New diagnostic applications of prostate-specific antigen

E.P. DIAMANDIS

Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, 600 University Avenue, Toronto, Ontario M5G 1X5 and
Department of Clinical Biochemistry, University of Toronto, 100 College Street, Toronto, Ontario M5G 1L5, Canada

Introduction

PSA was first discovered in seminal plasma [1] isolated from prostatic tissue [2] and found elevated in the serum of patients with prostate cancer [3]. It is a 33-kDa single-chain glycoprotein. The PSA gene has extensive homology with the genes encoding proteases of the kallikrein family [4,5], serine proteases which are crucial to the processing of various polypeptide precursors to their bioactive forms. Other members of this family include tonin, which cleaves angiotensinogen to angiotensin II in the rat, γ-renin and the growth factor-processing enzymes γ-nerve growth factor and epidermal growth factor-binding protein in the mouse [6].

It is thought that, in the prostate gland, PSA is produced exclusively by the epithelial cells, and is present in semen at concentrations of around 1 g/L [7]. In the seminal fluid, PSA is involved directly in the liquefaction of the seminal coagulum by cleaving seminal vesicle proteins [8]. PSA has chymotrypsin-like enzymatic activity [7].

PSA molecular forms

PSA is present in normal, benign hyperplastic and malignant prostatic tissue and in metastatic prostatic carcinoma. In the serum of normal men, PSA levels are usually <4 ng/mL. The serum PSA is present in two molecular forms, as free PSA with a molecular weight of ≈ 33 kDa and as PSA bound to proteinase inhibitors, predominantly α_1-antichymotrypsin (ACT) (≈ 100 kDa) and α_2-macroglobulin (A2M) (≈ 800 kDa) [9,10]. Current PSA immunoassays only measure the free PSA and the PSA-ACT complex; in serum, the ratio free PSA:PSA-ACT is about 1:4. The molecular forms of PSA in serum can be detected after separation on gel filtration columns [11]. Free PSA can now be measured directly with specific immunoassays [12,13].

Tissue-specificity of PSA – early reports

PSA is one of the most useful tumour markers because of its tissue-specificity. It is widely used for screening, diagnosis and monitoring of patients with prostate cancer. In the last few years, several reports have challenged the absolute tissue-specificity of PSA. Papotti et al. [14] reported in 1989 that some apocrine sweat gland carcinomas and some rare apocrine breast carcinomas stained positively for PSA when polyclonal antibodies were used in immunohistochemical stains. However, none of these tumours stained positively when monoclonal PSA antibodies were used and the authors concluded that the positive staining was due to cross-reactivity of the polyclonal antibodies. Importantly, none of the most common invasive breast ductal carcinomas was found positive by either antibody [14]. More recently, McLachlin and Srigley reported two cases of mature cystic teratomas of the ovary in females that contained prostatic tissue and stained positively for PSA [15]. Pummer et al. [16] reported increased levels of immunoreactive PSA in the sera of women with renal cell carcinoma, but these findings were explained as artefacts of the polyclonal antibody-based immunoassay used, as a more specific monoclonal antibody-based assay was free of such apparent interference. More recently, Van Krieken presented evidence that PSA is rarely produced by salivary gland neoplasms [17] and we confirmed this finding [18].

Several other groups have shown PSA to be present in the periurethral and perianal glands [19–21]. Considered together, these results suggest that certain tumours or tissues other than the prostate can produce PSA, although this event is extremely rare.

PSA in female serum

As women have no prostate, it was assumed for many years that women do not produce PSA in any tissue and that PSA is not present in the female blood circulation. However, PSA has been immunohistochemically localized to the female periurethral glands. This finding led Wernert et al. to propose that the 'female prostate' is the periurethral glands, as 67% of them stained positively for PSA and appeared histologically similar to the prostate gland before puberty [22]. With newly developed, highly sensitive assays for PSA, it was found that some females have measurable serum levels of PSA. However, the tissue origin of this PSA is still not known. We currently believe that female serum PSA arises from the breast (see below). In a study of 1061 sera from healthy

and hospitalized women, we have shown that 1.5% of them had serum PSA levels >0.10 ng/mL [23]. These PSA-positive sera were associated with women aged >50 years. We propose that steroid hormones (e.g. androgens, progestins or glucocorticoids) stimulate the target organ (female breast, periurethral glands or an unknown organ) to produce and release PSA into the circulation.

