Non-specific Cross-Reacting Antigen 50/90 Is Elevated in Patients with Breast, Lung, and Colon Cancer


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ABSTRACT

A total of 22 genes have been identified in the carcinoembryonic antigen (CEA) gene family. The protein products of this gene are highly homologous and include CEA, biliary glycoprotein, non-specific cross-reacting antigen 50/90 (NCA 50/90), NCA 95, and pregnancy-specific β-glycoprotein. We used a monoclonal antibody with high affinity to develop a specific enzyme-linked immunosorbent assay (ELISA) method for NCA 50/90 in serum and plasma. Our calibrators were based on affinity-purified recombinant protein from a baculovirus expression system. No significant reactivity with purified CEA, recombinant NCA 95, or recombinant biliary glycoprotein was found by Western blot analysis or in the ELISA method. Only 1 of 15 sera from pregnant women (chorionic gonadotropin > 1000 ng/ml) was positive in the NCA 50/90 ELISA, suggesting that this method does not detect pregnancy-specific glycoprotein. A cutoff value of 18 ng/ml was established based on the 95% value of serum and plasma from 147 healthy volunteers. Only 3 of 31 serum and plasma samples from patients with clinically active breast cancer were elevated above the cutoff value, but 44% of 136 samples from patients with clinically active breast cancer were positive. NCA 50/90 measurements were elevated in 7 of 25 patients with active breast cancer whose CEA and CA 15-3 values were below cutoff, and NCA 50/90 values did not correlate with CEA in breast cancer. In addition, we found sensitivities of 70, 39, and 42% for lung cancer, colon cancer, and leukemia, respectively. The sensitivity for non-small cell lung cancer was 85%, however, compared to 50% for small cell lung cancer. Serum from leukemia patients showed an overall sensitivity of 43%, but 71% (10 of 14) sera from patients with chronic myelogenous leukemia were positive compared to, for example, chronic lymphocytic leukemia where 0 of 7 sera had NCA 50/90 values above the cutoff. These studies suggest that NCA 50/90 may have clinical utility in the management of patients with a variety of cancers.

INTRODUCTION

NCAs are members of the CEA gene family which form a part of the immunoglobulin supergene family (reviewed in Refs. 1 and 2). The NCA subfamily was postulated to contain as many as 12 forms based on biochemical and immunochemical evidence (3). Molecular cloning analysis, however, has led to the identification of 22 members of the CEA gene family (1), only 2 of which are predicted to be NCAs based on domain structure and anchorage to the membrane via a glycosyl phosphatidylinositol linkage. NCA sequences described to date include NCA 50/90 (4-6) and NCA 95 (7). Seven lines of evidence have shown that the NCA 50/90 gene encodes forms previously described as NCA, NCA 55, and TEX (5, 8). Each of these forms share the identical peptide sequence and differ only in their carbohydrate content. NCA 95 is the product of a distinct gene and is expressed in cells of the granulocytic lineage (7, 9). Another potential member of the NCA subfamily is NCA 2, a Mr 160,000 glycoprotein from human meconium and feces which has immunological cross-reactivity with antibodies to CEA (10). NH₂-terminal amino acid sequencing, however, has demonstrated complete homology of the first 30 residues of NCA 2 with CEA, suggesting that this is a cleavage product of CEA (11). Molecular cloning analysis of NCA 50/90 predicts a 108-residue amino terminal region, or N domain, which is homologous to immunoglobulin variable regions, suggesting a binding function for this region of the NCA molecule (4-6). This region is followed by a single 178-amino acid region with high homology to immunoglobulin constant domains; the 24-amino acid COOH-terminal domain is thought to be processed posttranslationally with the addition of a phosphatidylinositol glycan anchor (12, 13). Members of the CEA gene family are believed to function as adhesion molecules because of (a) homology with the neural cell adhesion molecule, N-CAM (5); (b) homotypic and heterotypic intercellular adhesion (14, 15); and (c) binding of CEA and NCA to human strains of Escherichia coli (16). Recent findings have further suggested that the adhesive properties of CEA may be used by cancer cells to enhance attachment to sites of distant metastasis (17).

