Biomaterials 150 (2018) 14–24

Contents lists available at ScienceDirect

Biomaterials

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

MTA promotes chemotaxis and chemokinesis of immune cells through distinct calcium-sensing receptor signaling pathways

Fengjiao Chang [a, 1], Jin Man Kim [a, 1], Youngnim Choi [b], Kyungpyo Park [a, *]

[a] Department of Physiology, School of Dentistry, Seoul National University and Dental Research Institute, Seoul, South Korea
[b] Department of Immunology and Molecular Microbiology, School of Dentistry, Seoul National University and Dental Research Institute, Seoul, South Korea

ARTICLE INFO

Article history:
Received 7 July 2017
Received in revised form
28 September 2017
Accepted 2 October 2017
Available online 4 October 2017

Keywords:
Mineral trioxide aggregate
Calcium sensing receptor
Immune cell
Chemotaxis
Chemokinesis

ABSTRACT

Mineral trioxide aggregate (MTA) has been introduced as a choice material for regenerative dentistry. To date, the diverse biological activities of MTA, including its anti-inflammatory effects, have been extensively discussed. However, there is limited insight into the link between MTA and immune cell migration. In this study, we report the role of MTA in enhancing both chemotactic and chemokinetic immune cell migration through distinct signaling pathways. By using versatile live imaging techniques, we demonstrated that MTA-mediated CaSR activation induced diverse downstream pathways to govern cell migratory capacity. In this context, Cdc42 generates cytoskeleton-driven cellular protrusions to steer directional cell migration (chemotaxis) whereas Ca^{2+}-calmodulin dependent myosin light chain kinase induces cell contractility that plays an important role in speeding up the average migration speed (chemokinesis). Our findings illuminate an unrecognized role for MTA and the related CaSR signaling network in immune cell migration, providing evidence that can drive development of novel approaches to immunological therapy.

1. Introduction

Immune cell migration is a critical dimension for host defense. The initiation of a protective immune response is dependent on both the spatial and temporal distribution of immune cells. Cells leave the bloodstream and migrate into damaged tissues by the mediation of pro-inflammatory cytokines, adhesion molecules, chemokines, local growth factors, and interaction with other cells, which switch the migration patterns in a random or directed fashion [1,2]. Chemokinesis is generally defined in a random fashion, where cells sense inflammatory signals that stimulate their migration speed or other motile properties without affecting the direction of migration to reach surrounding targets with more efficiency. Factors that increase directionality during chemokinesis are known to promote chemotaxis, which refers that cells are informed the accurate information on prey location, target pathogens in sites of inflammation [1,3].

To mobilize diverse immune cells to where they are needed is essential for immune system function in promoting immune defense during different infectious conditions. Neutrophils act as leader cells and must sense, prioritize, and integrate all inflammatory signals into a migration response to defend against infection through phagocytosis and degranulation [4]. Monocytes can further differentiate into macrophages or dendritic cell populations and be recruited to sites of inflammation to mediate the clearance of viral, bacterial, and fungal infections [5]. T cells modify their migration patterns to follow or find cognate antigen on the surface of antigen-presenting cells (APCs) and other recently activated immune cells within an inflammatory microenvironment [6]. When an APC is recognized, a stable conjugate is rapidly formed and cooperates to fully activate the T cell [7]. By contrast, the impaired migratory potential of immune cells is a likely contributor to immunodeficiency. *Mycobacterium tuberculosis* may delay the migration of dendritic cells and subsequent T cell activation, resulting in pulmonary tuberculosis (TB) [8]; feline immunodeficiency virus (FIV) impairs the recruitment of neutrophils *in vivo* [9]. Developing evidence points to the pivotal role of cell migration in the immune system.

