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Differential expression of epidermal growth factor-related proteins in human colorectal tumors

(colon cancer/mRNA expression/cripto/amphiregulin/HER3)

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ABSTRACT **Amphiregulin (AR) and cripto are proteins that are structurally related to epidermal growth factor (EGF) and transforming growth factor α (TGF-α). AR is also functionally related to this family of growth regulatory molecules and is able to bind and activate the 170-kDa EGF receptor (EGFR). Human EGFR-3 (HER3)/ERBB3 is a recently identified protein related to the EGFR that is widely expressed in breast carcinomas and is a candidate receptor for EGF-like growth factors. Differential expression of these putative ligands and receptors in transformed cells suggests that they may function in an autocrine manner to regulate tumor cell growth. Specific mRNA transcripts for TGF-α [4.8 kilobases (kb)], AR (1.4 kb), cripto (2.2 kb), and HER3 (6.2 kb) were expressed in a majority of human colon cancer cell lines. HER3 mRNA was detected in 55% of primary or metastatic human colorectal carcinomas but in only 22% of normal colon mucosa and 32% of normal liver samples. In contrast, cripto and AR mRNA were expressed in 60–70% of primary or metastatic human colorectal cancers but in only 2–7% of normal human colonic mucosa. Immunostaining also detected AR protein in primary and metastatic colorectal tumors but not in normal colon or uninvolved liver. These findings suggest that cripto and AR may be useful markers to discriminate between normal and malignant colonic epithelium and may provide a selective growth advantage for colorectal carcinomas.**

Colorectal cancer is one of the most frequently occurring cancers in humans, accounting for ≈14% of all types of cancer (1). Activation of specific protooncogenes, such as *KRAS* by point mutation and inactivation of tumor suppressor genes like p53, *DCC* (deleted in colorectal cancer), and *MCC* (mutated in colorectal cancer), occur during the progression from normal colon epithelium to invasive carcinoma (2–8). In fact, it appears that cumulative mutations in at least four or five genes are required for colorectal tumor formation (2). In addition to these changes, human colon cancer cell lines and primary colon tumors produce several positive and negative growth regulators including transforming growth factor α (TGF-α), TGF-β, insulin-like growth factor I (IGF-I), IGF-II, and platelet-derived growth factor (9–17). Receptors for a number of these ligands are also expressed on these cells, suggesting that an autocrine mechanism of growth control may be operative in colon cancer (9, 12, 18, 19).

Recently, several additional members of the epidermal growth factor (EGF)/TGF-α family have been identified, including cripto, rat and human amphiregulin (AR), and heparin-binding EGF-like growth factor (20–26). Cripto mRNA is expressed in undifferentiated mouse and human embryonal carcinoma cells, and its 2.2-kilobase (kb) transcript predicts a primary translation product of 188 amino acids, including a 37-amino acid region that shares significant structural homology with other members of the EGF/TGF-α family (20, 21). AR is a secreted heparin-binding growth factor originally isolated from a human breast carcinoma cell line (22, 23). The 1.4-kb transcript for AR encodes a 252-amino acid transmembrane precursor that is proteolytically processed to release the 78-amino acid secreted molecule (24). AR shares significant structural and functional homology with EGF and TGF-α. Except for cripto, all of these proteins have been shown to bind the 170-kDa EGF receptor (EGFR). However, the EGFR is a member of a larger family of transmembrane receptor tyrosine kinases (27). Other members include human EGFR-2 (HER2) (*erbB-2*) and HER3 (*erbB-3*), neither of which has a well-characterized ligand (28–30). The 6.2-kb HER3 mRNA has been detected in a variety of human fetal and adult tissues as well as in a small panel of human tumor cell lines (29). In this report, we have analyzed several human colon cancer cell lines and a cohort of primary and metastatic colorectal tumors for mRNA expression specific for TGF-α, cripto, AR, and HER3. A majority of colon cancer cell lines express TGF-α, cripto, AR, and HER3. In addition, cripto and AR, but not HER3, are differentially expressed in the majority of primary and metastatic colorectal tumors compared with normal colon and liver tissue obtained from the same patient.

MATERIALS AND METHODS

Human Colon Cancer Cell Lines. GEO, MOSER, JVC, CBS, HCT116, and FET are colon cancer cell lines established from primary human colorectal adenocarcinomas by M. Brattain (Baylor College of Medicine, Houston, TX) and maintained as described (31, 32). SW620, SW480, LS174T, and WIDR were obtained from the American Type Culture Collection.

