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Increased mRNA expression of a laminin-binding protein in human colon carcinoma: Complete sequence of a full-length cDNA encoding the protein

(laminin receptors/tumor markers/epithelial cells/extracellular matrices/metastasis)

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ABSTRACT **Reliable markers to distinguish human colon carcinoma from normal colonic epithelium are needed particularly for poorly differentiated tumors where no useful marker is currently available. To search for markers we constructed cDNA libraries from human colon carcinoma cell lines and screened for clones that hybridize to a greater degree with mRNAs of colon carcinomas than with their normal counterparts. Here we report one such cDNA clone that hybridizes with a 1.2-kilobase (kb) mRNA, the level of which is ≈9-fold greater in colon carcinoma than in adjacent normal colonic epithelium. Blot hybridization of total RNA from a variety of human colon carcinoma cell lines shows that the level of this 1.2-kb mRNA in poorly differentiated colon carcinomas is as high as or higher than that in well-differentiated carcinomas. Molecular cloning and complete sequencing of cDNA corresponding to the full-length open reading frame of this 1.2-kb mRNA unexpectedly show it to contain all the partial cDNA sequence encoding 135 amino acid residues previously reported for a human laminin receptor. The deduced amino acid sequence suggests that this putative laminin-binding protein from human colon carcinomas consists of 295 amino acid residues with interesting features. Containing only two cysteine residues, the protein does not have consensus sequences for asparagine-linked glycosylation, amphipathic α-helix, or the N-terminal leader signal sequences for entry into endoplasmic reticulum, although hydrophobic segments for potential membrane associations exist. There is an unusual C-terminal 70-amino acid segment, which is trypsin-resistant (no lysine or arginine) and highly negatively charged (13 aspartic plus glutamic residues). Within this segment are five repeats of (Asp/Glu)-Trp-(Ser/Thr); two of these are nearly tandem repeats of Thr-Glu-Asp-Trp-Ser-Ala-Xaa-Pro.**

Colon carcinoma is a leading cause of cancer death in humans. Over 140,000 new cases of colon carcinoma are expected in 1988 in the United States; more than 70,000 of these patients will die of this disease. Current diagnostic techniques find less than half of the patients early enough to ensure effective control by surgery. In particular, for patients with poorly differentiated colon carcinomas, there is virtually no marker available for detection and monitoring because such a malignancy does not express carcinoembryonic antigen, the marker often useful for well- and moderately-differentiated colon carcinomas (1).

Little is known about the transformation of normal colon epithelial cells to the cancerous state and the differentiation of colon carcinoma cells at the molecular level. More than 100 human colon carcinoma-derived cell lines have been established (2). Although these cell lines share the common characteristics of human colonic epithelial origin, they may be quite different in lineage, degree of differentiation, tumorigenicity, and histology of tumor induced in nude mice, as well as in their transformed phenotype in culture (2, 3). These cell lines are potentially a rich source for revealing markers. One approach we have undertaken to identify potential molecular markers for human colon carcinomas is to construct cDNA libraries from these cell lines and to screen for specific clones that hybridize with mRNAs more abundant in colon carcinomas than in their normal counterparts. Our particular interest is in those mRNAs also increased in poorly differentiated colon carcinomas.

Here we report the identification of a 1.2-kilobase (kb) mRNA that is ≈9-fold more abundant in a human colon carcinoma than in adjacent normal colonic epithelium. Molecular cloning of a cDNA corresponding to a full-length coding region‡ and complete sequencing reveal the presence of a partial sequence encoding 135 amino acid residues previously reported for human laminin receptor. The deduced amino acid sequence suggests that our laminin-binding protein from human colon carcinomas consists of 295 amino acid residues with several unusual features.

