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Intercellular adhesion molecule-1 mediates murine colon adenocarcinoma invasion

*Kenton Howard, BS, Karen K. Lo, MD, Lihua Ao, BS, Fabia Gamboni, PhD, Barish H. Edil, MD, Richard Schulick, MD, and Carlton C. Barnett Jr., MD**

Department of Surgery, Denver Health Medical Center, University of Colorado, Denver Campus, Denver, Colorado

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ABSTRACT

Background: Intercellular adhesion molecule-1 (ICAM-1) modulates cell–cell adhesion and is a receptor for cognate ligands on leukocytes. Upregulation of ICAM-1 has been demonstrated in malignant transformation of adenomas and is associated with poor prognosis for many malignancies. ICAM-1 is upregulated on the invasive front of pancreatic metastases and melanomas. These data suggest that the upregulated ICAM-1 expression promotes malignant progression. We hypothesize that the downregulation of ICAM-1 will mitigate tumor progression.

Methods: Mouse colon adenocarcinoma cells (MC38) were evaluated for the expression of ICAM-1 using Western immunoblot analysis. Short hairpin RNA (shRNA) transduction was used to downregulate ICAM-1. Tumor invasion determined *via* a modified Boyden chamber was used as a surrogate of tumor progression examining MC38 cells, MC38 ICAM-1 knockdowns, and MC38 transduced with vehicle control. The cells were cultured in full media for 24 h and serum-starved for 24 h. A total of 5×10^4 cells were plated and allowed to migrate for 24 h using full media with 10% fetal bovine serum as a chemoattractant. Inserts were fixed and stained with crystal violet. Blinded investigators counted the cells using a stereomicroscope. Statistical analysis was performed by analysis of variance with Fischer protected least significant difference and a *P* value of <0.05 was considered statistically significant.

Results: ICAM-1 was constitutively expressed on MC38 cells. Transduction with anti–ICAM-1 shRNA vector downregulated ICAM-1 protein expression by 30% according to the Western blot analysis ($P < 0.03$) and decreased ICAM-1 messenger RNA expression by 70% according to the reverse transcription–polymerase chain reaction. shRNA knockdown cells had a significant reduction in invasion >45% ($P < 0.03$). There were no significant differences between the invasion rates of MC38 and MC38 vehicle controls.

Conclusions: Downregulation of ICAM-1 mitigates MC38 invasion. These data suggest that targeted downregulation of tumor ICAM-1 is a potential therapeutic target.

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* *Corresponding author*. Department of Surgery, Denver Health Medical Center, University of Colorado, Denver Campus, 777 Bannock Street, Denver, CO 80204. Tel.: +1 303 436 5402; fax: +1 303 436 6572.

E-mail address: carlton.barnett@ucdenver.edu (C.C. Barnett).

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1. Introduction

Intercellular adhesion molecule-1 (ICAM-1), a member of the immunoglobulin superfamily, is a cell surface glycoprotein expressed by multiple cells, but prominently on endothelia [1]. Previous studies have shown that ICAM-1 plays an important role in neutrophil adhesion, migration, and facilitation of respiratory burst [2]. Of particular importance is the interaction between the neutrophil membrane protein, β2-integrin, and ICAM-1 on cell membrane surfaces [2]. More recently, it has been observed that ICAM-1 is highly expressed on the leading edge of many types of neoplastic cells, such as gastric carcinoma, melanoma, and pancreatic cancer [3,4]. In the case of gastric and pancreatic carcinomas, increased circulating levels and increased surface expression of ICAM-1 on tumor cells are associated with a poor prognosis [3,4].

It has previously been shown that binding ICAM-1 leads to structural changes in the cytoskeleton of endothelia that separate tricellular junctions [5]. Furthermore, the tumor microenvironment contains ample binding sites for multiple adhesion molecules to augment cancer progression [6].

Based on these data, we hypothesize that tumor-associated ICAM-1 mediates tumor progression in the absence of immune cells. To test this hypothesis, ICAM-1 was downregulated using short hairpin RNA (shRNA) and immune cell–independent tumor invasion was examined *in vitro*.

