SPANDIDOS PUBLICATIONS

ONCOLOGY LETTERS 9: 2886-2894, 2015

Relative mRNA expression of prostate-derived E-twenty-six factor and E-twenty-six variant 4 transcription factors, and of uridine phosphorylase-1 and thymidine phosphorylase enzymes, in benign and malignant prostatic tissue

LUCIANE ROSTIROLA CAVAZZOLA[1*], GUSTAVO FRANCO CARVALHAL[2*], CANDIDA DEVES[1], DAIANA RENCK[1], RICARDO ALMEIDA[2] and DIÓGENES SANTIAGO SANTOS[1*]

[1]Center for Research on Molecular and Functional Biology, Pontifical Catholic University of Rio Grande do Sul; [2]Department of Urology, School of Medicine, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul 90619-900, Brazil

Received July 15, 2014; Accepted March 10, 2015

DOI: 10.3892/ol.2015.3093

Abstract. Prostate cancer is the most frequent urological tumor, and the second most common cancer diagnosed in men. Incidence and mortality are variable and appear to depend on behavioral factors and genetic predisposition. The prostate-derived E-twenty-six factor (PDEF) and E-twenty-six variant 4 (ETV4) transcription factors, and the thymidine phosphorylase (TP) and uridine phosphorylase-1 (UP-1) enzymes, are reported to be components of the pathways leading to tumorigenesis and/or metastasis in a number of tumors. The present study aimed to analyze the mRNA expression levels of these proteins in prostatic cancerous and benign tissue, and their association with clinical and pathological variables. Using quantitative reverse transcription polymerase chain reaction, the mRNA expression levels of PDEF, ETV4, TP and UP-1 were studied in 52 tissue samples (31 of benign prostatic hyperplasia and 21 of prostate adenocarcinomas) obtained from patients treated by transurethral resection of the prostate or by radical prostatectomy. Relative expression was assessed using the Δ-CT method. Data was analyzed using Spearman's tests for correlation. $P<0.05$ was considered to indicate a statistically significant difference. The results revealed that PDEF, ETV4, UP-1 and TP were expressed in 85.7, 90.5, 95.2 and 100% of the prostate cancer samples, and in 90.3, 96.8, 90.3 and 96.8% of the benign samples, respectively. PDEF and ETV4 exhibited a significantly higher relative expression level in the tumor samples compared with their benign counterparts. The relative expression of TP and UP-1 did not differ significantly between benign and cancerous prostate tissues. The relative expression of TP was moderately and significantly correlated with the expression of ETV4 in the benign tissues. The relative expression of UP-1 was significantly lower in T3 compared with T1 and T2 cancers. These findings indicate that PDEF, ETV4, TP and UP-1 are typically expressed in benign and malignant prostatic tissues. Further studies are necessary to define the role of these proteins as therapeutic targets in prostate cancer.

Correspondence to: Mrs. Luciane Rostirola Cavazzola, Center for Research on Molecular and Functional Biology, Pontifical Catholic University of Rio Grande do Sul, 6,681 Ipiranga Avenue, Building 92A, Porto Alegre, Rio Grande do Sul 90619-900, Brazil
E-mail: lurostirola@gmail.com

*Contributed equally

Key words: thymidine phosphorylase, uridine phosphorylase-1, prostate-derived E-twenty-six factor, E-twenty-six variant 4, prostate cancer, E-twenty-six transcription factor, relative expression

Introduction

Prostate cancer is the most common non-cutaneous malignant neoplasm, and the second leading cause of cancer mortalities in men. The majority of prostate cancers are sporadic, and etiological factors are largely unknown (1-3). Certain molecular pathways are important for the normal and pathological functioning of prostate cells, including those of the androgen receptor, estrogen receptor, transforming growth factor-β, insulin-like growth factor type 1 and phosphatidylinositol-4,5-biphosphate 3-kinase/protein kinase B (PI3K/Akt) (1,4,5).

The E-twenty-six (ETS) family of transcription factors are known to regulate various biological processes in benign and malignant tissues, including cell proliferation, differentiation, metastasis and angiogenesis (1,6-9). The prostate-derived ETS factor (PDEF) is limited to tissues that are of epithelial origin and are hormone-regulated, such as prostate, breast, salivary glands, ovaries, colon, airways and stomach tissues (7,10-15). In the prostate, PDEF is predominantly expressed in the luminal epithelium, acting as an activator of the transcription of prostate-specific antigen (PSA), either in the androgen-sensitive or androgen-independent setting (16-18). ETS variant 4 (ETV4) may be involved in chromosomal translocations in human

prostate cancer, and in the activation of metalloproteinases, which are relevant in the processes of cell migration and tissue invasion, eventually leading to metastasis. ETV4 is overexpressed in a variety of cancers; in prostate cancer, ETV4 may be detected at any stage of the disease, and is typically associated with a poorer prognosis (8,19,20).

Thymidine phosphorylase (TP) and uridine phosphorylase-1 (UP-1) are different isoforms of the same enzyme. TP acts in a reversible way in the formation of thymidine and thymine. The conversion of thymidine into thymine generates 2-D-deoxyribose, which may affect a number of cellular functions, including the promotion of angiogenic factors (21-26). UP-1 acts in a reversible way in the transformation of uridine into uracil (27-29). These enzymes have various biological functions. UP-1 affects the activation and catabolism of numerous analogous nucleosides employed in anticancer chemotherapy, including fluorouracil (30).

Understanding the role of these molecules may provide novel insights into the diagnosis, staging, prognosis and follow-up of prostate cancer patients. The present study aimed to describe the relative expression of these enzymes and transcription factors in benign and malignant prostatic tissues obtained during transurethral resection of the prostate (TURP) or radical prostatectomy (RP).

Materials and methods

Population and tissue samples. A total of 52 tissue samples (31 of benign prostatic hyperplasia and 21 of prostatic adenocarcinoma) were analyzed. All samples were obtained from patients who underwent surgical treatment by TURP or RP at Hospital São Lucas of the Pontifical Catholic University of Rio Grande do Sul (Porto Alegre, Brazil). Prostatic tissue was obtained from specimens of RP, TURP or simple prostatectomy (SP) for benign disease. All patients received a summary of the study protocol and signed an informed consent form prior to surgery. The research protocol was registered with the National Committee for Research Ethics in Brazil (protocol no. 15212413.10000.5336).