The clinical utility of serial measurements of CEA in the management of patients with colorectal, breast, and other types of solid tumors is well established. In contrast, no clinical value has been established for the measurement of other members of the CEA gene family in cancer patients. In fact, numerous reports have suggested that CEA assays are plagued by problems with cross-reactivity due to nonspecific factors (18, 19), implying that because other CEA gene family members are expressed in some normal tissues, they may confuse results with the tumor-specific marker, CEA. Despite these limitations, several studies have established that NCA may serve as a useful tumor biomarker, especially in leukemia (20-22). Previously published studies on the clinical utility of NCA in cancer patients, however, have used antibodies with uncertain specificity relative to homologous proteins of the CEA family. In this study, we used recombinant proteins to characterize a monoclonal antibody with high affinity binding to NCA 50/90 but with no detectable binding to CEA, NCA 95, BGP, NCA 2, and PSG. We used this MAb in an ELISA format to compare levels of NCA 50/90 in normal individuals to those of patients with a variety of cancers.

MATERIALS AND METHODS

Monoclonal Antibodies. NCA was purified from human spleen as described (23). On sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blots, this material contained NCAs of Mr 45,000-95,000. BALB/c mice were immunized with 50 μg of an emulsion of purified NCA and Freund's complete adjuvant. Spleen cells from hyperimmune animals were fused with Ag8 myeloma cells (CRL 1580; American Type Culture Collection, Rockville, MD) using standard methods (24), and resulting hybridomas were screened by a sandwich ELISA for binding to biochemically purified NCA 50/90 but not to CEA or BGP (25). Positive cultures were cloned, rechecked for antigenic specificity by ELISA, expanded by growth in mouse ascites.
the a-fetoprotein. The relative electrophoretic mobility of a stretch of six histidine residues, also at the carboxyl terminus of the molecule (27). These constructs were cloned into pVL1393 by polymerase chain reaction and expressed using recombinant baculovirus phage to infect Spodoptera frugiperda (SF9) cells. Supernatant fluids from SF9 cells were affinity-purified using a zinc-imidoacetate-Sepharose column as described (27). The purified recombinant NCA 50/90 has been found to migrate on Western blots with a slightly higher molecular weight than NCA 50/90 derived from human spleen (not shown). This may be due to differences in glycosylation. The 228.2 monoclonal antibody recognizes both the recombinant and biochemically purified forms of NCA 50/90 and also binds to NCA 50/90 following complete deglycosylation by N-glycanase, which suggests that the 228.2 epitope is on the polypeptide portion of the NCA 50/90 molecule. The concentration of NCA 50/90 and NCA 95 was determined by the BCA protein assay (Pierce Chemical Co., Rockford, IL). For use as a calibrator in the NCA 50/90 ELISA, purified recombinant NCA 50/90 was diluted in TBST [25 mm Tris, pH 7.5–150 mm NaCl-0.05% Tween 20-0.1% Na3PO4-5% BSA] as described below. NCA 2 purified from human meconium was purchased from Crystal Chem. BGPa, BGPh, BGPc, and BGPd, and possibly other forms of BGP (25, 28). A detergent-soluble phase was collected and purified by binding to a lectin and expressed using recombinant baculovirus phage to infect Spodoptera frugiperda (SF9) cells. Supernatant fluids from SF9 cells were affinity-purified using a zinc-imidoacetate-Sepharose column as described (27). The purified recombinant NCA 50/90 has been found to migrate on Western blots with a slightly higher molecular weight than NCA 50/90 derived from human spleen (not shown). This may be due to differences in glycosylation. The 228.2 monoclonal antibody recognizes both the recombinant and biochemically purified forms of NCA 50/90 and also binds to NCA 50/90 following complete deglycosylation by N-glycanase, which suggests that the 228.2 epitope is on the polypeptide portion of the NCA 50/90 molecule. The concentration of NCA 50/90 and NCA 95 was determined by the BCA protein assay (Pierce Chemical Co., Rockford, IL). For use as a calibrator in the NCA 50/90 ELISA, purified recombinant NCA 50/90 was diluted in TBST [25 mm Tris, pH 7.5–150 mm NaCl-0.05% Tween 20-0.1% Na3PO4-5% BSA] as described below. NCA 2 purified from human meconium was purchased from Crystal Chem.