Human Tissues. Human tumor tissues and adjacent normal noninvolved colon or liver tissues were collected through the Cooperative Human Tissue Network (Columbus, OH). The specimens were frozen in liquid nitrogen immediately after surgery and stored at −70°C until RNA extraction. Seventy-eight human primary and metastatic colorectal adenocarcinomas and 69 matched human normal colon or liver tissues from the same patients were analyzed. Of the samples, 44

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Abbreviations: EGF, epidermal growth factor; EGFR, EGF receptor; TGF, transforming growth factor; HER, human EGFR; AR, amphiregulin.

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were primary colon adenocarcinomas (Dukes grade A–C) and 34 were liver metastases from colon adenocarcinomas. Patient age ranged from 29 to 88 years with a mean age of 62.

RNA Isolation and Northern Blot Analysis. Total cellular RNA was extracted by lysis in guanidine thiocyanate and centrifuged over a cesium chloride cushion (33). Poly(A)$^+$ RNA was obtained by absorption of total cellular RNA to and elution from an oligo(dT) cellulose column (type III; Collaborative Research). Ten micrograms of poly(A)$^+$ RNA was electrophoresed through a denaturing 1.2% agarose/2.2 M formaldehyde gel. Ethidium bromide staining of the gels showed that each lane contained an equivalent amount of RNA. The gels were then transferred to Biotrans nylon membranes (ICN) and hybridized to the following ^{32}P-labeled nick-translated cDNA probes: pTGF-C1, a 406-base-pair (bp) *Eco*RI/*Apa* I restriction fragment derived from a human TGF-α cDNA clone (34); 2B3, a 900-bp *Eco*RI/*Eco*RI human cripto cDNA insert containing the full protein coding sequence (20); an 1100-bp *Eco*RI/*Eco*RI fragment containing the coding region of the human AR cDNA (24); H3EH1.7, a 1700-bp *Eco*RI/*Hin*dIII cDNA fragment encoding most of the extracellular domain, the transmembrane domain, and part of the intracellular tyrosine kinase domain of the human HER3 (30); or a 770-bp human β-actin cDNA probe (Oncor, Gaithersburg, MD).

Immunostaining for AR Expression. Frozen sections (10 μm) from tissue biopsies were fixed in 4% paraformaldehyde/phosphate-buffered saline (PBS, 0.15 M NaCl/5.6 mM Na_2HPO_4/1.06 mM KH_2PO_4, pH 7.4) for 30 min, washed in PBS three times (10 min each), and blocked with normal human serum diluted 1:5 in PBS. First-step monoclonal antibodies were mouse antibody 6RIC raised against a 41-amino acid peptide corresponding to the cysteine-rich region of human AR, or P117, a mouse myeloma control antibody. The antibodies were diluted to 25 μg/ml in a diluent containing 10% normal human serum and 3% normal goat serum in PBS (10% NHS/3% NGS). The first-step antibodies (100 μl per slide) were incubated for 16 hr at 4°C. The slides were washed three times with PBS (10 min each) and treated with the second-step antibody, goat anti-mouse IgG (Sternberger Monoclonals, Baltimore, MD) diluted 1:100 in 10% NHS/3% NGS. After a 1-hr incubation and washing as described above, the sections were incubated for 1 hr with a third-step antibody, monoclonal mouse peroxidase anti-peroxidase antibody (mPAP; Sternberger Monoclonals) diluted 1:100 in 10% NHS/3% NGS. After washing, the slides were treated again with step 2 and 3 antibodies as described above. Finally, the slides were developed with freshly prepared 0.05% 3,3-diaminobenzidine tetrahydrochloride and 0.01% hydrogen peroxide in 0.05 M Tris buffer (pH 7.6) for 8 min. Further exposure of the sections to a 1% OsO_4 solution in distilled water for 20 min intensified the staining. The sections were then rinsed in water, air dried, and viewed by water immersion and differential interference contrast microscopy.

