Differential Immunohistochemical Detection of Amphiregulin and Cripto in Human Normal Colon and Colorectal Tumors

Toshiaki Saeki, Kurt Stromberg, Chen-Feng Qi, William J. Gullick, Eiichi Tahara, Nicola Normanno, Fortunato Ciardiello, Nicholas Kenney, Gibbes R. Johnson, and David S. Salomon

Laboratory of Tumor Immunology and Biology, Division of Cancer Biology and Diagnosis and Centers, National Cancer Institute, NIH, Bethesda, Maryland 20892 [T. S., C-F. Q., N. N., N. K., D. S. S.]; Division of Cytokine Biology, Food and Drug Administration, Bethesda, Maryland 20892 [K. S., G. R. J.]; Molecular Oncology Laboratory, Imperial Cancer Research Fund, Hammersmith Hospital, London, England W12 OHB [W. J. G.]; Department of Pathology, Hiroshima University Hospital, School of Medicine, 1.2.3. Kasumi, Minaiku, Hiroshima, Japan [F. C.]

ABSTRACT

Thirty-six primary human colorectal tumors, 43 noninvolved colon samples that were adjacent to either carcinomas or adenomas, 22 adenomas, and nine normal colon specimens were immunohistochemically examined for the presence and localization of two epidermal growth factor-related peptides, amphiregulin (AR) and cripto. Within the primary tumors, 18 (50%) showed moderate levels of AR expression. Approximately 60% of the tubular and tubulovillous adenomas were positive for AR expression, whereas only 15% of the adjacent, noninvolved colon mucosa expressed AR. A greater proportion of well-differentiated tubules (71%) were positive for AR expression than were poorly differentiated tumors (18%). All of the nine normal colon specimens were positive. Consequently, AR expression appeared to be associated with both normal and malignant epithelial cells that were more differentiated. The distribution of cripto expression was different. Seventy-nine% of the colon tumors expressed cripto with a frequency of expression that was approximately equivalent between well-differentiated and poorly differentiated tumors. Approximately 86% of the tubulovillous adenomas, but only 43% of the tubular adenomas, were positive for cripto expression. In contrast, whereas AR was expressed in normal colon specimens, none of these tissues expressed cripto, and only 12% of the noninvolved normal colon samples adjacent to tumors or adenomas were positive for cripto. Cripto expression therefore appeared related to neoplasia. These data suggest that AR and cripto may be functioning as potential autocrine and/or paracrine growth factors in the colon and that the differential expression of cripto may serve as a potential tumor marker for colorectal carcinogenesis.

INTRODUCTION

Colorectal cancer is an example of multistage carcinogenesis in which there is a defined sequence of pathological stages ranging from premalignant adenomas to carcinoma in situ (1). In general, large villous adenomas have a higher probability of converting to frank adenocarcinoma than either the smaller tubulovillous or tubular adenomas (2). A number of somatic gene alterations that may be involved in the genesis and progression of colon cancer have been identified (3, 4). These genetic changes include activation of specific protooncogenes such as Ki-ras, LOH,2 or point mutations in tumor suppressor genes, such as p53, that arise at discrete histological stages during this transition process (5, 6). Protooncogenes can directly code for proteins that are growth factors or growth factor receptors, or they can indirectly regulate the expression of various growth factors or of proteins that are involved in the intracellular signal transduction pathway(s) for growth factors, which suggests that there may be some functional significance for selective genetic alterations in these genes (7, 8). In this respect primary human colon carcinoma elaborates several different growth factors, including TGF-α, TGF-β1 and -2, IGF-I, IGF-II, and platelet-derived growth factor (9–15). Since colon cancer cells also express specific cell surface receptors for EGF/TGF-α, TGF-β, IGF-I, and IGF-II, these growth factors may regulate some aspect of colon tumor growth through potential autocrine pathways (12, 13, 16, 17). However, expression of some of these growth factors is not restricted to malignant colonic epithelial cells because growth factors such as TGF-α and TGF-β are also found in normal epithelial cells along the gastrointestinal tract (9, 10).

