Life Sciences 149 (2016) 138–145

Contents lists available at ScienceDirect

Life Sciences

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

Effect of alpha 2,6 sialylation on integrin-mediated adhesion of breast cancer cells to fibronectin and collagen IV

Ye Yuan [a,b], Larry Wu [c,1], Siqi Shen [d], Shiyong Wu [a,b,*], Monica M. Burdick [c,**]

[a] Edison Biotechnology Institute, Ohio University, Athens, OH, United States
[b] Department of Chemistry and Biochemistry, Ohio University, Athens, OH, United States
[c] Department of Chemical and Biomolecular Engineering, Ohio University, Athens, OH, United States
[d] College of Life Sciences, Capital Normal University, Beijing, China

ARTICLE INFO

Article history:
Received 7 January 2016
Received in revised form 17 February 2016
Accepted 18 February 2016
Available online 20 February 2016

Keywords:
Cell adhesion
Extracellular matrix
Integrins
N-acetylneuraminic acid
Breast neoplasms

ABSTRACT

Aims: To determine the role of sialylation on α5β1 and α2β1 integrins in the regulation of adhesion between breast cancer cells and extracellular matrix (ECM).
Main methods: Static cell adhesion assays were performed to quantify avidity of breast cancer cells to ECM. The effects of sialidases on α2,6 sialylation was assessed by flow cytometry using biotin conjugated *Sambucus nigra* lectin. Lectin affinity assays were used to determine expression of α2,6 sialylated integrins. Cell migration and invasion were investigated by wound healing and transwell invasion assays.
Key findings: α2, α5 and β1 integrins had considerable α2,6 sialylation on MDA-MB-231 cells, whereas signals from MCF-7 cells were undetectable. Cleavage of α2,6 sialylation increased adhesion of MDA-MB-231 cells to ECM, while adhesion of MCF-7 cells was unaffected, consistent with the latter's lack of endogenous α2,6 sialylated surface integrins. Neither surface expression of α2β1 and α5β1 integrins, nor activated β1 integrin, changed in MDA-MB-231 cells after sialidase treatment. However, sialidase treatment did not have significant impact on migration or invasion of MDA-MB-231 cells.
Significance: Cell adhesion is an important early step of cancer metastasis, yet the roles of sialylation in regulating integrin-mediated breast cancer cell adhesion in comparison to migration and invasion are not well-understood. Our data suggest desialylation of α2,6-sialylated integrins increases adhesion, but not migration or invasion, of MDA-MB-231 cells to ECM without altering integrin expression. It should be considered that α2,6 sialylation may play different roles in regulating cell adhesion of different cancer cells when developing potential therapeutics targeting α2,6 sialylation.

Published by Elsevier Inc.

1. Introduction

The avidity of integrins to their ligands is not only affected by surface expression level, but also regulated by glycosylation. Integrins, glycoproteins composed of α and β subunits, are known receptors of extracellular matrix (ECM) [14]. The interactions between integrins and ECM have been shown to mediate diverse physiological functions such as cell adhesion, development and carcinogenesis [6]. As a basic function carried out by integrins, adhesion to ECM on endothelial and stromal cells is thought to participate in cancer cell metastasis by facilitating extravasation of circulating cancer cells [17]. In addition, integrin mediated adhesion also contributes to the resistance of cancer cells to chemotherapeutic drugs [3] and ionizing radiation [12]. The function of integrins is not only regulated by surface protein expression, but is also controlled by signaling pathways, which confer conformation changes between low and high affinity states [4,5].

The alteration of integrin sialylation is also an important regulator of cell adhesion, especially sialylation of β1 integrin. Mitogen-activated protein kinase/ERK kinase (MEK) activation in U937 myeloid cells induced hyposialylation of β1 integrin and increased the avidity of U937 cell adhesion to fibronectin [28]. In contrast, upregulation of ST6Gal-I in metastatic colon cancer cells led to the elevation of α2,6 sialylated β1 integrin, yet also enhanced cell adhesion, migration and metastasis [27,30]. Ras activation increased α2,6 sialylation of β1 integrin of HD3 colonocytes, and desialylation by sialidase inhibited cell adhesion to collagen I [26]. Application of a fluorinated sialic acid analogue has shown great potential in reducing B16F10 melanoma cell adhesion to ECM and tumor growth in mice, which is encouraging for developing drugs targeting hypersialylation in cancer [7]. In light of the importance of α2,6 sialylation of integrins in the regulation of adhesion of these types of cells, we sought to determine the role of α2,6 sialylation of

