Journal of Clinical Laboratory Analysis 9:375–379 (1995)

Immunoreactive Prostate-Specific Antigen in Lung Tumors

Michael Levesque,[1,2] He Yu,[1,2] Mario D'Costa,[2,3] Latif Tadross,[3] and Eleftherios P. Diamandis[1,2]

[1]Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, [2]Department of Clinical Biochemistry, University of Toronto, and [3]Department of Laboratory Medicine, St. Joseph's Health Centre, Toronto, Ontario, Canada

Prostate-specific antigen (PSA) is a glycoprotein produced by the epithelial cells of the prostate. PSA is currently used clinically to diagnose and monitor prostate carcinoma. In previous work we have demonstrated that 30% of breast tumors and, more rarely other tumors, contain significant amounts of PSA. PSA appears to be a favorable prognostic indicator in breast cancer. Here, using a sensitive assay, we demonstrated for the first time that lung adenocarcinomas and squamous cell carcinomas also contain PSA. PSA in lung tumor extracts was present mainly in its 33 KDa form (free PSA), at levels measurable by commercial methods. The presence of PSA was associated more closely with male patients and adenocarcinomas. The physiological role of PSA in lung tissue and the prognostic significance of PSA in lung cancer remain to be determined. These and our previous data as well as reports by other groups support the view that PSA is a ubiquitous biochemical marker of steroid hormone action.

Key words: prostate specific antigen, cancer, growth factors, serine proteases, tumor markers, lung cancer

INTRODUCTION

Prostate-specific antigen (PSA) is a 33 KDa serine protease present at high levels in semen. It was originally thought that this protein is secreted exclusively by the epithelial cells of the prostate gland (1). However, more recently it has been demonstrated that PSA could also be found in the periurethral and perianal glands and very rarely in tumors of the salivary glands (2). We found that PSA is present in 30% of female breast tumors and that its production in these tumors is associated with the presence of steroid hormone receptors (3,4). We observed a significant advantage in both disease-free and overall survival of breast cancer patients whose tumors are PSA positive (unpublished data). In addition, we were able to reproduce the phenomenon of PSA production by breast tumors using breast cancer cell lines stimulated by various steroid hormones. We have further demonstrated that the normal breast can also produce PSA and secrete it into the milk during lactation, post-pregnancy. Recently, we also found that some tumors of the colon, liver, parotid, adrenal, and ovary contain small amounts of immunoreactive PSA. These data have recently been reviewed (5).

This work examines lung tumor extracts for the presence of immunoreactive PSA. We studied 57 lung tumor extracts from 53 patients (four patients had two samples each). In addition, we studied one patient with primary prostate cancer which had metastasized to the lung. For PSA analysis we used a highly sensitive and specific procedure described in detail elsewhere (6). Our data suggest that immunoreactive PSA is present in a significant proportion of lung tumor extracts.

MATERIALS AND METHODS

Tumor Specimens and Extraction Procedure

The primary and metastatic lung tumors used in this study were collected at St. Joseph's Health Centre, Toronto, Canada. Diagnosis of lung cancer was established by pathological examination in all cases. The tumor tissue was immediately stored in liquid nitrogen after surgical resection, transported to the laboratory, and subsequently stored at –80°C until extraction was performed (up to 8 months). Lung tumors were extracted and analyzed for PSA as previously described (3,6).

The PSA content of each tumor was expressed as nanograms of PSA per milligram of total protein. High-performance liquid chromatography (HPLC) analysis was also performed as described previously (6).

Received April 12, 1995; accepted April 14, 1995.

Address reprint requests to Dr. E.P. Diamandis, Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, 600 University Avenue, Toronto, Ontario M5G 1X5, Canada.

Statistical Analysis

The chi-square test was used to determine the statistical significance of differences in distributions and all chi-square values and the corresponding *P* values were calculated by the statistical software SAS (SAS Institute Inc., Cary, NC). When the number of patients in one or more categories was too small we compared the categories with the Fisher's exact test (two-tailed).

