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Genetics

Carcinoembryonic antigen family: Expression in a mouse L-cell transfectant and characterization of a partial cDNA in bacteriophage λgt11

(gene transfection/fluorescence-activated cell sorting/chromosome 19/tumor antigen/cancer diagnosis)

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ABSTRACT **Genomic DNA and mRNA from the adenocarcinoma cell line LoVo were used to generate L-cell transfectants and a bacteriophage λgt11 cDNA clone that express epitopes of carcinoembryonic antigen (CEA). Primary and secondary L-cell transfectants expressing CEA were selected with a fluorescence-activated cell sorter (FACS). These transfectants, including some clones that were selected for high-level CEA expression by multiple rounds of FACS sorting, express a surface protein of 150 kDa that reacts with all anti-CEA antibodies tested. In parallel, a cDNA library of LoVo poly(A)$^+$ RNA was constructed in λgt11 and fusion proteins were screened with polyclonal antisera against CEA. One positive clone, λcLV7, was identified that hybridized specifically to transfectant DNA. The nucleic acid sequence of the cDNA insert (cLV7) contained two regions of extensive internal homology, with greater than 70% identity at the amino acid level. cLV7 hybridized to three mRNA species of LoVo cells and to a predominant mRNA of the CEA-expressing transfectants. Hybridization of cLV7 to restriction endonuclease-digested genomic DNA of colon carcinoma cells, normal human cells, and human–mouse somatic cell hybrids revealed the presence of multiple hybridizing bands, one of which was present in transfectant cells. These CEA-related sequences are not rearranged in tumors and, by somatic cell hybrid analysis, were mapped to human chromosome 19.**

Carcinoembryonic antigen (CEA) was originally identified as a tumor-associated antigen of the digestive tract by Gold and Freedman (1). It was subsequently characterized as a 180-kDa glycoprotein containing 50–60% carbohydrate by weight. The antigen is developmentally regulated during embryogenesis and is subsequently reexpressed during the development of several tumor types of epithelial origin. Although present on the surface of cells expressing it, CEA is also released by many tumors into extracellular fluid. Numerous immunoassays have been developed to measure serum concentrations of CEA, and these tests are widely used for clinical monitoring of cancer patients.

There is extensive biochemical evidence for the existence of a family of 6–10 molecules that share epitopes with CEA (2). Normal fecal antigen 2 (NFA-2) (3), for example, is almost indistinguishable from CEA, whereas other family members, such as normal crossreacting antigen (NCA) (4), are readily distinguished biochemically and immunologically yet share epitopes with CEA. Because of these crossreactive antigens, CEA immunoassays do not necessarily measure only the CEA released by tumors. Limited protein sequencing of some of the CEA-related antigens has revealed a high degree of amino-terminal sequence homology between molecules (5). However, the large size and extensive glycosylation of CEA-related molecules have made complete protein sequence analysis difficult.

As an alternative to protein sequencing, we have directed our efforts at molecular cloning to obtain primary sequence information on the CEA-related antigens, and we describe our initial results in this paper. Using genomic DNA from the adenocarcinoma cell line LoVo, we used gene transfer and fluorescence-activated cell sorter (FACS) selection to isolate L-cell transfectants that express one of the CEA-related antigens. In parallel, using mRNA from LoVo, we used the λgt11 expression system to isolate a partial cDNA that contains sequences homologous to those transfected into L cells. The cDNA clone was then used to deduce a protein sequence, to establish the presence of a CEA-related sequence family, and to map all of these homologous sequences to human chromosome 19.

MATERIALS AND METHODS

Cells. The human carcinoma cell lines LoVo (CCL 229) and LS174T (CCL 188) and a human colon mucosal cell line (HCMC; CCL 239) were obtained from the American Type Culture Collection and were maintained in Dulbecco's Modified Eagle's Medium (GIBCO) with 10% fetal bovine serum (KC Biological, Lenexa, KS). Epstein–Barr virus-transformed lymphoid cells GM1989 and GM1445 were obtained from the Human Genetic Mutant Cell Repository (Camden, NJ) and maintained in RPMI 1640 (GIBCO) with 10% fetal bovine serum. Mouse L cells were deficient in thymidine kinase (TK; EC 2.7.1.21) (6) or hypoxanthine phosphoribosyltransferase (HPRT; EC 2.4.2.8) (7). Cotransfection of TK$^-$ L cells with genomic DNA and the herpes simplex virus TK gene was performed as described (8). Somatic cell hybrids were produced by the fusion of primary human fibroblasts with HPRT$^-$ L cells and were selected and characterized by established methods (9).

