Inhibition of Adhesion, Invasion, and Metastasis by Antibodies Targeting CEACAM6 (NCA-90) and CEACAM5 (Carcinoembryonic Antigen)

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Abstract
CEACAM5 and CEACAM6 are overexpressed in many cancers and are associated with adhesion and invasion. The effects of three monoclonal antibodies targeting different epitopes on these antigens (NH2-terminal [MN-3] and A1B1 domains [MN-15] shared by CEACAM5 and CEACAM6 and the A3B3 domain [MN-14] restricted to CEACAM5) were evaluated in migration, invasion, and adhesion assays in vitro using a panel of human pancreatic, breast, and colon cancer cell lines, and in the GW-39 human colon micrometastasis model in vivo. MN-3 Fab' and MN-15 Fab' were both effective at inhibiting cell migration. MN-15 Fab' treatment inhibited invasion, reducing cell penetration through an extracellular matrix (ECM). MN-3 Fab' also decreased invasion but was less effective than MN-15 Fab' in four of five cell lines. All three monoclonal antibody (mAb) Fab's decreased adhesion of tumor cells to endothelial cells by 49% to 58%. MN-15 Fab' but not MN-3 or MN-14 Fab's induced a decrease in adhesion of three of six cell lines to the ECM protein, fibronectin, but adhesion to vitronectin, laminin, collagen-I, and collagen-IV was not affected. In vivo studies showed that treatment with MN-3 Fab' or MN-15 Fab' of mice implanted with GW-39 human colon cancer cells increased their survival (P < 0.025 and P < 0.01, respectively). These studies show that antibody Fab's that target either CEACAM5 or CEACAM6 affect cell migration, cell invasion, and cell adhesion in vitro, and that MN-15 and MN-3 Fab's have antitumor effects in vivo, resulting in improved survival of mice with metastases. Thus, blocking the N and A1B1 domains of CEACAM5/CEACAM6 can impede the metastatic process. (Cancer Res 2005; 65(19): 8809-17)

Introduction
The eradication of metastatic disease is crucial for achieving survival in most patients with cancer. The metastatic process consists of a series of sequential steps, including invasion of extracellular matrix (ECM), extravasation into vessels, transport in the circulation, adhesion to endothelial cells in a new tissue, extravasation through the vessel wall, and migration and proliferation in response to organ-specific factors at the new site (1). This article deals with the development of an antibody-based therapeutic approach to impede metastasis.

Antigenic sites on CEACAM5 and CEACAM6 have been characterized, and panels of antibodies recognizing specific epitopes have been generated (32, 33). Three subdomains in the N region that are required for intercellular homotypic adhesion have been identified by site-directed deletions and point mutations (34). Peptides have been developed to these regions in an effort to block adhesion (35). The concentration of peptide required to block cell aggregation was 1 mg/mL or a 25-fold higher molar concentration than the complete antibody, despite the predicted
greater penetration of a smaller peptide. This inefficient dose requirement could be due to instability of the small peptide or due to its low affinity. We postulated that using monovalent antibody fragments would be a more effective approach for targeting these domains, because stability of the mAb will likely be greater and the affinity higher than corresponding peptides. Our studies have primarily been done with monovalent fragments of the antibodies of interest, rather than intact IgGs, because the single binding arm of the Fab will prevent complexation of tumor cells, which might occur with a bivalent IgG. In addition, it allowed us to study the efficacy of a CEACAM5 or CEACAM6 on a panel of tumor cell lines and have determined the effects of several antibodies targeting different epitopes of CEACAM5 and CEACAM6 for their ability to affect tumor cell migration, invasion, and cell adhesion in vitro. We have also evaluated the therapeutic potential of these antibody Fabs in controlling metastasis and survival of mice bearing a CEACAM5/CEACAM6-expressing human colonic carcinoma.

