Significance of the TMPRSS2:ERG gene fusion in prostate cancer

ZHU WANG1*, YULIANG WANG2*, JIANWEN ZHANG1, QIYI HU1, FAN ZHI1, SHENGPING ZHANG1, DENGQI MAO1, YING ZHANG1 and HUI LIANG1

1Department of Urology, People’s Hospital of Longhua New District of Shenzhen and Affiliated Shenzhen Longhua Hospital of Southern Medical University, Shenzhen, Guangdong 518109; 2Department of Urology, Peking University People’s Hospital, Beijing 100044, P.R. China

Received November 4, 2016; Accepted June 28, 2017

DOI: 10.3892/mmr.2017.7281

Abstract. The transmembrane protease serine 2:v-ets erythroblastosis virus E26 oncogene homolog (TMPRSS2:ERG) gene fusion is common in prostate cancer, while its functional role is not fully understood. The present study aimed to investigate the significance of the TMPRSS2:ERG gene fusion in human prostate cancers using bioinformatics tools. Comprehensive alteration analysis of TMPRSS2 and ERG in 148 different human cancer studies was performed by cBioPortal, and the mRNA expression level of the ERG gene was evaluated using Oncomine analysis. Furthermore, lentiviral short hairpin (sh)RNA-mediated knockdown of TMPRSS2:ERG was performed to study the impact of ERG silencing on cell proliferation and cell cycle distribution in prostate cancer cells. The results demonstrated that the TMPRSS2 and ERG genes were mostly altered in prostate cancer, and the most frequent alteration was gene fusion. Oncomine analysis demonstrated that the ERG gene was significantly upregulated in prostate clinical samples compared with the normal prostate gland in four independent datasets, and a positive association was observed between potassium inwardly-rectifying channel subfamily J member 15, down syndrome critical region gene 4, potassium inwardly-rectifying channel subfamily J member 6 and ERG gene expression. There were 272 mutations of the ERG gene identified in the cBioPortal database; among the mutations, 2 missense mutations (R367C and P401H) were regarded as functional mutations (functional impact score >1.938). Furthermore, the present study successfully knocked down ERG gene expression through a lentiviral-mediated gene silencing approach in VCaP prostate cancer cells. The ERG mRNA and protein expression levels were both suppressed significantly, and a cell-cycle arrest at G1/S phase was observed after ERG gene silencing. In conclusion, these bioinformatics analyses provide novel insights for TMPRSS2:ERG fusion gene study in prostate cancer. Target inhibition of ERG expression could significantly cause cell growth arrest in prostate cancer cells, which could be a potentially valuable target for prostate cancer treatment. However, the precise mechanism of these results remains unclear; therefore, further studies are required.

Introduction

Prostate cancer is one of the most frequent malignancies and the most common leading cause of cancer-associated death in men all over the world, particularly in developed countries (1,2). In the past decades, prostate specific antigen (PSA) was the only widely-used serum biomarker for prostate cancer. However, due to the extensive use of serum PSA testing, the prostate cancer-specific mortality has increased significantly, which results in over-diagnosis or over-treatment (3). Multiple technologies have been applied to identify novel prostate cancer biomarkers in tissues and blood of patients. Nevertheless, no biomarker has been identified to replace the routine use of PSA at present.

Recently, gene fusion transcripts of transmembrane protease serine 2 (TMPRSS2):v-ets erythroblastosis virus E26 oncogene homolog (ERG), also termed TMPRSS2:ERG or T2E, have been identified as promising urinary novel biomarkers in prostate cancer (4,5). A study in 2005 demonstrated that up to 55% prostate cancer cases were identified to have ERG over-expression, using a novel biostatistical method called cancer outlier profile analysis (6). Furthermore, the overexpression of ERG is in the majority of tumors driven by fusion of the ERG gene with TMPRSS2, which are both located on chromosome 21 (7). TMPRSS2 is a prostate-specific and androgen-response gene that encodes a protein belonging to the serine protease family, which functions in prostate carcinogenesis and relies on gene fusion with ETS transcription factors, such as ERG and ETV1 (8). ERG is an oncogene that encodes a member of the erythroblast transformation-specific family of transcription factors (9), which is a key regulator of
cell proliferation, differentiation, angiogenesis, inflammation and apoptosis. The TMPRSS2:ERG gene fusion is the most frequent genomic alteration in prostate cancer cases and results in overexpression of the transcription factor ERG (10), which is present in both early- and late-stage prostate cancer (castration-resistant prostate cancer, CRPC) (6,11).

