Salivary Duct Carcinoma Secreting Prostate-Specific Antigen

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Prostate-specific antigen (PSA) is a 30 kDa glycoprotein serine protease that shows high tissue specificity for prostatic tissue, both benign and malignant. However, recent reports have shown that a variety of normal and neoplastic tissue types express PSA immunohistochemically. In addition, rare instances of the secretion of PSA by nonprostatic cancers have been reported in the literature. The authors present a case of salivary duct carcinoma associated with elevated serum levels of PSA. Both the primary tumor and metastases stained positively with anti-PSA monoclonal antibodies, but were negative with antibodies directed against prostate-specific acid phosphatase. Elevated serum PSA levels were confirmed with three different immunoassay methods. A peak serum level of 140 μg/L was measured and this correlates with levels of PSA associated with metastatic prostatic carcinoma. High performance liquid chromatography with a molecular sieve column characterized the serum PSA into both free protein (approximately 20%) and protein bound to α-1-antichymotrypsin (PSA-ACP) (approximately 80%). Molecular weights of the free PSA and PSA-ACP subfractions were 27–31 kDa and 100–110 kDa, respectively. (Key words: Salivary duct carcinoma; Prostate-specific antigen; Tumor marker) Am J Clin Pathol 1996; 106:242–247.

Prostate-specific antigen (PSA) is a single chain glycoprotein with a molecular weight of 30 kDa and is a member of the kallikrein family of proteases. Prostate-specific antigen is produced by the epithelial cells lining the acini and ducts of the prostate gland. It is also found in benign hyperplastic prostatic epithelium and prostatic adenocarcinoma.

Prostate-specific antigen is recognized as a very useful tumor marker because of its high tissue specificity. However, many groups have reported immunoreactivity for PSA in a variety of normal and neoplastic tissue types. These include rare apocrine sweat gland carcinomas, apocrine breast carcinomas, salivary gland neoplasms, colon carcinomas, pancreatic acinar cell carcinoma, primary ovarian carcinoma, adenocarcinoma of Skene’s (paraurethral) gland, biliary tract carcinomas, and bladder carcinomas. Moreover, PSA has been immunohistochemically demonstrated in normal axillary and perineal apocrine sweat glands, in addition to the paraurethral and perianal glands.

Serum PSA levels are routinely used to monitor patients with prostate cancer and facilitate the diagnosis of the latter. However, several recent reports have described elevation in serum PSA in a variety of nonprostatic malignancies.

We present a patient with salivary duct carcinoma metastatic to bone that is associated with marked elevation in serum PSA.

CASE REPORT

A 49-year-old white man presented with a right submandibular mass and multiple, enlarged cervical lymph nodes in January 1993. Five months later, the patient underwent a right modified neck dissection for a preoperative diagnosis of metastatic carcinoma of the right neck. The right submandibular mass was diagnosed as a salivary duct carcinoma involving the submandibular gland and adjacent adipose tissue with 10 of 13 regional lymph nodes positive for metastatic carcinoma. A bone scan revealed multiple bony metastases in the vertebral column.

Due to the unusual presentation of a salivary duct adenocarcinoma with bony metastases the possibility of metastatic prostatic carcinoma was considered. Despite a PSA level of 60 μg/L in June 1993, rectal examination, prostatic ultrasound, and a six quadrant biopsy of the prostate gland were negative for malignancy.

Subsequently, adjuvant 5-fluorouracil and levamisole chemotherapy was administered from July 1993 to January 1994 for back pain and neurologic symptoms related to the bony metastases. Figure 1 shows the serum PSA level during the patient’s clinical course. In March 1994 the serum PSA level sharply rose to 140 μg/L and acute spinal cord compression (T11-T12 level) and paraplegia developed in the following month.

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Salivary Carcinoma Secreting PSA

Due to the sustained elevation in the PSA level (Fig. 1), a trial of hormonal therapy with diethylstilbestrol and cyproterone was administered in May 1994.

The patient was admitted to Vancouver Hospital and Health Sciences Centre in June 1994, complaining of dyspnea, cough, and fever. Sputum culture was positive for Aspergillus and bronchoscopy showed invasive pulmonary aspergillosis. Despite initiation of amphotericin B therapy, he died 23 days after admission.

