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 periluminal 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-

Received 5/22/96; revised 9/12/96; accepted 10/9/96.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported in part by NIH Grant DK/CA 47650, the Koch Foundation, and the CaPCure Foundation. D. A. S. was supported in part by NIH Training Grant CA09501.

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.
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) 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-BSA. Negative controls (Fig. 1a). Blocking of endogenous biotin revealed intense cytoplasmic staining, exhibited identical reaction patterns with class-matched primary antibody substitution and negative controls (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.

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. Lymphoid elements did not exhibit immunoreactivities. The 18 osseous metastases of prostate carcinoma were divided between cases with and without detectable PSMA ex-
regarded as artifactual, since controls in biotin-blocked sections confirm this finding. The identification of rare PSMA-expressing cells in the colonic crypts represents a new finding. The morphology and immunohistochemical characteristics of these cells indicate a neuroendocrine origin. The significance of this finding is not clear, but it parallels the recent report by Carter et al. (3) of a carboxypeptidase involved in central nervous system glutamate metabolism with remarkable homology to PSMA. The finding of PSMA expression in the duodenum is consistent with the previous detection of PSMA mRNA transcripts in small bowel (13) and of PSMA in small bowel protein extracts (14). Additionally, monoclonal antibody 7E11-C5 has recently been shown to precipitate a molecule with folate hydrolase activity from prostate carcinoma cell line extracts (15), a finding which parallels the known high level of folate hydrolase activity in duodenal mucosa. Folate hydrolase is a carboxypeptidase and, like the brain enzyme, liberates glutamate as a reaction product. The possible function of PSMA as a folate hydrolase in the duodenum and in the prostate is currently under investigation. PSMA mRNA transcripts were also identified in central nervous system (13). However, immunohistochemically detectable PSMA expression was not seen in either cerebral cortex or cerebellum in the present study. This may represent expression of the alternatively spliced molecular form of PSMA (PSMA2) lacking the epitope recognized by 7E11-C5 or expression at a specific brainstem or ganglionic locus not analyzed.

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...
that the interaction of tumor with the metastatic site has an effect on tumor phenotype (16).

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 neovascularure, ranging from conventional cellular toxins to peptide-based prodrg 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 peritumoral capillaries must be examined in other malignancies to establish the range of this phenomenon.

ACKNOWLEDGMENTS

The Memorial Sloan-Kettering Cancer Center is an NIH-designated George M. O’Brien Urology Research Center.

REFERENCES


Prostate-specific membrane antigen expression in normal and malignant human tissues.

D A Silver, I Pellicer, W R Fair, et al.


Updated version  Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/3/1/81

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.