Biochimica et Biophysica Acta 1801 (2010) 23–30

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbalip

Lysophosphatidic acid mediates migration of human mesenchymal stem cells stimulated by synovial fluid of patients with rheumatoid arthritis

Hae Young Song [a], Mi Jeong Lee [a], Min Young Kim [a], Kyung Hye Kim [a], Il Hwan Lee [a], Sang Hun Shin [a], Jung Sub Lee [b,*], Jae Ho Kim [a,*]

[a] Medical Research Center for Ischemic Tissue Regeneration, the Medical Research Institute, School of Medicine, Pusan National University, Busan, Republic of Korea
[b] Department of Orthopaedic Surgery, School of Medicine, Pusan National University, Busan, Republic of Korea

ARTICLE INFO

Article history:
Received 20 March 2009
Received in revised form 17 July 2009
Accepted 26 August 2009
Available online 3 September 2009

Keywords:
Lysophosphatidic acid
Mesenchymal stem cell
Migration
Synovial fluid
Arthritis

ABSTRACT

Migration of mesenchymal stem cells plays a key role in regeneration of injured tissues. Rheumatoid arthritis (RA) is a chronic inflammatory disease and synovial fluid (SF) reportedly contains a variety of chemotactic factors. This study was undertaken to investigate the role of SF in migration of human bone marrow-derived mesenchymal stem cells (hBMSCs) and the molecular mechanism of SF-induced cell migration. SF from RA patients greatly stimulated migration of hBMSCs and the SF-induced migration was completely abrogated by pretreatment of the cells with the lysophosphatidic acid (LPA) receptor antagonist Ki16425 and by small interfering RNA- or lentiviral small hairpin RNA-mediated silencing of endogenous LPA_1/Edg2. Moreover, SF from RA patients contains higher concentrations of LPA and an LPA-producing enzyme autotoxin than normal SF. In addition, SF from RA patients increased the intracellular concentration of calcium through a Ki16425-sensitive mechanism and pretreatment of the cells with the calmodulin inhibitor W7 or calmodulin-dependent protein kinase II inhibitor KN93 abrogated the SF-induced cell migration. These results suggest that LPA-LPA_1 plays a key role in the migration of hBMSCs induced by SF from RA patients through LPA_1-dependent activation of calmodulin-dependent protein kinase II.

1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by the destruction of articular cartilage and adjacent bone tissues [1] and disordered synovial microenvironment, including infiltration of inflammatory cells, hyperplasia of stromal cells, and tissue scarring [2]. The pathological events are mediated by a complex interplay of pro-inflammatory cytokines and mediators produced in the joint tissues or synovium of patients with RA [3]. It has been reported that synovial fluid (SF), which nourishes articular cartilage and lubricates articular joint surfaces [4], contains various growth factors and chemotactic factors which play a key role in the pathogenesis of RA [5].

Mesenchymal stem cells or multipotent stromal cells (MSCs) can be isolated from a variety of tissues, including bone marrow, adipose tissue, peripheral blood, articular cartilage, and synovial tissue [6–10]. MSCs possess self-renewal capacity, long-term viability, and differentiation potential toward diverse cell types, such as adipogenic, osteogenic, chondrogenic, and myogenic lineages [7,8,11,12], suggesting potential application of MSCs for regenerative medicine. MSCs have been reported to reside in the SF of patients with arthritis [13] and accumulate in the synovium of collagen-induced arthritis animal model [14]. However, the molecular identities of the factors involved in the chemotactic migration of MSCs into the synovium have not been clarified.

Lysophosphatidic acid (LPA) is a naturally occurring bioactive lipid belonging to the family of phospholipid growth factors, present in micromolar concentrations in serum and biological fluids and in higher concentrations at sites of inflammation [15]. It has been reported that SF from RA patients contains LPA and autotaxin (ATX) [16], which is involved in the generation of LPA by hydrolyzing lysophosphatidylcholine [17]. ATX has been reported to exist in body fluids under pathological conditions, including ascetic fluid from ovarian cancer patients [18], serum from patients with chronic hepatitis C [19], and synovial fluid from RA patients [16,20], suggesting a potential role of LPA and ATX in the pathogenesis of RA. LPA is involved in a variety of

Abbreviations: RA, rheumatoid arthritis; SF, synovial fluid; MSCs, mesenchymal stem cells; LPA, lysophosphatidic acid; ATX, autotaxin; $[Ca^{2+}]_i$, intracellular concentration of calcium; hBMSCs, human bone marrow-derived mesenchymal stem cells; α-MEM, α-minimum essential medium; PTX, pertussis toxin; PDGF-BB, platelet-derived growth factor-BB; 1-oleoyl-LPA, 1-oleoyl-*sn*-glycero-3-phosphate; S1P, sphingosine-1-phosphate; OA, osteoarthritis; RT-PCR, reverse transcription–polymerase chain reaction; siRNA, small interfering RNA; shRNA, small hairpin RNA; RA-SF, SF from RA patients; CaMK II, calmodulin-dependent protein kinase II