**Biotinylation of Goat Antibody to CEA.** Affinity-purified goat polyclonal anti-CEA antibody (BiosPacific) was placed into 1.3 ml of 0.1 M NaHCO3 (pH 8.5) at a concentration of 1.0 mg/ml. To this was added 18.5 µl of a 10-µg/ml solution of NHS-LC-biotin (Pierce) in deionized water to give a 50:1 molar excess of biotin:antibody. After incubation at 0°C for 4 h, the biotinylated antibody was passed over a buffer-exchange column using 10 mM phosphate (pH 7.4)-150 mM NaCl and stored at 4°C with 0.1% thimerosal as preservative.

NCA 50/90-specific Immunoassay. A sandwich ELISA was configured using the 228.2 monoclonal antibody as the solid phase capture antibody and the biotinylated polyclonal anti-CEA as the reporter antibody. Ninety-six-well ELISA plates (Immulon 4; Dynatech) were coated with 100 µl of 228.2 monoclonal antibody at 5 µg/ml in 0.1 M NaHCO3 (pH 9.0) and incubated overnight at 4°C. Wells were emptied, and unreacted sites on the plates were quenched by the addition of 200 µl of TBST with 5% BSA (fraction V; Sigma) followed by a 1-h incubation at 37°C. Wells were washed six times with TBST, and 25 µl of either NCA 50/90 calibrators diluted in TBST-5% BSA-0.1% thimerosal or 25 µl of patient sample were added. An equal volume of 50 mM HEPES (pH 7.0)-500 mm NaCl-200 µg/ml mouse IgG-50 µg/ml gentamycin-0.1% (w/v) NaN3 (sample diluent) was added to each well, and the plates were incubated for 2 h at 37°C. After washing six times, a 100-µl volume of a 0.3 µg/ml solution of goat anti-CEA-biotin in 50 mM HEPES (pH 7.0)-150 mM NaCl-1 mM MgCl2-H2O-0.1 mM ZnCl2-5% BSA-50 µg/ml gentamycin-0.1% Na3PO4 (conjugate diluent) was added to all wells and incubated for 1 h at 37°C. The wells were washed an additional six times, and 100 µl of streptavidin conjugated to alkaline phosphatase (Pierce) diluted 1:5000 in conjugate diluent was added. After a 1-h incubation at 37°C, the plates were washed 12 times with TBST and incubated with 100 µl of p-nitrophenyl phosphate in diethanolamine substrate buffer (Pierce) for 30 min. The reaction was stopped with 100 µl 1 N NaOH and absorbance at 405 nm minus absorbance at 490 nm, was as determined using a microplate reader (Thermomax).
Molecular Devices Corp.). The amount of NCA 50/90 was determined for each test sample by comparison with the calibrator standard curve using a nonlinear spline curve fit program.

**Patient Samples.** Serum was prepared from blood drawn from normal, healthy volunteers by Hudson Valley Blood Services (Valhalla, NY). Plasma samples were obtained from healthy volunteers at Miles, Inc. (Tarrytown, NY) and from Antibody Systems, Inc., (Bedford, TX). Plasma samples from patients with inactive or active cancer were obtained from Dianon Systems (Stratford, CT). Serum samples from cancer patients were obtained from M. D. Anderson Cancer Center (Houston, TX). Patient disease status was determined from information supplied by attending physicians, and the results of diagnostic tests such as x-ray, bone scan, and liver scans, as well as results of testing for tumor biomarkers such as CEA, lipid-associated sialic acid, CA 15–3, and CA 19–9 were used. All patients designated as “active” had cancer which was classified by their physicians as progressive, stable, or responding to therapy and also had elevated values for at least one in vitro tumor biomarker. All patients designated as “inactive” had disease which was classified as NED, stable, or in remission, with levels of at least two in vitro tumor biomarkers within the normal range. Similarly, all patients labeled NED were classified as NED or in remission by their physicians and had normal levels of one or more tumor biomarkers. Samples were obtained from patients with breast, colon, and lung cancer classified as stages I–IV. Lung cancers and leukemias were subclassified based on histological data.

