An immunoperoxidase technique to aid in the differential diagnosis of prostatic carcinoma

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The differentiation of carcinomas arising in the prostate, bladder, and rectum may be a diagnostic problem when a neoplasm demonstrates extensive local invasion of adjacent organs. With the advent of enzyme-labeled antibody techniques, the specific identification of these neoplasms is potentially within the grasp of the pathologist.[1,2] However, the value of these techniques is limited by the specificity of available antibodies. Cross-reactivity, if not detected before clinical application, could result in diagnostic error.

We describe an immunoperoxidase method[3] to demonstrate the presence of prostatic acid phosphatase in neoplasms of prostatic origin, with the use of an antibody raised in rabbits against this enzyme. The potential application of this technique to differential diagnosis is discussed and the importance of in-house testing of these antibodies is demonstrated.

Materials and methods

The following immunochemicals were used in this study to examine paraffin-embedded tissue fixed in zinc-substituted Zenker's solution: (1) rabbit anti-human prostatic acid phosphatase, (2) sheep anti-rabbit immunoglobulin, and (3) peroxidase-rabbit antiperoxidase complex (PAP). All

antibodies were obtained from Immulok.

Tissue from 54 cases was examined. Four-micron sections of tissue, two per slide, were cut and dried on albuminized slides at 60 C overnight. The sections were deparaffinized in xylene and graded alcohols and washed in phosphate-buffered saline (PBS), pH 7.2 (FTA hemagglutination buffer, BBL) until miscible. Endogenous peroxidase activity was consumed by a 3-minute incubation in 3% hydrogen peroxide followed by a PBS wash. Sections were then incubated with normal sheep serum for 20 minutes to block nonspecific binding of the sheep anti-rabbit secondary antibody. The slides were then wiped to separate the two tissue sections and to remove any excess sheep serum. Rabbit anti-human prostatic acid phosphatase was applied to one of the tissue sections and PBS to the other with care taken to prevent mixing of the solutions overlying the two tissue sections. Slides were incubated in a moist chamber for 30 minutes, and rinsed in PBS to remove the primary antibody. Sheep anti-rabbit antibody was next applied to both tissue sections and incubated as previously described. Following a brief PBS wash, rabbit PAP complex was applied to both tissue sections and the incubation step was again repeated. The slides were washed in PBS once more, and flooded with aminoethylcarbazole (AEC), 12 mg, in 5 μl dimethyl ormamide and 50 ml 0.02 acetate buffer, pH 5.2, containing 40 μl of 30% H_2O_2 for 20 minutes at ambient temperature. The slides were then washed in PBS for more than 5 minutes. After hematoxylin counterstaining, the slides were mounted and coverslipped with 90% glycerol in PBS.

Table. Results of staining with immunoperoxidase

Histologic diagnosis	Total no.	Immunoperoxidase staining	
		No. positive	No. negative
Prostatic adenocarcinoma	7	7	0
Benign prostatic hyperplasia	2	2	0
Rectal carcinoid	6	6	0
Transitional cell carcinoma of bladder*	9	0	9
Rectal adenocarcinoma	3	0	3
Infiltrating duct carcinoma of breast	2	0	2
Fibrocystic disease of breast	4	0	4
Adenocarcinoma of lung	2	0	2
Squamous cell carcinoma of lung	2	0	2
Renal cell carcinoma	3	0	3
Gastric adenocarcinoma	2	0	2
Pancreatic adenocarcinoma	1	0	1
Pancreatic apudoma†	3	0	3
Pleomorphic adenoma, parotid‡	3	0	3
Adenoid cystic carcinoma, parotid	1	0	1
Hairy cell leukemia, spleen	2	0	2
Gaucher's disease, bone marrow	1	0	1
NL seminal vesicle	1	0	1
Total	54	15	39

* Two cases examined were secondary in prostate and had focal positive staining in areas of necrosis.

† Occasional cells in normal islets of Langerhans stained positively.

‡ Scattered individual cells are positive.

