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DIFFERENTIAL EXPRESSION OF CARCINOEMBRYONIC ANTIGEN AND NONSPECIFIC CROSSREACTING ANTIGEN GENES IN HUMAN COLON ADENOCARCINOMAS AND NORMAL COLON MUCOSA

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The level of mRNA for carcinoembryonic antigen (CEA) and nonspecific crossreacting antigen (NCA) in human colon adenocarcinomas and normal colon mucosa was analyzed by Northern blot hybridization using as probes ^{32}P-labeled CEA cDNA and synthetic oligodeoxyribonucleotides specific to CEA and NCA mRNA sequences. The major 3.5-kb mRNA and a minor 4.2-kb mRNA are shown to be CEA-specific and expressed in both tissues, albeit at slightly different degrees, suggesting that the expression of CEA is regulated post-transcriptionally. Another minor mRNA of 2.9 kb is NCA-specific and expressed predominantly in cancerous tissues, suggesting its usefulness as a marker for colon cancer.

Key words: Carcinoembryonic antigen — Nonspecific crossreacting antigen — mRNA — Expression — Hybridization

Although carcinoembryonic antigen (CEA) has been widely used as a tumor marker for colonic cancer, its absolute tumor specificity and its role, if any, in tumor formation or progression is still unclear, because of the presence of many immunologically closely related CEA-like antigens in normal tissues, such as NCA or NCA-1,[1] NCA-2,[2] tumor-extracted CEA-related antigen,[3] various normal fecal antigens (NFA-1, NFA-2 and NFCA)[4] and biliary glycoprotein-I.[5] Recent progress in cloning cDNAs and genes for CEA[6–8] and NCA[9–11] clearly revealed highly homologous but distinct primary amino acid sequences for both antigens and the existence of about 10 homologous genes, each of which may encode one of the CEA-like antigens, and this constitutes the CEA gene family.

In order to elucidate the mechanisms of tumor-specific expression of CEA, we studied the level of mRNA expression in colon adenocarcinomas and normal tissues by Northern blot hybridization using cDNA probes corresponding to different domains of CEA and synthetic 17-mer oligodeoxyribonucleotide probes specific to either CEA or NCA mRNA.

The cDNA insert of the clone pCEA55-2[6] was digested with various restriction endonucleases as described in the legend to Fig. 1 and fractionated by electrophoresis through 2% agarose gel. After recovery from the gel, the fragments were separately radiolabeled by nick-translation using [^{32}P]dATP. The 17-mer oligodeoxyribonucleotide probes complementary to the cDNA sequences described in Fig. 1 were chemically synthesized and radiolabeled with [^{32}P]phosphate at the 5′ ends.

Poly(A)$^+$ RNA prepared[12] from colon carcinomas and normal colon mucosae, the latter having been dissected from tissues 2 to 3 cm apart from the cancerous lesions, from six patients were electrophoresed through 1% agarose gel containing formaldehyde, transferred to nitrocellulose filters and hybridized with the radiolabeled probes. The hybridization with the cDNA fragment probes was for 20 hr at 42° in 100m*M* PIPES buffer (pH 6.8) containing 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin, 5 × SSC, 50% formamide, 0.2 m*M* EDTA and 100 μg/ml salmon sperm DNA.[13] The hybridization with the oligonucleotide probe was for 20 hr at 50° in 50m*M* phosphate buffer (pH 7.0) containing 0.9*M* NaCl,

