Prostate-specific Membrane Antigen Expression in Normal and Malignant Human Tissues

David A. Silver, Inmaculada Pellicer, William R. Fair, Warren D. W. Heston, and Carlos Cordon-Cardo

Urology Service, Department of Surgery [D. A., W. R. F., W. D. W. H.] and Division of Molecular Pathology, Department of Pathology [I. P., C. C. C.], Memorial Sloan-Kettering Cancer Center, New York, New York 10021

ABSTRACT

Prostate-specific membrane antigen is a type II membrane protein with folate hydrolase activity produced by prostatic epithelium. The expression of this molecule has also been documented in extraprostatic tissues, including small bowel and brain. In the present study, an extensive immunohistochemical analysis was performed on a panel of well-characterized normal and malignant human tissues to further define the pattern of prostate-specific membrane antigen (PSMA) expression.

Detectable PSMA levels were identified in prostatic epithelium, duodenal mucosa, and a subset of proximal renal tubules. A subpopulation of neuroendocrine cells in the colonic crypts also exhibited PSMA immunoreactivity. All other normal tissues, including cerebral cortex and cerebellum, had undetectable levels of PSMA. Thirty-three of 35 primary prostate adenocarcinomas and 7 of 8 lymph node metastases displayed tumor cell PSMA immunostaining. Eight of 18 prostate tumors metastatic to bone expressed PSMA. All of the other nonprostatic primary tumors studied had undetectable PSMA levels. However, intense staining was observed in endothelial cells of capillary vessels in peritumoral and endotumoral areas of certain malignancies, including 8 of 17 renal cell carcinomas, 7 of 13 transitional cell carcinomas, and 3 of 19 colon carcinomas.

Extraprostatic PSMA expression appears to be highly restricted. Nevertheless, its diverse anatomical distribution implies a broader functional significance than previously suspected. The decrease in PSMA immunoreactivity noted in advanced prostate cancer suggests that expression of this molecule may be linked to the degree of tumor differentia-

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2 To whom requests for reprints should be addressed, at Department of Pathology, Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021. Phone: (212) 639-7746; Fax: (212) 794-3186.

3 The abbreviation used is: PSMA, prostate-specific membrane antigen.

MATERIALS AND METHODS

Tissues. Normal and neoplastic formalin-fixed, paraffin-embedded tissue samples were obtained from the Department of Pathology at the Memorial Sloan-Kettering Cancer Center. Thirty-five primary prostate adenocarcinoma specimens were evaluated as well as 8 metastases to lymph nodes and 18 metastases to bone. Table 1 summarizes the clinical characteristics of these tumors in terms of pathological stage and prior treatment. Additional primary tumors included 17 renal cell carcinomas, 13 bladder transitional cell carcinomas, and 19 colon adenocarcinomas.

Antibodies. Mouse monoclonal antibody CYT-351 (clone 7E11-C5) (Cytogen, Princeton, NJ) was used as the primary antibody. This clone is derived from the original hybridoma reported by Horoszewicz et al. (7). Secondary antibodies consisted of biotinylated horse anti-mouse polyclonal IgG (Vector Laboratories, Inc., Burlingame, CA). The proper concentration of each reagent was determined by titration experiments prior to staining.

Immunohistochemistry. An avidin-biotin peroxidase method was used. Sections were deparaffinized, and endogeneous peroxidase activity was blocked in 1.0% hydrogen perox-
allowed to cool. In some cases, endogenous biotin was blocked with an avidin-biotin blocking kit (Vector Laboratories). Normal horse blocking serum (Organon Teknika Corp., West Chester, PA) at a 1:10 dilution in 2% PBS-BSA (Sigma Chemical Co., St. Louis, MO) was applied for 30 min to minimize background staining. Primary antibody at 2 μg/ml in 2% PBS/BSA was applied after suction removal of horse serum, and sections were incubated overnight in a wet chamber at 4°C. Sections were washed and biotinylated secondary antibodies were applied for 30 min (1:500 dilution). Sections were washed and avidin-biotin peroxidase complexes (Vector Laboratories) diluted 1:25 in PBS were applied for 30 min. Sections were then immersed in a solution of 0.05% diaminobenzidine tetrachloride and 0.01% hydrogen peroxide in 0.5% Triton X-100-PBS to accomplish the chromogen reaction. After extensive washing, sections were counterstained with hematoxylin, dehydrated, and mounted. Cases were considered positive if at least 20% of the malignant component demonstrated immunoreactivity. Positive control antibodies to normal antigen components present in specific cell types included CD45 (DAKO Corp., Carpinteria, CA) diluted 1:500 in 2% PBS-BSA and chromogranin (DAKO Corp.) diluted 1:20,000 in 2% PBS-BSA. Negative controls were conducted by substitution of primary antibodies with non-immune serum.