Numerous studies have evaluated the significance of TMPRSS2-ERG in prostate cancer patients with varying results (12), some of which indicated that the fusion gene is not an important predictor of prostate cancer mortality and recurrence (13), while other studies demonstrated that TMPRSS2:ERG fusion was associated with an increased risk of prostate cancer mortality (13-16). The present study examined the expression pattern of the TMPRSS2:ERG fusion gene in human pan-cancers, including prostate cancer, by using the publically available data from cBioPortal. Based on these findings, the present study specifically analyzed the ERG alterations, mRNA expression, mutations and interaction networks in several prostate cancer datasets. Furthermore, the functional role of ERG in prostate cancer cells was examined by lentiviral-mediated knockdown approaches. The present study provides novel insights for the TMPRSS2:ERG fusion gene study in prostate cancer.

Materials and methods

Determination of TMPRSS2 and ERG alterations across different cancer types. The frequency of TMPRSS2 and ERG gene alterations (including mutations, deletions, copy number gains and amplifications) was performed across multiple cancer types using the cBioPortal for Cancer Genomics database (www.cbioportal.org), which contains 147 common cancer studies with the details of almost 23,000 patients. All searches were performed according to the online instructions of cBioPortal.

Oncomine database analysis. ERG mRNA expression levels in prostate cancer were compared with its matched normal tissues by using The Cancer Genome Atlas (TCGA) datasets in the Oncomine database (www.oncomine.org). The threshold used to obtain the most significant probes of the queried gene for each microarray data included a two-fold difference in mRNA expression levels.

ERG gene silencing by short hairpin (sh)RNA in VCaP cells. The prostate cancer cell line VCaP which obtained from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China) and was cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Total RNA was reverse-transcribed into cDNA using a PrimeScript™ RT reagent kit (Takara Bio, Inc., Otsu, Japan) according to the manufacturer's instructions. The temperature protocol that was used was as follows: At 37°C for 15 min and at 85°C for 5 sec. Quantitative polymerase chain reaction (qPCR) was used to evaluate the ERG silencing effect at the mRNA level using SYBR Premix Ex Taq (Takara Bio, Inc.) according to the manufacturer's instructions. The thermocycling conditions that were used were as follows: At 95°C for 30 sec, followed by 40 cycles at 95°C for 15 sec, at 60°C for 20 sec and at 72°C for 30 sec. The following oligonucleotide primers were used: ERG, forward 5'-ATCGGATA TTGGCGCACTC‑3’; reverse 5'-GTGCAATGTCGCGGAG GA-3’; and β-actin, forward 5'-GGACTTTTGCAAGAGAT GG-3’ and reverse 5'-AGCAGTCGGTTGGCGTACAG-3’. The relative gene expression data were assayed using the comparative Cq method as described previously (17,18).

Total cellular proteins were extracted from 70-80% confluent cultured cells after 48 h transfection using ice cold lysis buffer (20 mM HEPES, 0.1% SDS, 1 mM EDTA, 1 mM EGTA, 10 mM monothioglycerol, 1 mM PMSF, 5 mM leupeptin, 0.25 M sucrose). Protein concentration was determined using a bicinchoninic acid protein assay kit (Thermo Fisher Scientific, Inc.). Equal amounts of extracted protein samples (30 µg) were separated by standard 12% SDS-PAGE and transferred onto polyvinylidene difluoride membranes. Following blocking with 5% non-fat dry milk at room temperature for 1 h, membranes were probed with optimally diluted primary antibodies at 4°C overnight, then incubated with a horseradish peroxidase-conjugated secondary antibody (cat no. ab6721; 1:5000; Abcam, Cambridge, MA, USA) at room temperature for 1 h. Protein bands were visualized with enhanced chemiluminescence western blot reagents (GE Healthcare Life Sciences, Little Chalfont, UK) as described previously (19). Blots were semi-quantified by densitometry using ImageJ software version 2.0 (National Institutes of Health, Bethesda, MD, USA). Primary antibodies used were as follows: Anti-ERG monoclonal antibody (cat no. ab92513; Abcam, Cambridge, MA, USA; 1:1,000) and anti-β-actin (cat no. 4970; Cell Signaling Technology, Inc., Danvers, MA, USA; 1:1,000).