Antisera. Polyclonal and monoclonal antibodies against CEA were obtained from Dako (Santa Barbara, CA), Hybritech (San Diego, CA), and Zymed Laboratories (San Francisco, CA). Antisera to native CEA (Scripps Laboratories, San Diego, CA) and to reduced and alkylated CEA (10) were prepared in New Zealand White rabbits (Hazelton Research Animals, Denver, PA). CEA-expressing L-cell transfectants were used to immunize C3H/HeJ × BALB/ByJ mice (The Jackson Laboratory) by published methods (11).

Immunoassays. Transfectant cells were removed from flasks by incubation with Dulbecco's phosphate-buffered saline containing 0.03% ethylenediaminetetraacetic acid (EDTA) and then were labeled by indirect immunofluores-

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Abbreviations: CEA, carcinoembryonic antigen; FACS, fluorescence-activated cell sorter; HPRT, hypoxanthine phosphoribosyltransferase; TK, thymidine kinase.

cence as described (12). Fluorescence analysis and sterile cell sorting (13) were performed with a FACS IV (Becton Dickinson FACS Systems). A sandwich enzyme-linked immunosorbent assay (ELISA) using native CEA was performed by coating 96-well plates with rabbit polyclonal anti-CEA IgG (Dako) (14). Binding of mouse antibodies to CEA was detected by using peroxidase-conjugated rabbit anti-mouse immunoglobulin (Miles Scientific, Naperville, IL) with tetramethylbenzidine as substrate.

Cell Surface Labeling and Immunoprecipitation. LoVo and 23.4 8^+ cells were surface-labeled using lactoperoxidase and $Na^{125}I$ (15). Cells were lysed in phosphate-buffered saline containing 1% (vol/vol) Triton X-100, 10 mM benzamidine, 10 mM EDTA, and 1 mM diisopropyl fluorophosphate. Immunoprecipitation was performed with rabbit-anti-CEA (Dako) by the method of Krangel *et al.* (16), and radioactive proteins were resolved by $NaDodSO_4$/PAGE (17) and visualized by fluorography at −70°C.

RNA Isolation and cDNA Cloning. Cytoplasmic RNA was isolated (18) and poly(A)$^+$ RNA was prepared by the method of Aviv and Leder (19). Double-stranded DNA complementary to 5 μg of LoVo poly(A)$^+$ RNA was synthesized (20) by using random [$(pdN)_6$] and oligo(dT) primers. Dephosphorylated *Eco*RI arms of λgt11 (Promega Biotec, Madison, WI) were ligated with an equimolar amount of cDNA and then packaged *in vitro*, using a commercially available extract (Stratagene, San Diego, CA). After infection of *Escherichia coli* Y1090 (r_K^- m_K^+) cells, a library of 1.2×10^6 recombinant phage was obtained and used for further screening.

cDNA Screening and Sequencing. Recombinant proteins of the cDNA library were induced and screened using a mixture of commercial CEA antibody (Dako) and rabbit antisera generated against native and also reduced and alkylated CEA. Positive plaques were visualized after incubation with mouse anti-rabbit IgG conjugated with alkaline phosphatase (Promega Biotec). Plaques positive after three rounds of immunoscreening were purified, amplified, and used for minilysate DNA preparation (21). cDNA inserts from λgt11 recombinants were excised and subcloned into M13mp8 or -mp9 phage cloning vector for primer-extended single-stranded DNA sequencing by the dideoxy chain-termination method (22).

Nucleic Acid Blot Analysis. Total cytoplasmic or poly(A)$^+$ RNA was denatured, electrophoresed in a 1% agarose/2.2 M formaldehyde gel, and transferred to nitrocellulose membrane (23). Filter-blotted RNAs were hybridized at 68°C with randomly primed ^{32}P-labeled (24) cLV7 cDNA under standard hybridization conditions. Denatured RNA markers ranged in size from 0.3 to 9.5 kilobases (kb) (Bethesda Research Laboratories). Cell DNAs were digested with either *Bam*HI or *Eco*RI endonuclease, electrophoresed in 1% agarose gels, and transferred, after partial acid depurination, to nitrocellulose sheets according to Southern (25). For hybridization with ^{32}P-labeled cLV7, conditions were either 50% (vol/vol) formamide/5× SSPE at 42°C or 2× SSPE at 68°C. Posthybridization washes were in 0.1× SSPE at 68°C. SSPE (1×) is 0.18 M NaCl/10 mM sodium phosphate, pH 7.0/1 mM EDTA.

