ARTICLE IN PRESS

Biochemical and Biophysical Research Communications xxx (2016) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Periostin supports hematopoietic progenitor cells and niche-dependent myeloblastoma cells *in vitro*

Satowa Tanaka [a], Akio Maekawa [a], Leo Matsubara [a], Azusa Imanishi [a], Masaya Yano [a], Robert G. Roeder [b], Natsumi Hasegawa [a], Shigetaka Asano [c], Mitsuhiro Ito [a, b, c, *]

[a] *Laboratory of Hematology, Division of Medical Biophysics, Kobe University Graduate School of Health Sciences, 7-10-2 Tomogaoka, Suma-ku, Kobe 654-0142, Japan*
[b] *Laboratory of Biochemistry and Molecular Biology, The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA*
[c] *Research Organization for Nano & Life Innovation, Waseda University, 3-4-1 Okubo, Shinjuku-ku, Tokyo 159-8555, Japan*

ARTICLE INFO

Article history:
Received 28 August 2016
Accepted 1 September 2016
Available online xxx

Keywords:
Bone marrow stromal cells
Hematopoietic niche
Extracellular matrix protein
Myeloblastoma cells
Mediator transcriptional coregulator complex

ABSTRACT

The expression of extracellular matrix protein periostin (POSTN) was attenuated in *Med1*$^{-/-}$ mouse embryonic fibroblasts (MEFs), which exhibited a decreased capability to support hematopoietic progenitor cells (HPCs) *in vitro*. When bone marrow (BM) cells were cocultured with mitomycin C-treated *Med1*$^{+/+}$ MEFs, or OP-9 or MS-5 BM stromal cells, in the presence of anti-POSTN antibody, the growth of BM cells and number of long-term culture-initiating cells (LTC-ICs) were attenuated. When BM cells were cocultured with *Med1*$^{-/-}$ MEFs in the presence of recombinant POSTN, the growth of BM cells and the number of LTC-ICs were restored. Moreover, antibody-mediated blockage of stromal cells-derived POSTN markedly reduced the growth and cobblestone formation, a leukemic stem cell feature, of stromal cell-dependent MB-1 myeloblastoma cells. POSTN was expressed both in BM cells and variably in different BM stromal cells. Expression in the latter cells was increased by physical interaction with hematopoietic cells. The receptor for POSTN, integrin αvβ3, was expressed abundantly in BM stromal cells. The addition of recombinant POSTN to BM stromal cells induced intracellular signaling downstream of integrin αvβ3. These results suggest that stromal cell POSTN supports both normal HPCs and leukemia-initiating cells *in vitro*, at least in part, indirectly by acting on stromal cells in an autocrine or paracrine manner.
© 2016 Elsevier Inc. All rights reserved.

1. Introduction

The maintenance and differentiation of hematopoietic stem/progenitor cells (HSPCs) is strictly controlled by a bone marrow (BM) microenvironment called the hematopoietic niche, and thereby postnatal hematopoietic homeostasis is maintained. The mesenchymal stem cells (MSCs) and related stromal cells within the periarteriolar and perisinusoidal niches are now regarded as the major niche components. The molecular basis of communication between mesenchymal and hematopoietic cells, involving niche factors that restrain or expand HSPCs, has been extensively investigated [reviewed in [1–4]]. Many of the major known niche factors, including CXC chemokine ligand 12 (CXCL12), stem cell factor (SCF) and transforming growth factor (TGF)-β among others, are produced by mesenchymal stem or stromal cells, while megakaryocytes, which constitute another niche, also provide CXCL4, TGF-β and fibroblast growth factor (FGF)1/2.

The Mediator, composed of about 31 subunits, is a master transcriptional coregulator complex that is essential for global transcription governed by RNA polymerase II [reviewed in [5]]. Among the Mediator subunits, MED1 acts as a specific coactivator for activators that include nuclear receptors [reviewed in [6,7]]. We previously reported that *Med1*$^{-/-}$ mouse embryonic fibroblasts (MEFs) have an attenuated ability to support hematopoietic progenitor cells (HPCs) relative wild-type MEFs, and that the attenuated expression of full-length osteopontin or FGF7 in *Med1*$^{-/-}$ MEFs is responsible for the observed phenotype. Thus, osteopontin and FGF7, produced by BM mesenchymal stromal cells, have been identified as important niche factors [8,9].

