Biochimica et Biophysica Acta 1853 (2015) 431–444

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Urokinase type plasminogen activator mediates Interleukin-17-induced peripheral blood mesenchymal stem cell motility and transendothelial migration

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A R T I C L E   I N F O

Article history:
Received 2 July 2014
Received in revised form 18 November 2014
Accepted 19 November 2014
Available online 27 November 2014

Keywords:
Interleukin-17
Peripheral blood mesenchymal stem cells
Urokinase type plasminogen activator
Migration
Transendothelial migration

A B S T R A C T

Mesenchymal stem cells (MSCs) have the potential to migrate toward damaged tissues increasing tissue regeneration. Interleukin-17 (IL-17) is a proinflammatory cytokine with pleiotropic effects associated with many inflammatory diseases. Although IL-17 can modulate MSC functions, its capacity to regulate MSC migration is not well elucidated so far. Here, we studied the role of IL-17 on peripheral blood (PB) derived MSC migration and transmigration across endothelial cells. IL-17 increased PB-MSC migration in a wound healing assay as well as cell mobilization from collagen gel. Concomitantly IL-17 induced the expression of urokinase type plasminogen activator (uPA) without affecting matrix metalloproteinase expression. The incremented uPA expression mediated the capacity of IL-17 to enhance PB-MSC migration in a ERK1,2 MAPK dependent way. Also, IL-17 induced PB-MSC migration alongside with changes in cell polarization and uPA localization in cell protrusions. Moreover, IL-17 increased PB-MSC adhesion to endothelial cells and transendothelial migration, as well as increased the capacity of PB-MSC adhesion to fibronectin, in an uPA-dependent fashion. Therefore, our data suggested that IL-17 may act as chemotropic factor for PB-MSCs by incrementing cell motility and uPA expression during inflammation development.

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1. Introduction

Cell migration is a complex process involving many both intra- and extracellular components, as well as the signaling events linking them. Due to their ability to preferentially migrate to sites of inflammation and tissue injury, and their immunomodulatory properties, mesenchymal stem cells (MSCs) have great potential for tissue regeneration and cellular therapy [1]. However, the molecular signals that guide MSCs to target tissues remain to be fully elucidated. One of the most remarkable, but least understood findings is the ability of MSCs to migrate from bone marrow or peripheral blood into damaged tissues to promote regeneration [2,3]. Although MSCs can be recruited to damaged or inflamed tissue by inflammatory cytokines [1], the molecular and cellular mechanisms mediating the recruitment of MSCs are not well understood so far.

Various inflammatory and autoimmune diseases, such as rheumatoid arthritis, psoriasis or inflammatory myopathies, include infiltration of Th17 cells into the tissue and high production of Interleukin-17 cytokines (IL-17) [4]. IL-17A, a founding member of the cytokine family has been known to regulate the pro-inflammatory responses by acting on different stromal cells, stimulating them to secrete other soluble and membrane-bound factors, among which are IL-6, G-CSF, GM-CSF, SCF, and NO. In addition, stromal cells, including bone marrow (BM) MSCs, express high levels of IL-17 receptor A (IL-17RA) and IL-17 has been shown to affect the proliferation and differentiation of MSCs [5,6]. Also, IL-17 affects MSC differentiation by inhibiting adipogenesis and myogenesis while promoting osteogenesis [7,8]. Nevertheless, the roles which IL-17 can have in MSC physiology are under research, and little is known about its involvement in MSC migration and homing to inflamed tissue.

