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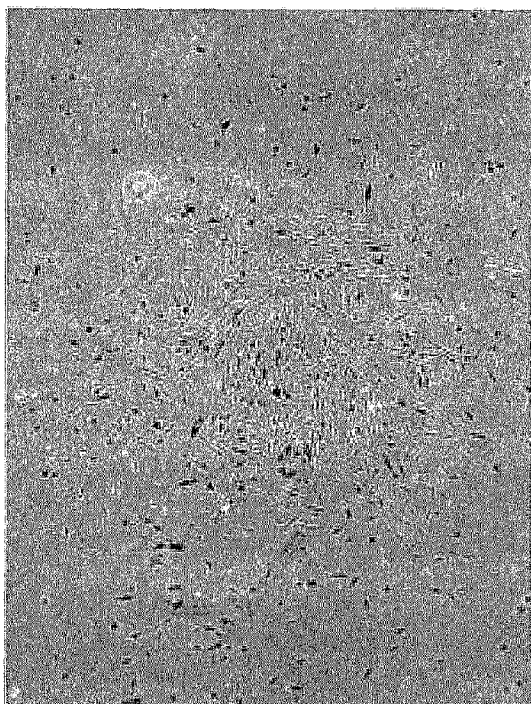
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(54) Title: BIOMARKER FOR PURIFICATION OR IDENTIFICATION OF MESENCHYMAL STEM CELLS AND METHODS OF PURIFICATION OF MESENCHYMAL STEM CELLS BY USING IT

[Fig. 7]



(57) Abstract: The present invention is related to biomarker for purification and identification of mesenchymal stem cells and methods for purification of mesenchymal stem cells by using it. The present invention provides composition, apparatus and kit for separation or identification of MSCs comprising anti-fibroblast activation protein α (FAP α). Also, the present invention provides the method for identification and purification of MSCs. In detail, the present invention provides the method for identification of MSCs comprising the steps of: contacting anti-FAP α antibody with test cell; detecting the forming of antigen-antibody complex of FAP α of the above test cell and the above anti-FAP α antibody. And the present invention provides the method for separation and purification MSCs comprising the steps of: forming antigen-antibody complex by contacting liquid biological media having MSC with anti-FAP α antibody; and collecting the above antigen-antibody complex.

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【DESCRIPTION】**【Invention Title】**

BIOMARKER FOR PURIFICATION OR IDENTIFICATION OF MESENCHYMAL STEM CELLS AND METHODS OF PURIFICATION OF MESENCHYMAL STEM CELLS BY USING IT

【Technical Field】

<1> The present invention is related to fibroblast activation protein α (FAP α), a surface protein marker for purification or identification of mesenchymal stem cells from human bone marrow, peripheral blood, umbilical cord blood and adipose tissue, and methods for purification of mesenchymal stem cells by using it.

【Background Art】

<2> Mesenchymal stromal cells (MSCs), also popularly termed mesenchymal stem cells [1], are stored in bone marrow (BM) but are released in response to mobilization signals into circulation to maintain homeostasis of connective tissues. In recent years they have gained widespread popularity in the fields of cell and immune therapy due to their multi-faceted functionalities, which include tissue regeneration, hematopoietic support and immunomodulation [2-4]. However, their swift development into clinically useful products has been impeded in large part by the lack of definitive markers. Although a couple of glycolipid markers, SSEA-4 [5] and GD2 [6], have been shown to identify MSCs within BM, no single protein marker has thus far been proven sufficient to uniquely distinguish MSCs from other cell types. To unambiguously identify human MSCs, therefore, one requires an analysis of multiple surface protein markers, the optimal combination of which has been proposed by the International Society for Cellular Therapy to be CD14⁻ (or CD11b⁻) CD19⁻ (or CD79 α ⁻) CD34⁻ CD45⁻ CD73⁺ CD90⁺ CD105⁺ HLA-DR⁺ [7].

<3> We have recently performed DNA microarray-based differential gene

<4> expression profiling to identify the molecular signature specific to human MSCs [8,9]. The articles report many genes which prefer expressing in MSCs, contrary to peripheral blood (PB)-derived mononuclear cell. However,

there has been no report to show that FAP α is expressed in MSCs specifically, so that MSCs can be identified.

<5> PCT/JP2004/002457 (filed February 27, 2004.) provides marker and method of distinguishing, separating or identifying MSCs. Also, it provides probe for distinguishing the above marker gene of MSCs and PCR primers for amplifying the gene of test cell, polypeptide markers for identifying MSCs consisting of polypeptides which are encoded by MSC marker genes, antibodies for identifying the above polypeptide markers attaching to them specifically, probes for identifying MSC marker genes, and method of distinguishing, separating MSCs using the antibodies attaching to polypeptide markers specifically. However, statement about FAP α as a marker for purification or identification of MSCs is not disclosed.

<6> During a bioinformatic survey of MSC-enriched genes, a gene for fibroblast activation protein α (FAP α), also called seprase, has caught our attention, because it has been known to encode an integral membrane protein with a large globular ectodomain [10] whose expression, however, is suppressed in normal adult tissues [11-14]. This finding raises the possibility that MSCs may be among very few, if any, cell types in the adult human body that express FAP α .

<7> In the present invention, therefore, we have investigated whether FAP α can serve as a surface protein marker to identify and isolate MSCs from human BM and, possibly, from other cell sources.

<8>

【Disclosure】

【Technical Problem】

<9> An object of the present invention is to provide a method for separating and identifying MSCs from human bone marrow, umbilical cord blood, peripheral blood, and adipose tissue.

<10> Further, it is another object of the present invention to provide a composition, an apparatus, or a kit for separating and identifying MSCs specifically.

【Technical Solution】

<11> To achieve the above objects, the present invention provides a composition for separating or identifying MSCs comprising an anti-fibroblast activation protein α (FAP α) antibody. The above antibody can be a monoclonal antibody, a polyclonal antibody, a single-chain antibody or a recombinant antibody. To detect binding between the above antibodies and MSC specific FAP α easily, the above antibodies can be linked to a ligand, a magnetic bead, an enzyme and the like. The above ligand can be, but not limited to, biotin, avidin or streptavidin. The above enzyme can be, but not limited to, luciferase, peroxidase or β -galactosidase. Person having ordinary skill in the art can choose an appropriate ligand, bead and enzyme to detect the antibodies.

<12> The present invention provides an apparatus for separation of MSCs comprising an anti-FAP α antibody. To separate the anti-FAP α antibody and FAP α complex, an antibody linked to a fluorescent material and a cell sorter, an antibody linked to a magnetic bead and a magnetic apparatus, or an antibody linked to a specific ligand and a column for the separation can be used.

<13> The present invention provides a kit for identifying MSC comprising an anti-FAP α antibody. To identify the anti-FAP α antibody and FAP α complex, an antibody linked to a fluorescent material and a fluorescence spectrometer, an antibody linked to a magnetic bead and a magnetic apparatus, or a specific enzyme and a substrate thereof can be used. The above kit can be a protein chip for identifying MSC comprising an anti-FAP α antibody, specifically.

