Oncogenic c-Ki-ras but Not Oncogenic c-Ha-ras
Up-regulates CEA Expression and Disrupts Basolateral Polarity in Colon Epithelial Cells*

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Colon carcinomas commonly contain mutations in Ki-ras4B, but very rarely in Ha-ras, suggesting that different Ras isoforms may have distinct functions in colon epithelial cell biology. In an earlier study we had demonstrated that oncogenic Ki-ras4BVal-12, but not oncogenic Ha-ras4BVal-12, blocks the apicobasal polarization of colon epithelial cells by preventing normal glycosylation of the integrin β1 chain of the collagen receptor. As a result, only the Ki-ras mutated cells exhibited altered cell to substratum attachment, whereas mutation of either Ras isoform activated mitogen-activated protein kinases. We have now asked whether intercellular adhesion proteins implicated in establishing basolateral polarity in colon epithelial cells are modulated by oncogenic Ki-ras4BVal-12 proteins but not oncogenic Ha-ras4Val-12 proteins. The embryonic adhesion protein carcinoembryonic antigen (CEA) was up-regulated on the mRNA and protein levels in each of three stable Ki-rasVal-12 transfectant lines but in none of three stable Ha-rasVal-12 transfectant lines. The elevated protein levels of CEA in Ki-ras4BVal-12 transfectant cells were decreased by blocking expression of Ki-ras4BVal-12 with antisense oligonucleotides. N-cadherin levels were decreased in only the Ki-ras transfectants, whereas E-cadherin levels were unchanged. Immunohistochemical analysis demonstrated that Ki-ras4BVal-12 transfectant cells did not polarize into cells with discrete apical and basal regions and so could not restrict expression of CEA to the apical region. These unpolarized cells displayed elevated levels of CEA all along their surface membrane where CEA mediated random, multilayered associations of tumor cells. This aggregation was both calcium-independent and blocked by Fab' fragments of anti-CEA monoclonal antibody col-1. Trafficking of the lysosomal cysteine protease cathepsin B may also be disrupted when cell polarity cannot be established. Ki-ras4BVal-12 transfectant cells expressed 2-fold elevated protein levels of the lysosomal cysteine protease cathepsin B but did not up-regulate cathepsin B mRNA expression. One function of oncogenic c-Ki-Ras proteins in colon cancer progression may be to up-regulate CEA and thus to prevent the lateral adhesion of adjacent colon epithelial cells that normally form a monolayer in vivo.

Studies in tissue culture have shown that cellular polarization may require both E-cadherin-mediated cell-to-cell contact and integrin-mediated cell-substratum interactions (1). E-cadherin is critical to the formation of the basolateral domain, whereas the orientation of the apicobasal axis depends on integrin-mediated associations with the substratum. Orientation of the apical-basal axis in Madin-Darby canine kidney cells is known to depend on integrin-mediated interactions with the growth surface. When grown in suspension, Madin-Darby canine kidney cells aggregate to form cysts with the apical domain facing outward. These cysts reverse their polarity when collagen is added to the culture medium causing apical markers to disappear from the external domain and reappear on the luminal face (2). Collagen interaction with integrins on the basal surface of cells is believed to orient the apical-basal axis, since addition of blocking antibody to β1 integrin prevents this polarity reversal (3).

HD6-4 colon carcinoma cells provide a unique model to study the effects of oncogenes on cellular polarization. HD6-4 cells polarize apicobasally by up-regulating their α2β1 integrin collagen receptors (4) and basolaterally by up-regulating membrane levels of E-cadherin 35-fold and membrane levels of the desmosomal protein desmoglein 16-fold (5). HD6-4 cells then differentiate into a specialized epithelial cell, the mucus granule producing colon goblet (5). This cell polarization can occur although HD6-4 cells contain inactivating mutations in the tumor suppressor genes p53 and APC. HD6-4 cells, however, have wild-type ras genes (6). Most colon cancer cells are rounded and unpolarized. As roughly 50% of colon cancers cases exhibit activating mutations in ras genes, mutations in ras may be one mechanism for loss of cell polarization. The vast majority of ras mutations in colon cancers are in Ki-ras, with only a few percent in N-ras and very uncommonly in Ha-ras (7, 8). The selection of Ki-ras mutations in the genesis of colon cancers suggested that mutations in this ras isoform might block colon epithelial cell apicobasal or basolateral polarization.