EGF and TGF-α belong to a larger family of proteins of diverse functions that include other growth factors, such as vaccinia virus growth factor; cell–cell adhesion molecules, such as the Notch and Delta proteins of Drosophila; and some more distantly related serine proteases and extracellular matrix-associated proteins (18). A common feature of all of these proteins is the presence of single or multiple EGF-like repeats in the extracellular domain that consist of six cysteine residues spaced at defined intervals that are capable of forming three intramolecular disulfide bonds (19). Two new members of the EGF/TGF-α family have been identified recently, AR and cripto (20–23). AR is a 78- or 84-amino acid, glycosylated protein that is initially synthesized as a 252-amino acid transmembrane-associated precursor and that is encoded by a 1.4-kilobase transcript. AR can bind to the EGF receptor and can either stimulate or inhibit cell growth, depending upon the concentration and the nature of the target cell (20, 24, 25). A cripto-specific 2.2-kilobase mRNA was originally identified in undifferentiated human embryonal carcinoma cells and codes for a protein of 188 amino acids that contains a 37-amino acid region that shares structural homology with other members of the EGF/TGF-α family (22). Cripto can function as a dominantly acting oncogene, since overexpression of the cripto gene can lead to the transformation of epithelial cells and fibroblasts in vitro (22, 23). It is unknown whether cripto can function as a growth modulator, since a recombinant protein has not yet been produced nor has the protein been purified. There is little information relating to the distribution and level of expression of AR or cripto in primary human tumors. We have recently found that approximately 68% and 50% of primary human colorectal tumors express specific cripto and AR mRNA transcripts, respectively (26). In contrast, only 3 to 15% of adjacent noninvolved colonic mucosa express the mRNAs for these two proteins. However, neither normal colon from healthy individuals nor colonic adenomas were analyzed in this prior study nor was it possible to localize at the cellular level which cell type, if any, was expressing these proteins. Consequently, to ascertain if this differential expression of AR and cripto mRNA between malignant and normal colon tissue is reflected by a
difference in the amount of AR and cripto protein that might be produced in these tissues, we have analyzed, using immunochemistry, a panel of primary human colorectal tumors, noninvolved colonic tissues adjacent to tumors or adenomas, adenomas of both tubulovillous and tubular histology, and normal colonic tissue obtained from noncancer patients for AR and cripto protein expression.

MATERIALS AND METHODS

Cell Lines and Human Tissues. GEO and JVC human colon cancer cell lines were obtained from Dr. Michael Brattain (Baylor College of Medicine, Houston, TX) and were maintained in McCoy's Medium 5A containing 10% fetal calf serum as previously described (27). MCF-7 clone E3 human breast cancer cells were obtained from Dr. Samuel Brooks (Michigan Cancer Foundation, Detroit, MI) and cultured in a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F-12 medium, supplemented with 10% fetal calf serum as previously described (28). Paraffin blocks of formalin-fixed tissues representing 36 primary colorectal tumors (Dukes' Grade A to C) with adjacent noninvolved colon and 9 normal colons were obtained from the Department of Surgical Pathology of the George Washington University Medical Center, Washington, DC, and from the University of Alabama Medical Center, Birmingham, AL. Twenty-two adenomas with adjacent noninvolved colon tissue were obtained from the Department of Gastroenterology, University of Hiroshima, Hiroshima, Japan, following colonoscopy.