Periostin (POSTN), first identified in osteoblasts and later found to be expressed more widely, is the fasciclin family extracellular

* Corresponding author. Laboratory of Hematology, Division of Medical Biophysics, Kobe University Graduate School of Health Sciences, 7-10-2 Tomogaoka, Suma-ku, Kobe 654-0142, Japan.
E-mail address: itomi@med.kobe-u.ac.jp (M. Ito).

http://dx.doi.org/10.1016/j.bbrc.2016.09.008
0006-291X/© 2016 Elsevier Inc. All rights reserved.

ARTICLE IN PRESS

matrix (ECM) protein that is induced by TGF-β, together with its paralog BIGH3. It interacts with other ECM proteins such as fibronectin, type I collagen and tenascin-C, and constitutes the structural basis for tissues [10]. POSTN also induces intracellular signaling through its receptor, integrin αvβ3, and contributes to some pathological conditions such as scar formation after myocardial infarction, and, as a malignant niche factor, cancer cell migration, infiltration and survival [11].

Postn, *Cxcl12* and *Ccl9* are the direct targets of the transcriptional activator early B-cell factor (EBF) within mouse OP-9 BM stromal cells [12]. POSTN, produced by OP-9 cells, has recently been reported to be required for optimal B lymphopoiesis *in vitro* [13], suggesting that POSTN, as well as CXCL12, are B cell-specific niche factors within the BM microenvironment. Moreover, increased expression of POSTN within BM stromal cells might correlate with myelofibrosis, leading to the interesting hypothesis that POSTN might be a niche factor for clonal expansion in some form of chronic myeloproliferative diseases [reviewed in [14]].

In this study we show that POSTN expression is attenuated in $Med1^{-/-}$ MEFs, and that POSTN produced by BM mesenchymal stromal cells is required for optimal support of both normal HPCs and leukemia-initiating cells *in vitro*. We propose that POSTN may be a bona fide niche factor for both normal and malignant hematopoiesis.

2. Methods

2.1. Cell culture

Stable lines of $Med1^{+/+}p53^{-/-}$ and $Med1^{-/-}p53^{-/-}$ MEFs in C57BL6 background are described elsewhere [8]. MEFs, mouse BM-derived MSCs obtained from GIBCO, and 293T cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS). The OP-9 cells, distributed by RIKEN BRC through the National Bio-Resource of the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT), and MS-5 cells [15] were maintained as described [8]. The niche-dependent MB-1 myeloblastoma cells were maintained by coculturing with mitomycin C (MMC)-treated OP-9 cells as described [16,17].

2.2. BM cell culture and colony-forming assays

Mouse BM cells in α-modified Eagle's medium with 20% FBS were plated on a dish for several hours, and adherent cells (non-hematopoietic cells and macrophages) were eliminated. These BM cells (1×10^6) were cocultured with MMC-treated MEFs, or OP-9 or MS5 cells on 12-well plates, in MyeloCult M5300 (Stem Cell Technologies, Canada) in the absence or presence of recombinant (r) mouse (m) POSTN, or 0.5 μg/ml anti-mPOSTN rabbit polyclonal antibody (Ab) (H-300: sc-67233; Santa Cruz Biotechnology) or normal rabbit IgG (Medical & Biological Laboratories). In some experiments, transwell (12-well; Corning) was used to exclude the effects of physical interaction between BM cells and stromal cells.

For colony-forming assays, half of the medium was replaced with fresh medium every week for 4 weeks. Cells were trypsinized, harvested and cultured in complete methylcellulose medium (MethoCult M3434; Stem Cell Technologies) for all types of colonies at 37 °C for 14 days, and the colonies were counted [8,9].