Cell proliferation assay. Cell proliferation was assessed by MTT assay according to the manufacturer’s protocol (Roche Diagnostics GmbH, Mannheim, Germany). The formed formazan crystals were dissolved by adding 100 µl/well acidic SDS buffer [10% SDS, 0.16% (6 mol/l) HCI and 5% isobutyl alcohol] and incubating overnight in a CO2-free incubator at 37°C. The optical density (OD) absorbance was measured in a microplate reader (Perkin Elmer Victor3 1420 Multilabel Plate Counter, PerkinElmer, Inc., Waltham,
MA, USA). Experiments were repeated three times, and data were represented as the mean of five-replicate wells ± standard error.

Cell cycle analysis. Cell cycle analysis of control and ERG-silenced VCaP cells from 3 independent biological replicates were collected. The cells were washed in PBS, and then fixed in 70% ethanol for 30 min at -20°C. The fixed cells were washed three times, resuspended in PBS containing 10 µg/ml of RNase A for 30 min, and then incubated with 10 µg/ml propidium iodide (PI) for 30 min in the dark. Subsequently, the samples were used for DNA flow cytometry (ALTRA cell sorting system, Beckman Coulter, Inc., Brea, CA, USA) analysis. For each measurement, at least 15,000 cells were acquired. Analysis of cell cycle was performed with ModFit LT2 software version 2.0 (Verity Software House, Inc., Topsham, ME, USA).

STRING analysis. STRING software (https://string-db.org/) (20) was used to generate the network of predicted associations for ERG protein. The network was set in evidence mode, in which the associations of the proteins were predicted based on up to 7 different evidences (the presence of fusion evidence, neighborhood evidence, co-occurrence evidence, text-mining evidence, database evidence and co-expression evidence).

Statistical analysis. GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA) was used for statistical analyses. All analysis was performed using the unpaired Student’s t-test or analysis of variance followed by a post hoc Tukey test for multiple comparisons. The data were presented as mean ± standard deviation. P<0.05 was considered to indicate a statistically significant difference.

Results

Determination of TMPRSS2 and ERG gene alterations across different human cancer types. By pan-cancer analysis, it was demonstrated that the TMPRSS2 and ERG alterations (including mutations, deletions and amplifications) were mainly observed in one breast cancer study and most of the prostate cancer studies (Fig. 1A). In the breast cancer xenografts study (21) TMPRSS2 and ERG were notably altered in 51.7% of 29 cases, among which 48.3% (14 cases) were amplification. Only one case contained a deep deletion in ERG gene in the breast cancer study mentioned above.

However, in prostate cancer studies, the most frequent alteration of TMPRSS2 and ERG was gene fusion (Fig. 1B). Amplification, missense mutation and deep deletion were less frequently observed. Studies in prostate adenocarcinoma (22,23) showed that TMPRSS2 and ERG were altered in over 47% in prostate cancers, and 46% of them were gene fusion. Moreover, two metastatic prostate cancer datasets (24,25) demonstrated that 42 and 49% of the patients had ERG gene fusion (Fig. 1B).

Although there was less frequent mutation than gene fusion observed in TMPRSS2 and ERG, some of them may serve important roles in prostate cancer progression. There were 272 mutations of the ERG gene identified in the eBioPortal database; among the mutations, 2 missense mutations (R367C and P401H) were regarded as functional mutations (Functional impact score >1.938). The details of missense mutations of TMPRSS2 and ERG with high mutation assessor score are presented in Fig. 2A and B.

ERG gene is overexpressed in prostate cancer clinical samples. Given the high-frequent alterations of TMPRSS2 and ERG observed in prostate cancer studies, the mRNA expression profile of ERG in prostate cancer in four independent datasets were analyzed using Oncomine analysis. Notably, ERG mRNA expression levels were significantly upregulated in prostate cancer cases compared with their normal tissues in all four independent datasets (Fig. 2C). Furthermore, the co-expression gene of ERG in a cohort of 230 patients with prostate cancer was also evaluated (Taylor Prostate) (26) in the Oncomine database, as well as the interaction networks by STRING (Fig. 3B). The most correlated gene of ERG was potassium inwardly-rectifying channel subfamily J member 6 (KCNJ6), potassium inwardly-rectifying channel subfamily J member 15 (KCNJ15) and down syndrome critical region gene 4 (DSCR4; Fig. 3A).

