspension volume, a cell concentration, or a cell number. The culturing process may be performed using a known method in the art related to culturing of stem cells.

In the method, the stem cell may include at least one selected from the group consisting of an iPS cell, an embryonic stem cell, and an adult stem cell. The adult stem cell may be selected from the group consisting of an MSC, an adipose-derived stem cell, an endothelial stem cell, and a hematopoietic stem cell. The MSC may be derived from a mammal, for example, a human. The MSC may include at least one selected from the group consisting of a BM-MSC, an UCB-MSC, an adipose-derived MSC, an embryonic yolk sac-derived MSC, a placenta-derived MSC, a skin-derived MSC, a peripheral blood-derived MSC, a muscle-derived MSC, a liver-derived MSC, a nervous tissue-derived MSC, a periosteum-derived MSC, an umbilical cord-derived MSC, a fetal membrane-derived MSC, a synovium-derived MSC, an amniotic membrane-derived MSC, a meniscus-derived MSC, an anterior cruciate ligament-derived MSC, an articular chondrocytes-derived MSC, and an MSC separated and/or cultured from other tissues including MSCs. The method may be performed in vitro or in vivo.

The method includes measuring a product expressed from the culture. The measuring may be performed using a known method. For example, the measuring may be performed by quantitative PCR when the product is RNA. On the other hand, the measuring may be performed by ELISA when the product is protein. The product may be RNA or protein.

The method may include identifying a material regulating stem cell activity, for example, activity of an MSC, from the measured product. The activity of the stem cell, for example, the MSC, may be a differentiation activity. The method may further include comparing the amount of the measured product with the amount of a product obtained through a control experiment. The control experiment may be a negative or positive control experiment. The control experiment may be performed by culturing a stem cell, for example, an MSC, by not using a lesion tissue or in the presence of a normal tissue instead of a lesion tissue and measuring a product expressed from the culture.

The method may include, when the amount of the product is larger than that of the control, determining that the lesion tissue positively regulates the stem cell activity, for example, activity of the MSC. The method may include, when the amount of the product is smaller than that of the control, determining that the lesion tissue negatively regulates the stem cell activity, for example, activity of the MSC. The differentiation may be a differentiation into a tissue corresponding to a lesion tissue.

For example, when the lesion tissue is a joint fluid, it may differentiate into a chondrocyte.

According to another embodiment of the present invention, there is provided a method of increasing an expression of at least one selected from the group consisting of TSP-2 and HB-EGF from a stem cell, the method including pellet culturing a stem cell.

The pellet culturing of the stem cell may be performed in a state where the stem cell is aggregated tri-dimensionally. For example, the pellet culturing may be performed by centrifuging a cell-containing suspension to form a precipitated cell pellet and culturing the pellet. In this regard, an initial cell concentration used in the culturing may be $5x10^5$ cells/ml to $5x10^7$ cells/ml. The centrifuging process may be performed at 350 g to 1500 g for 5 to 30 minutes. The stem cell may be selected from the group consisting of an iPS cell, an embryonic stem cell, and an adult stem cell. The adult stem cell may be selected from the group consisting of an MSC, an adipose-derived stem cell, an endothelial stem cell, and a hematopoietic stem cell. The MSC may be derived from a mammal, for example, a human. The MSC may include at least one selected from the group consisting of a BM-MSC, an UCB-MSC, an adipose-derived MSC, an embryonic yolk sac-derived MSC, a placenta-derived MSC, a skin-derived MSC, a peripheral blood-derived MSC, a muscle-derived MSC, a liver-derived MSC, a nervous tissue-derived MSC, a periosteum-derived MSC, an umbilical cord-derived MSC, a fetal membrane-derived MSC, a synovium-derived MSC, an amniotic membrane-derived MSC, a meniscus-derived MSC, an anterior cruciate ligament-derived MSC, an articular chondrocytes-derived MSC, and an MSC separated and/or cultured from other tissues including MSCs.

Advantageous effects

A composition including TSP-2, according to an embodiment of the present invention, may stimulate differentiation of a cell, for example, an MSC, into a chondrocyte.

According to one or more embodiments of the present invention, there is provided a method of identifying a capability of a cell, for example, an MSC, to differentiate into a chondrocyte, by using TSP-1, TSP-2, or IL-17BR, whereby a chondrogenic differentiation capability of the MSC may be efficiently indentified.

According to one or more embodiments of the present invention, there is provided a method of differentiating a cell, for example, an MSC, into a chondrocyte,

by using TSP-1, TSP-2, or IL-17BR, whereby the cell, for example, the MSC, may be efficiently differentiated into a chondrocyte.

According to an embodiment of the present invention, there is provided a method of differentiating a cell, for example, an MSC, into a lesion tissue cell, whereby the cell, for example, the MSC, may be efficiently differentiated into a lesion tissue cell.

According to an embodiment of the present invention, there is provided a method screening a material regulating cell activity, for example, activity of an MSC, whereby a material regulating the cell activity, for example, activity of the MSC, may be efficiently screened.

Brief description of the drawings

FIG. 1 illustrates images showing results of respectively differentiating 7 types of umbilical cord blood mesenchymal stem cell (UCB-MSC), i.e., C1, C2, C3, C4, C5, C6, and C7, cultured in differentiation media for 4 weeks;

FIG. 2 is a graph showing an expression amount of mRNA of thrombospondin 2 (TSP-2) of an UCB-MSC cultured in a chondrogenic differentiation medium;

FIGS. 3 and 4 are graphs showing the amounts of TSP-2 in a culture supernatant, obtained by enzyme-linked immunosorbent assay (ELISA);

FIG. 5 illustrates an image showing a pellet size of an UCB-MSC cultured in the presence or absence of TSP-2;

FIG. 6 is a graph showing TSP-2 expressed in a culture supernatant of each of 6 types of UCB-MSC cultured in a chondrogenic differentiation medium;

FIG. 7 illustrates images showing chondrogenic differentiation of an UCB-MSC and a bone marrow mesenchymal stem cell (BM-MSC) in vitro;

FIG. 8 is a graph showing capabilities of an UCB-MSC and a BM-MSC to differentiate into chondrogenic lineage;

