EXPERIMENTAL CELL RESEARCH 316 (2010) 2402–2413

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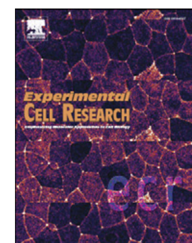

Research Article

Isolation and characterization of a large soluble form of fibronectin that stimulates adhesion, spreading, and alignment of mouse erythroleukemia cells☆

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ARTICLE INFORMATION

Article Chronology:
Received 19 August 2009
Revised version received 25 May 2010
Accepted 30 May 2010
Available online 12 June 2010

Keywords:
Cell adhesion factor
erythroid
erythroleukemia
fibronectin
MEL cells

ABSTRACT

Fibronectin (FN) is a major component of the extracellular matrix which plays important roles in a variety of cellular processes including cell adhesion, and migration. The soluble cellular form of FN has a monomer molecular weight of approximately 250 kDa, and generally exists as a dimer of 500 kDa. We have isolated a different form of soluble FN from mouse breast cancer cell line SC115 conditioned medium (CM) and purified it to homogeneity as evidenced by both native polyacrylamide gel electrophoresis (PAGE) and sodium dodecyl sulfate PAGE. It still exhibits a monomeric form of about 250 kDa while its form in the CM is stable and soluble with an apparent tetrameric molecular weight in the range of 800–1000 kDa. This form of FN is a potent cell adhesion factor (AF) that induces adhesion to polystyrene, elongation, spreading, alignment or "track" formation, and migration of mouse erythroleukemia cells. Column fractions homogeneous for AF protein were able to stimulate 10% cell adhesion at concentrations of 23 ng/ml and 1.9 ng/cm^2. Purified AF induced 50% cell adhesion at 94 ng/ml and 7.5 ng/cm^2. AF also increased the migration of human aortic smooth muscle and vascular endothelial cells. However, this form of FN differs from other forms as it does not bind tightly to either gelatin or heparin. Studies of this AF should shed light on adhesion of cells to extracellular matrix molecules and on cell migration, both of which are critical in several biological processes such as wound healing, metastasis, matrix formation and structure, and organ development.

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☆ In memoriam: This article is dedicated to Bonnie Anderson Bray (1929–2007), a fibronectin researcher.

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Abbreviations: Ab, antibody; AF, adhesion factor; APRT, adenine phosphoribosyl transferase; cFN, cellular FN; CM, conditioned medium; D-MEM, Dulbecco's minimal essential medium; EDTA, ethylene diaminetetraacetic acid; EGTA, ethylene glycol-bis(2-aminoethylether)-N,N,N′N′-tetraacetic acid; ESI-MS, electrospray ionization-mass spectrometry; FBS, fetal bovine serum; FGF, fibroblast growth factor; FN, fibronectin; G-CSF, granulocyte colony-stimulating factor; HAVSMC, human aortic vascular smooth muscle cells; HUVECs, human umbilical vein endothelial cells; IGF-I, insulin-like growth factor-I; IL-6, interleukin-6; LC-MS/MS, liquid chromatography-tandem mass spectroscopy; MEL, mouse erythroleukemia; PDGF, platelet-derived growth factor; pFN, plasma FN; TGF-α, transforming growth factor-α; TNF-α, tumor necrosis factor- α

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doi:10.1016/j.yexcr.2010.05.034

Introduction

Cell adhesion molecules are protein factors that play important roles in numerous cellular processes [1–3]. Some of these processes include tissue repair, embryogenesis, blood clotting, would healing, cell adhesion and cell migration [1–3]. Cell adhesion molecules are located on the cell surface and are involved in binding to other cells and/or to extracellular matrix [1–3]. Cell surface adhesion molecules are also capable of transmitting information from the extracellular milieu to the cell [1–3]. The adhesive characteristics of cells grown in culture are modulated by different cell adhesion factors. FN is a glycoprotein that is an important extracellular adhesion molecule that exists as a soluble cellular disulfide-linked heterodimer or in insoluble fibrils in the extracellular matrix or as a soluble disulfide-linked heterodimer in the plasma [4]. The importance of FN in cell adhesion and migration has been documented in several contexts [4–7].

Mouse erythroleukemia (MEL) cells are erythroid cells transformed by Friend leukemia virus. They were the first vertebrate cells shown to spontaneously undergo differentiation *in vitro* and this erythrodifferentiation can be further augmented by the addition of a variety of inducing agents [8,9]. MEL cells are generally spherical with erythroblastoid morphology and grow in suspension in tissue culture. Interestingly, studies of Demsey and Grimley first reported the derivation of a surface-adherent subline of MEL 175 cells that were originally grown in suspension [10]. This surface-adherent subline of clone MEL 175 represented a stable variant that exhibited “lightly adherent cells” that resulted from a spontaneous mutation during the course of passaging the cells [10]. In another study, Benedetto et al. [11] adapted line MEL 175 to become adherent by culturing it on a layer of fibroblasts. They also showed that the addition of human plasma FN (pFN) increased this adhesion. The cell line that we used in this study, MEL 179 or c179, was also derived from MEL 175 for a different reason that was not related to adhesiveness, but rather as a line deficient in APRT in order to perform chromosome exchange with globin-expressing human cells [12].

