Urol Res (2003) 31: 183–187
DOI 10.1007/s00240-003-0313-6

ORIGINAL ARTICLE

John Varkarakis · Charalambos Deliveliotis
Diamandis Sideris · Nikolaos Trakas
Aris Giannopoulos

Preoperative nested reverse transcription-polymerase chain reaction for prostate specific membrane antigen predicts non-organ confined disease in radical prostatectomy specimens

Received: 14 August 2002 / Accepted: 20 March 2003 / Published online: 23 April 2003

Abstract The aim of this study was to access the utility of the reverse transcriptase-polymerase chain reaction (RT-PCR) assay for prostatic specific membrane antigen (PSMA) in predicting non-organ confined (NOC) disease in final radical prostatectomy (RP) specimens. Nested RT-PCR for PSMA was performed on the blood of 33 patient candidates for RP, 20 patients with untreated metastatic disease and 20 healthy men. The final pathology report on the 33 RP patients was compared with the RT-PCR results and Partin nomograms. In the RP group, 4/18 patients with confined disease and 9/15 with NOC disease had positive RT-PCR assays. Sensitivity, specificity, positive and negative predictive values for RT-PCR were 60%, 77.7%, 69% and 70%, respectively. The Partin tables for this group of patients showed a sensitivity, specificity, positive and negative predictive values of 75%, 71%, 60% and 83%, respectively. *P*-values for the Partin tables and the RT-PCR assay were respectively 0.014 and 0.037. RT-PCR of PSMA has an independent predictive value and could help predict NOC disease in clinically localized prostate cancers, but is still less efficient than Partin tables.

Keywords RT-PCR · PSMA · Prostate cancer

J. Varkarakis (✉) · C. Deliveliotis · N. Trakas
A. Giannopoulos
Department of Urology, School of Medicine,
Athens University, Athens, Greece
E-mail: medvark3@yahoo.com
Tel.: +30-210-7247278
Fax: +30-210-7250476

D. Sideris
Department of Biochemistry and Molecular Biology,
School of Biology, Athens University, Athens, Greece

J. Varkarakis
Vas Sofias 62A, P.O.B. 11528, Athens, Greece

Introduction

Under-staging of clinically localized prostate cancer is common, and in some cases reaches 50% [17] due to the inadequacy of current staging tools in terms of the accurate determination of the local extent of this disease.

Introduction of reverse transcriptase-polymerase chain reaction (RT-PCR) technology and the discovery of a prostate specific marker such as prostate specific antigen (PSA), allowed the detection of prostate cancer cells expressing PSA [15, 30]. This assay has been used in various tissues (lymph nodes, bone marrow, serum) [3]. RT-PCR for PSA in blood has been used in the preoperative staging of clinically localized prostate cancer with equivocal results [8, 10, 22, 27]. The discovery of prostatic specific membrane antigen (PSMA) [18], a new prostate specific marker, and knowledge of its nucleotide sequence [25], allowed some investigators to apply RT-PCR technology for the detection of prostate cancer cells in the circulation [9, 12]. PSMA is currently being utilized as an immunoscintigraphic target using the antibody conjugate CYT-356 (ProstaScint; Cytogen, Princton, N.J.) and has been shown to have clinical value in detecting occult prostate cancer.

We evaluated whether RT-PCR of PSMA can predict non-organ confined disease and whether it is more accurate than Partin tables.

Materials and methods

Patient selection

After informed consent was obtained, we performed a RT-PCR assay for PSMA in the serum of 53 patients with prostate cancer and 20 healthy male controls, without known prostatic disease. Patients with prostate cancer were divided in two groups. The first consisted of 33 patients with clinically organ confined prostate cancer (cT1c-cT2). All 33 men underwent radical retropubic prostatectomy (RRP) without any neoadjuvant therapy. The second group consisted of 20 patients with newly confirmed metastatic disease without any previous treatment. All patients were staged by digital rectal examination (DRE), PSA, transrectal ultrasound,

computerized tomography, and a bone scan. Metastatic patients were considered to be those with a positive bone scan, or an excessively elevated PSA value (>100 ng/ml) [20]. Candidates for radical prostatectomy had a serum PSA level <20 ng/ml and had clinically localized disease. Radical prostatectomy and metastatic patients had mean ages of 63.7 years (range 55–72) and 70.2 years (range 60–88) respectively, while mean PSA values were 8.6 ng/ml (range 2.4–18.6) and 301.1 ng/ml (range 21–2,000 ng/ml) in the two groups. Healthy controls had a mean age of 60.25 (range 32–80) and a mean PSA value of 2.4 ng/ml (0.5–6.7 ng/ml). Patients with PSA >4 ng/ml had a previous negative prostate biopsy.

