Studies on the Control of Gene Expression of the Carcinoembryonic Antigen Family in Human Tissue

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ABSTRACT

The control of expression of genes of the carcinoembryonic antigen family was investigated in 22 specimens of malignant and nonmalignant human colorectal tissues. These surgical specimens included seven colonic adenocarcinomas that were compared with normal adjacent colonic mucosal tissues from the same individual. mRNA preparations from all colonic tissues expressed three bands of 3.5, 3.0, and 2.6 kilobases on Northern blots probed with carcinoembryonic antigen (CEA) complementary DNA probe while normal liver and spleen were negative. The major band of 3.0 kilobases was 6 to 10 times more intense in the colon tumor specimens than in the matched normal mucosa. However, the tumor/normal ratios of immunoreactive CEA in these pairs varied from 2- to greater than 100-fold. Furthermore, there was no direct proportionality between mRNA levels and gene product expression, suggesting that the known variations in CEA expression in human colorectal tissues result from both transcriptional and posttranscriptional control mechanisms. Southern blots of DNA from these specimens did not reveal any gene rearrangements or amplifications accompanying expression. Finally, Southern blots of DNA digested with methylation-sensitive endonucleases and probed with a genomic DNA fragment upstream of CEA gene coding regions demonstrated that CEA expression is correlated with a decreased level of methylation in the 5’ region of the CEA gene.

INTRODUCTION

CEA is widely used as a human tumor marker in the management of bowel cancer (1) and was first defined by Gold and Freedman in 1965 as an antigenic component in cancers derived from gastrointestinal tract epithelium (2). CEA is a membrane glycoprotein with a molecular mass of about 180,000 and consists of a single polypeptide chain (M, 72,800) with multiple carbohydrate side chains.

CEA is a member of a large family of immunologically related glycoproteins that vary in size and tissue distribution (for reviews, see Refs. 3 and 4). The question as to whether all these CEA family members represent different proteins, or a limited number of different proteins with variable glycosylation, has been partially resolved by studies with cDNA clones for CEA and NCA isolated by our (5) and other laboratories (6-10). The predicted translation products derived from the nucleotide sequences of CEA and NCA cDNA consist of a processed signal sequence of 34 amino acids, an amino-terminal domain of 107 amino acids for both CEA and NCA, 3 homologous internal domains (each of 178 amino acids) for CEA and one for NCA, and a hydrophobic carboxy domain of 27 amino acids for CEA and 25 amino acids for NCA. The coding sequences of CEA and NCA cDNA are, overall, 90% homologous (9). However, large differences between these two CEA family members reside in the 3’ untranslated sequences of their mRNAs which are unrelated. The use of portions of the CEA and NCA cDNA clones as specific hybridization probes has shown the existence of CEA and NCA transcripts in colon carcinoma, NCA transcripts in breast tumors and NCA, and a third CEA-like transcript in chronic myelogenous leukemia cells (11, 12). These observations have shown that the existence of a family of CEA-like molecules, defined immunologically, is partially due to a more limited family of different proteins coded by different genes.

The development of a radioimmunoassay for circulating CEA (13) demonstrated that serum CEA concentrations were elevated above normal control levels in a wide range of malignancies including breast, lung, gastric, and ovarian cancer, in a number of nonmalignant diseases including alcoholic cirrhosis, gastrointestinal inflammatory diseases, and in smokers (14). The availability of cDNA clones for CEA family members now allows investigation of the molecular basis for aberrant CEA expression in these diseases.

We report here the analysis of normal and diseased human colorectal tissues for expression of CEA and NCA genes. Our results support a major control of CEA expression at the level of transcription which is inversely correlated with the degree of DNA methylation upstream of the relevant genes and a potential component of posttranscriptional control.

MATERIALS AND METHODS

Purification of RNA and Northern Blot Analysis. A variety of human tissues were obtained from surgical specimens and frozen in liquid nitrogen generally within 15 min after resection. They were stored at -70°C until processed. Approximately 0.5 g of each sample were then reduced to a powder with a mortar and pestle precooled in liquid nitrogen. The pulverized tissue was suspended in a solution of 4.0 M guanidinium thiocyanate, and total RNA was isolated by the procedure of Chirgwin et al. (15).