RESULTS

Table 2 summarizes immunoreactivities identified in the normal tissues studied. In normal and hyperplastic prostate glands, staining was either weak and luminal or absent. In several tissues, the immunohistochemical procedure routinely utilized was modified to include blocking of endogenous biotin to avoid false-positive reactions. Renal tubules, initially noted to display intense cytoplasmic staining, exhibited identical reaction patterns with class-matched primary antibody substitution and negative controls (Fig. 1a). Blocking of endogenous biotin abolished the background cytoplasmic staining and revealed immunoreactivity that was reproducibly restricted to a subset of proximal tubules (Fig. 1b). A similar situation was encountered in the gastrointestinal tract, with intense staining of the duodenal and colonic mucosa. Blocking of endogenous biotin revealed persistent immunoreactivity limited to the duodenal brush border (Fig. 1c). Rare cells in the deepest portions of the colonic crypts were immunoreactive (Fig. 1d); these had a morphology and distribution similar to those of chromogranin-positive cells in serial sections (data not shown), implying a possible neuroendocrine origin.

Table 3 summarizes immunoreactivities identified in the tumors studied. Significant PSMA expression was detectable in 33 of 35 primary prostate tumors. The pattern of staining varied with the degree of differentiation, with the most intense and homogeneous reactivity located at the luminal site of the glands in well-differentiated tumors (Fig. 2a). Immunoreactivity was more heterogeneous in less well-differentiated lesions (Fig. 2b). Considerable heterogeneity of expression within the same tumor was noted in most cases. No immunoreactivity was present in prostatic stromal elements, including blood vessels.

Similarly, seven of eight prostate carcinomas metastatic to lymph nodes expressed detectable PSMA levels (Fig. 2c). In the majority of cases, the staining pattern was reminiscent of that observed in poorly differentiated primary tumors, without any noticeable subcellular orientation. In one case, pseudogland formation was present with intense reactivity at the luminal site. Staining within a metastatic deposit was less heterogeneous than that in the primary tumors, with cells virtually all positive or all negative. Lympoid elements did not exhibit immunoreactivities. The 18 osseous metastases of prostate carcinoma were divided between cases with and without detectable PSMA ex-
Expression of PSMA by a subset of renal tubules cannot be controlled sections utilizing a class-matched primary antibody. PSMA expression in normal tissues, with several distinctions.

DISCUSSION

None of the 17 renal cell carcinomas, 13 bladder transitional cell carcinomas, and 19 colon adenocarcinomas evaluated showed detectable PSMA levels in the tumor cells. Stromal components were similarly negative, except for some blood vessels. Capillary endothelial cell immunoreactivity restricted to the region of the tumor was noted in 8 of 17 renal cell carcinomas (Fig. 3a), in 7 of 13 transitional cell carcinomas (Fig. 3b), and in 3 of 19 colon tumors (Fig. 3, c and d). Capillaries located in normal tissue adjacent to the tumors were not immunoreactive. Considerable heterogeneity of expression was evident, with virtually all peritumor capillaries positive in some cases and only a few capillaries positive in others. Blocking of endogenous biotin did not change this result, and it was not seen in control sections utilizing a class-matched primary antibody.