RESULTS

Isolation of L-Cell Transfectants Expressing CEA. TK^- L cells were cotransfected with genomic DNA from the human carcinoma line LoVo and with a plasmid containing the herpes simplex virus TK gene. Approximately 20,000 hypoxanthine/aminopterin/thymidine (HAT)-resistant colonies were recovered, separated into four pools, and labeled by indirect immunofluorescence using a mixture of anti-CEA antibodies. The brightest 0.5% of the cell populations were selected by FACS, expanded in cell culture, and resorted (13). A population of CEA-positive cells was recovered from one of the four pools of transfectants, and single cells were cloned by FACS for further analysis. DNA from one of these clones was used to repeat the transfection and selection procedures, and CEA-positive transfectants were isolated from 20,000 HAT-resistant secondary clones.

Among the positive secondary transfectants were cells that expressed elevated levels of surface CEA. This cell population, 23.4, was repeatedly sorted for high-level antigen expression. After eleven FACS selections, a stable "amplificant" was isolated and cloned (23.4 11+). This transfectant displays ≈20 times the surface fluorescence of cloned primary and secondary transfectants (Fig. 1).

Cell Surface Expression of CEA on Transfectants. The CEA-like surface antigens expressed by the L-cell transfectants were analyzed with a battery of antibodies including commercially available rabbit polyclonal anti-CEA antibodies (Dako and Zymed) and mouse monoclonal anti-CEA antibodies (Hybritech and Zymed). All antibodies reacted strongly with cloned primary, secondary, and amplified transfectants but not with parental L cells (data not shown). Surface antigens expressed by an amplified secondary transfectant (23.4 8+) and by the parental LoVo cell were immunoprecipitated with anti-CEA antibodies and resolved by $NaDodSO_4$/PAGE. Cell surface antigens of 180 kDa and 150 kDa were specifically immunoprecipitated from LoVo and from the amplified transfectant, respectively (data not shown). Finally, mouse antisera were generated against 23.4 11+ cells and were tested for reactivity with native CEA by ELISA. As demonstrated in Fig. 2, this immunization produced a high-titer response to CEA.

Characterization of a CEA cDNA Clone. A cDNA library was prepared in λgt11 from LoVo poly(A)$^+$ RNA. Approximately 1.2×10^6 independent recombinants were screened, and one of these, designated λcLV7, was found to synthesize a β-galactosidase fusion protein that reacted with rabbit polyclonal anti-CEA antibodies. The cDNA insert (cLV7) of 900 base pairs was inserted into M13mp8 or -mp9 phage cloning vector for primer-extended single-stranded DNA sequencing. From the cLV7 cDNA sequence and the amino acid sequence encoded by the only open reading frame (Fig. 3), it is apparent that a region of 294 base pairs (98 amino acids) exists as a homologous repeat within the cDNA clone. These repeat regions are >80% homologous at the nucleic acid level. In addition, alignment of amino acids 9–107 with 187–285 provides 74% identity (72/98) of residues without the need for additions or deletions.

Analysis of CEA-Related mRNAs. Poly(A)$^+$ RNA was isolated from cell lines, resolved by gel electrophoresis, and hybridized with cLV7 (Fig. 4). Three bands of molecular size

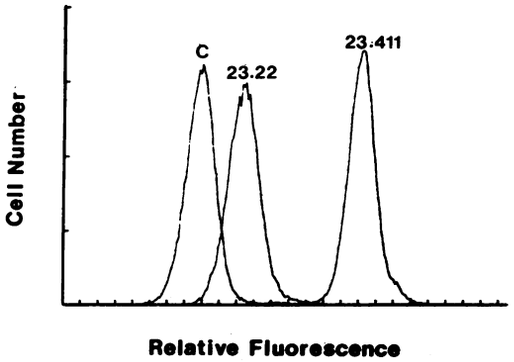

FIG. 1. FACS analysis of CEA expression by an L-cell secondary transfectant (23.22), an amplified secondary transfectant (23.4 11+), and TK-transfected L cells (C). Cells were stained by indirect immunofluorescence, using a rabbit polyclonal antibody (Dako), and analyzed for relative logarithmic fluorescence with the FACS 440 (13).