FIG. 9 illustrates images showing chondrogenic differentiation of 10 types of BM-MSC and 10 types of UCB-MSC analyzed on a sixth week after chondrogenic differentiation induction, according to an embodiment of the present invention;

FIG. 10 illustrates images showing a difference in chondrogenesis capability between an UCM-MSC and a BM-MSC on the sixth week after chondrogenic differentiation induction;

FIG. 11 is a graph showing expression results of TSP-2 under monolayer and pellet culturing conditions in the presence of a growth factor combination;

FIG.12 is a graph showing an expression level of TSP-2 according to the types of UCB-MSC;

FIG. 13 is a graph showing measurement results of expression amounts of TSP-2 obtained by pellet culuring a C3 UCM-MSC and a C5 UCM-MSC for 3 days;

FIG. 14 illustrates graphs showing the amount of TSP-2 expressed by an UCB-MSC under differentiation and dedifferentiation conditions;

FIGS. 15 through 17 are graphs showing an expression amount of a marker protein of an UCB-MSC cultured in the presence of TSP-2;

FIG. 18 illustrates graphs showing a degree of chondrogenic differentiation of an UCB-MSC cultured in a chondrogenic medium under TSP-2 expression-inhibiting conditions;

FIG. 19 is a graph showing a level of TSP-2 in blood plasma of a normal person and a patient with osteoarthritis;

FIGS. 20 and 21 are graphs showing expression amounts of TSP-1 of an UCB-MSC in the presence of a joint fluid of a patient with arthritis;

FIG. 22 is a graph showing results of analyzing the amount of mRNA of interleukin 17B receptor (IL-17BR) obtained by lysing an UCB-MSC differentiated into cartilage by a real time polymerase chain reaction (RT-PCR);

FIG. 23 illustrates graphs showing measurement results of mRNA of heparin-binding epidermal growth factor-like growth factor (HB-EGF) in an UCB-MSC cultured in the presence of a joint fluid of a patient with arthritis;

FIG. 24 is a diagram showing an expression amount of HB-EGF in an UCB-MSC cultured under chondrocyte death conditions;

FIG. 25 illustrates images showing observation results of a rabbit-derived chondrocyte cultured in the presence of HB-EGF; and

FIG. 26 is a graph showing results of pellet culturing an UCB-MSC and a BM-MSC.

Best mode for carrying out the invention

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

**Example 1: Identification of secretory proteins specifically induced in an UCB-MSC by joint fluid of patient with arthritis**

To identify a material regulating cartilage regeneration and cartilage inflammation produced by an UCB-MSC, a joint fluid of a patient with arthritis was added to a medium with an UCB-MSC being cultured therein to reach a final concentration of 20% (v/v), and then a resulting product was further cultured for 3 hours. The obtained culture supernatant was used as an analysis sample. In addition, as a control, an UCB-MSC culture cultured in a state where the joint fluid was not added thereto and/or a medium including 20% (v/v) joint fluid in which an UCB-MSC was not cultured were used. The joint fluid was obtained from a patient with degenerative arthritis.

Proteins expected to be included in each obtained culture or control sample were labeled with a detectable marker. The marker was biotin, and the biotin was detected by fluorescent detection of a complex formed by specific binding between the biotin and fluorescence-labeled streptavidin. Next, a protein chip with antibodies respectively binding to 507 secretory proteins immobilized thereon was treated with each sample (RayBiotech, Inc., RayBio$^{TM}$ Biotin Label-based Human Antibody Array I; Cat# AAH-BLG-1-2) to react together according to manufacturer guidelines. After the reaction, an excitation light of 532 nm was irradiated to the protein chip using a laser scanner (Axon Genepix Scanner 4000B) and a radiation light was detected at 635 nm. By comparing the obtained detection signal with a reference detection signal obtained from a control, the concentration of each protein in the sample was determined.

As a result of analysis, when the UCB-MSC was cultured in the presence of a joint fluid of a patient with arthritis, TSP-1, TSP-2, IL-17BR, and HB-EGF significantly increased, compared with the case where the UCB-MSC was cultured in the absence of a joint fluid of a patient with arthritis.

**Example 2: Association of chondrogenic differentiation of UCB-MSC with TSP-2**

In the present example, association of the chondrogenic differentiation of an UCB-MSC with TSP-2 was evaluated. In addition, it was evaluated whether TSP-2 induced an UCB-MSC to differentiate into a chondrocyte.

**1) Chondrogenic differentiation capability of types of UCB-MSC**

First, the chondrogenic differentiation capabilies of various types of UCB-MSC were confirmed. Each type of UCB-MSC was pellet cultured in a chondrogenic differentiation medium. The chondrogenic differentiation medium was a high glucose DMEM containg 50 μg/ml of ascorbate, 0.1 μM dexamethasone, 40 μg/ml of L-proline, 100 μg/ml of pyruvate, 10 ng/ml of TGF-β3, 500 ng/ml of BMP-6, 50mg ITS+/ml, and 50 μg/ml of gentamicin. An initial cell concentration was $5x10^5$ cells/ml, and the culturing was performed in 15 ml polypropylene tube for 4 weeks. The medium was changed twice weekly, and a pellet was immobilized with 4% paraformaldehyde contained in paraffin, and cut to a piece with 5㎛ thickness. The piece was stained with Safranin-O to detect an anionic proteoglycan.

FIG. 1 shows images of results of respectively differentiating 7 types of UCB-MSC, i.e., C1, C2, C3, C4, C5, C6, and C7 in a differentiation medium for 4 weeks, according to an embodiment of the present invention. Referring to FIG. 1, it is confirmed that C1 and C2, which was classified to have good chondrogenic differentiation capabilities, each have cross-sections having round lacunae with distinct borders satisfactorily formed entirely thereon. In this regard, the lacunae are markers allowing confirmation of the presence of cartilage. In addition, C3 and C4, which was classified to have medium chondrogenic differentiation capabilities, each have cross-sections having small lacunae with distinct borders entirely or partially formed thereon. In the cases of C5, C6, and C7, which was classified to have poor chondrogenic differentiation capabilities, lacunae structures are barely formed. This indicates that the UCB-MSC has different differentiation capabilities due to genetic differences among individuals and differences in processes of collecting umbilical cord blood.