Laminin, a major constituent of basement membrane from almost all tissues, is a large glycoprotein consisting of three polypeptide chains, A chain (400 kDa), B1 chain (210 kDa), and B2 chain (200 kDa) in a cross-shaped structure (4–6). This glycoprotein has been implicated in a wide variety of biological processes. Most relevant to colon cancer, laminin has been linked to cell adhesion, morphogenesis, mitogenesis, differentiation, and metastasis (7). A proteolytic fragment of laminin (8) and a pentapeptide of Tyr-Ile-Gly-Ser-Arg from the B1 chain have been identified to have cell-attachment activity (9). Intriguingly, this pentapeptide inhibits the metastasis of a melanoma in mice (10). Many of the effects of laminin may be mediated through a laminin receptor, a 67-kDa cell-surface glycoprotein (11–13), although two additional laminin receptors have recently been identified with molecular masses of 180 kDa (14) and 110 kDa (14, 15). Colon carcinomas and many other carcinomas have abundant unoccupied, as well as occupied, laminin receptors (16–19). Some poorly differentiated colon carcinoma cells contain more laminin receptors in their cytoplasm than do some well-differentiated ones (20). Furthermore, poorly differentiated cells adhere significantly better on laminin substrata than do well-differentiated cells (21).

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Abbreviations: nt, nucleotide(s); ORF, open reading frame.

‡The sequence reported in this paper is being deposited in the EMBL/GenBank data base (IntelliGenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03799).

MATERIALS AND METHODS

Tissues and Cell Lines. Surgical specimens of primary human colon carcinomas and adjacent normal colon tissues were obtained from the Department of Surgery at New England Deaconess Hospital. Immediately after surgery, normal colonic epithelium was dissociated from muscle and connective tissues under a dissection microscope and placed in liquid nitrogen (22). Human colon carcinoma cell lines, Clone A and Clone D (subclones of DLD-1), were from D. Dexter (DuPont), MIP101 was from N. Zamcheck (Mallory Institute), CX-1 was from S. Bernal (Dana–Farber Cancer Institute), RCA, HCT-116b, GLY, and Moser were from M. Brattain (Baylor College of Medicine), and HT-29, CL 187 LS 180, CCL 220.1 COLO 320HSR, CCL 222 COLO 205, CCL 224 COLO 201, CCL 227 SW620, CCL 228 SW480, CCL 229 LoVo, CCL 233 SW1116, CCL 234 SW1463, CCL 237 SW948, and HTB 39 SK-CO-1 were all from the American Type Culture Collection. All these cell lines are well characterized and documented as carcinoma lines and are of human colon origin. All cells were grown in 50% Dulbecco's modified Eagle's medium (GIBCO) and 50% RPMI 1640 medium (GIBCO) supplemented with 10% fetal calf serum (M. A. Bioproducts, Walkersville, MD) and 1% Nutridoma NS (Boehringer Mannheim) at 37°C in 5% CO_2 and 100% humidity.

Preparation of RNA. Total cellular RNAs from cultured cells were isolated by the methods of Cox (23) and Strohman *et al.* (24). Total cellular RNAs from surgical specimens were prepared according to Chirgwin *et al.* (25); it was crucial that normal and tumor tissues were ground into sand-like particles in liquid nitrogen before extraction with guanidine isothiocyanate.

Construction of pBR322 Clone A cDNA Library. Poly(A)$^+$ RNAs from Clone A cells were purified by two passages through a poly(U)-Sephadex column as described by Haff *et al.* (26). The first strands of the cDNAs were synthesized by avian myoblastosis virus reverse transcriptase (Molecular Genetics Resources, Tampa, FL) using oligo-(dT) as a primer; the second strands were synthesized with DNA polymerase I according to Wickens *et al.* (27). cDNAs were treated with S1 nuclease, tailed with dCTP, and fractionated on a Bio-Gel A-50 column. Only dC-tailed cDNAs larger than 400 base pairs (bp) were pooled and hybridized to *Pst* I-digested poly(G)-tailed pBR322 plasmids. *Escherichia coli* HB101 was transformed according to Dagert and Ehrlich (28). The complexity of this library is $\approx 5 \times 10^4$ clones per μg of cDNA.