RESULTS

Expression of TGF-α, Cripto, AR, and HER3 mRNA in Human Colon Cancer Cell Lines. TGF-α expression has been found in a variety of human tumor cell lines and human primary tumors (35). Specifically, TGF-α mRNA and protein can be detected in a large number of human colon cancer cell lines (9, 10, 14, 32). As shown in Fig. 1*A*, a 4.8-kb mRNA species for TGF-α was detected in the poly(A)$^+$ RNA obtained from 9 of the 10 human cancer cell lines examined in this study after hybridization with the labeled human TGF-α cDNA. In addition, a minor 1.6-kb TGF-α mRNA species could also be observed in some of these cell lines such as SW480, SW620, and MOSER. The relative levels of TGF-α mRNA detected in the majority of the colon cancer cell lines correlate with the amount of TGF-α protein that is produced and secreted by these cell lines (refs. 10, 14, and 32; D.S.S., F.C., and K. Mulder, unpublished results). To determine whether other members of the EGF/TGF-α gene family are expressed in human colon cancer cells, Northern blots containing poly(A)$^+$ RNA from the same colon cancer cell lines were analyzed for expression of specific mRNA transcripts for cripto and AR. A 2.2-kb cripto-specific mRNA was found in GEO, CBS, SW620, and LS174T cells (Fig. 1*B*) and in SW480 and FET cells (data not shown). The highest levels of cripto mRNA expression were detected in GEO, SW620, and LS174T cells. Furthermore, a 1.4-kb AR-specific mRNA could be found in GEO, MOSER, CBS, HCT116, and LS174T colon cancer cells, with the highest levels of expression found in MOSER, HCT116, and LS174T cells (Fig. 1*C*). Because HER3 is a candidate receptor for EGF-like ligands, we also measured its level of expression. In fact, all of the colon cancer cell lines that were examined expressed HER3-specific mRNA (Fig. 1*D*). GEO, CBS, SW620, and LS174T cells exhibited the highest levels of HER3 mRNA. Furthermore, all colon cancer cell lines with the exception of JVC expressed at least two or more of these four transcripts. In addition, cripto and HER3 were coexpressed at high levels in GEO, CBS, SW620, and LS174T cells while AR and HER3 were coexpressed in mRNA at high levels in GEO, CBS, and LS174T cells.

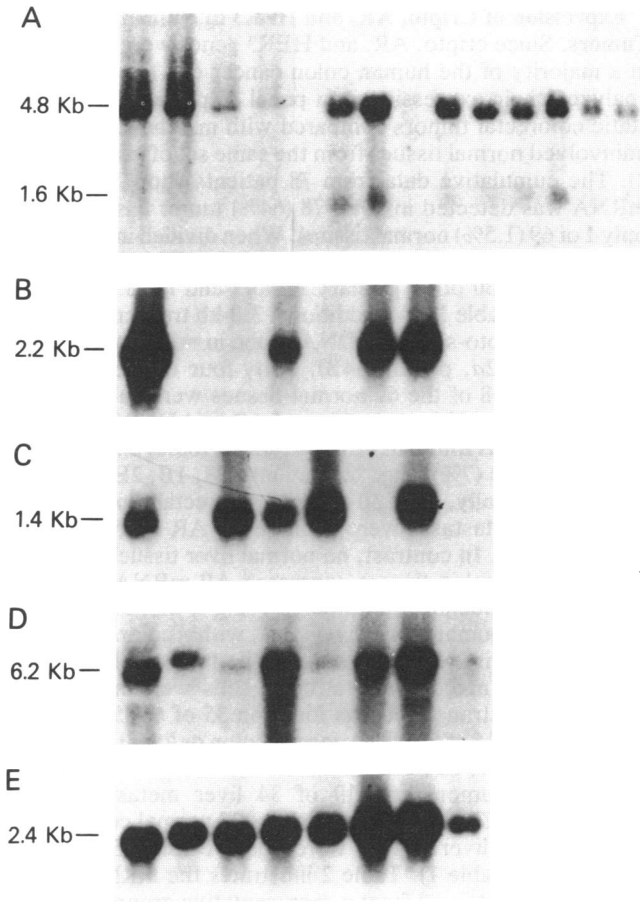

FIG. 1. Northern blot analysis of mRNA expression for TGF-α (*A*), cripto (*B*), AR (*C*), HER3 (*D*), and β-actin (*E*) in human colon cancer cell lines. Ten micrograms of poly(A)$^+$ RNA per lane was loaded for each sample. (*A*) From left to right: WIDR xenograft, WIDR cell line, LS174T xenograft, LS174T cell line, SW480, SW620, JVC, CBS, HCT116, MOSER, MOSER-R, GEO, and FET cell lines. (*B–E*) From left to right: GEO, JVC, MOSER, CBS, HCT116, SW620, LS174T, and WIDR cell lines.