RESULTS

The distribution of PSA concentration in the 57 lung tumor extracts was as follows: 26 of 57 extracts (46%) had PSA <0.0050 ng/mg; 31 of 57 extracts (54%) had PSA ≥0.0050 ng/mg; 14 of 57 extracts (25%) had PSA ≥0.010 ng/mg; 10 of 57 extracts (18%) had PSA ≥0.020 ng/mg, and 6 of 57 extracts (11%) had PSA ≥0.030 ng/mg. The latter cutoff was used to classify 30% of breast tumors as positive for PSA (3,4). One lung tumor metastatic from a prostate primary lesion contained about 4,000 ng of PSA per milligram of total protein. Four lung tumor extracts were also analyzed by a commercial PSA assay (IMx, Abbott Laboratories, Abbott Park, IL). The results by our method were (in μg/L) 0.32, 0.38, 2.7, and 4.7 and by the IMx were 0.40, 0.50, 3.1, and 4.9, respectively.

In order to further strengthen our proposal that lung tumors have the ability to produce PSA, we have established the molecular weight of the immunoreactive species measured by our ultrasensitive assay, using HPLC as previously described (6). For this experiment, we examined two lung tumor extracts with a PSA concentration of 0.85 and 4.7 μg/L, respectively, and one lymph node extract with a PSA concentration of 2.65 μg/L from a patient with primary lung cancer metastatic to the lymph nodes. The results are shown in Figure 1. The extract from the patient with metastatic lung cancer from a prostate cancer primary lesion was also analyzed for comparison. This latter specimen gave two immunoreactive peaks: at fractions 30–31, corresponding to a molecular weight of ~100 KDa and at fractions 40–41, corresponding to a molecular weight of ~33 KDa (data not shown). The peak with a molecular weight of ~100 KDa corresponds to PSA bound to α_1-antichymotrypsin (PSA-ACT). This was confirmed with an assay that measures specifically the PSA-ACT complex (6). This peak represented less than 2% of total PSA; the peak with a molecular weight of ~33 KDa corresponds to free PSA and represented more than 98% of total PSA (data not shown).

The primary lung tumor extract shown in Figure 1A contains a major peak with a molecular weight of ~33 KDa (free PSA) and a minor peak with a molecular weight of ~100 KDa (PSA-ACT) present at levels <1% of total PSA (Fig. 1B). Similar results were seen for the second primary lung tumor extract (Fig. 1C,D) and the lymph node extract from the patient with primary lung cancer metastatic to the lymph nodes (Fig. 1E,F).

In Figure 1 another weak immunoreactive peak around fractions 20–21, corresponding to molecular weights of 500–800 KDa, was also seen. The nature of this peak is unknown. However, in previous studies with prostate cancer patient sera, we have seen a similar peak and speculated that it may represent PSA bound to α_2-macroglobulin (A2M) (6). This PSA complex is weakly recognized by our assay. If this peak indeed represents PSA-A2M, its actual concentration may be much higher than the concentration shown in the graph.

We further examined if the presence of PSA in lung tumors is associated with patient sex. The results are shown in Table 1. At any cutoff level, males tended to have higher PSA levels. The association between PSA presence in lung tumors and histological type is also shown in Table 1. At all cutoff levels, there were more PSA-positive samples with a diagnosis of adenocarcinoma than squamous cell carcinoma although the difference was not statistically significant. Such differences were more pronounced at higher PSA cutoff levels.

DISCUSSION

We recently reported the presence of PSA in 30–40% of female breast tumors (3) and demonstrated that such an expression is mediated by steroid hormone receptors (4). We have also shown that PSA is produced by metastatic breast tumors and by the normal breast during pregnancy. PSA is present in the milk and serum of lactating women. It appears that the presence of PSA in breast tumors is associated with improved patient disease-free and overall survival (5).

We recently examined if other tumors could also produce PSA. In a study involving 43 tumors of 11 different tissues, we have demonstrated that at least some ovarian, liver, kidney, adrenal, colon, and parotid tumors contain small amounts of immunoreactive PSA (7). The immunoreactive PSA from breast tumors was fully characterized at the mRNA level. We amplified mRNA using reverse transcription-polymerase chain reaction (RT-PCR) with primers specific for the PSA gene. The PCR product was sequenced and found to be identical to the sequence of cDNA derived from prostatic tissue (8).

In this paper we have examined 57 lung tumor extracts from 53 patients with primary lung cancer and have shown that many of them contain small amounts of immunoreactive PSA. When a cutoff level of PSA of 0.030 ng/mg is used, as we have done for our breast tumor studies, 11% of the patients were found positive for PSA immunoreactivity. This positivity rate is second only to breast tumors (30% positivity at this cutoff level) (4). In our previous study of many different tumor types (7), only one of seven colon tumors and none of any other tumor type had PSA ≥0.030 ng/mg. If the cutoff is lowered, more lung tumors are classified as PSA positive.