Screening the Plasmid cDNA Library. The individual transformed *E. coli* colonies were transferred onto a master agar plate and two other filter plates containing tetracycline (29). Colonies were arranged in a grid pattern; each colony was picked and plated on each of the three plates in an identical position. The colonies on the nitrocellulose filters were lysed with alkali and fixed. ^{32}P-labeled first-strand cDNAs generated from poly(A)$^+$ RNAs of Clone A or CX-1 cells were used for colony hybridization. A total of 1500 colonies was screened.

RNA Gel Blot Hybridization. Total RNAs (15 μg) extracted from tissues and cultured cells were subjected to electrophoresis in agarose gels containing formaldehyde and transferred to GeneScreen*Plus* (DuPont) according to Seed (30). Recombinant plasmids were prepared by alkaline lysis and CsCl gradient centrifugation (29). Plasmid DNA was purified and digested with *Pst* I. After electrophoresis, the cDNA insert was eluted with LID/X filter syringes (Xydex; Gaithersburg, MD), extracted with phenol/chloroform, concentrated by ethanol precipitation, and suspended in Tris/EDTA buffer (29). Nick-translated, ^{32}P-labeled cDNA insert was used to hybridize blots on GeneScreen. Specific activities of the DNA probes were ≈ 2–5×10^8 cpm/μg of DNA. Densitometry was done with a LKB UltroScan XL Laser densitometer (LKB).

Screening of Phage cDNA Library. Construction of a CX-1 cDNA λgt10 phage library will be described elsewhere (unpublished results). ^{32}P-labeled cDNA insert from a 8-2V clone (a clone found to hybridize with an mRNA more abundant in carcinoma than in normal epithelium, see *Results*) was used to screen the CX-1 λgt10 phage library (29). Approximately 10,000 plaques were screened.

DNA Sequencing. The M13 dideoxynucleotide chain-termination method was used (31). Both cDNA clones, 8-2V and J-9, were sequenced from both directions with the M13 universal primer or with specifically synthesized 17-base oligonucleotides. M13 was from New England Biolabs and Sequenase sequencing kit was from United States Biochemical (Cleveland). Synthetic oligonucleotides were provided by P. H. Sayre in the laboratory of E. Reinherz (Dana–Farber Cancer Institute).

Computer-Assisted Sequence Analysis. A computer search of a DNA sequence data base was performed at the Molecular Biology Computer Research Resource/Harvard School of Public Health at Dana–Farber Cancer Institute by S. Russo, D. Faulkner, and T. Smith. Hydropathic analysis was done at Boston Biomedical Research Institute by J. S. Hong.

RESULTS

Identification of 8-2V Plasmid Clone. To search for cellular mRNAs that are more abundant in human colon carcinoma than in normal counterparts, we constructed a cDNA library in plasmid pBR322 using poly(A)$^+$ mRNA isolated from a poorly differentiated human colon carcinoma cell line, Clone A. Initial screenings were done by hybridizing 1500 plasmids with cDNAs generated from mRNAs of Clone A as well as CX-1, a well-differentiated human colon carcinoma cell line. Whereas the great majority of plasmids hybridized equally between the two cell lines, 10 clones attracted our attention because they hybridized to a greater degree with cDNAs from Clone A than with those from CX-1. cDNA inserts in these clones were then used to compare the levels of mRNA between normal human colonic epithelial tissues and a colon carcinoma of the same patient by blot hybridization of RNA gels. One clone, 8-2V, hybridized with a 1.2-kb mRNA that was more abundant in the carcinoma than in adjacent normal epithelium (Fig. 1). Equal amounts of total RNAs (15 μg)