Nonprostatic PSA

The reports which challenged the absolute tissue-specificity of PSA attracted little interest because of their rarity. However, using ultrasensitive immunological assays for PSA, we recently reported that PSA is very often present in female breast tumour cytosolic extracts [24,25]. At the threshold level of 0.015 ng of PSA per mg of total protein, half of breast tumours were positive for PSA [25]; at 0.030 ng/mg, the positivity was 30% and some female breast tumours contained PSA levels >50 ng/mg. The molecular weight of PSA in the female breast tumours was identical to that of seminal PSA and free serum PSA (≈ 33 kDa). The enzymatic activity of PSA has not as yet been tested in breast tumour extracts. Molecular characterization of breast-tumour PSA mRNA with RT-PCR and nucleic-acid sequencing has shown that this mRNA is identical in sequence with PSA mRNA from prostatic tissue [26]. Association analysis for 1275 tumours has shown that PSA was associated with the presence of progesterone receptor (PR) but not oestrogen receptor (ER) [25]. Premenopausal women and women with early stage cancer are more frequently PSA-positive than postmenopausal women or women with late-stage disease. Survival analysis has shown that patients with breast tumours producing PSA live longer and relapse less frequently [27,28]. Thus, PSA is a new favourable prognostic indicator in female breast cancer. A tissue culture system that was developed with female breast cancer cell lines has shown that PSA production in these cell lines was mediated through the action of PR and androgen receptors (AR), mineralocorticoid receptors (MR) and glucocorticoid receptors (GR), but not ER [29]. These data are in accord with the mechanism of gene regulation by steroid hormone receptors [30].

Subsequent to our studies on breast cancer, Clements and Mukhtar reported that PSA is present in normal endometrial tissue [31]. These authors speculated that PSA may play a role as a local regulator of uterine function but a substrate for this enzyme is currently unknown. Further studies by our group have shown that PSA is a very ubiquitous enzyme. For example, we have found that a normal female breast can produce large amounts of PSA in subjects who receive progestin-

containing oral contraceptives [32]. Moreover, after pregnancy, the normal breast produces PSA and secretes it into the milk of lactating women [33]. Some milk contains >300 ng/mL of PSA while some contains only traces. Breast discharge fluid contains more PSA than any other female or male fluid except for seminal plasma; we have found levels up to 4000 ng/mL (unpublished data). We also found PSA in breast cystic fluid [34] and in amniotic fluid [35]. In amniotic fluid, PSA concentration increases with gestation from weeks 11–21 and levels off or declines slowly afterwards [35]. Cases of fetuses with various congenital abnormalities associated with abnormal PSA levels in amniotic fluid have been identified recently by our group [35].

A more recent survey of various tumours has revealed that at least some ovarian, liver, kidney, adrenal, colon, parotid and lung tumours produce PSA [36,37]. As many of these tissues are known to contain steroid hormone receptors, these findings have led us to speculate that any tissue which contains steroid hormone receptors has the ability to produce PSA, provided that the cognate-stimulating steroid hormones are also available. The stimulating hormones could be exogenously administered or endogenously released by the adrenals or the ovaries. The AR, GR, MR and PR have the ability to regulate PSA gene expression through the same hormone-response element present in DNA [29,30].

Physiological role of PSA in nonprostatic tissue

The physiological role and importance of the presence of PSA in many normal, benign or malignant tissues, extracts and fluids is currently unknown. PSA in all tissues and fluids examined, except serum, is predominantly present as a 33-kDa monomer. Complexes with proteinase inhibitors also exist, but at much lower concentrations. We propose that PSA acts enzymatically upon one or more substrate(s) and modifies their action in a fashion similar to the function of the other proteinases of the kallikrein family. Such substrates remain to be identified. Recent data on prostatic tissue could be extrapolated to support the view that PSA may be involved in growth regulation of mammary and other tissues. The sequence of PSA shows extensive homology with γ-nerve growth factor (56%), epidermal growth-factor-binding protein (53%) and α-nerve growth factor (51%) [38]. In addition, PSA can enzymatically digest IGF binding protein III (IGFBP-3). This activity is thought to regulate IGF-1 concentration because digestion of IGFBP-3 by PSA releases biologically active IGF-1 [39]. Findings by other groups further support the hypothesis that PSA is a regulator of IGFBP-2 and IGFBP-3 in patients with prostate cancer [40]. Killian et al. have recently found that PSA has mitogenic activity, presum-

ably due to activation by PSA of latent transforming growth factor β (TGF-β) and through the modulation of cell adhesion [41]. Others have shown that PSA binds and inactivates protein-C inhibitor [42].