**RESULTS**

**Specificity of the 228.2 Mab.** The antigenic specificity of the 228.2 Mab was determined by Western blotting (Fig. 1). The 228.2 Mab reacts specifically with NCA 50/90 and not with other proteins related to CEA. The reactivity of the 228.2 Mab with the high molecular weight band of M, 110,000 (Fig. 1A, Lane 3) probably represents the formation of sodium dodecyl sulfate-stable protein dimers. The reactivity of the 228.2 Mab with NCA 50/90 is approximately 1 X 10^9 M^-1. The reproducibility of the association rate constant was obtained from the relationship \( k_{assoc}/k_{dissoc} \) from four data sets obtained on two occasions using two different BIAcore instruments. As shown in Table 1, the affinity of the 228.2 Mab for NCA 50/90 in breast, lung, and colon cancers was quantitated as relative uptake at ligand concentrations of 8–80 nM (Fig. 2A). For determination of kinetic constants, it may be assumed that binding of ligand to antibody is monovalent and free NCA 50/90 bound at time t, and Rmax is the maximum amount of NCA 50/90 bound by the antibody at saturation. From a linear plot of \( dR/dt \) versus R (Fig. 2B), a slope \( (k_a) \) was determined such that \( dR/dt = -(k_{assoc}C + k_{dissoc})R + k_{assoc}C R_{max} \). From a plot of \( k_a \) versus total concentration of ligand (Fig. 2C), the association rate can be derived from the slope, and the dissociation rate constant is derived from the Y-intercept. An estimate of the equilibrium affinity constant was obtained from the relationship \( K_A = k_{assoc}/k_{dissoc} \). These values were determined from four data sets obtained on two occasions using two different BIAcore instruments. As shown in Table 1, the affinity of the 228.2 Mab for NCA 50/90 is approximately 1 X 10^9 M^-1. The reproducibility of the derived constants is excellent with run to run variability of less than 10%.

**Characterization of the NCA 50/90 ELISA.** The standard curve presented in Fig. 3 demonstrates a nonlinear increase in absorbance as a function of NCA 50/90 concentration, which necessitated the use of a nonlinear spline curve fit program to convert raw patient data to NCA 50/90 concentrations. The interassay and intrassay CVs for the NCA 50/90 ELISA were tested using control preparations of recombinant NCA 50/90 diluted in horse serum with 0.1% thimerosal as preservative. Results in Table 2 show that the interassay CVs (<6.6%) and intrassay CVs (<4.3%) for two levels of control were very good.

**Table 1 Kinetic and affinity constants for the binding of NCA 50/90 to Mab 228.2**

<table>
<thead>
<tr>
<th>Constant</th>
<th>Mean</th>
<th>SD</th>
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<tr>
<td>( k_{assoc} ) (nM^-1·s^-1)</td>
<td>( 4.24 \times 10^5 )</td>
<td>( 4.15 \times 10^4 )</td>
</tr>
<tr>
<td>( k_{dissoc} ) (s^-1)</td>
<td>( 2.88 \times 10^{-4} )</td>
<td>( 1.89 \times 10^{-6} )</td>
</tr>
<tr>
<td>( K_A ) (nM)</td>
<td>( 1.48 \times 10^8 )</td>
<td>( 1.53 \times 10^8 )</td>
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</table>

**Fig. 2.** Measurement of the kinetic and affinity constants of the 228.2 Mab for recombinant NCA 50/90 for breast cancer. A, relative response following addition of NCA 50/90 at various concentrations at t = 600 sec and withdrawal of ligand at t = 1200 sec. The kinetics of dissociation were used to determine \( dR/dt \) versus R shown in B, and the slope of the linear portion of these plots gave a value for \( k_a \), which was plotted versus initial concentration of NCA 50/90 in C. The slope of the line in C gave an estimate of the association rate constant, \( k_{assoc} \), and the dissociation rate constant, \( k_{dissoc} \), was derived from the Y-intercept.