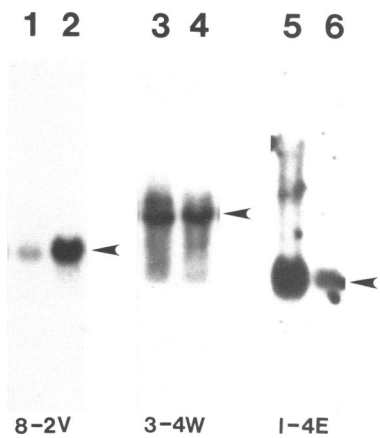

FIG. 1. Blot hybridization of RNAs (15 μg) from a human colon carcinoma and adjacent normal epithelium obtained from surgery with ^{32}P-labeled cDNA inserts from three plasmid clones. Lanes 1, 3, and 5 used total RNAs from normal human colonic epithelium; lanes 2, 4, and 6 used total RNAs from colon carcinoma. Lanes 1 and 2 were hybridized with 8-2V clone; lanes 3 and 4 were hybridized with 3-4W clone; and lanes 5 and 6 were hybridized with 1-4E clone. Arrowheads indicate the major species of RNA hybridized—1.2 kb, 1.7 kb, and 0.7 kb, left to right.

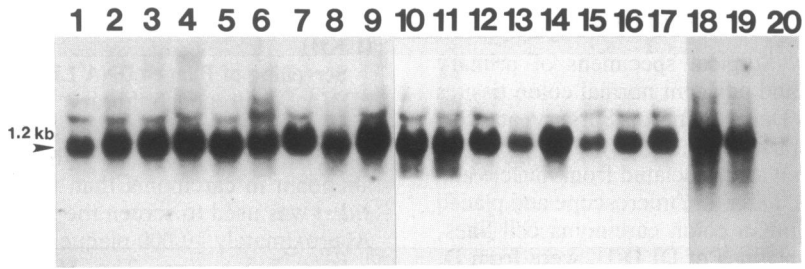

FIG. 2. Blot hybridization of RNAs (15 μg) from twenty human colon carcinoma cell lines with ^{32}P-labeled cDNA insert from 8-2V clone. Lanes: 1, HT-29; 2, CX-1; 3, RCA; 4, CCL 222 COLO 205; 5, CCL 220.1 COLO 320HSR; 6, CCL 228 SW480; 7, HCT-116b; 8, HTB 39 SK-CO-1; 9, Clone A subclone of DLD-1; 10, Clone D subclone of DLD-1; 11, MIP101; 12, CCL 229 LoVo; 13, GLY; 14, Moser; 15, CCL 233 SW1116; 16, CCL 224 COLO 201; 17, CCL 237 SW948; 18, CL 187 LS 180; 19, CCL 227 SW620; and 20, CCL 234 SW1463. Arrowhead indicates the major hybridized species of RNA, 1.2 kb in length.

from carcinoma and normal tissues were compared. Densitometric analysis suggested that the level of this mRNA was ≈9-fold greater in the carcinoma than in normal epithelium. To rule out the possibility that this difference resulted from an unequal loading of total RNAs, the same GeneScreen filter used for hybridization with 8-2V cDNA was hybridized sequentially with two other cDNA clones, the sequences of which are distinct from that of clone 8-2V. Clone 1-4E hybridized with a 0.7-kb RNA, which is far more abundant in normal colonic epithelium than in carcinoma tissue, whereas clone 3-4W hybridized with a 1.7-kb RNA equally abundant in normal and tumor tissues. (Sequences of these two cDNA clones will be presented elsewhere.) These results suggested that the 1.2-kb mRNA, which hybridized with clone 8-2V, encoded a polypeptide potentially useful for distinguishing colon carcinoma from normal colonic epithelium.

Blot Hybridization of Total RNAs from Various Cell Lines with 8-2V cDNA Clone. To investigate whether increased expression of 1.2-kb mRNA that hybridizes with clone 8-2V in colon carcinoma cells is a general phenomenon, particularly in poorly differentiated cell lines, 20 human colon carcinoma cell lines were examined by blot hybridization of an equal amount of total RNAs (15 μg) with ^{32}P-labeled probe. Fig. 2 shows that essentially all human colon carcinoma cell lines tested expressed significant levels of this mRNA except CCL 234 SW1463, a mucin-producing, well-differentiated, rectal adenocarcinoma cell line. Correlation between the level of this 1.2-kb mRNA and the degree of