Collection and storage of tissue samples. The surgical team collected the tissue samples during prostatic surgery (TURP, simple prostatectomy or RP). Prior to the transfer of the material to the Department of Pathology, the surgeon selected the tissue samples for the molecular analyses. In cancer cases, the prostate was sectioned at the most suspicious area, and a separate tissue sample was sent for conventional pathology in order to confirm that the tissue sent for molecular analysis was cancerous and not benign prostatic tissue. For the isolation of total RNA, the specimens were immediately stored in sterile flasks (Eppendorf®; Eppendorf Ltd., Stevenage, UK) with an RNA stabilization reagent (RNAlater™ RNA Stabilization Reagent, Ambion Life Technologies, Austin, TX, USA) at -80˚C.

Isolation and purification of mRNA. Total RNA was isolated from ~30 mg of each tissue sample using affinity chromatography and on-column DNAse I treatment according to the manufacturer's protocol (RNeasy® Protect Mini kit; Qiagen, Inc., Valencia, CA, USA), and stored at -80˚C.

Optical density. The concentration of RNA in the samples was analyzed by spectrophotometry determined at 260 nm (1DO=30 μg/ml), calculated using the following equation: A260 x (1DO) x (dilution factor) = concentration (μg/ml or ng/μl). RNA purity was assessed by the ratio of absorbance at 260 and 280 nm. Samples with the A260/280<1.40, or those that required more than total RNA of 8.4 μl to acquire a concentration of 500 ng/μl for the subsequent step, were excluded and reprocessed up to three times. The purified RNA was stored at -80˚C.

Reverse transcription and quantitative polymerase chain reaction (qPCR). cDNA was synthesized from 500 ng of total RNA isolated using a reaction mixture containing random primers, dNTPs, reverse transcription buffer, MultiScribe™ reverse transcriptase (50 U/μl) and nuclease-free H_2O + RNA (of each patient); the final concentration obtained was ~25 ng/ml cDNA. The reaction mixtures were subjected to 25˚C for 10 min, 48˚C for 30 min, and 95˚C for 5 min in a thermal cycler (TC-412, Techne®, Duxford, Cambridge, UK). The synthesized cDNA was stored at -20˚C.

qPCR was performed in duplicate using 96-well plates. Each well contained 2 μl (50 ng) cDNA sample, 9.25 μl of MilliQ water, 12.5 μl Universal PCR Master Mix (TaqMan) with fluorescence marker (TaqMan® Gene Expression Assay-on-Demand, Applied Biosystems Life Technologies, Foster City, CA, USA) with the FAM-MGB dye (Assay-on-Demand, Applied Biosystems Life Technologies), 1.25 μl β-actin gene (Applied Biosystems Life Technologies) for the endogenous control, or 1.25 μl target gene (TP/UP1/ETV4/PDEF; Applied Biosystems Life Technologies). The final volume of each reaction was 25 μl per well. The amplification conditions were divided into the following stages: Stage 1, 30˚C for 2 min; stage 2, 95˚C for 10 min; stage 3, 50 cycles of 95˚C for 15 s each; stage 4, 60˚C for 1 min. Samples containing β-actin and with the genes of interest were amplified in parallel for the normalization of reverse transcription reactions.

Statistical analysis. Statistical analyses were conducted using SPSS version 21 (IBM SPSS, Armonk, NY, USA). Relative expression of the molecules of interest was assessed using the Δ-CT method (31), which indicated the variation of the target genes in the tissue relative to a calibrator (i.e., the normal control tissue or benign tissue). Data is expressed as the geometric mean. Data were analyzed using non-parametric tests and Spearman's correlation coefficients. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Population and tissue samples. The relative expression of the different enzymes and ETS transcription factors (PDEF, ETV4, TP and UP-1) was determined in the prostatic tissue of 52 males treated at Hospital São Lucas. Among these, 31 samples were from patients with benign prostatic hyperplasia undergoing TURP or simple prostatectomy, and 21 tissue specimens came from patients with prostatic adenocarcinoma who underwent RP.

The mean age of the patients with prostatic hyperplasia was 66.4±10.4 years, whilst the mean age of the patients with

Table I. Associations between the benign and malignant prostatic tissue samples, with regard to the geometric mean relative expression levels of TP, UP-1, PDEF and ETV4.

Gene	Mean relative expression		P-value[a]
	Benign tissue (n=31)	Malignant tissue (n=21)	
TP	0.0074	0.0066	0.79
UP-1	0.0013	0.0016	0.49
PDEF	0.0311	3.1696	<0.001
ETV4	0.0045	0.0276	<0.001

[a]Spearman's correlation test. TP, thymidine phosphorylase; UP-1, uridine phosphorylase-1; PDEF, prostate-derived E-twenty-six factor; ETV4, E-twenty-six variant 4.

prostatic adenocarcinoma was 62.9±8.2 years; no significant difference in age was identified between these groups (P=0.190).

Relative expression of TP, UP-1, PDEF and ETV4 in benign and malignant tissue. Table I shows the geometric means of the relative expression levels of the enzymes and ETS transcription factors assessed in the samples of benign and malignant prostatic tissue. No statistically significant difference was identified in the relative expression of TP or UP-1 between the benign and malignant tissues. However, the relative expression of PDEF and ETV4 was significantly higher in the malignant tissues compared with the benign tissues (Table I).

Association of the relative expression of TP, UP-1, PDEF and ETV4 with preoperative PSA levels. Tables II and III show the association between the geometric mean of the relative expression levels of the genes studied and the geometric mean of the pre-operative PSA levels in samples of benign or malignant prostatic tissue. There was no statistically significant difference between the geometric means of the relative expression levels of the different enzymes and ETS transcription factors in the samples of benign and malignant prostatic tissue, with regard to the geometric mean of the pre-operative PSA level.