Peptide Synthesis and Polyclonal Antibodies. A 17-mer synthetic peptide (CPPSFYGRNCEHDVRKE) that corresponds to amino acid residues 97 to 113 in the human cripto protein and that represents the COOH terminus of the 37-amino acid EGF-like region was prepared by solid-phase synthesis as previously described (29). This region was selected because it is related in sequence to the COOH-terminal 17 amino acids of TGF-α, which was used previously to raise polyclonal (30) and monoclonal (31) antibodies for immunocytochemical staining. The peptide was coupled with KLH using glutaraldehyde (29). The KLH-coupled synthetic peptide was then used to immunize rabbits. Serum titers were monitored periodically by ELISA and an antisera, designated CR-1, was obtained following the last of three immunizations. CR-1 reacted strongly in an ELISA using plates coated with the peptide immunogen, but failed to recognize either TGF-α or AR at dilutions that were routinely used for immunocytochemistry. In addition, CR-1 was able to detect a Mr. 32,000 MS2-cripto fusion protein derived from Escherichia coli following Western blot analysis. A 19-mer synthetic peptide (VKFPQKNTESENTSDPKKR) that corresponds to NH2-terminal amino acid residues 8 to 26 in the human AR protein was synthesized as previously described (29). The peptide was conjugated to KLH and was then used to generate monospecific antiserum in rabbits. The IgG fraction was obtained by affinity chromatography, designated AR-Abl IgG, and characterized as previously described (25). Affinity-purified AR-Abl anti-AR antibody reacted with the 19-mer synthetic peptide antigen. No immunoperoxidase staining after reaction with the anti-cripto CR-1 antibody at an appropriate concentration or dilution of the appropriate preimmune rabbit serum or IgG and/or by absorbing the primary antibody for 2 h at 37°C with 20 μg/ml of the 17-mer cripto peptide for the CR-1 antibody or with 2 μg/ml of the 19-mer AR peptide for the affinity-purified AR-Abl antibody. The number of positive cells per slide was stratified into three groups based on the percentage of positive cells: Group 1, <33%; Group 2, 33 to 67%; and Group 3, >67%. Semiquantitative scores ranging from 1 to 9 for specific staining for each specimen were obtained by multiplying the staining intensity by the number of the group that represented the percentage of positive cells within each specimen as previously described (32). A score of zero represents no specific staining observed.

RESULTS

AR was originally isolated from the conditioned medium of TPA-treated MCF-7 cells (24). In addition, we have previously demonstrated that specific mRNA transcripts for AR and cripto could be detected in several human colon cancer cell lines such as GEO, whereas others such as JVC failed to express mRNA for either of these two proteins (26). MCF-7 cells that had been treated with 10 nM TPA for 48 h (24), GEO, and JVC cells were therefore reacted with the antipeptide rabbit antibodies to ascertain if these antibodies could specifically detect any immunoreactive AR or cripto protein in these cells after formalin fixation. Intense cytoplasmic staining for AR was detected in TPA-treated MCF-7 cells and in GEO cells but not in JVC cells after reaction with 10 μg/ml of the AR-Abl anti-AR antibody, which was found to be optimal for demonstrating specific immunoperoxidase staining (data not shown). This staining could be effectively abolished after preabsorption of the antibody with the 19-mer synthetic peptide antigen. Likewise, GEO but not JVC cells exhibited intense cytoplasmic staining after reaction with the anti-cripto CR-1 antibody at an optimal 1:400 dilution (data not shown). This immunoreactivity could also be eliminated by pretreatment of the antibody with the 17-mer synthetic peptide antigen. No immunoperoxidase staining could be detected in any of these different cells when the appropriate preimmune serum or IgG was used at comparable concentrations to the postimmune serum.

Using optimal concentrations of the anti-AR or anti-cripto antibodies as determined on the human tumor cell lines, formalin-fixed paraffin-embedded human colon tissues were then examined immunohistochemically for the expression of AR and cripto. Of the 36 primary human colon carcinomas that were examined, 18 (50%) were specifically reactive with the
anti-AR antibody (Table 1). When the tumors were segregated into different groups based on their degree of differentiation, well-differentiated tumors had the highest frequency of AR expression with 71.4% (10 of 14) showing specific staining. Moderately differentiated tumors exhibited an intermediate frequency with 54.5% (6 of 11), showing reactivity while only 18.2% (2 of 11) of the poorly differentiated tumors reacted with the AR-Abl antibody. Thus, there was a correlation between AR expression and the degree of differentiation of the carcinoma. No relationship was found between AR expression and Dukes' grade or tumor location.