**(2) Association of chondrogenic differentiation capability with TSP-2**

UCB-MSC types having different chondrogenic differentiation capabilities were each cultured in a chondrogenic differentiation medium for 1 week, and the amount of mRNA of TSP-2 was measured from the cultured cell by real time-PCR (RT-PCR) using a total RNA as a template and a TSP-2-specific primer.

FIG. 2 is a graph showing an expression amount of mRNA of TSP-2 of an UCB-MSC cultured in a chondrogenic differentiation medium, according to an embodiment of the present invention. Referring to FIG. 2, TSP-2 was expressed in the largest amount in a C1 (or C2) UCB-MSC having a high chondrogenic

differentiation capability, while the expression of TSP-2 was weak in a C5 (C6 or C7) UCB-MSC having a low chondrogenic differentiation capability.

In addition, UCB-MSC types having chondrogenic differentiation capabilities were each cultured in a chondrogenic differentiation medium, and the concentration of TSP-2 in the obtained culture supernatant was analyzed by ELISA according to time.

FIGS. 3 and 4 are graphs showing the amount of TSP-2 in a culture supernatant by ELISA, according to embodiments of the present invention. Referring to FIGS. 3 and 4, a high level of TSP-2 was expressed in a C1 or C2 UCB-MSC having a high chondrogenic differentiation capability (refer to FIG. 3), while a very low level of TSP-2 was expressed in a C5, C6, or C7 UCB-MSC (refer to FIG. 4).

**(3) Activity of TSP-2 to induce chondrogenic differentiation**

An UCB-MSC was pellet cultured in a chondrogenic differentiation medium containing 10 ng/ml of isolated and purified human TSP-2 protein (R&D System, Minneapolis, MN, USA), and a pellet size thereof was measured. As the UCB-MSC differentiates into a chondrocyte, the synthesis of extracellular matrix (ECM) increases, and thus the pellet size represents a degree of chondrogenic differentiation.

FIG. 5 is an image showing a pellet size of an UCB-MSC cultured in the presence or absence of TSP-2, according to an embodiment of the present invention. In FIG. 5, the pellet size of the control was 258526.070 $\mu m^2$, and, when the chondrogenic differentiation medium containing 10 ng/ml of TSP-2 was used, the pellet size was 3.49 times greater than that of the control, i.e., 901919.431 $\mu m^2$. As illustrated in FIG. 5, the pellet size of the UCB-MSC increased by TSP-2, which indicates that TSP-2 induces chondrogenic differentiation.

**Example 3: Expression level of TSP-2 according to chondrogenic differentiation capability**

In the present example, an expression level of TSP-2 of an UCB-MSC according to its chondrogenic differentiation capability was measured. First, C3, C4 and C5 UCB-MSCs were each cultured in a chondrogenic differentiation medium under the same conditions for 7 days to induce chondrogenic differentiation. Relative chondrogenic differentiation capabilities of the C3, C4 and C5 UCB-MSCs

were previously confirmed by an experiment, and satisfied the condition of C3 > C4 > C5. Next, TSP-2 in the obtained culture supernatant was measured by ELISA.

FIG. 6 is a graph showing TSP-2 expressed in a culture supernatant of each of 3 types of UCB-MSC cultured in a chondrogenic differentiation medium, according to an embodiment of the present invention. Referring to FIG. 6, the C5 UCB-MSC, which was classified to have the lowest chondrogenic differentiation capability, secreted 72 pg/ml of TSP-2 per $1X10^5$ cells in a state before chondrogenic differentiation induction (naive state), secreted 1.2 ng/ml of TSP-2 per $1X10^5$ cells on the third day after chondrogenic differentiation induction, and secreted 0.550 ng/ml of TSP-2 per $1X10^5$ cells on the seventh day after chondrogenic differentiation induction. Thus, an UCB-MSC expressing TSP-2 to a larger amount than that of TSP-2 expressed by the C5 UCB-MSC may be selected as an UCB-MSC suitable for use in chondrogenic differentiation.

**Example 4: Chondrogenesis capabilities of UCB-MSC and BM-MSC**

An in vitro chondrogenesis experiment was performed using a UCB-MSC and a BM-MSC each derived from about 10 different human donors.

**(1) Preparation of UCB-MSC and BM-MSC**

An umbilical cord blood (UCB) sample was obtained from the umbilical vein of deliveries under informed maternal consent. A bone marrow aspirate was obtained from an iliac crest of each donor under consent of each donor. Adherent and spindle-shaped mesenchymal stem cell (MSC)-like mononuclear cells were isolated from human BM and UCB through the same process. The following properties of the adherent and spindle-shaped MSC-like mononuclear cells obtained from the two origins were confirmed: (1) stemness (proliferativeness), (2) adhesion, (3) spindle shape, (4) cell surface antigens using flow cytometry, and capability to differentiate into mesenchymal tissue such as bone and cartilage.

A cell surface antigen phenotype of the adherent and spindle-shaped MSC-like mononuclear cells obtained from the two origins, confirmed to satisfy the requirements of (1) through (3), was negative for CD14, CD34 and CD45 (hemapoietic marker) and HLA-DR (class II marker), while it was positive for CD29, CD44, CD73, CD105 and CD90 (MSC marker) and HLA-ABC (class I marker). Since a fibroblast also expresses the same set of surface antigens as described above and is an adherent, spindle-shaped, proliferative cell, the properties of the

MSC-like mononuclear cells were further confirmed to confirm appropriate differentiation potential of a MSC into mesenchymal tissue such as bone and cartilage.

