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Immunocytochemical Evaluation of Human Esophageal Neoplasms and Preneoplastic Lesions for β-Chorionic Gonadotropin, Placental Lactogen, α-Fetoprotein, Carcinoembryonic Antigen, and Nonspecific Cross-Reacting Antigen[1]

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ABSTRACT

Human esophageal neoplasms were studied in comparison to normal, uninvolved, and preneoplastic human esophageal epithelium for the presence of human chorionic gonadotropin (HCG), human placental lactogen (HPL), α-fetoprotein (AFP), carcinoembryonic antigen (CEA), and nonspecific cross-reacting antigen (NCA) using the unlabeled antibody peroxidase-antiperoxidase technique.

HCG immunoreactivity was identified in 10 of 33 squamous cell carcinomas (33%), in 1 of 6 adenocarcinomas (17%), and 1 of 6 preneoplastic esophageal lesions (17%); while 9 of 33 squamous cell carcinomas (33%) and 1 of 6 adenocarcinomas (17%) contained immunoreactive AFP. Immunoreactive HPL was detected in 6 of 33 squamous cell carcinomas (20%), but in none of the adenocarcinomas. Neither AFP nor HPL immunoreactivity was identified in the 6 hyperplastic lesions which were studied. When stained with an antiserum that was able to detect both CEA and NCA, 27 of 33 squamous cell tumors (82%) and 6 of 6 adenocarcinomas (100%) showed positive immunostaining reactions. Of these, 8 squamous cell carcinomas and 1 adenocarcinoma were subsequently shown to contain only NCA immunoreactivity, while 19 squamous cell carcinomas and 5 adenocarcinomas contained both NCA and CEA immunoreactivity. NCA immunoreactivity alone was identified in 3 of 6 preneoplastic lesions and NCA and CEA immunoreactivity in 1 of 6 preneoplastic lesions. None of the markers was detected in 8 specimens of normal esophageal epithelium which were studied as controls, nor in 6 specimens of uninvolved esophageal epithelium obtained from patients with esophageal cancer.

Most tumors expressed 2 or 3 markers, and some tumors were identified which expressed up to 4 of the 5 markers investigated. Only 3 tumors failed to express any of the markers studied.

No association was found between the degree of tumor differentiation and presence or absence of HCG immunoreactivity. However, HPL immunoreactivity was more common in poorly differentiated squamous cell carcinomas. In contrast, immunoreactive AFP was more common in well-differentiated squamous cell carcinomas than in other tumor types. Similarly, both CEA and NCA were more frequently expressed in well-differentiated squamous cell carcinomas, adenosquamous carcinomas, and adenocarcinomas than in less differentiated tumors.

Our results suggest that HCG, HPL, AFP, CEA, and NCA are tumor-associated antigens in esophageal cancer. Therefore, they could be of value in screening tests for esophageal neoplasms and could be useful in subclassification of esophageal neoplasms.

INTRODUCTION

Immunocytochemical techniques provide a sensitive and specific means of localizing a diverse array of antigens in fixed, paraffin-embedded tissue (1–4). Localization of certain antigens in neoplastic tissue may have important clinical and histopathological applications. For example, the identification of antigens peculiar to or differentially expressed by certain tumors could provide markers for the early detection of neoplasms and could add a valuable dimension to present histopathological diagnosis if they were found to correlate with histopathological features and/or clinical behavior of neoplasms. This could lead to classification schemes based on the functional activities of tumor cells that may be more clinically relevant than those based solely on morphological criteria.

While marker expression has been explored and characterized in a variety of neoplastic tissues, very little investigation has centered upon tumors of esophageal origin. Esophageal neoplasms account for only 10% of all cancers of the gastrointestinal tract, but they are responsible for 4% of all cancer deaths in the United States and are associated with a 5-yr survival rate of 7% or less (5). The high mortality rate associated with these neoplasms is primarily related to the fact that tumor invasion is widespread at the time of diagnosis. However, there are techniques for detecting cancer of the esophagus early in its course. As an example, esophageal brushing to obtain cells for cytological examination is in wide use in China, where cancer of the esophagus is common (6). This, or some other method of obtaining esophageal cells, combined with testing the cells for neoplasm-associated markers, could improve the prognosis of this devastating disease. In this paper, we report the results of our evaluation of malignant and preneoplastic esophageal epithelium for HCG,[4] HPL, AFP, CEA, and NCA using the PAP technique (7).

MATERIALS AND METHODS

Tissue. Thirty-nine malignant esophageal neoplasms were evaluated in the present study. Thirty-three of these were obtained through the Divisions of Surgical Pathology at several hospitals (University of Maryland Hospital, South Baltimore General Hospital, Maryland General Hospital, Loch Raven Veterans Administration Medical Center, and the Washington, DC Veterans Administration Medical Center). Six cases including samples of uninvolved, preneoplastic (including areas of hyperplasia and basal cell atypia), and neoplastic esophageal tissue collected at the time of surgical resection for esophageal cancer were obtained from Dr. Ren Sheng Chen (Jinan University Medical College, Department of Pathology, Guangzhou, Peoples Republic of China). Tumors were fixed in either mixed aldehydes (4% formaldehyde-1% glutaraldehyde) or in neutral buffered formalin. They included 26 squamous cell carcinomas, 7 adenosquamous carcinomas, and 6

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[2] Submitted in partial fulfillment of the requirements for the degree, Doctor of Philosophy, from the Department of Pathology, University of Maryland School of Medicine.

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[4] The abbreviations used are: HCG, β-subunit of human chorionic gonadotropin; HPL, human placental lactogen; AFP, α-fetoprotein; CEA, carcinoembryonic antigen; NCA, nonspecific cross-reacting antigen; PAP, horseradish peroxidase:anti-horseradish peroxidase; NGS, normal goat serum; DAB, 3,3′-diaminobenzidine tetrahydrochloride; PBS, phosphate-buffered saline (0.1 M NaCl:0.01 mM Na_2HPO_4:0.003 M KH_2PO_4, pH 7.4).

