Cancer Letters 344 (2014) 62–73

Contents lists available at ScienceDirect

Cancer Letters

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

Roles for GP IIb/IIIa and αvβ3 integrins in MDA-MB-231 cell invasion and shear flow-induced cancer cell mechanotransduction

Fenglong Zhao, Li Li, Liuyuan Guan, Hong Yang, Chunhui Wu, Yiyao Liu *

Department of Biophysics, School of Life Science and Technology, University of Electronic Science and Technology of China, Chengdu 610054, Sichuan, PR China

ARTICLE INFO

Article history:
Received 8 June 2013
Received in revised form 10 October 2013
Accepted 18 October 2013

Keywords:
MDA-MB-231 cells
Shear stress
PI3K/Akt pathway
Integrin
NF-κB

ABSTRACT

Adhesion of cancer cell to endothelial cells and the subsequent trans-endothelial migration are key steps in hematogenous metastasis. However, the molecular mechanisms of cancer cell/endothelial cell interaction under hemodynamic shear flow and how shear flow-induced cancer cell mechanotransduction are yet to be fully defined. In this study, we identified that the integrins of both platelet glycoprotein IIb/IIIa (GP IIb/IIIa) and αvβ3 were crucial for hematogenous metastasis of human breast carcinoma MDA-MB-231cells. The cell migration and invasion were studied by using Millicell cell culture insert system. The numbers of invaded MDA-MB-231 cells significantly increased by thrombin-activated platelets and reduced by eptifibatide, a platelet inhibitor. Meanwhile, RGDWE peptides, a specific inhibitor of αvβ3 integrin, also inhibited MDA-MB-231 cell invasion. We further used a parallel-plate flow chamber to investigate MDA-MB-231 cell adhesion under flow conditions. Alike in static condition, the adhesion capability of MDA-MB-231 cells to endothelial monolayer was also significantly affected by GP IIb/IIIa and αvβ3 integrins. The expression of matrix metalloproteinase-2 (MMP-2), MMP-9 and αvβ3 integrin in MDA-MB-231 cells were up-regulated after low shear stress exposure (1.84 dynes/cm^2, 2 h). Moreover, we also demonstrated that low shear stress induced a sustained activation of p85 (a regulatory subunit of PI3K) and Akt. Pre-treating MDA-MB-231 cells with the specific PI3K inhibitor of LY294002 abolished the shear stress induced-Akt activation, and the expression of MMP-2, MMP-9, vascular endothelial growth factor (VEGF) and αvβ3 integrin were also down-regulated. Immunofluorescence assay showed that low shear stress also induced αvβ3 integrin clustering and nuclear factor-κB (NF-κB) activation. Interestingly, shear stress-induced activation of Akt and NF-κB was attenuated by LM609, a specific antibody of αvβ3 integrin. It suggests that αvβ3 integrin might be as a mechanosensor to trigger both PI3K/Akt and NF-κB signaling pathways. Taken together, these results establish that GP IIb/IIIa and αvβ3 integrins are essential mediators, and provide insight into how shear stress-induced αvβ3 integrin activation and the downstream pathways for contribution to MDA-MB-231 cell adhesion, migration and invasion.

1. Introduction

Hematogenous metastasis is the mainly leading cause of death in breast carcinoma patients. A better understanding of the underlying molecular and cellular mechanisms is crucial for the development of effective treatment for metastatic breast cancer. Hematogenous metastasis is a complex process involving the activities of both tumor cells and host cells [1]. The process starts with the intravasation of cancer cells into the blood stream in the primary tumor lesion. The cancer cells then travel in the blood stream, where they interact with various blood cells, and finally they adhere to endothelial cells somewhere in the peripheral vessel walls. After extravasation, they enter the connective tissue and form a new metastatic lesion [1,2]. Adhesion of circulating cancer cells to endothelium and the subsequent trans-endothelial migration are critical steps in this process. Therefore, identifying the precise molecular mechanisms which mediate cancer cell invasion is necessary for us to design specific treatment to prevent breast cancer metastasis.

It has been well established that cell adhesion and invasion are mediated by a variety of transmembrane proteins, including integrins, cadherins, selectins, and intercellular adhesion molecules. Among these adhesion molecules, integrins and their downstream signaling pathways have been extensively studied [3–5]. Integrins, a widely expressed family of transmembrane adhesion receptors, are important family of cell-surface adhesion molecules that mediate interactions of cell–cell or cell–extracellular matrix (ECM). The αvβ3 integrin is predominantly, although not exclusively, found in cancer cells and neovessels [6]. To date, the aberrant expression of αvβ3 integrin has been demonstrated on various kinds of cancer

* Corresponding author. Tel.: +86 28 8320 3353; fax: +86 28 8320 8238.
E-mail address: liuyiyao@hotmail.com (Y. Liu).