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                                        -37 CTGGATTCCCGTCGTAACTTAAAGGGAAATTTTCACA   -1
                                                                  ***
ATG TCC GGA GCC CTT GAT GTC CTG CAA ATG AAG GAG GAG GAT GTC CTT AAG TTC CTT GCA   60
Met Ser Gly Ala Leu Asp Val Leu Gln Met Lys Glu Glu Asp Val Leu Lys Phe Leu Ala   20

GCA GGA ACC CAC TTA GGT GGC ACC AAT CTT GAC TTC CAG ATG GAA CAG TAC ATC TAT AAA  120
Ala Gly Thr His Leu Gly Gly Thr Asn Leu Asp Phe Gln Met Glu Gln Tyr Ile Tyr Lys   40
                                        ::::----------::::--::::::---::::::::
AGG AAA AGT GAT GGC ATC TAT ATC ATA AAT CTC AAG AGG ACC TGG GAG AAG CTT CTG CTG  240
Arg Lys Ser Asp Gly Ile Tyr Ile Ile Asn Leu Lys Arg Thr Trp Glu Lys Leu Leu Leu   60
----------::::
GCA GCT CGT GCA ATT GTT GCC ATT GAA AAC CCT GCT GAT GTC AGT GTT ATA TCC TCC AGG  240
Ala Ala Arg Ala Ile Val Ala Ile Glu Asn Pro Ala Asp Val Ser Val Ile Ser Ser Arg   80

AAT ACT GGC CAG AGG GCT GTG CTG AAG TTT GCT GCT GCC ACT GGA GCC ACT CCA ATT GCT  300
Asn Thr Gly Gln Arg Ala Val Leu Lys Phe Ala Ala Ala Thr Gly Ala Thr Pro Ile Ala  100
                                        ^^^^^^^^^^^-------^^^^^^^^
GGC CGC TTC ACT CCT GGA ACC TTC ACT AAC CAG ATC CAG GCA GCC TTC CGG GAG CCA CGG  360
Gly Arg Phe Thr Pro Gly Thr Phe Thr Asn Gln Ile Gln Ala Ala Phe Arg Glu Pro Arg  120

CTT CTT GTG GTT ACT GAC CCC AGG GCT GAC CAC CAG CCT CTC ACG GAG GCA TCT TAT GTT  420
Leu Leu Val Val Thr Asp Pro Arg Ala Asp His Gln Pro Leu Thr Glu Ala Ser Tyr Val  140

AAC CTA CCT ACC ATT GCG CTG TGT AAC ACA GAT TCT CCT CTG CGC TAT GTG GAC ATT GCC  480
Asn Leu Pro Thr Ile Ala Leu Cys Asn Thr Asp Ser Pro Leu Arg Tyr Val Asp Ile Ala  160
                            +++
ATC CCA TGC AAC AAC AAG GGA GCT CAC TCA GTG GGT TTG ATG TGG TGG ATG CTG GCT CGG  540
Ile Pro Cys Asn Asn Lys Gly Ala His Ser Val Gly Leu Met Trp Trp Met Leu Ala Arg  180
         +++                                <<<<<<<<<<<>>>>>>>>>>>
GAA GTT CTG CGC ATG CGT GGC ACC ATT TCC CGT GAA CAC CCA TGG GAG GTC ATG CCT GAT  600
Glu Val Leu Arg Met Arg Gly Thr Ile Ser Arg Glu His Pro Trp Glu Val Met Pro Asp  200
                                                  ::::-----------::::
CTG TAC TTC TAC AGA GAT CCT GAA GAG ATT GAA AAA GAA GAG CAG GCT GCT GCT GAG AAG  660
Leu Tyr Phe Tyr Arg Asp Pro Glu Glu Ile Glu Lys Glu Glu Gln Ala Ala Ala Glu Lys  220
---::::::---::::::::::----------::::                    ^^^^^^^^^^^^-------
GCA GTG ACC AAG GAG GAA TTT CAG GGT GAA TGG ACT GCT CCC GCT CCT GAG TTC ACT GCT  720
Ala Val Thr Lys Glu Glu Phe Gln Gly Glu Trp Thr Ala Pro Ala Pro Glu Phe Thr Ala  240
^^^^---^^^^      225
ACT CAG CCT GAG GTT GCA GAC TGG TCT GAA GGT GTA CAG GTG CCC TCT GTG CCT ATT CAG  780
Thr Gln Pro Glu Val Ala Asp Trp Ser Glu Gly Val Gln Val Pro Ser Val Pro Ile Gln  260