An autopsy was performed. The patient's cause of death was attributed to angio-invasive pulmonary aspergillosis secondary to chemotherapeutic immunosuppression for metastatic carcinoma. Multiple bony metastases to the vertebra, pelvis, ribs, clavicles, and the humerus were present. There was no evidence of residual or recurrent carcinoma in the neck or metastases to other organs. The prostate gland was grossly unremarkable and microscopic examination of the entire gland showed no evidence of carcinoma or prostatic intraepithelial neoplasia.

**MATERIALS AND METHODS**

**Pathology**

Tissue from the initial right radical neck dissection and the autopsy were available for review. Representative tissue sections were formalin-fixed, processed routinely, and embedded in paraffin. All tissues were stained with hematoxylin and eosin, and examined by light microscopy. Additional immunoperoxidase stains were performed on the representative sections from the right radical neck dissection and the autopsy.

**Immunohistochemistry**

Representative sections of the submandibular gland tumor, cervical lymph nodes, and bony metastasis from the autopsy were stained using the avidin-biotin complex technique with the following antibodies: S-100 (monoclonal 1:100, Dako, Carpinteria, CA), muscle specific actin (MSA) (monoclonal 1:100, Dako), B72.3 (monoclonal 1:1000, Signet, Dedham, MA), Keratin (KER) (polyclonal 1:4000, Dako), carcinoembryonic antigen (CEA) (monoclonal 1:200, Dako), epithelial membrane antigen (EMA) (monoclonal 1:200, Dako), gross cystic disease fluid protein (GCDFP) (monoclonal 1:100, Signet), prostate-specific acid phosphatase (PSAP) (monoclonal 1:400, Dako), and PSA (monoclonal 1:1000, Incstar, Stillwater, MN).

**Serum Analysis**

Intermittent samples of the patient’s serum were analyzed from June 24, 1993 to May 17, 1994, inclusive, using the IMx system (Abbott Laboratories, Abbott Park, IL). This system uses a mouse, monoclonal capture anti-PSA Ab and a goat, polyclonal signal anti-PSA Ab. Multiple serum specimens were also analyzed using the Hybritech (San Diego, CA) Tandem PSA kit which is an immunoradiometric assay employing two monoclonal antibodies.

A serum sample drawn from the patient on July 27, 1993 was analyzed with the ultrasensitive time-resolved immunofluorometric assay (TR-IFA). Time-resolved immunofluorometric assay uses mouse monoclonal (MBPO405) capture and rabbit polyclonal (PBP0101) signal anti-PSA antibodies (Medix Biotech, Foster City, CA). Molecular size characterization analysis of this serum sample was performed. One hundred μL of this serum was fractionated and separated with high performance liquid chromatography by using a Bio-Sil SEC-250 molecular sieve column, 600 × 7.5 mm (Bio-Rad Labs, Richmond, CA), a Model FRAC-100 fraction collector (Pharmacia, Uppsala, Sweden), and was analyzed with a Shimadzu system (Shimadzu, Kyoto, Japan) as previously described. Fractions were analyzed for PSA using the TR-IFA.

**RESULTS**

**Pathologic Findings**

The right modified radical neck dissection specimen contained a 1.5 cm diameter, firm, ill-defined, white
mass arising in the right submandibular gland, which extended into the surrounding adipose tissue. Several enlarged lymph nodes ranging from 0.5 to 2.0 cm in diameter, including a coalesced mass of lymph nodes measuring $2 \times 1.5 \times 1.5$ cm, surrounded the mass.

Histologic examination of the submandibular gland mass (Fig. 2) and 10 of 13 upper jugular nodes showed poorly differentiated adenocarcinoma. Focal cribriform areas with central necrosis and extensive perineural invasion were present. The tumor cells had abundant pale eosinophilic cytoplasm and large nuclei with prominent nucleoli. These histologic features are characteristic of salivary duct carcinoma.  

The bony metastases from the autopsy contained metastatic carcinoma of the same histologic pattern as the adenocarcinoma of the submandibular gland.