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adenocarcinomas. The squamous carcinomas included 4 well-differentiated, 7 well- to moderately differentiated, 4 moderately differentiated, 4 moderate to poorly differentiated, and 7 poorly differentiated (4 with spindle cell component) tumors. Preneoplastic and uninvolved esophageal specimens were fixed in neutral buffered formalin. In addition, 8 specimens of normal human esophagus obtained at the immediate autopsy of young trauma victims were studied as controls. Normal tissue was fixed in 4% formaldehyde-1% glutaraldehyde.

Tissue used as immunochemical controls included sections of formalin fixed specimens of hepatocellular carcinoma and colon adenocarcinomas for AFP and CEA, respectively, and formalin or Bouin's-fixed human placenta for HCG and HPL. Normal spleen was used as an immunocytochemical control for NCA and also for absorption of CEA antiserum.

Reagents. Antisera raised in rabbits to HCG, HPL, AFP, and CEA were obtained as purified immunoglobulin fractions from Dakopatts a/s Copenhagen, Denmark (United States distributor, Accurate Chemical and Scientific Corp., Westbury, NY).

Goat antiserum to rabbit IgG was used as the secondary antibody in the PAP procedure. It was obtained from Sternberger-Meyer Immunocytochemicals (Jarrettsville, MD), as was normal rabbit serum (control serum). Affinity purified PAP produced in rabbits, normal rabbit IgG (control serum), and NGS and α-1-fetoprotein standard serum were obtained from Dakopatts. DAB was obtained from Sigma Chemical Co. (St. Louis, MO). Human chorionic gonadotropin, human placental lactogen, and CEA were purchased from Calbiochem-Behring Corp. (LaJolla, CA). The antisera, normal rabbit IgG, and the PAP were stored at 4°C. Goat antiserum to rabbit IgG and normal rabbit serum were stored in aliquots at −70°C.

Immunostaining Procedure. Multiple 5-μm sections were cut from each block of tissue, cleared of paraffin in 4 changes of mixed xylenes, and placed in absolute methanol containing 3% H_2O_2 for 30 min to ablate endogenous peroxidase activity. The tissue sections were then rehydrated by passage through a series of alcohol solutions of decreasing concentration and finally placed in PBS. After this, they were treated for 30 min with undiluted NGS to suppress nonspecific staining and then exposed sequentially at room temperature to the following reagents. (*a*) Primary antiserum consisted of anti-HCG (1:200 dilution) for 18 to 22 h or 1:200 dilutions of anti-HPL, anti-AFP, or anti-CEA for 1 h. As controls the antisera were replaced with similar dilutions of normal rabbit serum, PBS, or with absorbed antisera (see below). (*b*) Secondary antibody consisted of goat anti-rabbit IgG (1:40 dilution) for 30 min. (*c*) The last reagent was PAP (1:100 dilution) for 30 min. All dilutions were made in PBS containing 3% NGS. After each incubation tissue sections were washed 3 times (each change, 5 min) with 0.05 M Tris buffer (pH 7.6).

Antibody localization in the tissue was detected by placing the sections in a filtered, freshly prepared solution of DAB (0.05% in 0.05 M Tris buffer, pH 7.6, containing 0.03% H_2O_2) for 5 to 10 min. After treatment, the tissue sections were washed in PBS followed by tap water, lightly counterstained with hematoxylin, dehydrated, and mounted in Coverbond (Scientific Products, Inc., McGaw Park, IL).

Absorbed Antisera. Specificity of immunostaining was confirmed by absorption of antisera with purified antigens. Anti-HCG was absorbed by adding 250 μg of purified HCG per ml to a 1:200 dilution of anti-HCG, followed by incubation at 4°C for 1 h. The absorbed antiserum was tested using tissue sections of normal human placenta.

HPL antiserum was absorbed by addition of 17.8 μg of purified HPL per ml to a 1:200 solution of anti-HPL followed by overnight incubation at 4°C. Sections of human placenta were used to test the absorbed antiserum.

Absorption of AFP antiserum was accomplished by addition of an equal volume of α-1-fetoprotein control serum to a 1:100 solution of anti-AFP followed by overnight incubation at 4°C. Tissue sections from human fetal liver and an AFP-positive liver tumor were used to test the absorbed antiserum.

CEA antiserum was rendered specific for CEA by depleting its reactivity toward NCA by absorption with normal human spleen (8). For this purpose, 250 mg of acetone-dried human spleen tissue were added to the antiserum (1:200 dilution in PBS plus 3% NGS) and stirred gently for 1.5 to 2 h at room temperature. The suspension was then filtered and used in the PAP procedure. The effectiveness of the absorption was tested using sections of spleen. Tissue sections from a CEA-positive colon adenocarcinoma were also tested to confirm that the absorbed antiserum was still able to detect CEA.

Anti-CEA was absorbed by addition of 5 μg of purified CEA per ml to a 1:200 solution of CEA antiserum which had previously been absorbed with spleen tissue. Absorption was carried out overnight at 4°C. The effectiveness of absorption was determined using sections of a colon adenocarcinoma.

RESULTS

Human Chorionic Gonadotropin

Neoplastic Esophagus. The HCG antiserum used in this study stained the syncytial trophoblast cells of human placenta (Fig. 1*a*), which synthesize HCG. Specificity of the antiserum was demonstrated by absorption of the antiserum with purified HCG which abolished the immunostaining of these cells (Fig. 1*b*).