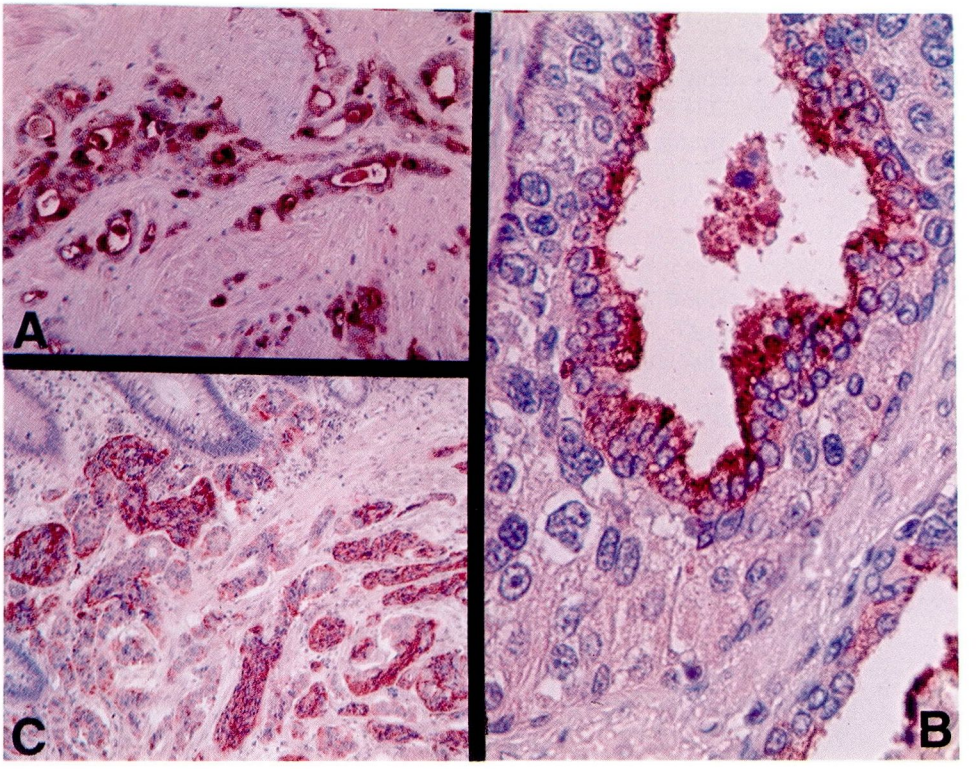

Fig 1 A-C. Photomicrographs demonstrating immunostaining of prostatic acid phosphatase: prostatic carcinoma, prostate (1A), transitional cell carcinoma of bladder invading prostate (1B), carcinoid tumor of rectum (1C). The invading transitional cell carcinoma (1B) fails to stain, whereas the normal prostatic ducts are positive. All sections are counterstained with hematoxylin.

At each step in the procedure, tissue sections were examined for evidence of drying. Those with dry sections were discarded and restained.

Results

The results of immunostaining for prostatic acid phosphatase are summarized in the *Table*. Positive staining for prostatic acid phosphatase was present diffusely in a cytoplasmic location in all six prostatic carcinomas examined (*Fig. 1A*), in both cases of benign prostatic hypertrophy, and in the normal prostate tissue present in these sections. Stromal staining was absent or minimal and easily differentiated from true positive immunostaining. Prostatic concretions, ducts, and acini were positive. Neoplastic urothelium in all nine transitional cell carcinomas of the bladder failed to demonstrate any staining of the neoplasm. Sections of two of these neoplasms were from intraprostatic areas of the invasive neoplasm, and in each instance in viable portions of the tumor the neoplastic transitional cells remained negative, whereas the normal prostate epithelium stained positively (*Fig. 1B*). However, in areas of necrosis within the prostate in these two cases, the necrotic tissue stained positively, probably secondary to the release of acid phosphatase from necrotic prostatic

glands. In addition, all six cases of rectal carcinoid tumors showed diffuse cytoplasmic immunostaining with negative staining of the overlying rectal mucosa (*Fig. 1C*). The remainder of the neoplasms examined failed to stain by this technique. However, scattered cells within the normal pancreatic islets demonstrated diffuse cytoplasmic staining and scattered normal breast ducts demonstrated positive staining of the glycocalyx along the luminal border as well as intracytoplasmic staining. The normal seminal vesicle failed to stain as did tissue sections of hairy cell leukemia in the spleen and Gaucher's disease involving the bone marrow.

Discussion

The differentiation of carcinomas of the prostate from carcinomas arising in the adjacent bladder or rectum is generally not a diagnostic problem when the neoplasm remains localized to its organ of origin. However, when the tumor invades contiguous organs, the diagnosis may be more difficult. Dysplastic changes in the urothelium or rectal mucosa adjacent to the neoplasm may aid in the differential diagnosis. However, these changes may not be present. Elevations of serum prostatic acid phosphatase likewise may be helpful, but unfortunately if significant neoplastic invasion of the prostate occurs with destruction of normal parenchyma, elevations of the serum enzyme levels may be misleading. For this reason we examined the potential application of the peroxidase-antiperoxidase immunoperoxidase method, utilizing unlabeled antibody to human prostatic acid phosphatase.