Specific immunoperoxidase staining for AR was detected in the tumor cells but not in the surrounding stroma, smooth muscle, or capillary endothelial cells (Fig. 1A to C). No staining was detected in sections that had been incubated with the preimmune IgG (Fig. 1A) or with AR-Abl IgG that had been preabsorbed with the peptide antigen (data not shown). Adenomatous polyps also expressed AR, and approximately 64% (14 of 22) of the polyps showed reactivity with the AR-Abl IgG. No significant difference was observed in the frequency of AR expression between relatively benign tubular adenomas (66.6%) and the more aggressive tubulovillous adenomas (60%). Within the moderately and well-differentiated carcinomas and in the adenomas, immunospecific staining was localized to cells of the luminal surface of the glands and was often observed in cytoplasmic inclusion bodies within these cells (Fig. 1B and C). A heterogeneity of AR expression was also observed in both carcinomas (Fig. 1B) and adenomas. The frequency of AR expression in histologically normal colon mucosa that was adjacent to either a carcinoma or adenoma was relatively low with only 15% of the tissues expressing a weak level of AR protein. In contrast, all nine colon tissue samples that were obtained from noncancer patients exhibited moderate levels of AR expression. Immunoperoxidase staining was generally limited to surface columnar epithelial cells and goblet cells of the luminal mucosa (Fig. 1D, E). No cells within normal crypts reacted with the AR-Abl antibody to AR.

The relative distribution and frequency of cripto expression in colon tissue were different from those of AR (Table 2). Twenty-four of the 36 carcinomas were also analyzed for cripto expression. Within this smaller group, 79.2% (19 of 24) of the primary tumors showed moderate cytoplasmic staining with the anti-cRIPTO CR-1 antibody (Fig. 2B and C) and no staining with the preimmune serum (Fig. 2A). Unlike the distribution of AR, there was only a marginal difference in the frequency of cripto expression when the tumors were histologically stratified according to the degree of differentiation. Ninety-two% (12 of 13) of the well-differentiated tumors versus 60% (3 of 5) of the poorly differentiated tumors expressed cripto. However, as was the case with AR expression, marked heterogeneity was observed in the intensity of the immunoperoxidase staining and in the percentage of tumor cells that were stained with the CR-1 antibody within any one specimen. Similarly, specific immunoperoxidase staining was observed in the carcinoma cells of the tumors, whereas very little antibody reactivity was detected within the stroma or with vascular elements. In addition, normal appearing colonic mucosa that was adjacent to carcinomas was also generally negative (Fig. 2B). Of the total number of polyps, 57% (12 of 21) stained with the CR-1 antibody. Most importantly, 85.7% (6 of 7) of tubulovillous adenomas reacted with the antibody compared with 42.9% (6 or 14) of the more benign tubular adenomas. No relationship was found between cripto expression and adenoma size or the degree of dysplasia within a given adenoma specimen. The intensity of staining in the adenomas was slightly less than the intensity of staining that was observed in the carcinomas and was preferentially restricted to cells of the luminal surface of the glands (Fig. 2D). Unlike AR expression, none of the normal colon tissues obtained from noncancer patients expressed cripto, and only 12.2% (5 of 41) of normal colon mucosa adjacent to carcinomas or adenomas expressed this protein. Coexpression of mRNA for AR and cripto occurs in a majority of individual primary and metastatic colorectal carcinomas (26). To determine if this coexpression is also reflected in the distribution of AR and cripto protein, colon tissue samples were stratified on this basis (Table 3). Expression of both cripto and AR (CR+/AR+) occurred in 41.7% of both the carcinomas and adenomas that were analyzed for both proteins. Approximately 50% of the moderately and well-differentiated carcinomas exhibited this phenotype, whereas none of the poorly differentiated carcinomas were positive for both AR and for cripto. Nearly 86% of the tubulovillous adenomas were positive for both proteins compared with only 28.6% of the tubular adenomas. In contrast, only 7.3% of normal colon tissue adjacent to either carcinoma or adenoma exhibited this phenotype. The distribution of tissues that failed to express both proteins was opposite to the pattern that was found in tissues that expressed both proteins. For example, an absence of expression of both proteins (CR-/AR-) was found more frequently in epithelium adjacent to a tumor or polyp (80.4%) than in adenomas or carcinomas (23.8% and 16.6%, respectively). In addition, a higher percentage of more poorly differentiated carcinomas failed to express both proteins compared with well-differentiated carcinomas (40% versus 7.7%, respectively).