Table 3 PSMA expression in tumor tissues

<table>
<thead>
<tr>
<th>Carcinoma</th>
<th>No. studied</th>
<th>Tumor cells</th>
<th>Neovascularity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate, primary</td>
<td>35</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>Prostate, metastatic</td>
<td>8</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Lymph node</td>
<td>18</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Bone</td>
<td>17</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Renal cell</td>
<td>13</td>
<td>0</td>
<td>3</td>
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</table>

The present study confirms results from previous analyses with respect to the immunohistochemical detection of PSMA expression in primary and metastatic prostate cancer. Horoszewicz et al. (7) described immunoreactivity in frozen prostate tissues, including nine of nine normal prostates, nine of nine primary prostatic carcinomas, and two of two lymph node metastases. Lopes et al. (8) compared staining patterns of 7E11-C5 and the radionuclide-labeled immunoconjugate CYT-356 in frozen prostate tissues. They noted immunohistochemical...
Fig. 2 PSMA expression in prostatic carcinoma. Intense PSMA immunoreactivity in the glandular epithelium located mainly at the luminal site of a well-differentiated primary tumor (a). More homogeneous cytoplasm and membrane immunostaining of a poorly differentiated primary tumor (b). PSMA expression by tumor cells of lymph node metastasis. Note the absence of staining in lymphoid elements (c). Osseous metastasis showing a heterogeneous pattern of PSMA immunoreactivity (d). a–c, ×200; d, ×400.

Fig. 3 PSMA expression by neovascular capillary endothelial cells in peritumoral areas of selected primary epithelial malignancies. Renal cell carcinoma (a), transitional cell carcinoma of the urinary bladder (b), and colonic adenocarcinoma (c and d). Note the intense immunostaining of endothelial cells, whereas tumor cells had undetectable PSMA levels. a and d, ×400; b and c, ×200.

detection of PSMA by 7E11-C5 in an unspecified number of normal prostates and in 10 primary prostatic carcinomas. Wright et al. (9) found PSMA immunoreactivity in all normal prostates analyzed, 157 of 165 primary tumors, and 72 of 79 lymph node metastases.

With respect to prostate cancer metastatic to bone, Wright et al. (9) noted that all of the seven cases examined expressed PSMA. This is at variance with the current study, in which PSMA expression could be detected in only 8 of 18 osseous metastases (Table 2). This difference may be due to sample size or it may be related to the degree of differentiation and extensive prior treatment (androgen deprivation, radiation, chemotherapy) of the lesions analyzed. It is also possible that some bone metastases express the alternatively spliced form of PSMA (PSMA') lacking the epitope recognized by 7E11-C5. Additionally, although down-regulation of PSMA mRNA expression in response to androgen has been demonstrated in vitro, with the greatest expression noted at castrate levels of androgen (13), PSMA detection in the present study was lowest in the group of patients failing androgen deprivation. These patients represent those with tumor progression to osseous metastases despite hormonal manipulation. These findings support the hypothesis
PSMA expression was not detected in a variety of primary epithelial tumors. The lack of PSMA in renal cell carcinomas is of interest, in view of its expression in a subset of proximal tubules. It is known that renal cell carcinomas, specifically clear cell and granular cell carcinomas, are derived from proximal epithelial cells. The undetectable PSMA levels in the renal cell carcinomas analyzed may be due to the loss of PSMA during malignant transformation. Alternatively, the lack of PSMA in the renal tumors studied may indicate that they are derived from cells not displaying the PSMA-positive phenotype. Similarly, the cells which express PSMA in colonic crypts are of neuroendocrine derivation. Since these cells are not the precursors of colonic adenocarcinomas, the lack of PSMA staining in tumor cells from these neoplasms is not unexpected.

An important finding of the present study is the novel demonstration of PSMA expression by neovascular capillary endothelium in the peritumoral areas of a variety of epithelial malignancies. The significance of this finding in terms of the function of PSMA is presently unclear; however, it may have therapeutic implications. Humanized anti-PSMA antibodies could be used to deliver a variety of agents aimed at destroying neovascularature, ranging from conventional cellular toxins to peptide-based produg activators. Additionally, analysis with RNase protection techniques has demonstrated the presence of PSMA mRNA in both healthy and diseased prostate tissue (13). Further understanding of the PSMA gene’s control mechanisms may be useful in the development of promoter-driven gene therapy for both benign and malignant prostate diseases.

In summary, PSMA appears to be highly expressed in normal prostate tissue as well as primary and nodally metastatic prostate cancer. In the present study, 40% of prostate cancers metastatic to bone expressed PSMA. Examination of normal tissues revealed PSMA expression in prostate epithelium, duodenal mucosa, a subset of renal tubules, and certain neuroendocrine cells in colonic crypts. Carcinomas arising in the bladder, kidney, and colon do not appear to express PSMA. PSMA expression by peritumor capillaries must be examined in other malignancies to establish the range of this phenomenon.

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