**Fig. 3.** Measurement of the kinetic and affinity constants of the 228.2 Mab for recombinant NCA 50/90 for breast cancer. A, relative response following addition of NCA 50/90 at various concentrations at t = 600 sec and withdrawal of ligand at t = 1200 sec. The kinetics of dissociation were used to determine \( dR/dt \) versus R shown in B, and the slope of the linear portion of these plots gave a value for \( k_a \), which was plotted versus initial concentration of NCA 50/90 in C. The slope of the line in C gave an estimate of the association rate constant, \( k_{assoc} \), and the dissociation rate constant, \( k_{dissoc} \), was derived from the Y-intercept.
We measured the specificity of the NCA 50/90 ELISA by the addition of purified proteins with high amino acid homology to NCA 50/90. The data in Fig. 5 show that CEA, NCA 95, and BGP give no significant reactivity in this method. In addition to interferences from members of the CEA gene family, a potential for cross-reactivity with serum protein inhibitors stems from observations that biochemically purified CEA and NCA may associate with molecules with amino acid homology to α-1-antichymotrypsin and α-1-antitrypsin (31, 32). We found no reactivity of purified α-1-antichymotrypsin, α-1-antitrypsin, α-2-macroglobulin, α-2-antiplasmin, and antithrombin III in the NCA 50/90 ELISA (data not shown). We also tested this method for cross-reactivity with an additional member of the CEA gene family, PSG (33), which is elevated in the serum of pregnant women. We tested reactivity to this glycoprotein by examining sera from 15 pregnant women with human chorionic gonadotropin values ranging from 2,200 to 79,000 (normal cutoff for human chorionic gonadotropin, 10). As can be seen in Fig. 6, only one patient showed an NCA 50/90 value above the cutoff value of 18 ng/ml, which demonstrates that the NCA 50/90 ELISA does not detect PSG.

We then measured NCA 50/90 values in samples from 31 breast cancer patients undergoing treatment who were clinically free of cancer and found that 3 of 31 (10%) of the values were above the cutoff value (Fig. 8). In contrast, 44% of samples from 136 patients with active breast cancer had NCA 50/90 values >18 ng/ml, which demonstrates that NCA 50/90 is elevated in the serum and plasma of some patients with breast cancer. We also tested plasma from 26 breast cancer patients who had active cancer by clinical examination but whose levels of CEA and CA 15–3 were below cutoff for those markers (5 ng/ml and 35 U/ml, respectively). Of the 26 such patients, 7 (27%) had NCA 50/90 values in excess of 18 ng/ml. These results suggest that the addition of NCA 50/90 to a panel of current breast cancer biomarkers may provide improved sensitivity.

Because NCA 50/90 has high amino acid homology to CEA, it may be expected that elevations of NCA 50/90 in cancer patients would correlate with increases in CEA values. To test this, we compared the values for NCA 50/90 and CEA in 143 patients under treatment for breast cancer, of which 32 were positive for CEA and 40 were positive for NCA 50/90. The results (Fig. 9) demonstrate that the levels of NCA 50/90 and CEA are correlated only poorly (r² = 0.47). This suggests that increases in blood levels of NCA 50/90 occur in a population of breast cancer patients which is different from that with increases in levels of CEA. Although the number of patients in this study with elevated values for CEA and NCA 50/90 is small, these data suggest that measurement of NCA 50/90 in blood may be of additional clinical value to currently used biomarkers.

We also measured the level of NCA 50/90 in the serum and plasma of patients diagnosed with colon cancer, lung cancer, and leukemia. The level of NCA 50/90 is elevated in 39% of 155 patients with active colon cancer (Fig. 10), which contrasts with 84% positive CEA values in the same patient population. These data support the results with breast cancer which suggest that NCA 50/90 may recognize a unique population of cancer patients compared to CEA, even though both of these glycoproteins are synthesized coordinately by some cancer cells (34, 35). While NCA levels were elevated in 70% of all lung cancer patients tested, 3 of 14 patients with clinically inactive lung cancer showed NCA 50/90 values above cutoff (Fig. 10). A subset of samples were from lung cancer patients with histological data which allowed separation into small cell and non-small cell lung cancers. Results showed that NCA 50/90 levels were increased in 85% of patients with non-small cell lung cancer but only in 50% of patients with small cell lung cancer (Table 3). In leukemia patients, NCA 50/90 values were above cutoff levels in 42% of all patient samples. However, when these were subclassified into various types of leukemic disease, it was clear that NCA 50/90 levels are increased only in some types. For example, 10 of 14 patients with active chronic myelogenous leukemia were positive, and 4 of these patients had NCA 50/90 values ≥ 70 ng/ml. In contrast, only 4 of 10 patients with acute myelocytic leukemia and 4 of 22 with chronic lymphocytic leukemia had NCA 50/90 levels above 18 ng/ml.