Expression of Cripto, AR, and HER3 in Human Colorectal Tumors. Since cripto, AR, and HER3 genes were expressed in a majority of the human colon cancer cell lines, we next analyzed their expression in a panel of primary and metastatic colorectal tumors compared with matched samples of uninvolved normal tissues from the same set of patients (Fig. 2). The cumulative data from 78 patients show that cripto mRNA was detected in 50 of 78 (64%) tumor tissues and in only 1 of 69 (1.5%) normal tissues. When divided into primary and metastatic colorectal cancers, cripto mRNA expression was found in 30 of 44 primary tumors and in 21 of 34 liver metastases (Table 1). An additional 3.0-kb transcript hybridized to the cripto-specific cDNA probe in ≈10% of the colon tumors (Fig. 2*a*, patient 34B). Sixty-four of the 78 tumor samples and 58 of the 69 normal tissues were subsequently analyzed for mRNA expression of AR and HER3. A 1.4-kb AR mRNA was found in 42 of 64 tumors (66%) and in 4 of 58 normal tissues (7%) (Fig. 2*b*, patients 1A, 1B, 2E, 11A, and 34B). Specifically, 15 of 30 primary colorectal tumors and 27 of 34 liver metastases were positive for AR mRNA expression (Table 1). In contrast, no normal liver tissue and only 4 of 27 normal colon tissues expressed AR mRNA. Immunocytochemical localization studies using a monoclonal antibody against human AR showed that, within colon carcinoma biopsies, AR is expressed only in the tumor cells (Fig. 3). Both normal mucosa and stromal cells were negative. A 6.2-kb HER3 transcript was found in 35 of 64 (55%) tumor tissues and in 16 of 58 (28%) normal colon or liver tissues (Fig. 2*c*, patients 1A, 1B, 2D, 2E, and 34B). In this regard, 16 of 30 primary tumors and 19 of 34 liver metastases were expressing HER3 mRNA, while 6 of 27 normal colon and 10 of 31 normal liver tissues were positive for HER3 mRNA expression (Table 1). Table 2 illustrates the mRNA expression profile obtained from a representative group of individual patients with primary or metastatic disease. In the majority of both primary and metastatic colorectal tumors, there is coexpression of two or more of these three genes. More specifically, either cripto or AR was expressed in the HER3-positive tumors. For one individual (patient 47), both primary and metastatic tissues exhibited the same pattern of expression of cripto, AR, and HER3.

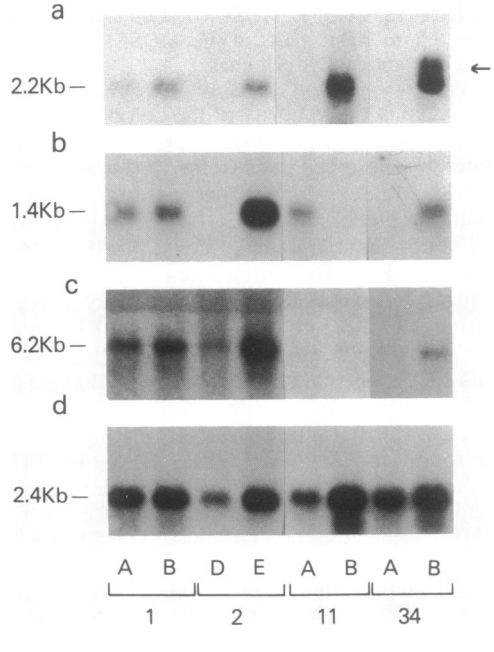

FIG. 2. Northern blot analysis of mRNA expression for cripto (*a*), in which arrow designates 3.0-kb transcript, AR (*b*), HER3 (*c*), and β-actin (*d*) in human primary and metastatic colorectal tumors obtained from four patients. (*a*) Normal noninvolved colon tissue. (*b*) Primary colon carcinoma. (*c*) Normal liver. (*d*) Liver metastasis. Ten micrograms of poly(A)$^+$ RNA per lane was loaded for each sample.