<14> The present invention also provides a method for identifying MSC comprising the steps of: contacting an anti-FAP α antibody with a test cell; and detecting the formation of antigen-antibody complex of FAP α on the test cell and the FAP α antibody. The detecting step can be performed by, but not limited to, radio immuno assay (RIA), ELISA, immuno fluorescence assay, color particle hybridization assay, or chemiluminescent molecular hybridization assay.

<15> The present invention also provides a method for separating and purifying MSCs comprising the steps of: forming an antigen-antibody complex by contacting liquid biological media having MSC with an anti-FAP α antibody; and collecting the antigen-antibody complex. The collecting step can be performed by using any one of ligands selected from the group consisting of biotin, avidin and streptavidin, or using a combination of a magnetic bead and a magnetic apparatus.

<16>

【Description of Drawings】

<17> Fig. 1 is tandem MS coverage map of FAP α sequence. The bold italic sequences designate the locations of twelve peptides whose sequences are determined by tandem MS analysis (~24% coverage). A boxed sequence at the N-terminus corresponds to a single α -helical transmembrane domain of FAP α .

<18> Fig. 2 is RT-PCR analysis indicating preferential FAP α expression on BM derived MSCs over MNCs. BM-MSC; bone marrow (BM) derived mesenchymal stromal cell (MSC), BM-MNC; bone marrow (BM) derived mononuclear cell (MNC).

<19> Fig. 3 is Flow cytometry analysis demonstrating exclusive FAP α expression on the cell surface of MSCs (a shaded profile) but not on MNCs (an open profile).

<20> Fig. 4 is MTC (multiple tissue cDNA) panel screening showing null *FAP α* expression in human blood fractions.

<22> Fig. 5 is RT-PCR analysis demonstrating strong *FAP α* expression on BM, UCB and AT-derived MSCs, but not on UCB derived MNCs and AT cells. BM-MSC: bone marrow (BM) derived mesenchymal stromal cell (MSC), UCB-MSC: umbilical cord blood (UCB) derived mesenchymal stromal cell (MSC), AT-MSC: adipose tissue (AT) derived mesenchymal stromal cell (MSC), UCB-MNC: umbilical cord blood (UCB) derived mononuclear cell (MNC), AT: adipose tissue.

<23> Fig. 6 is RT-PCR analysis showing deficient *FAP α* expression in human cancer cell lines.

<24> Fig. 7 is photomicrograph of the cell cultured after FAP-immunoselection for 3 weeks (magnification 40X). Scattered tiny white dots

indicate microbeads with mean diameter of 4-5 μm .

<25> Fig. 8 is flow cytometry analysis demonstrating that FAP-positive cell culture is negative to CD45, but positive to CD73, CD90 and CD105. Cells stained with only secondary antibody were used as negative control (an open profile).

<26> Fig. 9 is showing differentiation potential for bone, cartilage and fat of FAP-positive cell culture which was measured by staining with Oil Red O, Alizarin Red S and Alcian Blue, respectively.

<27> Fig. 10 is RT-PCR analysis of genes related to differentiation in undifferentiated (U) and differentiated (D) FAP-positive cell. Differentiation for bone was measured by not only increasing expression of genes encoding products related to bone such as secreted phosphoprotein 1 (SPP1), alkaline phosphatase, liver/bone/kidney (ALPL) and bone γ -carboxyglutamate protein (BGLAP), but also indicating positive to Alizarin Red S staining, indicator for Ca accumulation. Differentiation for fat was measured by Oil Red O staining and increasing expression of genes encoding proteins related to fat including lipoprotein lipase (LPL), fatty acid binding protein 4, adipocyte (FABP4) and peroxisome proliferator-activated receptor γ (PPARG). Also differentiation for cartilage through micromass pellet culture including TGF β s was measured by indicating positive to Alcian Blue and increasing expression of genes encoding products related to cartilage such as collagen type X α 1 (COL10A1), sex determining box region Y-box 9 (SOX9) and aggrecan (ACAN).

<28>

【Best Mode】

<29> According to the first aspect of the present invention, there is provided a composition comprising an anti-FAP α antibody for separating or identifying MSCs.

<30> "Fibroblast activation protein α (FAP α)" of the present invention is a homodimeric integral membrane gelatinase belonging to serine protease family. This protein is considered to participate in interaction between

epithelium and mesenchymal cell or growth of fibroblast during development, tissue repairing and carcinogenesis of epithelium. NCBI accession number for the sequence of the gene is NM_004460, and NP_004451, AAH26250, etc. for the sequence of the protein.

<31> "Antibody" of the present invention includes, besides antibodies ordinarily existing in a living body, molecules having at least one antigen binding site made from H chain of antibody, L chain of antibody or combination thereof. For example, it comprises a peptide having only variable region of H chain, a Fab having one set of H chain fragment and a L chain fragment, a (Fab')₂ having two sets of H chain fragment and L chain fragment, "ScFv", a single chain antibody having H chain fragment and L chain fragment combined tandemly on the same peptide, etc.

<32> The above antibody can be a monoclonal, a polyclonal, or a single or a recombinant antibody. The above antibodies can be linked to a ligand, a magnetic bead, an enzyme or etc. to detect the binding between the antibodies and the MSC specific FAP α easily. The above ligand can be, but not limited to, biotin, avidin or streptavidin. The above enzyme can be, but not limited to, luciferase, peroxidase or β -galactosidase. Person having ordinary skill in the art can choose an appropriate ligand, bead and enzyme to detect the antibodies.

<33>

<34> According to the second aspect of the present invention, there are provided an apparatus for separating MSCs and a kit for identifying MSCs using an anti-FAP α antibody.

<35> The apparatus for separating MSCs comprising an anti-FAP α antibody of the present invention can use an antibody linked to a fluorescent material and a cell sorter, an antibody linked to a magnetic bead and a magnetic apparatus, or an antibody linked to a specific ligand and a column to separate the complex of the anti-FAP α antibody and FAP α .

<36> The kit for identifying MSC comprising an anti-FAP α antibody of the present invention can use an antibody linked to a fluorescent material and a

fluorescence spectrometer, an antibody linked to a magnetic bead and a magnetic apparatus, or a specific enzyme and a substrate thereof to detect the complex of the anti-FAP α antibody and FAP α . The above kit can be a protein chip for identifying MSC comprising an anti-FAP α antibody, specifically.

<37>

<38>

Shapes of a column packed with a water insoluble carrier, a flask packed with a water insoluble carrier or a flask case are exemplified as the shapes of the vessel used in the present invention. A FAP α positive cell sorter can be produced by combination of insoluble carriers fixing the above shapes of vessel to an antibody directly or indirectly. Also, pump eluting cell suspension, saline solution, etc. can be adapted to the above apparatus to increase efficiency of the sorter.

<39>

When the antigen is fixed to a water-insoluble carrier, it is useful to combine by interposing a spacer between them. Also, a water insoluble carrier and a compound can be combined through a molecule (spacer) of arbitrary length, according to demands. The details about spacer are disclosed in some references [See, for example, "Affinity Chromatography" K. Kasai, etc., Tokyo Kagaku Dojin Publishing (1991) p.105-108]. Examples of spacer are polymethylene chain and polyethyleneglycol chain, etc.. Preferably, the length of spacer is below 500Å and more preferably it is below 200Å. Using agarose as a water insoluble carrier is one example of the methods to combine compound with a water insoluble carrier through a spacer. Another example is as follows: attaching hydroxyl group of agarose to one isocyanate group of hexamethylene diisocyanate which is used as spacer by chemical reaction, and then attaching amino, hydroxyl or carboxyl group, etc. of antibody to the other isocyanate group by chemical reaction.