In response to tissue damage MSCs are mobilized from bone marrow to the blood circulation and "invade" the target tissue [1,2]. A key requirement for cells to reach distant target sites is their ability to traverse the protein fibers of the extracellular matrix (ECM) which are present between cells of all tissue types [9]. To overcome these matrix barriers, migrating cells require specific proteolytic enzymes. One of the most important enzymes which enables cell migration is urokinase plasminogen activator (uPA). uPA is a proteolytic enzyme which belongs to the group of trypsin-like enzymes. uPA mediates the proteolytic cleavage of

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http://dx.doi.org/10.1016/j.bbamcr.2014.11.025
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plasminogen to give plasmin [10,11]. Plasmin, a protease with a broad spectrum of activity, is able to directly break down the components of the ECM, such as fibrin, fibronectin, laminin, collagen IV and the protein backbone of the proteoglycans [11]. Biosynthesis and activity of uPA are associated with the invasive capacity of various cell types such as leukocytes, endothelial cells, and metastasizing tumor cells. In addition to traversing the ECM, during their migration through the body, MSCs need to pass through the endothelium in order to enter the target tissue. Regarding their mobilization to the sites of inflammation, MSCs probably hold properties similar to immune cells. Hence, the well described transendothelial migration of leukocytes can serve as a starting model for MSC transendothelial migration [12]. Certain proinflammatory cytokines, such as IL-1β and TNF-α are known to increase the migratory and adhesion capacity of MSCs in vivo, by up-regulating adhesive proteins and receptors for some chemokines in these cells [1]. Although IL-17 is present in damaged or inflamed tissues and uPA recently has been implicated in migration and tropism to tumor microenvironment [13–15], the capacity of IL-17 to induce peripheral blood MSC (PB-MSC) migration and endothelium transmigration, as well as the involvement of uPA in these processes, was not elucidated so far. Therefore, in this work, we have examined whether IL-17, as a proinflammatory cytokine, is able to modulate the expression of uPA in PB-MSCs, and its importance in PB-MSC motility, endothelial cell adhesion and transendothelial migration.

2. Material and methods

2.1. Cell culture

The PB-MSC isolation was previously described [16]. Briefly, peripheral blood mononuclear cells (PBMC) were isolated from the peripheral blood of healthy donors by density gradient centrifugation on lymphocyte separation media (PAA Laboratories, Linz, Austria), and plated in cell culture flasks in growth medium (GM) consisting of 10% fetal bovine serum (FBS) and 100 units/ml Penicillin/Streptomycin (both from PAA Laboratories) in high glucose Dulbecco's Modified Eagle's Medium (DMEM, Sigma-Aldrich, St Luis, MO, USA) and cultured in a humidified atmosphere at 37 °C with 5% CO_2. The medium was replaced twice a week and nonadherent cells were discarded. Adherent, fibroblast-like cells were detached and seeded in a new flask in GM. Following the first confluence, cells were passaged regularly, and after three passages a homogenous cell culture was obtained. PB-MSCs obtained displayed a multipotent capacity of mesenchymal differentiation, since under appropriate conditions they differentiated to osteogenic, chondrogenic, adipogenic, and myogenic lineages. In addition, they were fully characterized and displayed a normal karyotype [16].

Human microvascular endothelial cells (HMEC cell line) were kindly provided by Dr. C. Bernabeu (CIB, CSIC, Spain). Cells grown in 0.2% gelatin-coated flasks in endothelial cell (EC) medium consisting of 50% DMEM, 50% Ham's F12 (Sigma-Aldrich), 1 μM hydrocortisone (Galenika, Belgrade, Serbia), 20 ng/ml EGF (R&D Systems, Minneapolis, MN, USA) and 5 μl/ml ECGS (Sigma-Aldrich). Myoblast C2C12 cell line was purchased from American Type Culture Collection (ATCC, CRL-1772) and cultured in DMEM supplemented with 10% fetal. The prostatic adenocarcinoma derived PC-3 cell line was obtained from ATCC (CRL-1435) and grown in Hams F12:DMEM (1:1) supplemented with 10% FBS.