When the HCG antiserum was applied to esophageal tumors, 33% (10 of 33) of the squamous cell carcinomas was stained (Table 1). The staining was, in most instances, of weak to moderate intensity and involved single cells or small groups of cells scattered throughout the tumor. Variation in the staining intensity of the tumor cells was common. The keratinized areas of one well-differentiated squamous cell carcinoma were weakly stained in addition to isolated tumor cells (Fig. 2). One poorly differentiated squamous cell carcinoma showed a more generalized pattern of staining, with many tumor cells showing weak cytoplasmic staining and a few tumor cells having moderately intense cytoplasmic staining. All but one of the adenocarcinomas (6 cases) that were studied were unreactive with HCG antiserum; the exceptional tumor contained isolated cells with moderately strong cytoplasmic staining. The immunostaining of tumors with HCG antiserum was abolished when the antiserum was absorbed with HCG (Fig. 2, *inset*; Fig. 3, *inset*).

Normal, Uninvolved, and Preneoplastic Esophagus. Epithelial tissue from 8 normal esophagi and 6 cases of uninvolved esophageal epithelium were not stained with HCG antiserum. However, among the 6 preneoplastic lesions studied, one hypertrophic focus adjacent to a well- to moderately differentiated squamous cell carcinoma contained 2 small groups of cells with

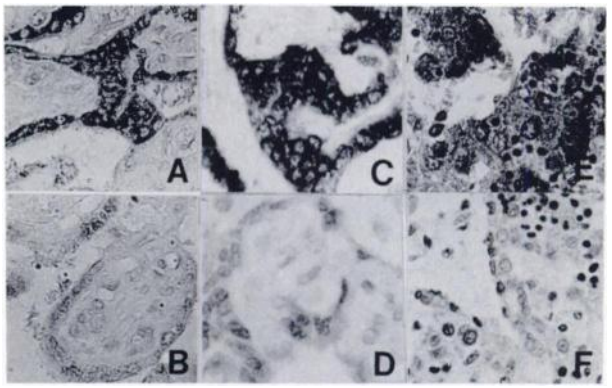

Fig. 1. *A*, immunostaining of placenta. β-HCG antiserum used in the present study specifically stained the syncytial trophoblast cells of human placenta. In *B*, immunostaining of placenta was abolished following absorption of the antiserum with HCG. *C*, HPL immunostaining of placenta. HPL antiserum used in the present study specifically stained the syncytial trophoblast cells of human placenta. In *D*, immunostaining was abolished following absorption of the antiserum with HPL. *E*, immunoperoxidase localization of AFP immunoreactivity in a human liver tumor. In *F*, absorption of the antiserum with AFP control serum eliminated the staining. × 250.

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Table 1 *Immunostaining reactions of human esophageal neoplasms with HCG, HPL, AFP, and CEA antisera*

Antiserum	No. of specimens with positive staining stratified by tissue differentiation[a]				
	Squamous cell carcinomas				
	W-WMD[c] (11)	M-MPD (8)	P-PDS (7)	Adeno[d] (7)	Adenocarcinomas (6)[b]
HCG	5 (46)[e]	1 (13)	2 (29)	2 (29)	1 (17)
HPL	1 (9)	2 (25)	3 (43)	0 (0)	0 (0)
AFP	4 (36)	1 (13)	1 (14)	3 (43)	1 (17)
CEA, absorbed[f]	9 (82)	4 (50)	1 (14)	5 (71)	5 (83)
CEA, unabsorbed[g]	11 (100)	7 (88)	3 (43)	6 (86)	6 (100)

[a] In order to simplify tabulation of results, the well- and well- to moderately differentiated squamous cell carcinomas and the moderately and moderate to poorly differentiated squamous cell carcinomas have been combined to form 2 groups, respectively. In the text the former will be referred to as well differentiated and the latter as moderately differentiated.

[b] Numbers in parentheses (table boxhead), total number of tumors for each histopathological classification.

[c] W-WMD, well, well-moderately differentiated; M-MPD, moderately, moderate-poorly differentiated; P-PDS, poorly differentiated, poorly differentiated with spindle cell component; Adeno, adenosquamous carcinoma.

[d] Adenosquamous carcinomas showed evidence of glandular elements with mucin secretion as demonstrated by Alcian blue-periodic acid-Schiff staining combined with areas of squamous differentiation.

[e] Numbers in parentheses (table body), percentage.

[f] CEA antiserum absorbed with spleen tissue. Detects CEA alone; specificity of stain confirmed by further absorption with purified CEA antigen.

[g] CEA antiserum not absorbed with spleen tissue; detects both CEA and NCA.

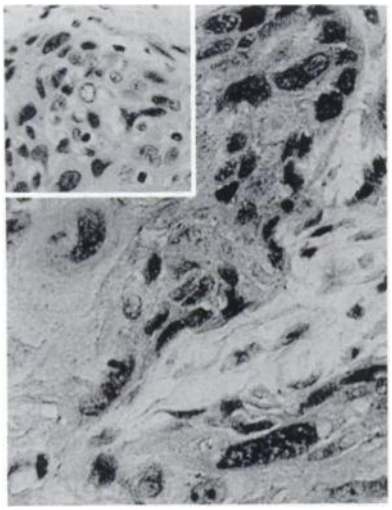

Fig. 2. β-HCG immunoreactivity in esophageal squamous cell carcinoma. Scattered cells are moderately immunostained. Immunostaining was not observed following absorption of the antiserum with HCG (*inset*). × 325.

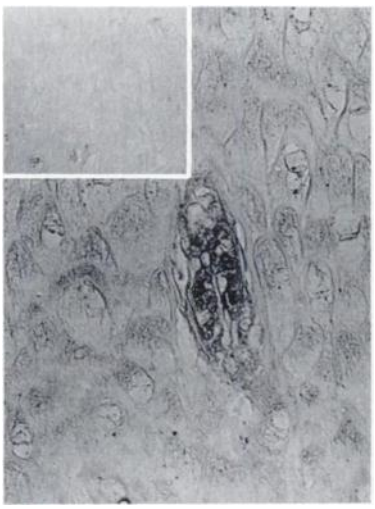

Fig. 3. Immunoperoxidase localization of β-HCG in a hyperplastic esophageal lesion. One group of cells is stained. Staining is abolished following absorption of the antiserum with HCG (*inset*). No counterstain. × 325.