The presence of immunoreactive PSA in some lung tumor extracts was verified with a widely used automated assay for

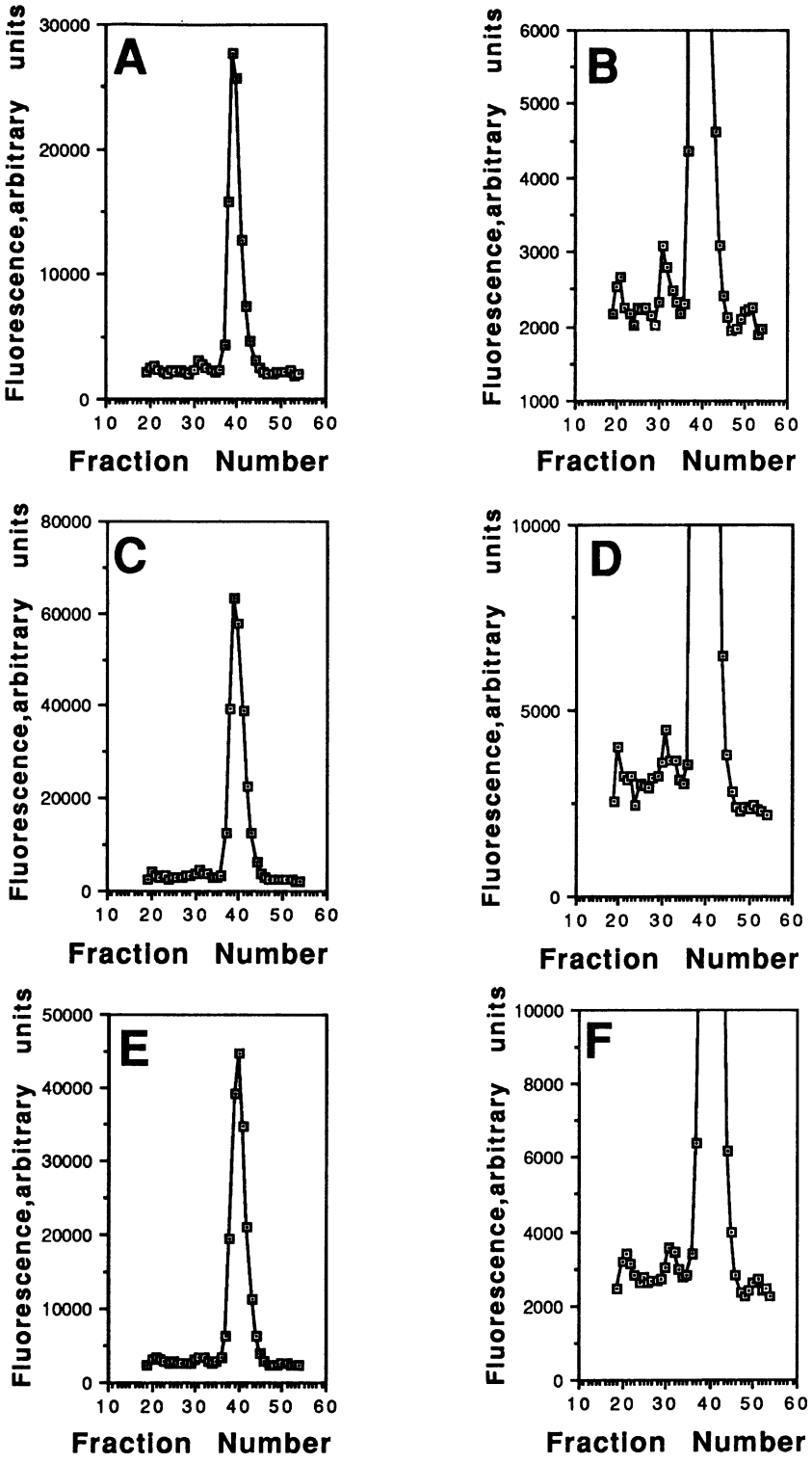

Fig. 1. **A:** HPLC of a primary lung tumor extract from a male patient with poorly differentiated primary lung adenocarcinoma. After injecting 300 μL of extract, fractions were collected and analyzed for PSA. The PSA-ACT complex elutes at fraction 31 ± 1 and free PSA at fraction 40 ± 1 (data established with male serum and purified seminal PSA, but not shown). The HPLC column was calibrated with a molecular weight standard eluting at fractions 21 (660 KDa), 29 (160 KDa), 33 (44 KDa), 43 (17 KDa), and 52 (1.4 KDa). Flow rate was 0.5 mL/min. **B:** The fluorescence scale was expanded to show the existence of two small peaks at fractions 21 (unknown identity) and 31 (PSA-ACT). **C,D:** HPLC of a primary lung tumor extract from a male patient with moderately differentiated squamous cell lung carcinoma. Other information as in A and B. **E,F:** HPLC of a lymph node extract from a female patient with moderately differentiated squamous cell lung carcinoma metastatic to the regional lymph nodes. Other information as in A and B.

TABLE 1. Relationship Between PSA Content of Lung Tumors and Patient Sex or Histological Type

	Patient sex			Histological type		
PSA (ng/mg)[a]	Male (%)	Female (%)	*P*	Adenocarcinoma (%)	Squamous cell carcinoma (%)	*P*
<0.005	9 (30)	17 (74)		10 (37)	11 (52)	
≥ 0.005	21 (70)	6 (26)	0.002*	17 (63)	10 (48)	0.29*
< 0.010	19 (63)	21 (91)		18 (67)	18 (86)	
≥ 0.010	11 (37)	2 (9)	0.019*	9 (33)	3 (15)	0.13*
< 0.020	21 (70)	23 (100)		20 (74)	20 (95)	
≥ 0.020	9 (30)	0 (0)	0.003**	7 (26)	1 (5)	0.06**
< 0.030	25 (83)	23 (100)		23 (85)	20 (95)	
≥ 0.030	5 (17)	0 (0)	0.061**	4 (15)	1 (5)	0.12**

[a]Data for primary lung tumors only. Lymph node data were not included for this analysis.
*Data analysis with chi-square test.
**Data analysis with Fisher's exact test (two tailed).

PSA which is commercially available. Furthermore, HPLC analysis has shown that the molecular weight of the immunoreactive species is identical to the molecular weight of free PSA (Fig. 1). We provided evidence that traces of PSA-ACT complexes also exist in these extracts. Similar findings were reported for breast tumors (3,4).