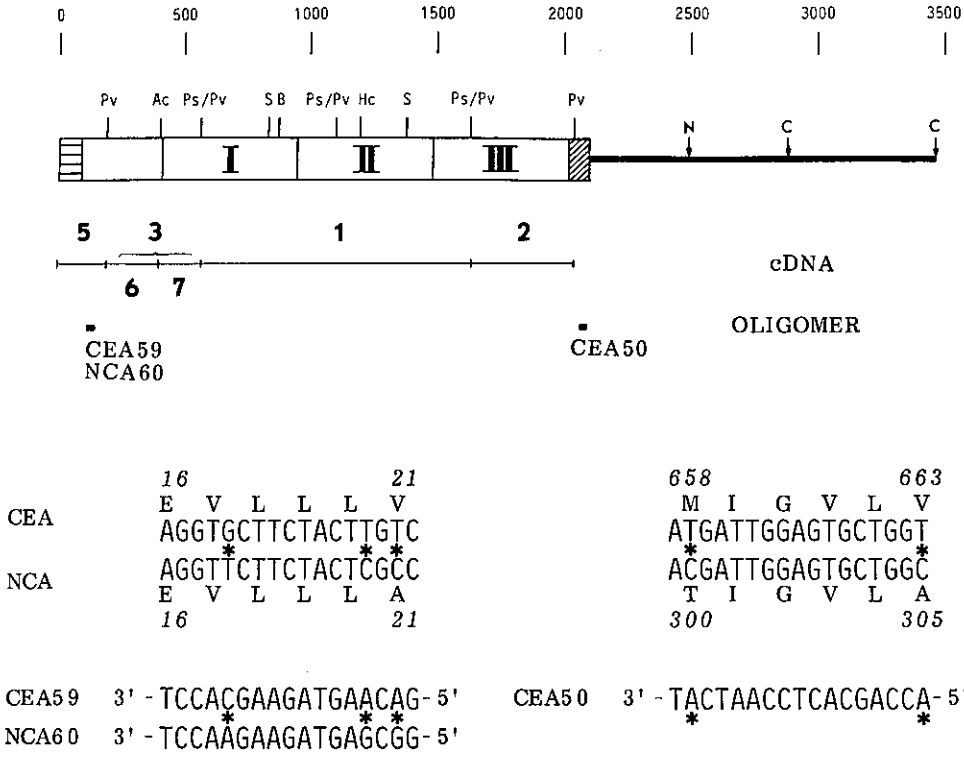

Fig. 1. Structures of CEA cDNA and probes. Coding regions and untranslated region of the cDNA are shown by boxes and a thick horizontal line, respectively. The length of the cDNA starting from the initiation codon is shown by the nucleotide numbers at the top of the figure. Only restriction enzyme sites relevant to this work are shown; Pv, *Pvu*II; Ac, *Acc*I; Ps, *Pst*I. Arrows N and C indicate the poly(A) addition sites for mRNA of NCA and CEA, respectively. The horizontal line below the cDNA scheme shows the regions covered by cDNA (—) and oligodeoxyribonucleotide (■) probes whose designations are indicated. Nucleotide and deduced amino acid sequences of CEA and NCA corresponding to the regions covered by the oligodeoxyribonucleotide probes and the sequences of the probes are shown.

5m*M* EDTA, 0.3% $NaDodSO_4$ and 100 μg/ml salmon sperm DNA.[14]

The figures in the upper panels in Fig. 2 depict Northern blot analysis of RNA from normal and cancerous colon tissues of a single patient using cDNA probes complementary to different regions of CEA mRNA. Comparison of short- and long-exposure autoradiograms obtained using ^{32}P-labeled CEA6 probe confirmed the previous observation[6] that there are four CEA-related mRNAs, i.e. a major 3.5-kb mRNA and less abundant 4.2-, 2.9- and 1.6-kb mRNAs, in both normal and cancerous colon tissues of a patient. However, in the case of cancerous tissue, hybridizable RNA migrating faster than 2.9-kb mRNA is very dispersed and forms almost no discrete band. In the case of this patient the expression of 4.2- and 3.5-kb mRNA in normal tissue greatly exceeds that in cancerous tissue. It was noted that probes containing sequences corresponding to the N-terminal domain gave more distinct bands than probes corresponding to repetitive domains (Fig. 2, upper panel).

When tissues from five more patients were examined with probe 3, which contains sequences corresponding to parts of both the N-domain and domain I (Fig. 1), it was found that the degree of expression of CEA-related gene(s) differs from patient to patient