PDEF expression. The relative expression level of the ETS transcription factor PDEF was determined by qPCR in 28/31 (90.3%) samples from patients with benign hyperplasia and in 18/21 (85.7%) samples from patients with prostatic adenocarcinoma. The geometric mean relative expression level of PDEF was 0.0311 in the samples of benign prostatic hyperplasia and 3.1696 in the samples of prostatic adenocarcinoma (a 102-fold difference between the two tissue types). This difference was statistically significant (P<0.001) (Table I; Fig. 1).

The association between the values of the geometric mean pre-operative PSA level and the geometric mean relative expression level of PDEF in the benign and malignant tissues is shown in Tables II and III, respectively. In the cases of benign hyperplasia, there was no association between the geometric mean relative expression level of PDEF and the geometric mean pre-operative PSA level (Spearman's correlation coefficient = 0.040; P=0.853). Similarly, no significant association was identified between the mean PDEF expression level and the mean pre-operative PSA level in the cases of prostatic adenocarcinoma (Spearman's correlation coefficient = -0.273; P=0.391).

Among the cases of adenocarcinoma of the prostate, no association was identified between the pre-operative Gleason

Table II. Association between the geometric mean relative expression levels of TP, UP-1, PDEF and ETV4 and the geometric mean pre-operative PSA values in benign prostatic tissue samples.

Gene	PSA (n=31)[a]	
	r_s	P-value
TP	0.037	0.857
UP-1	0.375	0.710
PDEF	0.040	0.853
ETV4	0.160	0.434

[a]Spearman's correlation test. TP, thymidine phosphorylase; UP-1, uridine phosphorylase-1; PDEF, prostate-derived E-twenty-six factor; ETV4, E-twenty-six variant 4; PSA, prostate-specific antigen; r_s, Spearman's correlation coefficient.

Table III. Association between the geometric mean relative expression levels of TP, UP-1, PDEF and ETV4 and the geometric mean pre-operative PSA values in malignant prostatic tissue samples.

Gene	PSA (n=21)[a]	
	r_s	P-value
TP	0.461	0.084
UP-1	0.059	0.840
PDEF	-0.273	0.391
ETV4	-0.055	0.859

[a]Spearman's rank correlation test. TP, thymidine phosphorylase; UP-1, uridine phosphorylase 1; PDEF, prostate-derived E-twenty-six factor; ETV4, E-twenty-six variant 4; PSA, prostate-specific antigen; r_s, Spearman's correlation coefficient.

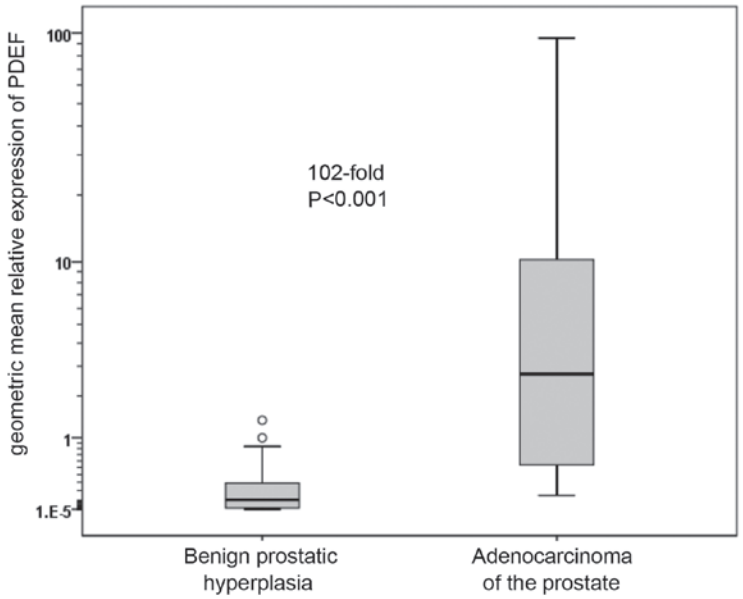

Figure 1. Correlation between the geometric mean of the relative expression level of PDEF in patients with adenocarcinoma of the prostate and the geometric mean of the relative expression level of PDEF in patients with benign prostatic hyperplasia. PDEF, prostate-derived E-twenty-six factor.

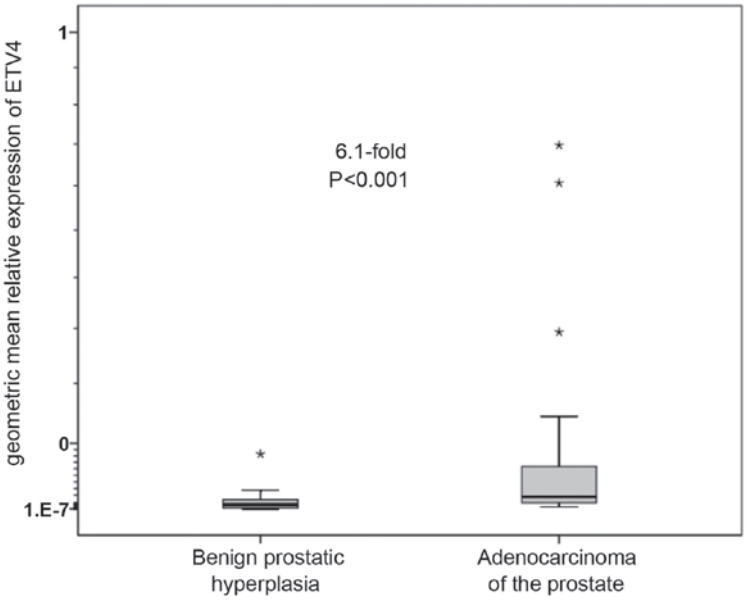

Figure 2. Correlation between the relative expression level of the geometric mean of ETV4 in patients with prostatic adenocarcinoma and the geometric mean of the relative expression level of the geometric mean of ETV4 in patients with benign prostatic hyperplasia. ETV4, E-twenty-six variant 4.

score and the relative PDEF mRNA expression level (P>0.05); there was also no correlation between TNM classification and the relative expression level of PDEF (P>0.05).