Statistical analysis of the data revealed that most tumors containing PSA belong to male patients (Table 1). None of the 23 tumor extracts from female patients contain PSA ≥0.020 ng/mg; 9 of 30 tumors belonging to male patients have PSA ≥0.020 ng/mg ($P = 0.003$). We have not measured steroid hormone receptor levels in the lung tumors studied. However, we have previously shown that in breast cancer, PSA production is mediated by steroid hormone receptors which are known to be present in many different tissues (4,7). Furthermore, with our tissue culture system, which mimics the phenomenon of PSA production by breast tumors in vitro, we have shown that androgens are potent mediators of PSA production (9). We speculate that in the case of lung tumors, androgens produced by the testes in males and by the ovaries in females stimulate PSA production through the androgen receptor. This and other possibilities are currently under investigation.

Both adenocarcinomas and squamous cell carcinomas contain immunoreactive PSA. However, adenocarcinomas were found to contain PSA more frequently and at higher levels than the squamous cell type (Table 1).

In this paper we have shown that the levels of PSA in the lung tumor extracts are relatively very low, at least 10^4 times lower than the PSA levels in extracts obtained from a prostatic cancer which metastasized to the lung. The possibility that the lung cancer extracts contain PSA because of contamination from serum PSA present in tumor vasculature is unlikely for the following reasons. First, some of the PSA-positive lung tumors were derived from women whose serum is known to have no PSA or very little PSA (6). One case of a PSA-positive lung tumor from a female patient is presented in Figure 1E. Second, in all cases examined, PSA in the tumor extracts was present mainly in its 33 KDa form (>98%). The major form of serum PSA is the PSA-ACT complex (~80%) with a molecular weight of ~100 KDa (6).

Subsequent to our reports on breast tumors (3,4), Clements and Mukhtar (10) recently reported PSA presence in normal endometrium. Our extensive data previously published (5) and those of Clements and Mukhtar (10) strongly suggest that many different tissues have the ability to produce PSA. Our group has additionally found PSA in amniotic fluid, in the normal breast, and in breast cyst fluid (5).