We have presented evidence in a recent study (1) that only mutations in the Ki-ras gene, not the Ha-ras gene, disrupt colon epithelial cell apicobasal polarity through a specific effect on β1 integrin maturation. We made stable transfectants of HD6-4 cells using minigene constructs of the huge cellular Ki-ras gene, both wild-type and constructs activated by mutation at valine 12. We compared these transfectants with those made using Ha-ras mutated at valine 12. The mutated Ha-ras and Ki-ras genes were expressed on the protein level and each activated the mitogen-activated protein kinases erk1 and erk2, which were correlated with increased cell proliferation. How-

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ever, only the mutated Ki-ras gene blocked normal glycosylation of β1 integrin, leading to accumulation of an aberrant β1 integrin. The aberrant β1 integrin was transported to the cell surface where it mediated a looser attachment to collagen I. Antisense oligonucleotides to Ki-ras restored normal glycosyltransferase activity to the Ki-ras transfectants that regained the capacity to make mature β1 integrin.1 These studies demonstrated that the blocked glycosylation of β1 integrin was the direct result of the functioning of the oncogenic Ki-ras gene and not some nonspecific effect of transfection. HD6-4 colon epithelial cells bind to collagen I as a first step in polarization, followed by differentiation to mucin-producing goblet cells (4). Imperfect binding to this substratum could prevent normal apicolateral polarization.

Selection of colon adenoma cells with oncogenic Ki-ras mutations is seen during the growth of benign tumors (10), and this selection is maintained in the transition to malignancy and during further progression of the cancer cells to highly invasive and metastatic states (11, 12). Tumor cells freed from tight cell-to-cell and cell-to-substratum adherence because of the synthesis of aberrant β1 integrin could have a selective advantage in both growth and invasion and become the dominant cell type within a tumor. In the current study, we have asked whether the oncogenic Ki-ras 4BVal-12 gene can disrupt colonic epithelial cell polarity in a second way by targeting intercellular adhesion proteins and whether any such effect is restricted to the Ki-ras gene and is not mediated by oncogenic Ha-ras8Val-12.

**EXPERIMENTAL PROCEDURES**

**Materials**—[3H]Simeionethane was obtained from NEN Life Science Products, protein A-Sepharose from Pharmacia Biotech Inc., polynvlinide difluoride transfer paper Immobolin-P was obtained from Millipore, N-cadherin monoclonal antibody clone GC-4 mouse IgG1 isotype was purchased from Sigma, monoclonal antibody clone 36 to E-cadherin was purchased from Bio摘, monoclonal antibody clone GC-4 mouse IgG1 isotype for E-cadherin was purchased from Bioagen, monoclonal antibody clone Y13–259, which reacts with the p21 translational products of the Ha-, Ki-, and N-cadherin human homologues, and monoclonal antibody CA10 to cathepsin B from Oncogene Science. Col-I monoclonal antibody to CEA2 (13) was a gift of Dr. Jeffrey Schlom, NCI. Phosphorothioate oligodeoxynucleotides were a gift of Dr. Brett Monia, Isis Pharmaceuticals (Carlsbad, CA). Isis-2503 is a 20-mer of random sequence. Isis-13177 is a 20-mer targeted to the initiation codon of c-Ha-ras mRNA (TTC-CTG-ATC-GCT-CAT-CAG-GG) (14), Isis-6957 is a 20-mer targeted to the 5′-UTR of Ki-ras (CAG-TGC-CTG-CCG-GCG-CCG-CGC-CCG-CGG) (15), Isis-13177 is a 20-mer of random sequence. The HD6 human colon carcinoma cell line was cloned from the HT29 cell line and recloned as the HD6-4 line immediately before transfection. The parental and transfectant lines were maintained in Dulbecco’s modified Eagle’s medium containing 7% fetal bovine serum or ITS-Dulbecco’s modified Eagle’s medium, as described (4).