Materials and Methods

Antibody production. MN-15 binds to the A1B1 domain (Gold group 4) and MN-3 binds to the N domain (Gold group 3) found on both CEACAM5 and CEACAM6. MN-14 binds to the A3B3 domain (Gold group 3) only found on CEACAM5 (ref. 36; Fig. 1). These antibodies and their Fab' fragments, including the nonspecific Ag8 Fab' and anti-o-fetoprotein IgG, were supplied by Immunomedics, Inc. (Morris Plains, NJ). MN-3 and MN-15 were used as murine mAbs, whereas MN-14 was included in its humanized form, hMN-14 or labetuzumab (37).

Cell culture. All cell lines were maintained in monolayer culture having RPMI 1640 supplemented with 10% fetal bovine serum (FBS) plus 100 units/mL penicillin, 100 units/mL streptomycin, 4 mmol/L glutamine, and 1% nonessential amino acids and grown at 37°C in 95% air and 5% CO2. Cells were harvested using 1% trypsin and counted by hemocytometer. Viability was determined by trypan blue exclusion.

CEACAM5 and CEACAM6 expression. Cells were harvested from culture and aliquoted into FACScan tubes containing 2 mL Dulbecco's PBS with 0.2% sodium azide and 1% appropriate blocking serum. Cells were incubated with 10 μg/mL of hMN-14 anti-CEACAM5 in PBS (+ NaNO3 + 1% blocking serum) for 1 hour on ice, pelleted (1,440 rpm for 5 minutes), and supernatant decanted. Some tubes were then incubated with 10 μg/mL murine MN-15 IgG or MN-3 IgG (to determine CEACAM6 expression) for 1 hour on ice and pelleted. Neither MN-15 nor MN-3 bind CEACAM5 once MN-14 is bound so that only CEACAM6 is detected by MN-15 or MN-3. Second antibody conjugated with FITC (FITC-goat-anti-human for CEACAM5 determination and FITC-goat-anti-mouse for CEACAM6 determination) was added (1:500 secondary antibody in PBS + NaNO3 + 1% blocking serum) for 30 minutes on ice in the dark. Cells were pelleted, washed twice with 2 mL ice-cold PBS + NaNO3 and resuspended in 1.5% parafomaldehyde. Fluorescence-1 was read on a Becton Dickinson (San Jose, CA) flow cytometer. Percentage of cells that were positive and mean channel fluorescence (MCF) were recorded. CEACAM5 and CEACAM6 expression on GW-39 tumor xenografts was determined by immunohistochemistry using a similar approach of hMN-14 followed by biotinylated GAH to detect CEACAM5 and preincubation with hMN-14 followed by mMN-15 or mMN-3 and biotinylated GAm second antibody to detect CEACAM6 on paraffin sections.

Spontaneous migration assay. Glass coverslips were placed in 100 × 15 mm Petri dishes and UV sterilized overnight. Suspensions of cancer cells (2 × 10^5 cells/mL) were prepared from 80% to 90% confluent monolayers. Cells were plated on each coverslip and incubated for 2 to 5 days to reach 70% to 80% confluence. Two diagonal cell-free paths (“wounds”) were created by dragging a sterile yellow pipette tip across the surface. Monolayers were rinsed several times to remove floating cells and 4 mL of fresh medium were added back in the absence or presence of antibody IgG or Fab' (10 μg/mL) and incubated at 37°C. After 18 to 24 hours, the medium was removed and coverslips stained with 1 mL Wright-Giemsa for 1 minute. The stain was washed off with distilled water, air-dried, and mounted onto slides with Cytoseal 60. Repopulation of the wound space was evaluated by counting the number of cells that migrated into the wound area in 10 representative fields. Regions of migration were photographed for documentation (38, 39).