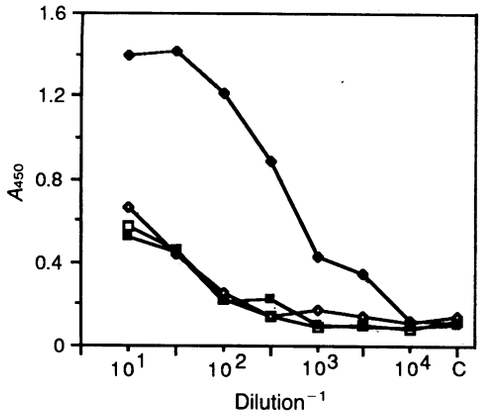

FIG. 2. Reactivity of anti-transfectant (23.4 11+) antibodies with CEA. Rabbit polyclonal anti-CEA IgG (Dako) was used to immobilize native CEA. Serial dilutions of normal serum (◇, □) or antiserum to transfectant 23.4 11+ (◆, ■) were added and the binding of mouse IgG was determined by reaction with peroxidase-conjugated rabbit anti-mouse immunoglobulin. □ and ■, background binding of normal serum immunoglobulin and immune serum immunoglobulin, respectively, in the absence of added CEA. ◇ and ◆, binding of normal serum immunoglobulin and immune serum immunoglobulin, respectively, in the presence of 50 ng of CEA per well. C (control) on the abscissa indicates infinite dilution (i.e., no serum).

4.0 kb, 3.6 kb, and 3.0 kb were detected in LoVo poly(A)$^+$ RNA (Fig. 4*A*, lane c; Fig. 4*B*, lane b), of which the 3.6-kb species comprised ≈75%. No hybridization could be detected to mRNA from a nontransformed, nontumorigenic colon mucosal cell line (HCMC), Epstein–Barr virus-transformed lymphoblasts (GM1989 and GM1445), thymic epithelium, or placenta (Fig. 4*A*). In contrast to the bands seen in LoVo, a predominant band at 3.9 kb and minor bands at 3.7 kb and 2.2 kb were detected in the amplified transfectant 23.4 11+ (Fig. 4*B*, lane a).

cLV7 Sequences in Tumor, Normal, and Transfectant Cells. Restriction enzyme-digested genomic DNA from cells and cell lines was resolved by gel electrophoresis and hybridized with cLV7 (Fig. 5). At least seven major bands and some minor ones were detected in human genomic DNA cleaved with either *Eco*RI or *Bam*HI restriction endonuclease. In contrast, only one band was detected in each digest of the transfectant lines. The cLV7 cDNA hybridized to a large (≈20-kb) *Eco*RI segment and to a 5.3-kb *Bam*HI segment in transfectant DNA. The intensity of hybridization to these segments correlated with increasing CEA expression in amplified secondary transfectants (Fig. 5). No differences in sizes or intensities of DNA segments were detected in the hybridization patterns of normal and tumor DNA (Fig. 5, XY vs. LoVo and LS174T).

Chromosome Mapping of cLV7 Sequences. A human–mouse somatic cell hybrid panel (26) was used to map cLV7-hybridizing sequences to a human chromosome. Hybrid-cell DNA was digested with *Bam*HI, fractionated by gel electrophoresis, and hybridized with cLV7. Of the eight somatic cell hybrids tested, only three (IIIMA9-4, IIIMA9-13, and VIMA9-14B) contained human sequences that hybridized to the cDNA probe (Fig. 6). Specific crossreactive hybridization to mouse (A9 cell line) DNA was detected in all of the human–mouse hybrids. Identical results were obtained when the hybrids were digested with *Eco*RI and probed with cLV7 cDNA (data not shown). All of the hybridizing bands segregate concordantly in the hybrid lines, indicating that they are syntenic. Correlation of this hybridization pattern with the partial human genomes in the hybrid cells (26) allows the unambiguous assignment of the CEA-related sequences to human chromosome 19.

DISCUSSION

Using two expression systems, we isolated DNA sequences encoding CEA or a related family member. A functional genomic gene was introduced by transfection into mouse L cells. These transfectants contained transcribed DNA sequences that hybridized to a partial cDNA (cLV7) that was isolated by expression in λgt11. This result demonstrated the convergence of our independent experimental approaches. The cDNA is homologous to at least seven distinct restriction segments in the human genome. If each of these represents a functional gene, as in the transfectant that shows a single hybridizing segment with cLV7, then these segments could account for the large number of CEA-related antigens that have been identified by biochemical studies (2). Somatic cell genetic analysis localized all of these hybridizing sequences to human chromosome 19, suggesting the existence of a CEA gene locus consisting of multiple, homologous genes.