During our studies with MEL 179, we observed that 5–30% of the cells were elongated or spindle-shaped. These cells became adherent to the polystyrene tissue culture vessel, and had a fibroblastoid-appearance. We also noted that some of the MEL 179 cells established themselves into straight lines of cells in a “head-to-tail” fashion, in double lines of cells (tracks), or in straight lines of multiple cells (chords of cells). In addition, we also observed that other MEL cell lines that had been cloned in several different laboratories [13–15] also have moderate levels of spontaneously adherent cells. These observations prompted us to undertake studies to identify potential factors responsible for the adherence of MEL 179 cells. We identified an adhesion factor (AF) that was related to FN, but with properties different from other forms of FN. The AF that we characterized is a soluble form of mouse cellular FN that still exhibits a monomeric form of 252 kDa while the native form even after purification to homogeneity remains stable and soluble with an apparent tetrameric molecular weight in the range of 800–1000 kDa. Such a tetrameric form of FN has not been previously identified. This AF potently induces cell migration and cell adherence to polystyrene. However, it differs from other isolated forms of FN not only in its size and activities, but also in its lack of ability to bind tightly to either gelatin or heparin.

Materials and methods

Reagents

Human fibroblast growth factor (FGF-1) was a kind gift of Dr. Mitchell Goldfarb, Mount Sinai Medical Center, NY, human FGF-2 (155 amino acids) was from Collaborative Research, Inc.; human FGF-8b (FGF-8) and human pFN were from Sigma-Aldrich (St. Louis, MO); transforming growth factor α (TGF-α), tumor necrosis factor-α (TNF-α), insulin growth factor-I (IGF-I), and interleukin-6 (IL-6) were obtained from Pepro Tech, Inc. Rocky Hill, NJ; platelet-derived growth factor (PDGF)-AA (shorter form) and PDGF-BB were from Zymogen Laboratories, San Francisco, CA; granulocyte colony stimulating factor (G-CSF) was from AMGEN, Thousand Oaks, CA; gelatin sepharose 4B and heparin sepharose CL-6B were from Amersham Biosciences, Piscataway, NJ; high molecular weight blue dextran was from Sigma-Aldrich, and the high molecular weight native marker kit for native acrylamide gels was from Amersham Biosciences. All other reagents were from Sigma-Aldrich unless otherwise noted.

Cell culture

The MEL cell lines that we used in this study have been described previously: MEL 179 [12], DS-19 and DS19-sc9, a subclone of DS19 [16], 585S [17], the line used by Hug et al. [18], designated here as “Hug cells”, and line 745A [19]. SC115 mouse breast cancer cells [20], HL60 human granulocytic leukemia cells, human breast cancer cells (ZR751, MCF-7, MCF7/Adr, T47-D, and MDA-MB231), K562 human erythroleukemia cells, and human aortic smooth muscle cells (HAVSMC) were obtained from American Type Culture Collection, Manassas, VA, rat secondary aortic smooth muscle cells from Dr. Bin Liu, and human umbilical endothelial cells (HUVECs) were purchased from Lonza Walkersville, Inc.

All murine cells (MEL 179, Hug, mouse breast cancer, and rat secondary aortic smooth muscle cells), unless specifically noted, were maintained in L-glutamine-containing Dulbecco's MEM medium (D-MEM) (Gibco BRL, Gaithersburg, MD) and 10% vol/vol fetal bovine serum (FBS) (HyClone Laboratories, Inc., Logan, UT). DS19 and DS19-sc9 cells were maintained similarly except 15% FBS was utilized. HUVECs were grown in EGM-2 [21] supplemented with the components supplied in the Bullet Kit (Lonza) according to the manufacturer's directions. Other human cells (breast cancer cell lines ZR751, MCF-7, MCF7/Adr, T47-D, and MDA-MB231, K562 human erythroleukemia cells, and HAVSMC human aortic smooth muscle cells) were grown in RPMI 1640 (Gibco) medium containing 10% vol/vol heat-inactivated FBS. SC115 cells were passaged routinely after standard trypsin treatment by seeding at 2×10^6 cells per 10 ml complete medium in petri dishes. All cells were grown at 37 °C in a humidified CO_2 environment. Cell counts were performed in a Z1 particle counter (Beckman Coulter Co., Miami, FL).

CM preparation

Initial CM preparations were obtained from confluent SC115 cultures by collecting the supernatant medium that was passed in aliquots of 5 ml or less through HT Tuffryn membrane 0.2 μm pore-size filters (PALL Life Sciences, Ann Arbor, MI). The supernatant

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