Table I. shRNA sequences targeting the ERG gene.

| shRNA duplex | Sequence (5’-3’) |
|-------------|-----------------|
| ERG-shRNA1  | CCGGTTCATATCAAGGAAGCCTTTATCAGATAGGCCCTTCCTTGATATGAGCTTTTTTAAATAAAAAGCCATATCAGAAGGAGCCTTCCTTGATATGAGCTTTTTAATAAAAAAACCCACAAGAAGATGAACCTTCTCTTGAAAAAGTCATCTTTCTTGAGGGTTG |
| ERG shRNA2  | CCGGTTCACCCACAGAAGATGAACTTTTCAAGAGATGTCTCTCTGTTGCCTTTTTATGTTGTTTAAATTTTTTTTTTTTTT |
| EEG-shRNA3  | CCGGTGATGATGTTGATAAAGCCTTTATCATACATCATCTTTTTAATTTTTT |

ERG, v-ets erythroblastosis virus E26 oncogene homolog; shRNA, short hairpin RNA.
shRNA specifically reduces ERG expression in VCaP cells. To study the role of TMPRSS2:ERG in this context of preexisting genetic alterations, lentiviral-mediated shRNA was used to knock down ERG gene expression in VCaP cells.
Figure 2. Missense mutations and mRNA expression of TMPRSS2 and ERG in prostate cancer. Missense mutations of (A) ERG and (B) TMPRSS2 in prostate cancer. (C) mRNA expression profile of ERG gene in four independent prostate cancer studies using Oncomine analysis. TMPRSS2, transmembrane protease serine 2; ERG, v-ets erythroblastosis virus E26 oncogene homolog.
that are known to harbor the TMPRSS2:ERG gene fusion (11). Three pairs of shRNA of ERG were designed; both RT-qPCR and western blot analysis demonstrated that shRNA1-ERG exhibited the highest knockdown efficiency compared with the scramble control. The mRNA expression level of ERG was decreased by >79% and the protein expression level was reduced >93% in the shRNA-ERG1 viral-infected VCaP cells (Fig. 4A, P<0.05). In addition, there was no significant changes in cell morphology observed in the shRNA-ERG infected cells (Fig. 4B).
Knockdown of ERG in VCaP cells inhibits cell proliferation through cell cycle arrest. Upon the shRNA-mediated knockdown of ERG in VCaP cells, cell proliferation was determined by MTT assay and the cell cycle distribution was

Figure 4. shRNA mediate ERG gene silencing and functional studies in prostate cancer cells. (A) shRNA-ERG knockdown efficiency studied by reverse transcription-quantitative polymerase chain reaction and western blotting. (B) Phenotype characterization of VCaP cells after ERG silencing by shRNA-ERG. (C) Flow cytometric analysis of VCAP cells and (D) % of cells in each phase of the cell cycle after ERG gene silencing. (E) Cell proliferation analysis using MTT methods. Data are presented as the mean ± standard deviation of three independent experiments. *P<0.05 vs. blank control or negative control. shRNA, short hairpin RNA; TMPRSS2, transmembrane protease serine 2; ERG, v-ets erythroblastosis virus E26 oncogene homolog.
assessed by flow cytometry. The results demonstrated that specific knockdown of the ERG gene in prostate cancer cells could cause G2/G0 cell cycle arrest (Fig. 4C and D) and significantly inhibit cell proliferation (Fig. 4E) compared with the scramble virus-infected controls in VCaP cells.

Discussion

The discovery of fusion genes involving the TMPRSS2 promoter region with ERG coding DNA sequences in $\geq$50% of prostate cancer cases has provide a significant insight for exploration of useful biomarkers for prostate cancer study and clinical treatment (11,27). However, the prognostic value of TMPRSS2:ERG gene fusion is a hotly debated topic in the current literature (13,28). The present study analyzed the TMPRSS2 and ERG gene expression and alteration in multi-cancer types by using cBioPortal, and indicated that these genes were mostly altered in prostate cancer, and the most frequent alteration was gene fusion, which was consistent with previous studies (11). Notably, some missense mutations with high mutation assessor score were identified in the TMPRSS2 and ERG gene, which may serve important roles in the gene fusion process and prostate cancer development.