The CEA-related molecule expressed by the L-cell transfectants reacted with all anti-CEA antibodies tested and induced the production of anti-CEA antibodies in immunized mice (Fig. 2). The antigen amplification in some of the secondary transfectants has facilitated both biochemical and genetic studies of this gene. The transfectant antigen has a molecular mass of ≈150 kDa, distinctly smaller than the 180-kDa CEA expressed on LoVo cells. This size difference could be due to different glycosylation or processing by mouse L cells (27) or due to the transfection of a CEA-related gene. It is clear that increased cell surface expression of the transfectant antigen results from a quantitative increase in gene copy number (Figs. 1 and 5). A genomic *Eco*RI segment of ≈20 kb and a *Bam*HI segment of 5.3 kb each contain the cLV7-homologous sequences in the transfectant, and these sequences have been recovered from a genomic phage library of transfectant 23.4 6+ (M.E.K., J.T.H., and T.R.B., unpublished data).

The cLV7 cDNA has been sequenced and the amino acid sequence of its only open reading frame has been deduced. The most striking feature of the sequence is the existence of internal repeats with a high degree of both nucleic acid and amino acid homology. Other significant structural features include six cysteine residues, which are regularly spaced within the sequence at intervals of 40–49 amino acids (Fig. 3). The disulfide loops potentially formed by these residues, and the homology domains in which they reside, are similar to those of the immunoglobulin superfamily members. In two of the three potential loops, there are found tyrosine and glycine residues at positions −2 and −4, respectively, from the second cysteine. These features are similar to those of other immunoglobulin family members such as Thy-1 (28), α_1B-glycoprotein (29), polymeric-immunoglobulin receptor (30), and the neural cell adhesion molecule (31). There are 12 potential sites for asparagine-linked oligosaccharides (Fig. 3), consistent with the high degree of N-linked glycosylation observed in CEA. In spite of the various potential similarities to members of the immunoglobulin superfamily, only short stretches of DNA (20–30 base pairs) or amino acid homology was observed upon searching the GenBank or the Protein Identification Resource (Dayhoff) data bases.*

There are at least three CEA-related mRNA species expressed in colon carcinoma (LoVo) cells (Fig. 4*A*). The apparent sizes of these messages are 4, 3.6, and 3 kb, with the 3.6 kb band being the predominant in intensity. In contrast, a major 3.9-kb mRNA species was detected in transfectant 23.4 11+ cells. The difference in sizes between LoVo and

*National Institutes of Health (1986) Genetic Sequence Databank: GenBank (Research Systems Div., Bolt, Beranek, and Newman, Cambridge, MA), Tape Release 40.0; and Protein Identification Resource (1986) Protein Sequence Database (Natl. Biomed. Res. Found., Washington, DC), Release 10.

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1          10         20         30         40         50         60         70         80         90
*          *          *          *          *          *          *          *          *          *
GG GGT TTA CAC AAC CAC CAC CCC ATC AAA CCC TTC ATC ACC AGC AAC AAC TCC AAC CCC GTG GAG GAT GAG GAT GCT GTA GCC TTA ACC TGT
   Gly Leu His Asn His His Pro Ile Lys Pro Phe Ile Thr Ser Asn Asn Ser Asn Pro Val Glu Asp Glu Asp Ala Val Ala Leu Thr CYS

         100        110        120        130        140        150        160        170        180
         *          *          *          *          *          *          *          *          *
GAA CCT GAG ATT CAG AAC ACA ACC TAC CTG TGG TGG GTA AAT AAT CAG AGC CTC CCG GTC AGT CCC AGG CTG CAG CTG TCC AAT GAC AAC
Glu Pro Glu Ile Gln Asn Thr Thr Tyr Leu Trp Trp Val Asn Asn Gln Ser Leu Pro Val Ser Pro Arg Leu Gln Leu Ser Asn Asp Asn

         190        200        210        220        230        240        250        260        270
         *          *          *          *          *          *          *          *          *
AGG ACC CTC ACT CTA CTC AGT GTC ACA AGG AAT GAT GTA GGA CCC TAT GAG TGT GGA ATC CAG AAC GAA TTA AGT GTT GAC CAC AGC GAC
Arg Thr Leu Thr Leu Leu Ser Val Thr Arg Asn Asp Val Gly Pro Tyr Glu CYS Gly Ile Gln Asn Glu Leu Ser Val Asp His Ser Asp

         280        290        300        310        320        330        340        350        360
         *          *          *          *          *          *          *          *          *
CCA GTC ATC CTG AAT GTC CTC TAT GGC CCA GAC GAC CCC ACC ATT TCC CCC TCA TAC ACC TAT TAC CGT CCA GGG GTG AAC CTC AGC CTC
Pro Val Ile Leu Asn Val Leu Tyr Gly Pro Asp Asp Pro Thr Ile Ser Pro Ser Tyr Thr Tyr Tyr Arg Pro Gly Val Asn Leu Ser Leu