Patient blood samples

All patient blood samples were collected 30 days after the DRE and biopsies were performed. For surgical candidates, samples were obtained 1 day before the operation. From each patient and control, 10 cc of whole blood samples were collected in four tubes with EDTA and transported for RNA isolation to the laboratory within 2 h. All samples were transferred in crushed ice, maintaining the temperature at 4°C without freezing it. Samples were diluted in two volumes of 9 g/l NH_4Cl and then shaken overnight at 4°C.

Cell culture

The human lymph node-derived LNCaP cell line was used as a positive control in the experiment. The cells were cultured in RPMI medium that contained 10% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin and 250 ng/ml fungizone (GIBCO-BRL, Life Technologies).

Determination of assay sensitivity

To determine the sensitivity of our PCR assay, LNCaP cells were serially diluted into whole blood obtained from a healthy normal male volunteer. Serial dilutions of the LNCaP cells was performed and each different dilution was added to a different tube of whole blood at concentrations of 0, 1, 10,10^2,10^3,10^4,10^5,10^6 LNCaP cells per 10 cc normal whole blood. These samples were then processed in the manner described as above. Samples were stored overnight at −20°C for later RNA extraction.

RNA isolation

Total cellular RNA was isolated from whole blood using the QIAamp RNA Blood Mini Kit (Qiagen), according to the manufacturers instructions.

Reverse transcription reaction and cDNA synthesis

We used 3 μg of total RNA for the synthesis of the first strand of cDNA with Superscript II reverse transcriptase (GIBCO-BRL, Life Technologies), according to the manufacturers instructions. Briefly, RNA and random hexamers (GIBCO-BRL, Life Technologies) or downstream outer PSMA primer (10 pmol/μl) was first denatured for 5 min at 65°C, chilled on ice for 1 min, and then incubated for 1 h at 42°C in 20 μl of a reaction mixture containing 4 μl 5× first strand buffer (0.25 mol/l Tris-HCl, pH 8.3, 0.375 mol/l KCl, 15 mmol/l $MgCl_2$), 250 mmol/l dNTP mix (Boehringer Mannheim, Germany), 10 mmol/l dithiothreitol and 200 U of superscript II reverse transcriptase.

Oligonucleotide primers

We used the two pairs of PSMA primers constructed by Israeli et al. [9]: (1) the outer upstream primer $PSM_{1368–1390}$: 5′ CAG ATA TGT CAT TCT GGG AGG TC 3′ and the downstream primer $PSM_{1995–2015}$: 5′ AAC ACC ATC CCT CCT CGA ACC 3′, yielding a 647-bp PCR product, and (2) the PSMA inner upstream primer PSM_{1689-}: 5′ CCT AAC AAA AGA GCT GAA AAG CCC 3′ and the downstream primer PSM_{1923}: 5′ ACT GTG ATA CAG TGG ATA GCC GCT 3′, yielding a 234-bp product for the nested PCR. We also used the actin primers used by Degushi et al. [1]: with the upstream primer (BA1: 5′ACA-ATGAGCTGC GTGTGGCT 3′) and the downstream primer (BB1: 5′TCTCCTTAATGTCACGCACGA 3′) yielding a 372-bp PCR product, in order to rule out degraded RNA. If samples failed to yield amplified products for β-actin RNA, they were omitted.

Polymerase chain reaction

A total of 2.5 μl of the cDNA served as the starting material for the outer primer PCR reaction. The 50 μl of PCR mix consisted of 5 μl 10×PCR buffer (200 mmol/l Tris-HCl, pH 8.4, and 500 mmol/l KCl, pH 8.8), 1 μM of each primer, 250 mmol/l dNTPs, 10 mmol/l β-mercaptoethanol, 2 mmol/l $MgCl_2$, and 1 U of Taq DNA polymerase (Promega). PCR was carried out in a MJ Research DNA thermal cycler according to the following program: 94°C for 1 min (3 min for the first cycle); 58°C for 1 min; and 72°C for 1 min (10 min for the last cycle) for 40 cycles. Then, 2.5 μl of the first PCR reaction products were used as the template for the nested PCR reaction. Finally, 10–15 μl of each PCR reaction mix was run on 2% agarose gel, stained with ethidium bromide, and photographed.