**(2) Confirmation of chondrogenic differentiation capability and property of each type of MSC**

A BM-MSC or a UCB-MSC was pellet cultured in a chondrogenic differentiation medium for 6 weeks to induce chondrogenesis. As the chondrogenic differentiation medium, a high glucose Dulbecco's modified Eagle Medium (DMEM) supplemented with 500 ng/ml bone morphogenetic protein-6 (BMP-6) (R&D System, Minneapolis, MN, USA), 10 ng/ml tranforming growth factor-β3 (TGFβ3) (Sigma), ITS+ Premix (6.25 ㎍/ml insulin, 6.25 ㎍/ ml transferrin, 6.25 ng/ ml selenious acid, 1.25 mg/ ml BSA, and 5.35 mg/ml linoleic acid, 1:100 dilution, Becton Dickinson), 100 nM dexamethasone (Sigma), 50 ㎍/ml of ascorbate-2-phosphate, 40 ㎍/ml of L-proline (Sigma), and 100 ㎍/ml of pyruvate (Sigma) was used. The chondrogenic differentiation medium is commonly used by one of ordinary skill in the art of chongrogenesis (see "Pellet Culture" in Materials and Methods of PNAS, Vol.99, No. 7, pp.4397-4402(2002); "Chondrogenesis" in MATERIALS AND METHODS of Stem cells, 20(2002): 530-41). It is known that the UCB-MSC and the BM-MSC easily differentiate into a chondrocyte.

An MSC at 4 to 6 passages was separated with trypsin, and then was suspended to $5x10^5$/ml in the chondrogenic differentiation medium. Next, the suspension was added into a 15 ml polypropylene tube, and the MSC was centrifuged at 500 g for 5 minutes to form a pellet. The obtained pellet was cultured. The medium was changed twice weekly, and the pellet was immobilized with 4% paraformaldehyde contained in a paraffin according to time, and cut to a piece with 5㎛ thickness. The piece was stained with Safranin-O to detect an anionic proteoglycan. In addition, the piece was subjected to type II collagen immunostaining. The chondrogenic differentiation was determined according to whether pellets having around shape were formed in a pellet culture, whether cartilage-specific proteoglycan existed in counter-staining by Safranin-O or Hematoxylin, and whether type II collagen existed in type II collagen immunostaining.

FIG. 7 illustrates images showing in vitro chondrogenic differentiation of an UCB-MSC and a BM-MSC, according to an embodiment of the present invention. In FIG. 7, pellets a, c and e respectively represent Safranin-O staining results of the

UCB-MSC obtained 1 week (a), 3 weeks (c), and 6 weeks (e) after in vitro chondrogenesis, and pellets b, d and f respectively represent Safranin-O staining results of the BM-MSC obtained 1 week (a), 3 weeks (c), and 6 weeks (e) after in vitro chondrogenesis. In addition, g and h respectively represent type II collagen immunostaining results of the UCB-MSC and the BM-MSC obtained 6 weeks after in vitro chondrogenesis.

Safranin-O-specific orange-red staining was more distinct in the UCB-MSC obtained 6 weeks after in vitro chondrogenesis than in the BM-MSC obtained 6 weeks after in vitro chondrogenesis (refer to e and f). In addition, collagen II immunostaining (indicated by arrows in FIG. 7) was more distinct in the UCB-MSC obtained 6 weeks after in vitro chondrogenesis than in the BM-MSC obtained 6 weeks after in vitro chondrogenesis (refer to g and h).

1 week after chondrogenesis induction, the UCB-MSC and the BM-MSC did not show distinct differences in the Safranin-O-specific orange-red staining. 3 weeks after chondrogenesis induction, the BM-MSC did not show any cartilage form, while the UCB-MSC began to exhibit a chondrocyte form. That is, in the case of the UCB-MSC, perichondrium-like cells were observed outside the pellet, an extra-cellular matrix began to be secreted inside the pellet, and the UCB-MSC began to be weakly positive for Safranin-O staining. 6 weeks after chondrogenesis induction, the BM-MSC showed the same chondrogenesis degree as that of the 3-week UCB-MSC, while the UCB-MSC showed a typical chondrogenesis tissue form. To confirm whether a functioning, normal chondrocyte is formed, collagen II immunostaining is performed, and as a result, brown-colored positive staining may be observed, as indicated by arrows in FIG. 7. Comparing the UCB-MSC with the BM-MSC, the UCB-MSC exhibits more positive than the BM-MSC, which indicates that the UCB-MSC has better chondrogenesis capability.

In conclusion, as illustrated in FIG. 6, the chondrogenesis capability of the UCB-MSC is significantly better than that of the BM-MSC.

FIG. 8 is a graph showing capabilities of an UCB-MSC and a BM-MSC to differentiate into chondrogenic lineage, according to an embodiment of the present invention. An experiment for determining capabilities of the UCB-MSC and the BM-MSC to differentiate into the chondrogenic lineage was performed as follows. First, an MSC at 4 to 6 passages was separated with trypsin, and then was suspended to $5x10^5$ cells/ml in a chondrogenic differentiation medium. Next, the

suspension was added into a 15 ml polypropylene tube, and the MSC was centrifuged at 500 g for 5 minutes to form a pellet. The obtained pellet was cultured. The medium was changed twice weekly.

Referring to FIG. 8, 7 of 10 UCB-MSC samples (70%) had a capability to differentiate into the chondrogenic lineage, while 5 of 10 BM-MSC samples (50%) had a capability to differentiate into the chondrogenic lineage. 6 weeks after chondrogenic differentiation, the size of a pellet area of the UCB-MSC (n=7, 1450123.7±24256.9 $\mu m^2$) ($p<0.02$) was much bigger than the size of a pellet area of the BM-MSC (n=5, 346531.3±87396.6 $\mu m^2$). Pellet areas and areas positive for Safranin-O were measured by i-solution software (IM Technology, Doosan, Daejeon).

FIG. 9 illustrates images showing chondrogenic differentiation of 10 types of BM-MSC and 10 types of UCB-MSC analyzed on the sixth week after chondrogenic differentiation induction, according to an embodiment of the present invention. Referring to FIG. 9, cartilage proteoglycan-specific orange-red staining by Safranin-O was distinct in 7 types of UCB-MSC (A panel-7 from an upper panel and 2 from a lower panel), while it was distinct in 5 types of BM-MSC (B panel-5 from the upper panel). That is, 70% of the total types of UCB-MSC differentiated into the chondrogenic lineage, while only 50% of the total types of BM-MSC differentiated into the chondrogenic lineage.