         370        380        390        400        410        420        430        440        450
         *          *          *          *          *          *          *          *          *
TCC TGC CAT GCA GCC TCT AAC CCA CCT GCA CAG TAT TCT TGG CTG ATT GAT GGG AAC ATC CAG CAA CAC ACA CAA GAG CTC TTT ATC TCC
Ser CYS His Ala Ala Ser Asn Pro Pro Ala Gln Tyr Ser Trp Leu Ile Asp Gly Asn Ile Gln Gln His Thr Gln Glu Leu Phe Ile Ser

         460        470        480        490        500        510        520        530        540
         *          *          *          *          *          *          *          *          *
AAC ATC ACT GAG AAG AAC AGC GGA CTC TAT ACC TGC CAG GCC AAT AAC TCA GCC AGT GGC CAC AGC AGG ACT ACA GTC AAG ACA ATC ACA
Asn Ile Thr Glu Lys Asn Ser Gly Leu Tyr Thr CYS Gln Ala Asn Asn Ser Ala Ser Gly His Ser Arg Thr Thr Val Lys Thr Ile Thr

         550        560        570        580        590        600        610        620        630
         *          *          *          *          *          *          *          *          *
GTC TCT GCG GAC GTG CCC AAG CCC TCC ATC TCC AGC AAC AAC TCC AAA CCC GTG GAG GAC AAG GAT GCT GTG GCC TTC CAC TGT GAA CCT
Val Ser Ala Asp Val Pro Lys Pro Ser Ile Ser Ser Asn Asn Ser Lys Pro Val Glu Asp Lys Asp Ala Val Ala Phe His CYS Glu Pro

         640        650        660        670        680        690        700        710        720
         *          *          *          *          *          *          *          *          *
GAG GCT CAG AAC ACA ACC TAC CTG TGG TGG GTA AAT GGT CAG AGC CTC CCA GTC AGT CCC AGG CTG CAG CTG TCC AAT GGC AAC AGG ACC
Glu Ala Gln Asn Thr Thr Tyr Leu Trp Trp Val Asn Gly Gln Ser Leu Pro Val Ser Pro Arg Leu Gln Leu Ser Asn Gly Asn Arg Thr

         730        740        750        760        770        780        790        800        810
         *          *          *          *          *          *          *          *          *
CTC ACT CTA TTC AAT GTC ACA AGA AAT GAC GCA AGA GCC TAT GTA TGT GGA ATC CAG AAC TCA GTG AGT GCA AAC CGC AGT GAC CCA GTC
Leu Thr Leu Phe Asn Val Thr Arg Asn Asp Ala Arg Ala Tyr Val CYS Gly Ile Gln Asn Ser Val Ser Ala Asn Arg Ser Asp Pro Val

         820        830        840        850        860
         *          *          *          *          *
ACC CTG GAT GTC CTC TAT GGG CCG GAC ACC CCC ATC ATT TCC CCC CCC CC
Thr Leu Asp Val Leu Tyr Gly Pro Asp Thr Pro Ile Ile Ser Pro Pro
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FIG. 3. Sequence of cLV7 cDNA and its deduced amino acid sequence. Arrows above the sequence indicate the beginnings and ends of the homology domains. Potential N-glycosylation sites are underlined, and cysteine residues are shown in uppercase type.

transfectant mRNAs suggests that the CEA-like antigen of the transfectant should be distinguishable from the CEA expressed by LoVo cells, an expectation consistent with immunoprecipitation data. Although it is unlikely that more than one gene is present in the secondary transfectants, multiple hybridizing poly(A)$^+$ RNA bands were observed (Fig. 4*B*). It is possible that genes truncated during the transfection and amplification process are transcribed, resulting in additional mRNA species. Alternatively, both the transfectant mRNAs and the multiple message species in LoVo may represent products of differential RNA splicing.

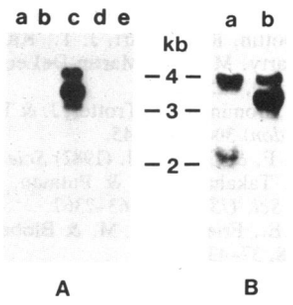

FIG. 4. Cellular expression of CEA-related mRNA. One microgram of poly(A)$^+$ RNA was electrophoresed in each lane before blotting and hybridization with ^{32}P-labeled cLV7 cDNA. (*A*) Poly(A)$^+$ RNA was isolated from freshly prepared cell pellets of lymphoblastoid cell lines GM1445 (lane a) and GM1989 (lane b), colon adenocarcinoma line LoVo (lane c), and colon mucosal line HCMC (lane d). RNAs from thymus epithelium (lane e) and placenta (lane f) were obtained from Clontech Laboratories (Palo Alto, CA). (*B*) Poly(A)$^+$ RNA was isolated from frozen cell pellets of transfectant 23.4 11+ (lane a) and colon carcinoma LoVo (lane b).