**Antisense Oligonucleotide Treatment**—Treatment of Ki-ras and Ha-ras transfectants was essentially as described (15). Cells were seeded per well at 2 × 10^4 in 6-well plates. 24 h later the cells were washed with pre-warmed serum-free ITS-Dulbecco’s modified Eagle’s medium and then incubated in this medium with a fixed ratio of oligonucleotide to Lipofectin (2.4 μL of Lipofectin per 100 μM oligonucleotide) for 4 h. The oligonucleotide-containing medium was then replaced with normal growth medium and growth continued for 48 h to allow Ras turnover plus reduced Ras mRNA levels to result in reduced Ras protein level.

**Immunodetection**—50 μg of cell lysate proteins were blotted onto polyvinylidene difluoride membranes after separation on 7% SDS-polyacrylamide gel electrophoresis. The blots were blocked in PBS containing 4% bovine serum albumin and 0.05% Tween 20 (blocking buffer) for 1 h at room temperature, incubated for 1 h with 1:500 dilution of col-1 and CEA, 1 μg/ml anti-cathepsin B, 1:2000 dilution of E-cadherin antibody, a 1:100 dilution of N-cadherin antibody, or 5 μg/ml pan-ras antibody, washed once in blocking buffer, then incubated for 1 h with rabbit anti-mouse IgG (H and L chains, Cappel/Cooper Biomedical) at 1:500 in blocking buffer. The blots were then washed once in blocking buffer and either detected by incubation for 30 min with 1 μCi/ml ^32^P-protein A followed by washing and autoradiography or by using enhanced chemiluminescence.

**Ras Immunoprecipitation**—The method is essentially the same as that described previously (16). Cells were cultured in 100-mm dishes until subconfluent, and cells were prelabeled overnight with [3H]Simeionethane (625 μCi/ml, 1220 Ci/mmol) and lysed in 0.5 ml of buffer consisting of 0.5% Nonidet P-40, 50 mM Tris-HCl, pH 7.5, 20 mM MgCl_2, 150 mM NaCl, 1 mM Na_3PO_4, pH 7.4, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 10 μg/ml benzamidine, 10 μg/ml soybean trypsin inhibitor, and 1 mM phenylmethylsulfonyl fluoride. 10% of monolocal antibody Y13–259 was added and incubation proceeded for 1 h. p21 ^14-Val^13–259 complexes were precipitated by addition of 10 μl of rabbit anti-rat IgG (Cappel) (about 20 μg) per mg of total protein, which had been prebound for 1 h with 60 μl of protein A-Sepharose, and then cleared of unbound antibody by centrifugation, followed by incubation for 10 min with 10 μl of 2% bovine serum albumin. The p21 ^14-Val^13–259 complexes and rabbit anti-rat IgG prebound to protein A and saturated with bovine serum albumin were incubated for an additional hour and washed two times with lysis buffer and three times with TBST (25 mM Tris, pH 8, 125 mM NaCl, 0.025% Tween 20) before analysis.

**Northern Analysis for CEA and Cathepsin B mRNA Analysis**—20 μg of total RNA from each cell line was electrophoresed in a 0.8% agarose-formaldehyde gel. 0.5% agarose-formaldehyde gel was stained with 0.5 μg/ml ethidium bromide to visualize the RNA. Northern analysis was performed on normal horse serum (Life Technologies, Inc.) in PBS containing 2% bovine serum albumin (PBS diluent) and then incubated in antibody col-1 at a 1:50 dilution in PBS overnight at 4°C. After 3 washes in PBS diluent, 1:67 dilution of biotinylated horse anti-mouse IgG in PBS diluent was added for 30 min, followed by ABC reagent and detection using the ABC kit manufacturer’s instructions. Slides were counterstained with Harris’ hematoxylin.