Endothelial cell adhesion assay. Human umbilical vein endothelial cells (HUVEC; Cambrex, San Diego, CA.) were grown in collagen-coated dishes in EGM Media in a humidified atmosphere with 5% CO2 at 37°C. At passages 2 to 5, cells were plated at a density of 4 × 10^4 cells per well in 96-well plates 24 hours before the assay. Interleukin-1β (IL-1β, 1 ng/mL; Hoffmann-La Roche, Nutley, NJ) was added 4 hours before the assay. At the start of the study, the medium with the IL-1β was removed and fresh DMEM with 1% bovine serum albumin (BSA) added and incubated for 30 minutes. Fresh medium without antibodies or with 10 μg/mL of MN-15 Fab', MN-3 Fab', or Ag8 Fab' was added. Tumor cells (1 × 10^6 cells/mL) prelabeled overnight with ^3H-thymidine (100 μl per well using 1.0 μCi/μL) were added to HUVEC cultures and incubated for 30 minutes at 37°C with rotation in medium with 20% FBS. Samples were washed thrice with PBS to remove unattached cells. Attached cells were solubilized with 0.1 N NaOH and radioactivity was measured in a liquid scintillation counter. The cpm attached/total cpm added (attaching potential) was determined.

Adhesion to extracellular matrix proteins assay. The assay was done using the Cytomatrix screening kit from Chemicon (Kit #ECM205, Temecula, CA). The kit contains 96-well plates with strips coated with fibronectin, vitronectin, laminin, collagen-I, or collagen-IV. Subconfluent cell cultures were used for these studies. Cells (1 × 10^5 cells/mL) were seeded onto coated substrate and incubated at 37°C for 1 hour in a CO2
incubator in PBS containing Ca\textsuperscript{2+}/Mg\textsuperscript{2+} (200 µL per well). Adherent cells were fixed and stained. The plate was washed to remove unadhered cells and stained with 100 µL per well of MTS (Cell Titer Aq 96, Promega, Madison, WI) for 5 minutes at room temperature. Excess stain was removed using absorbance readings (A\textsubscript{570 nm}).

**Collagen-based invasion assay.** The assay was done using Chemicon (Kit #ECM551). Tumor cells at 80% confluence and were serum-starved for 18 to 24 hours before the assay was used. Cells were harvested with 5 mL of 2 mmol/L EDTA/PBS per 100-mm dish and incubated at 37°C for 5 to 15 minutes. Cells were collected into 10 to 20 mL of quenching medium (serum-free DMEM containing 5% BSA) to inactivate trypsin/EDTA from the harvesting buffer. Cells were pelleted, resuspended in quenching medium (1 × 10\textsuperscript{6} cells/mL), and appropriate antibody Fabs were added to individual cell aliquots. Cell invasion potential was determined as follows. Baseline invasion and invasion in the presence of a chemoattractant (10% FBS) were measured after a 72-hour incubation period at 37°C in a 5% CO\textsubscript{2} incubator. The bottom side of the collagen-coated polycarbonate membrane insert of the invasion chamber was placed in 400 µL of cell stain for 20 minutes at room temperature and washed. The dye was extracted and transferred into a 96-well microtiter plate for colorimetric measurements (A\textsubscript{560 nm}).

**In vivo therapy studies.** Female athymic nude mice (6 to 8 weeks old) were purchased from Taconic (Germantown, NY). Therapy studies were done using our CEACAM5+/CEACAM6+ GW-39 intrapulmonary micro-metastasis model, GW-39iv (40–42). The GW-39 human colonic carcinoma has been maintained as a serially transplanted signet-ring cell cancer line since 1966 (42). Stock s.c. GW-39 tumors grown in nude mice were used to

### Table 1. CEACAM5 and CEACAM6 expression in tumor cell lines

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Abbreviation: ND, not determined.
Results

