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Influence of surfaces modified with biomimetic extracellular matrices on adhesion and proliferation of mesenchymal stem cells and osteosarcoma cells

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ABSTRACT

Preparation of surfaces modified with biomimetic extracellular matrices (ECMs) is important for investigation of the interaction between ECMs and cells. In the present study, surfaces modified with ECMs from normal somatic cells, stem cells and tumor cells were prepared by cell culture method. The ECMs derived from bone marrow-derived mesenchymal stem cells (MSCs), dermal fibroblasts (FBs), osteoblasts (OBs) and MG63 osteosarcoma cells were deposited on the surfaces of cell-culture polystyrene plates (TCPS). The ECMs from different cell types had different compositions. The effects of the ECM-deposited surfaces on the adhesion, spreading and proliferation of MSCs and MG63 human osteosarcoma cells were dependent on the type of both ECMs and cells. The surfaces deposited with ECMs from MSCs, FBs and OBs promoted cell adhesion more strongly than surfaces deposited with ECMs from MG63 cells and TCPS. Compared to TCPS, the ECM-deposited surfaces promoted proliferation of MSCs while they inhibited the proliferation of MG63 cells.

1. Introduction

Cells in tissues and organs are surrounded by extracellular matrices (ECMs). Interaction between cells and ECMs plays an important role in the regulation of cell functions such as cell adhesion, migration, proliferation and differentiation [1–3]. ECMs are composed of various proteins and proteoglycans, and their compositions change with different cell types and phenotypes [4–8]. Abnormal ECM dynamics may lead to dysregulation of cell functions and in some cases may result in pathological processes including cancer [4,9]. Therefore, precisely controlled ECMs models are necessary for investigation of the interaction between ECMs and cells, specially stem cells and tumors. Tumors are heterogeneous tissues, which contain tumor cells and tumor involved normal cell types, such as fibroblasts [10] and mesenchymal stem cells (MSCs) [11,12]. Beside these cells, a wide variety of ECM components produced by stromal cells and tumor cells form a specific microenvironment for the regulation of both tumor cells and stromal cell behaviors [13–16]. Therefore, investigation of the effect of stromal and tumor cells derived ECMs on stromal and tumor cell behavior is important for understanding the interaction between cells and ECMs during tumor progression.

Surface coating of one or a few ECMs components is a simple and convenient method to prepare a biomimetic surface for investigation of ECMs functions. It has been widely used to investigate the effects of ECMs components on cell adhesion, proliferation and differentiation [17–22]. Although the coating method is good for investigating the effect of a specific or a combination of a few proteins form ECMs, it is not possible to mimic the real ECMs surrounding cells because of the complexity of ECMs. Instead of coating method, surface deposition of ECMs from cultured cells has been developed to prepare biomimetic ECMs [20–22].

In the present study, cell culture method was used to create biomimetic ECMs to investigate the effects of ECMs derived from different cell types on the functions of stem cells and tumor cells. Human bone marrow-derived mesenchymal stem cells (MSCs), fibroblasts (FBs), osteoblasts (OBs) and MG63 osteosarcoma cells

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were used to prepare their respective ECMs. They were used to study their effects on the adhesion, spreading and proliferation of MSCs and MG63 cells.

2. Materials and methods

2.1. Cells and cell culture

Human bone marrow mesenchymal stem cells (MSCs), normal human dermal fibroblasts (FBs), normal human osteoblasts (OBs) and MG63 human osteosarcoma cells were used to prepare the ECM substrates. MSCs and OBs at passage 2 were purchased from Lonza (Walkersville, MD). FBs at passage 2 and MG63 (cell line) were purchased from Cascade Biologics (Invitrogen, Portland, OR and JCRB cell bank (Japan), respectively. The four types of cells were seeded in 75 cm^2 tissue culture flasks and sub-cultured in their respective maintenance media under an atmosphere of 5% CO_2 at 37 °C. MSCs were sub-cultured in MSCGM™ medium (Lonza, Walkersville, MD). OBs were sub-cultured in OBM™ medium (Lonza, Walkersville, MD). FBs were sub-cultured in Medium 106 with low serum growth supplement (Sigma, St. Louis, MO). MG63 cells were cultured in Minimum Essential Medium (MEM, Sigma, St. Louis, MO) supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY), 100 units/ml penicillin, 100 mg/l streptomycin, 584 mg/l glutamine and 0.1 mM nonessential amino acid. All the cells were sub-cultured twice to get enough cell number before usage for next experiments.