**DISCUSSION**

More than 100,000 individuals each year develop colorectal cancer, and it accounts for approximately 14% of all types of cancer (2, 33). Early diagnosis when the tumor has not invaded is crucial for successful treatment because the depth of tumor invasion relates directly to prognosis (2). Therefore, identifying novel proteins that are preferentially expressed in premalignant adenomas and in carcinomas that may facilitate detection of this malignancy is important. A number of glycoproteins and mucin-like proteins, such as CEA and TAG-72, have been identified and have been demonstrated to be tumor-associated antigens, which are useful in the diagnosis, localization, and possible therapy of colorectal cancer (34, 35). Overexpression of growth factors and/or their receptors may also be of clinical and biological significance in particular carcinomas. For example, overexpression of the EGF receptor occurs in approxi-
approximately 40 to 50% of breast tumors that are estrogen receptor negative and is generally associated with reduced survival (36). High levels of expression of TGF-α and EGF receptor also occur in a majority of adenocarcinomas of the lung, and coexpression of both proteins is correlated with a poor prognosis (37). More importantly, EGF receptors can be detected in 80 to 90% of colorectal carcinomas, suggesting that EGF-related peptides may be operating through this system to regulate some aspects of colon tumor growth or differentiation (38, 39). The present study demonstrates that AR and particularly cripto, two new members of the EGF/TGF-α family, are expressed in colon tissues at a relatively high frequency and may therefore fulfill some of these characteristics, as potential tumor markers and/or as autocrine or paracrine growth factors.

AR has been previously detected in many human breast, ovarian, and pancreatic carcinoma cell lines as well as in several normal human tissues including placenta, ovary, breast, testis, lung, and kidney (21, 24). More recently, expression of AR mRNA has been detected in approximately 50% of primary human colorectal tumors but in only 15% of noninvolved colon mucosa that was adjacent to tumors (26). These values agree fairly well with the frequency of AR protein expression that can be found in these tissues by immunohistochemistry (Table 1). Expression of AR was found in all normal colon mucosa

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![Image](https://cancerres.aacrjournals.org/content/53/10/3470/fig1)

**Fig. 1.** Immunoperoxidase staining of formalin-fixed paraffin-embedded colon tissues using purified anti-amphiregulin AR-Ab1. In A and B, well-differentiated adenocarcinoma reacted with preimmune AR-Ab1 IgG at 10 μg/ml (A) or postimmune IgG at 10 μg/ml (B), × 200; in C, columnar epithelial cells of well-differentiated adenocarcinoma stained with AR-Ab1 postimmune IgG, × 1000; in D and E, normal colon epithelium from a noncancer patient reacted with preimmune AR-Ab1 IgG (D) or postimmune AR-Ab1 IgG (E), × 400.
samples that were obtained from noncancer patients, and the level of expression was approximately equivalent to the level that was found in the well-differentiated carcinomas. These results suggest that AR may perform some normal function in the colon. In particular, the localization of AR in more differentiated surface columnar epithelial and goblet cells and not in the proliferative cells of the crypts in normal mucosa further suggests that AR may be associated with differentiation and not growth alone. The relatively high frequency of expression in well-differentiated tumors compared with poorly differentiated tumors supports this possibility. In addition, in many carcinomas luminal reactivity with the anti-AR antibody was observed in glandular structures, suggesting that AR might be secreted. Secreted AR may be functioning as an autocrine and/or paracrine growth factor in vivo. In this regard, GEO colon cancer cells, which are derived from a well-differentiated carcinoma, secrete AR that, in turn, acts as an external autocrine growth factor for these cells in vitro. Our finding that AR is frequently expressed by well-differentiated colon carcinomas suggests that AR may function as an autocrine growth factor for these types of carcinomas. In contrast to the presence of moderate levels of AR in normal colonic mucosa, low or undetectable levels of AR expression were detected in colonic mucosa that was adjacent to either tumors or polyps. These results agree with immunostaining data accrued from a previous study where AR protein could not be detected in mucosa adjacent to a small number of carcinomas that were examined (26). These results further suggest that epithelial cells in the vicinity of premalignant or malignant lesions may be abnormal and may be in a period of transition. This is probably the case, since a recent study has detected a high incidence of histologically aberrant crypts that represent potential preneoplastic lesions in the colonic mucosa of patients with colon cancer (40). Furthermore, enhanced staining with monoclonal antibodies against CEA or against the p21WAF1 protein, two colorectal tumor markers, was detected in epithelium adjacent to carcinomas but not in more distant normal mucosa, again suggesting that these cells may be slightly dysplastic (35, 41).