<40>

The water insoluble carrier of the present invention exists as solid phase in an aqueous solution at room temperature and it may have any shape, for example, spherical, cuboid, plane, chip, fibrous, flat membrane, sponge, hollow fiber type, etc.. Among them, spherical, particle or fibrous types are

preferable considering fine packing, homogeneous distribution of antibody on surface, substantially available space and fluidity of cell suspension.

<41> The water insoluble carrier can be made from an inorganic compound or an organic compound as far as it maintains antibodies on the surface. However, a high molecular organic compound is more preferable because the amount of exudate is small when it contacts with cell suspension and control of shape is easier. For example, vinyl compounds such as polypropylene, polystyrene, polymetachrylate ester, polyacrylate ester, polyacrylacid or polyvinyl alcohol, or polymer or copolymer of derivative thereof; polyamide compound such as nylon 6 or nylon 66, etc.; polyester compound like polyethylene therephthalate, etc.; polysaccharide compound derived from plant like cellulose, etc.. Also, the above carrier can be obtained easily by combining with magnet.

<42> The water insoluble carrier of the present invention can be modified and hydrophilicity can be imparted to the surface thereof. It is possible to impart hydrophilicity to the surface of the water insoluble carrier by fixing the proteins derived from a living body such as albumin, globulin, etc. and introducing synthetic functional group. The above synthetic functional group includes polyethyleneglycol chain having 2-1500 repeating units, hydroxyl group, amide group, ether group and ester group. Polyethyleneglycol chain and hydroxyl group can combine together or exist independently. Examples of monomer having polyethyleneglycol chain which impart hydrophilicity are as follows: terminal methoxymetachrylate such as methoxydiethyleneglycol metachrylate, methoxytriethyleneglycol metachrylate, etc.; terminal methoxyachrylate such as methoxydiethyleneglycol achrylate, methoxytriethyleneglycol achrylate, etc. and metachrylate and achrylate having terminal hydroxyl group to which hydrogen atom is attached instead of methyl group; or monomers having at least one synthetic functional group and polyethyleneglycol chains of 2-1500 repeating units at the terminus. Polyethyleneglycol derivatives having 2-1500 repeating units are also included when they are attached to the carrier directly. Example of monomers

having other groups imparting hydrophilicity is, but not limited to, vinylpyrrolidon. Also, one and above synthetic functional group can exist. The synthetic functional group includes the functional group which can synthesize independently or in combination with other functional groups, for example, but not limited to, carbon-carbon multiple bonds such as vinyl group, acetylene group, diene group, etc. and ring structures such as epoxy group, oxatane group, etc..

<43> Non-ionic functional group may also exist with the above functional group. The examples are as follows: non-ionic functional group, particularly amide group aiming increase of hydrophilicity such as dimethyl amide group, diethyl amide group, diisopropyl amide group, etc.; aromatic polyester chain such as polyethylene terephthalate chain, polybutylene terephthalate chain, etc. or polyester chain like aliphatic polyester chain; polyether chain such as methyleneglycol chain and propyleneglycol chain; non-ionic hydrophilic functional group such as polycarbonate chain, or ordinary non-ionic functional groups such as alkyl chain, fluoroalkyl chain, allyl chain, etc. to impart hydrophobicity. The above functional groups can coexist. However, non-ionic hydrophilic functional groups provides good result.

<44> All known methods including covalent bond, ionic bond, graft method by radiation or plasma; physical absorption; embedding; or insolubilization of a precipitate to the surface can be used as the method for modifying the above water insoluble carrier and imparting hydrophilicity on the surface. Therefore, known methods such as graft-synthesis of high molecular compound or monomer thereof using radiation or plasma, or formation of covalent bond can be used preferably to modify the surface (See. JP patent publication 1-249063 and 3-502094).

<45> The methods for fixing antibody to the surface of the water insoluble carrier are as follows: binding the antibody on the surface of the water insoluble carrier covalently by chemical method or graft method using radiation or electron beam; or attaching the antibody to the functional group located on the surface of the water insoluble carrier by covalent bond

through chemical means. Among them, the method using covalent bond through the functional group is preferable because there is no elution of antibody when it is used. If the water insoluble carrier has coating layer, it is possible to make antibody insoluble on the surface of the layer.

<46> Halogenocyan method, epichlorohydrin method, bisepoxide method, bromoacetyl bromide method, halogenation acetamide method, etc. can be used as the method of obtaining activation group for fixing the antibody to the water insoluble carrier or the surface of coating layer thereof. Specifically, amino, carboxyl, hydroxyl, thiol, acid anhydride, succinyl amide, substitutive halogen, aldehyde, epoxy, tricyl group, etc. can be used. Particular, bromocyan method, N-hydroxysuccinimide, halogenation acetamide method are preferable considering the easiness in fixing the antibody.

<47> N-hydroxymethylchloroacetamide, N-hydroxymethylfluoroacetamide, N-hydroxymethylbromoacetamide, N-hydroxymethyliodoacetamide, etc. are examples of the haloacetaminomethylation agents used in the activation group. Among them, N-hydroxymethylbromoacetamide and N-hydroxymethyliodoacetamide are preferable in the aspects of economical efficiency and stability. Fluoro group or chloro group can be introduced to the water insoluble carrier by introducing the activation group, and then changing to iodine group or bromo group easily by treating with potassium iodide or potassium bromide solution. Any strong acids, particularly proton acids can be used as acid catalysts used for producing the above water insoluble carrier to which the above activation group is introduced. However, not limited to, derivatives of sulfonic acid such as trifluoromethane, methane, benzene, toluene, etc.; and Friedel-Crafts catalysts such as sulphuric acid, zinc chloride, aluminum chloride, tin tetrachloride, etc. are preferable.

<48>

<49> According to the third aspect of the present invention, there is provided a method for separating and identifying MSCs using the anti-FAP α antibody. More specifically, the method comprises the steps of: contacting anti-FAP α antibody with test cell; and detecting the formation of antigen-

antibody complex of FAP α in the above test cell and the above FAP α antibody.

<50> Immunoassay using the antigen-antibody reaction can be used as the method of detecting the formation of the above antigen-antibody complex. Among the immunoassay, ELISA is the most common method. Radio ImmunoAssay (RIA), Fluorescence Immuno Assay (FIA), Enzyme Immuno Assay (EIA, ELISA), color particle hybridization assay, or chemiluminescent molecular hybridization assay, etc. are known as the above ELISA method.

<51> Also, the present invention provides the method for separating and purifying MSCs comprising the steps of: forming antigen-antibody complex by contacting liquid biological media having MSC with anti-FAP α antibody; and collecting the above antigen-antibody complex. In the step of collecting the above antigen-antibody complex, any one of ligands selected from the group consisting of biotin, avidin and streptavidin bead and magnetic apparatus can be used.