FIG. 10 illustrates images showing a difference in chondrogenesis capability between an UCM-MSC and a BM-MSC on the sixth week after chondrogenic differentiation induction, according to an embodiment of the present invention. FIG. 10 more clearly shows a difference between cartilage pellets produced from the UCB-MSC and cartilage pellets produced from the BM-MSC, wherein the same number of the UCB-MSC and the BM-MSC were cultured for 6 weeks under the same chondrogenic conditions. Referring to FIG. 9, the cartilage pellet produced from the UCB-MSC is obviously much bigger than the cartilage pellet produced from the BM-MSC. In addition, a cartilage-specific proteoglycan matrix was more abundant and distinct in the UCB-MSC-derived cartilage pellet with chondrocyte-like cells surrounding lacuna than in the BM-MSC-derived cartilage pellet. This indicates that the UCB-MSC has superior chondrogenesis capability to that of the BM-MSC under the same in vivo chondrogenic conditions.

Such results verify that the chondrogenic differentiation capability of the UCB-MSC is statistically significantly higher than the BM-MSC. Due to such a fact,

MSCs may have significantly different differential cellular features, although MSCs are named the same. That is, this indicates that identical MSCs may also be classified as different cell types. In the present embodiment, a differentiation test was performed to test a difference in MSC cell types, wherein the differentiation depends on (1) the identity of MSCs different than terminally-differentiated fibroblasts and (2) in particular, the origin and age of a source tissue from which each type of MSC was isolated.

The same chondrogenic medium was used for the UCB-MSC and the BM-MSC. In addition, a growth factor combination contained in the medium is introduced for chondrogenesis of the BM-MSC and is well-known in the art (Biochemical and Biophysical Research Communications 320 (2004): Abstract on pp. 914-919, "Cell culture" and "Pellet culture" of Materials and Methods). Thus, specific medium conditions used in the present embodiment do not preferably affect the chondrogenic ability of the UCB-MSC. In conclusion, the UCB-MSC has superior in vitro chondrogenic activity to that of the BM-MSC.

**Example 5: Identification of inducer of expression of TSP-2 in UCB-MSC**

In the present example, an inducer of expression of TSP-2 in a UCB-MSC was identified by varying culture conditions.

First, a UCB-MSC being monolayer cultured was treated with trypsin to be separated, and was suspended to a concentration of $5x10^5$ cells/ml in a serum-free DMEM, and the resulting product was cultured for 24 hours. The medium used was a DMEM (containing 100 nM dexamethasone, 50 $\mu g$/ml of ascorbate-2-phosphate, 40 $\mu g$/ml of L-proline, and 100 $\mu g$/ml of pyruvate) and a DMEM supplemented with a growth factor selected from 10 ng/ml of TGF-β3 (Sigma), 500 ng/ml of BMP-6 (R&D System, Minneapolis, MN, USA), and ITS+ (6.25 $\mu g$/ml of insulin, 6.25 $\mu g$/ml of transferrin, 6.25 $\mu g$/ml of selenious acid, 1.25 mg/ml of BSA, and 5.35 mg/ml of linoleic acid, 1:100 dilution, Becton Dickinson). The UCB-MSC was monolayer cultured or pellet cultured. In the case of pellet culturing, the suspension was centrifuged at 500 g for 5 minutes to form a cell pellet, and the obtained cell pellet was cultured.

After the obtained culture supernatant was collected, a cell lysate was obtained, and a level of mRNA of TSP-2 of the UCB-MSC was measured by using a real-time polymerase chain reaction (RT-PCR) using a total RNA extracted from the cell lysate

as a template.

FIG. 11 is a graph showing expression results of TSP-2 under monolayer and pellet culturing conditions in the presence of a growth factor combination, according to an embodiment of the present invention. Referring to FIG. 11, the expression of TSP-2 significantly increased under the pellet culturing conditions. In addition, from the results illustrated in FIG. 11, it was confirmed that the growth factor did not affect the expression of TSP-2.

**Example 6: Selection of cell types suitable for use in chondrogenesis**

A UCB-MSC was cultured in a medium that did not induce chondrogenesis, and it was confirmed whether an expression amount of TSP-2 was associated with a chondrogenic differentiation capability of the UCB-MSC.

In particular, C3 and C5 UCB-MSCs were monolayer cultured and pellet cultured in a serum-free DMEM (containing 100 nM dexamethasone, 50 $\mu g$/ml of ascorbate-2-phosphate, 40 $\mu g$/ml of L-proline, and 100 $\mu g$/ml of pyruvate) to a concentration of $5x10^5$ cells/ml. The culturing conditions were the same as those in Example 5. The expression amount of TSP-2 in the obtained culture supernatant was measured by ELISA. In addition, the culturing conditions of a BM-MSC were also the same as those of the UCB-MSC.

FIG.12 is a graph showing an expression degree of TSP-2 according to the types of UCB-MSC, according to an embodiment of the present invention. In FIG. 12, C3 and C5 represent UCB-MSC cell types, and naive and pellet, respectively represent monolayer culturing for 24 hours and pellet culturing for 24 hours. Optical microscope observation results of C3 and C5 during the culturing process and after the culturing process are the same as those of C3 and C5 of FIG. 1.

Referring to FIG. 12, the monolayer cultured C5 (naive) expressed 33 to 72 pg/ml of TSP-2 per $1.0x10^5$ cells, while the pellet cultured C5 (pellet) expressed 163 to 550 pg/ml of TSP-2 per $1.0x10^5$ cells. It was previously confirmed that the chondrogenesis capability of the C3 UCB-MSC was better than that of the C5 UCB-MSC. Thus, whether a cell has a high chondrogenesis capability may be determined by comparing an expression amount of TSP-2 of the cell with an expression amount of TSP-2 of a reference cell, for example, a C5 UCB-MSC. For example, when a 1-day monolayer cultured (naive) reference cell expresses TSP-2 to an amount higher than 33 to 72 pg/ml per $1.0x10^5$ cells, or when a 1-day pellet

cultured reference cell expresses TSP-2 to an amount higher than 163 to 550 pg/ml per $1.0x10^5$ cells, it may be determined that the chondrogenesis capability of the reference cell is higher than that of the C5 UCB-MSC. Such a method may be used to select MSCs suitable for use in chondrogenesis.