Table 1. Summary of mRNA expression of cripto, AR, and HER3 in human colorectal samples

	Colon		Liver	
	Normal mucosa	Primary carcinoma	Normal tissue	Metastasis from colon carcinoma
Cripto	1/38 (2.6%)	30/44 (68.2%)	0/31 (0)	21/34 (61.8%)
AR	4/27 (14.8%)	15/30 (50%)	0/31 (0)	27/34 (79.4%)
HER3	6/27 (22.2%)	16/30 (53.3%)	10/31 (32.3%)	19/34 (55.9%)

DISCUSSION

TGF-α is a potent mitogen that is frequently elaborated by a variety of human tumor cell lines and by primary human carcinomas (35, 36). For this reason, TGF-α has been circumstantially implicated as an autocrine growth factor for tumor cells that are also expressing a sufficient complement of functional EGFRs (32, 35, 36). In contrast, relatively little information is available on the expression in specific types of human tumors of other newly discovered members of the EGF/TGF-α and EGFR protein families such as cripto, AR, and HER3. The present study demonstrates that mRNA transcripts for TGF-α, cripto, AR, and HER3 are expressed in a majority of human colon cancer cell lines and in primary and metastatic human colorectal tumors. In addition, the results of this study show that unlike HER3, cripto and AR are preferentially expressed in primary colorectal tumors and liver metastases compared with normal colon or liver tissues. Furthermore, a small subset of colon tumors expressed an additional 3.0-kb cripto-related transcript. This larger transcript may be the result of differential splicing or polyadenylylation of the cripto mRNA, or it may represent another cripto-related gene (21).

This study is in agreement with previous findings demonstrating that TGF-α is synthesized and secreted by several human colon cancer cell lines and primary human colon tumors (9–17). TGF-α is also expressed in the gastrointestinal tract, including stomach, small intestine, and colon (12, 13). Coexpression of TGF-α and EGFR occurs in several colon cancer cell lines, primary colon carcinomas, adenomas, and normal colon mucosa, suggesting that TGF-α may function as a potential autocrine growth factor in both normal and transformed colonic epithelial cells (10, 12, 14, 34, 37, 38).

Cripto mRNA expression has a more restricted distribution since it is only found in undifferentiated mouse and human embryonal carcinoma cells, midgestation mouse embryos, adult mouse brain and lung, and human hepatomas, but not in a panel of other normal adult mouse or human tissues (ref. 20; R.D., D. S. Liscia, and M.G.P., unpublished results). In contrast, specific mRNA transcripts for both AR and HER3 are present in a variety of normal human tissues including placenta, keratinocytes, lung, kidney, and several human breast cancer cell lines (24, 29, 39), whereas cripto mRNA was not detected in a panel of normal and malignant breast tissues (G. Colletta, F.C., and D.S.S., unpublished results). In the present study, cripto, AR, and HER3 mRNA were found to be expressed in a major proportion of both colon tumor cell lines and primary and metastatic colorectal carcinomas. These findings suggest that there may be coordinate expression of certain members of the EGF/TGF-α and EGFR gene families in human colon tumors and that the