2. Materials and methods

2.1. Cell culture and reagents

The murine colon adenocarcinoma cell line MC38 was obtained from the Division of Cancer Treatment and Diagnosis Tumor Repository, National Cancer Institute, Frederick, MD, in association with the National Institutes of Health. All culture media were purchased from Life Technologies Corporation, Grand Island, NY. Cells were grown and maintained at 37°C in 5% carbon dioxide and RMPI-1640 containing 10% fetal bovine serum and 1% penicillin–streptomycin. During serum starvation, the cells were incubated in Roswell Park Memorial Institute-1640 containing 0.5% fetal bovine serum and 1% penicillin–streptomycin.

2.2. Western blot analysis

MC38 cells were plated in six-well culture plates with 200,000 cells/well in full media for 24 h and then serum-starved for 12 h before lysis. Cells were lysed with mammalian protein extraction reagent lysis buffer (Thermo Fisher Scientific, Rockford, IL) and mixed in a 3:1 ratio with a Laemmli buffer/BME solution. Cell lysate protein density was equalized using mass spectrometry before beginning the immunoblotting. Proteins were resolved using 4%–20% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Bio-Rad Laboratories, Hercules, CA). The proteins were transferred to nitrocellulose membranes, blocked, and probed with goat anti–ICAM-1 monoclonal antibody (mAb) as well as with rabbit anti–GAPDH mAb (Santa Cruz Biotechnology, Dallas, TX). GAPDH was used to assess overall protein density of the cell lysates. Blocking solution contained 5% nonfat milk in 1× phosphate-buffered saline (PBS) with 0.1% Tween-20. The ICAM-1 antibodies were diluted in 5% nonfat milk and 1× PBS with 0.1% Tween-20. After incubation with horseradish peroxidase–conjugated secondary antibody (Santa Cruz Biotechnology), signal was detected using West Dura chemiluminescent substrate (Thermo Fisher Scientific).

2.3. Short hairpin RNA knockout of ICAM-1

MC38 cells were plated into six-well culture plates in full media for 24 h before viral infection (day 1). On day 2, the media were removed and replaced with full media containing polybrene (8 μg/mL). Lentiviral particles containing the shRNA with the ICAM-1 knockdown plasmids were added to half of the wells, whereas other wells received the lentiviral particles containing plasmids with nontargeting control, which will serve as a vehicle control (all lentiviral particles were acquired from Functional Genomics Facility, University of Colorado at Boulder, Boulder, CO). The lentiviral particle used to knockdown ICAM-1 was the single clone (TRCN0000065923) that has been validated by Sigma-Aldrich (St. Louis, MO), whereas the vehicle control was a nontargeting control (SHC002). Cells were incubated overnight, then the media containing polybrene and lentiviral particles were replaced with full media. Starting on day 4, full media containing puromycin (5 μg/mL) were used. The puromycin in this concentration kills untransduced MC38 cells, allowing selection of stable MC38 viral transductants. ICAM-1 protein density was assessed in these two cell groups alongside MC38 control cells according to the Western blot analysis protocol explained previously.

2.4. Real-time polymerase chain reaction

Untransduced MC38 cells, MC38 cells transduced with vehicle control, and MC38 cells transduced with ICAM-1 knockdown were grown in six-well plates at a density of 200K and 300K cells/well with full media, overnight. Messenger RNA (mRNA) was collected using Qiagen (Valencia, CA) mini RNA prep kit. All cells were analyzed for mRNA expression of ICAM-1 using quantitative real-time reverse transcriptase–polymerase chain reaction as previously described (Abcam, Cambridge, MA). mRNA for ICAM-1 was normalized to mRNA for GAPDH in the same samples (Abcam) and untransduced MC38 cells were designated a relative value of 1.

2.5. Cell invasion assay

The invasion chamber apparatus we used was BD BioCoat Matrigel Matrix (BD Biosciences, San Jose, CA). The three types of cells were plated for culture in six-well plates, according to the cell culture protocol described previously: an MC38 control cell group, a lentiviral MC38 vehicle control group, and the MC38 cell group with shRNA ICAM-1 knockdown. The cells were cultured in full media for 24 h and then serum-starved for 24 h in preparation for introduction into a 24-well Boyden-style chamber assay. A total of 5×10^4 cells were

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