Recently, Fichtner *et al.* [43], using an *in vivo* rat bladder model, found that PSA released kinin-like substance(s) after proteolysis of seminal vesicle fluid, and this substance(s) was able to stimulate smooth muscle contraction. This finding supplements previous investigations which showed that PSA has chymotrypsin-like activity and is able to digest seminal vesicle proteins like seminogelins and fibronectin, thus causing seminal liquefaction.

Our detection of PSA in breast, colon, ovarian, parotid, kidney, lung and liver tumours, 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. Instead, PSA should be regarded as a molecule which could be produced by cells bearing steroid hormone receptors under conditions of steroid hormone stimulation. The biological role of PSA in normal tissues, in tumours and during pregnancy raises numerous questions which will only be answered by further investigation.

Diagnostic value of serum PSA in women

In a recent paper [44], we examined whether total PSA in female serum has any diagnostic value for breast cancer. We concluded that there were no substantial differences in total serum PSA between normal women and women with breast cancer. However, more recent data [45 and patent pending] have shown that PSA subfractions may have diagnostic value. In normal women, most serum PSA is in the complexed form (PSA-ACT). In women with breast cancer, a substantial proportion exists as free PSA. Based on these findings, we developed a model for PSA regulation by breast cancer cells (Fig. 1).

Ultrasensitive PSA assays and PCR

Most of the new data on PSA have been generated with assays that have detection limits of < 0.01 ng/mL [11,46]. Currently, the most sensitive PSA assay available commercially is the IMMULITE® assay [46],
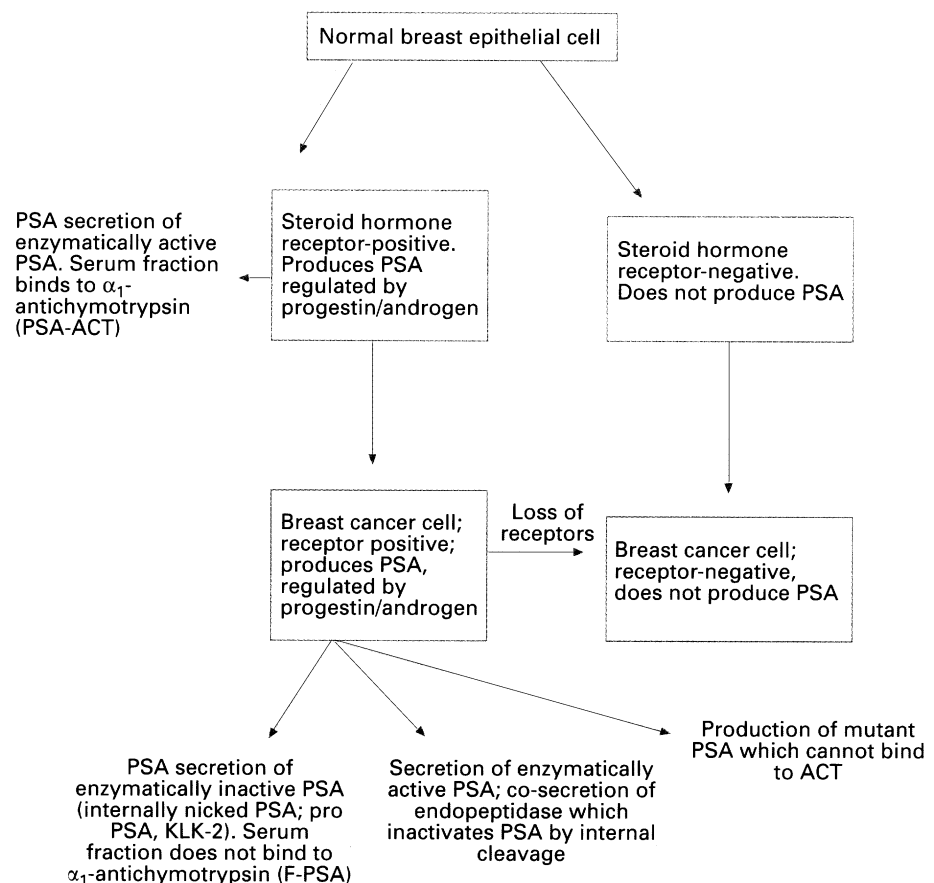
Fig. 1. PSA is produced by steroid hormone receptor-positive breast epithelial cells under regulation by progestins/ androgens. Normal epithelial cells produce and secrete enzymatically active PSA which binds to α_1-antichymotrypsin when it enters the general circulation. Breast tumours seem to produce and secrete enzymatically inactive PSA (either internally 'nicked' PSA, pro-PSA or KLK-2) which cannot bind to α_1-antichymotrypsin when it enters the general circulation.