HCG immunoreactivity (Fig. 3; Table 2). One cell group exhibited immunostaining of moderate intensity, while the other showed weak immunostaining. Interestingly, some of the HCG

Table 2 *Summary of marker distribution in neoplastic and preneoplastic esophageal lesions by individual case as determined by immunocytochemistry*

Case	Histopathological classification	Neoplastic esophageal lesions by the following marker(s)					Preneoplastic esophageal lesions by the following marker(s)				
		HCG	HPL	AFP	CEA	NCA	HCG	HPL	AFP	CEA	NCA
1	WD[a]	+	−	−	+	+	−	−	−	−	+
2	WD	+	−	−	+	+	−	−	−	+	+
3	W-MD	+	−	−	+	+	+	−	−	−	+
4	M-PD	+	+	−	−	+	−	−	−	−	−
5	M-PD	−	−	−	+	+	−	−	−	−	+
6	M-PD	−	−	−	+	+	−	−	−	−	−

[a] WD, well-differentiated squamous cell carcinoma; W-MD, well-moderately differentiated squamous cell carcinoma; M-PD, moderate-poorly differentiated squamous cell carcinoma.

reactive cells in the preneoplastic lesion were more strongly stained than were the majority of cells with HCG immunoreactivity within the tumor itself.

Placental Lactogen

Neoplastic Esophagus. The antiserum to HPL specifically stained the syncytial trophoblast cells of human placenta (Fig. 1*c*). The immunostaining of these cells was abolished following absorption of the antiserum with purified HPL (Fig. 1*d*).

The majority of esophageal carcinomas were unreactive with HPL antiserum. None of the adenosquamous carcinomas and none of the adenocarcinomas were stained with HPL antiserum. Two well- to moderately differentiated squamous cell carcinomas were positive for HPL (Fig. 4). One of these 2 tumors contained areas that were poorly differentiated based on examination of tissue samples from other regions of the tumor. In addition, 3 poorly differentiated and 1 moderate to poorly differentiated squamous cell carcinomas showed HPL immunoreactivity (Table 1). In these tumors staining intensity varied from weak to moderate (Fig. 5). Variation in the intensity of cytoplasmic staining was also noted among the tumor cells of individual tumors. The staining of these tumors with HPL antiserum was consistent with the presence of HPL in some tumor cells, since it was abolished by absorption of the antiserum with purified HPL (Fig. 4, *inset*; Fig. 5, *inset*).

Normal, Uninvolved, and Preneoplastic Esophagus. Normal, uninvolved, and preneoplastic esophageal epithelium showed no immunoreactivity when tested with HPL antiserum.

α-Fetoprotein

Neoplastic Esophagus. The antiserum to AFP stained human fetal liver cells and cells of a liver carcinoma (Fig. 1*e*). The

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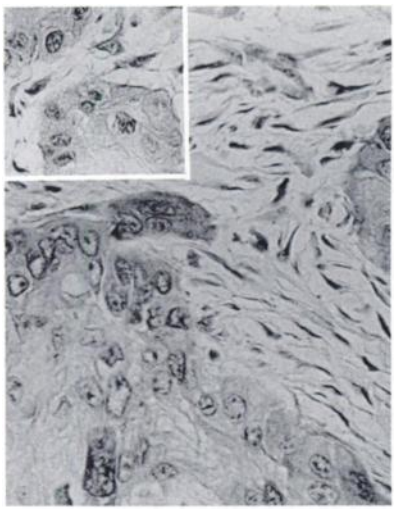

Fig. 4. HPL immunoreactivity in a well- to moderately differentiated squamous cell carcinoma. Some cells are moderately stained. Following absorption of the antiserum with purified HPL, staining is abolished (*inset*). × 325.

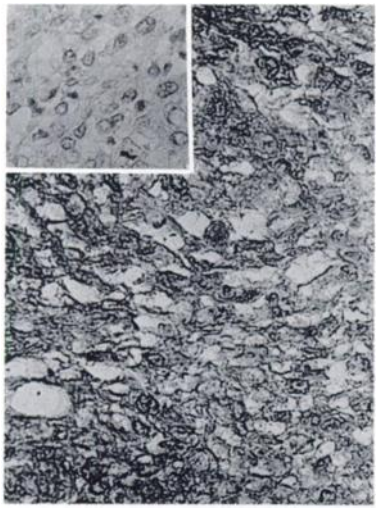

Fig. 5. Immunoperoxidase localization of HPL immunoreactivity in a poorly differentiated squamous cell carcinoma. Most of the tumor cells are strongly immunostained with HPL antiserum. Immunostaining was abolished following absorption of the antiserum with purified HPL (*inset*). × 325.

staining was abolished following absorption of the antiserum with human α-1-fetoprotein control serum (Fig. 1*f*).

Immunoreactive AFP was detected in 30% (9 of 33) of the esophageal squamous cell carcinomas that were evaluated, but in only 1 of 6 adenocarcinomas (Table 1). AFP immunoreactivity was more common in well-differentiated tumors than in less-differentiated ones. Staining of tumors was weak to moderate in intensity and usually involved isolated tumor cells (Fig. 6), although one poorly differentiated squamous cell carcinoma contained many positive cells (Fig. 7). None of the tumors was stained when normal rabbit serum replaced AFP antiserum in the PAP procedure (Fig. 6, *inset*; Fig. 7, *inset*). The immunoreactive material was dispersed evenly throughout the cytoplasm of tumor cells. AFP immunoreactivity was also observed in the keratinized areas of some positive tumors.

