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Focal positive prostate-specific membrane antigen (PSMA) expression in ganglionic tissues associated with prostate neurovascular bundle: Implications for novel intraoperative PSMA-based fluorescent imaging techniques[☆]

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Abstract

Objective—Prostate specific membrane antigen (PSMA) is primarily expressed in glandular prostatic tissue and is frequently utilized to detect primary or metastatic prostatic adenocarcinoma (CaP). A purported novel application of PSMA detection is the intraoperative real-time identification of CaP using radioimmunosintigraphy to define the extension of the surgical resection. Considering that PSMA expression has been reported in other tissues, we evaluated its immunoexpression in prostatic neurovascular bundle elements to assess the convenience and safety of the aforementioned procedure.

Materials and methods—Twenty consecutive specimens of radical prostatectomy (RP) were retrieved from our surgical pathology archives. PSMA immunoexpression (Clone 3E6, DAKO) was assessed in a representative section from each specimen containing neurovascular bundle elements.

Results—PSMA expression was documented in 20/20 of examined CaP slides. Most cases exhibited an apical/cytoplasmic or cytoplasmic with membranous accentuation pattern of staining. Focal weak to moderate cytoplasmic staining was detected in associated ganglionic tissue in 3/15 of the examined RP. In all cases, staining was cytoplasmic, less extensive, and weaker than the pattern observed in CaP. None of the peripheral nerve sheath cells or lymphovascular components of the examined neurovascular bundles were positive for PSMA.

Conclusions—We found focal positive PSMA expression in the ganglionic cells of the prostatic neurovascular bundle. Our results suggest that the radioimmunosintigraphic detection of radiolabeled PSMA antibodies might not be entirely specific for prostatic cells; this observation

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must be taken into account should an intraoperative PSMA-based fluorescent imaging technique be used to define the extension of the surgical procedure.

Keywords

Prostate specific membrane antigen (PSMA); Prostate carcinoma; Radioimmunoscinigraphy; Radiolabeled antibodies; Fluorescent imaging technique; Radical prostatectomy

1. Introduction

Prostate specific membrane antigen (PSMA) is a trans-membrane glycoprotein that is primarily expressed in glandular prostate tissue. Besides its application in the differential diagnosis of purported prostatic tumors using tumor tissue samples, PSMA is frequently utilized to detect prostatic adenocarcinoma (CaP) recurrences or metastasis to lymph nodes, soft tissues, or bone using radioimmunoscinigraphic detection of radiolabeled PSMA antibodies [1–4]; in addition, considering the putative advantages of a PSMA-targeted therapy in hormone-resistant and disseminated CaP [2,5–9], there is a strong interest in defining the tissue lineage specificity of PSMA expression. A real time intra-operative imaging technique using a newly developed low molecular weight urea-based compound that binds to PSMA and fluoresces in the NIR spectrum is being pursued at our institution. Such intraoperative techniques could help decrease the rate of positive surgical margins at radical prostatectomy (RP). In the current study, we evaluated PSMA expression in neurovascular bundle elements including peripheral nerve, ganglion, and lymphovascular tissue in order to assess the feasibility of the above intraoperative technology.

2. Materials and methods

Twenty consecutive RP specimens were retrieved from our surgical pathology archives. PSMA immunoexpression was assessed in a representative section from each specimen containing neurovascular bundle elements using a PSMA monoclonal antibody (Clone 3E6, DAKO, Carpinteria, CA). PSMA expression was evaluated by 2 pathologists. An intensity (1: weak; 2: moderate; 3: strong) and extent (1: <25%; 2: 25%–75%; 3: >75%) score was assigned in each RP for available CaP, neurovascular bundle elements, and associated ganglion tissue.

3. Results

As expected, PSMA expression was documented in 100% (20/20) of examined CaP tumors. Most cases exhibited an apical/cytoplasmic or cytoplasmic with membranous accentuation pattern of staining (Fig. 1A). Focal weak to moderate cytoplasmic staining was detected in associated ganglionic tissue in 3/15 (20%) of the examined RP (Fig. 1B). In all cases, staining was cytoplasmic, less extensive, and weaker than the pattern observed in CaP. None of the peripheral nerve sheath cells or lymphovascular components of the examined neurovascular bundles were positive for PSMA (1C and D). Data is summarized in Table 1.