Twenty μg of each total RNA preparation were electrophoresed using 1.1 M formaldehyde in 1.5% agarose gels (16). The gels were stained in 20 μg/ml of acridine orange:10 mM sodium phosphate (pH 7.7) for 30 min and were visualized under UV light to ensure that equivalent amounts of RNA were present in all lanes. The RNA was transferred to Hybond-N nylon membranes (Amersham, Arlington Heights, IL). Detection of RNA with random primer ³²P-labeled cDNA probes (17) was done by hybridization for 18 h at 42°C in 5× SSPE, 5× Denhardt’s solution (1× Denhardt’s solution is 0.02% bovine serum albumin:0.02% Ficoll:0.02% polyvinyl pyrolidone), 50% formamide, 10% dextran sulfate, 50 μg/ml of heat-denatured salmon testis DNA, and 10⁶ cpm/ml of radioactive probe. Membranes were washed to a final stringency of 0.1× SSPE for 15 min at room temperature and then autoradiographed at -70°C with intensifier screens.

Purification of DNA and Southern Blot Analysis. Approximately 0.5 g of tissue were reduced to powder as above and resuspended in a solution of 20 mM Tris (pH 8.0), 50 mM EDTA, and 10 mM NaCl. Sodium N-lauroylsarcosine and proteinase K were added to a final concentration of 0.5% and 100 μg/ml, respectively, and the lysates were incubated at 50°C for 3 h. Digests were extracted with phenol and chloroform and ethanol precipitated, and the DNA was redissolved in

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5 The abbreviations used are: CEA, carcinoembryonic antigen; NCA, nonspecific cross-reacting antigen; cDNA, complementary DNA; SSPE, 0.18 M sodium chloride;10 mM sodium phosphate (pH 7.7);1 mM EDTA; SSC, standard saline citrate.
15 mM Tris (pH 7.5):0.1 mM EDTA. Restriction enzymes (6 units/μg) were used under conditions recommended by the suppliers. A series of enzyme concentrations and times was used to ensure that HpaII digests had proceeded to completion.

Samples of restriction digests were electrophoresed through 1% agarose gels and transferred to Hybond-N membranes, after partial acid depurination (16). Detection of restriction fragments with random primer-32P-labeled cDNA probes (17) was done by hybridization for 18 h at 42°C in 5× SSPE, 5× Denhardt's solution, 0.5% sodium dodecyl sulfate, 10% dextran sulfate, 50% formamide, 50 μg/ml of heat-denatured salmon testis DNA, and 2.5 × 106 cpm/ml of radioactive probe. Membranes were washed to a final stringency of 0.1× SSC (1× SSC is 0.15 M NaCl, and 0.015 M sodium citrate, pH 7.0) for 15 min at 65°C and autoradiographed at -70°C with intensifier screens.

CEA Assay. Tissues were suspended in phosphate-buffered saline by passing them through a sieve and disrupted by three 10-s sonication bursts using an immersion probe. The sonicate was assayed for CEA with the CEA double monoclonal antibody clinical kit (Abbott CEA-EIA Monoclonal, Abbott Laboratories, North Chicago, IL). Internal standards allowed calculation of the amount of CEA; the quantity of CEA was then normalized to the amount of protein in the sonic extracts as measured by the Bio-Rad protein assay.

RESULTS

In order to investigate the control of CEA expression in normal and malignant colonic tissues, we analyzed the patterns and relative levels of CEA and NCA gene expression in 22 specimens of such tissues obtained at surgical resection. These included nine samples of colonic adenocarcinoma, six of which were paired with their adjacent normal mucosas obtained at a distance from the tumor in the same surgical specimen, two specimens of liver metastases of colonic adenocarcinoma obtained with adjacent normal liver, two specimens of colon involved by ulcerative colitis, one by diverticulosis, one colonic polyp, and two samples of normal colonic mucosa from specimens removed at surgery for trauma. Paired samples of colonic adenocarcinomas and adjacent normal colon mucosas are indicated by corresponding Roman numerals in the figures and table. The RNA samples were analyzed by the Northern blot technique using three different cDNA probes derived from a human colon carcinoma cell line (LS180) cDNA library (11): (a) a probe containing the 5′ untranslated region and the complete coding sequences for CEA but lacking almost all of the Alu-like element and 3′ untranslated region typical of a full-length CEA cDNA clone (clone 31 of Ref. 5); (b) the NcoI-BamHI fragment of this clone comprising the leader, amino-terminal region, and most of the first repeat. Probes (a) and (b) detect both CEA and NCA mRNA species; (c) the 3′ EcoRI fragment of NCA cDNA which represents almost all of the 3′ untranslated sequences specific to NCA and which does not hybridize with CEA mRNA.