**Culture on Transwells**—Cells were cultured on Costar transwells with 2 ml of medium under the layer and 1.5 ml above the layer for 2 weeks postconfluence with media changes 3 times a week, fixed, and analyzed for CEA production by immunocytochemistry as above.

**Aggregation Assay**—Cells were harvested by gentle trypsinization for 3 min with 0.025% trypsin, 0.01% EDTA in 0.15 μl NaCl. Cells were washed once with 10 ml EDTA in 1 × PBS, pelleted, and resuspended at 2 × 10^5/ml in 5 ml EDTA, 1 × PBS followed by disaggregation by repeated forceful passages through a transfer pipette. 1-ml aliquots in a 15 × 100-mm test tube were shaken gently for the indicated times at a 45° angle in an orbital shaker at room temperature. A sample was taken gently with a Pasteur pipette and counted visually using a hemacytometer. For each determination, duplicate aggregation experiments were performed. 5 ml CaCl_2 was added to the aggregation experiments performed in the presence of calcium. Anti-CEA monoclonal antibody col-1 and control isotype monoclonal antibody MOPC-21 at 1 μg/ml were digested with papain as described (5). 100 μg/ml Fab’ fragments were added to each aggregation assay.

**RESULTS**

**Effects of Ki-ras 4BVal-12 but not Ha-ras 8Val-12 on N-cadherin and CEA Expression in Colon Epithelial Cells**—Transfections of HD6-4 colon epithelial cells had been performed with a mini-gene construct of the cellular Ki-ras 4B gene modified at codon 12 to valine (Ki-rasG12V), an expression plasmid encoding a mini-gene construct of the wild-type cellular Ki-ras 4B gene, and a cellular Ha-rasG12V expression plasmid. We isolated three independent transfectant clones expressing the Ki-ras 4BVal-12 oncogene: 4V, 4V1, and 4V2; three independent transfectant clones expressing the Ha-rasG12V oncogene: H15, H18, and H25; and transfectant clone 4G1, expressing the...
transfected wild-type Ki-ras4B gene. The transfected oncogenes were expressed as active proteins, which had been identified by Western blotting with pan-ras \(^{Val-12}\) antibody, and these oncogenic proteins bound elevated levels of GTP. Whereas the Ki-ras transfectants displayed elevated levels of Ki-Ras proteins and the Ha-ras transfectants displayed elevated levels of Ha-Ras proteins, as expected, 1 all the transfectants expressed equal amounts of total Ras proteins as shown by Western blotting of cell lysates with a pan-Ras antibody (Fig. 1A) or by immunoprecipitation from \(^{35}S\)-prelabeled cells (Fig. 1B). Possibly expression of the transfected oncogenic Ras proteins down-regulated levels of endogenous Ras proteins.

In an earlier study, we observed that the culture of HD6-4 cells in low calcium medium prevented both apicobasal and basolateral polarization (5). When HD6-4 cells polarized, levels of E-cadherin increased 35-fold and mediated lateral adherence between adjacent cells to form the HD6-4 monolayer. Cells form lateral attachments and polarize so that their nuclei are found at the basal end of the cell, and CEA expression as identified by immunocytochemis-

expression of oncogenic Ki-ras unexpectedly led to decreased levels of the adult adhesion protein N-cadherin in each of the three transfected lines (Fig. 3).

We then examined CEA expression by Northern analysis. Transcripts representing CEA (3.0 kb) and a CEA family member called NCA at 2.6 kb were detected in each cell line (Fig. 4). Each of the three transfected clones expressing the Ki-ras\(^{4B}G^{12V}\) oncogene: 4V, 4V1, and 4V2, expressed elevated levels of CEA mRNA and decreased levels of NCA mRNA compared with each of the three independent transfected clones expressing the Ha-ras\(^{G12V}\) oncogene: H15, H18, and H25, and the parental line (Fig. 3). Thus, expression of oncogenic Ki-ras elevated CEA expression at the transcriptional level. Oncogenic Ha-Ras proteins in the Ha-transfectant cells were expressed at roughly equivalent protein levels to the oncogenic Ki-Ras proteins in the Ki-transfectant cells as assayed by Western blotting 1 (Fig. 1). Thus, the inability of oncogenic Ha-Ras proteins to increase CEA transcription in these colon epithelial cells was not due to low levels of expression of Ha-Ras proteins.