Statistical analysis

Sensitivity, specificity, positive and negative predictive values were calculated for the ability of the RT-PCR assay to predict organ from non-organ confined disease preoperatively. The same parameters were also evaluated for the Partin tables, using as a cut-off value a 50% probability of the nomograms to correctly predict the final pathological stage of the patient. All statistical analysis was done using commercially available statistical software.

Results

The mRNA of PSMA from an average of one LNCaP cell diluted in 10^6 mononuclear cells could be detected after nested RT-PCR. When this assay was performed on 20 metastatic patients, 19/20 (95%) were found positive. The same assay done on 20 healthy male controls was negative in all cases (100%). Among 33 patients undergoing radical prostatectomy, 18 (54.5%) had organ-confined disease (15 pT_{2a} and three pT_{2b}) while 15 (45.5%) had non-organ confined disease (ten pT3a, four pT3b, one pT3c). Four of 18 patients with organ-confined disease had positive RT-PCR assays, while 9/15 patients with non-confined disease were positive using the same assay. All clinical and pathological characteristics of the patients with prostate cancer are presented in Table 1. Patients with metastatic, non-organ confined disease, organ confined prostate cancer and healthy males presented a positive RT-PCR for PSMA in 95%, 60%, 22.2%, and 0% of cases, respectively.

The sensitivity and specificity of RT-PCR in the group of patients undergoing radical prostatectomy was 60% and 77.7%, respectively, while the corresponding positive and negative predictive values were 69% and

Table 1 Clinical and pathological characteristics of patients with prostate cancer

Radical prostatectomy patients								
No.	Age	PSA (ng/ml)	Clinical TNM	Preoperative Gleason score	Extraprostatic extension	Seminal vesicle invasion	p TNM	RT–PCR
1	66	8.1	T1c	7	–	–	T2a	+
2	66	12.4	T2b	8	+	–	T3b	–
3	69	8	T2b	7	+	–	T3a	+
4	64	9	T1c	7	+	–	T3a	+
5	72	5.4	T1c	4	–	–	T2a	+
6	65	7.2	T1c	6	–	–	T2a	–
7	63	8.2	T1c	6	+	–	T3a	–
8	55	12.5	T2b	7	+	–	T3a	+
9	65	11	T1c	7	–	–	T2a	–
10	67	10	T2a	7	+	–	T3a	+
11	68	10	T1c	6	–	–	T2a	–
12	68	14	T2a	7	+	–	T3a	+
13	56	15	T1c	6	–	–	T2a	+
14	58	7	T2a	8	+	–	T3a	–
15	62	5	T1c	5	–	–	T2a	+
16	64	5.6	T2b	7	+	+	T3c	–
17	59	8.7	T1c	7	+	–	T3a	+
18	70	2.3	T2b	7	+	–	T3b	–
19	62	3	T2a	7	–	–	T2a	–
20	70	13	T1c	5	–	–	T2b	–
21	59	9.4	T1c	7	+	–	T3b	+
22	72	17	T2a	7	–	–	T2a	–
23	64	18.6	T2b	6	–	–	T2b	–
24	60	7	T1c	6	–	–	T2a	–
25	59	8.1	T2b	7	+	–	T3b	+
26	60	7	T1c	7	–	–	T2a	–
27	62	7.2	T1c	7	–	–	T2a	–
28	69	3.2	T2a	6	+	–	T3a	–
29	65	4.5	T1c	6	–	–	T2α	–
30	63	6	T1c	7	–	–	T2α	–
31	56	7.8	T2a	6	–	–	T2β	–
32	70	8	T2b	7	+	–	T3α	+
33	56	4	T1c	5	–	–	T2α	–
Patients with metastatic prostate cancer								
1	72	950	M	9				+
2	75	100	T4N2	8				+
3	80	21	M	9				+
4	67	370	M	10				+
5	70	62	M	7				+
6	73	2000	M	10				–
7	60	150	T4	8				+
8	79	500	T4N2	8				+
9	88	120	T4N2	8				+
10	74	88	M	9				+
11	68	130	M	7				+
12	70	100	M	9				+
13	69	500	M	10				+
14	70	394	T4N2	9				+
15	72	102	M	8				+
16	68	68	M	7				+
17	66	120	T4N1	9				+
18	65	39	M	8				+
19	69	56	M	7				+
20	78	152	M	8				+

70%. RT-PCR could predict pathological stage ($P = 0.0377$). The use of Partin tables for this patient group presented a sensitivity, specificity, negative and positive predictive values of 75%, 71%, 83% and 60%, respectively. Prediction of the final pathology using Partin tables was statistically significant ($P = 0.014$) (Table 2).

