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# Anti-adhesive functions of CD43 expressed on colon carcinoma cells through the modulation of integrins

Weon Seo Park [a,b], Hae Jung Kim [a], Geon Kook Lee [b], Hyeon S. Son [c,d], Youngmee Bae [e,*]

[a] Hematologic Malignancies Branch, National Cancer Center, 809 Madu-dong, Ilsan-gu, Goyang, Gyeonggi 411-769, Republic of Korea
[b] Department of Pathology, National Cancer Center, 809 Madu-dong, Ilsan-gu, Goyang, Gyeonggi 411-769, Republic of Korea
[c] Laboratory of Computational Biology & Bioinformatics, Institute of Health and Environment, Graduate School of Public Health, Seoul National University, 599 Gwanangno, Gwanak-gu, Seoul 151-742, Republic of Korea
[d] Interdisciplinary Graduate Program in Bioinformatics, College of Natural Science, Seoul National University, 599 Gwanangno, Gwanak-gu, Seoul 151-742, Republic of Korea
[e] Department of Parasitology and Tropical Medicine, Seoul National University College of Medicine and Institute of Endemic Diseases, Seoul National University Medical Research Center, Seoul 110-799, Republic of Korea

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ABSTRACT

CD43 has conflicting roles in both pro- and anti-adhesive function in cell-to-cell adhesion in hematopoietic cells. We examined the role of CD43 glycoprotein in a colorectal carcinoma cell line. We expressed human CD43 antigen on HT-29 cells, a colon adenocarcinoma cell line, and compared the adhesion to the extracellular matrix with that of mock-transduced cells in vitro. CD43 expression inhibited the adhesion to extracellular matrix, such as collagen type IV and laminin. As the expression of β1 integrin was downregulated in CD43-expressing HT-29 cells, the anti-adhesive effect of CD43 might be implicated in its expression. Our findings suggest that the anti-adhesive function of CD43 in colon carcinoma cells plays a role in the tumorigenesis and metastasis of colorectal carcinoma cells.



## Introduction

CD43 (also known as sialophorin or leukosialin) is a cell-surface glycoprotein that is expressed in a variety of hematopoietic lineage cells, including T lymphocytes (T cells) (Carlsson and Fukuda, 1986; Shelley et al., 1990). The extracellular domain of CD43 contains more than 80 serine or threonine moieties, most of which are O-glycosylated (Carlsson and Fukuda, 1986; Remold-O'Donnell et al., 1987; Shelley et al., 1990). This abundant glycoprotein seems to play multiple roles in regulating leukocyte migration and activation. The CD43 molecule has appeared to have anti-adhesive function in various experimental and physiological cellular interactions (Cyster et al., 1991; Remold-O'Donnell et al., 1986). CD43 has a rod-like structure that is thought to extend 45 nm from the cell membrane (Sperling et al., 1998), and strong anionic charges due to the extensive sialylation of attached O-glycans. By contrast, it has also been suggested that the CD43 molecule plays a role in pro-adhesive function (Brown et al., 1981; Fabbi et al., 1999; Shelley et al., 1989).

Only a few reports have examined the expression of CD43 antigen outside hematopoietic cell lineages. Some have unambiguously demonstrated expression of CD43 in the human colon carcinoma cell line COLO 205 at the protein and mRNA levels (Wong et al., 1990). Some researchers found that CD43 was expressed in all colon adenomas and in about 50% of adenocarcinomas, suggesting an important role for CD43 in colon tumorigenesis (Sperling et al., 1995). They suggested that aberrant CD43 expression during colon tumorigenesis could impair apoptosis and this might be involved in the development of colon cancer (He and Bevan, 1999; Sperling et al., 1995).

The β1 integrin subunit associates with multiple α-subunits to form transmembrane adhesion receptors for extracellular matrix proteins, including collagen, fibronectin, vitronectin, and laminin (Rocca et al., 1996). Once present at the cell surface, mature integrins anchor the plasma membrane to the actin cytoskeleton and promote cell signaling (McEvoy et al., 1997a; Park et al., 1991). Altered cell surface integrin expression may be particularly important in cancer, affecting various aspects of cancer metastasis (McEvoy et al., 1997b; Stockton et al., 1998).

Based on these findings, we examined the role of CD43 in colon cancer adhesion in vitro. We found that CD43-expressing cells detached from the culture plate and their capacity to adhere to extracellular matrix, such as laminin and collagen IV, was decreased. Adhesion to collagen type IV is inhibited by the synthetic peptide DGEA. The CD43-expressing colon cancer cell line showed decreased β1 integrin on the cell surfaces. Our findings suggest that high expression of CD43 on the cell surface inhibits cellular adhesion to laminin and collagen IV, and this inhibition might be caused by decreased expression of β1 integrin.

* Corresponding author at: Department of Parasitology and Tropical Medicine, Seoul National University College of Medicine 28 Yongon dong, Chonro gu, Seoul 110-799, Korea. Fax: +82 2 740 8345.
*E-mail address:* ymbae@snu.ac.kr (Y. Bae).

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## Materials and methods

### *Antibodies and chemicals*

A monoclonal antibody (mAb) against CD43 was produced in our laboratory, as previously described (Ardman et al., 1992). MAbs against CD44, CD49a, and CD29 were purchased from DiNonA (Seoul, Korea). Laminin, poly-L-lysine, and collagen type IV were purchased from Sigma (St. Louis, MO).

### *Cell culture*

HT-29 cells (American Type Cell Collection, HTB-38, VA) were cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma Chemicals, St. Louis, MO) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mM glutamine, 100 μg/ml penicillin, and 50 U/ml streptomycin at 37 °C in a humidified 5% $CO_2$ incubator.