4. Discussion

PSMA, a type II transmembrane protein with folate hydrolase and neuropeptidase functions, has a high specificity for benign and malignant prostatic tissue [4]. The reported immunohistochemical positivity of PSMA in CaP ranges from 57% to 100% [6,10] with most positive cases showing strong cytoplasmic staining. Various patterns of PSMA immunoexpression have been described, including apical, apical/cytoplasmic, cytoplasmic with membranous accentuation, and cytoplasmic only. The first 3 patterns of staining are more specific for CaP [4]. In addition, the vast majority of metastatic CaP maintain strong PSMA immunoexpression [4,6]. However, despite some early claims, PSMA expression is also observed in a variety of tumor types besides those arising from the prostatic glandular epithelium. In a recent tissue microarray (TMA)-based study of 2,174 malignant tumors, Mhawech-Fauceglia et al. found PSMA expression in 5.4% of non-CaP tissue samples [4], including squamous cell carcinomas (8.7%), adenocarcinomas (15.5%), and isolated cases of small cell carcinoma of lung, thyroid carcinoma, papillary renal cell carcinoma, hepatocellular carcinoma, cutaneous basal cell carcinoma, lymphoepithelioma of larynx, sarcomatoid carcinoma of bladder, malignant mesothelioma, and glioblastoma multiforme. Interestingly, 17% of bladder urothelial carcinomas were PSMA positive. In all non-CaP positive cases, PSMA staining in tumor cells exhibited an exclusive cytoplasmic pattern. Other previous studies also found PSMA immuno-expression in some malignant tumors, including positivity in urothelial carcinomas [11,12]. Mhawech-Fauceglia *et al.* also investigated 846 premalignant and benign tumors and found no PSMA expression in any of these lesions [4]. However, in a recently published study, we found cytoplasmic PSMA staining in 11/11 schwannomas from different anatomical sites [13]; the striking discordances between our and Mhawech-Fauceglia's *et al.* series might be related to the differences in the anti-PSMA antibodies used. In the same study, we also identified PSMA positivity in the endothelium of intralesional neovessels, an observation that is consistent with previous studies [4].

Cytoplasmic PSMA expression has also been detected in normal tissues, including endometrial glands, breast epithelium, kidney tubules, urothelium, glial cells, pancreatic islets and ducts, epithelium of the gastrointestinal tract (esophagus, stomach, small intestine, and colon), skeletal muscle, testis, ovary, fallopian tube, adrenal glands, and liver [4,11,14,15]. PSMA positivity in ganglionic cells of the gastrointestinal tract as well as negativity in nerve and endothelial cells have been reported previously, in agreement with our current results [4,11].

Besides its application in diagnostic pathology, PSMA detection has several potential clinical uses, including the real-time evaluation of surgical margins during RP. Considering that up to one-third of all CaP patients treated by RP may have positive surgical margins (PSM) [16] and taking into account that these are more likely to experience recurrence, undergo radiation therapy, and die of disseminated disease [17,18], the development of methods to intraoperatively detect PSM and locoregional tumor spread is of vital importance to improve the surgical management of individuals with prostate cancer. Several monoclonal antibodies have been designed to target PSMA [9] and they have showed promising results for PET imaging of metastatic prostate cancer [5]. Recently, a urea-based agent (YC-27),

conjugated to a carbocyanine NIR fluorophore (IRDye 800CW; LI-COR Biosciences, Lincoln, NE), was able to identify an in vivo PSMA-positive prostate cancer cell line (PC3-PIP) but not a PSMA-negative cell line (PC3-Flu) after intravenous injection in a nude mouse [19]. This promising technique is currently under evaluation in our institution using animal models. In this regard, the recognition of the PSMA patterns of immunoexpression could offer some clues on both the clinical usefulness and limitations of this technique.

In summary, we found focal positive PSMA expression in the ganglionic cells of the prostatic neurovascular bundle. In all cases, immunoexpression was cytoplasmic, focal, and less intense compared with PSMA staining in prostatic tumor cells. Our results suggest that the radioimmunoscentigraphic detection of radiolabeled PSMA antibodies might not be entirely specific for prostatic cells, and this observation must be taken into account should this intraoperative technique be used to define the extension of the surgical procedure.