CAA TTC CCT ACT GAA GAC TGG AGC GCT CAG CCT GCC ACG GAA GAC TGG TCT GCA GCT CCC  840
Gln Phe Pro Thr Glu Asp Trp Ser Ala Gln Pro Ala Thr Glu Asp Trp Ser Ala Ala Pro  280
           **********************---****     **********************---****
ACT GCT CAG GCC ACT GAA TGG GTA GGA GCA ACC ACT GAC TGG TCT TAA GCTGTTCTTGCATAG  +15
Thr Ala Gln Ala Thr Glu Trp Val Gly Ala Thr Thr Asp Trp Ser Stop

GCTCTTAAGCAGCATGGAAAAATGGTTGATGGAAAATAAACATCAGTTTCTAAAAAAAAAAAAAAAAAAAA
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FIG. 3. Nucleotide sequence and deduced amino acid sequence of J-9 cDNA clone. 8-2V cDNA sequence is located within the sequence shown here (nt 162–829). Partial sequence of a 67-kDa human laminin receptor previously reported by Wewer *et al.* (32) is found here with exact identity (starting amino acid residue 161). Sequences of interest are indicated (see *Deduced Amino Acid Sequence* for details). Dashes in :, ^, and * marked sequences indicate differences from similarly marked sequences.

differentiation is not apparent, although all poorly differentiated cell lines (CCL 220.1 COLO 320HSR, HCT-116b, Clone A, and MIP101) expressed high levels of the mRNA and those that expressed lower levels tend to be the well-differentiated cell lines (CCL 233 SW1116 and GLY, both high carcinoembryonic antigen producers). On the basis of equal total RNAs, the level of this 1.2-kb mRNA in colon carcinoma cell lines is in the same range as that of surgical tissue of colon carcinoma shown in Fig. 1.

Nucleotide Sequence of 8-2V cDNA Clone. The cDNA insert in the 8-2V clone has 668 bp. Its sequence from nucleotide (nt) 162–829 is shown in Fig. 3. A computer search of the DNA sequence data base revealed that clone 8-2V contains a stretch of 350 nt with a sequence identical with the partial cDNA sequence of human laminin receptor reported by Wewer *et al.* (32). The 2H5 monoclonal antibody is known to bind the laminin receptor on immunoblots, to react with the purified laminin receptor on enzyme-linked immunoassays, to prevent the binding of laminin to either cells or plasma membrane, and to block the adhesion of cells to amnion basement membrane enriched in laminin (32–34). The epitope recognized by this antibody is present in the deduced amino acid sequence. Furthermore, the predicted polypeptide contains the 20-amino acid stretch recognized by the polyclonal antibodies that inhibit laminin-mediated cell attachment (20). Taken together, it seems highly likely that the product of 1.2-kb mRNA hybridized with 8-2V cDNA would also bind laminin.

Molecular Cloning and Sequence of cDNA Corresponding to 1.2-kb mRNA Hybridized with 8-2V cDNA Clone. ^{32}P-labeled cDNA insert of clone 8-2V was then used to clone cDNAs with longer inserts from a CX-1 cDNA λgt10 phage library. One cDNA clone, J-9, contained an insert of ≈1 kb. The complete cDNA sequence of this insert is shown (Fig. 3). 8-2V cDNA sequence from Clone A cell line is completely located within this sequence of cDNA. Computer-assisted analysis revealed an open reading frame (ORF) starting from the first ATG and terminating at TAA as indicated. This ORF is preceded by an in-frame stop codon at position −18 and ANN immediately before the first ATG (35). Signal sequences for polyadenylylation are also present.