Elevated Levels of CEA Shown to Be Caused By Oncogenic Ki-ras Gene By Use of Gene-specific Antisense Oligonucleotides—Blocking transcription of the transfected oncogenic Ki-ras gene in 4V2 cells blocked expression of CEA. A 4-h treatment with oligonucleotides (see “Experimental Procedures”) was followed by a 48-h chase to allow turnover of the endogenous Ki-Ras mRNA and protein. In each of three experiments a decrease in CEA protein abundance was seen in 4V2 cells treated with antisense oligonucleotides directed to the Ki-ras gene (A) compared with 4V2 cells treated with random sequence oligonucleotides (R) or untreated cells (C) (Fig. 5). Two concentrations of oligonucleotides were used. 100 \(\mu\)M of the antisense oligonucleotide decreased CEA expression marginally, whereas 200 \(\mu\)M almost completely blocked CEA expression although CEA expression was unaltered by either concentration of the random sequence oligonucleotide. Thus, the increase in CEA expression seen in each of three independently cloned transfected lines is not due to some extraneous event in the establishment of these lines but is directly due to the functioning of the oncogenic c-Ki-ras gene.

CEA in Unpolarized Ki-ras Transfectants Is Expressed All Along the Membrane—The HD6-4 parental line and the HD6-4G1 line grow as monolayers when grown in vitro on a transwell that allows feeding through the basal surface as occurs in vivo (Fig. 6, panels 1–3). Cells form lateral attachments and polarize so that their nuclei are found at the basal end of the cell, and CEA expression as identified by immunocytochemis-

\(\text{Fig. 1. A, Western blot of lysates from oncogenic Ha-ras transfectants (H15, H18, H25), oncogenic Ki-ras transfectants (4V1, 4V2, 4V), wild-type Ki-ras transfectant 4G1, and parental line HD6-4 showing equal expression of Ras proteins in all lines. B, SDS-polyacrylamide gel electrophoresis gels showing that equal concentrations of Ras proteins were immunoprecipitated from \(^{35}S\)-prelabeled parental HD6-4, wild-type Ki-ras control cells HD6-4G1, mutated Ki-ras containing HD6-4V cells, and mutated Ha-ras containing H18 cells. + lanes, immunoprecipitations with anti-ras antibody Y13–259; unmarked lanes, control immunoprecipitations with normal rat serum. }\) marks the position of Ras proteins. Molecular weight markers are 15.7, 18.9, 29.1, 44.5, 67.5, 103, and 202 kDa, indicated by dashes on the left.

\(\text{Fig. 2. Western blot showing similar abundance of E-cadherin in parental HD6-4, Ki-ras}^{Val-12}\text{ transfectant 4V2 cells, Ha-ras}^{Val-12}\text{ transfectant H18 cells, and wild-type Ki-ras}^{Gly-12}\text{ transfectant 4G1 cells.}