2.2. Preparation of cell-derived ECMs

To prepare the ECMs from different types of cells, the sub-cultured MSCs, FBs and OBs were seeded on tissue culture polystyrene (TCPS) plates at a density of 10,000 cells/cm^2 and cultured for 2 weeks. The sub-cultured MG63 cells were seeded on TCPS at a density of 5000 cells/cm^2 and cultured for 5 days. After the cells became confluent, cellular components were removed while ECMs deposited on the surfaces of TCPS plates were kept on the surfaces. Confluent cells were treated with 0.5% Triton X-100 and 20 mM NH_4OH in PBS at 37 °C for 5 min, followed with treatment with DNase I (100 μg/ml, Roche Applied Science) and RNase A (100 μg/ml, Nacalai Tesque, Kyoto, Japan) at 37 °C for 1 h [7]. All reagents used for decellularization were sterilized by passing through sterilized 0.22 μm filter (Millipore, Bedford, MA). The matrices were washed with sterilized Milli-Q water 6 times before using for cell culture. Removal of cellular components by decellularization treatment was confirmed by staining of actin cytoskeleton and cell nuclei. The cells before and after decellarization were fixed with 4% paraformaldehyde for 10 min and treated with 0.2% Triton X-100 for 2 min. To block the non-specific interaction, the samples were incubated with 1% BSA in PBS at room temperature for 30 min. Actin filaments were visualized by incubation with phalloidin-Alexa 488 (Invitrogen, Carlsbad, CA) at room temperature for 20 min. Cell nuclei were visualized by staining with 4′,6-diamidino-2-phenylindole (DAPI, Invitrogen, Carlsbad, CA), at room temperature for 10 min. The stained samples were observed under an Olympus BX51 fluorescence microscope with a DP-70 CCD camera (Olympus, Tokyo, Japan). Deposition of ECMs on the surfaces of TCPS plates was confirmed by Coomassie Brilliant Blue (CBB) staining. CBB staining solution (Nacalai Tesque, Kyoto, Japan) was added to the decellularized samples and held at room temperature for 5 min. After being washed with PBS for 6 times, the stained samples were observed under an optical microscope (Olympus, Tokyo, Japan).

2.3. Immunohistological staining of deposited ECM

Type I collagen, fibronectin, biglycan, decorin, versican and laminin α-4 in the deposited ECMs were investigated by immunohistological staining. At first, the deposited ECMs after decellularization were fixed with 4% paraformaldehyde (Wako, Osaka, Japan) at room temperature for 15 min and then incubated with 0.1 M glycine at room temperature for 30 min. The samples were further incubated in 1% bovine serum albumin (BSA, Sigma, St. Louis, MO) solution at room temperature for 30 min to block the non-specific interaction of antibodies during following experiments. Subsequently, the samples were incubated with primary antibodies as following: rabbit monoclonal anti-collage I, mouse monoclonal anti-fibronectin, rabbit polyclonal anti-biglycan, rabbit polyclonal anti-decorin, rabbit polyclonal anti-versican and rabbit polyclonal anti-laminin α-4 (Santa Cruz Biotechnology, Santa Cruz, CA) in 1% BSA solution at room temperature for 2 h. After being washed with PBS for 3 times, the samples were incubated with peroxidase-conjugated anti-rabbit IgG antibody or peroxidase-conjugated anti-mouse IgG antibody (Dako, Carpinteria, CA) at room temperature for 1 h. Finally, the samples were incubated with AEC+high sensitivity substrate chromogen (Dako, Carpinteria, CA) as a colorimetric substrate at room temperature for 30 min to visualize the peroxidase-labeled proteins. The stained samples were observed under an optical microscope (Olympus, Tokyo, Japan).

2.4. Culture of MSCs and MG63 cells on the ECM-deposited surfaces

MSCs and MG63 cells were seeded to each well of the ECM-deposited TCPS plates at a density of 20,000 cells/cm^2 and cultured in their respective media under an atmosphere of 5% CO_2 at 37 °C. The bare TCPS plates were used as controls. MSCs were cultured in DMEM supplemental with 10% fetal bovine serum (FBS, Gibco, Grand Island, NY), 4500 mg/l glucose, 584 mg/l glutamine, 100 units/ml penicillin, 100 mg/l streptomycin, 0.1 mM nonessential amino acid, 1 mM sodium pyruvate, 0.4 mM proline and 50 mg/l ascorbic acid. MG63 cells were cultured in MEM (Sigma, St. Louis, MO) supplemented with 10% fetal bovine serum (Gibco, Gaithersburg, MD), 100 units/ml penicillin, 100 mg/l streptomycin and 584 mg/l glutamine. After being cultured for 30 min, the cells were washed twice with warm PBS to remove non-adhered cells from the wells of the ECM-deposited TCPS plates. The adhered cells were collected by being incubated with 0.2% collagenase and 5% trypsin in PBS at 37 °C for 1 h. The number of the adhered cells was counted by using a hemocytometer. At each time point, three samples were used to count the number of adhered cells. Cell spreading and morphology were investigated by examining F-actin cytoskeleton and cell nuclei. After one day of culture, the cells were fluorescently stained with fluorescein isothiocyanate (FITC)-conjugated phalloidin and DAPI. The stained cells were observed under an Olympus BX51 fluorescence microscope with a DP-70 CCD camera.

2.5. Proliferation of MSCs and MG63 cells on the ECM-deposited surfaces

MSCs and MG63 cells were seeded to each well of the ECM-deposited TCPS plates at a density of 5000 cells/cm^2 and 2000 cells/cm^2, respectively. The media were the same as described above. The cells were cultured in their respective culture media described above under an atmosphere of 5% CO_2 at 37 °C for 1, 2 and 4 days. After cell culture, the cells were detached by treatment with 0.2% collagenase and 5% trypsin in PBS at 37 °C for 1 h. The detached cells were collected and cell number was counted by a