Deduced Amino Acid Sequence. This ORF may encode a 32,817-Da polypeptide with 295 amino acid residues. The deduced amino acid sequence of Fig. 3 reveals some interesting features. At the N terminus no leader signal sequence for entry into endoplasmic reticulum reported for numerous cell surface proteins is found. By hydropathy plots, the N-terminal two-thirds contains some hydrophobic segments that may serve as a signal sequence or provide membrane anchoring (Fig. 4). No consensus sequence of Asn-Xaa-(Ser/Thr) for N-linked glycosylation is present. There are only two cysteines, separated by 14 amino acid residues. No arginine or lysine is found after amino acid residue 225; thus, a complete digestion by trypsin of this polypeptide should lead to a 70-amino acid, C-terminal domain of a highly acidic polypeptide (13 asparagine plus glutamic residues) without a positively charged residue (Fig. 3).

Unusual sequences include two repeats of Thr-Glu-Asp-Trp-Ser-Ala-Xaa-Pro [264–271 and 273–280; (*) Fig. 3], noted previously (32); two repeats of Ala-Ala-Ala-Xaa-Xaa-Ala [91–96 and 216–221; (∧) Fig. 3]; three repeats of Lys-Glu-Glu (11–13, 212–214, and 224–226), with two of them in Lys-Glu-Glu-Gln-Xaa-Xaa-Xaa-Glu (212–219) and Lys-Glu-Glu-Xaa-Gln-Xaa-Glu (224–230); two repeats of (Asp/Glu)-Xaa-Xaa-Xaa-(Glu/Asp)-Xaa-Tyr-Xaa-Tyr-(Lys/Arg)-Xaa-Xaa-Xaa-(Asp/Glu) [31–44 and 196–209; (:) Fig. 3]; six repeats of (Asp/Glu)-(Asp/Glu)-hph; and one symmetrical sequence of Leu-Met-Trp-Trp-Met-Leu [173–178; (<◇>), Fig. 3].

DISCUSSION

The search for markers that may distinguish both poorly and well-differentiated human colon carcinomas from normal colonic epithelium has been hampered by the difficulty of growing normal human colonic epithelial cells in culture for comparison (2). An alternative is to isolate proteins, glycolipids, RNA, DNA, from normal colonic epithelium and carcinoma tissue of surgical specimens for comparison, ideally from the same individuals. The limiting factor for this approach is the vast number of cellular components requiring analysis. To narrow the range of cellular components for investigation, we adopted the convenience of using a cDNA library to screen for mRNAs that are more abundant in colon carcinoma than in adjacent normal colonic epithelial tissues. Using this approach we have identified a 1.2-kb mRNA that hybridized with the cDNA insert in pBR322 plasmid designated as 8-2V. Significantly, the increased levels of this mRNA are detected in both poorly differentiated and well-differentiated colon carcinoma cell lines.

Whether this 1.2-kb mRNA encodes 67-kDa laminin receptor awaits further investigation. The size of this mRNA is much smaller than the 1.7-kb mRNA previously described for