<52> The methods for separating only MSCs specifically using antibody comprise the method separating with cell sorter after marked with fluorescent antibody, the method fixing the anti-FAP α antibody which is compatible with MSC specific FAP α on the water insoluble carrier and then combining with MSC directly or indirectly, the method using immunoabsorption column and immunomagnetic bead, etc..

<53> The method for separating the cell marked with fluorescent antibody comprises the steps of: incubating cell mixture solution with monoclonal antibody marked with fluorescence which can recognize membrane antigen expressed on the target cell; irradiating laser beam to the treated cell using cell sorter, etc.; and separating the cell radiating fluorescence by attaching to the antibody.

<54> The method fixing monoclonal antibody on the water insoluble carrier is the cell separating method performed by attaching the above antigen positive cell to the monoclonal antibody fixed to the carrier or apparatus directly or indirectly.

<55> International patent publication number WO 87-04628 disclosed the method using the monoclonal antibody to the antigen existed on the surface of target cell membrane by fixing it directly on the surface of the sorter, and the method incubating cell mixture solution with monoclonal antibody attaching to membrane antigen on target cell and then treating ligand such as anti-immunoglobulin antibody attaching to the antibody on the cell surface in fixed cell sorter. In the above methods, separation was done on the petri dish fixed with the antibody and the cell mixture was poured into the the petri dish fixed with the first antibody and the antibody was incubated to bind to a membrane antigen on the target cell. After incubating, unbounded cell was eliminated and separated by washing the petri dish.

<56> Immunoabsorption column method is an method that an ligand, such as an antibody to an membrane antigen of a target cell, is fixed to surface of a bead and the bead is packed in a column, and then cell separation is carried out on the column.

<57> International patent publication WO 91-16116 disclosed a method separating a target cell by using strong bonding between biotin and avidin, which method comprises the steps of: incubating biotin marked antibody, which is against the membrane antigen on the surface of a target cell, with a cell mixture solution, thereby forming cell-antibody-biotin bonding; and then passing them through multiporus polyacrylamide gel column packed avidin fixed bead.

<58> In immunomagnetic bead method, target cell is marked with magnetic bead by incubating cell mixture solution with magnetic beads having an antibody, and then, the marked cell is separated from unmarked cells by using magnetic apparatus.

<59>

<60> For example, biotin or magnetic bead is prepared to bind to an antibody which will be fixed to cell, a material being easily bound to biotin or magnetic bead such as avidin or magnetic beads is reacted to a water-insoluble materials, thereby water-insoluble carrier bound with the cell

could be easily collected. Also, a target cell could be selectively separated by reacting FAP- α antibody with a target hematopoietic stem cell, reacting with water-insoluble carrier having anti-immunoglobulin antibody, and then cleaning or collecting insoluble carrier. If an antibody fixed to magnetic bead, a water-insoluble carrier, is used, it could separated more efficiently by using magnet. Shapes of vessel packed with water-insoluble carrier according to present invention could be a flask or flask case packed with water-insoluble carrier. Various apparatus for separating hematopoietic stem cell could be produced by directly or indirectly combining the above vessel with insoluble carrier fixed with antibody. Also, these apparatus could be improved to be more efficient by adapting pump which elutes cell suspension, saline solution, etc..

<61>

【Mode for Invention】

<62>

Reference will now be made in greater detail to a preferred embodiment of the invention, an example of which is illustrated in the accompanying drawings. In the drawings and specification, typical exemplary embodiments of the invention have been disclosed, and although specific terms are employed, they are used in a generic and descriptive sense only and are not for the purposes of limitation, the scope of the invention being set forth in the following claims.

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<64>

Example 1. Selective expression of FAP α in bone marrow (BM)-derived mesenchymal stem cell (MSC)

<65>

<66>

(1) Cell Preparation

<67>

Cryopreserved human BM-derived and peripheral blood-derived mononuclear cells (MNCs) were purchased from Lonza Inc. (Allendale, NJ) and umbilical cord blood (UCB) and adipose tissue (AT) were purchased from local hospitals. UCB was centrifuged immediately at 200 x g for 10 min to obtain mononuclear cells of buffy coat layer which is used in the present experiment. AT was

treated with collagenase I (Sigma, St Louis, MO) at room temperature for about 1 hour, and then centrifuged at 200 x g for 10 min to obtain mononuclear cells of stromal vascular fraction which is used in the present experiment. All human cancer cell lines were obtained from Korean Cell Line Bank (Seoul, Korea).

<68>

<69>

(2) RT-PCR analysis

<70>

Human peripheral blood (PB) fractions multiple tissue cDNA (MTC) panel was obtained from Clontech Laboratories Inc. (Mountain View, CA). For all other cell samples, total RNA was extracted using Trizol Reagent and reverse-transcribed to cDNA. Primers used in the present invention are shown in table 1 below.

<71>

【Table 1】

Primers used in the RT-PCR

Gene	Sequence	Product (bp)	Annealing temperature (°C)
<i>FAPα</i>	F 5' -CAAGTGGCAAGTGGGAGG-3' (SEQ ID No.1)R 5' -GGTTTTTCAGATTCTGATA-3' (SEQ ID No.2)	1152	57
<i>SPP1</i>	F 5' -ATCACCTGTGCCATACCAGT-3' (SEQ ID No.3)R 5' -CACATCGGAATGCTCATTGC-3' (SEQ ID No.4)	663	50
<i>ALPL</i>	F 5' -CTGGTAGGCGATGTCCTTA-3' (SEQ ID No.5)R 5' -ACGTGGCTAAGAATGTCATC-3' (SEQ ID No.6)	475	50
<i>BGLAP</i>	F 5' -CTCACACTCCTCGCCCTA-3' (SEQ ID No.7)R 5' -CCTGAAAGCCGATGTGGT-3' (SEQ ID No.8)	262	50
<i>LPL</i>	F 5' -GAGATTTCCTGTATGGCACC-3' (SEQ ID No.9)R 5' -CTGCAAATGAGACACTTCTC-3' (SEQ ID No.10)	276	61
<i>FABP4</i>	F 5' -GGCCAAACCCAACCTGA-3' (SEQ ID No.11)R 5' -GGGCGCCTCCATCTAAG-3' (SEQ ID No.12)	167	60
<i>PPARG</i>	F 5' -GCTGTTATGGGTGAACTCTG-3' (SEQ ID No.13)R 5' -ATAAGGTGGAGATGCAGGTTC-3' (SEQ ID No.14)	350	62
<i>COL10A1</i>	F 5' -GCCCAAGAGGTGCCCTGGAATAC-3' (SEQ ID No.15)R 5' -CCTGAGAAAGAGGAGTGGACATAC-3' (SEQ ID No.16)	703	57
<i>SOX9</i>	F 5' -AGACAGCCCCCTATCGACTTC-3' (SEQ ID No.17)R 5' -TGCTGCTTGGACATCCACAC-3' (SEQ ID No.18)	230	63
<i>ACAN</i>	F 5' -TTCCCTGAGGCCGAGAAC-3' (SEQ ID No.19)R 5' -GGGCGGTAATGGAACACAAC-3' (SEQ ID No.20)	194	65
<i>ACTB</i>	F 5' -AGAAAATCTGGCACCACACC-3' (SEQ ID No.21)R 5' -CCATCTCTTGCTCGAAGTCC-3' (SEQ ID No.22)	435	57

<72> *FAP α* , fibroblast activation protein α ; *SPP1*, secreted phosphoprotein 1; *ALPL*, alkaline phosphatase; *BGLAP*, bone γ -carboxyglutamate protein; *LPL*, lipoprotein lipase; *FABP4*, fatty acid binding protein 4; *PPARG*, peroxisome proliferator-activated receptor γ ; *COL10A1*, collagen type X α 1; *SOX9*, sex determining box region Y-box 9; *ACAN*, aggrecan; *ACTB*, β -actin.