Based on the standard for selecting cells suitable for use in chondrogenesis, the reference cell may be appropriately selected by one of ordinary skill in the art.

As illustrated in FIG. 12, when a stem cell was pellet cultured, the expression of TSP-2 significantly increased.

FIG. 13 is a graph showing measurement results of expression amounts of TSP-2 obtained by pellet culuring a C3 UCM-MSC and a C5 UCM-MSC for 3 days, according to an embodiment of the present invention. Referring to FIG. 13, the expression amount of TSP-2 of the C5 UCM-MSC having a low chondrogenic differentiation capability was smaller than that of the C3 UCM-MSC having a high chondrogenic differentiation capability even as a culturing time increases.

Thus, the expression amount of TSP-2 is associated with the chondrogenic differentiation capability of the UCB-MSC, and the chondrogenic differentiation capability of MSCs may be predicted by measuring the expression amount of TSP-2.

FIG. 26 is a graph showing results of measuring an expression level of TSP-2 after an UCB-MSC and a BM-MSC are pellet cultured, according to an embodiment of the present invention. Referring to FIG. 26, the UCB-MSC expressed a significantly higher level of TSP-2 than the BM-MSC. This indicates that the chondrogenic differentiation degree of the UCB-MSC is better than that of the BM-MSC.

The results illustrated in FIG. 26 were obtained as follows. First, an UCB-MSC and a BM-MSC that were being monolayer cultured were treated with trypsin to be separated, and the UCB-MSC and the BM-MSC were each suspended to a concentration of $5x10^5$ cells/ml in a serum-free DMEM, and cultured for 24 hours. The medium used was a DMEM (containing 100 nM dexamethasone, 50 $\mu$g/ml of ascorbate-2-phosphate, 40 $\mu$g/ml of L-proline, and 100 $\mu$g/ml of pyruvate). Each cell was pellet cultured, and centrifuged at 500 g for 5 minutes to form a cell pellet, and the obtained cell pellet was cultured for 24 hours. The obtained culture supernatant was collected, and the expression level of TSP-2 was measured by ELISA.

**Example 7: Expression of TSP-2 by UCB-MSC under chondrogenic differentiation and dedifferentiation conditions**

To confirm association of an expression amount of TSP-2 with chondrogenic differentiation, the expression amount of TSP-2 by an UCB-MSC was measured under chondrogenic differentiation and dedifferentiation conditions.

**(1) Expression of TSP-2 by chondrogenic progenitor cell under chondrogenic differentiation condition**

A chondrogenic progenitor cell was separated from a limb bud of a mouse embryo. $4x10^7$ cells/ml of the separated chondrogenic progenitor cell was resuspended in a medium (containing DMEM/F-12 (2:3), 10%(v/v) FBS, 50 ㎍/ml of streptomycin, and 50 units/ml of penicillin), and each of 15 ㎕ of the resuspended chondrogenic progenitor cell was dropped into a culture dish to be attached thereto in an independent spot form. Next, the chondrogenic progenitor cell in a spot form was cultured for 6 days to induce each spot to differentiate into a chondrocyte. An expression amount of TSP-2 was measured by using RT-PCR using a total RNA isolated from the cell as a template.

FIG. 14 illustrates graphs showing the amount of TSP-2 expressed by a chondrogenic progenitor cell or a chondrocyte under differentiation and dedifferentiation conditions, according to an embodiment of the present invention. Referring to A of FIG. 14, the expression amount of TSP-2 increased with a culturing time under differentiation conditions.

**(2) Expression of TSP-2 by chondrocyte under chondrogenic dedifferentiation condition**

A chondrocyte was separated from a knee joint of a 2-week-old rabbit. The separated chondrocyte was cultured in a medium containing a DMEM, 10%(v/v) FBS, and 50 ㎍/ml of gentamicin in the presence of 5 ng/ml of interleukin-1β (IL-1β) to induce dedifferentiation. IL-1β is a pro-inflammatory cytokine that dedifferentiates a chondrocyte, resulting in loss of the properties of the chondrocyte. The expression amount of TSP-2 was measured by RT-PCR using a total RNA isolated from the cell as a template.

Referring to B of FIG. 14, the expression amount of TSP-2 decreased with a culturing time under dedifferentiation conditions.

From the results described above, it is confirmed that the expression of TSP-2 is associated with chondrogenic differentiation and dedifferentiation of a chondrocyte.

**Example 8: Chondrogenic differentiation induction of UCB-MSC by**

**TSP-2**

An UCB-MSC was cultured in the presence of TSP-2 to induce chondrogenic differentiation. The medium used was the chondrogenic culture medium described above. A recombinant TSP-2 (R&D System, Minneapolis, MN, USA) was added to the medium in an amount of 10 ng/ml to 500 ng/ml, and the resultant was pellet cultured. An initial concentration of the UCB-MSC was $5x10^5$ cells/ml. After the culturing process, a RT-PCR using a total RNA isolated from the cell as a template and using primers specific to a chondrocyte marker (for example, type II collagen (Col IIA1), aggrecan (Acan), Sox-9 and TSP-2); and hypertrophic chondrocyte and bone markers (for example, Col IA1 and Col XA1) was performed to measure an expression amount of mRNA of these markers.

FIGS. 15 through 17 are graphs showing an expression amount of a marker protein of an UCB-MSC cultured in the presence of TSP-2, according to embodiments of the present invention. Referring to FIGS. 15 through 17, the expressions of type II collagen (Col IIA1), aggrecan (Acan), and Sox-9 increased depending on the concentration thereof 1 week after the chondrogenic differentiation induction, while the expressions of Col 1A1 and Col XA1 decreased or were not exhibited with a culturing time.

Thus, it is confirmed that externally added TSP-2 stimulates chondrogenic differentiation of the UCB-MSC.