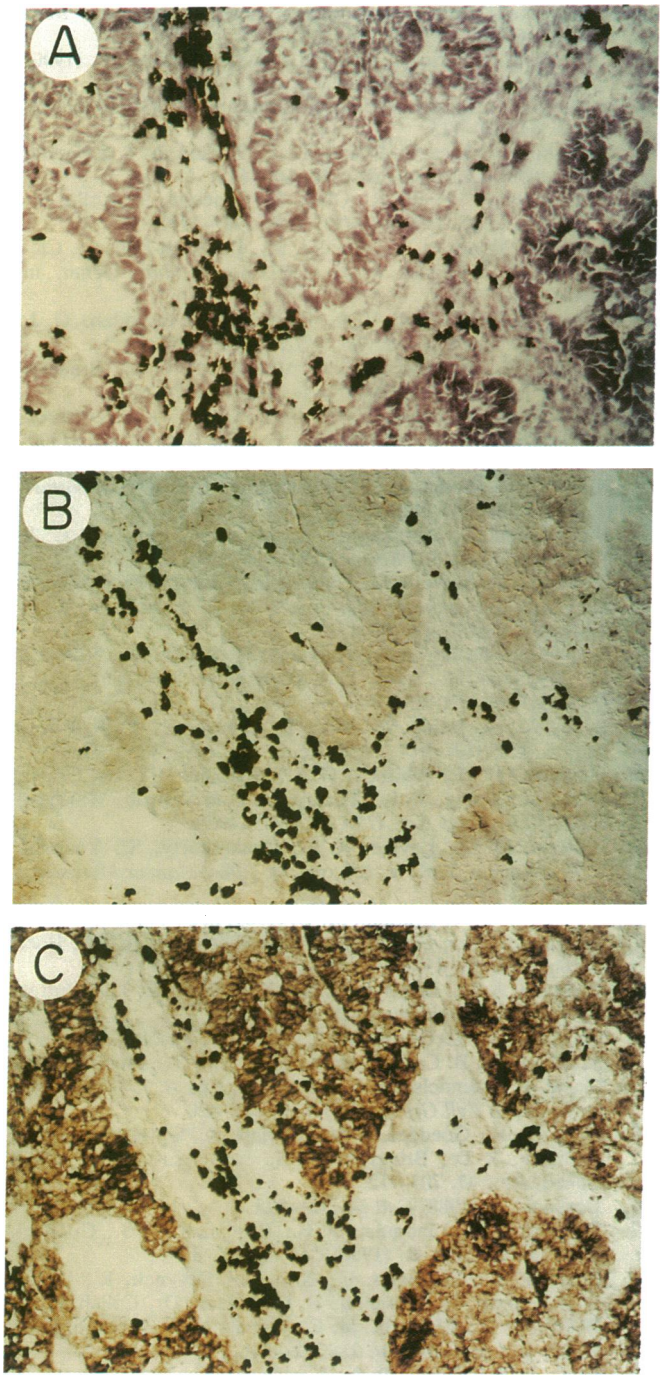

FIG. 3. Immunolocalization of AR in colon carcinoma. Serial frozen sections of a colon carcinoma biopsy were stained with a control (*A* and *B*) or specific monoclonal antibody to AR (*C*). One of the control slides (*A*) was stained with hematoxylin and eosin. Note that the antibody to AR specifically stained the tumor islands, while the surrounding stroma was not stained. Some cells within the stroma have endogenous peroxidase activity and are stained in *A–C*.

HER3 membrane-associated p160 tyrosine kinase may function as a potential receptor for a bifunctional growth modulator such as AR and/or a putative growth modulator such as cripto.

Cumulative genetic alterations are found in a majority of human colorectal tumors and probably contribute to tumor development (2). Genes that are frequently altered, either by point mutation or by allelic deletion, include the *KRAS* protooncogene on chromosome 12p11 and the p53 tumor suppressor gene on chromosome 17p13 (3–5, 40). Other

Table 2. mRNA expression of cripto, AR, and HER3 in individual colorectal cancer patients

Patient	Cripto	AR	HER3	β-Actin
		Colon		
1A	+	+	++	+++
1B	++	+	++	+++
11A	−	++	−	+++
11B	++	−	−	+++
12A	−	−	+	+++
12B	++	++	++	+++
14A	−	+	−	+++
14B	−	−	−	+++
19A	−	−	−	+++
19B	++	++	+	+++
34A	−	−	−	+++
34B	+++	+	+	+++
35A	−	+	−	+++
35B	+++	+++	++	+++
43A	−	−	−	+++
43B	−	−	−	+++
47A	−	−	−	+++
47B	++	+	++	+++
47C	++	+	++	+++
51A	−	−	+	+++
51B	+++	++	++	+++
		Liver		
2D	−	−	+	+++
2E	++	+++	+++	+++
6D	−	−	−	+++
6E	++	++	+	+++
9D	−	−	−	+++
9E	+	−	−	+++
15D	−	−	−	+++
15E	−	−	−	+++
24D	−	−	−	+++
24E	−	++	++	+++
36D	−	−	−	+++
36E	++	+++	++	+++
37D	−	−	−	+++
37E	++	++	++	+++
52D	−	−	+	+++
52E	++	++	++	+++
62D	−	−	−	+++
62E	−	+++	−	+++
63D	−	−	−	+++
63E	+	+++	+	+++

A, normal colon tissue; B, primary colon carcinoma; C, liver metastasis from primary colon carcinoma; D, normal liver; E, liver metastasis from primary colon carcinoma. The relative levels for each gene mRNA expression were determined by comparison to β-actin mRNA expression levels in each tissue sample.

chromosomal regions that are frequently deleted in colorectal tumors include 5q and 18q, which contains the cell adhesion mediator-related *DCC* gene (7). Each of these genetic alterations can occur at discrete stages in the progression of colon cancer proceeding from benign tubular adenomas through villous adenomas to frank carcinomas. For example, loss of heterozygosity on chromosome 5q21 at the *FAP* locus, which contains the guanine nucleotide binding protein-related *MCC* gene, is a relatively early event that occurs during the transition from normal colon to benign adenomas; in contrast, inactivation of both p53 alleles by deletion and by point mutation generally occurs at a later stage during the conversion of villous adenomas to frank carcinoma (2, 6, 8, 40). Changes in the level of expression of cripto and AR can also be added to this list of genes that might contribute to the onset or progression of colon cancer. The differential expression of cripto and AR in transformed versus normal colonic epithelia