Table 2 Cripto immunoreactivity in human colon tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>No. of immunopositive</th>
<th>Av. score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcinoma</td>
<td>19/24 (79.2)*</td>
<td>5.4 [1-9]*</td>
</tr>
<tr>
<td>Well differentiated</td>
<td>12/13 (92.3)</td>
<td>6 [1-9]</td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>4/6 (66.7)</td>
<td>1 [1]</td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>3/5 (60.0)</td>
<td>9 [9]</td>
</tr>
<tr>
<td>Adenoma</td>
<td>12/21 (57)</td>
<td>1.03 [1-2]</td>
</tr>
<tr>
<td>Tubulovillous</td>
<td>6/7 (85.7)</td>
<td>1.2 [1-2]</td>
</tr>
<tr>
<td>Tubular</td>
<td>6/14 (42.9)</td>
<td>1 [1-3]</td>
</tr>
<tr>
<td>Normal mucosa</td>
<td>0/3 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Mucosa adjacent to carcinoma</td>
<td>5/41 (12.2)</td>
<td>1 [1]</td>
</tr>
<tr>
<td>or adenoma</td>
<td></td>
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* Numbers in parentheses, percentage of total that were positive for each group.

** Numbers in brackets, range of scores.

A specific 2.2-kilobase human cripto mRNA has been detected in only a limited number of tissues such as undifferentiated mouse and human embryonal carcinoma cells, human colon cancer cells, and midgestation mouse embryos (22, 26). The high incidence of immunoreactivity that was detected in the present study for cripto expression in primary colorectal tumors (79%) is in accord with mRNA expression data that were accrued from two separate studies where approximately 68% and 82% of colon tumors were positive using Northern blot analysis (26, 42). Cripto has a cellular distribution that was similar to AR, since a moderate level of immunostaining for cripto predominantly localized to the luminal surface of the glands in well-differentiated tumors. Both AR and cripto are also expressed in most adenomas. Adenomas represent premalignant lesions that have a tendency to become malignant depending upon their size and histopathology. In general, villous or large tubulovillous adenomas have a higher probability of converting to frank carcinoma than do smaller tubular adenomas. Whereas AR is markedly expressed in normal colon epithelium and moderately expressed in tubular and tubulovillous adenomas, cripto is not expressed in normal colon mucosa and is only expressed in tubulovillous and tubular adenomas. More importantly, a much greater proportion of tubulovillous adenomas are reactive with the anti-crypto antibody than are tubular adenomas (86% compared with 43%), suggesting that a graded increase in the expression of cripto may be one correlate with the development of colon carcinoma. Furthermore, onset in the expression of cripto may be a relatively early event like LOH of the MCC gene, a potential tumor suppressor gene, or activation of the Ki-ras gene, which can occur in a clonal population of cells during the transition from dysplastic epithelium to adenoma or from small benign adenomas to large locally invasive adenomas (4, 43). The low level of cripto expression that was detected in histologically normal mucosa adjacent to carcinomas or to adenomas supports this possibility and is in reasonable agreement with the mRNA expression data in that cripto mRNA was detected in only 3% of mucosa adjacent to carcinomas (26). Because cripto is preferentially expressed in primary colorectal carcinomas and in adenomas and because it shares structural homology with TGF-α and other members of the EGF family, it may regulate the growth of these cells through a potential autocrine and/or paracrine pathway. Therefore, one question that needs to be addressed once a recombinant or purified protein is available is whether criterio can function as an autocrine growth factor for GEO cells. More over, both TGF-α (10, 12) and AR are expressed in normal glands in well-differentiated tumors. Whereas AR is markedly expressed in normal colon epithelium and moderately expressed in tubular and tubulovillous adenomas, cripto is not expressed in normal colon mucosa and is only expressed in tubulovillous and tubular adenomas. More importantly, a much greater proportion of tubulovillous adenomas are reactive with the anti-crypto antibody than are tubular adenomas (86% compared with 43%), suggesting that a graded increase in the expression of cripto may be one correlate with the development of colon carcinoma. Furthermore, onset in the expression of cripto may be a relatively early event like LOH of the MCC gene, a potential tumor suppressor gene, or activation of the Ki-ras gene, which can occur in a clonal population of cells during the transition from dysplastic epithelium to adenoma or from small benign adenomas to large locally invasive adenomas (4, 43). The low level of cripto expression that was detected in histologically normal mucosa adjacent to carcinomas or to adenomas supports this possibility and is in reasonable agreement with the mRNA expression data in that cripto mRNA was detected in only 3% of mucosa adjacent to carcinomas (26). Because cripto is preferentially expressed in primary colorectal carcinomas and in adenomas and because it shares structural homology with TGF-α and other members of the EGF family, it may regulate the growth of these cells through a potential autocrine and/or paracrine pathway. Therefore, one question that needs to be addressed once a recombinant or purified protein is available is whether cripto can function as a growth factor for colonic epithelial cells and, if so, whether it interacts with the EGF receptor or a unique cell surface receptor molecule.