<74> (3) Flow cytometry

<75> Flow cytometry was performed on Guava PCA-96 Flow cytometer (Guava Technologies, Hayward, CA, USA) using mouse monoclonal antibodies to *FAP α* , CD45, CD74, CD90 and CD105 (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) and Alexa488-conjugated goat anti-mouse IgG (Invitrogen, Carlsbad, CA,

USA) as secondary antibody to the murine antibodies. All analyses were performed on Guava express software (Guava Technologies Inc.) and De Novo software (De Novo software Inc., Thornhill, ON, Canada).

<76>

<77>

(4) Result

<78>

Prior to undertaking the analysis of *FAP* α expression in various human cells, we carefully examined human MSCs' membrane proteomic data [15]. It was found that FAP was among the ectodomain-containing membrane-bound proteins identified with high sequence coverage (Fig. 1), which led us to consider this type II membrane protein as a prime surface protein marker candidate.

<79>

First we compared *FAP* α expression between BM-derived MSCs and MNCs by RT-PCR, to address the possibility that FAP α can serve as a selective marker for MSCs within BM. Fortunately it turned out that *FAP* α was highly expressed in BM-derived MSCs, but not in MNCs (Fig. 2). Ensuing flow cytometry analysis also demonstrated that FAP α was strongly expressed on MSCs' surface, but negligibly on MNCs (Fig. 3). These results suggest that MSCs may be the only *FAP* α -expressing cell population within BM stroma.

<80>

We then extended the RT-PCR analysis to further investigate *FAP* α expression in other human blood cells. An MTC (multiple tissue cDNA) panel screening revealed that *FAP* α was expressed in neither resting nor activated CD8+ (effector T lymphocytes), CD4+ (helper T lymphocytes), CD14+ (myeloid cells) or CD19+ (B lymphocytes) blood fractions (Fig. 4), indicating that FAP α -expressing cells, if any, in the human bloodstream would belong to none of these hematopoietic lineage cells.

<81>

Next we investigated whether *FAP* α expression is unique to MSCs derived from BM, or is common to other MSC populations derived from umbilical cord blood (UCB) and adipose tissue (AT). The results show that all three MSC populations expressed similarly high levels of *FAP* α (Fig. 5), although their background MNC populations did not (Figs. 2, 4 and 5). These findings indicate that *FAP* expression can uniquely distinguish MSCs not only from BM, but also from other cell sources, such as PB, UCB and AT.

<82> Interestingly, but not surprisingly, a variety of human cancer cell lines, such as Hek293, THP-1, Jurkat, SH-SY5Y and CaCo-2, were also found not to express *FAP* α (Fig. 6). *FAP* α was slightly expressed in HeLa cells, but this was in line with prior reports showing that *FAP* α is often up-regulated in epithelial cancers [12-14], from which HeLa cells are derived. All these observations, taken together, suggest that *FAP* α expression may be tightly suppressed in most, if not all, human cells other than MSCs.

<83>
<84> Example 2. Slective separation of MSC using monoclonal anti-*FAP* α antibody

<85>
<86> We could separate *FAP*- α expression cell from mononuclear cells extracted from not only cryopreserved BM and PB-derived but also umbilical cord blood (UCB) and adipose tissue (AT) using magnetic bead immunoselection having monoclonal anti-*FAP* α antibody and verify that the separated cells are pure MSCs using the cell surface antigen analysis.

<87>
<88> (1) Immunoselection

<89> About 25 μ l of Dynabeads Pan mouse IgG (including 10 millions of beads) (DynaI Biotech, Oslo, Norway) was mixed with 0.5 μ g of anti-*FAP* α mouse monoclonal antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA) and reacted for 30 min at room temperature. And then it was reacted with 2×10^7 mononuclear cells extracted from bone marrow, peripheral blood, umbilical cord blood and adipose cell, respectively at 4°C for 20 min. We obtained cells expressing *FAP* α attached to beads using external magnet, rinsed with a saline solution twice, poured into culture flask with basic culture solution, and then observed cell proliferation for 3 weeks.

<90>
<91> (2) Differentiation

<92> *FAP*-immunoselected cells were spreaded and cultured on differentiation medium for fat, bone, and cartilage (Lonza Inc.) according to the

manufacturer's instructions. Differentiation was confirmed by Oil Red O, Alizarin Red S and Alcian Blue staining, respectively.

<93>

<94> (3) Result

<95> We attempted to isolate FAP-expressing cells from cryopreserved MNCs by magnetic bead immunoselection with a monoclonal anti-FAP α antibody. One immunoselected FAP α positive cell per about 4×10^6 cryopreserved mononuclear cells had been attached to the bottom of culture dish for one to several days. When the cells were attached, they maintained morphologically similar to MSC more and more and started to proliferate quickly for a few weeks after that, forming very homogeneous fibroblastic cell colonies respectively (Fig. 7). Flow cytometry result indicated that the above attached cells were negative to CD45, a pan leucocyte marker, but positive to CD73, CD90 and CD105, MSC-related surface markers (Fig. 8). Furthermore we found that the cells developed into bone, adipose and cartilage easily from the results that the cells are positive to the histochemical staining when they were cultured on the other culture media (Fig. 9) and expression level of the developmental marker genes was increased (Fig. 10). These observations led us to conclude that the above cells are real MSC individuals.

<96>

【Industrial Applicability】

<97> FAP α protein of the present invention is very useful as a surface protein marker for separating and identifying MSCs from human bone marrow, peripheral blood, umbilical cord blood and adipose cell. By using magnetic bead immunoselection having monoclonal anti-FAP α antibody, MSCs having FAP α can be separated specifically and quickly. Human MSC can be developed into the tissue cells related to the mesoderm like bone, cartilage, muscle and adipose, etc.. Furthermore they are recognized as one of the most applicable cells to wide areas because they can help hematosiis of blood stem cell or regulate immune reaction. The above stem cell is in the limelight as a promising cell therapeutics because it has several advantages. For example,

it has less legal or ethical problems than other stem cells, but has excellent self-proliferative potential. Also, it can be cryopreserved for a long time without affecting characteristics of stem cell and it has totipotency close to pluripotency. Furthermore it shows high stability after gene manipulation.

<98> Therefore, FAP α has high industrial applicability in cell therapy based on the MSC by separating and identifying MSC easily from human bone marrow, peripheral blood, umbilical cord blood and adipose cell. And it can also be used in the cell or molecular biological research and help to elucidate major cell signaling pathways related to self-proliferation and development researches.