may be secondary to these chromosomal aberrations. However, no structural alterations have been identified at either of these loci in colorectal carcinomas. It will be important to ascertain whether tubular or villous adenomas also express these two genes, and whether they are expressed to the same level and frequency as that observed in primary and metastatic colon tumors. In this respect, preliminary studies have shown that AR protein can be immunocytochemically detected in a majority of primary colorectal tumors and at a lower frequency in tubular or villous adenomas or in normal colon mucosa (T.S. and G. Johnson, unpublished data). This study demonstrates that cripto and AR may be important in the pathogenesis of colorectal cancer and can serve as useful markers for metastatic disease.

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1. Augenlicht, L. H. (1989) *Cell and Molecular Biology of Colon Cancer* (CRC, Boca Raton, FL).
2. Fearon, E. R. & Vogelstein, B. (1990) *Cell* **61**, 759–767.
3. Bos, J. L., Fearon, E. R., Hamilton, S. R., Verlaan-de Vries, M., van Boom, J. H., van der Eb, A. J. & Vogelstein, B. (1987) *Nature (London)* **327**, 293–297.
4. Nigro, J. M., Baker, S. J., Preisinger, A. C., Jessup, J. M., Hostetter, R., Cleary, K., Bigner, S. H., Davidson, N., Baylin, S., Devilee, P., Glover, T., Collins, F. S., Weston, A., Modali, R., Harris, C. C. & Vogelstein, B. (1989) *Nature (London)* **342**, 705–708.
5. Rodrigues, N. R., Rowan, A., Smith, M. E. F., Kerr, I. B., Bodmer, W. F., Gannon, J. V. & Lane, D. P. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 7555–7559.
6. Leppert, M., Dobbs, M., Scambler, P., O'Connell, P., Nakamura, Y., Stauffer, D., Woodward, S., Burt, R., Hughes, J., Gardner, E., Lathrop, M., Wasmuth, J., Laloulel, J.-M. & White, R. (1987) *Science* **238**, 1411–1413.
7. Fearon, E. R., Cho, K. R., Nigro, J. M., Kern, S. E., Simons, J. W., Ruppert, J. M., Hamilton, S. R., Preisinger, A. C., Thomas, G., Kinzler, K. W. & Vogelstein, B. (1990) *Science* **247**, 49–56.
8. Kinzler, K. W., Nilbert, M. C., Vogelstein, B., Bryan, T. M., Levy, D. B., Smith, K. J., Preisinger, A. C., Hamilton, S. R., Hedge, P., Markham, A., Carlson, M., Josyln, G., Groden, J., White, R., Miki, Y., Miyoshi, Y., Nishisho, I. & Nakamura, Y. (1991) *Science* **251**, 1366–1370.
9. Coffey, R. J., Goustin, A. S., Mangelsdorf Soderquist, A., Shipley, G. D., Wolfshohl, J., Carpenter, G. & Moses, H. L. (1987) *Cancer Res.* **47**, 4590–4594.
10. Watkins, L. F., Brattain, M. G. & Levine, A. E. (1988) *Cancer Lett.* **40**, 59–70.
11. Liu, C., Woo, A. & Tsao, M.-S. (1990) *Br. J. Cancer* **62**, 425–429.
12. Markowitz, S. D., Molkentin, K., Gerbic, C., Jackson, J., Stellato, T. & Willson, J. K. V. (1990) *J. Clin. Invest.* **86**, 356–362.
13. Malden, L. T., Novak, U. & Burgess, A. W. (1989) *Int. J. Cancer* **43**, 380–384.
14. Anzano, M. A., Rieman, D., Prichett, W., Bowen-Pope, D. F. & Greig, R. (1989) *Cancer Res.* **49**, 2898–2904.
15. Culouscou, J.-M., Remacle-Bonnet, M., Garrouste, F., Marvaldi, J. & Pommier, G. (1987) *Int. J. Cancer* **40**, 646–652.
16. Tricoli, J. V., Rall, L. B., Karakousis, C. P., Herrera, L., Petrelli, N. J., Bell, G. I. & Shows, T. B. (1986) *Cancer Res.* **46**, 6169–6173.