CEACAM5 and CEACAM6 expression in cell lines. Flow cytometric analysis of CEACAM5 and CEACAM6 expression in a panel of 31 to 33 commonly used solid tumor cell lines revealed that only 6 of 29 (20.7%) expressed significant amounts of CEACAM5, whereas 16 of 30 (53.3%) lines were positive for CEACAM6 (Table 1). Two cancer cell lines were CEACAM5+/CEACAM6+ (Moser and LNCAP), four were CEACAM5+/CEACAM6+, and 12 were CEACAM5−/CEACAM6+. The CEACAM6+ cell lines included 7 of 10 breast cancers, one of four ovarian cancers, three of four colon cancers, three of four pancreatic cancers, one of six prostate cancers, and two of four non–small cell lung cancer lines. Many of these tumor lines had >95% of cells expressing the CEACAM6 antigen and, in some cases, expression was very high (MCF-7, ZR-75-30, 702 for BxPC3, and 476 for CaPAN-1). In contrast, the most positive CEACAM5-expressing lines had <60% of cells expressing the antigen, with a MCF-7.12.

Effect of anti-CEACAM5 and anti-CEACAM6 antibodies on cell migration. Using the wound-healing assay, we assessed cell migratory activity in vitro in a panel of cell lines. LS174T and HT-29 cell lines showed the most migratory activity, whereas ZR75-30, MCF-7, and BxPC3 cells showed very little migration (results not shown). Compared with an irrelevant antibody, MN-3 and MN-15 intact IgG and Fab' all reduced migration in both cell lines. In HT-29, the number of migrating cells per field decreased from 14.9 ± 9.1 to 6.1 ± 0.9 and 3.7 ± 1.6 cells with MN-3 IgG and Fab', respectively, and to 5.5 ± 1.8 and 5.4 ± 0.8 with MN-15 IgG and Fab', respectively (P < 0.01 for all comparisons of mAb treatment with untreated cells; n = 10 for each arm; Fig. 2). In LS174T Studies, untreated cells had 38.8 ± 10.9 migrating cells per field, whereas cells treated with MN-3 IgG or Fab' had 21.4 ± 5.2 and 18.1 ± 1.6 migrating cells, respectively (P < 0.001 versus untreated), and cells treated with MN-15 IgG or Fab' had 20.3 ± 0.1 and 21.6 ± 1.2 migrating cells, respectively (P < 0.001 versus untreated). Thus, blocking either the N or A1B1 domain of CEACAM6 with either an intact IgG or a monovalent Fab inhibited migration in vitro.

Effect of MN-15 and MN-3 on tumor cell adhesion to endothelial cells. Because both CEACAM5 and CEACAM6 are known to have an adhesion role, we evaluated whether blocking these antigens reduces adhesion to endothelial cells. In the CEACAM5−/CEACAM6− MCA38 murine colon cancer line, neither MN-3 Fab' targeting the N domain nor MN-15 Fab' targeting the A1B1 domain of CEACAM6 had an effect on tumor cell–endothelial cell adhesion (Fig. 3). In contrast, both antibodies reduced endothelial cell binding of MCA38ce (a human CEA-targeted line) from 11.68 ± 0.77% to 6.42 ± 2.1% (MN-3) and 5.53 ± 1.15% (MN-15), being significant (P < 0.05) for both Fab's. Both antibodies induced a 49% to 58% adhesion-inhibition in four other cell lines (P < 0.01 for MN-3 Fab' on MCF-7, HT-29, and BT-20; P < 0.02 for MN-15 on the same three lines; and P < 0.05 for both Fab's on Moser cell adhesion to endothelial cells). An isotype-matched irrelevant antibody Fab' (Ag8) did not affect tumor cell–endothelial cell adhesion nor did the MN-14 anti-CEA Fab'. The magnitude of the antiadhesion effect did not seem strictly correlated with the amount of CEACAM5 or CEACAM6 expressed, because MN-15 Fab' resulted in a greater decrease in adhesion in HT-29 cells (48%) than with MCF-7 cells (41%) that express much more CEACAM6.