Normal, Uninvolved, and Preneoplastic Esophagus. AFP immunoreactivity was not detected in normal, uninvolved, or preneoplastic esophageal epithelium.

Carcinoembryonic Antigen

Neoplastic Esophagus. The CEA antiserum used in our study detected both CEA and NCA immunoreactivity; therefore, specific demonstration of CEA in tissue required absorption of the antiserum with spleen tissue to remove its reactivity toward NCA. The effectiveness of the absorption was tested with histological sections of spleen. These were treated with CEA antiserum before and after absorption with spleen tissue. In the former case, numerous NCA-containing granulocytes throughout the spleen were intensely stained (Fig. 8*a*), whereas after absorption the staining of granulocytes was abolished (Fig. 8*b*). In contrast, tissue sections from a CEA-positive colon adenocarcinoma were intensely stained with spleen-absorbed antiserum (Fig. 8*c*). However, immunostaining of the colon adenocarcinoma was abolished when the spleen-absorbed antiserum was further absorbed with purified CEA (Fig. 8*d*), thereby demonstrating its specificity.

Thirty-three squamous cell carcinomas and 6 adenocarcinomas were examined with CEA antiserum with and without absorption with spleen tissue. The results are compared in Table 1. Most of the squamous cell carcinomas (27 of 33; 82%) and all of the adenocarcinomas (6 of 6) were stained when treated with CEA antiserum that had not been absorbed with splee

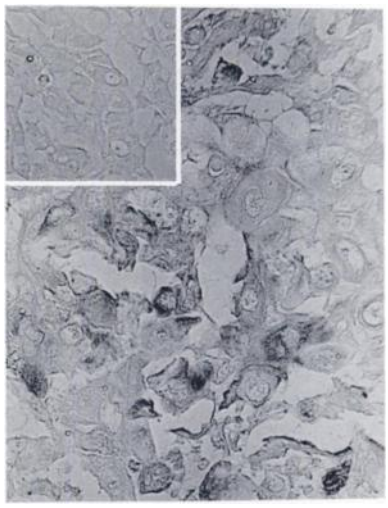

Fig. 6. AFP immunoreactivity in an esophageal carcinoma. Some neoplastic cells of this well- to moderately differentiated squamous cell carcinoma are moderately stained with AFP antiserum, while others remain unstained. In the *inset*, esophageal neoplasm is not stained by absorbed AFP antiserum. No counterstain. × 250.

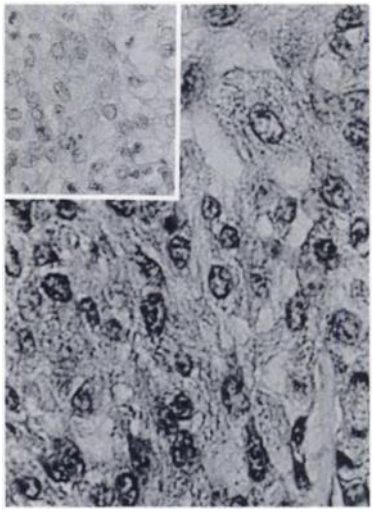

Fig. 7. Localization of AFP immunoreactivity in a poorly differentiated squamous cell carcinoma. This tumor also contained HPL immunoreactivity (Fig. 5). In the *inset*, the tumor is not stained by normal rabbit serum. × 325.

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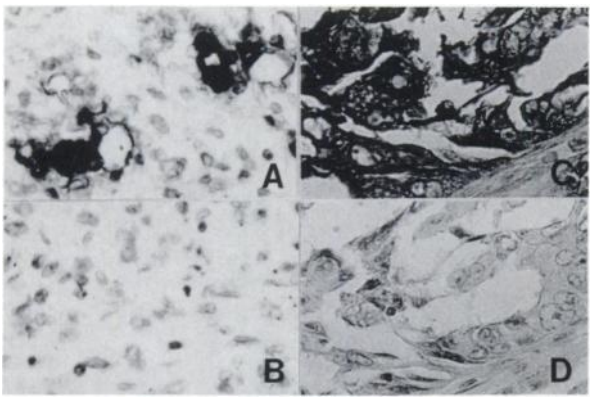

Fig. 8. *A*, immunoperoxidase localization of NCA in normal human spleen. When treated with CEA antiserum, granulocytes scattered throughout the spleen are intensely stained. In *B*, immunostaining is abolished when CEA antiserum is absorbed with human spleen. *C*, immunoperoxidase localization of CEA in colonic carcinoma. The immunostaining of the colon carcinoma with spleen-absorbed CEA antiserum is intense. In *D*, staining is abolished following absorption of the spleen-absorbed antiserum with CEA. × 250.

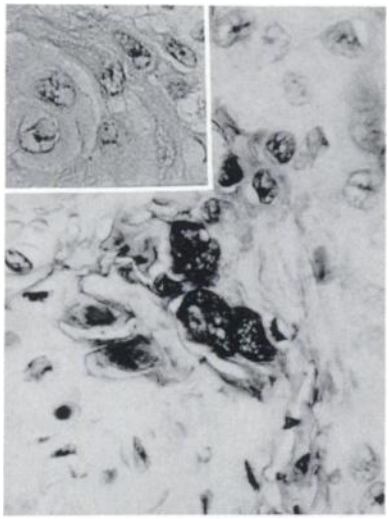

Fig. 9. Immunoperoxidase localization of NCA immunoreactivity in an esophageal carcinoma. When treated with CEA antiserum, some esophageal tumors showed positive staining of neoplastic cells. Immunostaining was related to NCA immunoreactivity, since it was completely abolished when CEA antiserum was depleted of NCA reactivity by absorption with acetone-dried human spleen (*inset*). × 250.