Discussion

RT-PCR involves the detection of cells at an mRNA level and is obviously associated with many technical challenges. The difficulties encountered are reflected in the conflicting results presented by numerous studies

Table 2 Statistical analysis of RT-PCR and Partin nomogramsfor predicting non-organ confined disease in radical prostatectomy specimens

	No. patients	Sensitivity %	Specificity %	Negative predictive value %	Positive predictive value %	*P* value (χ-square test with continuity correction)
RT-PCR	33	60	77.7	70	69.2	0.037
Partin tables	33	75	71	83	60	0.014

evaluating the efficacy of RT-PCR in staging clinically localized prostate cancer [10, 14, 15, 22], but also by false positive assays in healthy controls [11, 16], or false negative tests in clearly metastatic patients [33]. In addition, differences in the technical parameters of this assay mean that the results from various other papers are not comparable [3]. The different components and concentrations of PCR mixtures, the differences in the number of cycles performed by each researcher and the various primers used are only part of the variation in the RT-PCR reactions presented [3]. More cycles than needed may make the assay more sensitive than necessary. This must also be taken in consideration from the moment that PSA and PSMA molecules are expressed in various non-prostatic cell lines in minimum quantities [6, 24, 31]. Therefore, false positive results may occur if our assays are so sensitive that they can detect even minimal quantities of PSA and PSMA produced by non-prostatic cells. We had 100% negative RT-PCR in healthy controls, comparable with most other studies [2, 9, 12]. This confirms the fact that our assay was not too sensitive as it did not have false positives among the healthy controls.

It is crucial to use the appropriate primers for each mRNA we want to amplify when using RT-PCR. Saimoto et al. [26] compared the two most common primers constructed by Loric et al. [12] and Israeli et al. [9], and found that those of Loric et al. were more efficient. In fact Milion et al. [16], using Israeli's primers, had many positive healthy controls. This was due to the presence of leaky promoters that illegitimately transcribed parts of the DNA that had nucleotide homology with the PSMA gene. Nevertheless, Mao et al. [13], using the same primers as Loric, had RT-PCR for PSMA signals in another 12 lines of tumor cells, confirming the possibility of a leaky promoter for these primers as well. Okegawa et al. [21] used Loric primers for PSMA and found comparable results to us although we used Israeli et al.'s primers instead. The essential part of primer construction is knowledge of the exact DNA sequence of the PSMA gene, which in some papers seems not to be clear since more than one variant for PSMA exist [28].

Another cause of false positive results may be contamination of samples with DNA different from the target DNA. Therefore specialized personnel must perform the RT-PCR reactions in appropriate laboratory settings. We did not have any false positive healthy control, a fact that confirmed that the conditions under which RT-PCR was performed protected our samples from contamination.

Prompt isolation of the mRNA target is essential, as it is prone to rapid degradation by the lytic enzymes of other cells [3]. Therefore, all samples were handled within a period of 2 h. We did not have any case of a negative signal from the internal molecule which we used in order to evaluate the integrity of the mRNA of our samples.

False negative samples are possible in metastatic patients and may be explained by the fact that some cancer cells in advanced disease are so undifferentiated that they can not express the prostate specific marker [33]. We attribute to this phenomenon our only negative RT-PCR in the metastatic group, which interestingly had a PSA value of 2,000 ng/ml.

We performed all our RT-PCRs on patients who had not had any prostatic manipulation for at least 30 days. This was done in order to avoid the intermittent presence of prostatic cells in the circulation due to mechanical injury of the prostate, a fact that could lead to false positive reactions. There are reports that during transurethral resection of the prostate, prostate biopsy and radical prostatectomy, the shedding of prostate cells occurs, converting negative RT-PCRs to positive ones for a short period of time [7, 19, 23].

We also avoided any kind of neoadjuvant therapy as it has been postulated that RT-PCR for PSA becomes negative after hormone therapy [29]. Despite the fact that PSMA is considered to be expressed better in hormone refractory states, we do not know how this protein behaves in localized disease, and therefore evaluated the RT-PCR for PSMA in patient candidates for radical prostatectomy, without giving them any previous neoadjuvant therapy [5].