Effect of anti-CEACAM5 and anti-CEACAM6 antibodies on tumor cell adhesion to extracellular matrix proteins. In addition to tumor cell binding to endothelial cells, these cells can also bind ECM proteins. The extent of tumor cell binding to ECM proteins varies among different cell lines. MCF-7 bound well to four of five proteins (A500 nm > 1.1) except laminin (A500 nm = 0.2 ± 0.04), whereas ZR75-30 attached weakly to all five ECM proteins evaluated (A500 nm < 0.45). MDA-468 bound quite well to collagen-I and collagen-IV (A500 nm = 1.25 ± 0.07 and 0.97 ± 0.03, respectively) but not as well to fibronectin, vitronectin, or...
on the day after transplantation. This design simulates the effect of
properties, we pretreated GW-39 tumor cell suspensions with each
native effects yet showed significant anti-invasive and antiadhesive
immunohistochemistry (Fig. 6, 39 expresses both CEACAM5 and CEACAM6, as shown by
tumor cell invasion in vitro in five cell lines that expressed CEACAM5, CEACAM6, or both antigens. MN-14
anti-CEA (CEACAM5) had no effect on tumor cell invasion in most
cell lines. MDA-231 expresses neither antigen, and its invasion was
not reduced by either MN-3 or MN-15 IgG or Fab’. For the five
antigen-positive lines, both the intact IgG and the monovalent Fab’
given an antibody were equally effective. For example, MN-15
Fab’ reduced cell invasion of LS174T, MCF-7,ZR75-30, BxPC3, and
CoPan-1 cells by 30% (P < 0.02), 77% (P < 0.01), 49% (P < 0.01), 44% (P < 0.01), and 73% (P < 0.02), respectively. The effect of MN-3 Fab’
on the same five lines was a reduction in invasion of 3% (P = NS),
47% (P < 0.01), 59% (P < 0.01), 0% (P = NS), and 55% (P < 0.05),
respectively. Thus, the A1B1 domain of CEACAM6 seems to be
a more important target than the N domain for the process of tumor
cell invasion.

Effect of MN-3, MN-15, and MN-14 Fabs on tumor cell invasion. Specific invasion in response to FBS as a chemo-
attraction was 1.9-fold (LS174T) to 7.4-fold (BxPC3) higher than in the absence of FBS (Fig. 5). MN-15 Fab’ was more effective than
MN-3 Fab’ at reducing tumor cell invasion in vitro in five cell lines that expressed CEACAM5, CEACAM6, or both antigens. MN-14
anti-CEA (CEACAM5) had no effect on tumor cell invasion in most
all cell lines. MDA-231 expresses neither antigen, and its invasion was
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a more important target than the N domain for the process of tumor
cell invasion.

Effect of MN-3, MN-15, and MN-14 Fab’ on survival of mice bearing GW-39 intrapulmonary colonic micrometastases. GW-39
expresses both CEACAM5 and CEACAM6, as shown by
immunohistochemistry (Fig. 6, top). Based on the in vitro results
suggesting that anti-CEACAM6 antibodies had limited antiprolifer-
ating effects yet showed significant anti-invasive and antiadhesive
properties, we pretreated GW-39 tumor cell suspensions with each
of the antibody fragments (10 µg/mL) for 15 minutes before i.v.
injection of 30 µL of cells and then dosed with 100 µg of antibody
on the day after transplantation. This design simulates the effect of
a continuous exposure to antibody that would be available at any
time that a cell from a primary tumor might initiate the metastatic cascade. The results presented in Fig. 6 illustrate that both MN-15
and MN-3 Fabs increased median survival time (>77 and 77 days,
respectively; P < 0.001 versus untreated cells) of these mice,
whereas hMN-14 Fab’ (49 days) did not affect survival significantly
(42 days for untreated mice). Although the study was continued
until mice were near death, rather than sacrificing them at a
defined time after treatment and counting the number of lung
nodules, median survival time should correlate with amount of
lung metastatic disease. The results with hMN-14 Fab’ in this study
are similar to what we reported recently for hMN-14 F(ab)2 in
this metastatic model (16), indicating that targeting the A3B3
domain does not affect median survival in this model, whereas
targeting the N and A1B1 domains shared by CEACAM5 and
CEACAM6 do affect metastasis and host survival when the
respective Fabs are used.