The differential expression of cripto in primary colon carcinomas suggests that this protein may be a potential colon cancer marker. Although cripto is expressed in primary colorectal carcinomas and not in normal colon mucosa from noncancer patients, as a solitary marker, cripto fails to distinguish among tumors based on their degree of differentiation. In contrast, AR, as a marker of differentiation, does not differentiate between well-differentiated malignant and normal colon tissue. However, when both proteins are coexpressed a clear segregation can be observed between well-differentiated carcinomas or moderately differentiated carcinomas where both proteins are present in approximately 50% of these pathological tissues and in poorly differentiated carcinomas where coexpression of both proteins is not detected. This may be biologically and clinically significant. For example, poorly differentiated colorectal tumors are more aggressive than well-differentiated tumors with respect to the ability to metastasize (44, 45). In fact, the degree of differentiation is an independent prognostic marker for survival (2, 46). Therefore, stratification of tumor subtypes based upon the coexpression of AR and cripto may be diagnostically useful in complementing histological grading as a pathological criterion. In addition, several colon carcinoma cell lines that differ in their degree of differentiation have been characterized with respect to their mitogenic response to exogenous EGF and to their production of TGF-α (11). Well-differentiated or moderately differentiated colon carcinoma cell lines require EGF for monolayer growth, whereas poorly differentiated carcinoma cells do not. The poorly differentiated colon carcinoma cells also synthesize and secrete higher levels of TGF-α than the more well-differentiated colon carcinoma cells that then might function as an autocrine growth factor for these cells (11). AR production with respect to histological grade is just the reverse. Therefore, two EGF-related peptides, TGF-α and AR, might be regulating the proliferation of different histological subsets of colon tumors through an autocrine mechanism. This may be the case, since the anchorage-dependent growth of two human colon cancer cell lines, GEO and DLD-1, can be significantly inhibited by anti-TGF-α-neutralizing antibodies or anti-EGF receptor-blocking antibodies (47, 48). In addition, AR may also function as an autocrine growth factor for GEO cells. Moreover, both TGF-α (10, 12) and AR are expressed in normal colon mucosa, suggesting that these two peptides may also be important growth factors for nonneoplastic colon epithelial cells, whereas cripto has more restricted distribution of expression and may be a clinically relevant tumor marker and may perform a role in normal to adenoma to carcinoma transformation. Collectively, these data suggest that changes in the level of expression of cripto and possibly AR in specific colonic epithelial cell populations may be one of several changes that are important in the pathogenesis and diagnosis of colon cancer. For example, it has been demonstrated that a minimum of at least four to five genetic alterations is required for the initiation of colon cancer and that it is probably the cumulative effect of these events rather than the sequence of their appearance that may be important in the onset and/or progression of this disease (3, 4). It will, therefore, be important to ascertain if changes in the level of expression of cripto and AR in colon tissues are secondary to any genetic alterations such as amplifications, deletions, or rearrangements.

REFERENCES

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