ETV4 expression. The relative expression level of ETV4 was determined by qPCR in 30/31 (96.8%) samples from the patients with benign hyperplasia and in 19/21 (90.5%) samples from the patients with adenocarcinoma of the prostate. The geometric mean relative expression level of ETV4 was 0.0045 in the samples of benign prostatic hyperplasia, and 0.0276 in the samples of prostatic adenocarcinoma; the mean relative expression level in the patients with prostatic adenocarcinoma was 6.1-fold greater than that of the patients with benign prostatic hyperplasia. This difference was statistically significant (P<0.001) (Table I; Fig. 2).

In the cases of benign hyperplasia, there was no correlation between the mean relative expression level of ETV4 and the mean pre-operative PSA level (Spearman's correlation coefficient = 0.160; P=0.434; Table II). In the cases of prostatic adenocarcinoma, there was also no correlation between the mean relative expression level of ETV4 and the mean pre-operative PSA level (Spearman's correlation coefficient = -0.055; P=0.859; Table III).

Among the cases of prostatic adenocarcinoma, no association was found between the Gleason score and the relative ETV4 expression level (P>0.05). Additionally, there was no

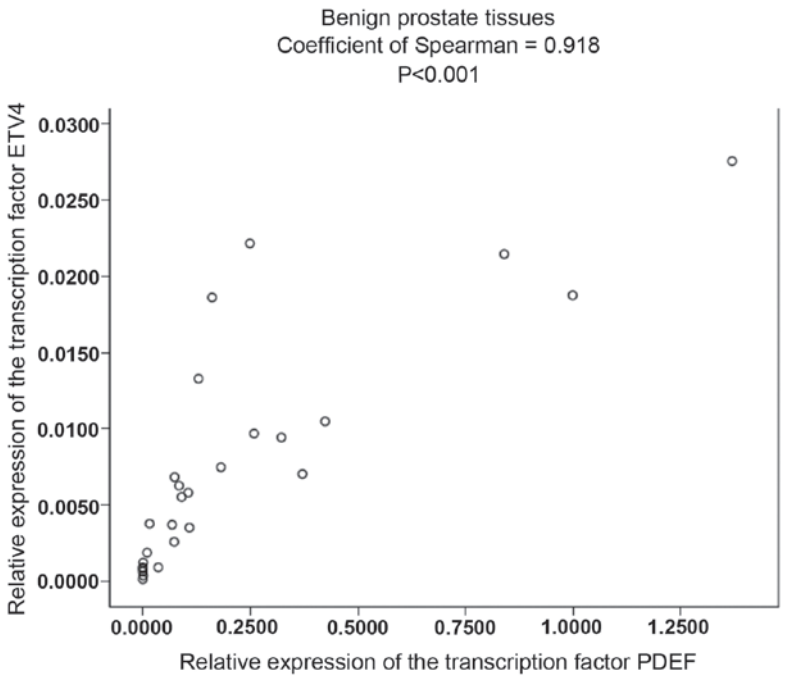

Figure 3. Correlation between the relative expression level of the PDEF transcription factor and the relative expression level of the ETV4 transcription factor in benign prostate tissues. PDEF, prostate-derived E-twenty-six factor. ETV4, E-twenty-six variant 4.

association between the TNM classification and the relative ETV4 expression level (P>0.05).

TP expression. qPCR was used to determine the relative expression level of TP in 30/31 (96.8%) samples from the patients with benign hyperplasia and in all samples from the patients with prostatic adenocarcinoma (21/21; 100%). The mean relative expression level of TP was 0.0074 in the samples of benign prostatic hyperplasia and 0.0066 in the prostatic adenocarcinoma samples (12% lower in adenocarcinoma compared with benign prostatic hyperplasia). This difference was not statistically significant (P=0.79) (Table I).

In the cases of benign hyperplasia, there was no significant correlation between the mean relative expression level of TP and the mean pre-operative PSA level (Spearman correlation coefficient = 0.037; P=0.857; Table II). In the cases of prostate adenocarcinoma, there was a moderate correlation between the mean relative expression level of TP and the mean pre-operative PSA level (Spearman's correlation coefficient = 0.461), however, this difference was not statistically significant (P=0.084; Table III). No association was identified between Gleason score and TNM classification, and the relative expression level of TP (P=NS).

UP-1 expression. The relative expression level of UP-1 was determined by qPCR in 28/31 (90.3%) samples from the patients with benign hyperplasia and in 20/21 (95.2%) samples from the patients with prostatic adenocarcinoma. The mean relative expression level of UP-1 was 0.0013 and 0.0016 in the samples of benign prostatic hyperplasia and prostatic adenocarcinoma, respectively (26% higher in prostatic adenocarcinoma samples). However, there was no statistically significant difference between the means in these two tissue types (P=0.49) (Table I).

In the cases of benign hyperplasia, a moderate correlation was identified between the mean relative expression level of UP-1 and the mean pre-operative PSA level (Spearman's correlation coefficient = 0.375), however, this association was not statistically significant (P=0.071; Table II). In the cases of prostatic adenocarcinoma, the relative expression level of UP-1 was not found to be correlated with the mean pre-operative PSA level (Spearman's correlation coefficient = 0.059; P=0.840; Table III).

Among the adenocarcinoma cases, Gleason score was not associated with the relative expression level of UP-1 (P>0.05). However, the relative expression level of UP-1 differed significantly between T3 samples (0.0008) and T1/T2 samples (0.0024), exhibiting 65%/2.84-fold lower expression in the T3 samples compared with the T1/T2 samples (P=0.032).

Association between the expression levels of the enzymes and transcription factors studied. The relative expression level of the TP and UP-1 enzymes was significantly correlated in the benign prostatic hyperplasia and prostatic adenocarcinoma tissue samples. The relative expression levels of these enzymes in benign tissue showed a strong Spearman coefficient (0.620); in malignant tissues, the association was also strong, with a Spearman coefficient of 0.574. These associations were statistically significant in the cases of benign hyperplasia and prostatic adenocarcinoma (P<0.001 and P=0.008, respectively).

The relative expression level of TP was demonstrated to be moderately associated with the relative expression level of the PDEF and ETV4 transcription factors in the samples of benign prostatic hyperplasia. In this tissue type, the Spearman coefficient for TP and PDEF expression was determined to be 0.351; this association was not statistically significant (P=0.067). However, for TP and ETV4, this association was statistically significant (Spearman's coefficient = 0.394; P=0.035).