17. Lambert, S., Vivario, J., Boniver, J. & Gol-Winkler, R. (1990) *Int. J. Cancer* **46**, 405–410.
18. Murthy, U., Anzano, M. A. & Grieg, R. G. (1989) *Int. J. Cancer* **44**, 110–115.
19. Moorghen, M., Ince, P., Finney, K. J., Watson, A. J. & Harris, A. L. (1990) *Anticancer Res.* **10**, 605–612.
20. Ciccodicola, A., Dono, R., Obici, S., Simeone, A., Zollo, M. & Persico, M. G. (1989) *EMBO J.* **8**, 1987–1991.
21. Dono, R., Montuori, N., Rocchi, M., De Ponti-Zilli, L., Ciccodicola, A. & Persico, M. G. (1991) *Am. J. Hum. Genet.*, in press.
22. Shoyab, M., McDonald, V. L., Bradley, J. G. & Todaro, G. J. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6528–6532.
23. Shoyab, M., Plowman, G. D., McDonald, V. L., Bradley, J. G. & Todaro, G. J. (1989) *Science* **243**, 1074–1076.
24. Plowman, G. D., Green, J. M., McDonald, V. L., Neubauer, M. G., Disteche, C. M., Todaro, G. J. & Shoyab, M. (1990) *Mol. Cell. Biol.* **10**, 1969–1981.
25. Kimura, H., Wolfgang, H. F. & Schubert, D. (1990) *Nature (London)* **348**, 257–260.
26. Higashiyama, S., Abraham, J. A., Miller, J., Fiddes, J. C. & Klagsbrun, M. (1991) *Science* **251**, 936–939.
27. Carpenter, G. & Wahl, M. I. (1990) in *Peptide Growth Factors and Their Receptors I*, eds. Sporn, M. B. & Roberts, A. B. (Springer, Berlin), pp. 69–171.
28. Yamamoto, T., Ikawa, S., Akiyama, T., Semba, K., Nomura, M., Miyajima, N., Saito, T. & Toyoshima, K. (1986) *Nature (London)* **319**, 230–234.
29. Kraus, M. H., Issing, W., Miki, T., Popescu, N. C. & Aaronson, S. A. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 9193–9197.
30. Plowman, G. D., Whitney, G. S., Neubauer, M. G., Green, J. M., McDonald, V. L., Todaro, G. J. & Shoyab, M. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 4905–4909.
31. Brattain, M. G., Levine, A. E., Chakrabarty, S., Yeoman, L. C., Willson, J. K. V. & Long, B. (1984) *Cancer Metastasis Rev.* **3**, 177–191.
32. Mulder, K. M. & Brattain, M. G. (1989) in *Cell and Molecular Biology of Colon Cancer*, ed. Augenlicht, L. H. (CRC, Boca Raton, FL), pp. 45–67.
33. Chirgwin, J. M., Przybyla, A. E., McDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299.
34. Ciardiello, F., McGeady, M. L., Kim, N., Basolo, F., Hynes, N., Langton, B. C., Yokozaki, H., Saeki, T., Elliott, J. W., Masui, H., Mendelsohn, J., Soule, H., Russo, J. & Salomon, D. S. (1990) *Cell Growth Differ.* **1**, 407–420.
35. Derynck, R., Goeddel, D. V., Ullrich, A., Gutterman, J. U., Williams, R. D., Bringman, T. S. & Berger, W. H. (1987) *Cancer Res.* **47**, 707–712.
36. Derynck, R. (1988) *Cell* **54**, 593–595.
37. Rosenthal, A., Lindquist, P. B., Bringman, T. S., Goeddel, D. V. & Derynck, R. (1986) *Cell* **46**, 301–309.
38. Shankar, V., Ciardiello, F., Kim, N., Derynck, R., Liscia, D. S., Merlo, G., Langton, B. C., Sheer, D., Callahan, R., Bassin, R. H., Lippman, M. E., Hynes, N. & Salomon, D. S. (1989) *Mol. Carcinogen.* **2**, 1202–1216.
39. Cook, P. W., Mattox, P. A., Keeble, W. W., Pittelkow, M. R., Plowman, G. D., Shoyab, M., Adelman, J. P. & Shipley, G. D. (1991) *Mol. Cell. Biol.* **11**, 2547–2557.
40. Baker, S. J., Fearon, E. R., Nigro, J. M., Hamilton, S. R., Preisinger, A. C., Jessup, J. M., van Tuinen, P., Ledbetter, D. H., Barker, D. F., Nakamura, Y., White, R. & Vogelstein, B. (1989) *Science* **244**, 217–221.