\(\text{Fig. 3. Upper panel, Western blots showing increased CEA protein abundance in each of three Ki-ras}^{Val-12}\text{ but not in any of three Ha-ras}^{Val-12}\text{ colon epithelial cell transfectants compared with parental HD6-4 cells. Middle panel, Western blot showing decreased N-cadherin protein abundance in Ki-ras}^{Val-12}\text{ but not Ha-ras}^{Val-12}\text{ colon epithelial cell transfectants. Lower panel, Western blot showing increased cathepsin B protein in Ki-ras}^{Val-12}\text{ but not Ha-ras}^{Val-12}\text{ colon epithelial cell transfectants. Abundance of mature single chain of cathepsin B at 31 kDa is shown.}\)
Expression of Ki-ras4B val12 Leads to Use of CEA as an Adhesion Molecule—Rounded, unpolarized HD6-4V cells express CEA all along their cell membrane (Fig. 6) and thus can use CEA to aggregate (Fig. 7). Cultures of the parental HD6-4 line, the wild-type Ki-ras control HD6-4G1 line, and the oncogenic Ki-ras HD6-4V line were dissociated to single cells. The capacity of each cell preparation to aggregate was tested by performing the aggregation under permissive conditions in calcium-containing medium, which would allow adherence through cadherins and would not block adherence through CEA (Fig. 7C). In these preparations about 70% of the parental and wild-type Ki-ras transfectants and 50% of the HD6-4V line aggregated. A parallel aggregation experiment was carried out in medium without calcium. Under these conditions only calcium-independent adhesion could occur such as that mediated by CEA, whereas cadherin-mediated adhesion that requires calcium would be blocked. Cells were gently agitation in calcium-free medium in the presence of Fab fragments of an isotype control antibody, MOPC-21. Neither the parental HD6-4 cells nor the wild-type ras transfectant HD6-4G1 cells were able to aggregate in the absence of calcium (<10%) showing that they did not use their apical cell surface CEA (Fig. 7A). However, half of the unpolarized HD6-4V cells capable of aggregation in vitro (Fig. 7C) used their cell surface CEA molecules to aggregate (Fig. 7A). CEA was shown to be the adhesion molecule as HD6-4V cells were able to form aggregates in the presence of the isotype control antibody (Fig. 7A) but not in the presence of Fab’ fragments of a monoclonal antibody to CEA, col-1 (Fig. 7B).

Expression of Ki-ras4B val12 but Not Ha-ras val12 Leads to Increased Expression of Cathepsin B—When assayed for in vitro invasion through a collagen I coated modified Boyden chamber (17), HD6-4V cells exhibited over double the invasion capability as the HD6-4G1 control transfectant line (28 ± 9 versus 11 ± 3 invasive cells per field, n = 8, mean ± S.E.). We wondered whether the oncogenic Ki-ras gene had increased expression of a protease associated with colon cancer invasion. We investi-
gated expression of the lysosomal protease cathepsin B as transfection of the c-Ha-ras oncogene into MCF-10 human breast epithelial cells increased the protein level and the membrane association of cathepsin B (18). Analysis by Western blotting (Fig. 3) demonstrated an increase in expression of mature single chain cathepsin B protein of 31 kDa in each of the three transfectant clones expressing the Ki-ras4BVal-12 oncogene: 4V, 4V1, and 4V2, compared with each of the three independent transfectant clones expressing the Ha-rasG12V oncogene: H15, H18, and H25, and the parental line, as well as the wild-type Ki-ras transfectant (data not shown). However, the increase in cathepsin B expression was post-transcriptional as HD6-4V cells exhibited no increase in cathepsin B mRNA expression level compared with control cells (Fig. 8), which is similar to the post-transcriptional regulation observed in Ha-ras transformed MCF-10 human breast epithelial cells (18). Two cathepsin B transcripts were seen in colon epithelial cells, one of 4 kb and one of 2 kb. Duplicate experiments showed no increase in either 4-kb or 2-kb cathepsin B transcripts in HD6-4V cells compared with wild-type Ki-ras transfectant 4G1 cells or parental cells, normalized to 18 S rRNA levels by densitometry analysis. Thus, cathepsin B expression is controlled by post-transcriptional mechanisms in both breast cancer cells transfected with oncogenic Ha-ras (18) and in colon cancer cells transfected with oncogenic Ki-ras (this study). Cathepsin B is present in vesicles in the apical region of normal colon epithelial cells and some benign tumor cells, but with progression to carcinoma, vesicles staining for cathepsin B are redistributed to the basal pole of the cell just inside the basal membrane adjacent to the underlying basement membrane (19). Altered intracellular trafficking of cathepsin B and its membrane association may be caused by ras-induced morphological changes in both Ha-ras-transfected breast cancer cells (18) and in Ki-ras-transfected colon cancer cells (this study).