Normal breast epithelial cell

PSA secretion of enzymatically active PSA. Serum fraction binds to α_1-antichymotrypsin (PSA-ACT)

Steroid hormone receptor-positive. Produces PSA regulated by progestin/androgen

Steroid hormone receptor-negative. Does not produce PSA

Breast cancer cell; receptor positive; produces PSA, regulated by progestin/androgen

Loss of receptors

Breast cancer cell; receptor-negative, does not produce PSA

PSA secretion of enzymatically inactive PSA (internally nicked PSA; pro PSA, KLK-2). Serum fraction does not bind to α_1-antichymotrypsin (F-PSA)

Secretion of enzymatically active PSA; co-secretion of endopeptidase which inactivates PSA by internal cleavage

Production of mutant PSA which cannot bind to ACT

with a functional sensitivity of $\approx 0.005-0.01$ ng/mL, and our latest PSA assay, which has a functional sensitivity of 0.001 ng/mL [46]. Using these assays we, and others, have shown that prostate cancer relapse after radical prostatectomy can be detected much earlier than by using less sensitive assays [47,48]. RT-PCR for PSA mRNA can detect a single prostate cell [49–52], and is useful for detecting micrometastases in lymph nodes [51] and cancer cells in the blood [50,52,53]. I anticipate that such assays will be applied to breast cancer in the near future.

References

1 Hara M, Inorre T, Fukuyama T. Some physico-chemical characteristics of gamma-seminoprotein, an antigenic component specific for human seminal plasma. *Jap J Legal Med* 1971; **25**: 322–6

2 Wang MC, Valenzuele LA, Murphy GP, Chu TM. Purification of human prostate specific antigen. *Invest Urol* 1979; **17**: 159–63

3 Papsidero LD, Wang MC, Valenzuela LA, Murphy GP, Chu TM. A prostate antigen in sera of prostatic cancer patients. *Cancer Res* 1980; **40**: 2428–32

4 Riegman PHJ, Klaassen P, Van der Korput JAGM, Romijn JC, Trapman J. Molecular cloning and characterisation of novel prostate antigen cDNAs. *Biochem Biophys Res Commun* 1988; **155**: 181–8

5 Schedlich LJ, Bennetts BH, Morris BJ. Primary structure of a human glandular kallikrein gene. *DNA* 1987; **6**: 429–37

6 Clements JA. The glandular kallikrein family of enzymes: tissue-specific expresion and hormonal regulation. *Endocrine Rev* 1989; **10**: 393–419

7 Oesterling JE. Prostate specific antigen: a critical assessment of the most useful tumor marker for adenocarcinoma of the prostate. *J Urol* 1991; **145**: 907–23

8 Lilja H. A kallikrein-like serine protease in prostatic fluid leaves the predominant seminal vesicle protein. *J Clin Invest* 1985; **76**: 1899–903

9 Stenman U-H, Leionen J, Alfthan H, Rannikko S, Tuhkanen K, Alfthan O. A complex between prostate-specific antigen and α_1-antichymotrypsin is the major form of prostate-specific antigen in serum of patients with prostatic cancer: assay of the complex improves clinical sensitivity for cancer. *Cancer Res* 1991; **51**: 222–6