Transcription of the CEA Gene Family in Human Colonic Tissues. We have previously shown that polyadenylate-containing RNA from the human adenocarcinoma cell line LS-180 shows three bands of 3.5, 3.0, and 2.6 kilobases on Northern blots when probed with CEA cDNA (5). Furthermore, high, medium, and low CEA-producing subclones of this line all demonstrated the same band pattern, while the relative intensities correlated with the level of CEA protein expression. As noted in Fig. 1, the various colonic samples analyzed, whether normal or malignant, all expressed this same triplet pattern, while the normal liver RNA preparation was entirely negative. It is notable that the absolute amounts of CEA mRNA varied widely among the nine colonic adenocarcinomas tested with a range of 6-fold to 20-fold more than the LS-180 cell line used as internal control. More significantly, six pairs of colonic tumors compared with adjacent normal tissues from the same individual demonstrated a wide range of relative amounts of mRNA expression with one pair having approximately the same intensities of the 3.0-kilobase bands in tumor and normal preparations (e.g., Fig. 1, cf. Lanes 3 and 2), and five pairs having a 6- to 10-fold more intense 3.0-kilobase band in the tumor (e.g., Fig. 1, cf. Lanes 5 and 4).

In addition to relative intensities differing in these tissues, we noted that, whereas the ratios of the 3.5- and 3.0-kilobase species tended to be constant from one specimen to another, the intensity of the 2.6-kilobase band was relatively higher in the malignant colonic specimens. This is best appreciated in Fig. 1 (bottom) in which the same Northern blot was washed to remove the CEA probe and rehybridized with the NCA-specific probe. All specimens except for the normal liver expressed the 2.6-kilobase species though it was consistently more intense in

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the adenocarcinomas. Thus, first, the 3.5-kilobase and 3.0-kilobase message species are likely transcripts from the same or coordinately regulated genes, while the control of the NCA gene is independent of these. Next, malignant colonic cells demonstrate higher expression of the NCA gene than do the corresponding normal cells. It should be noted that we have not entirely eliminated the possibility of contamination of the tumor RNA samples with mRNA from putative inflammatory cells often associated with tumors, so that some of the NCA transcripts could have derived from these cells. It is nevertheless unlikely that all of the NCA transcripts could have been derived from such cells, as the cloned cell line LS-180 also produces NCA transcripts (Fig. 1).

CEA Gene Product Determinations. Given the wide range of CEA mRNA concentrations in different colonic tumors and the inconstancy in relative amounts when comparing matched pairs of tumors and normal tissues, we compared the relative amounts of CEA gene product expression in these tissues. Thus, immunoreactive CEA was measured in sonicates of five colonic tumors and the adjacent normal tissues, and the corresponding relative amounts of the 3.0-kilobase mRNA bands were estimated (Table 1). Note that the amount of CEA extracted from the malignant colon tissues varied from 130 ng to 9600 ng per mg of total protein. The normal colon tissues showed a narrower range of amounts (40 to 200 ng/mg of protein). The ratio of gene product expression in matched pairs of tumor/normal colons varied considerably from 2-fold to as high as 113-fold greater in the tumor. Of considerably greater interest is the relative amounts of the 3.0-kilobase mRNA bands were estimated (Table 1). Note that the amount of CEA extracted from the malignant colon tissues varied from 130 ng to 9600 ng per mg of total protein. The normal colon tissues showed a narrower range of amounts (40 to 200 ng/mg of protein). The ratio of gene product expression in matched pairs of tumor/normal colons varied considerably from 2-fold to as high as 113-fold greater in the tumor. Of considerably greater interest is the comparison between immunoreactive CEA levels, on the one hand, and those of the corresponding mRNAs. Thus, the pair demonstrating 2-fold more CEA product in the tumor had a 10-fold greater amount of mRNA in the tumor, while the tumor/normal pair showing 59-fold more product had a tumor/normal mRNA ratio of eight. Thus, in these human surgical specimens, in sharp contrast to the cell line subclones that we have analyzed, there is no direct proportionality between message levels and gene product expression. These results can be summarized to suggest that the known variations in CEA expression in human colon tumors and normal tissues result from both transcriptional and posttranscriptional control mechanisms.

Mechanism of Transcriptional Control of CEA Genes. In view of the large variation in the level of CEA transcripts within the set of tissues analyzed, we then asked whether gene amplification could be at least in part responsible for increased expression. Next, because of the demonstrated homology of CEA with the immunoglobulin supergene family (18), it was also of interest to determine whether somatic rearrangement of CEA genes could underlie increased transcriptional expression. We therefore compared the genomic organization of CEA genes in transcriptionally active (colonic metastasis) and nonactive tissues (normal liver) from the same individual. Fig. 2A illustrates a Southern blot of genomic DNA from such sources digested with four different restriction endonucleases and probed with the NcoI-BamHI CEA cDNA probe; Fig. 2B is the same blot probed with the NCA-specific cDNA probe. There are no evident changes in restriction fragment patterns or band intensities between tumor and liver DNA in either case. Thus, there is no evidence to support the occurrence of CEA or NCA gene rearrangements or amplification in concert with gene expression. Finally, similar analyses have been carried out using DNA prepared from 15 different individuals, and we have not observed any variations that would suggest gene polymorphism within the CEA gene family.