* Correspondence to: S. Wu, Edison Biotechnology Institute, Ohio University, Athens, OH 45701, United States.
** Correspondence to: M. M. Burdick, Department of Chemical and Biomolecular Engineering, Ohio University, Athens, OH 45701, United States.
E-mail addresses: wus1@ohio.edu (S. Wu), burdick@ohio.edu (M.M. Burdick).
[1] Current address: College of Medicine and Life Sciences, University of Toledo, Toledo, OH, United States.

http://dx.doi.org/10.1016/j.lfs.2016.02.071
0024-3205/Published by Elsevier Inc.

integrins in regulating adhesion and invasion of breast cancer cells to ECM. Our data indicate that integrins are highly α2,6 sialylated on MDA-MB-231 cells but not on MCF-7 cells. Desialylation of integrins increases adhesion of MDA-MB-231 cells to ECM without alteration of integrin expression. Moreover, the desialylation-increased adhesion does not correlate with the migration and invasiveness of the cells.

2. Materials and methods

2.1. Cell culture

The breast cancer cell line MDA-MB-231 was kindly provided by Dr. Jianjian Li (University of California Davis Cancer Center, CA), and the breast cancer cell line MCF-7 was obtained from the American Type Culture Collection (Manassas, VA). MDA-MB-231 cells were cultured in minimal essential medium (MEM) (Corning, Manassas, VA), containing 10% fetal bovine serum (Atlanta Biologicals, Lawrenceville, GA), 1 mM sodium pyruvate, 1× non-essential amino acids (NEAA) and 1% penicillin and streptomycin. MCF-7 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin.

2.2. Antibodies and reagents

Sialidase from *Vibrio cholerae* [1] (VC, broad substrate specificity) was purchased from Roche (Indianapolis, IN). *Clostridium perfringens* sialidase [8,32] (CP, specificity of cleavage: α2,3 > α2,6 sialylation) and *Arthrobacter ureafaciens* sialidase [11,25] (AU, specificity of cleavage: α2,6 > α2,3 sialylation) were purchased from Sigma-Aldrich (St. Louis, MO) and Prozyme (Hayward, CA), respectively. An ECM screening kit containing fibronectin and collagen IV pre-coated strips was obtained from EMD Millipore (Billerica, MA). The following anti-human primary antibodies were purchased from BD Biosciences (San Jose, CA): CD29 (Mab13) mAb, CD15 (HI98) mAb, CD15s (CSLEX1) mAb, and CD49b (12F1) mAb. The following anti-human primary antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA): CD49e (JBS5) mAb, CD49e (H-104) polyclonal antibody, CD49b (H-293) polyclonal antibody and CD29 (N-20) polyclonal antibody. Anti-activated integrin β1 antibody (HUTS-4) was purchased from EMD Millipore (Billerica, MA). Biotin conjugated *Sambucus nigra* lectin (SNA) was obtained from EY labs (San Mateo, CA). Corresponding isotype control antibodies, fluorescein isothiocyanate (FITC) conjugated secondary antibodies, and FITC-conjugated streptavidin were purchased from BD Biosciences. Horse radish peroxidase (HRP) conjugated secondary antibodies were obtained from Santa Cruz Biotechnology. Streptavidin agarose resin was obtained from Thermo Scientific (Rockford, IL).

2.3. Western blotting and lectin affinity assay

For western blotting, MDA-MB-231 and MCF-7 cells were first lysed in 2% NP-40 buffer containing protease inhibitor on ice. Supernatant was collected by centrifugation. Proteins (100 μg) were separated in 8% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) resolving gel and transferred to a nitrocellulose membrane (Pall, Port Washington, NY). After blocking for 1 h with 5% non-fat milk in Tris-buffered saline with Tween (TBS-T) at room temperature, the membrane was incubated with anti-CD49b (H293), anti-CD49e (H-104), or anti-CD29 (N-20) antibodies at 4 °C overnight. After washing with TBS-T three times, the membrane was incubated with corresponding HRP conjugated secondary antibodies for 30 min at room