* Corresponding authors. J.H. Kim, Department of Physiology, School of Medicine, Pusan National University, Yangsan 626-813, Gyeongsangnam-do, Republic of Korea. J.S. Lee, Department of Orthopaedic Surgery, School of Medicine, Pusan National University, Busan 602-739, Republic of Korea.
E-mail addresses: jungsublee@pusan.ac.kr (J.S. Lee), jhkimst@pusan.ac.kr (J.H. Kim).

1388-1981/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbalip.2009.08.011

physiological and pathophysiological responses including wound healing, production of angiogenic factors, chemotaxis, neointima formation, tumor cell invasion, metastasis, and cell cycle progression [21,22]. The biological functions of LPA are mediated through several G protein-coupled receptors, i.e., LPA_1/Edg2, LPA_2/Edg4, LPA_3/Edg7, LPA_4/p2y9/GPR23, LPA_5/GPR92, GPR87, and p2y10 [23–26]. Activation of LPA receptors mediates the biological responses through activating multiple signaling pathways involving intracellular concentration of calcium ($[Ca^{2+}]_i$), ERK, and phosphatidylinositol-3-kinase [21,22]. We have previously reported that LPA in malignant ascites from patients with ovarian cancer induces migration of human adipose tissue-derived MSCs through LPA_1-dependent mechanism [27], suggesting a key role of LPA in the migration of MSCs. However, it is still elusive whether LPA plays a key role in the migration of MSCs induced by SF from RA patients.

In the present study, we sought to explore whether SF from patients with RA can induce migration of human bone marrow-derived MSCs (hBMSCs). We demonstrated for the first time that LPA plays a pivotal role in the migration of hBMSCs stimulated by SF from RA patients through LPA_1 receptor-mediated activation of calmodulin-dependent protein kinase II (CaMK II).

2. Materials and methods

2.1. Materials

α-Minimum essential medium (α-MEM), phosphate-buffered saline (PBS), trypsin, fetal bovine serum, M-MLV reverse transcriptase, and Lipofectamine plus™ reagent were purchased from Invitrogen (Carlsbad, CA). Pertussis toxin (PTX) was from BIOMOL (Plymouth Meeting, PA). Human platelet-derived growth factor-BB (PDGF-BB) was purchased from R&D Systems (Minneapolis, MN). Fluo-4-AM was from Molecular Probes, Inc. (Eugene, OR). 1-Oleoyl-*sn*-glycero-3-phosphate (1-oleoyl-LPA), sphingosine-1-phosphate (S1P), fatty acid-free bovine serum albumin, W7, KN92, KN93, and Ki16425 were purchased from Sigma-Aldrich (St. Louis, MO). Universal LPA assay kit was purchased from Echelon Biosciences, Inc. (Salt Lake City, UT).

2.2. Collection of synovial fluid

SF was obtained with the patient's consent, as approved by the Institution Review Board of Busan National University Hospital. SF was obtained from 11 patients with RA (7 male, 4 female; mean age of 59 ± 10 years) and 10 patients with osteoarthritis (OA) (5 male, 5 female; 61 ± 13 years) during therapeutic arthrocentesis. SF from normal donors was obtained postmortem from 10 organ donors (4 male, 6 female; mean age of 55 ± 15 years) without joint diseases. SF was transferred to heparin-treated tubes, transported immediately to the laboratory, and centrifuged at $3000 \times g$ for 10 min at 4 °C to remove possible inflammatory cells and blood cells. Aliquots of the supernatants were used immediately or stored at −80 °C for future analysis. SF aliquots were either used immediately or subjected to one freeze–thaw cycle. To prevent ATX-mediated generation of LPA during preparation of SF, all steps were carried out at 4 °C. To denature proteins in SF, an aliquot (200 μl) of SF or 1-oleoyl-LPA was heated at 95 °C for 5 min and centrifuged at 15,000 rpm for 5 min to remove denatured proteins, and the supernatants were collected.