Many studies exist for RT-PCR of PSA in staging of prostate cancer; the same is not true for the PSMA molecule. We found that patients with metastatic, non-organ confined disease, confined prostate cancer and healthy males presented a positive RT-PCR for PSMA in 95%, 60%, 22,2%, and 0%, respectively. Zhang et al. [32] and Grasso et al. [4] had comparably high positive results (91%) for metastatic patients as well as for organ confined disease patients (22% vs 23% and 43%, respectively). Both groups, like us, agreed that RT-PCR for PSMA could predict pathological stage, a conclusion that was not found by Sokoloff et al. [27], nevertheless, this group had a 16% positive assay rate for patients with localized disease, which is close to our result. This might be explained by the low sensitivity of their assay, which only found 39.4% of metastatic patients to be positive.

The existing preoperative staging tools for prostate cancer are not optimal. Partin tables present the most accurate way of predicting the final pathological stage of

prostate cancer using clinical stage, Gleason score, and preoperative PSA. Every new diagnostic tool has to be compared with these tables. In order for RT-PCR, a complex assay available only in very specialized laboratories, to be useful as a preoperative staging tool, it must be better than the Partin nomograms. In this study, the specificity of our RT-PCR for PSMA was higher than the specificity demonstrated by the Partin tables, but its sensitivity was worse. When evaluating the statistical significance of these two staging tools, the Partin tables had a $P = 0.014$ which is better than the $P = 0.037$ of our RT-PCR assay. Therefore RT-PCR of PSMA can preoperatively predict the pathological stage of the RP specimen, but does not offer more accurate staging than the Partin nomograms.