Important studies in recent years clarified the significance of the TMPRSS2:ERG gene fusion in prostate cancer, and most of them indicated that the presence of the fusion gene product denotes an unfavorable outcome (7,15,29). The most direct consequence for the TMPRSS2:ERG gene fusion was the significant upregulation of the ERG gene, which is not normally expressed in prostate epithelia (30), and is likely to be involved in prostate cancer development by enhancing tumor angiogenesis (31). The high expression of ERG in prostate cancer is associated with advanced tumor stage, shorter survival time, high Gleason score and metastasis (12).

Full-length ERG is a 486 amino-acid 54 kDa transcription factor, and contains an ETS DNA-binding domain and a pointed domain (32,33). Normally, ERG is highly expressed in the embryonic mesoderm and endothelium and serves a critical role in the formation of the vascular system and the urogenital tract, and in bone development (34-36). Abrupt expression of the ERG gene has a major impact on cell invasion (37) and metastasis (38), as well as the differentiation of prostate epithelium (39). The ERG gene is the first demonstration of constitutive oncogene activation in prostate cancer; however, the functional consequences and mechanisms of the TMPRSS2:ERG gene fusion are not fully understood. In particular, the co-expression genes and interaction networks have not been characterized.

Recently, interest in the TMPRSS2:ERG fusion gene in prostate cancer remains high, which is supposed to be a novel biomarker, therapy target, diagnostic and prognostic indicator in prostate cancer (7,36). Therefore, the present study also surveyed ERG gene expression by Oncomine analysis, based on RNA-Seq data, which demonstrated that the ERG gene was significantly increased in four independent prostate cancer study datasets. Based on these findings, the present study designed specific shRNA of the ERG gene for loss-of-function study. It was demonstrated that ERG gene silencing could significantly inhibit prostate cancer cells proliferation, and induce G0/G1 cell cycle arrest in prostate cancer cells. These results suggested that not only the alteration of TMPRSS2 and ERG gene could be a specific marker in prostate cancer, but also could be a potential therapy target in prostate cancer. However, the exact mechanism remains unclear; therefore, further studies are required to illustrate the signaling pathways involved in this progression.

Acknowledgements

The present study was supported by Shenzhen Science and Technology Program Basic Research Project (grant no. JCYJ20150402144905865).