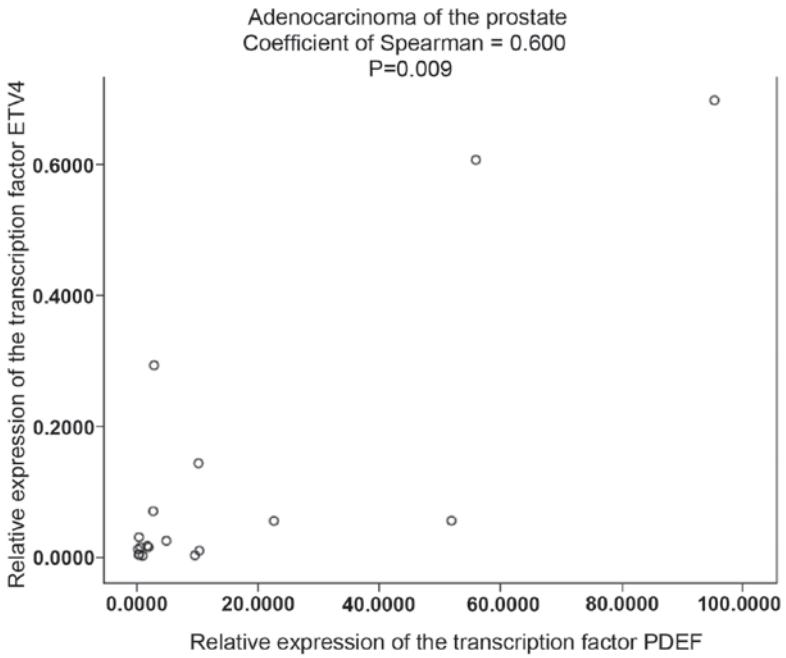

Figure 4. Correlation of the relative expression level of the PDEF transcription factor and the relative expression level of the ETV4 transcription factor in prostate adenocarcinoma tissues. PDEF, prostate-derived E-twenty-six factor. ETV4, E-twenty-six variant 4.

In the benign and malignant tissues, the relative expression level of PDEF was significantly associated with the expression level of ETV4 (Figs. 3 and 4). The relative expression level of PDEF and ETV4 in the benign tissues showed an extremely strong Spearman's coefficient, equal to 0.918 (P<0.001); for malignant tissues, a Spearman's coefficient of 0.600 was determined (P=0.009).

Discussion

In the present study, qPCR was utilized to assess the relative expression levels of TP, UP-1 and the ETS transcription factors PDEF and ETV4. qPCR is a standard technique used in the laboratory, and has also been used clinically for the detection of tumor markers and for the expression analysis of small samples, with a detection capability on the order of picograms and good accuracy. qPCR has been universally adopted due to its superiority with respect to speed, sensitivity, reproducibility and availability of reagents and instrumentation compared with other methods, such as northern blotting (32,33).

ETS transcription factors are considered to be challenging therapeutic targets due to their poor enzymatic activity, the complex regulation of their target genes, and their dependence on a wide network of connections and partnerships necessary for their proper functioning (1). In the current study, the expression of PDEF was found to be associated with that of ETV4 in benign and malignant prostatic tissues (P<0.001 and P<0.001, respectively). The mean relative expression level of PDEF was 102 times higher in the malignant tissues compared with the benign tissues, while ETV4 expression was 6.09 times higher in the malignant tissues compared with the benign tissues. Numerous studies have reported that the expression of PDEF decreases as the tumor becomes more aggressive and invasive, correlating with tumor grade and stage; this has been reported in prostate, breast, ovarian and colon cancer (7,10-12,34). However, the converse has also been observed in prostate, breast and ovarian cancer, suggesting a tumor suppressor activity for PDEF (7,10,35-38). The correlation coefficient of the expression of the two transcription factors in the benign tissues was strong, with P<0.001; in malignant tissue, this value indicated an even stronger correlation, with P=0.009. This indicates that these transcription factors are multifunctional, and may act via metalloproteinases, leading to migration, cell invasion and metastasis.

It is important to note that PDEF mRNA expression levels, although precise, may not necessarily reflect the level of expression of the protein in the laboratory or in clinical samples (7,39). Studies have suggested that PDEF is regulated at the transcriptional and post-translational levels via miRNAs (7,10,40).

The incidence of prostate cancer is associated with age, race, diet, environmental pollution and other factors, including a higher rate of gene fusions of ERG, ETV1, ETV4 and ETV5 with other genes (TMPRSS2, SLC45A3, C15orf21, CANT1, EST14, FOXP1, HERVK17, FLJ35294, HERV-K, ACSL3, NDRG1, DDX5, HNRPA2B1, KLK2) (41). It is now well established that PEA3 transcription factors are involved in prostate tumors and Ewing's sarcoma, as well as in other tumors, as a result of chromosomal translocations with ETV4 (8). Among the key features often found to be dysregulated in advanced prostate cancer, the PI3K and Ras pathways are altered in 40% of primary tumors and in 90% of metastatic tumors, and the combined signaling activity of these two pathways promotes the metastasis of prostate cancer through activation of ETV4 (42,43). In a study of breast cancer in humans, a positive association was observed between the overexpression of ETV4 and HER2/neu overexpression, tumor grade and greater recurrence rates (8). However, two other studies identified no correlation between ETV4, breast cancer and pathological clinical adverse effects (8,44,45).

No consensus has been reached on whether ETV4 stimulates or represses the Her2/neu pathway. It has been proposed that this difference may be due to the use of different cell lines between studies, and that overexpression of ETV4 may replace ETS factors that are most active in the promoter region of the gene Her2/neu, or may sequester certain co-activators causing the Her2/neu gene to be repressed rather than stimulated. Currently there is no consensus regarding whether the gene fusions of ERG, ETV1, ETV4 and ETV5 with other genes is correlated with more aggressive prostate cancer, or whether prostate tumors with gene translocations are more or less lethal than those without gene translocations (8,46).