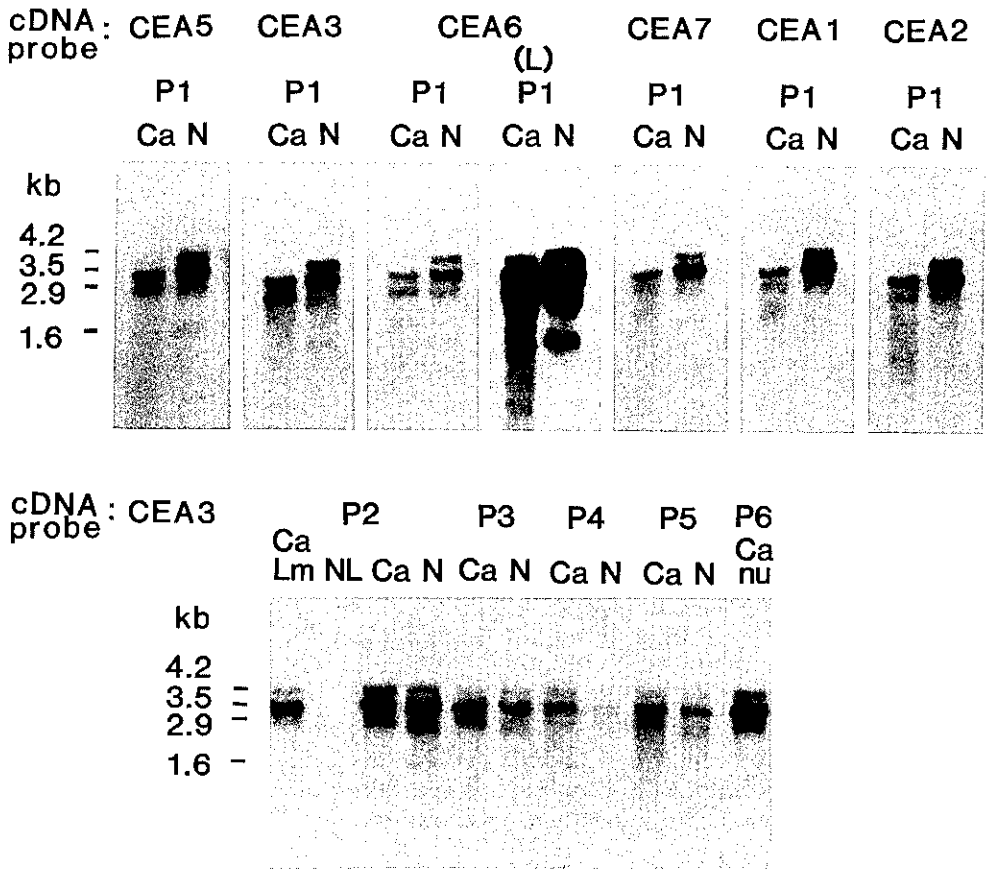

Fig. 2. Northern hybridization of poly(A)$^+$ RNA from human colon carcinomas and normal tissues with CEA cDNA probe. Poly(A)$^+$ RNAs (2 μg each) extracted from colon carcinomas and normal mucosa of patients 1 to 6 were fractionated by electrophoresis, transferred to nitrocellulose filters and hybridized with ^{32}P-labeled fragments of CEA cDNA as described in the text. P1–P6, patient 1–patient 6, respectively; Ca, colon carcinoma; N, normal colon mucosa; CaLm, liver metastasis of colon carcinoma; NL, normal liver tissue; Canu, colon carcinoma transplanted to and maintained in nude mice; (L), long exposure. The designation of CEA cDNA probes is indicated in Fig. 1.

(Fig. 2, lower panels). Contrary to the cases of patient 1 (see above) and patient A,[6] cancerous colon tissues expressed more CEA-related mRNA than normal tissues (Fig. 2), as reported by others.[7] Especially noteworthy is that normal colon tissues had a rather diffused band migrating ahead of the major 3.5-kb mRNA, some of which migrated faster than the 2.9-kb mRNA of cancerous tissues and banded at about 2.6-kb (Fig. 2, P2-N). On the other hand, although normal tissues showed a faint but discrete band at 1.6-kb, cancerous tissues often gave a very diffused pattern ahead of the 2.9-kb mRNA and had scarcely any discrete band at 1.6 kb. Liver metastasis had a similar expression pattern, although the ratio of 3.5-kb mRNA to other CEA-related mRNA is more than in the primary colon cancer. Normal liver from the same patient showed almost no expression of any of the CEA-related genes hybridizable to the probe (Fig. 2, P2). A colon carcinoma maintained in nude mice had a similar expression pattern to that of freshly removed cancerous tissues (Figs. 2 and 3).