0304-3835/$ - see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.canlet.2013.10.019

including metastasis human melanoma, breast, prostate, and glioblastoma tumor cells. As shown previously by several groups, αvβ3 integrin has been shown to contribute to the establishment and growth of pulmonary metastatic melanoma lesions [7], and to increased invasiveness of cutaneous melanomas from the epidermis to the dermis [8] and of human breast cancer cells in nude mice [9]. It was also reported that its expression could up-regulate cdc2 to modulate prostate cancer cell migration [10]. All these evidence lead to the confirmation that αvβ3 integrin plays an important role in cancer cell metastasis.

The specific events determining tumor cell interactions with endothelial cells during hematogenous metastasis are well defined [11], whereas the contributions of other cell types, such as platelets and leukocytes, in this process are less well understood. Recently, both clinical and experimental evidences point to a role of platelets in the spread of cancer [12–14]. Interestingly, clinical statistics showed that half of cancer patients have accompanying platelet activation and thrombosis, as well as activated platelet number elevation [15,16]. GP IIb/IIIa integrin is the most abundant receptor on the platelet surface. It participates in hemostasis by bridging platelet/platelet interactions via the ligand, fibrinogen [17]. Recent investigations demonstrated that activated platelets as the mediators to link hemostasis and hematogenous metastasis [18]. Circulating tumor cells also contain various membrane receptors that can bind directly to platelets and mediate tumor cell-platelet binding and activate platelets. Although there are a few primary pre-clinic studies which suggest GP IIb/IIIa integrin has contributed to cancer metastasis, how GP IIb/IIIa mediates this process under dynamic fluid stress has not been confirmed.

Increasing a number of evidence indicate that shear stress plays a critical role in hematogenous metastasis of human breast cancer cells. Circulating tumor cells are continuously exposed to shear stress in the bloodstream. However, the pathways by which such stimulation influence integrin function in cancer cells are not well understood. Although mechanical stimuli activating the PI3K/Akt pathway in endothelial and other cells has been well defined [19–21], whether shear stress activating αvβ3 integrin to trigger PI3K/Akt signaling is incompletely elucidated. In some cellular systems, conversion of integrins to the high affinity state was triggered by PI3K activation. Integrin activation may in turn contribute to the adhesive and invasive properties of cancer cells. It was reported that pressure induced the adhesion of colon cancer cells via a Src-dependent PI3K/Akt pathway [22]. Other studies demonstrated that integrin activation enhanced the expression and activity of MMPs, and promoted cell migration [23].

In the present study, we firstly investigated the potential roles of GP IIb/IIIa and αvβ3 integrins in adhesion and invasion of human breast carcinoma MDA-MB-231 cells, then characterized whether αvβ3 integrin acted as a mechanosensor to trigger the PI3K/Akt signal pathway under low shear stress exposure. We found that GP IIb/IIIa and αvβ3 integrins facilitated MDA-MB-231 cell adhesion and invasion, and further assessed low shear stress-induced PI3K/Akt signaling to increase the adhesiveness and metastatic potential of MDA-MB-231 cells via αvβ3 integrin activation. In addition, Low shear stress-activated PI3K/Akt signaling also up-regulated the mRNA expression of MMP-2, MMP-9, VEGF, and αvβ3 integrin. Moreover, shear stress could trigger αvβ3 integrin clustering on the membrane and NF-κB activation. Taken together, low shear stress could regulate breast cancer cell metastasis through αvβ3 integrin-mediated mechanotransduction via the activation of PI3K/Akt pathway and NF-κB. These results identified that inhibition of platelet activation and low shear stress-induced PI3K/Akt signaling may have a therapeutic value in the prevention of cancer hematogenous metastasis.