The hybridization of cLV7 to genomic DNA suggests an upper limit of seven to nine CEA-related genes on chromosome 19 (Figs. 5 and 6). However, the number of CEA-related molecules could be even larger if differential RNA splicing occurs within this sequence family. Using cLV7 as probe, we have isolated complete cDNAs from LoVo and transfectant mRNAs (T.R.B., J.T.H., A. Kretschmer, and M.E.K., unpublished data). These sequences will allow an examination of message processing and will facilitate functional studies of these molecules in epithelial cell differentiation and transformation. We have thus far established that gross rearrangement or amplification of the CEA gene does not necessarily precede or accompany colon cell oncogeny (Fig. 5).

The expression of CEA-related antigens on mouse cells has facilitated the production of specific antibodies in immunized syngeneic mice (Fig. 2). As complete cDNAs for additional CEA family members are cloned and expressed in L cells, it should be possible to generate monoclonal antibodies that

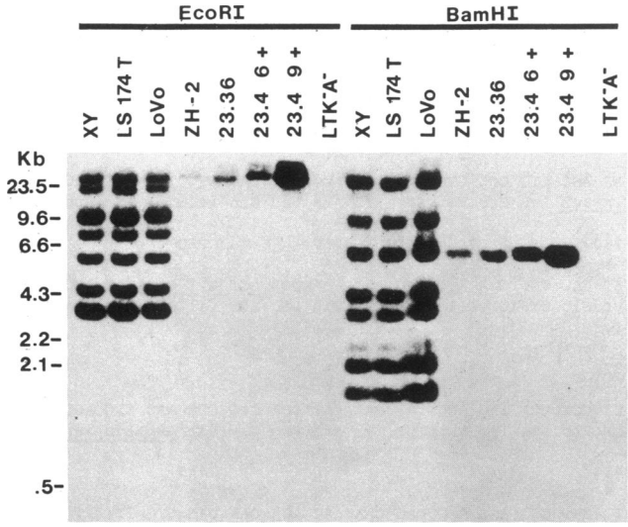

FIG. 5. DNA blot analysis of cLV7 sequences in tumor, normal, and transfectant genomes. DNA samples (10 μg) were digested to completion with restriction endonuclease *Bam*HI or *Eco*RI, electrophoresed, blotted, and probed with ^{32}P-labeled cLV7 insert. Samples are XY (normal male), LS174T and LoVo (colon carcinoma), ZH-2 (primary transfectant), 23.36 (secondary transfectant), 23.4 6+ (secondary transfectant selected six times), 23.4 9+ (secondary transfectant selected nine times), and LTK$^-$ A$^-$ (TK$^-$ L-cell line used for transfection). Markers at left indicate positions of *Hin*dIII fragments of phage λ DNA, run in parallel to provide size markers.

recognize conformational epitopes. It is also anticipated that comparisons of the primary sequences of CEA and related antigens will reveal unique sequences, which can be used as synthetic peptide immunogens to generate sequence-specific antibodies (32). The availability of reagents to distinguish the various CEA-related antigens would increase the reliability and sensitivity of CEA detection for cancer diagnosis.

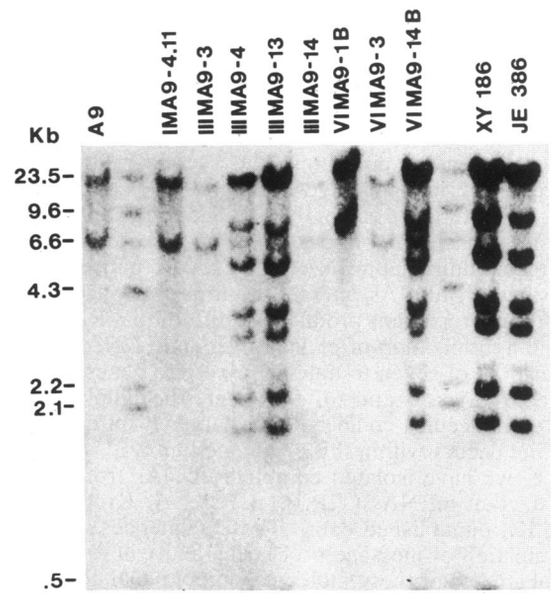

FIG. 6. DNA blot analysis of cLV7 sequences in mouse, human, and human–mouse hybrid cell lines. DNA samples (10 μg) were digested to completion with *Bam*HI, electrophoresed, blotted, and probed with ^{32}P-labeled cLV7 insert. In addition to hybrid cell lines (26), samples include the mouse cell line A9 (mouse parent line for the hybrids) and XY186 and JE386 (normal males). Unlabeled lanes contained *Hin*dIII-digested phage λ DNA used as size markers.