**Table 2 Precision of the enzyme immunoassay method for NCA 50/90**

<table>
<thead>
<tr>
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<th>High control</th>
</tr>
</thead>
<tbody>
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<td></td>
</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
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<td>Coefficient of variation (%)</td>
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</table>

**Fig. 3. Typical standard curve for the NCA 50/90 ELISA. Each point was tested in duplicate.**
DISCUSSION

The Western blots presented in Fig. 1 demonstrate that the 228.2 Mab recognizes NCA 50/90 but does not bind to CEA, BGP, NCA 95, or NCA 2. The specificity of the 228.2 Mab for NCA 50/90 is reflected in the high binding affinity (Fig. 2; Table 1). Although the affinity of the 228.2 Mab for recombinant NCA 50/90 is high, the binding parameters for native NCA 50/90 were not measured in these studies. The 228.2 Mab does recognize native antigen as shown by reactivity with serum and plasma, reactivity with NCA purified from placenta and spleen in ELISA and Western blotting, and by histological reactivity with normal and cancerous tissue (data not shown). The sandwich ELISA was also specific for NCA 50/90, and no reactivity with PSG was found (Figs. 5 and 6).

Several previous reports have described MAbs to NCA (36-39). Although these antibodies do not recognize CEA, binding to BGP, NCA 95, and PSG was not determined. A recent publication described an NCA 50/90-specific Mab, but no data on the measurement of NCA 50/90 in the serum of cancer patients was presented (39). In our hands, the frequency of MAbs which bind NCA 50/90 but do not recognize closely related members of the CEA family are extremely rare. Since NCA 50/90 has 85% sequence homology with CEA (5), there may be very few epitopes on the NCA 50/90 molecule which are not expressed on any other CEA family members. In a previous study, progressive deletion of a complementary DNA clone, followed by site-directed mutagenesis, was used to identify an epitope on the NCA molecule which is reactive with an NCA-specific Mab (38). The epitope included residues 78-79 of the NCA 50/90 molecule, but these are conserved in BGP and PSG, suggesting that this Mab may cross react with other family members. It will now be of interest to compare the epitopes recognized by the various MAbs to NCA, as was done for 52 MAbs to CEA (40).

When NCA 50/90 levels were measured in serum and plasma from 147 healthy individuals, a total of 6 were above the 18 ng/ml cutoff. No further information is available on the individuals who donated the serum samples. However, collection of longitudinal samples from 22 of the 50 healthy plasma donors is currently in progress, and this group includes 3 individuals with elevated levels of NCA 50/90. It will be of interest to determine if healthy individuals show persistently elevated levels of NCA 50/90, or if the levels increase transiently and return to lower baseline values. The mean value for NCA 50/90 in

\[ y = 1.003x - 5.926 \]

\[ \text{y} = 1.003x - 5.926 \]

Fig. 4. Linearity dilution studies. A. Two test kit standards of 75 and 10 ng/ml and two patient sera spiked with NCA 50/90 at 200 ng/ml were serially diluted with the test kit zero standard and tested in the NCA ELISA. B. Pooled human serum was spiked with NCA 50/90 at 1000 ng/ml and was serially diluted with pooled human serum and tested in the NCA ELISA.
NCA 50/90 IN BREAST, LUNG AND COLON CANCER

Fig. 7. NCA 50/90 values in 97 serum and 50 plasma samples obtained from healthy volunteers.

Fig. 8. NCA 50/90 values in patients with breast cancer.

Fig. 9. Correlation study of CEA and NCA 50/90 in 143 patients with breast cancer. Patients with active and inactive cancer were included in this study.

serum from healthy donors was 11.5 ng/ml, which is somewhat higher than that for the mean value of CEA in serum (1–3 ng/ml). A high level of circulating NCA 50/90 in normal individuals could be due to release of NCA 50/90 from neutrophils or from other tissues which normally express low levels of this protein, such as colonic epithelium (42). It is important to note that 119 of 147 (81%) healthy individuals had NCA 50/90 values < 10 ng/ml. Since individual baseline values are more useful to monitor therapy than cutoff values, the relatively low normal range for NCA 50/90 suggests that this marker may be useful to manage patients undergoing therapy.