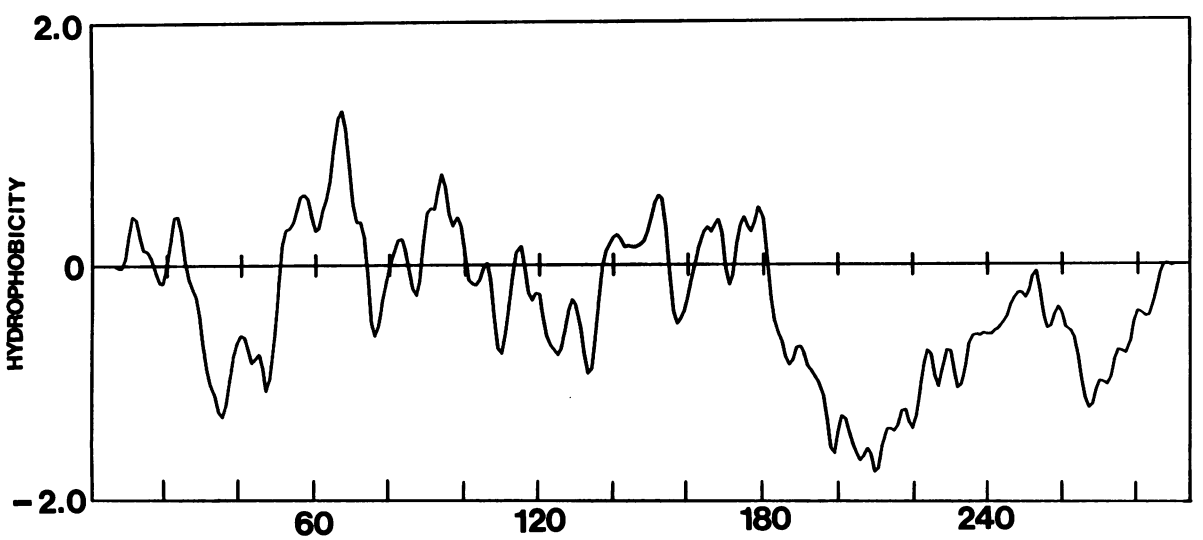

FIG. 4. Hydropathy plots of deduced amino acid sequences shown in Fig. 3 using the algorithm and hydropathy values of Kyte and Doolittle (36). Hydrophobicities were averaged over windows of 19 amino acids (37). Note that the N-terminal two-thirds appears to have some hydrophobic segments, whereas the C-terminal one-third is relatively hydrophilic.

laminin receptor (32). The ORF in the cDNA described here will only suffice for a polypeptide of 32,817 Da, far smaller than the 67-kDa protein described for laminin receptor. However, because all partial cDNA sequences reported for laminin receptor are included in the ORF described here, the product of 1.2-kb mRNA, if not the 67-kDa laminin receptor, is probably a laminin-binding protein. That the product of this 1.2-kb mRNA might be a laminin-binding protein is strongly supported by the fact that the coding region for an epitope in 67-kDa laminin receptor recognized by the monoclonal antibody 2H5, which blocks binding of laminin with its receptor, is present entirely in our sequence (32). Moreover, the C-terminal 20-amino acid synthetic peptide recognized by polyclonal antibodies that interfere with laminin-mediated cell attachment is also present at the C terminus of this sequence (20). Because there are other laminin receptors—one of 110-kDa molecular mass in both epithelial and neuronal cells (14, 15) and the other of 180 kDa in neuronal cells (14)—that a smaller laminin-binding protein of 32,817 Da could exist before posttranslational modification.

Although most blot hybridizations of RNA gels shown here revealed a predominant 1.2-kb band, minor bands of larger sizes did appear. Whether these bands relate to other laminin receptors needs investigation; we have, however, identified a 5.5-kb mRNA that hybridizes strongly with 8-2V cDNA in a human esophageal carcinoma cell line (to be reported elsewhere).

(Asp/Glu)-Xaa-Xaa-Xaa-(Glu/Asp)-Xaa-Tyr-Xaa-Tyr-(Lys/Arg)-Xaa-Xaa-Xaa-(Asp/Glu) in positions 31–44 and 196–209 is a notable repeat. If the 140-amino acid stretch flanked by the two cysteines is considered a linking domain, the two flanking domains would each have one of this repeat sequence. There is also another repeat, Ala-Ala-Ala-Xaa-Xaa-Ala-Xaa$^-$-Thr, one each in these two flanking domains. The C-terminal domain after residue 225 contains a 70-amino acid segment with 13 aspartic plus glutamic residues and without any lysine or arginine residues. This trypsin-resistant, highly acidic domain might mark laminin-binding proteins. Within this 70-amino acid domain are five repeats of (Asp/Glu)-Trp-(Ser/Thr), with two of them in nearly tandem repeats of Thr-Glu-Asp-Try-Ser-Ala-Xaa-Pro.

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