**DISCUSSION**

The mammalian ras gene family comprises a highly conserved gene family of 21 kDa GDP/GTP-regulated switches, which relay signals from receptor tyrosine kinases to multiple effectors including the mitogen-activated protein kinases, phosphatidylinositol-3-OH kinase, and GAP, among others. Three mammalian ras isoforms, Ki-, Ha- and N-ras, share many biochemical, structural, and functional characteristics including localization to the inner surface of the plasma membrane. Transforming ras mutations lock ras molecules in the active GTP-bound state and are found in about 40% of all human cancers. However, Ki-, Ha-, and N-ras must play different roles in transformation in different cell types. A comparison of the three human ras genes, each carrying a G12D mutation, demonstrated that oncogenic Ha-ras was more transforming in rat-2 and NIH3T3 fibroblasts, whereas oncogenic N-ras was more transforming in hematopoietic TF-1 cells (20). The vast majority of ras mutations in colon cancers are in Ki-ras with only a few percent in N-ras and none reported in Ha-ras (8). The selection of Ki-ras mutations in the genesis of colon cancers suggested that mutations in this ras isoform might have different effects on colon epithelial cells than mutations in Ha-ras.

In an earlier study we demonstrated that oncogenic Ki-ras4BVal-12, but not oncogenic Ha-rasVal-12, blocks the apico-basal polarization of colon epithelial cells by preventing normal glycosylation of the β chain of the collagen receptor α2β1 integrin. We now show that oncogenic Ha-rasVal-12 up-regulates expression of CEA on the mRNA and protein levels in colon epithelial cells and coincidentally down-regulates expression of the homophilic cell adhesion molecule N-cadherin. Ectopic expression of CEA in melanoma cells was shown to decrease transcription of another homophilic cell adhesion molecule, NCAM (21). Thus, CEA may function to selectively alter expression of other cell adhesion molecules. In an earlier study we found that preventing the expression of E-cadherin by culture in low calcium medium led to the up-regulation of CEA in colon epithelial cells (5). Possibly the cell can express either embryonic adhesion molecules like CEA or the cadherin class of calcium-dependent adhesion molecules. Up-regulation of CEA by either transfection (21) or in this study, expression of oncogenic Ki-ras4BVal-12 may inhibit transcription of one or more cadherins typically expressed by the cell. In the current study, N-cadherin, but not E-cadherin, was down-regulated by expression of CEA. Up-regulation of CEA by oncogenic Ki-ras was paralleled by down-regulation of a closely related homotypic adhesion molecule, NCA. NCA is also up-regulated in various cancer cells but apparently not by oncogenic Ki-ras.

CEA is a cell surface glycoprotein and intercellular adhesion protein that is often increased in abundance and unrestricted in spatial expression in cancer cells (22). CEA was originally described as an oncofetal protein in colon carcinoma cells. During the early weeks of gestation, the human embryonic intestine is multilayered and exhibits CEA expression in adjacent cell membranes when cells develop in multilayered arrays. At later times in gestation and in the adult, the colonic crypts form a palisaded monolayer and CEA is localized to the apical luminal membrane (23). In the current studies, HD6-4V cells could not polarize and expressed CEA molecules all over the cell surface. When HD6-4V cells aggregated in multilayered cell arrays, they expressed CEA at points of contact where CEA mediated intercellular adhesion, reproducing the embryonic phenotype.

CEA is known to enhance experimental metastasis (24), probably through its capacity to mediate cell adhesion. CEA injected intravenously into nude mice before injection of tumor cells with low metastatic potential enhanced metastasis by increasing the retention of such tumor cells within the liver and lungs 4 h after intrasplenic injection (25). A strong correlation was found between formation of metastases and early retention of tumor cells after injection. Cell aggregates formed by cell surface CEA molecules may more readily implant in
sites of metastasis than single cells. CEA mediated homotypic aggregation of LS-180 colon carcinoma cells (23) and indirectly mediated colon carcinoma attachment to collagen (9) probably because of its adhesive cell to cell properties. The familiar pattern of colon carcinoma cell multilayered growth may result, at least in part, from the adhesive properties of overexpressed and inappropriately localized CEA.

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