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PROSTATE-SPECIFIC ANTIGEN
A Clue for the Prostatic Origin of Metastasis

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ABSTRACT. The prostate-specific antigen is a recently purified glycoprotein which is present only in the prostatic gland. In order to confirm the usefulness of this protein in isolating prostatic carcinomas from so-called metastatic carcinomas of unknown primary site, we immunohistochemically studied 19 non-neoplastic prostatic tissue, 18 primary carcinomas of the prostate, and 32 non-prostatic adenocarcinomas. From our study, we concluded that PSA is highly specific for the prostatic carcinomas. The absence of PSA, however, does not necessarily rule out its prostatic origin when the tumor is poorly differentiated. In our laboratory, from now on, PSA will be applied routinely in cases of metastatic carcinomas of unknown origin.

Key words : Prostate-specific antigen — PAP — metastatic carcinomas of unknown origin

The primary site is hardly known solely from the histology of metastatic cancer particularly when it is poorly differentiated. Previously, we have tested how accurately we could determine the primary site merely from a hematoxylin-eosin stained section of metastatic cancer.[1] A mucin stain such as PB/KOH/PAS technique was also tried as an adjunct to identify the colonic cancer, but did not give better information.[2] We are still in pursuit of obtaining the way to know the primary site accurately in the variety of metastatic tumors.

Recently, prostate-specific antigen (PSA) has been purified[3,4] and its antibody was in use to isolate prostatic carcinoma by means of peroxidase anti-peroxidase technique in the tissue section.[5,6] It is a glycoprotein with about 34,000 mol. wt., distinct from precipitin band by gel diffusion studies with those of bladder, heart, intestine, kidney, liver, lung, pancreas, spleen, and urethra. Fortunately PSA antibody is now available commercially. Not infrequently we encounter the metastatic carcinoma of prostatic origin which has been submitted for histological study as carcinoma of unknown primary site. Out of 23,397 surgical materials experienced in our hospital for eight years until 1981, 65 cases were referred to be of unknown primary site on the clinical basis.[1] About 6% of them were revealed to be of prostatic origin. However, we have an impression that prostatic carcinomas are seen more frequently as carcinomas of unknown primary in practice, possibly because

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of clinical submission without any specification. The recognition of the primary site is important in carcinomas of the prostate, thyroid, breast and germ cells because there are relatively effective systemic treatments for these carcinomas[7] whereas those of other primary site only rarely influences the choice of treatment.[8]

In order to confirm the usefulness of this protein, we immunohistochemically studied 19 non-neoplastic prostatic tissues, 18 primary carcinomas of the prostate, and some other adenocarcinomas. A few similar reports[5,6] have been published, so far. Our result was essentially identical to the previous reports. This technique seemed superb to identify the cells of prostatic origin. The purpose of this communication is to describe our experience and to alert the clinicians in our hospital about the availability of this technique in the laboratory.

MATERIALS AND METHODS

Formalin-fixed paraffin-embedded tissue sections from neoplastic and non-neoplastic prostatic tissue as well as non-prostatic neoplasms were utilized for this study. Among these cases, 19 normal and/or hyperplastic prostatic tissue, 18 adenocarcinomas of the prostate, and one squamous cell carcinoma of the prostate were taken by the needle biopsy. Adenocarcinomas of the other sites, removed surgically, included breast (5), lung (5), urachus (1), rectum (5), stomach (6), pancreas (5), and thyriod (5). The hematoxylin-eosin stained sections were reviewed and those of prostatic carcinomas were graded according to the Gleason's pattern system.[9] His grading system[10] was not used because of limited amount of tissue sample and of comparison between the pattern and positivity. When two or more different patterns were recognized, each pattern was described separately and compared with its reactivity for PSA. The peroxidase-antiperoxidase technique was carried out as follows.