Discussion
A long-term goal of immunotherapy has been to induce antitumor responses against tumor-related antigens. Promising
results of several naked mAbs have been reported for solid tumors
and for lymphoma (43–46). Antibodies that directly perturb
signaling mechanisms have shown clinical benefit, such as those
directed against the extracellular domains of HER-2/neu, epidermal
growth factor receptor, and CD20 (47, 48). The studies in this article
suggest that instead of being directly therapeutic by immune
effector or direct cytostatic mechanisms, mAbs against CEACAM6
inhibit migration, invasion, and adhesion, thereby limiting meta-
tasis. The monovalent Fab’ form was used for most of our studies to
avoid effector cell function from the Fc region of the mAb and focus
on mechanisms implicated in the metastatic process.

CEACAM5 is overexpressed in a majority of carcinomas,
including those of the gastrointestinal, respiratory and genitouri-
inary systems, and the breast (2–6). Our results show that another
tumor family member, CEACAM6, may be as good, if not better, as
a target for solid tumor antimetastatic therapy. We have shown that
CEACAM6 is expressed in a larger percentage of solid tumor cell
lines than CEACAM5, and the high MCF on many of these lines

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**Figure 3. In vitro endothelial cell adhesion assay.** Percentage adhesion of various tumor cells with
varying amounts of expressed CEACAM5 and
CEACAM6 to HUVEC cells in the absence or
presence of MN-15 Fab’, MN-3 Fab’, or A68 Fab’
control. Cells were labeled with 1 µCi/mL of
3H-thymidine and added to HUVEC cultures and
incubated for 30 minutes at 37°C. Samples were
washed thrice with PBS to remove unattached cells.
Attached tumor cells were solubilized with 0.1 N
NaOH and radioactivity was measured in a
v-scintillation counter. The cpm attached/total
cpm added (attaching potential) was determined.
Results of a typical study are presented. Cell lines
used include BT-20 (CEACAM5+/CEACAM6+),
MCF-7 (CEACAM5+/CEACAM6+), HT-29
(CEACAM5+/CEACAM6+), Moser
(CEACAM5+/CEACAM6+), MCA38cea
(CEACAM5+/CEACAM6+), and MCA38
(CEACAM5+/CEACAM6+). Both MN-15 and
MN-3 induced a 49% to 58% inhibition in adhesion in
cell four lines (P < 0.01 for MN-3 Fab’ on MCF-7,
HT-29, and BT-20; P < 0.02 for MN-15 on the same
three lines; and P < 0.05 for both Fabs on Moser
adhesion to endothelial cells).

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suggests that more anti-CEACAM6 mAb can be delivered to these tumor cells. Thus, the MN-15 and MN-3 mAbs are useful for tumors that express CEACAM6 or CEACAM5, because they target epitopes that are shared by both antigens. These mAbs may therefore have advantages over mAbs like MN-14, which only target CEACAM5, or mAbs used by others (49) that are specific for only CEACAM6 and do not cross-react with CEACAM5. Our data are consistent with mAbs used by others (49) that are specific for only CEACAM6 and that are shared by both antigens. These mAbs may therefore have advantages over mAbs like MN-14, which only target CEACAM5, or that are shared by both antigens. These mAbs may therefore have advantages over mAbs like MN-14, which only target CEACAM5, or that are shared by both antigens. These mAbs may therefore have advantages over mAbs like MN-14, which only target CEACAM5.