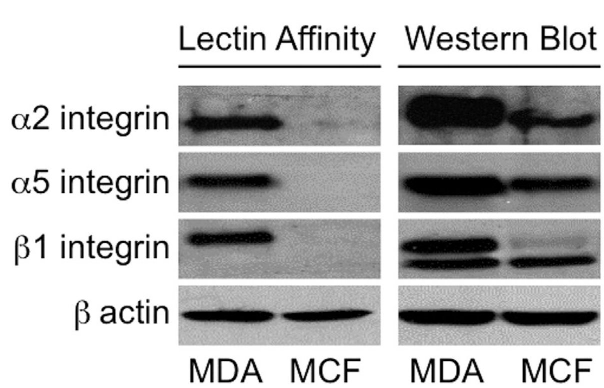

Fig. 1. Sialylation of relevant integrins on MDA-MB-231 and MCF-7 cells. For lectin affinity assay, 300 μg cell lysate of MDA-MB-231 and MCF-7 cells were harvested and incubated with 50 μg/mL biotinylated SNA at 4 °C overnight. Streptavidin agarose beads were then added for additional 4 h incubation at 4 °C. Beads were washed and boiled before loading to the gel. Loading control for lectin affinity assay was collected before adding biotinylated SNA. For western blotting, 100 μg cell lysate was loaded. This figure is representative of 3 independent experiments.

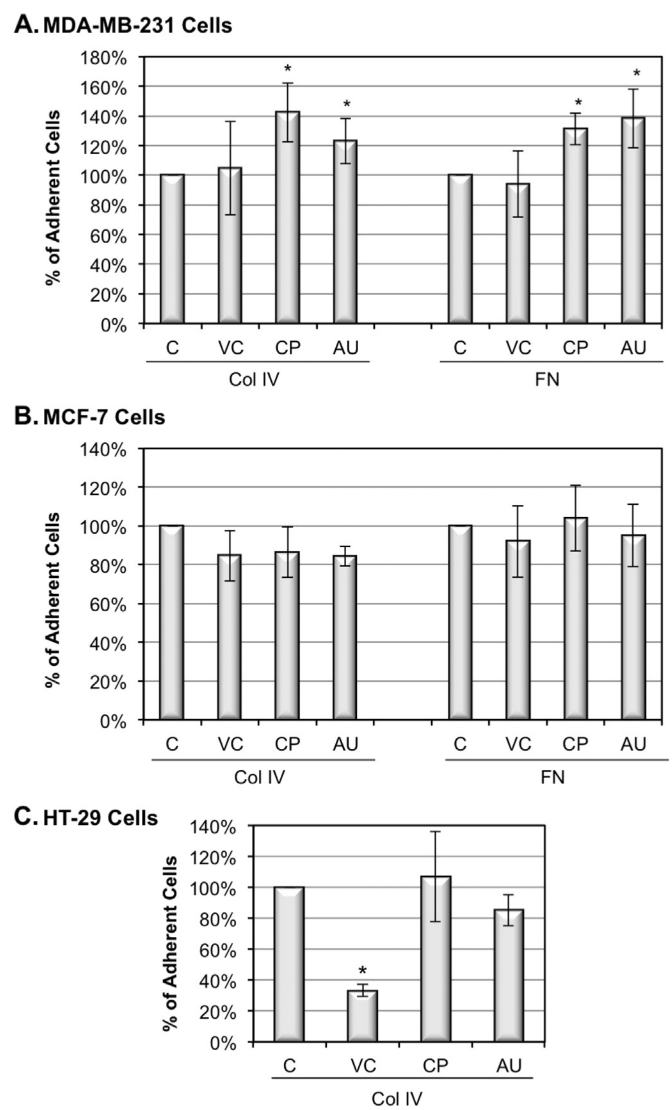

Fig. 2. Effect of sialidase on adhesion of MDA-MB-231 cells, MCF-7 cells and HT-29 cells to ECM. (A) MDA-MB-231 cells, (B) MCF-7 cells and (C) HT-29 cells were harvested and treated with 0.1 U/mL *V. cholera* (VC), *C. perfringens* (CP), and *A. ureafaciens* (AU) sialidase in DPBS for 30 min at 37 °C. Cells were seeded into each well of fibronectin (FN) or collagen IV (Col IV) pre-coated strips. Cells were washed and stained after 30 min incubation at 37 °C. Absorbance was normalized to the control cells incubated in DPBS without sialidase. Duplicate samples were prepared in each experiment. Data shown are the means ± SD, n = 5. *: $p < 0.05$ versus untreated control group.