A frequent accompaniment to increases in gene transcription is a decreased level of methylation of the DNA in the enhancer-promoter regions upstream of the transcribed element (19). Such methylation states at CCGG sites can be studied using the isoschizomeric restriction endonuclease pair HpaII and MspI, insofar as methylation at the second cytosine renders this sequence resistant to HpaII digestion but does not affect digestion with MspI. Methylation of the first cytosine, on the other hand, renders the CCGG sequence resistant to both enzymes. Genomic DNA from the cell lines expressing widely different levels of transcripts and CEA protein and from a series of different normal and tumor tissues with different levels of CEA transcripts was digested with HpaII and MspI and subjected to Southern analysis. An AvrII restriction fragment of a genomic CEA clone includes 1000 base pairs upstream of the sequences represented in our longest CEA cDNA clone (5). The results are shown in Fig. 3.

Fig. 3, Lanes 1 and 2, contains DNA from the LS-180-1 subclone cut with MspI and HpaII, respectively, and Lane 3 contains DNA from subclone 86/8 cut with HpaII as indicated. LS-180-1 cultures have a cell-associated CEA level of 1000 ng/mg of protein, while subclone 86/8 does not produce any detectable CEA (less than 0.1 ng/mg of protein); these differences are reflected in the levels of the CEA and NCA transcripts. Comparison of Lanes 2 and 3 shows that the upstream region of 86/8 CEA DNA is more methylated than that of the producing subclone LS-180-1; for example, intense bands of 2.1 kilobases, 760 base pairs and 680 base pairs, are evident in the latter but absent from the former. More importantly, the 2.7- and 0.68-kilobase fragments noted in the HpaII digest of LS-180-1 (Lane 2) correspond to fragments evident in the MspI digest (Lane 1). However, there are no bands in the 86/8 HpaII

*CEA and total protein were measured as described in "Materials and Methods."

**Relative amounts of CEA mRNA (3.0 kilobases) in Northern blots of tumor and corresponding normal mucosa were assessed from relative band intensities on timed autoradiography.

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Table 1 CEA gene product determinations

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EXPRESSION STUDIES OF CEA GENES

Fig. 2. Southern blot analysis of CEA and NCA sequences in human liver metastasis of colonic adenocarcinomas and normal liver. DNA samples (8 μg) were digested with restriction endonucleases, electrophoresed, blotted, and probed with 32P-labeled NcoI-BamHI fragment from CEA cDNA (Fig. 2A) or EcoRI 3' untranslated fragment of NCA (Fig. 2B), and filters were washed as described in "Materials and Methods" and autoradiographed.

Fig. 3. Loss of methylation in the 5' end region of the CEA gene expressing tissues and cell lines. DNA samples (8 μg) were digested with MspI or HpaII as indicated and were electrophoresed, through 1% agarose gels, blotted onto nylon membranes, and hybridized to the 32P-labeled Avril fragment of a genomic CEA clone which includes 1000 base pairs upstream of the sequences represented in our longest CEA cDNA clone. Filters were washed as described in "Materials and Methods" and autoradiographed. Southern blots of MspI digests of all the samples indicated in the figure were identical to each other and to that of LS-180 shown in Lane 1.

digest (Lane 3) corresponding to any MspI bands. Thus, in the cell subclones, CEA gene expression is correlated with decreased gene methylation. It is noteworthy that the DNA from LS-180-1 is partially methylated as well (Lane 2 versus Lane 1), consistent with the likelihood that only some and not all members of the CEA gene family are expressed in these cells and that some cytosine residues are methylated even in active genes.

A parallel analysis for DNA prepared from the surgical specimens is included in this figure which shows HpaII digests hybridized with the AvrII probe. First, the tissues that produce no CEA transcripts (normal liver and spleen; Lanes 4, 6, and 8) show very similar patterns and have no discrete bands corresponding to the MspI digest of LS-180-1. (MspI digests of all the tissue specimens have the same pattern as that of LS-
DISCUSSION

Initial studies confirmed the presence of CEA mRNA in normal colonic tissues (Fig. 1; Refs. 11 and 12); furthermore such levels were not consistent with many of the previous studies of antigen prevalence (3). We therefore undertook an examination of CEA gene expression in paired normal and malignant colon tissue specimens to investigate the putative mechanisms of gene control responsible.