**Immunohistochemical Findings**

Immunoperoxidase staining of the primary tumor (Fig. 3) and bony metastasis with anti-PSA showed strong cytoplasmic immunoreactivity. Tumor cells were also strongly positive for B72.3, EMA, and KER. Focal positivity was present for GCDFP, whereas PSAP, S-100, CEA, and MSA were negative.

**Serum Analysis**

Intermittent samples of the patient’s serum were analyzed from June 24, 1993 to May 17, 1994, inclusive, using the Abbott IMX system. The patient’s PSA level profile as measured by the IMX method is illustrated in Figure 1. Using this method a maximum level of serum PSA was measured at 140$\mu$g/L on March 22, 1994. Table 1 illustrates the elevated PSA levels obtained on multiple, previously frozen serum samples analyzed with the Hybritech Tandem PSA kit and these are compared to results obtained with the Abbott IMX system.

Ultrasensitive time-resolved immunofluorometric assay (TR-IFA) performed on the serum sample drawn

![Fig. 2. A. Salivary duct carcinoma exhibiting solid and cribriform architecture with perineural invasion (hematoxylin and eosin stain, ×40); B. Focal central (comedo) necrosis (arrow) (hematoxylin and eosin stain, ×125).](image)

![Fig. 3. Strong cytoplasmic PSA immunoreactivity in salivary duct carcinoma. Note perineural invasion (arrow) (peroxidase-conjugated streptavidin method, hematoxylin counterstained, ×300).](image)
TABLE 1. SERUM PROSTATE SPECIFIC ANTIGEN LEVELS AS DETERMINED BY IMX (ABBOTT) AND TANDEM PSA KIT (HYBRITEX) ASSAYS

<table>
<thead>
<tr>
<th>Sample Date</th>
<th>IMx (Abbott)</th>
<th>Tandem PSA kit* (Hybritex)</th>
</tr>
</thead>
<tbody>
<tr>
<td>June 1993</td>
<td>60</td>
<td>44.2</td>
</tr>
<tr>
<td>August 1993</td>
<td>50</td>
<td>41.8</td>
</tr>
<tr>
<td>December 1993</td>
<td>41</td>
<td>24.1</td>
</tr>
<tr>
<td>January 1994</td>
<td>54</td>
<td>28.3</td>
</tr>
<tr>
<td>March 1994</td>
<td>140</td>
<td>82.4</td>
</tr>
</tbody>
</table>

* PSA levels were measured on previously frozen serum samples on September 24, 1995.

from the patient July 27, 1993, revealed a PSA content of approximately 90 µg/L. This correlates with the IMX system measurement of the identical sample. Molecular size characterization analysis of PSA activity in this serum sample was performed. One hundred µL of this serum was fractionated and separated with high performance liquid chromatography using a molecular sieve column as previously described. The upper panel of Figure 4 shows major peaks eluting from the gel filtration column. Approximately 80% of the PSA immunoreactivity eluted with the first peak at an estimated molecular mass of 100–110 kDa. This corresponds to the molecular mass of PSA bound to α-1-antichymotrypsin (ACT). The remaining PSA immunoreactivity eluted in the second peak with an estimated molecular mass of 27–31 kDa which corresponds to the molecular weight of free PSA. Two previously described immunoreactive peaks of unknown identity eluted at fraction 20 (approximately 700 KDa) and fraction 25 (approximately 400 KDa) and these are illustrated in the lower panel of Figure 4.

DISCUSSION

Serum PSA levels are very useful as an adjuvant diagnostic tumor marker and for monitoring patients with prostatic adenocarcinoma. It is highly specific for prostatic tissue. However, numerous reports have found PSA immunoreactivity using polyclonal antibodies in a variety of nonprostatic normal and neoplastic tissue types. In addition, the occurrence of PSA positive prostate tissue in cystic teratomas of the ovaries have been described. However, no cases of nonprostate cancers immunoreactive with a monoclonal anti-PSA antibody have been reported.