2.3. Bromodeoxyuridine (BrdU) incorporation

DNA synthesis of cocultured hematopoietic cells in 24-well plates was measured by bromodeoxyuridine (BrdU) incorporation as described [8,9].

2.4. Quantitative RT-PCR

Total cellular RNA (0.5 μg), isolated with Isogen II (Toyobo), was reverse-transcribed with ReverTra Ace qPCR RT Master Mix with a gDNA Remover kit (Toyobo). Quantitative PCR (StepOnePlus Real-Time PCR system; Life Technologies) was performed for quantitation of various mouse and human mRNAs. Mouse or human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used for normalization of the results. The sequences of the primers used for amplification are available upon request.

2.5. Western blot analysis and ELISA

For western blot analysis, total cell lysates, separated by SDS-PAGE, were probed with polyclonal Abs against mouse focal adhesion kinase (FAK) (#3285; Cell Signaling), phospho-mFAK (#3283; Cell Signaling), mitogen-activated protein (MAP) kinase ERK1/ERK2 (Millipore), and phospho-MAP kinase ERK1/ERK2 (Tyr202/204) (#4370; Cell Signaling), and monoclonal Abs against mouse integrin αv/CD51 (NBP1-96739; Novus Biologicals), mouse integrin β3 (I19620; Transduction Laboratories), and β-actin (BioLegend). Chemiluminescence was detected by an ImageQuant LAS 4000mini (GE Healthcare).

For quantitation of mPOSTN protein, ELISA was performed by using Quantikine ELISA Mouse Periostin/OSF-2 Immunoassay kit (MOSF20; R&D Systems).

2.6. Statistical analysis

All numerical results are expressed as means ± SD. Data were evaluated using Student's *t*-test (for two groups) or one-way analysis of variance (ANOVA) (for more than two groups) for statistical comparisons. Two-way ANOVA was used for studies over prolonged time and, when significant, values for each time point were compared. $P < 0.05$ was considered significant. $P < 0.05$ and $P < 0.01$ were represented by * and **, respectively. Reproducibility of all data was confirmed by repeating the experiments more than twice.

3. Results

3.1. POSTN, downregulated in $Med1^{-/-}$ MEFs, mediates growth of BM cells in MEF-based coculture

MEFs have an early mesenchymal feature and are known to support HPCs *in vitro* [8]. We have previously reported that the $Med1^{-/-}$ MEFs have reduced ability to promote growth of BM cells and to support of HPCs *in vitro*, at least partly, through the reduced production of full-length osteopontin [8] and FGF7 [9]. Comparative microarray analysis of the $Med1^{+/+}p53^{-/-}$ and $Med1^{-/-}p53^{-/-}$ MEFs has revealed that *Postn* expression is also attenuated in $Med1^{-/-}$ MEFs (GEO accession number GSE22471) [8]. These findings and the proposed role for POSTN as a niche factor for rabbit and mouse B lymphopoiesis [13], prompted us to investigate whether POSTN also acts as a niche factor for other lineages of hematopoiesis.

We first examined the effect of POSTN on mitogenicity of BM cells cocultured with MEFs. When normal BM cells were cocultured with MMC-treated $Med1^{+/+}$ MEFs in the presence of anti-mPOSTN Ab, the number of BM cells was reduced compared to the control (Fig. 1A, left panel). These cells also showed reduced DNA synthesis (Fig. 1B, left panel), but the rate of cell death was comparable to that of the control as revealed by trypan blue staining. However, the number (Fig. 1A, right panel) and DNA synthesis level (Fig. 1B, right panel) of BM cells increased when cocultured with $Med1^{-/-}$ MEFs

Please cite this article in press as: S. Tanaka, et al., Periostin supports hematopoietic progenitor cells and niche-dependent myeloblastoma cells *in vitro*, Biochemical and Biophysical Research Communications (2016), http://dx.doi.org/10.1016/j.bbrc.2016.09.008

Download English Version:

https://daneshyari.com/en/article/5506645

Download Persian Version:

https://daneshyari.com/article/5506645

Daneshyari.com