10 Christensson A, Laurell CB, Lilja H. Enzymatic activity of prostate-specific antigen and its reactions with extracellular serine proteinase inhibitors. *Eur J Biochem* 1990; **194**: 755–63

11 Lilja H, Christensson A, Dahlén U et al. Prostate specific antigen in serum occurs predominantly in complex with α_1-antichymotrypsin. *Clin Chem* 1991; **37**: 1618–25

12 Pettersson K, Piironen T, Seppälä M et al. Free and complexed prostate specific antigen (PSA): in vitro stability, epitope map and development of immunofluorometric assays for specific and sensitive detection of free PSA and PSA-α_1-antichymotrypsin complex. *Clin Chem* 1995; **41**: 1480–8

13 Yu H, Diamandis EP. Ultrasensitive time-resolved immunofluorometric assay of prostate specific antigen in serum and preliminary clinical studies. *Clin Chem* 1993; **39**: 2108–14

14 Papotti M, Paties C, Peveri V, Moscuzza L, Bussolati G. Immunocytochemical detection of prostate-specific antigen (PSA) in skin adnexal and breast tissues and tumors. *Bas Appl Histochem* 1989; **33**: 25–9

15 McLachlin SM, Srigley JR. Prostatic tissue in mature cystic teratomas of the ovary. *Am J Surg Pathol* 1992; **16**: 780–4

16 Pummer K, Wirnsberger G, Purstner P, Stettner H, Wondshneider G. False positive prostate specific antigen values in the sera of women with renal cell carcinoma. *J Urol* 1992; **148**: 21–3

17 Van Krieken JH. Prostate marker immunoreactivity in salivary gland neoplasms. *Am J Surg Pathol* 1993; **17**: 410–14

18 James GK, Pudek M, Berean KW, Diamandis EP, Archibald BL. Salivary duct carcinoma secreting prostate specific antigen. *Am J Clin Pathol* 1996; **106**, 242–7

19 Kamoshida S, Tsutsumi Y. Extraprostatic localisation of prostate acid phosphatase and prostate specific antigen: distribution in cloacogenic glandular epithelium and sex-dependent expression in human anal gland. *Hum Pathol* 1990; **21**: 1108–11

20 Frazer HA, Humphrey PA, Burchette JL, Paulson DF. Immunoreactive prostatic specific antigen in male periurethral glands. *J Urol* 1992; **147**: 246–8

21 Iwakiri J, Grandbois K, Wehner N, Graves HCB, Stamey T. An analysis of urinary prostatic specific antigen before and after radical prostatectomy: evidence for secretion of prostate specific antigen by the periurethral glands. *J Urol* 1993; **149**: 783–6

22 Wernert N, Albrech M, Sesterhenn J et al. The 'female prostate'. Location, morphology, immunohistochemical characteristics and significance. *Eur Urol* 1992; **22**: 64–9

23 Yu H, Diamandis EP. Measurement of serum prostate specific antigen levels in females and in prostatectomised males with an ultrasensitive immunoassay technique. *J Urol* 1994; **153**: 1004–8

24 Diamandis EP, Yu H, Sutherland DJA. Detection of prostate specific antigen immunoreactivity in breast tumors. *Breast Cancer Res Treat* 1994; **32**: 291–300

25 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* 1994; **27**: 75–9

26 Monne M, Croce CM, Yu H, Diamandis EP. Molecular characterisation of prostate specific antigen mRNA expressed in breast tumors. *Cancer Res* 1994; **54**: 6344–7

27 Yu H, Diamandis EP, Levesque MA, Sismondi P, Zola P, Katsaros D. Ectopic production of prostate specific antigen by a breast tumor metastatic to the ovary. *J Clin Lab Anal* 1994; **8**: 251–3

28 Yu H, Giai M, Diamandis EP et al. Prostate specific antigen is a new favourable prognostic indicator for women with breast cancer. *Cancer Res* 1995; **55**: 2104–10

29 Yu H, Diamandis EP, Zarghami N, Grass L. Induction of prostate specific antigen production by steroids and tamox-

ifen in breast cancer cell lines. *Breast Cancer Res Treat* 1994; **32**: 301–10

30 Beato M. Gene regulation by steroid hormones. *Cell* 1989; **56**: 335–44

31 Clements A, Mukhtar A. Glandular kallikreins and prostate specific antigen are expressed in the human endometrium. *J Clin Endocrinol Metab* 1994; **78**: 1536–9