2.3. Cell culture

After informed consent, heparinized bone marrow cells were obtained from different individuals undergoing total hip arthroplasty and hBMSCs were isolated as previously described [28]. To isolate hBMSCs, mononuclear cells from bone marrow were separated by centrifugation in a Ficoll–Hypaque gradient (density = 1.077 g/cm^3; Sigma) and seeded at a concentration of 1×10^6 cells/cm^2. Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 in growth medium (α-MEM, 10% fetal bovine serum, 100 units/ml of penicillin, 100 μg/ml of streptomycin) until they reached confluence. The primary hBMSCs were subcultured in tissue culture dishes at a concentration of 2000 cells/cm^2. The hBMSCs were c-kit, CD34, and CD45 negative and greater than 90% CD29, CD44, CD90, and CD105 positive were used in the experiments.

2.4. Cell migration assay

Migration of hBMSCs was assayed using a Boyden chamber apparatus, as previously described [29]. Briefly, hBMSCs were harvested with 0.05% trypsin containing 0.02% EDTA, washed once, and suspended in α-MEM at a concentration of 2×10^5 cells/ml. A polycarbonate membrane filter with 8-μm pores of the disposable 96-well chemotaxis chamber (Neuro Probe, Inc., Gaithersburg, MD) was precoated overnight with 20 μg/ml rat-tail collagen at room temperature, an aliquot (50 μl) of hBMSCs suspension was loaded into the upper chamber, and test reagents were then placed in the lower chamber, unless otherwise specified. For elucidation of signaling pathways involved in the LPA-induced migration, the cells were preincubated with pharmacological inhibitors for 15 min before loading. After exposure of the cells to either LPA or SF in the absence or presence of inhibitors for 12 h at 37 °C, the filters were then disassembled, and the upper surface of each filter was scraped free of cells by wiping it with a cotton swab. The number of cells that had migrated to the lower surface of each filter was determined by counting the cells in four places under microscopy at ×100 magnification after staining with hematoxylin and eosin.

2.5. Reverse transcription–polymerase chain reaction (RT-PCR)

Total cellular RNA was extracted by the Trizol method (Invitrogen, Carlsbad, CA). For RT-PCR analysis, aliquots of 2 μg each of RNA were subjected to cDNA synthesis with 200 U of M-MLV reverse transcriptase and 0.5 μg of oligo (dT) 15 primer (Promega, Madison, WI). The cDNA in 2 μl of the reaction mixture was amplified with 0.5 U of GoTaq DNA polymerase (Promega, Madison, WI) and 10 pmol each of sense and antisense primers as follows: LPA_1 (384 bp product): sense 5′-TCTTCTGGGCCATTTTCAAC-3′, antisense 5′-TGCCTRAAGGTGGCGCT-CAT-3′; LPA_2 (780 bp product): sense 5′-CCTACCTCTTCCTCATGTTC-3′, antisense 5′-TAAAGGGTGGAGTCCATCAG-3′; LPA_3 (450 bp product): sense: 5′-GGAATTGCCTCTGCAACATCT-3′, antisense 5′-GAGTAGAT-GATGGGGTTCA-3′; LPA_4 (200 bp product): sense 5′-TACAACTTCAACC-GCCACTG-3′, antisense 5′-ATCCAGACACCAGCACACAC-3′; LPA_5/GPR92 (261 bp product): sense 5′-GTGCTGATGGTGATGGTGCT-3′, antisense 5′-TGTGAAGGAAGACAGAGAGTGG-3′; GPR87 (536 bp product): sense 5′-CCGTATGAGGTGAATGGACA-3′, antisense 5′-CCAAGGAACACGATG-GAAGT-3′; p2y10 (434 bp product): sense 5′-TCTTCTTCATCTGCTT-CACTCC-3′, antisense 5′-CTCTGCCTTCACCATCACAC-3′; glyceraldehyde-3-phosphate dehydrogenase (GAPDH): sense 5′-TCCATGACAACTTTGG-TATCG-3′, antisense 5′-TGTAGCCAAATTCGTTGTCA-3′. The thermal cycle profile was as follows: denaturation at 95 °C for 30 s, annealing at 54–55 °C for 30 s depending on the primers used, and extension at 72 °C for 40 s. Each PCR reaction was carried out for 30 cycles, and PCR products were size fractionated on 1.2% ethidium bromide/agarose gel and photographed under UV transillumination.

2.6. Transfection with small interfering RNA

Small interfering RNA (siRNA) duplexes were synthesized, desalted, and purified by Samchully Pharm. Co. Ltd. (Siheung, GyeongGi, Korea) as follows: LPA_1, 5′-GGACUUGGAAUCACUGUUUUU-3′ (sense) and 5′-AAACAGUGAUUCCAAGUCCUU-3′ (antisense). Nonspecific control siRNA (D-001206-13-05) was purchased from Dharmacon, Inc. (Chicago, IL). For siRNA experiments, hBMSCs were seeded on 60-mm