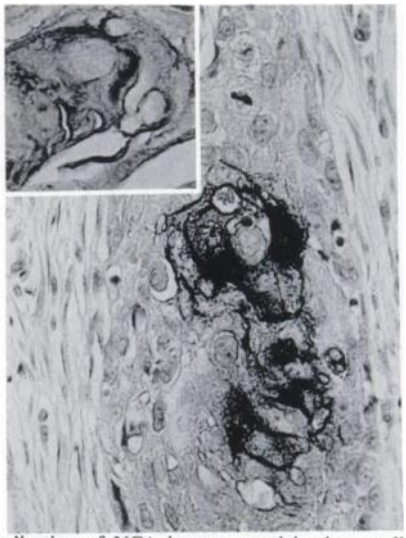

Fig. 10. Localization of NCA immunoreactivity in a well- to moderately differentiated squamous cell carcinoma. In the *inset*, CEA immunoreactivity is seen in the same tumor. Staining is less intense and involves fewer tumor cells. × 250.

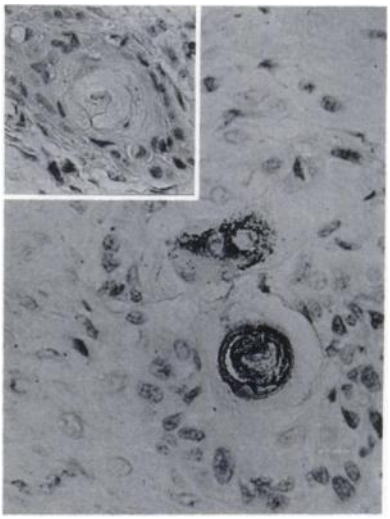

Fig. 11. CEA immunoreactivity in a well-differentiated squamous cell carcinoma. Intense immunostaining is present in a keratin pearl and 2 cells. Immunostaining is abolished when spleen-absorbed CEA antiserum is absorbed with purified CEA (*inset*). × 325.

tissue. This was consistent with the presence of NCA and/or CEA in such tumors. The staining intensity of tumor cells within positive tumors varied, but overall the majority of tumor cells were strongly stained. One adenocarcinoma and 8 squamous cell carcinomas that were stained with the unabsorbed antiserum (Fig. 9) were not stained following absorption of the antiserum with spleen tissue (Fig. 9, *inset*). This result showed that these tumors contained NCA but not CEA immunoreactivity. In the tumors that were stained with both antisera, staining was stronger and involved a greater number of cells when the tumors were treated with unabsorbed CEA antiserum (Fig. 10) compared to staining with spleen-absorbed CEA antiserum (Fig. 10, *inset*). Thus, the more intense staining obtained with the former appeared to represent the combined result of staining for both NCA and CEA.

Overall, 57% (19 of 33) of the squamous cell carcinomas was stained with spleen-absorbed CEA antiserum. Almost all well-differentiated squamous cell carcinomas were moderately to strongly stained, but only one poorly differentiated tumor was positive. Most of the adenosquamous carcinomas and slightly less than half of the moderately differentiated tumors were strongly to moderately stained. Immunoreactive tumor cells were often found in association with keratinized areas, but positive cells were not confined to these areas (Fig. 11). CEA immunoreactivity was also detected in some of the moderately or poorly differentiated squamous cell carcinomas in which keratinizing areas were either negative or were not present (Fig. 12). Approximately half of the tumors with CEA immunoreactivity showed cell membrane staining combined with cytoplasmic staining. The staining of cell membranes did not appear related to the degree of tumor differentiation, since it was observed in well-differentiated, moderately differentiated, and poorly differentiated squamous cell carcinomas, as well as in adenosquamous carcinomas. In tumors that showed staining of both keratinized areas and individual cells, the staining of the former was typically more intense than the latter. The staining of these tumors was abolished following absorption of the antiserum with purified CEA (Fig. 11, *inset*; Fig. 12, *inset*).

Immunoreactive CEA was also detected in almost all of the adenocarcinomas. Staining intensity varied from weak to

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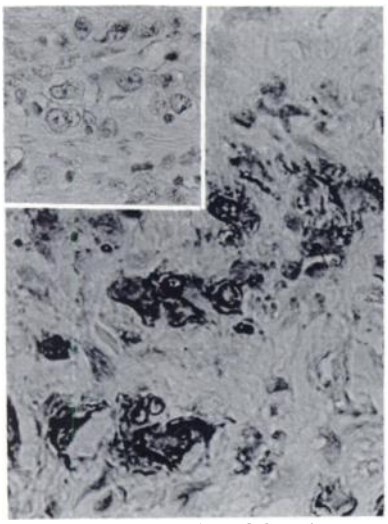

Fig. 12. Immunoperoxidase localization of CEA in a poorly differentiated squamous cell carcinoma. Most neoplastic cells are intensely immunostained. In the *inset*, the staining is abolished following absorption of the antiserum with CEA. × 325.

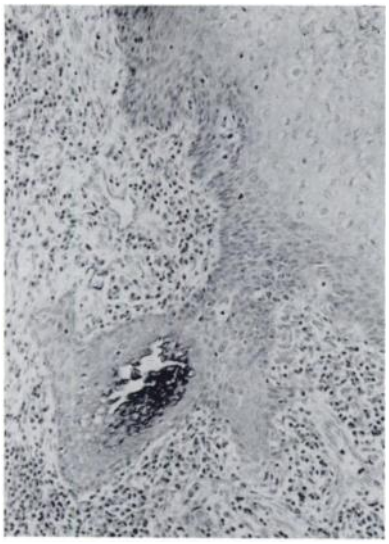

Fig. 13. NCA immunostaining in a small group of cells from an esophageal specimen containing hyperplastic foci and areas of basal cell atypia. The cells are intensely stained. × 130.

strong. All of the adenocarcinomas showed combined cell membrane and cytoplasmic staining of tumor cells.

