Urological Research 6, 211-214 (1978)

Urological Research

Immunoperoxidase Staining of Carcinoembryonic Antigen in Urinary Bladder Cancer

D. M. Goldenberg[1] and B. Wahren[2]

[1]Division of Experimental Pathology, Department of Pathology, University of Kentucky College of Medicine, Lexington, Kentucky, USA, and
[2]Department of Virology, National Bacteriological Laboratory and Radiumhemmet, Karolinska Hospital, Stockholm, Sweden

Summary. An immunoperoxidase study of the presence of carcinoembryonic antigen (CEA) in primary cancers of the urinary bladder, metastases to the bladder, non-malignant diseased bladder, and normal bladder tissues revealed that approximately 10 percent of the urothelial carcinomas (transitional cell and squamous cell types) contained detectable quantities of this antigen. The other tissues were devoid of stainable CEA. It thus appears that the incidence of positive CEA by immunoperoxidase staining of formalin-fixed, paraffin-embedded tumour tissue sections is much less than the frequency of blood or urinary CEA elevations in patients with urothelial cancer.

Key words: Urinary bladder cancer - Carcinoembryonic antigen - Immunoperoxidase.

The carcinoembryonic antigen (CEA) described by Gold and Freedman (3) as restricted to digestive tract cancers has since been shown to be present in diverse neoplasms, both benign and malignant, by a variety of methods, including immunocytochemistry (4, 5). Using a triple-layer, indirect immunoperoxidase method for staining CEA in conventional histopathology specimens, we have reported frequent positive reactions in carcinomas of the digestive system, bronchus, and uterine cervix (5). Among transitional cell carcinomas of the urinary bladder, only 10 percent of a small number of formalin-fixed, paraffin-embedded specimens showed CEA staining (5). The purpose of this study was to increase the number of bladder carcinoma specimens for a more detailed evaluation of the presence of CEA in this tumour type.

METHODS AND RESULTS

Fifty-nine paraffin-embedded tissue specimens were recut for immunoperoxidase staining of CEA by the method described elsewhere (5, 9). Briefly, a 1 : 300 dilution of the primary, goat anti-CEA antiserum was added to the tissue sections, incubated at 37°C for 10 min, washed in two 5-min changes of 0.01 M phosphate-buffered saline (pH 7.2), and then treated with rabbit antiserum to goat IgG (1 : 50 dilution), followed by goat antiserum against peroxidase (1 : 100 dilution). The same primary antiserum, neutralized with CEA, was used in an adjacent identical section on each slide as a negative control. The third antiserum applied was bound to horseradish peroxidase by adding the latter at a concentration of 100 µg/ml for 20 min at 37°C. Finally, the reaction was developed by adding 75 mg 3,3'-diaminobenzidine in 100 ml 0.05 M Tris buffer (pH 7.6) and 0.05 percent H_2O_2 at room temperature for 20 min. Thereafter, the sections were washed in phosphate-buffered saline, dehydrated in graded ethyl alcohol solutions and xylene, and mounted with Permount. Counterstaining of the sections to enhance morphological identification was achieved with haematoxylin.

In addition to primary cancers of the urinary bladder, metastases to the bladder, non-malignant diseased bladders, and normal bladder tissues were evaluated. Table 1 records the results with various bladder specimens tested for the presence of CEA by the immunoperoxidase method. The set of 35 transi-

0300-5623/78/0006/0211/$01.00

Table 1. Immunoperoxidase results for CEA in urinary bladder cancer

Histopathology	Pos/Total
Primary cancer (transitional cell carcinoma)	4/35
Primary cancer (squamous cell carcinoma)	1/10
Metastatic cancer to bladder	
From cervix	1/1
From caecum	1/1
Non-malignant diseased bladder tissue from patients with bladder carcinoma	0/2
"Normal" bladder tissue from patients with concurrent bladder carcinoma	0/2
Non-malignant diseased bladder tissue from patients without concurrent bladder carcinoma	0/3
Normal bladder	0/5