<99>

<100> [References]

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【CLAIMS】**【Claim 1】**

A composition for separating or identifying mesenchymal stem cell (MSC) comprising an anti-fibroblast activation protein α (FAP α) antibody.

【Claim 2】

The composition according to claim 1, wherein the antibody is a monoclonal antibody.

【Claim 3】

The composition according to claim 1, wherein the antibody is a polyclonal antibody.

【Claim 4】

The composition according to claim 1, wherein the antibody is a single chain antibody or a recombinant antibody.

【Claim 5】

The composition according to claim 1, wherein the antibody is an anti-fibroblast activation protein α (FAP α) antibody linked to a separation bead.

【Claim 6】

The composition according to claim 5, wherein the separation bead is a magnetic bead.

【Claim 7】

The composition according to claim 1, wherein the antibody is an anti-fibroblast activation protein α (FAP α) antibody linked to a separation ligand.

【Claim 8】

The composition according to claim 7, wherein the separation ligand is any one of ligands selected from the group consisting of biotin, avidin and streptavidin.

【Claim 9】

The composition according to claim 1, wherein the antibody is an anti-fibroblast activation protein α (FAP α) antibody linked to an enzyme.

【Claim 10】

The composition according to claim 9, wherein the enzyme is any one of enzymes selected from the group consisting of luciferase, peroxidase and β -galactosidase.

【Claim 11】

An apparatus for separating MSCs comprising:
an anti-FAP α antibody linked to a fluorescent material;
a vessel for contacting the antibody with a test cell; and
a cell sorter for separation of cells bound with the antibody through antigen antibody binding.

【Claim 12】

An apparatus for separating MSCs comprising:
an anti-FAP α antibody linked to a magnetic bead;
a vessel for contacting the antibody with a test cell; and
a magnetic apparatus for separating cells bound with the antibody through antigen antibody binding.

【Claim 13】

An apparatus for separating MSCs comprising:
an anti-FAP α antibody linked to any one of ligand selected from the group consisting of biotin, avidin and streptavidin;
a vessel for contacting the antibody with a test cell; and
a column for separating cells bound with the antibody through antigen antibody binding.

【Claim 14】

A kit for identifying MSCs comprising:
an anti-FAP α antibody linked to a fluorescent material;
a vessel for contacting the antibody with a test cell; and
a fluorescence spectrometer for detecting cells bound with the antibody through antigen antibody binding.

【Claim 15】

A kit for identifying MSCs comprising:

an anti-FAP α antibody linked to a magnetic bead;
a vessel for contacting the antibody with a test cell; and
a magnetic apparatus for detecting cells bound with the antibody through antigen antibody binding.

【Claim 16】

A kit for identifying MSCs comprising:
an anti-FAP α antibody linked to an enzyme;
a vessel for contacting the antibody with a test cell; and
an device for detecting cells bound with the antibody through enzyme substrate reaction.

【Claim 17】

A protein chip for identifying MSC comprising an anti-FAP α antibody.

【Claim 18】

A method for identifying MSC comprising the steps of:
contacting an anti-FAP α antibody with a test cell; and
detecting the formation of antigen-antibody complex of FAP α on the test cell and the anti-FAP α antibody.

【Claim 19】

The method according to claim 18, wherein the detecting step can be performed by any one of methods selected from the group of radio immuno assay (RIA), ELISA, fluorescence immuno assay (FIA), color particle hybridization assay, and chemiluminescent molecular hybridization assay.

【Claim 20】

A method for separating and purifying MSCs comprising the steps of:
forming an antigen-antibody complex by contacting liquid biological media having MSC with an anti-FAP α antibody; and
collecting the antigen-antibody complex.

【Claim 21】

The method according to claim 20, wherein the collecting step is performed by using any one of ligands selected from the group consisting of biotin, avidin and streptavidin.

【Claim 22】

The method according to claim 20, wherein the collecting step is performed by using a magnetic bead and a magnetic apparatus.

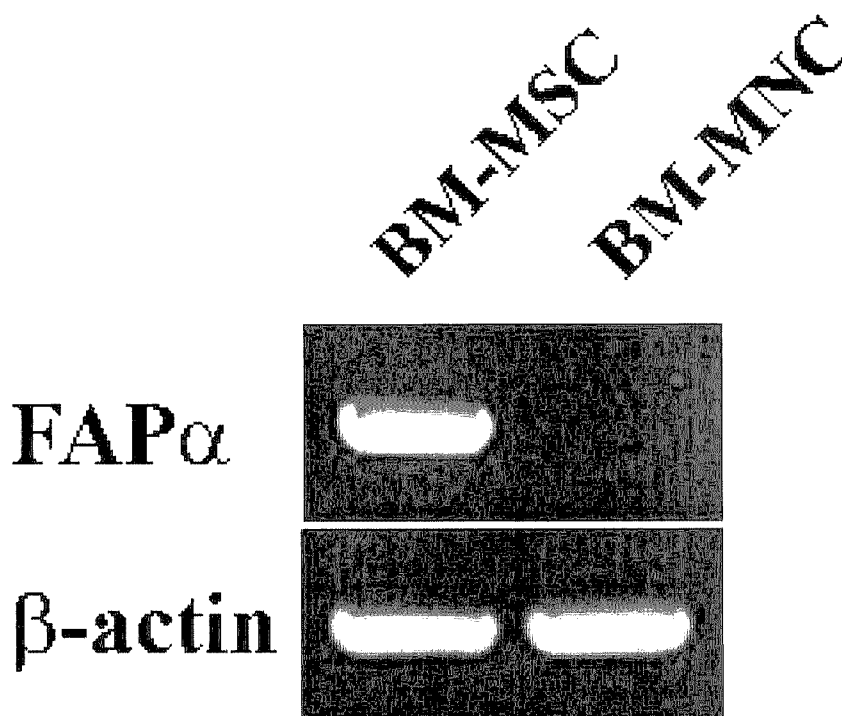
[Fig. 1]