Mineral trioxide aggregate (MTA) has been used in diverse dental treatments including root-end filling, pulp capping, and

* Corresponding author. Department of Physiology, School of Dentistry, Seoul National University and Dental Research Institute, 101 Daehak-ro, Jongno-gu, Seoul, 03080, South Korea.
E-mail address: kppark@snu.ac.kr (K. Park).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.biomaterials.2017.10.009
0142-9612/

perforation repair with promising results [10,11]. Due to its practical manner of application with pulpal and periapical tissues, the biological properties of this widely acceptable material have been generally discussed. MTA has biocompatible and bioactive properties and is able to improve the regeneration of damaged tissues to their pre-disease state [12]. Recently, the biological activities of MTA have been investigated at the cellular level, clarifying the mechanisms of MTA-induced tissue repair in biological systems. MTA modulates the proliferation, migration, and differentiation of cells in teeth and adjacent tissues (eg. odontoblast, osteoblasts, dental pulp stem cells, fibroblasts) [12–15]. Moreover, in pathological conditions, the most characteristic reaction that MTA induces is the reduction of inflammation of connective tissues by regulating cytokine production and release of immune cells [16]. Even though the important role of immune cell migration in inflammatory process has been noted, the effects of MTA on immune cell migration have yet to be identified.

MTA is a calcium silicate-based cement that has a high alkaline pH when contact with tissue fluid and slowly releases calcium ions after hydration. Our previous study showed that the calcium-sensing receptor (CaSR) acts as a core biological mediator for the modulation of extracellular Ca^{2+} and pH by MTA [17]. As a member of the G protein-coupled receptor (GPCR) family, CaSR functions as a general sensor for the extracellular change of various molecules such as cations (eg. Ca^{2+} and Sr^{2+}), pH, polyamines and L-amino acids and transfers the environmental changes into intracellular signaling pathways [18]. CaSR expression has been reported in diverse organs, including kidney, skin, and bone, and is linked with a wide range of physiological processes. Within the different CaSR-expressing cell types, this receptor regulates cellular functions including hormone secretion, differentiation, proliferation, apoptosis, and gene expression [19]. Additionally, CaSR existence in T lymphocytes and its activation could promote apoptosis and cytokine secretions are previously reported [20]. Therefore, CaSR has a universal function in the maintenance of biological balance including immune system. However, despite the broad range of CaSR functions, there is a lack of evidence supporting the role of CaSR in immune cell migration.

In this paper, we investigated the role of MTA in immune cell migration. We found that MTA could enhance immune cell migration in both chemotactic and chemokinetic manners. CaSR is a critical signaling integrator in these motile responses mediated by MTA. Moreover, we further identified distinct intracellular signaling cascades related to each response, thus elucidating the mechanisms of MTA-enhanced immune cell migration.

2. Materials and methods

2.1. Reagents and plasmids

The following reagents were used at the indicated concentrations in this experiment: 50–500 ng/ml SDF-1α (300-28A, Peprotech), 2 μg/ml Pertussis toxin (P7208, Sigma), 100 μM $LaCl_3$ (449830, Sigma), 5 μM NPS 2143 (3626, Tocris), 0.2–5 mM $SrCl_2$ (439665, Sigma), 1 mM ethylene glycol-bis(2-aminoethylether)-N,N,N′,N'-tetraacetic acid (EGTA, E4378, Sigma), 50 μM LY 294002 (L9908, Sigma), 10–100 μM NSC 23766 (SML0952, Sigma), 10–100 μM ZCL 278 (4794, Tocris), 10 μM U73122 (U6756, Sigma), 2 μM Ionomycin (I9657, Sigma), 20 μM ML-7 (I2764, Sigma), 50 μM blebbistatin (B0560, Sigma), and 10 μM Y27632 (1254, Tocris).