### *Transfection and retroviral transduction*

The coding sequence for the human *CD43* gene was amplified from a CD43 cDNA plasmid (recloned, provided by Dr. C. S. Shelley, MGH at Harvard Medical School) and used to construct the expression vector pRetro-CD43. 293GPG packaging cells, obtained as a gift from Dr R. C. Mulligan (Whitehead Institute, MIT, Cambridge), were used to produce retrovirus expressing human CD43. The 293GPG cells were transfected using the calcium phosphate precipitation method. After incubating the 293GPG cells with the coprecipitate of calcium phosphate and DNA encoding CD43 for 16–20 h, the medium was replaced with 5 ml of fresh solution and the cells were incubated for another 24 h. HT-29 cells were plated onto six-well plates the day before transduction. The viral supernatant, harvested from the transfected 293GPG cells and filtered through a 0.45-μm filter to remove cell debris, was add to the HT-29 cells.

### *Immunoblotting*

Cell extracts were fractionated by SDS-polyacrylamide gel electrophoresis (10%) under reducing conditions (Laemmli, 1970). The separated polypeptides were transferred electrophoretically to nitrocellulose paper (Hybond, Amersham, Little Chalfont, UK). The transferred membranes were incubated with primary antibodies (purified antibody at 1 μg/ml) that recognize CD43 epitopes, and then washed extensively. Immune complexes were tagged using goat anti-mouse antibodies conjugated with horseradish peroxidase (HRP), and visualized using an Enhanced Chemi-Luminescent kit (ECL, Amersham).

### *Immunofluorescence staining and flow cytometry*

Cells were stained with each primary mAb on ice for 30 min followed by secondary Ab (FITC-conjugated goat anti-mouse). The cells were washed with cold PBS twice in every step. In some experiments, dead cells were excluded by incubating cells with propidium iodide (PI) and then electronically gating PI-positive cells from the analysis. Flow cytometry was performed using a Becton Dickinson FACSCalibur. For intracellular staining, the cultured cells were harvested, fixed with 2% paraformaldehyde, and permeabilized with 0.3% (w/v) saponin in PBS. FITC-conjugated anti-CD29 was added. After incubation for 30 min, the cells were washed with permeabilization buffer and analyzed by flow cytometry.

### *Cell adhesion and inhibition assays*

Adhesion substrates were prepared by coating flat-bottomed 96-well microtiter plates (Nunclone; Nunc, Rockville, Denmark) overnight at 4 °C with 50 μl of collagen type IV and laminin at the indicated concentrations. The coated wells were subsequently saturated with 1% BSA in PBS for 1 h and then washed with PBS to block nonspecific adhesion. The human colon adenocarcinoma cell line HT29 was obtained in a single-cell suspension by treating subconfluent cell monolayers with 0.5 mM EDTA in PBS. After centrifugation, the cells were washed twice

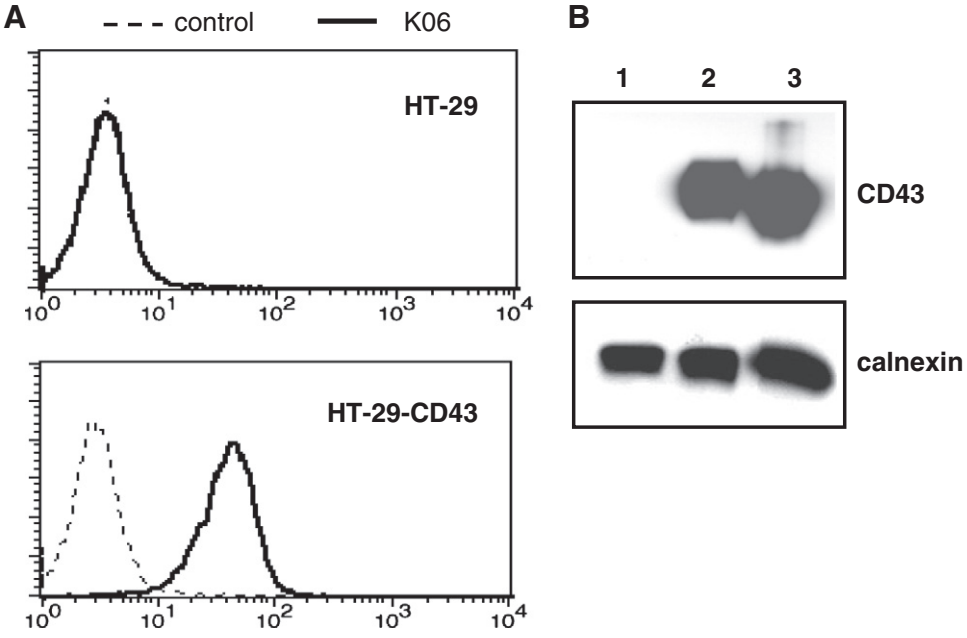


**Fig. 1.** CD43 expression on the cell surface of HT-29 colon adenocarcinoma cells. The HT-29 and CD43-transduced (HT-29-CD43) cells were stained with the anti-CD43 mAb K06. The CD43-transduced cells expressed CD43 on the cell surface well. These data are from three experiments. The mock-transduced cells did not express CD43. The CD43 expressed in the HT-29 cells showed slower mobility on SDS-PAGE than that in human thymocytes (B; lane 1, HT-29; 2, CD43-transduced HT-29; 3, human thymocytes). Parallel calnexin antibody staining was used as a control.