It is known that CEACAM6 is expressed on the surface of neutrophils, thus modulating adherence to endothelial-leukocyte adhesion molecule-1 on activated endothelial cells (24). We have shown that mAbs to different epitopes on CEACAM6 (N and A1B1 domains) affect cell adhesion with endothelial cells. There is also evidence that CEACAM5 can affect cell adhesion to endothelial cells (50) via activation of Kupffer cells and stimulation of IL-1α, tumor necrosis factor α, and IL-6 production. These cytokines then induce the expression of intercellular adhesion molecules on endothelial cells, thus increasing adhesion of tumor cells to endothelium. Our results have shown that MN-3 and MN-15 Fab's are more active than MN-14 Fab at reducing adhesion of CEACAM5+/CEACAM6+ tumor cells to endothelial cells. Thus, both the N and the A1B1 domains but not the A3B3 domain are involved in tumor cell to endothelial cell adhesion.

Tumor cells can adhere to other tumor cells, to endothelial cells, as well as to ECM proteins. We have found that the amount of adhesion to a panel of these proteins varied between cell lines and was not related to the type of tumor (e.g., breast and pancreatic) or to the amount of CEACAM5 or CEACAM6 expressed. Tumor cell interactions with ECM proteins are important for migration and invasion and therefore metastasis. For example, tumor cell interactions with fibronectin are involved in the development of secondary tumors inside the

![Figure 4. In vitro ECM adhesion assay. Percentage of adhesion of a panel of tumor cell lines with varying expression of CEACAM5 and CEACAM6 to ECM proteins: fibronectin, vitronectin, laminin, collagen-I, and collagen-IV proteins using the Cytomatrix screening kit from Chemicon. Attached cells were stained with MTS and MOSER 0.42](image)

<table>
<thead>
<tr>
<th>Table 2. Adhesion to fibronectin</th>
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<tr>
<td><strong>Cell line</strong></td>
</tr>
<tr>
<td>MCF-7</td>
</tr>
<tr>
<td>MDA-468</td>
</tr>
<tr>
<td>CalPAN-1</td>
</tr>
<tr>
<td>ZR75-30</td>
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<tr>
<td>BxPC3</td>
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<tr>
<td>MOSER</td>
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NOTE: Relative attachment (mean ± SD) recorded for triplicate samples.
*Cell lines used for these studies include MCF-7 (CEACAM5+/CEACAM6+), MDA-468 (CEACAM5+/CEACAM6+), CaPAN-1 (CEACAM5+/CEACAM6+), ZR75-30 (CEACAM5+/CEACAM6+), BxPC3 (CEACAM5+/CEACAM6+), and MOSER (CEACAM5+/CEACAM6+).
bone marrow stroma via the α5β1 integrin (51). Blocking adhesion with polypeptide fragments of heparin-binding domains of fibronectin inhibited metastasis (52). Similar results have been obtained with a peptide blocking tumor cell-laminin adhesion (53). Interestingly, only MN-15 (A1B1 domain) was able to reduce adhesion to fibronectin in three of six tumor cell lines tested. The percent inhibition in fibronectin adhesion in this panel of cell lines did not correlate with the amount of adhesion in untreated cell samples. mAbs targeting the N or A3B3 domain did not affect cell adhesion to fibronectin. In one report by Duxbury (49), mAb-mediated CEACAM6 cross-linking resulted in increased ECM adhesion. However, the targeted epitope was different from the ones studied here.

Active migration of tumor cells is a prerequisite of tumor cell invasion and metastasis. Adhesion molecules that increase invasion also enhance the migratory process (38). Overexpression of CEACAM6 has been reported to promote cellular invasiveness of pancreatic cancer (29). Agents that inhibit metastases often affect several steps including migration, adhesion, and invasion (39, 54–56). Because our data also suggest that CEACAM6 has a role in adhesion and invasion, it is important to also assess the ability of these mAbs to impede migration. We have shown that in cell lines with strong migratory tendencies, mAb blocking of CEACAM5 and/or CEACAM6, with MN-3 > MN-15, decreases the number of migrating cells. In our in vitro assay, the process of cell invasion, which involves adhesion to ECM and migration steps, was inhibited by MN-15 Fab’ > MN-3 Fab’, (suggesting that the A1B1 domain of CEACAM6 is more important for this step but that the N domain also plays a role.