References

1. Degushi T, Doi T, Ehara H, Ito S, Takahashi Y, Nishino Y, Kawamura T, Komeda H, Horie M, Kaji H, Shimokawa K, Tanaka T, Kawada Y (1993) Detection of micrometastatic prostate cancer cells in lymph nodes by RT-PCR. Cancer Res 53: 5350
2. Dumas F, Eschwege P, Blanchet P, Benoit G, Jardin A, Lacour B, Loric S (1996) Enhanced detection of hematogenous circulating prostatic cells in patients with prostatic adenocarcinoma using PSMA based nested RT-PCR assay. J Urol 155: 506A
3. Gomella LG, Raj GV, Moreno JG (1997) RT-PCR for PSA in the management of prostate cancer. J Urol 158: 326
4. Grasso Y, Gupta M, Levin H, Zippe C, Klein E (1998) Combined nested RT-PCR assay for PSA and PSMA in prostate cancer patients: correlation with pathological stage. Cancer Res 58: 1456
5. Gregorakis A, Holmes E, Murphy G (1998) PSMA current and future utility. Semin Urol Oncol 16: 2
6. Henke W, Jung M, Jung K (1996) Detection of PSA M-RNA in blood by RT-PCR does not exclusively indicate prostatic tumor cells. Clin Chem 42: 1499
7. Heung YM, Walsh K, Sriprasd S, Mulvin D, Sherwood RA (2000) The detection of prostate cells by the RT-PCR in the circulation of patients undergoing TUR-P. BJU Int 85: 65
8. Ignatoff M, Oefelein MG, Watkin W, Chmiel JS, Kaul KL (1997) Prostate specific antigen RT-PCR assay in preoperative staging of prostate cancer. J Urol 158: 1870
9. Israeli RS, Miller WHJr, Su SL, Samadi DS, Powell CT, Heston WDW, Wise GJ, Fair WR (1995) Sensitive detection of prostatic hematogenous micrometastasis using prostate-specific antigen (PSA) and prostate-specific membrane antigen (PSMA) derived primers in the polymerase chain reaction. J Urol 153: 573
10. Katz AE, Olsson CA, Raffo AJ, Cama C, Perlman H, Seaman E, O'Toole KM, McMahon D, Benson MC, Buttyan R (1994) Molecular staging of prostate cancer with the use of an enhanced reverse transcriptase-PCR assay. Urology 43: 765
11. Lintula S, Stenman U (1999) The expression of PSMA in peripheral blood leukocytes. J Urol 157: 1969
12. Loric S, Dumas F, Eschwge P, Blanchet P, Benoit G, Jardin A, Lacour B (1995) Enhanced detection of hematogenous circulating prostatic cells in patients with prostate adenocarcinoma by using nested RT-PCR assay based on prostate specific membrane antigen. Clin Chem 41: 1698
13. Mao H, Hoshi S, Takahashi T, Kaneda T, Wang J, Orikasa S (1998) Detection of PSA m-RNA from the peripheral blood and pelvic lymph nodes in-patients with prostatic cancer by means of RT-PCR. Nippon Hinyokika Gakkai Zasshi 89:596
14. Melchior SW, Corey E, Ellis WJ (1997): Early tumor cell dissemination in-patients with clinically localized carcinoma of the prostate. Clin Cancer Res 3: 249
15. Moreno JG, Croce CM, Fischer R, Monne M, Vihko P, Mulholland SG, Gomella LG (1992) Detection of hematogenous micrometastasis in patients with prostate cancer. Cancer Res 52: 6110
16. Milion R, Jacqmin D, Muller D, Guillot J, Eber M, Abecassis J (1999) Detection of PSA or PSMA circulating cells in prostatic cancer patients: clinical implications. Eur Urol 36: 278
17. Mukamel E, Hanna J, Dekernion JB (1987): Pitfalls in preoperative staging in prostate cancer. Urology 30: 318
18. Murphy GP, Ragde H, Kenny G (1995) Comparison of prostate specific membrane antigen and prostate specific antigen levels in prostate cancer patients. Anticancer Res 15: 1473
19. Ogawa O, Iinuma M, Sato K, Sasaki R, Shimoda N (1999) Circulating PSA m-RNA during radical prostatectomy in-patients with localized prostate cancer: with special reference to neoadjuvant hormonal therapy. Urol Res 27: 291
20. Ohori M, Goad JR, Wheeler TM (1994) Can radical prostatectomy alter the progression of poorly differentiated prostate cancer? J Urol 152: 1843
21. Okegawa T, Yoshioka J, Morita R, Nutahara K, Tsukada Y, Higashihara E (1998) Molecular staging of prostate cancer: comparison of nested reverse transcription-polymerase chain reaction assay using PSA versus PSMA as primer. Int J Urol 5: 349
22. Olsson CA, DeVries GM, Benson M, Raffo A, Buttyan R, Cama C, O'Toole K, Katz AE (1996) The use of RT-PCR for PSA assay to predict potential surgical failures before radical prostatectomy: molecular staging of prostate cancer. Br J Urol 77: 411
23. Price D, Clontz D, Woodard W, Kaufman J, Daniels J, Stolzenberg S, Teigland C (1998) Detection and clearance of prostate cells subsequent to ultrasound-guided needle biopsy as determined by multiplex nested reverse transcription polymerase chain reaction assay. Urology 52): 261
24. Renneberg H, Friedetzky A, Konrad L, Kurek R, Tun W, Aumuller G (1999) PSMA is expressed in various human tissues: implications for the use of PSMA RT-PCR to detect hematogenous prostate cancer spread. Urol Res 27: 23
25. Rinker-Shaffer CW, Hawkins AL, Su SL (1995) Localization and physical mapping of the prostate specific membrane antigen (PSMA) gene to human chromosome 11. Genomics 30: 105
26. Saimoto A, Saito S, Murai M (1999) PSMA derived primers in a nested RT-PCR for detecting prostatic cancer cells. Jpn J Cancer Res 90: 233
27. Sokoloff MH, Tso CL, Kaboo R, Nelson S, Ko J, Dorey F, Figlin RA, Pang SP, DeKernion J, Belldegrun A (1996) Quantitative PCR does not improve preoperative prostate cancer staging: a clinicopathological molecular analysis of 121 patients. J Urol 156: 1560
28. Su SL, Huang IP, Fair WR (1995) Alternatively spliced variants of prostate specific membrane antigen RNA: ratio of expression as a potential measurement of progression. Cancer Res 55: 1441
29. Su S, Heston W, Perrotti M, Cookson M (1997) Evaluating neoadjuvant therapy effectiveness on systemic disease: use of a RT-PCR of PSMA. Urology 49: 95
30. Vessela RL, Riley DE, Blouke KA, Arfman EW, Lange PH (1992) A sensitive method for detection of a prostate tumor cell marker using the polymerase chain reaction. J Urol 147 (Suppl): 441A
31. Yu H, Diamandis EP, Zarghami N, Grass L (1994): Induction of PSA by steroids and tamoxifen in breast cancer cell lines. Breast Cancer Res Treat 32: 219
32. Zhang Y, Zippe C, VanLente F (1997) Combined nested RT-PCR assay for PSA and PSMA in detecting circulating prostatic cells. Clin Can Res 3: 1215
33. Zippelius A, Kufer P, Honold G, Kollermann M, Oberneder R, Schlimok G, Pantel K (1997) Limitations of RT-PCR analyses for detection of micrometastatic epithelial cancer cells in bone marrow. J Clin Oncol 15: 2701