The physiological role of PSA production by breast, lung, and other tumors and tissues and during pregnancy is currently unknown. However, recent data on prostatic tissue offers clues that PSA may be involved in growth regulation (11–15). Our current and previous findings of PSA presence in lung, breast, colon, ovarian, parotid, kidney and liver tumors, stimulated normal breast, amniotic fluid, and breast milk and data presented by others for normal endometrium suggest that PSA can no longer be regarded as a specific prostatic marker and as a physiological molecule associated only with semen liquefaction. Given the new evidence that PSA may be a candidate growth factor or growth factor regulator, the biological role of PSA in normal tissues, tumors, and during pregnancy may be much more complex than thought and raises numerous questions which will only be answered by further investigation.

ACKNOWLEDGMENTS

This work was supported by a grant to E.P. Diamandis from the Ontario Section of the Canadian Breast Cancer Foundation and to M. D'Costa from the St. Joseph's Health Centre Foundation. We thank Dr. M.C. Patterson for encouragement and support.

REFERENCES

1. Armbruster DA: Prostate-specific antigen: Biochemistry, analytical methods, and clinical application. *Clin Chem* 39:181–195, 1993.

2. Van Krieken JH: Prostate marker immunoreactivity in salivary gland neoplasms. *Am J Surg Pathol* 17:410–414, 1993.
3. Diamandis EP, Yu H, Sutherland DJA: Detection of prostate specific antigen immunoreactivity in breast tumors. *Breast Cancer Res Treat* 33:301–310, 1994.
4. Yu H, Diamandis EP, Sutherland DJA: Immunoreactive prostate specific antigen levels in female and male breast tumors and its association with steroid hormone receptors and patient age. *Clin Biochem* 27:75–79, 1994.
5. Diamandis EP, Yu H: New biological functions of prostate specific antigen? *J Clin Endocrinol Metab* 80:1515–1517, 1995.
6. Yu H, Diamandis EP: Ultrasensitive time-resolved immunofluorometric assay of prostate specific antigen in serum and preliminary clinical studies. *Clin Chem* 39:2108–2114, 1993.
7. Levesque M, Yu H, D'Costa M, Diamandis EP: Prostate specific antigen expression by various tumors. *J Clin Lab Anal* 9:123–128, 1995.
8. Monne M, Croce CM, Yu H, Diamandis EP: Molecular characterization of prostate specific antigen mRNA expressed in breast tumors. *Cancer Res* 54:6344–6347, 1994.
9. Yu H, Diamandis EP, Zarghami N, Grass L: Induction of prostate specific antigen production by steroids and tamoxifen in breast cancer cell lines. *Breast Cancer Res Treat* 32:291–300, 1994.
10. Clements J, Mukhtar A: Glandular kallikreins and prostate specific antigen are expressed in the human endometrium. *J Clin Endocrinol Metab* 78:1536–1539, 1994.
11. Watt KWK, Lee PJ, M'Timkulu T, Chan WP, Loor R: Human prostate specific antigen: Structural and functional similarity with serine proteases. *Proc Natl Acad Sci USA* 83:3166–3170, 1986.
12. Cohen P, Graves HCB, Peehl DM, Kamarei M, Giudice LC, Rosenfeld RG: Prostate-specific antigen (PSA) is an insulin-like growth factor binding protein-3 protease found in seminal plasma. *J Clin Endocrinol Metab* 75:1046–1053, 1992.
13. Kanety H, Madjar Y, Dagan Y, Levi J, Papa MZ, Pariente C, Goldwasser B, Karasik A: Serum insulin-like growth factor-binding protein-2 (IGFBP-2) is increased and IGFBP-3 is decreased in patients with prostate cancer: Correlation with serum prostate-specific antigen. *J Clin Endocrinol Metab* 77:229–233, 1993.
14. Killian CS, Corral DA, Kawinski E, Constantine RI: Mitogenic response of osteoblast cells to prostate-specific antigen suggests an activation of latent TGF-β and a proteolytic modulation of cell adhesion receptors. *Biochem Biophys Res Commun* 192:940–947, 1993.
15. Espana F, Gilabert J, Estelles A, Romeu A, Asnar J, Cabo A: Functionally active protein C inhibitor/plasminogen activator inhibitor-3 (PCI/PAI-3) is secreted in seminal vesicles, occurs at high concentrations in human seminal plasma and complexes with prostate-specific antigen. *Thromb Res* 64:309–320, 1991.