**Example 9: Chondrogenic differentiation induction of UCB-MSC under TSP-2 expression-inhibiting conditions**

An UCB-MSC was cultured in a chondrogenic culture medium under TSP-2 expression-inhibiting conditions to induce chondrogenic differentiation.

Small interfering RNA (siRNA) (Bioneer, Daejeon, Korea, sense sequence: SEQ ID NO:9, anti-sense sequence: SEQ ID NO: 10) with a sequence complementary to that of mRNA of TSP-2 was added to a medium in a concentration of 33 nM to inhibit the expression of TSP-2. The medium used was the chondrogenic culture medium described above, and the UCB-MSC was pellet cultured. An initial concentration of the UCB-MSC was $5x10^5$ cells/ml, and the culturing process was performed for 7 days. The expression amount of TSP-2 was measured by using RT-PCR using a total RNA extracted from the UCB-MSC and using a TSP-2-specific primer, or the expression amount of TSP-2 in the obtained

culture supernatant was measured by ELISA. The expressions of Col IIA1 and aggrecan were measured by using a RT-PCR.

FIG. 18 illustrates graphs showing a degree of chondrogenic differentiation of an UCB-MSC cultured in a chondrogenic medium under TSP-2 expression-inhibiting conditions, according to an embodiment of the present invention. Referring to FIG. 18, in the UCB-MSC cultured under TSP-2 expression-inhibiting conditions, i.e., in the presence of siRNA of TSP-2, the expressions of the chondrocyte markers, i.e., Col IIA1 and aggrecan significantly decreased. This indicates that TSP-2 induces or stimulates the chondrogenic differentiation of the UCB-MSC. In FIG. 18, A shows results of measuring the concentration of TSP-2 by RT-PCR, B shows results of measuring the concentration of TSP-2 by ELISA, and C and D show RT-PCR results of Col IIA1 and aggrecan, respectively.

**Example 10: Level of TSP-2 in blood plasma of patient with osteoarthritis**

Blood was collected from 15 normal people and 28 patients with osteoarthritis, and the level of TSP-2 in each blood plasma was measured by ELISA.

FIG. 19 is a graph showing levels of TSP-2 in blood plasma of a normal person and a patient with osteoarthritis, according to an embodiment of the present invention. Referring to FIG. 19, the level of TSP-2 was higher in the blood plasma of the patient with osteoarthritis than in the blood plasma of the normal people. This indicates that the level of TSP-2 in blood may act as a marker for diagnosing arthritis. This also indicates that the level of TSP-2 in blood may act as a marker for diagnosing chondrogenic differentiation-related diseases, in addition to arthritis.

**Example 11: Expression of TSP-1 in UCB-MSC by joint fluid of patient with arthritis**

The effect of a joint fluid of a patient with arthritis on the expression of TSP-1 in an UCB-MSC was confirmed.

An UCB-MSC was cultured in the presence of a joint fluid of a patient with arthritis. The UCB-MSC was cultured in a medium containing MEM-α, 10%(v/v) FBS, and 50 $\mu g$/ml of gentamicin for 5 to 6 days. The joint fluid of joint cavity was added to the medium when the UCB-MSC was cultured to a level of 70-80% of the area of a culture container. The joint fluid of the patient with arthritis was added to a concentration of 20% (v/v) after the medium with the UCB-MSC being cultured

therein was changed to a medium containing MEM-α and 50 ㎍/ml of gentamicin, and the resultant was further cultured for 3 hours. The obtained culture was used as an analysis sample. In addition, as a control, an UCB-MSC culture cultured in a state where the joint fluid was not added thereto and/or a medium with the joint fluid added to a concentration of 20% (v/v) in which an UCB-MSC was not cultured were used. The joint fluid was obtained from a patient with degenerative arthritis.

FIGS. 20 and 21 are graphs showing expression amounts of TSP-1 of an UCB-MSC in the presence of a joint fluid of a patient with arthritis, according to embodiments of the present invention. In FIG. 20, MSC only represents that the UCB-MSC was cultured without the joint fluid, and JF#1, JF#2, and JF#3 respectively represent joint fluids of different patients, and results from a triplicate experiment. In FIG. 20, the expression amount of TSP-1 was measured by using a RT-PCR using a total RNA extracted from the UCB-MSC as a template and using a TSP-1-specific primer.

In FIG. 21, JF represents a joint fluid of a patient with arthritis. In FIG. 21, the expression amount of TSP-1 in the obtained culture supernatant of the UCB-MSC was measured by ELISA. Referring to FIG. 21, the UCB-MSC cultured in the presence of a joint fluid of a patient with arthritis expressed a larger amount of TSP-1 than that in the UCB-MSC cultured in a medium excluding the joint fluid of a patient with arthritis or in the culture obtained in a medium including only 20% of joint fluid of a patient with arthritis.

**Example 12: Association of IL-17BR with chondrogenic differentiation capability**

UCB-MSC types having different chondrogenic differentiation capabilites were pellet cultured in a chondrogenic differentiation medium for 1 week to induce chondrogenic differentiation. The amount of mRNA of IL-17BR was measured by using a RT-PCR using a total RNA obtained by lysing the UCB-MSC as a template.

FIG. 22 is a graph showing results of analyzing the amount of mRNA of IL-17BR obtained by lysing an UCB-MSC differentiated into cartilage by a RT-PCR, according to an embodiment of the present invention.

Referring to FIG. 22, an expression level of mRNA of IL-17BR varied according to the chondrogenic differentiation capability of the UCB-MSC. That is, the C2 and C3 UCB-MSC expressed mRNA of IL-17BR, and the degree of

chondrogenic differentiation capability was 8.9 times higher in the C2 UCB-MSC than in the C3 UCB-MSC. On the other hand, the C5 UCB-MSC having a low chondrogenic differentiation capability did not express mRNA of IL-17BR.

**Example 13: The effect of joint fluid of patient with arthritis on expression of HB-EGF**

An UCB-MSC was cultured in a medium to which a joint fluid of a patient with arthritis was added to a concentration of 10% (v/v) by using a method similar to that used in Example 1, for 6 hours, and the amount of mRNA of HB-EGF was measured by RT-PCR using a total RNA obtained from the UCB-MSC as a template. As a control, an UCB-MSC cultured under the same conditions described above, except that a medium did not include the joint fluid of a patient with arthritis, was used.