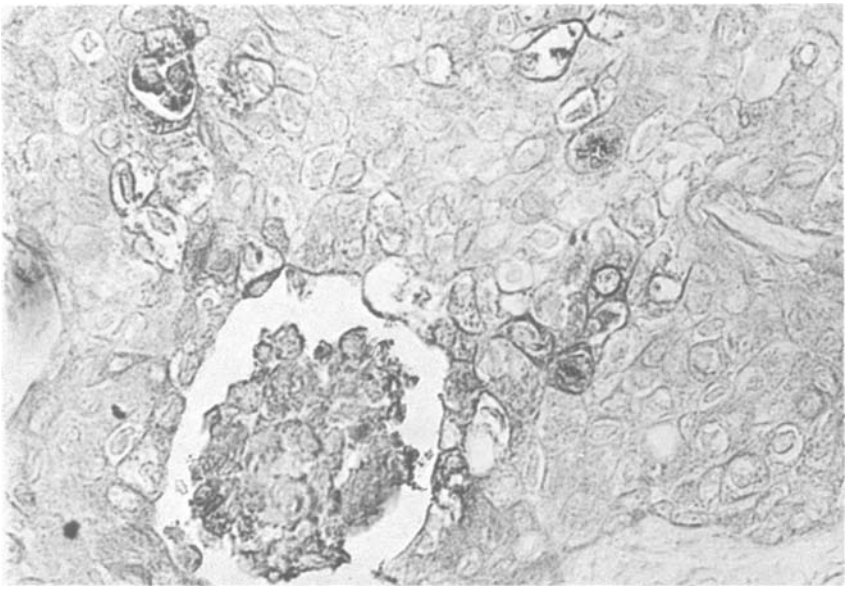

Fig. 1. Immunoperoxidase staining of CEA in formalin-fixed, paraffin-embedded section of a squamous cell carcinoma of the urinary bladder, indicating a peripheral staining reaction on selected tumour cells within the field shown. Mag. x 240

tional cell carcinomas included representatives of all clinical stages and histopathological grades, yet did not indicate any predilection for CEA among these 4 (11%) which were positive. Not all areas with malignant cells stained uniformly for CEA; instead, a heterogeneously staining population was seen. Also, both peripheral and cytoplasmic staining were found, although the former was more pronounced. Although only a small number of squamous cell carcinomas was examined, a similar percentage to that found positive in transitional cell carcinomas was found. Figure 1 shows the surface immunoperoxidase reaction in a squamous cell carcinoma specimen.

Cancers of the uterine cervix or of the

caecum which were metastatic to the bladder stained well for CEA, while bladder tissues from bladder cases apparently free of tumour did not reveal any CEA-immunoperoxidase reaction. Likewise, normal bladder tissues did not show CEA by immunoperoxidase. Hence, it appeared that only the malignant cells, either of urothelial or other origin, contained sufficient CEA for detection by immunoperoxidase.

DISCUSSION

CEA has been detected in elevated quantities in the urine (1, 2, 6-8, 10, 12, 14) and blood (2, 8, 10, 12, 13) of patients with urothelial cancers, and it is estimated that 42 percent of bladder cancer patients have raised plasma CEA titres (4). Since numerous factors control circulating CEA values, such as liver function, tumour concentration and size, presumably cell type and cell turnover, one could expect that malignant bladder tissue would have a higher incidence of detectable quantities of CEA than the corresponding extracellular compartments. This assumption, however, is not confirmed in this study, where only about 10 percent of the bladder cancers (transitional cell and squamous cell carcinomas) showed CEA by the immunoperoxidase test. Since the immunoperoxidase method is more sensitive than immunofluorescence (5), detecting 3.0 - 5.0 µg CEA per g tissue (5), this apparent discrepancy suggests that the CEA measured in urine or plasma of patients with urothelial cancers by radioimmunoassay may not be identical to that being detected (or not staining) in tissue specimens by immunoperoxidase. Obviously, the two different assays (radioimmunoassay and immunoperoxidase) have different levels of sensitivity. Nevertheless, we did expect a higher incidence of CEA staining in bladder cancer specimens than in fact experienced.