Normal, Uninvolved, and Preneoplastic Esophagus. Neither NCA nor CEA immunoreactivity was present in normal or uninvolved esophageal epithelium. However, weak to moderately strong staining of some esophageal epithelial cells was observed in 4 of the 6 preneoplastic esophageal specimens that were treated with unabsorbed CEA antiserum (67%) (Table 2).

All of the specimens contained hyperplastic foci and/or areas of basal cell atypia or hyperplasia. However, some of the immunostained cells were located in areas that could not be definitively classified as hyperplastic due to the plane of section of the tissue. The staining involved the cells of the basal cell layer and/or the cells immediately above the basal cell layer (Fig. 13). Most staining was cytoplasmic, although one specimen displayed both cell membrane and cytoplasmic staining. The immunostaining was abolished in 3 of 4 lesions when the CEA antiserum was absorbed with spleen tissue, showing that staining was due to NCA immunoreactivity. In the single exception the staining was not completely abolished, but the

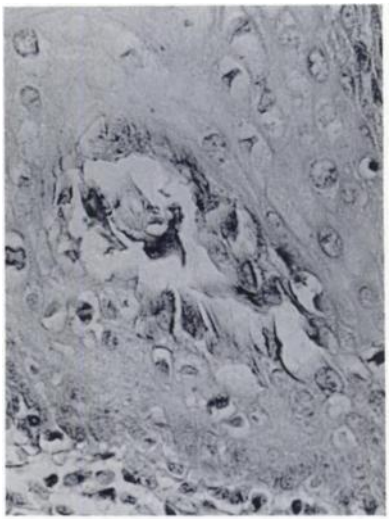

Fig. 14. The same lesion as seen in Fig. 13 after treatment with spleen-absorbed CEA antiserum. Staining is diminished. × 325.

number of immunoreactive cells was diminished (Fig. 14). The immunostaining of these reactive cells was abolished following treatment with spleen-absorbed CEA antiserum that had been further absorbed with purified CEA. Thus, some of the cells in this preneoplastic lesion contained CEA immunoreactivity in addition to NCA immunoreactivity. In all cases the corresponding neoplasms were stained for NCA and CEA.

DISCUSSION

In the present study human esophageal neoplasms and preneoplastic esophageal epithelium, as well as uninvolved and normal esophageal epithelium, were evaluated for HCG, HPL, AFP, CEA, and NCA immunoreactivity using immunohistochemistry. HCG is a glycoprotein hormone synthesized by the normal placenta and by a diverse group of malignant cells (9, 10). HCG has a molecular weight of 38,000 and consists of α- and β-subunits that are nonidentical and noncovalently bonded (11, 12). HPL is a polypeptide hormone with a molecular weight of 19,000 that is also synthesized by the syncytial trophoblasts of the placenta and is present in the circulation in large quantities only during pregnancy (13). Production of HPL has been documented in the case of trophoblastic neoplasms (14) as well as in some tumors of nontrophoblastic and nongonadal origin (15–18).

AFP is an oncofetal antigen that is normally found in high concentrations in fetal and maternal blood, but it also appears in adults having certain neoplastic diseases (19). It is a single chain sialylated glycoprotein with a molecular weight of about 67,000 (20). CEA is a glycoprotein with a molecular weight of approximately 200,000 (21). It was originally isolated from colon adenocarcinomas by Gold and Freedman (22), but it has since been found to be present in tumors of a variety of other epithelia (23–25). NCA is a M_r 100,000 glycoprotein antigen that is structurally similar to CEA, differing only in molecular weight and some antigenic determinants (8). NCA closely resembles a tumor-extracted CEA-related antigen found in a variety of tumors (26). NCA has also been isolated from some nonneoplastic tissues (8, 27).

HCG has not been identified in the sera of patients with esophageal neoplasms (9, 28, 29), but very few studies have evaluated HCG production by neoplasms of the esophagus. Hattori *et al.* (29) detected HCG in tissue extracts of 2 of 3 esophageal squamous cell carcinomas using radioimmunoassay.

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Table 3 *Summary of markers in esophageal carcinomas as determined by immunocytochemistry*

Marker(s)					No. of tumors with indicated marker(s) by histopathological diagnosis					
					Tumors with indicated markers[a]	Squamous cell carcinomas				
HCG	HPL	AFP	CEA	NCA		W-WMD[c] (11)	M-MPD (8)	P-PDS (7)	Adeno (7)	Adenocarcinomas (6)[b]
−	−	−	−	−	3 (8)[d]			3 (42)		
−	−	−	−	+	4 (10)		2 (25)		1 (14)	1 (17)
+	−	−	−	−	2 (5)			1 (14)	1 (14)	
−	+	−	−	+	1 (3)			1 (14)		
−	−	+	−	−	1 (3)		1 (13)			
−	−	+	−	+	2 (5)	2 (18)				
−	−	−	+	+	11 (28)	2 (18)	4 (50)		1 (14)	4 (68)
+	−	−	+	+	4 (10)	3 (27)			1 (14)	
−	+	−	+	+	2 (5)	1 (9)		1 (14)		
−	−	+	+	+	4 (10)	1 (9)			3 (43)	
+	+	−	−	+	1 (3)		1 (13)			
+	+	+	−	+	1 (3)			1 (14)		
+	−	+	+	+	2 (5)	1 (9)				1 (17)
+	+	−	+	+	1 (3)	1 (9)				

[a] Total number of tumors, 39.
[b] Numbers in parentheses (table boxhead), total number of tumors for each histopathological classification.
[c] W-WMD, well, well-moderately differentiated; M-MPD, moderately, moderate-poorly differentiated; P-PDS, poorly differentiated, poorly differentiated with spindle cell component; Adeno, adenosquamous carcinoma.
[d] Numbers in parentheses (table body), percentage.