In the present study, no statistically significant difference was identified between the relative expression levels of TP in benign and malignant prostatic tissues, despite the mean expression in the tumor tissues being 12% lower than that measured in the benign tissues. However, other studies have reported that the overexpression of TP may induce angiogenesis and tumor progression in organs such as the prostate, colon, pancreas, ovary, bladder, kidney, breast and stomach (21,47-49).

In addition, the current study found no statistically significant difference in the mean relative expression levels of UP-1 between benign and malignant prostatic tissues, although the mean expression of UP-1 was 26% higher in the tumor samples than in the benign samples. Studies have indicated that UP-1 is overexpressed in numerous tumors, including tumors of the breast, colon, kidney, lung, liver, ovary and intestine, in comparison to adjacent benign tissues; in certain studies, this difference was 2-3 times higher in tumors compared to normal tissue, and there were statistically significant differences in its expression in breast and colon tumors (27,50). However, negative results have also been reported (27,51).

The results of the present study indicated a moderate correlation between PSA levels and TP expression in the tumor samples, and also a moderate correlation between PSA levels and UP-1 expression in the benign tissues; however, these associations did not achieve statistical significance. The fact that benign and malignant prostatic tissues are associated with increased levels of serum PSA may explain these correlations and the lack of statistical significance in tumor and benign tissues with regard to pre-operative PSA levels (19,52,53).

In the present study, the relative expression of TP was strongly associated with the relative expression of UP-1 in the benign and tumor tissues ($P<0.001$ and $P=0.008$, respectively). This finding was expected, as the TP and UP-1 enzymes act on the nucleoside salvage pathway (54).

The relative expression of TP was moderately correlated with the relative expression of PDEF and also ETV4, the latter being statistically significant ($P=0.035$) in the benign prostatic tissues. We hypothesize that in benign, hyperplastic tissues, no significant changes occur in the concentrations of these enzymes and transcription factors or associated pathways.

In the prostatic carcinoma tissues, no correlation was found between the relative expression levels of TP and Gleason score or TNM classification, which is consistent with the majority of previous studies (55). In immunohistochemical studies of colorectal cancer, the expression of TP in stromal cells has been associated with a good prognosis; however, in breast cancer, increased TP expression was associated with a poor prognosis (56-58) It is likely that the expression of this enzyme varies according to the type of tissue, such as in tumor tissues infiltrated by macrophages, which exhibit an overexpression of TP (59).

The relative expression level of UP-1 showed a geometric mean 63% or 2.8 times lower in the T3 tumors compared with the T1 and T2 tumors ($P=0.032$); T3 tumors refer to those with extra-prostatic invasion, in generally larger tumors with increased vascularization. UP-1 is not associated with any angiogenic activity due to limited catalytic activity, which may explain its low levels in T3 disease (60,61).

It must be emphasized that there are numerous caveats limiting the conclusions that may be drawn with regard to these molecular mechanisms. All procedures involved, from the collection of the tissues to the final processing, may affect the quality of the biological sample. A number of variables may interfere at different stages of the study. Firstly, there may be variation during the surgical procedure, including anoxia and changes in local pH due to anesthesia, embolization or agglutination of arteries, sudden changes in systemic blood pressure and loss of intraoperative blood. All represent stress events that may alter the state of phosphorylation of various molecules, including that of TP and UP-1, and induce the activation or deactivation of molecular pathways. Tissue handling may also present challenges. This is performed by placing the sample as soon as possible into a sterile vial and DNase-free liquid under ice conditions; the temperature is extremely important, and storage must be at -80˚C after the sample has been frozen in liquid nitrogen. This ensures the transcription of genes and prevents the degradation of DNA and RNA (62).

The findings that PDEF and ETV4 were significantly more highly expressed in prostate cancer than in benign tissue warrants further studies to define the role of these transcription factors as potential therapeutic targets in prostate cancer.

References

1. Leitzmann MF and Rohrmann S: Risk factors for the onset of prostatic cancer: Age, location, and behavioral correlates. Clin Epidemiol 4: 1-11, 2012.
2. Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D: Global cancer statistics. CA Cancer J Clin 61: 69-90, 2011.
3. Felgueiras J, Silva JV and Fardilha M: Prostate cancer: The need for biomarkers and new therapeutic targets. J Zhejiang Univ Sci B 15: 16-42, 2014.
4. da Silva HB, Amaral EP, Nolasco EL, de Victo NC, Atique R, Jank CC, Anschau V, Zerbini LF and Correa RG: Dissecting major signaling pathways throughout the development of prostate cancer. Prostate Cancer 2013: 920612, 2013.
5. Frank SB and Miranti CK: Disruption of prostate epithelial differentiation pathways and prostate cancer development. Front Oncol 3: 273, 2013.
6. Cho JY, Lee M, Ahn JM, *et al*: Proteomic analysis of a PDEF Ets transcription factor-interacting protein complex. J Proteome Res 8: 1327-1337, 2009.
7. Steffan JJ and Koul HK: Prostate derived ETS factor (PDEF): a putative tumor metastasis suppressor. Cancer Lett 310: 109-117, 2011.
8. Oh S, Shin S and Janknecht R: ETV1, 4 and 5: an oncogenic subfamily of ETS transcription factors. Biochim Biophys Acta 1826: 1-12, 2012.
9. Hsu T, Trojanowska M and Watson DK: Ets proteins in biological control and cancer. J Cell Biochem 91: 896-903, 2004.
10. Johnson TR, Koul S, Kumar B, Khandrika L, Venezia S, Maroni PD, Meacham RB and Koul HK: Loss of PDEF, a prostate-derived Ets factor is associated with aggressive phenotype of prostate cancer: Regulation of MMP 9 by PDEF. Mol Cancer 9: 148, 2010.