References

1. Bander NH. Technology insight: Monoclonal antibody imaging of prostate cancer. *Nat Clin Pract Urol.* 2006; 3:216–25. [PubMed: 16607370]
2. Foss CA, Mease RC, Fan H, et al. Radiolabeled small-molecule ligands for prostate-specific membrane antigen: In vivo imaging in experimental models of prostate cancer. *Clin Cancer Res.* 2005; 11:4022–8. [PubMed: 15930336]
3. Karam JA, Mason RP, Koeneman KS, et al. Molecular imaging in prostate cancer. *J Cell Biochem.* 2003; 90:473–83. [PubMed: 14523981]
4. Mhawech-Fauceglia P, Zhang S, Terracciano L, et al. Prostate-specific membrane antigen (PSMA) protein expression in normal and neoplastic tissues and its sensitivity and specificity in prostate adenocarcinoma: An immunohistochemical study using multiple tumor tissue microarray technique. *Histopathology.* 2007; 50:472–83. [PubMed: 17448023]
5. Bander NH, Milowsky MI, Nanus DM, et al. Phase I trial of ¹⁷⁷lutetium-labeled J591, a monoclonal antibody to prostate-specific membrane antigen, in patients with androgen-independent prostate cancer. *J Clin Oncol.* 2005; 23:4591–601. [PubMed: 15837970]
6. Elsasser-Beile U, Buhler P, Wolf P, et al. Targeted therapies for prostate cancer against the prostate specific membrane antigen. *Curr Drug Targets.* 2009; 10:118–25. [PubMed: 19199907]
7. Henry MD, Wen S, Silva MD, et al. A prostate-specific membrane antigen-targeted monoclonal antibody-chemotherapeutic conjugate designed for the treatment of prostate cancer. *Cancer Res.* 2004; 64:7995–8001. [PubMed: 15520207]
8. Olson WC, Heston WD, Rajasekaran AK. Clinical trials of cancer therapies targeting prostate-specific membrane antigen. *Rev Recent Clin Trials.* 2007; 2:182–90. [PubMed: 18474004]
9. Tagawa ST, Beltran H, Vallabhajosula S, et al. Anti-prostate-specific membrane antigen-based radioimmunotherapy for prostate cancer. *Cancer.* 2010; 116:1075–83. [PubMed: 20127956]
10. Marchal C, Redondo M, Padilla M, et al. Expression of prostate specific membrane antigen (PSMA) in prostatic adenocarcinoma and prostatic intraepithelial neoplasia. *Histol Histopathol.* 2004; 19:715–8. [PubMed: 15168332]
11. Chang SS, Reuter VE, Heston WD, et al. Five different anti-prostate-specific membrane antigen (PSMA) antibodies confirm PSMA expression in tumor-associated neovasculature. *Cancer Res.* 1999; 59:3192–8. [PubMed: 10397265]
12. Gala JL, Loric S, Guiot Y, et al. Expression of prostate-specific membrane antigen in transitional cell carcinoma of the bladder: Prognostic value? *Clin Cancer Res.* 2000; 6:4049–54. [PubMed: 11051255]
13. Wang W, Tavora F, Sharma R, et al. PSMA expression in Schwannoma: A potential clinical mimicker of metastatic prostate carcinoma. *Urol Oncol.* 2009; 27:525–8. [PubMed: 18534872]

14. Kinoshita Y, Kuratsukuri K, Landas S, et al. Expression of prostate-specific membrane antigen in normal and malignant human tissues. *World J Surg.* 2006; 30:628–36. [PubMed: 16555021]
15. Silver DA, Pellicer I, Fair WR, et al. Prostate-specific membrane antigen expression in normal and malignant human tissues. *Clin Cancer Res.* 1997; 3:81–5. [PubMed: 9815541]
16. Campbell, MF.; Wein, AJ.; Kavoussi, LR. *Campbell-Walsh Urology.* Philadelphia, PA: Saunders-Elsevier; 2007.
17. Han M, Partin AW, Pound CR, et al. Long-term biochemical disease-free and cancer-specific survival following anatomic radical retropubic prostatectomy. The 15-year Johns Hopkins experience. *Urol Clin North Am.* 2001; 28:555–65. [PubMed: 11590814]
18. Wright JL, Dalkin BL, True LD, et al. Positive surgical margins at radical prostatectomy predict prostate cancer specific mortality. *J Urol.* 2010; 183:2213–8. [PubMed: 20399459]
19. Chen Y, Dhara S, Banerjee SR, et al. A low molecular weight PSMA-based fluorescent imaging agent for cancer. *Biochem Biophys Res Commun.* 2009; 390:624–9. [PubMed: 19818734]

Fig. 1. Patterns of PSMA expression. (A) Prostatic adenocarcinoma, Gleason score 3+4=7, with strong and diffuse PSMA immunoexpression in tumor cells. (B) Ganglionic cells showing focal, weak cytoplasmic PSMA expression. (C) Peripheral nerve sheath cells negative for PSMA expression; note the ganglionic cell (arrow) with weak cytoplasmic PSMA positivity. (D) Vascular element negative for PSMA expression. (Color version of figure is available online.)

Table 1
Patterns of PSMA immunoeexpression in prostatic adenocarcinoma (CaP) and neurovascular bundle elements

Tissue	PSMA+ (%)	PSMA Intensity (%)			PSMA Extent (%)		
		1	2	3	1	2	3
CaP	11/11 (100)	5 (45.4)	0 (0)	6 (54.5)	2 (18)	2 (18)	7 (64)
Nerve sheath cells	0/19 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Ganglion cells	3/15 (20)	2 (67)	1 (33)	0 (0)	3 (100)	0 (0)	0 (0)
Vessels	0/19 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)