Briefly, paraffin sections 3 μ thick were deparaffinized. The intrinsic peroxidase activity was blocked by 10% H_2O_2 in methanol for 30 min. The tissue was then incubated with 10% egg albumin for 30 min to block non-specific binding of immunoglobulins. Nextly, the tissue was incubated with rabbit antiprostate-specific antigen antibody (DAKO KIT) for 30 min at room temperature. After brief wash with 1% egg albumin, swine antirabbit immunoglobulin (1 : 30) was applied for 30 min. Then the tissue was incubated with rabbit peroxidase-antiperoxidase 1 : 200 for 30 min and stained with diaminobenzidine 0.02% (Sigma) for 5 min. Tissues were finally counterstained with hematoxylin for 1 min, rinsed with tap water, dehydrated, and mounted with permount. The presence of the brown stain of diaminobenzidine was taken as a positive reaction and graded as minimally, slightly, moderately, and strongly positive. The distribution of positivity was recorded as diffuse or focal both at cell and tissue levels.

RESULTS

I. Non-neoplastic prostatic tissue.

19 non-neoplastic tissue revealed diffuse peroxidase staining of prostatic glands except for inflamed and cystically dilated glands (Fig. 1. a,b,c). All the

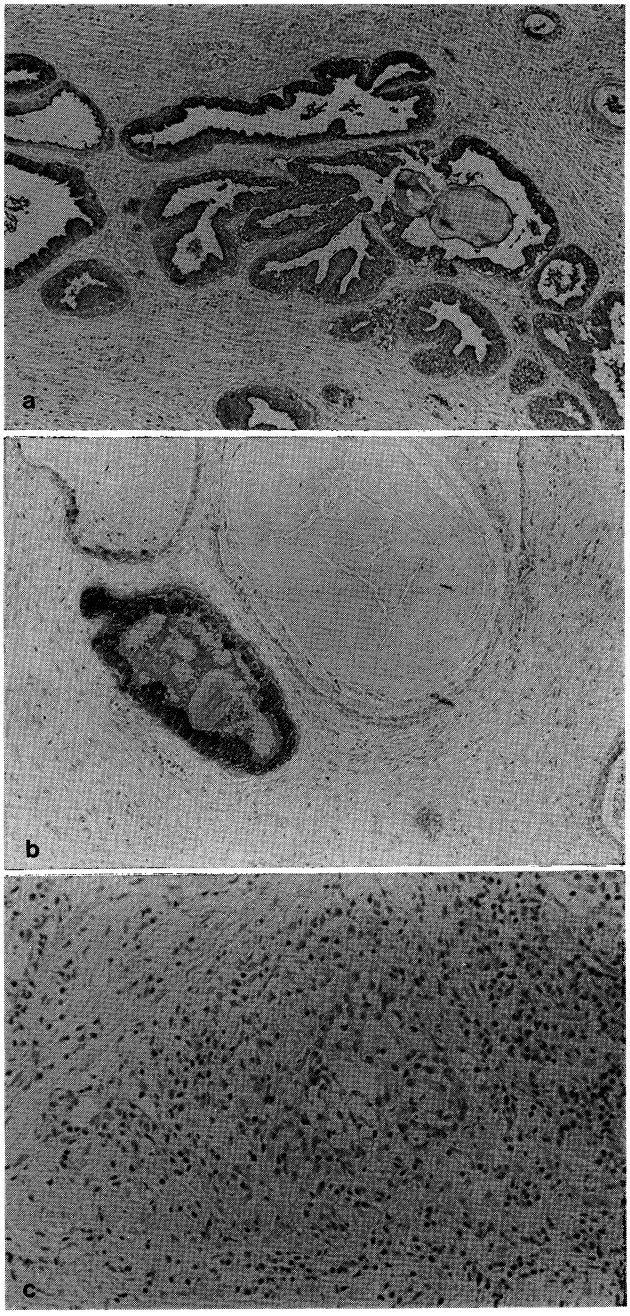

Fig. 1. Non-neoplastic prostatic tissue.
a) Glandular epithelia are positive for PSA. Note that prostatic concretions are PSA-negative. (PAP- Hematoxylin ×100)
b) The thin epithelia of cystically dilated glands are negative for PSA while adjacent glandular epithelia are positive. (PAP-Hematoxylin ×100)
c) Inflamed portion of the glands are also negative for PSA. (PAP-Hematoxylin ×200)