11. Feldman RJ, Sementchenko VI, Magda M, Fraig MM and Watson DK: Pdef expression in human breast cancer is correlated with invasive potential and altered gene expression. Cancer Res 63: 4626-4631, 2003.
12. Ghadersohi A, Odunsi K, Zhang S, Azrak RG, Bundy BN, Manjili MH and Li F: Prostate-derived Ets transcription factor as a favorable prognostic marker in ovarian cancer patients. Int J Cancer 123: 1376-1384, 2008.
13. Pal M, Koul S and Koul HK: The transcription factor sterile alpha motif (SAM) pointed domain-containing ETS transcription factor (SPDEF) is required for E-cadherin expression in prostate cancer cells. J Biol Chem 288: 12222-12231, 2013.
14. Paner GP, Luthringer DJ and Amin MB: Best practice in diagnostic immunohistochemistry: Prostate carcinoma and its mimics in needle core biopsies. Arch Pathol Lab Med 132: 1388-1396, 2008.
15. Horst D, Gu X, Bhasin M, Yang Q, Verzi M, Lin D, Joseph M, Zhang X, Chen W, Li YP, Shivdasani RA and Libermann TA: Requirement of the epithelium-specific Ets transcription factor Spdef for mucous gland cell function in the gastric antrum. J Biol Chem 285: 35047-3555, 2010.
16. Oettgen P, Dedo E, Sun Z, *et al*: PDEF, a novel prostate epithelium-specific ets transcription factor, interacts with the androgen receptor and activates prostate-specific antigen gene expression. J Biol Chem 275: 1216-1225, 2000.
17. Tsui KH, Chung LC, Feng TH, Chang PL and Juang HH: Upregulation of prostate-derived Ets factor by luteolin causes inhibition of cell proliferation and cell invasion in prostate carcinoma cells. Int J Cancer 130: 2812-2823, 2012.
18. Steffan JJ, Koul S, Meacham RB and Koul HK: The transcription factor SPDEF suppresses prostate tumor metastasis. J Biol Chem 287: 29968-29978, 2012.
19. Rahim S and Uren A: Emergence of ETS transcription factors as diagnostic tools and therapeutic targets in prostate cancer. Am J Transl Res 5: 254-268, 2013.
20. Tomlins SA, Rhodes DR, Perner S, *et al*: Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science 310: 644-648, 2005.
21. Nakajima Y, Madhyastha R and Maruyama M: 2-Deoxy-D-ribose, a downstream mediator of thymidine phosphorylase, regulates tumor angiogenesis and progression. Anticancer Agents Med Chem 9: 239-245, 2009.
22. Desgranges C, Razaka G, Rabaud M and Picard P, Dupuch F and Bricaud H: The human blood platelet: a cellular model to study the degradation of thymidine and its inhibition. Biochem Pharmacol 31: 2755-2759, 1982.
23. Friedkin M and Roberts D: The enzymatic synthesis of nucleosides. I. Thymidine phosphorylase in mammalian tissue. J Biol Chem 207: 245-256, 1954.
24. Brown NS, Jones A, Fujiyama C, Harris AL and Bicknell R: Thymidine phosphorylase induces carcinoma cell oxidative stress and promotes secretion of angiogenic factors. Cancer Res 60: 6298-6302, 2000.
25. Hotchkiss KA, Ashton AW and Achwartz EL: Thymidine phosphorylase and 2-deoxyribose stimulate human endothelial cell migration by specific activation of the integrins alpha 5 beta 1 and alpha V beta 3. J Biol Chem 278: 19272-19279, 2003.
26. Bronckaers A, Gago F, Balzarini J and Liekens S: The dual role of thymidine phosphorylase in cancer development and chemotherapy. Med Res Rev 29: 903-953, 2009.
27. Pizzorno G, Cao D, Leffert JJ, Russell RL, Zhang D and Handschumacher RE: Homeostatic control of uridine and the role of uridine phosphorylase: A biological and clinical update. Biochim Biophys Acta 1587: 133-144, 2002.
28. Cao D, Leffert JJ, McCabe J, Kim B and Pizzorno G: Abnormalities in uridine homeostatic regulation and pyrimidine nucleotide metabolism as a consequence of the deletion of the uridine phosphorylase gene. J Biol Chem 280: 21169-21175, 2005.
29. Cao D, Russell RL, Zhang D, Leffert JJ and Pizzorno G: Uridine phosphorylase (-/-) murine embryonic stem cells clarify the key role of this enzyme in the regulation of the pyrimidine salvage pathway and in the activation of fluoropyrimidines. Cancer Res 62: 2313-2317, 2002.
30. Kawamura K, Takiguchi N, Wada A, Takenobu H, Kimura H, Soda H, Nagata M, Asano T and Tagawa M: Up-regulated expression of the uridine phosphorylase gene in human gastric tumors is correlated with a favorable prognosis. Anticancer Res 26: 4647-4651, 2006.
31. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-deltadelta C(t)) method. Methods 25: 402-408, 2001.
32. Xu D, Li XF, Zheng S and Jiang WZ: Quantitative real-time RT-PCR detection for CEA, CK20 and CK19 mRNA in peripheral blood of colorretal cancer patients. J Zhejiang Univ SCIENCEB 7: 445-451, 2006.
33. Kheirelseid EA, Chang KH, Newell J, Kerin MJ and Miller N: Identification of endogenous control genes for normalization of real-time quantitative PCR data in colorectal cancer. Mol Biol 11: 1-13, 2010.
34. Moussa O, Turner DP, Feldman RJ, Sementchenko VI, McCarragher BD, Desouki MM, Fraig M and Watson DK: PDEF is a negative regulator of colon cancer cell growth and migration. J Cell Biochem 108: 1389-1398, 2009.
35. Gunawardane RN, Sgroi DC, Wrobel CN, Koh E, Daley GQ and Brugge JS: Novel role for PDEF in epithelial cell migration and invasion. Cancer Res 65: 11572-11580, 2005.
36. Rodabaugh KJ, Mhawech-Fauceglia P, Groth J, Lele S and Sood AK: Prostate-derived Ets factor is overexpressed in serous epithelial ovarian tumors. Int J Gynecol Pathol 26: 10-15, 2007.
37. Sood AK, Saxena R, Groth J, Desouki MM, Cheewakriangkrai C, Rodabaugh KJ, Kasyapa CS and Geradts J: Expression characteristics of prostate-derived Ets factor support a role in breast and prostate cancer progression. Hum Pathol 38: 1628-1638, 2007.
38. Ghadersohi A and Sood AK: Prostate epithelium-derived Ets transcription factor mRNA is overexpressed in human breast tumors and is a candidate breast tumor marker and a breast tumor antigen. Clin Cancer Res 7: 2731-2738, 2001.
39. Nozawa M, Yomogida K, Kanno N, Nonomura N, Miki T, Okuyama A, Nishimune Y and Nozaki M: Prostate-specific transcription factor hPSE is translated only in normal prostate epithelial cells. Cancer Res 60: 1348-1352, 2000.
40. Turner DP, Findlay VJ, Moussa O, Semenchenko VI, Watson PM, LaRue AC, Desouki MM, Fraig M and Watson DK: Mechanisms and functional consequences of PDEF protein expression loss during prostate cancer progression. Prostate 71: 1723-1735, 2011.
41. Wang JJ, Liu YX, Wang W, Yan W, Zheng YP, Qiao LD, Liu D and Chen S: Fusion between TMPRSS2 and ETS family members (ERG, ETV1, ETV4) in prostate cancers from northern China. Asian Pac J Cancer Prev 13: 4935-4938, 2012.
42. Aytes A, Mitrofanova A, Kinkade CW, *et al*: ETV4 promotes metastasis in response to activation of PI3kinase and Ras signaling in a mouse model ofadvanced prostate cancer. Proc Natl Acad Sci USA 110: E3506-E3515, 2013.
43. Taylor BS, Schultz N, Hieronymus H, *et al*: Integrative genomic profiling of human prostate cancer. Cancer Cell 18: 11-22, 2010.
44. Span PN, Manders P, Heuvel JJ, Beex LV and Sweep CG: E1AF expression levels are not associated with prognosis in human breast cancer. Breast Cancer Res Treat 79: 129-131, 2003.
45. Xia WY, Lien HC, Wang SC, *et al*: Expression of PEA3 and lack of correlation between PEA3 and HER-2/neu expression in breast cancer. Breast Cancer Res Treat 98: 295-301, 2006.
46. Clark JP and Cooper CS: ETS gene fusions in prostate cancer. Nat Rev Urol 6: 429-439, 2009.
47. Takebayashi Y, Akiyama S, Akiba S, Yamada K, Miyadera K, Sumizawa T, Yamada Y, Murata F and Aikou T: Clinicopathologic and prognostic significance of an angiogenic factor, thymidine phosphorylase, in human colorectal carcinoma. J Natl Cancer Inst 88: 1110-1117, 1996.
48. Imazano Y, Takebayashi Y, Nishiyama K, Akiba S, Miyadera K, Yamada Y, Akiyama S and Ohi Y: Correlation between thymidine phosphorylase expression and prognosis in human renal cell carcinoma. J Clin Oncol 15: 2570-2578, 1997.
49. Shimaoka S, Matsushita S, Nitanda T, Matsuda A, Nioh T, Suenaga T, Nishimata Y, Akiba S, Akiyama S and Nishimata H: The role of thymidine phosphorylase expression in the invasiveness of gastric carcinoma. Cancer 88: 2220-2227, 2000.
50. Liu M, Cao D, Russell R, Handschumacher RE and Pizzorno G: Expression, characterization, and detection of human uridine phosphorylase and identification of variant uridine phosphorolytic activity in selected human tumors. Cancer Res 58: 5418-5424, 1998.
51. Maehara Y, Sakaguchi Y, Kusumoto T, Kusumoto H and Sugimachi K: Species differences in substrate specificity of pyrimidine nucleoside phosphorylase. J Surg Oncol 42: 184-186, 1989.
52. Thompson IM, Ankerst DP, Chi C, Goodman PJ, Tangen CM, Lucia MS, Feng Z, Parnes HL and Coltman CA Jr: Assessing prostate cancer risk: Results from the Prostate Cancer Prevention Trial. J Natl Cancer Inst 98: 529-534, 2006.
53. Picard JC, Golshayan AR, Marshall DT, Opfermann KJ and Keane TE: The multi-disciplinary management of high-risk prostate cancer. Urol Oncol 30: 3-15, 2012.