2.2. Antibodies and reagents

Anti uPA, IL-17 and IL-17R rabbit antibodies (sc-14019, sc-7927 and sc-30175 respectively) were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies against MT1-MMP, pERK1,2, ERK1,2, pp38 and p38 were purchased from R&D Systems. For immunofluorescence analysis, anti α-tubulin, anti γ-tubulin, anti-mouse-FITC secondary antibody and DAPI were from Sigma-Aldrich. For F-actin staining, phalloidin coupled to TRITC (Sigma-Aldrich) was used. Recombinant human IL-17 and human recombinant TGF-beta were provided by R&D Systems. Epsilon aminocaproic acid (EACA) and doxycycline were from Sigma-Aldrich. 12-O-tetradecanoylphorbol-13-acetate (TPA), p38 inhibitor SB203580 and MEK1,2 inhibitor PD98059 were obtained from Calbiochem (Darmstadt, Germany), while uPA inhibitor, BC11 hydrobromide was purchased from Tocris Bioscience (Bristol, UK). Anti-uPA mouse Mab, SAM-3, was kindly provided by Dr. F. Castellino (University of Notre Dame, Indiana).

2.3. PBMSC immunophenotyping

To phenotype cell-surface antigens, third-passage cells were stained with fluorescein isothiocyannate (FITC)- or phycoerythrin (PE)-conjugated antibodies specific for the following human antigens CD90-PE, CD44-PE, CD73, CD11b (Biosource), CD45-FITC (R&D System), and CD105-PE (Invitrogen). Stained cells were analyzed using CyFlow CL (Partec, Munster, Germany). For each sample, at least 10,000 events were recorded.

2.4. Wound healing and invasion assays

Cell migration was analyzed by an in vitro wound healing assay. Briefly, 5×10^4 cells/well were seeded in 24 well plates and allowed to grow until confluence, when a scratch wound in the monolayer was made by a 200 μl pipette tip. After the cells were washed three times with PBS, they were allowed to migrate for additional 24 h in GM with the treatments indicated in Results. After the migration period, cells were fixed with ice-cold methanol and stained with 0.1% crystal violet. Cell migration into the scratch area was photographed using an inverted light microscope and quantified by TScratch software (Computational Science and Engineering Laboratory, Swiss Federal Institute of Technology, ETH Zurich, Zurich, Switzerland).

The migration capacity of PB-MSC was also evaluated in a Boyden chamber-based cell migration assay (Costar, Cambridge MA) with 8.0 μm-pore polycarbonate filters (Collaborative Research, Bedford, MA). Briefly, PB-MSCs were labeled with PKH26, according to manufacturer's instructions and seeded in the upper chamber (10^5 cells per transwell) in 100 μl of growth medium. Growth medium (0. 5 ml), with or without IL-17, as chemoattractant factor, and the indicated inhibitors, were added in the lower chamber. After 16 h, cells from the upper compartment were cleaned with a cotton swab to remove the nonmigrating cells. Cells attached to the bottom of transwells were fixed by immersing the transwells into 3.7% formaldehyde in PBS. After washing, transwells were turned upside down, mounted with a cover slip and cells were observed using an epifluorescence microscope. Red labeled cells from each sample were counted using ImageJ software in eight random fields per transwell insert.

2.5. Cell mobilization assay

Collagen was prepared as described previously [17]. Briefly, type I collagen was extracted from rat tail tendons in 0.2% acetic acid and 4.4 mg/ml solution was obtained. This solution was subsequently mixed with 0.34 N NaOH and 10 times concentrated DMEM in an 8:1:1 ratio and stored at 4 °C to initiate the gelling process. For cell mobilization assay, 1.2×10^6 PB-MSCs were embedded in 600 μl of collagen I (2.2 mg/ml) and four 25 μl drops were added per well in a 6 well plate. After 1 h of incubation in a humidified atmosphere at 37 °C with 5% CO_2, the cell–collagen drops gelled and 2 ml of GM with 0, 25 or 50 ng/ml IL-17 was added to each well. After a 5-day culture, cell mobilization from the collagen plugs into the surrounding plastic surface was monitored by phase-contrast microscopy. For the experiments using inhibitors, PD98059 (25 μM) or BC11 hydrobromide (50 μM) was added to GM 30 min prior to the addition of rhIL-17.

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