2. Materials and methods

2.1. Chemicals and reagents

Cell culture medium of L15 and RPMI 1640, penicillin, streptomycin and newborn calf serum (NCS) were purchased from Gibco (Grand Island, NY, USA). Water-soluble cholesterol and methyl-β-cyclodextrin (MBCD), trypsin, HAT (hypoxanthine, aminopterin, and thymidine) supplement, thrombin, matrigel, and gelatin were obtained from Sigma–Aldrich (St. Louis, MO, USA). Alexa Fluor 488-conjugated cholera toxin subunit B (CTxB) was purchased from Invitrogen Life Technologies (Carlsbad, CA, USA). 2-[4-(2-Hydroxyethyl)-1-piperazine] ethanesulfonic acid (HEPES) was supplied by Amresco (Cleveland, USA). 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI), PI3K inhibitor of LY294002, rabbit anti-human p65 polyclonal antibody, AlexFluo 555-labeled goat anti-mouse secondary polyclonal antibody, and Cy3-labeled goat anti-rabbit IgG were from Beyotime Institute of Biotechnology (Jiangsu, China). Monoclonal antibody against the integrin receptor αvβ3 (LM609) was obtained from Chemicon International (Temecula, CA, USA). Monoclonal antibody against the Akt, phosphor-Akt, p85, and phosphor-p85 were purchased from Cell Signaling Technology (Beverly, MA, USA), Human eptifibatide and arg-gly-asp-trp-glu peptides (RGDWE peptides) were purchased from Cali-bio (CA, USA) and SBS Genetech (Beijing, China), respectively. All other chemicals and solvents if not mentioned were of analytical grade and used as received without additional purification.

2.2. Cell culture

The human umbilical vein derived endothelial cell line, EA.hy926 (ATCC number CRL-2922) [24] was grown in cell culture medium RPMI 1640 containing 10% NCS, 20 mM HEPES, 2% $NaHCO_3$, 2% HAT, and 1% penicillin/streptomycin and cells were maintained in an incubator at 37 °C in 5% CO_2 atmosphere. This cell line, a hybridoma of human umbilical vein endothelial cells (HUVECs) and the human epithelial cell line A549, has been shown to retain a number of properties of endothelial cells, including the production of human factor VIII-related antigen. Cells were plated on glass cover slips at least 2 days before use in experiments.

Human breast cancer cell line of MDA-MB-231 (ATCC number HTB-26) was obtained from American Type Culture Collection (ATCC, Manassas, VA, USA) and grown in L15 culture medium supplemented with 10% fetal bovine serum (FBS, HyClone, Thermo Scientific, UT, USA), 100 mg/ml streptomycin and 100 units/ml penicillin in a humidified incubator at 37 °C and without CO_2 atmosphere. Prior to following experiments, cells were detached from 25-cm^2 culture flasks with 0.25% trypsin in phosphate buffered saline (PBS) (pH 7.4).

2.3. Platelet isolation

Platelet isolation was adopted from the reported method [13,25]. In brief, human blood was drawn by venipuncture from healthy volunteers into heparin sodium (1 U/mL) anticoagulant. Platelet-rich plasma (PRP) was prepared by centrifugation of whole blood at 120*g* for 10 min. Platelet-poor plasma (PPP) was obtained by further centrifugation of the blood at 500*g* for 10 min. The final platelet count of the PRP was adjusted to the desired levels (2×10^8/mL) with PPP. Specimens were stored at 37 °C in capped polypropylene tubes and used for the following experiments. The human platelet isolation experiment was approved by the institutional review boards and performed in accordance with the institutional guidelines for human and animal welfare (UESTC-EXP2012013).

2.4. Cell treatment with thrombin, eptifibatide, and RGDWE peptides

To potentiate platelet activation, platelets were treated for 30 min with thrombin (0.55 U/mL) before incubation with MDA-MB-231 cells. For inhibition studies, platelets and MDA-MB-231 cells were also treated for 30 min with eptifibatide (0.65 μg/mL) and RGDWE peptides (425 μg/mL) to block the GP IIb/IIIa and αvβ3 integrins, respectively. Control experiments were performed in which platelets or MDA-MB-231 cells were treated exactly as stated above but in the absence of thrombin, eptifibatide, or RGDWE peptides.

2.5. Cell adhesion and invasion in static conditions

For cell adhesion assay, MDA-MB-231 cells (3000 cells/well) were seeded in 96-well plate, which were pre-coated with matrigel (BD Biosciences) for overnight. Cells were allowed to adhere for 1 h. After carefully washing three times with warm PBS, adherent cells were stained with 0.05% (w/v) crystal violet after fixation with methyl alcohol for 30 min. At least five random fields (10× magnification) for each group were photographed under an inverted microscope (Nikon TE-2000U, Japan). The quantitative cell adhesion was determined by a microplate reader (Model 680, Bio-Rad, Philadelphia, PA, USA) at 495 nm to record the absorbance values [13].

A transwell system (8-μm pore size) was used to evaluate cell invasion. The membrane filter of the upper chamber was coated with 100 μL of gelatin (10%, w/v) and then was air-dried in an incubator (37 °C). Cells (1×10^5 in 500 μL serum-free medium) were added to the upper well, and 1.5 mL of culture medium with 10% FBS was added to the lower chamber. At the end of 24-hour incubation