32 Yu H, Diamandis EP, Monne M, Croce CM. Oral contraceptive-induced expression of prostate specific antigen in the female breast. *J Biol Chem* 1995; **270**: 6615–18

33 Yu H, Diamandis EP. Prostate specific antigen in milk of lactating women. *Clin Chem* 1994; **41**: 54–60

34 Diamandis EP, Yu H, Lopez-Otin C. Prostate specific antigen — A new constituent of breast cyst fluid. *Breast Cancer Res Treat* 1996; **38**: 259–64

35 Yu H, Diamandis EP. Prostate specific antigen immunoreactivity in amniotic fluid. *Clin Chem* 1995; **41**: 204–10

36 Levesque M, Yu H, D'Costa M, Diamandis EP. Prostate specific antigen expression by various tumors. *J Clin Lab Anal* 1995; **9**: 123–8

37 Levesque M, Yu H, D'Costa M, Tadross L, Diamandis EP. Immunoreactive prostate specific antigen in lung tumors. *J Clin Lab Anal* 1995; **9**: 375–9

38 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* 1986; **83**: 3166–70

39 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 Endoc Metab* 1992; **75**: 1046–53

40 Kanety H, Madjar Y, Dagan Y et al. 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 Endoc Metab* 1993; **77**: 229–33

41 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* 1993; **192**: 940–7

42 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* 1991; **64**: 309–20

43 Fichtner J, Graves HCB, Thatcher K, Yemoto C, Shortliffe LMD. Prostate specific antigen releases a kinin-like substance on proteolysis of seminal vesicle fluid that stimulates smooth muscle contraction. *J Urol* 1996; **155**: 738–42

44 Giai M, Yu H, Roagna R et al. Prostate specific antigen in serum of women with breast cancer. *Br J Cancer* 1995; **72**: 728–31

45 Melegos D, Diamandis EP. Diagnostic value of molecular forms of prostate specific antigen for female breast cancer. *Clin Biochem* 1996; **29**: 193–200

46 Ferguson RA, Yu H, Kalyvas M, Zammit S, Diamandis EP. Ultrasensitive detection of prostate specific antigen by a new time resolved immunofluorometric assay and the IMMULITE immunochemiluminescent third generation assay: potential applications in prostate and breast cancers. *Clin Chem* 1996; **42**: 675–84

47 Yu H, Diamandis EP, Prestigiacomo AF, Stamey TA. Ultrasensitive assay of prostate-specific antigen used for early detection of prostate cancer relapse and estimation of tumor doubling time after radical prostatectomy. *Clin Chem* 1995; **41**: 430–4

48 Randell EW, Yu H, Diamandis EP, Prestigiacomo AF, Stamey T. Early detection of prostate cancer relapse in post-radical prostatectomy patients using the IMMULITE third generation prostate specific antigen assay. *Can J. Oncol* 1997; in press

49 Galvan B, Christopoulos TK, Diamandis EP. Detection of prostate specific antigen mRNA by reverse-transcription polymerase chain reaction and time-resolved fluorometry. *Clin Chem* 1995; **41**: 1705–9

50 Diamandis EP, Yu H. Prostate cancer, prostate specific antigen and the polymerase chain reaction. *Clin Chem* 1995; **41**: 177–9

51 Deguchi T, Doi T, Ehara H et al. Detection of micrometastatic prostate cancer cells in lymph nodes by reverse transcriptase-polymerase chain reaction. *Cancer Res* 1993; **53**: 5350–4

52 Katz AE, Olsson CA, Raffo AJ et al. Molecular staging of prostate cancer with the use of an enhanced reverse transcriptase-PCR assay. *Urology* 1994; **43**: 765–75

53 Jaakkola S, Vornanen T, Leinone J, Rannikko S, Stenman U-H. Detection of prostatic cells in peripheral blood: correlation with serum concentrations of prostate specific antigen. *Clin Chem* 1995; **41**: 182–6

Author

E.P. Diamandis, Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, 600 University Avenue, Toronto, Ontario M5G 1X5; and Department of Clinical Biochemistry, University of Toronto, 100 College Street, Toronto, Ontario M5G 1L5, Canada.