All of the prostatic carcinomas examined stained positively in diffuse cytoplasmic distribution, whereas the bladder carcinomas and rectal adenocarcinomas were uniformly negative (*Table*). Normal prostate tissue in sections of benign prostatic hyperplasia demonstrated uniform positive staining, and in two cases distinctly contrasted with the negatively stained bladder carcinoma that had invaded the prostate. However, areas of tissue and tumor necrosis in these latter two cases demonstrated a positive immunologic reaction, presumably secondary to release of prostatic acid phosphatase by the necrotic prostate cells. This false-positive staining should caution pathologists from overinterpreting a positive reaction of tumor cells in areas of intraprostatic necrosis, a potential problem in needle biopsies where nonnecrotic tissue might not be present.

Although these findings support the usefulness of this procedure in differentiating prostatic carcinomas from rectal adenocarcinomas and bladder carcinomas, we were surprised to discover that all of the rectal carcinoid tumors we examined demonstrated positive cytoplasmic staining identical to that of the prostatic neoplasms. This cross-reactivity is probably secondary to contamination of the prostatic extract used to immunize the rabbit by argentaffin or argyrophil cells or both, which have been reported to be present in a normal prostate.[4,5] This is unfortunate, as rectal carcinoids may display a glandular pattern that is difficult to distinguish from prostatic tumors. The cross-reactivity of the immunoperoxidase staining therefore does limit somewhat the diagnostic usefulness of this particular antibody. With the use of histochemical staining for neurosecretory granules, the number of cases in which this diagnostic problem is not resolvable is probably small. Neurosecretory granules shown by electron microscopy would help differentiate be-

tween the two neoplasms. However, this is of limited practical use, especially if a single needle biopsy is performed to obtain a diagnosis. Although this technique is a useful adjunct to the pathologist's diagnostic regimen, it is not entirely specific for prostatic tissue.

On a broader scale, the cross-reactivity of this antibody with both prostatic glandular epithelium and rectal carcinoid tumor emphasizes the importance of in-house control testing by pathology laboratories of all immunoperoxidase antibodies to be used for clinical diagnostic purposes. The antibody we used in our study was supplied by a reputable company that does extensive work in the field of immunohistochemical techniques. Their antibody product is tested by consulting pathologists to demonstrate its monospecificity. The cross-reactivity of this particular antibody with rectal carcinoid tumors was not detected before marketing of this immunoperoxidase kit. If we had not tested the antibody against a variety of neoplasms, we might easily have made a diagnostic error. Manufacturers that produce antibodies should conduct extensive studies to demonstrate monospecificity. However, we think that it is the ultimate responsibility of the individual pathology laboratory to control test these products to assure optimal diagnostic accuracy.

Summary

The unlabeled immunoperoxidase technique and antibody to human prostatic acid phosphatase was used to study a variety of normal and neoplastic tissues. The technique was found to be a useful adjunct in the differential diagnosis of prostatic carcinoma versus transitional cell carcinoma of the bladder and rectal adenocarcinoma, but was not entirely specific, as cross-reactivity with rectal carcinoid tumors was documented. This study emphasizes the importance of controlled testing of these antibodies by pathology laboratories using immunoperoxidase techniques to assure accurate surgical pathologic diagnosis.

References

1. Nadji M, Tabei SZ, Castro A, Chu TM, Morales AR. Prostatic origin of tumors; an immunohistochemical study. Am J Clin Pathol 1980; **73:** 735–9.
2. Klacsmann P, Cantrell BB, Mendelsohn G, et al. Immunohistochemical localization of prostatic acid phosphatase and carcinoembryonic antigen in Stage A_2 carcinoma of the prostate. (Abstr) 1981; **44:** 32A–33A.
3. Taylor CR. Immunoperoxidase techniques; practical and theoretical aspects. Arch Pathol Lab Med 1978; **102:** 113–21.
4. Kazzaz BA. Argentaffin and argyrophil cells in the prostate. J Pathol 1974; **112:** 189–93.
5. Azzopardi JG, Evans DJ. Argentaffin cells in prostatic carcinoma; differentiation from lipofuscin and melanin in prostatic epithelium. J Pathol 1971; **104:** 247–51.