The sensitivity of NCA 50/90 ranged from 39–70% for various types of solid cancers and leukemia. The clinical utility of cancer biomarkers is currently limited to monitoring changes in the disease status of patients undergoing treatment. Because other biomarkers are well established for monitoring therapy, such as CEA in colon cancer and prostate-specific antigen in prostate cancer, it will be of primary importance to determine if NCA 50/90 adds clinical value to established markers for patient monitoring and management. The data (Fig. 8) suggest that approximately 25% of breast cancer patients with normal levels of CEA and CA 15–3 have elevated levels of NCA 50/90. In addition, the correlation study (Fig. 9) demonstrates that NCA 50/90 levels do not correlate with CEA levels in breast cancer, and similar results were found in lung and colon cancer (data not shown). We also found that the sensitivity of NCA 50/90 in 155 patients with active colon cancer was only 39% compared with 84% sensitivity for CEA in the same patient population. These data agree with previous studies which demonstrated that a proportion of colon cancers noncoordinately express mRNA for CEA and NCA 50/90 (34, 43). These data suggest that NCA 50/90 levels may be increased in a subset of patients which is different from the subset in which CEA is elevated. Further studies are required to determine if NCA 50/90 values correlate with other markers, such as CA 15–3 in breast cancer or CA 19–9 in gastrointestinal cancer. It may be that a panel composed of NCA 50/90, a mucin such as CA 15–3, and an oncogene product such as HER-2/neu will show elevations in at least one marker in a high proportion of breast cancer patients. In addition, it remains to be determined if changes in the serum or plasma levels of NCA 50/90 correlate with changes in disease status for patients undergoing therapy. Further studies will be required to address these issues.

NCA has historically been considered non-tumor specific and, therefore, not predictive of cancer burden or status (1, 42). This stems from observations that NCA is found in a variety of normal tissues including breast, colon, lung, prostate, ovary, and pancreas (1, 2, 42). More recent studies, however, have shown that NCA is a normal component of granulocytes (41), and a receptor for CEA and NCA has been described on the surface of macrophages and histiocytes (2). Taken together, these data suggest that NCA may not be synthesized in substantial amounts in normal epithelial tissues but may be detected immunologically in normal tissue sections due to the presence of contaminating neutrophils and histiocytes. This may explain the unexpected high apparent specificity of NCA 50/90 measurements in breast, colon, and lung cancer found in these studies.

In the studies reported here, the elevation of NCA 50/90 in the serum and plasma of cancer patients is approximately 10–100 fold lower than the elevations of CEA found in some of these same patients. If NCA 50/90 values in serum and plasma reflect release of NCA from tumor cells, then the NCA 50/90 values would be expected to increase to higher levels as tumor burden increases. The reasons for the lower values of NCA 50/90 compared to other mass biomarkers could be due to one of several factors. NCA 50/90 could bind to a serum protein resulting in decreased immunoreactivity in the ELISA. The results of dilution linearity studies (Fig. 4) suggest that this is not the case. Alternatively, the level of NCA mRNA may be lower in tumor tissues than for other mass markers; however, a recent study found that NCA mRNA was expressed in 28 of 32 colorectal tumors...
and was overexpressed relative to normal mucosa in 26 of these (34). To date, no quantitative comparison of mRNA expression for CEA and NCA in tumor tissue has been reported. Additionally, it is known that NCA can be released from the surface of tumor cells by glycosyl phosphatidylinositol phospholipase C, and CEA is released by glycosyl phosphatidylinositol phospholipases C and D and serum (44). It may be, however, that NCA and CEA are released from the cell surface by different mechanisms such that the amount of NCA released from the tumor cell surface is low relative to CEA. Members of the CEA gene family have also been shown to mediate homotypic and heterotypic cell adhesion (14, 15) and may have receptors on the surface of vascular endothelium which could bind differentially to soluble CEA family members. It is also possible that NCA in blood is bound to receptors on circulating macrophages. That seems unlikely since only mature macrophages carry NCA receptors, and peripheral blood contains primarily monocytes but very few activated macrophages. Experiments are currently in progress to better understand the differences in circulating levels of NCA and CEA in the blood of tumor patients.

Taken together, the results of this study suggest that NCA 50/90 is elevated in the serum and plasma of a proportion of cancer patients and that this population of patients is different from those with elevated levels of CEA. Future studies will be directed toward determining the clinical utility of NCA 50/90 in monitoring the success of primary therapy and in monitoring for recurrence of cancer. It will also be of interest to determine the added value of NCA 50/90 when compared with currently accepted cancer biomarkers and to measure the specificity of NCA 50/90 in noncancerous diseases such as chronic inflammatory disease, benign cancers, and other nonneoplastic pathologies.

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