FIG. 23 illustrates graphs showing measurement results of mRNA of HB-EGF in an UCB-MSC cultured in the presence of a joint fluid of a patient with arthritis, according to an embodiment of the present invention. In FIG. 23, C3 and C5 represent types of UCB-MSC, BM-MSC represents a bone marrow-derived mesenchymal stem cell, BEAS-2B represents a lung-derived bronchial epithelial cell, and JF represents a joint fluid. Referring to FIG. 23, the expression of HB-EGF in the UCB-MSC is significantly increased by the joint fluid of the patient with arthritis, while it is not significant in the BM-MSC and the BEAS-2B. The UCB-MSC expressed HB-EGF by the joint fluid of the patient with arthritis to an amount 2 times (C5 UCB-MSC) to 8.4 times (C3 UCB-MSC) larger than that of HB-EGF in the BM-MSC.

This indicates that the expression of HB-EGF is specifically induced in the UCB-MSC by the joint fluid of the patient with arthritis. This also indicates that the UCB-MSC may express a significantly larger amount of HB-EGF than that of HB-EGF in the BM-MSC.

In addition, an expression degree of HB-EGF by an UCB-MSC and in an UCB-MSC by the joint fluid of the patient with arthritis was measured. In this regard, C3 and C5 UCB-MSCs were used, and the joint fluids collected from 3 patients (JF1, JF5, and JF11) were used. The culturing conditions and measurement conditions of HB-EGF are the same as those described above in connection with FIG. 20. Table 1 shows results of analyzing an expression degree of HB-EGF by an UCB-MSC and in an UCB-MSC by joint fluids of patients with arthritis, by using RT-PCR.

<Table 1>

| HB-EGF | MSC | MSC+JF1 | MSC+JF5 | MSC+JF11 |
|---|---|---|---|---|
| C5 UCB-MSC | 1.00 | 9.80 | 26.60 | 9.20 |
| C3 UCB-MSC | 1.00 | 15.00 | 46.90 | 17.50 |

Referring to Table 1, when the UCB-MSC was cultured with the joint fluid of the patient with arthritis, it expressed HB-EGF to an amount 9.2 to 46.9 times larger than that of HB-EGF in the control.

**Example 14: Expression of HB-EGF by UCB-MSC under chondrocyte death conditions**

An expression degree of HB-EGF by an UCB-MSC was analyzed under chondrocyte death conditions. First, a chondrocyte was separated from a joint of a 2-week-old rabbit. The separated chondrocyte was cultured in a medium containing a DMEM and 10% (v/v) FBS in a 6-well plate for 5 days, and the chondrocyte being cultured was used in an experiment. The culturing of the UCB-MSC was performed in the presence of sodium nitroprusside (SNP) or the rabbit-derived chondrocyte for 24 hours. In this regard, 500 μM of SNP was added in the medium. SNP is a nitric oxide-producing compound, and is known to induce chondrocyte death. The addition of SNP is performed to simulate conditions arising in a patient with arthritis in vitro. In addition, the rabbit-derived chondrocyte and the UCB-MSC were respectively co-cultured on a lower portion and an upper portion of a transwell chamber (BD Falcon, San Jose, California, USA, Cell Culture inserts for 6-well plates, 0.4㎛, translucent PET membrane).

Whether HB-EGF is expressed or not was measured by performing immunoblotting such that the UCB-MSC was separated from the culture and lysed, and then an anti-HB-EGF antibody and an antibody specifically binding to an anti-HB-EGF antibody that were labeled with a fluorescence marker were used with respect to the same concentration of the lysate.

FIG. 24 is a diagram showing an expression amount of HB-EGF in an UCB-MSC cultured under chondrocyte death conditions, according to an embodiment of the present invention. Referring to FIG. 24, the UCB-MSC did not express HB-EGF when it was cultured under chondrocyte apoptosis conditions, while the UCB-MSC expressed HB-EGF when it was co-cultured with the rabbit-derived chondrocyte.

In addition, the rabbit-derived chondrocyte was cultured in the presence of HB-EGF, and then it was confirmed whether the rabbit-derived chondrocyte was protected. FIG. 25 illustrates optical miscroscopic images showing observation results of a rabbit-derived chondrocyte cultured in the presence of HB-EGF, according to an embodiment of the present invention. Referring to FIG. 25, the rabbit-derived chondrocyte died depending on the concentration of SNP in the control (upper portion), while the apoptosis of the rabbit-derived chondrocyte cultured in a medium containing 50 ng/ml of HB-EGF was inhibited depending on the concentration of SNP. This indicates that the apoptosis of the rabbit-derived chondrocyte caused by SNP is inhibited by HB-EGF.

The sequence list enclosed in the present specification is incorporated herein by reference.

## Claims

1. A composition for stimulating a umbilical cord blood mesenchymal stem cell (UCB-MSC) to differentiate into a chondrocyte, the composition including a UCB-MSC; and a pharmaceutically acceptable carrier,

wherein the UCB-MSC is an UCB-MSC expressing thrombospondin-2 (TSP-2) to an amount higher than 72 pg/ml/1.0x$10^5$ cells when monolayer cultured in a maintenance medium for 1 day, or an UCB-MSC expressing TSP-2 to an amount higher than 550 pg/ml/1.0x$10^5$ cells when pellet cultured in a maintenance medium.

2. The composition of claim 1, for use in treating or preventing at least one selected from the group consisting of a cartilage injury, cartilage degeneration, cartilage loss, a cartilage defect, and combinations thereof.

3. The composition of claim 1, wherein the composition is for use in treating or preventing arthritis.

4. Use of the composition of claim 1 in the preparation of a medicament for treating or preventing at least one selected from the group consisting of a cartilage injury, cartilage degeneration, cartilage loss, a cartilage defect, and combinations thereof.

5. Use of the composition of claim 1 in the preparation of a medicament for treating or preventing arthritis.