Another possible explanation for the low percentage of bladder cancers staining for CEA is that formalin fixation and paraffin embedding may result in a greater destruction of tissue CEA in urothelial cancers than in other tumour types, a situation already found for colonic polyps compared with adenocarcinomas (11). However, the paraffin blocks containing CEA-positive bladder cancers ranged from 6 months to 6 years of age post-fixation in formalin, so that antigen destruction because of tissue processing or storage could only play a minor role in influencing CEA staining in bladder tumours. It is therefore important to undertake a comparison of immunoperoxidase staining of fresh and formalin-paraffin treated bladder cancers, and also to obtain tissue CEA levels by radioimmunoassay. Furthermore, the good staining of CEA in exfoliated urothelial carcinoma cells by immunofluorescence (13) suggests that these specimens should be evaluated by immunoperoxidase after similar fixation procedures. Future research will have to determine whether immunocytochemical staining of CEA in tissue sections or exfoliated cells of urothelial cancer will be of any clinical value in the management of these cancer patients.

Acknowledgement. We thank Robert M. Sharkey for performing the immunocytochemical procedures in this study.

REFERENCES

1. Coombes, B.G., Hall, R.R., Laurence, D.J.R., Neville, A.M.: Urinary carcinoembryonic antigen (CEA)-like molecules and urothelial malignancy. A clinical appraisal. British Journal of Cancer 31, 35 (1975)
2. Edsmyr, F., Wahren, B., Zimmerman, R.: Measurement of urine and serum CEA - a help in the diagnosis and follow-up of bladder cancer. Läkartidningen 72, 3141 (1975)
3. Gold, P., Freedman, S.O.: Demonstration of tumour-specific antigens in human colonic carcinomata by immunological tolerance and absorption techniques. Journal of Experimental Medicine 121, 439 (1965)
4. Goldenberg, D.M.: Oncofetal and other tumour-associated antigens of the human digestive system. In: Current Topics in Pathology (Grundmann, E., Kirsten, W.H., eds), Vol. 63, Pathology of the Gastro-Intestinal Tract (Morson, B.C., ed.). p. 289. Berlin, Heidelberg, New York: Springer 1976
5. Goldenberg, D.M., Sharkey, R.M., Primus, F.J.: Carcinoembryonic antigen in histopathology: Immunoperoxidase staining of conventional tissue sections. Journal of the National Cancer Institute 57, 11 (1976)
6. Guinan, P., John, T., Sadoughi, N., Ablin, R.J., Bush, I.: Urinary carcinoembryonic-like antigen levels in patients with bladder carcinoma. Journal of Urology 111, 350 (1974)
7. Hall, R.R., Laurence, D., Darcy, D., Stevens, U., James, R., Roberts, S., Neville, A.M.: Carcinoembryonic antigen in the urine of patients with urothelial carcinoma. British Medical Journal 3, 609 (1972)
8. Hall, R.R., Laurence, D.J.R., Neville, A.M., Wallace, D.M.: Carcinoembryonic

antigen and urothelial carcinoma. British Journal of Urology 45, 88 (1973)
9. Primus, F. J., Wang, R. H., Sharkey, R. M., Goldenberg, D. M.: Detection of carcinoembryonic antigen in tussue sections by immunoperoxidase. Journal of Immunological Methods 8, 267 (1975)
10. Reynoso, G., Chu, T., Guinan, P., Murphy, G.: Carcinoembryonic antigen in patients with tumors in the urogenital tract. Cancer 30, 1 (1972)
11. Sharkey, R. M., Hagihara, P. F., Goldenberg, D. M.: Localization by immunoperoxidase and estimation by radioimmunoassay of carcinoembryonic antigen in colonic polyps. British Journal of Cancer 35, 179 (1977)
12. Wahren, B., Edsmyr, F., Zimmerman, R.: Measurement of urinary CEA-like substance. An aid in management of patients with bladder carcinoma. Cancer 36, 1490 (1975)
13. Wahren, B., Esposti, P., Zimmerman, R.: Characterization of urothelial carcinoma with respect to the content of carcinoembryonic antigen in exfoliated cells. Cancer (submitted for publication).
14. Wu, J. T., Madsen, A., Bray, P. F.: Quantitative measurements and chromatography of human urinary carcinoembryonic antigen activity. Journal of the National Cancer Institute 53, 1589 (1974)

D. M. Goldenberg, Sc. D., M. D.
Professor of Pathology
University of Kentucky College of Medicine
Lexington, Kentucky 40506
USA