54. Rampazzo C, Miazzi C, Franzolin E, Pontarin G, Ferraro P, Frangini M, Reichard P and Bianchi V: Regulation by degradation, a cellular defense against deoxyribonucleotide pool imbalances. Mutat Res 703: 2-10, 2010.
55. Roosild TP, Castronovo S, Villoso A, Ziemba A and Pizzorno G: A novel structural mechanism for redox regulation of uridine phosphorylase 2 activity. J Struct Biol 176: 229-237, 2011.
56. Kobayashi Y, Wada Y, Ohara T, Okuda Y, Suzuki N, Hasegawa K, Kiguchi K and Ishizuka B: Enzymatic activities of uridine and thymidine phosphorylase in normal and cancerous uterine cervical tissues. Hum Cell 20: 107-110, 2007.
57. Yasuno M, Mori T, Koike M, Takahashi K, Toi M, Takizawa T, Shimizu S, Yamaguchi T and Matsumoto H: Importance of thymidine phosphorylase expression in tumor stroma as a prognostic factor in patients with advanced colorectal carcinoma. Oncol Rep 13: 405-412, 2005.
58. Oppenheim JJ, Zachariae CO, Mukaida N and Matsushima K: Properties of the novel proinflammatory supergene intercrine cytokine family. Annu Rev Immunol 9: 617-621, 1991.
59. Takahashi Y, Bucana CD, Liu W, Yoneda J, Kitadai Y, Cleary KR and Ellis LM: Platelet-derived endothelial cell growth factor in human colon cancer angiogenesis: Role of infiltrating cells. J Natl Cancer Inst 88: 1146-1151, 1996.
60. Cao D, Ziemba A, McCabe J, Yan R, Wan L, Kim B, Gach M, Flynn S and Pizzorno G: Differential expression of uridine phosphorylase in tumors contributes to an improved fluoropyrimidine therapeutic activity. Mol Cancer Ther 10: 2330-2339, 2011.
61. Brown NS and Bicknell R: Thymidine phosphorylase, 2-deoxy-D-ribose and angiogenesis. Biochem J 334: 1-8, 1998.
62. Di Napoli A and Signoretti S: Tissue biomarkers in renal cell carcinoma: Issues and solutions. Cancer 115: 2290-2297, 2009.