The only reported cases of nonprostatic tumors associated with elevated levels of serum PSA are extrapulmonary small cell carcinoma, renal cell carcinoma, pulmonary adenocarcinoma, and adenocarcinoma of Skene’s (pararethral) gland. The extrapulmonary small cell carcinoma described by Freeman and Doolittle was negative for PSA and PAP by immunoperoxidase. They speculated that the serum PSA level of 278 µg/L was tumor related, either by the tumor cells secreting PSA or through tumor induction of PSA secretion by normal prostatic tissue. The pulmonary adenocarci-

FIG. 4. High performance liquid chromatography (HPLC) of a serum sample from a patient with salivary gland neoplasm. After injecting 100 µL of serum, fractions were collected and analyzed for PSA. The PSA-α-1-antichymotrypsin complex (ACT-PSA) elutes at fraction 29/−1 and free PSA at fractions 37/−1. The HPLC column was calibrated with a molecular weight standard solution eluting at fraction 20 (660 KDa), 27 (160 KDa), 33 (44 KDa), 39 (17 KDa) and 48 (1.4 KDa). Flow rate was 0.5 mL/min. In the lower panel the y-axis was expanded to reveal the presence of the other two immunoreactive peaks (arrows) at fraction 20 (approximately 700 KDa) and 25 (approximately 400 KDa) of unknown identity. These peaks were also previously described.
noma reported by Bilgami and colleagues was positive for PSA by immunohistochemistry using a polyclonal Ab, but negative with a monoclonal Ab against PSA. The serum level in this case was 26.3 μg/L and was confirmed with a murine monoclonal antibody assay (Tandem-ESPA, Hybritech).

Using only a polyclonal assay system for the assessment of PSA, serum levels can lead to erroneous high PSA levels. This was thought to be due to cross reactivity with proteins, such as ACT, that are known to form complexes with PSA. For example, Pummer and colleagues found 6 of 22 cases of females with renal cell carcinoma with measurable serum PSA using a polyclonal antibody assay system. Four of these six cases had PSA levels greater than 3.9 μg/L. On repeating the PSA measurement with a monoclonal assay on three patients, no PSA was demonstrated. The authors concluded that a substance secreted by the tumor was cross reacting with their polyclonal assay.

Paranephral (Skene’s) glands are considered the female homologue of the prostate gland. They are lined by a pseudostratified mucous-secreting epithelium that shows PSA immunoreactivity. The homology to prostate has led some investigators to evaluate expression of PSA in paranephral gland adenocarcinomas. In both tumors studied, cytoplasmic immunoreactivity for PSA was demonstrated. Serum PSA was measured in only one patient and a modest elevation to 5.9 μg/L was found.

Immunoreactivity for PSA has been previously reported in salivary gland neoplasms. Using a polyclonal antibody, van Krieken found PSA immunoreactivity in a variety of both benign and malignant salivary gland tumors, including all three cases of adenocarcinoma, not otherwise specified (NOS). None of the tumors in this series was identified as a salivary duct carcinoma. However, prostate-specific antigen immunoreactivity was demonstrated along the luminal aspect of the duct epithelial cells in normal salivary gland. In a separate study of salivary duct carcinomas, PSA was negative in 4 of 4 tumors. These findings suggest that PSA may be expressed by a minority of salivary duct carcinomas. Alternatively, the discrepancy between the latter study and the present case may be related to the fact that antibodies from different manufacturers were used.

Serum PSA elevation has not been previously reported in salivary gland carcinomas. Serum PSA was measured in one patient whose tumor demonstrated PSA immunoreactivity in the series of van Krieken and was found to be normal. This patient was treated with an antiestosterone with apparent tumor regression. Elevation in serum PSA in the case under discussion was confirmed on multiple serum specimens with three different immunoassays, including a murine monoclonal assay. Further analysis of the patient’s serum sample via ultrasensitive time-resolved immunofluorometric assay and high-performance liquid chromatography confirmed the presence of both PSA-ACT and free serum PSA subfractions with molecular mass identical to that seen in prostatic cancer. The proportion of free and PSA-ACT (20% and 80%, respectively) was also similar to that seen in patients with prostatic malignancy.

This case provides unequivocal evidence that PSA can be expressed and secreted by nonprostatic tumors at serum levels equivalent to those seen with metastatic prostate carcinoma. Although ectopic production of PSA is rare, it should be considered as a diagnostic possibility in patients with elevated serum levels of serum PSA in the absence of prostatic pathology.

REFERENCES


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