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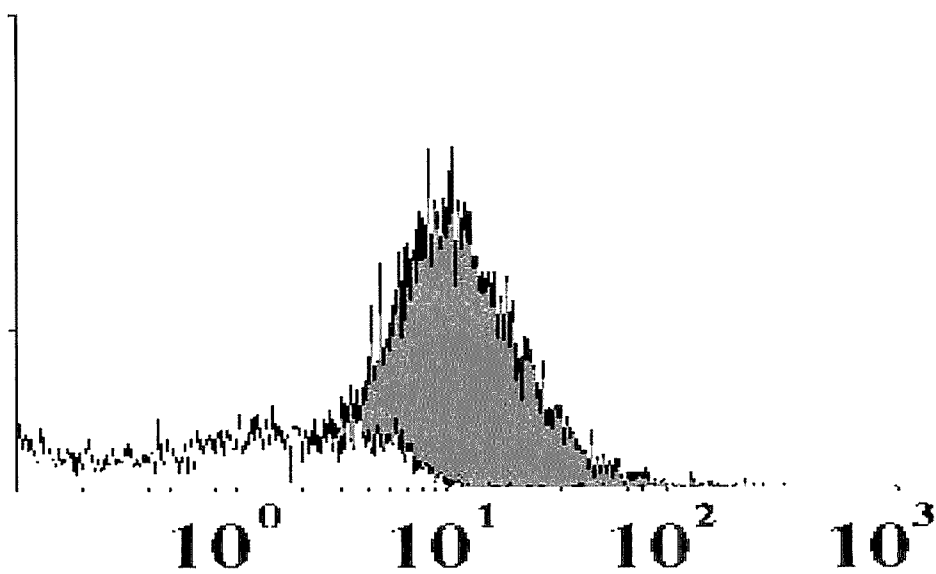
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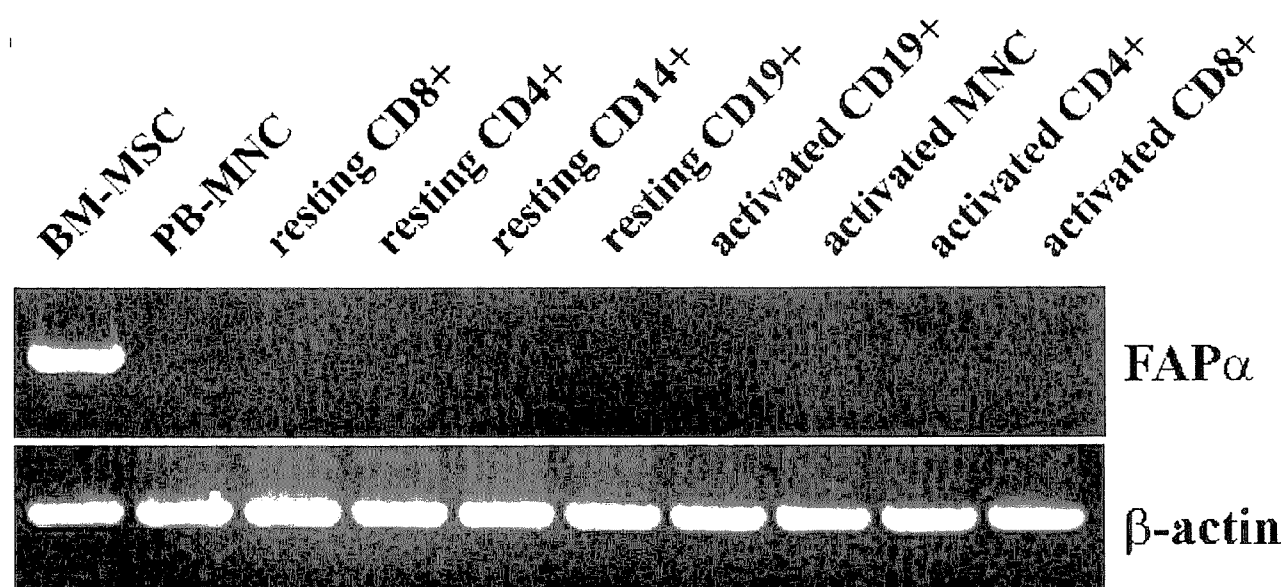
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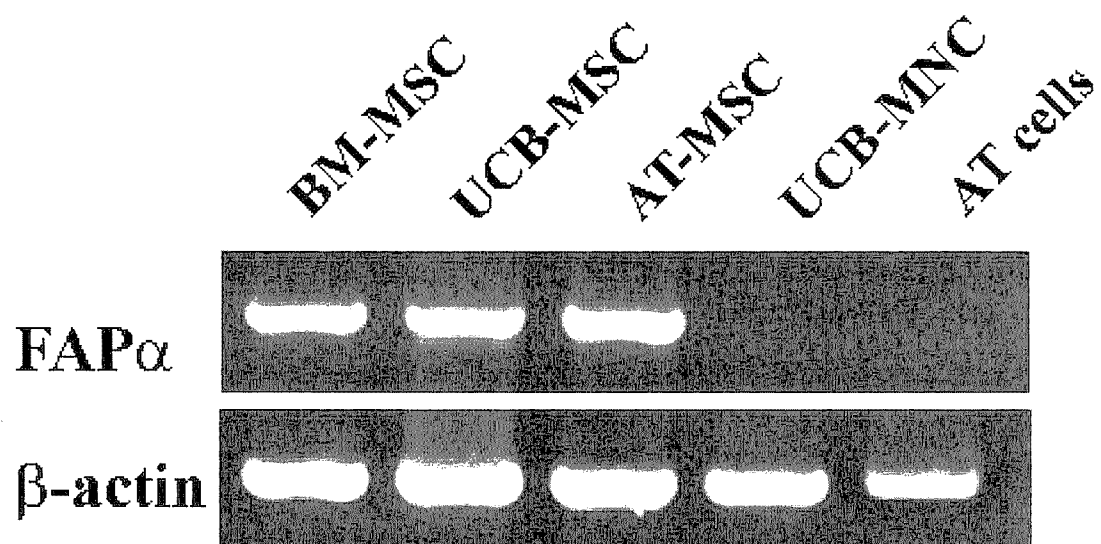
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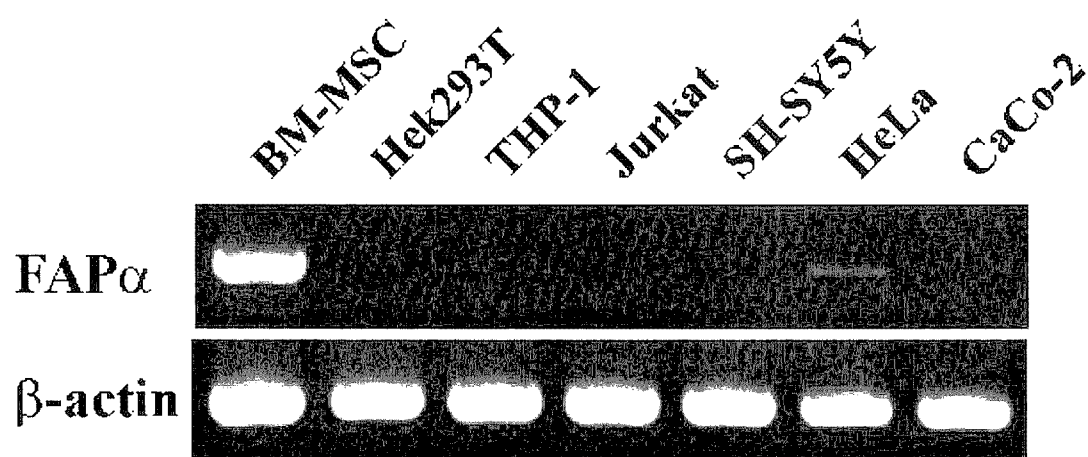
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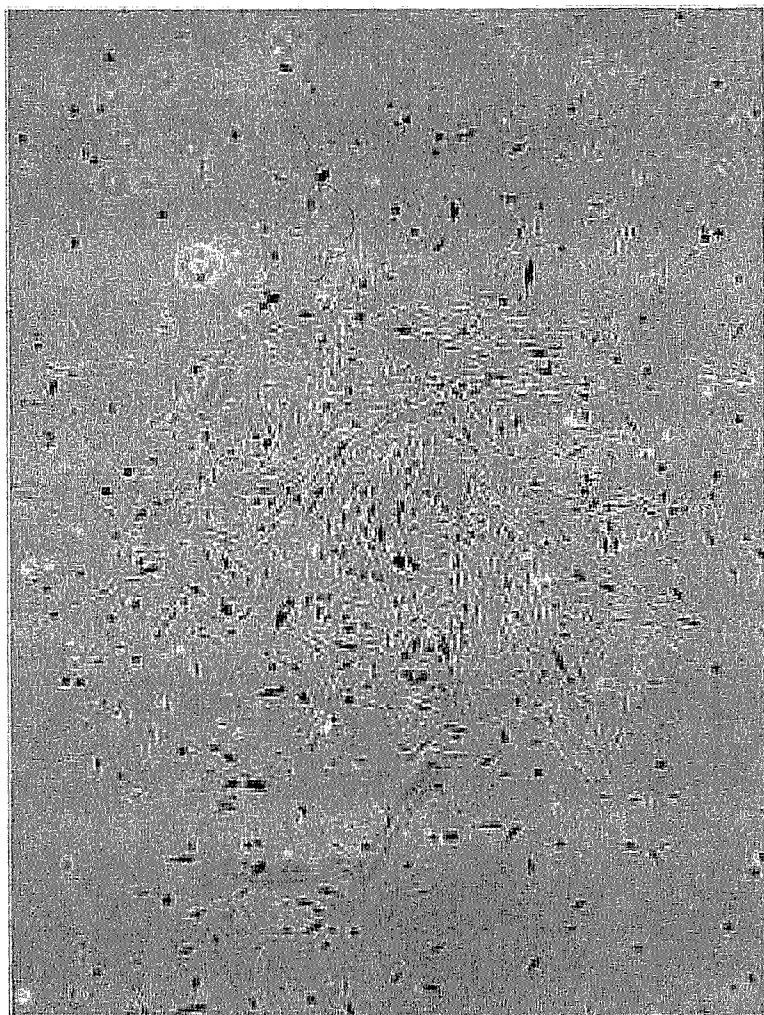
[Fig. 5]