References

1. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu QX and He J: Cancer statistics in China, 2015. CA Cancer J Clin 66: 115-132, 2016.
2. Siegel RL, Miller KD and Jemal A: Cancer statistics, 2016. CA Cancer J Clin 66: 7-30, 2016.
3. Velonas VM, Woo HH, dos Remedios CG and Assinder SJ: Current status of biomarkers for prostate cancer. Int J Mol Sci 14: 11034-11060, 2013.
4. Stephan C, Rittenhouse H, Hu X, Cammann H and Jung K: Prostate-Specific Antigen (PSA) Screening and New Biomarkers for Prostate Cancer (PCa). EHJFC 25: 55-78, 2014.
5. Teoh JY, Tsu JH, Yuen SK, Chan SY, Chiu PK, Lee WM, Wong KW, Ho KL, Hou SS, Ng CF and Yiu MK: Prognostic significance of time to prostate-specific antigen (PSA) nadir and its relationship to survival beyond time to PSA nadir for prostate cancer patients with bone metastases after primary androgen deprivation therapy. Ann Surg Oncol 22: 1385-1391, 2014.
6. Tomlins SA, Palanisamy N, Siddiqui J, Chinnaiyan AM and Kunju LP: Antibody-based detection of ERG rearrangements in prostate core biopsies, including diagnostically challenging cases: ERG staining in prostate core biopsies. Arch Pathol Lab Med 136: 935-946, 2012.
7. Hossain D and Bostwick DG: Significance of the TMPRSS2:ERG gene fusion in prostate cancer. BJU Int 111: 834-835, 2013.
8. Yu J, Ju Y, Mani RS, Cao Q, Brenner CJ, Cao X, Wang X, Wu L, Li J, Hu M, et al.: An integrated network of androgen receptor, polycomb, and TMPRSS2:ERG gene fusions in prostate cancer progression. Cancer Cell 17: 443-454, 2010.
9. Gasi Tandevelt D, Boormans J, Hermans K and Trapman J: ETS fusion genes in prostate cancer. Endocr Relat Cancer 21: R143-R152, 2014.
10. Kissick HT, On ST, Dunn LK, Sandra MG, Asara JM, Pellegrini KL, Noel JK and Arredouani MS: The transcription factor ERG increases expression of neurotransmitter receptors on prostate cancer cells. BMC Cancer 15: 604, 2015.
11. Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, Varambally S, Cao X, Tchinda J, Kuefer R, et al.: Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science 310: 644-648, 2005.
12. Hagglof C, Hammarsten P, Strömvall K, Sun XW, Varambally S, Cao X, Tchinda J, Kuefer R, et al.: The transcription factor ERG increases expression of neurotransmitter receptors on prostate cancer cells. BMC Cancer 15: 604, 2015.
13. Stephey MJ, Uran K, Yee KL, Li G, Yi S, et al.: TMPRSS2:ERG expression predicts prostate cancer survival and associates with stromal biomarkers. PLoS One 9: e86824, 2014.
14. Pettersson A, Graff RE, Bauer SR, Pitt MJ, Lis RT, Stack EC, Martin NE, Kunz L, Penney KL, Ligon AH, et al.: The TMPRSS2:ERG rearrangement, ERG expression, and prostate cancer outcomes: A cohort study and meta-analysis. Cancer Epidemiol Biomarkers Prev 21: 1497-1509, 2012.
15. Demichielis F, Fall K, Perren S, André O, Schmidt F, Setlur SR, Hoshida Y, Mosquera JM, Pavitan Y, Lee C, et al.: TMPRSS2:ERG gene fusion associated with lethal prostate cancer in a watchful waiting cohort. Oncogene 26: 4596-4599, 2007.
16. St John J, Powell K, Conley-Lacomb MK and Chinni SR: TMPRSS2:ERG Fusion gene expression in prostate tumor cells and its clinical and biological significance in prostate cancer progression. J Cancer Sci Ther 4: 94-101, 2012.
17. Attard G, Clark J, Ambroisine L, Fisher G, Kovacs G, Floth P, Berney D, Foster CS, Fletcher A, Gerald WL, et al.: Duplication of the fusion of TMPRSS2 to ERG sequences identifies fatal human prostate cancer. Oncogene 27: 253-263, 2008.
17. Xu L, Wang Z, Li XF, He X, Guan LL, Tuo JL, Wang Y, Luo Y, Zhong HL, Qiu SP and Cao KY: Screening and identification of significant genes related to tumor metastasis and PSMA in prostate cancer using microarray analysis. Oncol Rep 30: 1920-1928, 2013.

18. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.

19. Xu L, Wang Z, He SY, Zhang SF, Luo HJ, Zhou K, Li XF, Qiu SP and Cao KY: Bax-interacting factor-1 inhibits cell proliferation and promotes apoptosis in prostate cancer cells. Oncol Rep 36: 3513-3521, 2016.

20. Szklarczyk D, Franceschini A, Kuhn M, Simonovic M, Roth A, Minguez P, Doerks T, Stark M, Muller J, Bork P, et al.: The STRING database in 2011: Functional interaction networks of proteins, globally integrated and scored. Nucleic Acids Res 39 (Database Issue): D561-D568, 2011.

21. Eirew P, Steif A, Khattra J, Carver BS, Arora VK, Kaushik P, Cerami E, Reva B, Taylor BS, Schultz N, Hieronymus H, Sun Y, Jacobsen A, Sinha R, Larsson E, Gao JJ, Aksoy BA, Dogrusoz U, Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.

22. Petrovics G, Liu A, Shaheduzzaman S, Furusato B, Sun C, Chen Y, Nau M, Ravindranath L, Chen Y, Dobi A, et al.: Frequent overexpression of ETS-related gene-1 (ERG1) in prostate cancer transcripome. Oncogene 24: 3847-3852, 2005.

23. Young A, Palanisamy N, Siddiqui J, Wood DP, Wei JT, Chinnaiyan AM, Kunju LP and Tomlins SA: Correlation of urine TMPRSS2:ERG and PCA3 to ERG+ and total prostate cancer burden. Am J Clin Pathol 138: 685-696, 2012.

24. Grasso CS, Wu YM, Robinson DR, Cao X, Dhanasekaran SM, Khan AF, Quist MJ, Jing X, Lonigro RJ, Brenner JC, et al.: The mutational landscape of lethal castration-resistant prostate cancer. Nature 487: 239-243, 2012.

25. Gao JJ, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.