One of the notable advantages of MN-15 or MN-3 mAb therapy, compared with our previously reported results with MN-14 anti-CEA IgG (16), is the ability to target tumors that express either CEACAM6, CEACAM5, or both, whereas MN-14 can only be used for CEACAM5+ tumors. As shown in Table 1, many solid tumor lines express CEACAM6 but not CEACAM5 or express more CEACAM6 than CEACAM5. These tumor types are candidates for metastasis-directed mAb therapy with CEACAM6 mAbs.

An important consideration based on the in vivo experiments is the availability of mAb when cells first enter the circulation. MN-15 Fab’ and MN-3 Fab’ showed therapeutic efficacy if cells were exposed to mAb before the initiation of the metastatic process. However, if mAb was delivered after cancer cells had exited the vasculature and had begun to seed in the lung, mAbs alone were not therapeutic (data not shown). Therefore, to be clinically applicable, anti-CEACAM5/CEACAM6 mAbs would need to be available continuously, perhaps using implantable pumps, to maintain a desired level in the circulation at all times.

Figure 5. In vitro invasion assay. Percentage of invasion of a panel of human tumor cell lines with varying amounts of CEACAM5 and CEACAM6 through a polycarbonate membrane coated with ECM proteins over a 72-hour incubation period (Chemicon kit). Invasion in the absence of antibody Fab’ or in the presence of MN-3, MN-15, or nonspecific Ag8 Fab’ (10 μg/mL) was recorded. FBS in the lower chamber served as the chemottractant for invasion. Reduced invasion was noted in samples that did not have FBS. Cell lines used for these studies include LS174T (CEACAM5+/CEACAM6++), MCF-7 (CEACAM5−/CEACAM6+), ZR75-30 (CEACAM5−/CEACAM6++), BXPC3 (CEACAM5−/CEACAM6++), CaPAN-1 (CEACAM5+/CEACAM6++), and MDA-231 (CEACAM5−/CEACAM6−). MN-15 Fab’ reduced cell invasion of LS174T, MCF-7, ZR75-30, BXPC3, and CaPAN-1 cells by 30% (P < 0.02), 77% (P < 0.01), 49% (P < 0.01), 44% (P < 0.01), and 73% (P < 0.002), respectively. The effect of MN-3 Fab’ on the same five lines was a reduction in invasion of 3% (P = NS), 47% (P < 0.01), 59% (P < 0.01), 0% (P = NS), and 55% (P < 0.05), respectively.
Overall, the antimetastasis and mAb inhibition of adhesion, invasion, and migration is a technology that should be relatively nontoxic, not limited by issues of drug resistance, and easy to apply as an adjuvant with other standard and/or experimental therapy approaches. Because CEACAM6 is also expressed in normal lung, spleen, and granulocytes (57), the effect of anti-CEACAM6 mAb on normal tissues remains to be determined. In one report, CEACAM6-targeted immunotoxin therapy was effective in a tumor-bearing nude mouse model (58), but this model does not express CEACAM6 on normal tissues and can therefore not reliably assess whether mAb-toxin conjugates targeting CEACAM6 will be tolerated in humans.

In summary, we have shown that anti-CEACAM6/CEACAM5 mAb fragments devoid of effector cell functions and targeting the N and A1B1 domains of these antigens block migration, adhesion to endothelial cells and ECM, and invasion, and also increase the median survival of mice with intrapulmonary micrometastases of human colonic cancer.

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References

Inhibition of Adhesion, Invasion, and Metastasis by Antibodies Targeting CEACAM6 (NCA-90) and CEACAM5 (Carcinoembryonic Antigen)

Rosalyn D. Blumenthal, Hans J. Hansen and David M. Goldenberg


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