When an RNA blot was hybridized with oligonucleotide probes, two groups of mRNA species were clearly discernible (Fig. 3). One

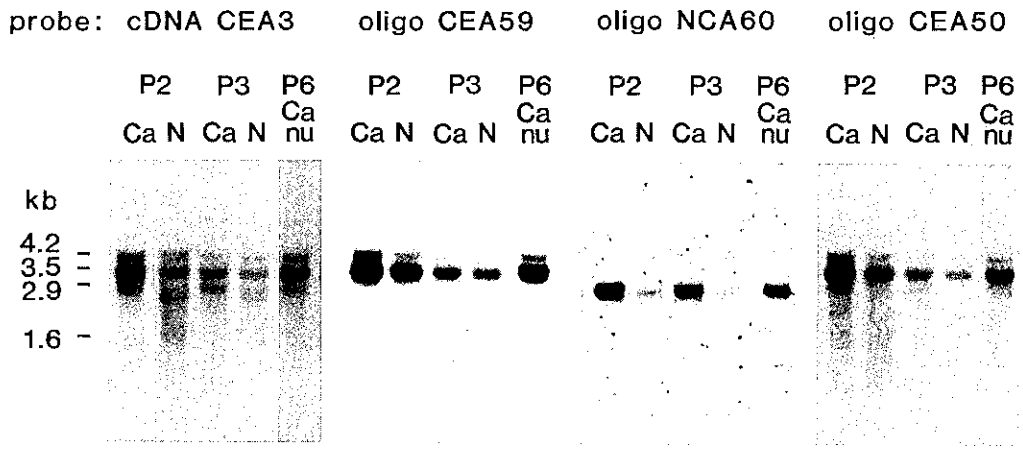

Fig. 3. Northern hybridization of poly(A)$^+$ RNA from human colon carcinomas and normal tissues with oligonucleotide probes specific to CEA or NCA. Poly(A)$^+$ RNAs (10 μg each) were fractionated by electrophoresis, transferred to nitrocellulose filters and hybridized with ^{32}P-labeld synthetic oligonucleotide probes as described in the text. RNA preparations are the same as those in Fig. 2. The designation of oligo probes are as in Fig. 1.

group hybridized with a CEA-specific probe, CEA 59, and consisted of the major 3.5-kb and the minor 4.2-kb mRNA species, in accord with our previous finding that there are two cDNA species encoding CEA which are about 0.7-kb different in size at their 3′-untranslated regions[6] (Fig. 1). The other group is 2.9-kb mRNA species which hybridized only with the NCA-specific probe, NCA60. The size fits well with that of the NCA mRNA predictable from our 5′-truncated cDNA clone for NCA[9] (Fig. 1). The two CEA mRNAs were expressed in carcinomas somewhat more than in normal colonic tissues of the same patients but the degrees of expression were more significantly different from patient to patient rather than from normal to cancerous tissues. On the other hand, significant and similar amounts of NCA mRNA were detected in three carcinomas but very little was expressed in normal colon mucosa of the same patients (Fig. 3). The CEA50 probe gave similar results to those obtained with CEA59. Higher background and detection of 2.9-kb mRNA in carcinoma of patient 2 (Fig. 3) may be caused by less specific nature of the probe to CEA mRNA (Fig. 1). The 2.6-kb and 1.6-kb mRNA species were not detected by either probe, suggesting that they are neither CEA mRNA nor NCA mRNA but are products of other CEA-related genes, of which 9–11 are known to exist.[6, 10]

In this report, we have shown that the expression of CEA and NCA genes is measurable at the transcriptional level and that significant amounts of CEA mRNAs are expressed in both normal and cancerous colon tissues. This conflicts with the results of immunological analyses indicating that the level of expression of CEA in normal colon mucosa is generally very low compared to that in cancerous tissues, suggesting the regulation of CEA expression at post-transcriptional step(s). Actually, our immunohistochemical analysis on formalin-fixed specimens prepared from the same tissues used for RNA blot analysis clearly showed significantly higher expression of CEA in cancerous tissues than in normal colon mucosae (data not shown). The rather specific expression of NCA mRNA suggests that it could be used as a marker for colon cancer. However, more studies on the expression of the NCA gene at transcriptional and translational levels in other tumors are clearly needed to determine the tumor specificity of the expression of the NCA gene. Furthermore, it is also necessary to clarify the

identity of the NCA encoded by the cDNA presently used, for it was suggested that there could be multiple genes for NCA[15] which might at least partly explain the discrepancy between the present results and the general belief that NCA is expressed in both cancerous and non-cancerous colon tissues. Despite these uncertainties, the NCA mRNA should be a useful molecule in studies of the control of tissue- and/or cancer-specific expression of the CEA gene family.

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