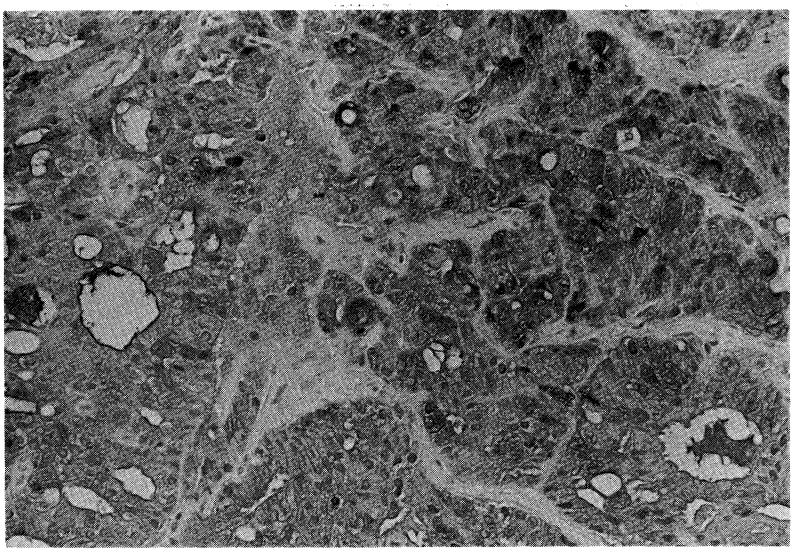

Fig. 2. Prostatic carcinoma of pattern 3.
The cytoplasm as well as materials within lumina is stained positively for PSA. (PAP-Hematoxylin ×200)

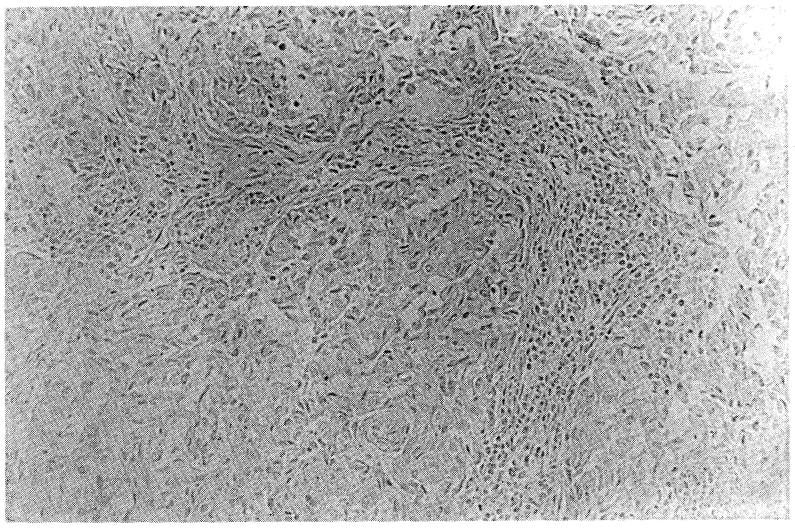

Fig. 3. Carcinoma of the lung.
The PSA is negative throughout. (PAP-Hematoxylin ×200)

glandular epithelia were not stained equally and a few negative cells were scattered. Generally, the cytoplasm was uniformly stained brown with granular appearance. Sometimes, however, they were globular and sometimes concentrated abluminally. Lumina may contain positive granular materials. Prostatic concretions were consistently negative for peroxidase staining. Inflamed ducts with neutrophilic infiltration were always negative. Ductular epithelia of cuboidal type were usually negative but minimally positive on occasion. The bladder and

urethral mucosa as well as colonic mucosa simultaneously embedded were always negative.