Note Added in Proof. Zimmermann et al. (33) and Oikawa et al. (34) have reported partial cDNA sequences for CEA that confirm the results presented here.

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1. Gold, P. & Freedman, S. O. (1965) *J. Exp. Med.* **122,** 467–481.
2. Shively, J. E. (1985) *CRC Crit. Rev. Oncol. Hematol.* **2,** 355–399.
3. Kuroki, M., Koga, Y. & Matsuoka, Y. (1981) *Cancer Res.* **41,** 713–720.
4. Grunert, F., AbuHarfeil, N., Schwarz, K. & von Kleist, S. (1985) *Int. J. Cancer* **36,** 357–362.
5. Engvall, E., Shively, J. E. & Wrann, M. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 1670–1674.
6. Kit, S., Dubbs, D., Pierarski, L. & Hsu, T. (1963) *Exp. Cell Res.* **31,** 297–303.
7. Littlefield, J. W. (1966) *Exp. Cell Res.* **41,** 190–194.
8. Kuhn, L. C., Barbosa, J. A., Kamarck, M. E. & Ruddle, F. H. (1983) *Mol. Biol. Med.* **1,** 335–340.
9. Kamarck, M. E., Barker, P. E., Miller, R. L. & Ruddle, F. H. (1984) *Exp. Cell Res.* **152,** 1–14.
10. Brew, K., Castellino, F. J., Vanaman, T. C. & Hill, R. L. (1968) *Proc. Natl. Acad. Sci. USA* **59,** 491–497.
11. Dorman, B. P., Shimizu, N. & Ruddle, F. H. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 2363–2367.
12. Kamarck, M. E., Barbosa, J. A. & Ruddle, F. H. (1982) *Somat. Cell Genet.* **8,** 385–402.
13. Kamarck, M. E. (1987) *Methods Enzymol.* **151,** in press.
14. Moudallal, Z. A., Altschuh, D., Briand, J. P. & VanRegenmortel, M. H. V. (1984) *J. Immunol. Methods* **68,** 35–43.
15. Poduslo, J. F., Greenberg, C. S. & Glick, M. C. (1972) *Biochemistry* **11,** 2616–2621.
16. Krangel, M. S., Orr, H. T. & Strominger, J. L. (1979) *Cell* **18,** 979–991.
17. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
18. Favaloro, J., Treisman, R. & Kamen, R. (1980) *Methods Enzymol.* **65,** 718–749.
19. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1408–1412.
20. Gubler, V. & Hoffman, B. J. (1983) *Gene* **25,** 263–269.
21. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 371–373.
22. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
23. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5201–5205.
24. Feinberg, A. P. & Vogelstein, B. (1984) *Anal. Biochem.* **137,** 266–267.
25. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
26. Nigro, J. M., Schweinfest, C. W., Rajkovic, A., Pavlovic, J., Jamal, S., Dottin, R. P., Hart, J. T., Kamarck, M. E., Rae, P. M. M., Carty, M. D. & Martin-DeLeon, P. (1987) *Am. J. Hum. Genet.* **40,** 115–125.
27. Newman, R., Domingo, D., Trotter, J. & Trowbridge, I. (1983) *Nature (London)* **304,** 643–645.
28. Williams, A. F. & Gagnon, J. (1982) *Science* **216,** 696–703.
29. Ishioka, N., Takahashi, N. & Putman, F. W. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 2363–2367.
30. Mostov, K. E., Friedlander, M. & Blobel, G. (1984) *Nature (London)* **308,** 37–43.
31. Hemperly, J. J., Murray, B. A., Edelman, G. M. & Cunningham, B. A. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 3037–3041.
32. Lerner, R. A., Green, N., Alexander, H., Lin, F.-T., Sutcliffe, J. G. & Shinnick, T. M. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 3403–3407.
33. Zimmermann, W., Ortlieb, B., Friedrich, R. & von Kleist, S. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 2960–2964.
34. Oikawa, S., Nakazato, H. & Kosaki, G. (1987) *Biochem. Biophys. Res. Commun.* **142,** 511–518.