The following plasmids were purchased from Addgene: pGP-CMV-GCaMP6s was a gift from Douglas Kim (Addgene plasmid # 40753) [21], pTriEx-mVenus-PA-Cdc42 and pcDNA3-EGFP-Cdc42(T17N) were gifts from Klaus Hahn (Addgene plasmid # 75263, 12601) [22,23], pcDNA3-EGFP was a gift from Doug Golenbock (Addgene plasmid # 13031), pcDNA3-EGFP-Rac1-T17N was a gift from Gary Bokoch (Addgene plasmid # 12982) [24], pEGFP-MRLC, pEGFP-MRLC T18D, S19D and pEGFP-MRLC T18A, S19A were gifts from Tom Egelhoff (Addgene plasmid # 35680, 35682, 35681) [25]. mCherry-Lifeact was generated by substitution of the fluorescent protein sequence of EGFP-Lifeact plasmid [26]. pTriEX-mVenus was cloned by removing the PA-Cdc42 sequence in the pTriEx-mVenus-PA-Cdc42 plasmid.

2.2. Cell culture and transfection

Human acute T-cell leukemia cell line Jurkat (clone E6-1, 40152), human monocytic cell line THP1 (40202), human neutrophil-like HL-60 (10240), and human U937 monocytes (21593.1) cell lines were purchased from the Korean cell line bank. All cells were cultured in RPMI-1640 (SH30027.01, Hyclone) supplemented with 10% FBS (SH30084.03, Hyclone), 1% sodium pyruvate (11360-070, Gibco) and 1% penicillin/streptomycin (15140-122, Gibco) at 37 °C with 5% CO_2. THP1 cells were differentiated for 2 days with Phorbol 12-myristate 13-acetate (100 ng/ml). HL-60 cells were differentiated in culture medium plus 1.3% DMSO [27], and the cells were harvested on day 5 for the migration test. U937 cells were stimulated to differentiate by 1 mM cyclic-AMP (D0260, Sigma) for 24 h as previously described [28]. Cells were used from 3 to 15 passages for all the experiments. Jurkat T cells were grown in culture medium without antibiotics 1 day prior to electroporation. Plasmid DNAs were used at a final amount of 0.5–1 μg. The usage of the Neon transfection system (MPK5000, Invitrogen) was performed according to the manufacturer's instructions. Transfected cells were plated on a 96-well glass bottom plate (0611129L2L, Matrical Bioscience) coated with fibronectin (F0895, Sigma). The cells were incubated for 24 h before each experiment.

2.3. Primary culture of $CD4^+$ T cells

ICR male mice at 8–15 weeks were used for the primary T cell culture. All the procedures were approved by the protocol of Seoul National University (SNU-150722-3-1). Mouse $CD4^+$ T cells were isolated from the spleen by negative selection as previously described [29]. Individual spleens were meshed through a 40 μm cell strainer (352340, BD Falcon) to release splenocytes into a 15 ml complete RPMI 1640 medium. Red blood cells were lysed using an ACK lysis buffer (155 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA, pH 7.4). The cell suspension was centrifuged (5 min at 1500 rpm at room temperature) and counted. $CD4^+$ T cells were enriched using a MACS $CD4^+$ T cell isolation kit (# 130-104-454, Miltenyi Biotec). Human PBMCs were isolated from buffy coats using Ficoll-Hypaque density gradients (Amersham Bioscience). $CD4^+$ T cells were purified by using anti-human CD4 magnetic particles (557767, BD Biosciences) according to the manufacturer's instructions. The protocol was approved by the Institutional Review Board of Seoul National University (No. S-D20150007).

2.4. Preparation of MTA solution

A total of 0.2 g of MTA powder (ProRoot MTA, Dentsply) was diluted with 20 ml RPMI-1640 or HEPES-buffered physiological salt solution (pH 7.4) containing 145 mM NaCl, 3.6 mM KCl, 1 mM $MgCl_2$, 1.3 mM $CaCl_2$, 5 mM D-glucose, and 10 mM HEPES [30]. The mixture was incubated for 24 h at 37 °C in a shaking water bath and then filtered through a 0.2 μm pore filter (16534-K, Sartorius).

2.5. Chemotaxis assay

Jurkat T cells, HL-60 cells, U937 cells (2×10^5) in serum-free