II. Neoplastic prostatic tissue.

19 specimens showed areas with pattern 1 in two cases, pattern 2 in 3 cases, pattern 3 in 9 cases, pattern 4 in 3 cases, and pattern 5 in one case. A case of squamous cell carcinoma of the prostate were included in this group. Cases with pattern 5 and squamous cell carcinoma were completely negative throughout. In the remaining cases, the peroxidase staining was essentially similar in their intensity and distribution in any pattern examined (Fig. 2). The cytoplasm was diffusely and moderately stained. In contrast to the normal glands, no predilection for the abluminal areas was noted. Occasionally, strongly positive cells were scattered. Lumina also contained strongly positive granular materials on occasion.

III. Non-prostatic malignancy.

All the cases with well, moderately and poorly differentiated adenocarcinoma of non-prostatic tissue were consistently negaive for PSA (Fig. 3).

DISCUSSION

The immunoperoxidase technique has gained the wide acceptance as a valuable tool in diagnostic pathology. This procedure has significant advantages over other histochemical and/or immunofluorescent technique. It does not require fresh frozen tissue, dark field, or special equipment. Instead, paraffin-embedded tissues may be utilized and excellent morphological features can be obtained. With this procedure any antigens, once purified, can be identified in the tissue section and consequently tumor cells may be revealed to have such antigens, thereby enabling the determination of cell origin or their characteristics.

Serum acid phosphatase is widely used as an adjunct to the diagnosis of prostatic carcinoma.[11,12] It is, however, elevated in only 75% of the patients with disseminated carcinoma of the prostate and it is not specific for this condition because occasionally benign prostatic processes such as hyperplastic, infarction and rectal examination may account for the elevation. In addition, elevated levels have been reported to occur in osteogenic sarcomas, Paget disease of the bone, multiple myeloma, chronic granulocytic leukemia and so forth.[13] Parallel to serum enzymatic studies, hisochemical staining for acid phosphatase has been developed. The enzymatic activity of acid phosphatase isoenzymes of the prostatic origin is resistant to formaldehyde but alcohol destroys the activity of this isoenzyme.[14] Therefore, it cannot be demonstrated by the enzyme histochemical methods in routinely processed paraffin-embedded materials, but requires the frozen tissue. Studies showed that 79 to 100% of primary prostatic carcinoma contained prostatic acid phosphatase.[15–17] This enzyme may therefore be detected by peroxidase-antiperoxidase technique.[15–17] However, this isoenzyme may cross-react with acid phosphatase of granulocytes, pancreas and breast. Another specific protein which is distinct from acid phosphatase has been purified and named as prostatic specific antigen (PSA).[3,4] This protein seems to be specific for the prostate only. For this reason, detection of PSA by peroxidase-antiperoxidase methods has started to be utilized in surgical

pathology.[5,6] In carcinoma tissues, the demonstration of PSA may determine the prostatic origin of the tumor. Two previous reports[5,6] emphasized its usefulness. Results suggested that it was of prostatic origin when the staining was positive. The absence of PSA in a poorly differentiated carcinoma, however, does not rule out the possibility of a prostatic carcinoma. Our result was in good agreement with the above. Further, our results indicated the intensities of the staining in prostatic carcinomas were not different in all patterns of Gleason's classification except for pattern 5 in which peroxidase staining was negative. Of interest was that inflamed glandular epithelia showed negative staining. Contrary to the previous reports,[5,6] prostatic concretions were always negative. No neoplastic or non-neoplastic cells of the other tissues exhibited peroxidase positivity.

we concluded that PSA is highly specific for the prostatic carcinomas but of course the absence of PSA in a poorly differentiated tumor of undetermined origin does not necessarily rule out its possibility. In our laboratory, from now on, PSA will be applied routinely in cases of metastatic carcinomas of unknown origin.

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