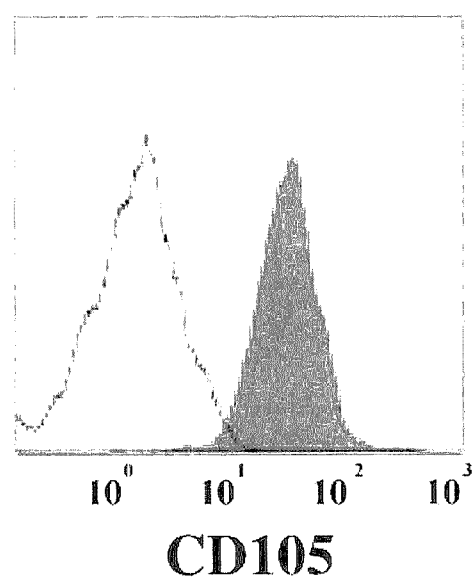
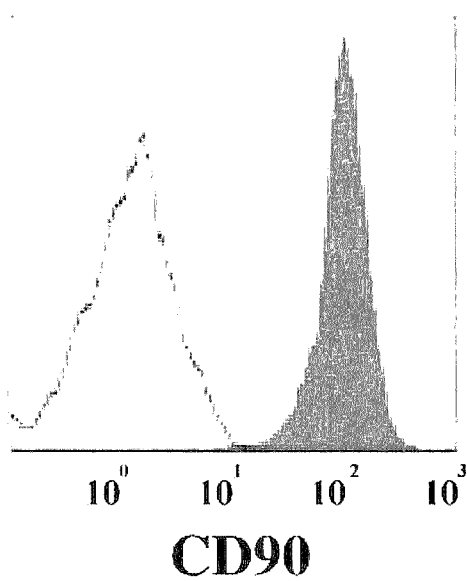
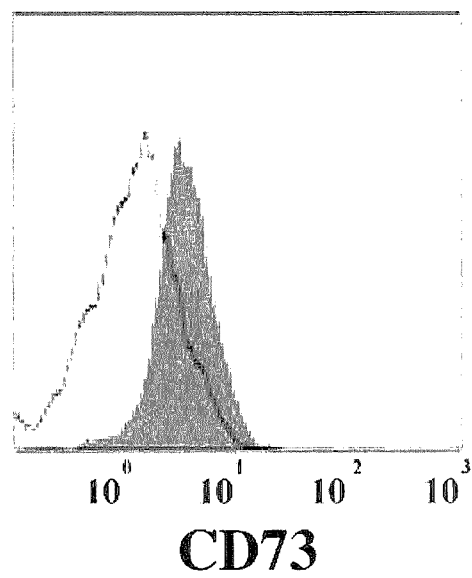
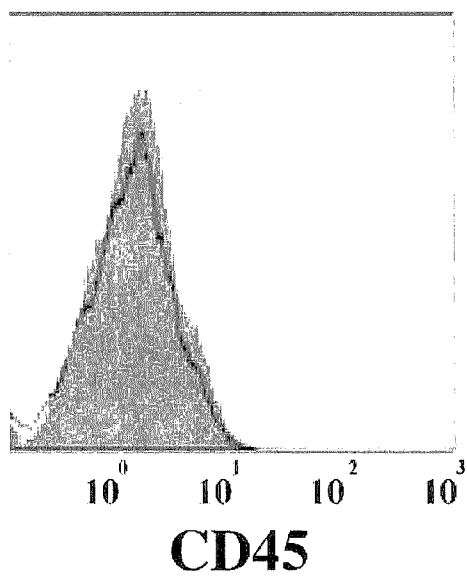
[Fig. 6]



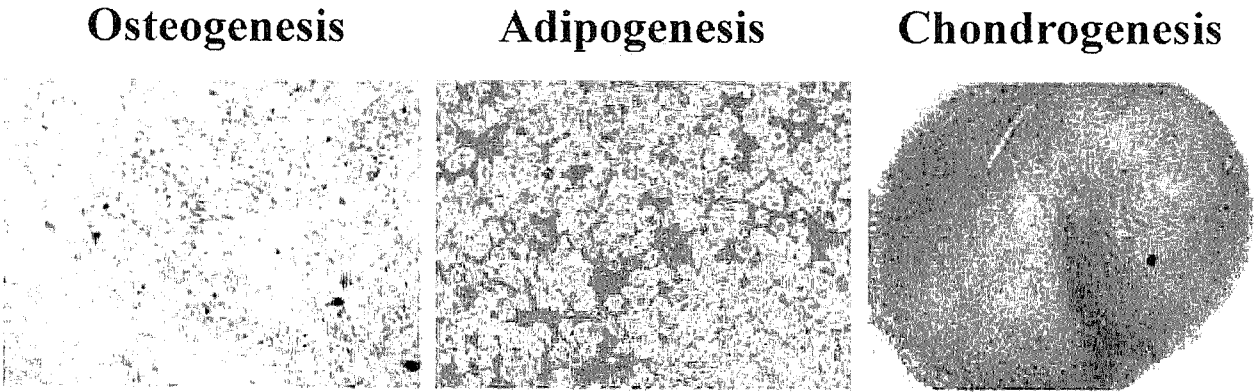
[Fig. 7]



[Fig. 8]



[Fig. 9]



[Fig. 10]