Our data indicate that multiple levels of control are involved in CEA expression. Thus, although all nine colonic adenocarcinomas tested exhibited the triplet mRNA pattern that we and others have noted previously, the actual specific mRNA levels varied over a 3-fold range within this set of samples.

The concentration of immunoreactive CEA in primary large bowel carcinomas was on the average 65 times higher than in the normal colon mucosa, although direct paired comparisons demonstrated a range of 2 to 113 times. A portion of this variability may result from variable "contamination" of the tumor specimens with nonmalignant inflammatory cells and debris, together with the sampling errors stemming from potential regional heterogeneity within the tumors. On the other hand, this degree of variation in CEA product expression between colonic tumors has been previously reported (3), and these results are in agreement with those of LoGerfo and Herter (20), Khoo (21), and Frischte and Mach (22) who have shown by immunological methods that the concentration of CEA-reactive material in normal colon mucosa was about 10 to 40 times lower than in primary large bowel carcinomas. As well, we have noted a 10,000-fold range of CEA expression in cellular clones obtained from the LS-180 and LS-174T adenocarcinoma cell lines in vitro. The noted variability in total CEA protein levels in the tumor specimens was far higher than the differences in levels of mRNA had led us to expect. Overall, there is no direct proportionality between the level of CEA mRNA detectable and the phenotypic protein product. Thus, there are elements of CEA genetic control that must operate posttranscriptionally affecting the efficiency of translation.

Our results clearly show that CEA mRNA is expressed in both normal and malignant colonic tissue. We cannot ascertain by these techniques whether the CEA RNAs expressed in normal and malignant tissues are completely identical, because minor differences in the nucleotide sequence cannot be detected with the Northern technique. On the other hand, CEA cDNA clones have been obtained from normal colon (6), human colon tumor (7), and colonic adenocarcinoma cell line (5); despite these different sources, no differences have been reported in the sequenced coding region. The only exception was reported by Kamarck et al. (8) who described a cDNA clone with many nucleotide sequence differences isolated from the LoVo cell line. There remains the possibility of finding differences in the 5' untranslated region, however, which has been previously shown to affect translational efficiency of eukaryotic mRNAs (23), as well as differences in posttranslational modifications such as glycosylation.

The ratios of the 3.5- and 3.0-kilobase CEA RNA are the same in all samples we examined. However, the ratio of the 2.6-kilobase NCA mRNA to 3.0-kilobase CEA mRNA was often greater in the tumor than in normal mucosa, as has been noted previously (11). This suggests that the regulatory mechanisms for production of the 2.6-kilobase NCA mRNA and 3.0-kilobase CEA mRNA are different, or at the least, independent.

The molecular basis of the observed increase in CEA transcript levels with malignancy was investigated. Because of the homology of CEA with the immunoglobulin supergene family (18), it was of interest to address the question whether CEA gene rearrangements could be correlated with expression and/or malignancy. We examined Southern blots of genomic DNA from colonic liver metastases and adjacent normal liver from two individuals digested with four different restriction enzymes and could not demonstrate rearrangements correlated with expression or translocations with malignancy. Also no evidence for CEA gene amplification correlated with expression could be found.

Since we have shown that DNA methylation plays a role in the expression of CEA gene in a series of clonal cell lines derived from a human colonic adenocarcinoma which shows widely different levels of CEA (Footnote 6; Fig. 3), we examined the level of methylation upstream of CEA genes in the malignant and normal tissues studied here. The results showed that CEA expression is correlated with a decreased level of methylation in the 5' region of the CEA gene as observed in other systems (19). The transcript levels were, however, on average 10-fold higher in the malignant tissues relative to the high producer cell line LS-180, although the degree of methylation was actually higher in the tissues. Thus, other mechanisms, such as variations in the levels of transacting transcriptional factors, must also be operative.

The CEA gene family contains a series of elements presumably derived from an ancestral common precursor and presents one type of complexity in coding for a large series of glycoproteins present in human tissues. In addition, there appear to be several different loci of control of expression of the members of the gene family with both transcriptional and posttranscriptional mechanisms playing a role accompanied by alterations of methylation of the DNA upstream from the coding regions. Finally, the data suggest that the control of expression by such mechanisms may be different in human tissues and in cell lines derived from these.

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REFERENCES

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