A single esophageal adenocarcinoma included in their study was negative. Our results also indicate that HCG is associated with some esophageal squamous cell carcinomas, as well as some adenocarcinomas and preneoplastic lesions. Thus, while HCG may not be a useful serological marker for esophageal neoplasms, it may have limited application as a cytological tumor marker.

In our study, HPL immunoreactive tumor cells were identified in 6 of 39 primary esophageal neoplasms. Expression of HPL was observed most frequently in poorly differentiated squamous cell carcinomas, suggesting a possible association between poorly differentiated esophageal tumors and production of HPL. This result expands previous reports of the presence of HPL in tissue of nontrophoblastic neoplasms, including tumors of lung, kidney, and stomach (30). Elevated serum levels of HPL have also been described in patients with colorectal cancer (31), gastric cancer (16), hepatocellular carcinomas (16, 32), breast cancer (18), cancer of the uterine cervix (15), and lung cancer (32). Our localization of HPL in some squamous cell carcinomas of the esophagus further emphasizes its potential as a tumor marker.

AFP production is usually associated with liver and nonseminomatous testicular cancers (33–35). However, a variety of other types of tumors have been found to release AFP into the peripheral blood (19, 36–38). Wahren *et al.* (39) reported elevated AFP serum levels in 33% of the patients (59 cases) with primary esophageal cancer whom they studied using radioimmunoassay. In agreement with the latter studies, we detected immunoreactive AFP in 30% of the esophageal carcinomas which were evaluated in our study. AFP was more frequently encountered in well-differentiated squamous cell carcinomas and adenosquamous carcinomas than in moderately and poorly differentiated squamous cell carcinomas and adenocarcinomas, suggesting an association with well-differentiated tumors.

An antiserum to human colonic CEA was used in the present study to search for NCA and CEA immunoreactivity in esophageal neoplasms and preneoplastic lesions. When tested with unabsorbed antiserum 82% of the squamous cell carcinomas and all of the adenocarcinomas were positive. When the positive tumors were reexamined with spleen-absorbed CEA-specific antiserum, 57% of the squamous cell cancers and 83% of the adenocarcinomas were found to have CEA immunoreactivity. Both NCA and CEA immunoreactivity were more common in well-differentiated squamous cell carcinomas, adenosquamous carcinomas, and adenocarcinomas than in less-differentiated tumors. In the former, intense CEA immunoreactivity was often found in areas of keratinization. This has also been observed in squamous cell carcinomas of the lung (40).

Our results differ from those of Goldenberg *et al.* (41), who failed to demonstrate CEA immunoreactivity in any of 10 esophageal squamous cell carcinomas examined using immunocytochemistry and CEA-specific antiserum, although 2 of 4 adenocarcinomas at the esophagogastric junction were found to be CEA positive. Our findings are, however, in agreement with serological studies of patients with esophageal cancer that have reported elevated levels of CEA in 59% (39) and 70% (42) of cases.

Of special interest was the detection of cells with NCA immunoreactivity in 4 of 6 preneoplastic esophageal lesions. One of the 4 NCA-positive lesions was found to contain CEA immunoreactivity as well. Greater numbers of NCA and CEA immunoreactive cells were present in the neoplastic portion of the specimen, which suggests that an increase in marker production may be associated with the evolution of the neoplasm. The finding that preneoplastic esophageal lesions contain NCA and occasionally CEA could prove to be useful in screening tests for esophageal cancer in view of the fact that normal esophageal epithelial cells are immunocytochemically negative for these markers. CEA and NCA have not previously been reported in preneoplastic esophageal lesions, although CEA has been detected in normal and dysplastic squamous epithelium in lung cancer patients (43), and it has been identified immunocytochemically in normal and dysplastic exfoliated squamous cells in sputum from patients with carcinoma of the lung and from patients at risk for, but having no evidence of, lung cancer (44).

Table 3 summarizes the distribution of markers in esophageal tumors in relation to histopathological diagnosis. The majority of squamous cell carcinomas were positive for 2 or more markers, while some tumors exhibited multiple markers. None of the squamous cell carcinomas in our study expressed all of the markers that were examined, but 4 tumors expressed 4 of the 5 markers that were studied.

All of the adenocarcinomas were positive for NCA and/or CEA. One tumor was positive for NCA alone, while another was positive for HCG, AFP, CEA, and NCA.

No association was observed between the degree of tumor

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differentiation and the occurrence of HCG immunoreactivity. However, HPL immunoreactivity was more commonly encountered in poorly differentiated squamous cell carcinomas than in other types of tumors. In contrast, AFP immunoreactivity was more often expressed by well-differentiated squamous cell carcinomas and by adenosquamous carcinomas than by tumors of other histological types. Both NCA and CEA immunoreactivity were more frequently detected in well-differentiated squamous cell carcinomas, adenosquamous carcinomas, and adenocarcinomas than in other types of tumors. In preneoplastic lesions, NCA was encountered most often in those lesions that were associated with well-differentiated tumors. The relationship of tumor marker production to tumor differentiation should be interpreted cautiously, however, since esophageal tumors are morphologically heterogeneous, with many tumors containing well, moderately, and poorly differentiated components.

Our study indicates that HCG, HPL, AFP, CEA, and NCA are produced by some esophageal neoplasms and that NCA and less commonly CEA and HCG are produced by some preneoplastic esophageal lesions. None of these markers was expressed by normal or uninvolved esophageal epithelium; therefore, they appear to be tumor-associated markers which could be useful in cytological screening tests for the identification of esophageal cancer cells. Furthermore, these markers, as well as others which may be identified in the future, could lead to a means of subclassifying esophageal tumors based on the presence or absence of specific antigens. An association may exist between certain tumor markers or combinations of markers and the biological and clinical behavior of esophageal tumors. Thus, immunocytochemical classification of esophageal neoplasms may provide more prognostic information than classification schemes based solely on morphology.

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