ANTICANCER RESEARCH *36*: 4787-4794 (2016)
doi:10.21873/anticanres.11037

Prognostic Significance of *TMPRSS2-ERG* Fusion Gene in Prostate Cancer

VLASTIMIL KULDA[1], ONDREJ TOPOLCAN[2], RADEK KUCERA[2], MICHAELA KRIPNEROVA[3], KRISTYNA SRBECKA[1], MILAN HORA[4], ONDREJ HES[5], JIRI KLECKA[6], VACLAV BABUSKA[1], MILENA ROUSAROVA[2], VERONIKA BENSON[7] and MARTIN PESTA[3]

Departments of [1]Medical Chemistry and Biochemistry, [3]Biology, [4]Urology and [5]Pathology, Faculty of Medicine in Pilsen, Charles University in Prague, Pilsen, Czech Republic; [2]Department of Nuclear Medicine – Immunoanalytic Laboratory, University Hospital in Pilsen, Pilsen, Czech Republic; [6]Urocentrum Plzen, Pilsen, Czech Republic; [7]Institute of Microbiology, v.v.i., Academy of Sciences of the Czech Republic, Prague, Czech Republic

Abstract. *Background/Aim: Current research of prostate cancer (PCa) offers a promising way of identifying patients with adverse prognosis who do benefit from radical treatment that can affect quality of life as resections are associated with numerous side-effects. The aim of our study was to evaluate the relationship of TMPRSS2-ERG fusion gene status, tumor tissue prostate-specific antigen (PSA), prostate cancer antigen 3 (PCA3), miR-23b, miR-26a and miR-221 expression levels in combination with preoperative serum PSA level to the risk of PCa recurrence after radical prostatectomy. Patients and Methods: The study group consisted of 108 patients who underwent radical prostatectomy. PSA was measured in peripheral blood collected preoperativelly. The expression of TMPRSS2-ERG transcript and the expression of miR-23b, miR-26a and miR-221 in formalin-fixed, paraffin-embedded (FFPE) tumor tissues was analyzed by reverse transcription (RT) real-time polymerase chain reaction (PCR). Results: Significantly shorter time to recurrence was observed in patients with high expression of TMPRSS2-ERG (p=0.0020). High levels of preoperative PSA (>10.0 ng/ml) proved to be marker of shorter time to recurrence (p=0.0153). The most promising marker of the risk of recurrence after radical prostatectomy was a combination of high level of preoperative serum PSA and high expression of TMPRSS2-ERG fusion transcript in tumor tissue (p=0.0001). Conclusion: A combination of high preoperative serum PSA and high expression of TMPRSS2-ERG could be promising in distinguishing those tumors that are aggressive and life-threatening.*

Correspondence to: Dr. Martin Pesta, Department of Biology, Faculty of Medicine in Pilsen, Alej Svobody 76, 326 00, Plzen, Czech Republic. Tel: +420 377593261, Fax: +420 377593209, e-mail: martin.pesta@lfp.cuni.cz

Key Words: Prostate cancer, *TMPRSS2-ERG*, PSA, PCA3, microRNA, prognosis.

Prostate cancer (PCa) belongs to the most commonly diagnosed cancers worldwide having a higher incidence in Western countries, which might be due to environmental and lifestyle factors, as well as the greater scale of prostate-specific antigen (PSA) screening in the developed countries. PCa has been proven to be a very heterogenous disease, with individual cases differing in both the speed of progression and overall prognosis (1). The main issue of PCa management is to distinguish those tumors that are subsequently progressing. This would make it possible to limit the number of patients undergoing radical surgery to cases of life-threatening tumors as radical prostatectomy is associated with numerous side-effects (2, 3). To assure radical treatment in cases of aggressive prostate cancer a set of markers reliably indicating the nature of the individual case needs to be identified.

Current research offers a promising way of identifying patients with adverse prognosis. It is based on the knowledge of the pathogenesis of PCa and on the role of the fusion gene *TMPRSS2-ERG* described and linked to PCa by Tomlin *et al.* in 2005 (4). The origin of the *TMPRSS2-ERG* oncogene can be traced to a recurrent rearrangement (translocation, interstitial deletion) on long arm of chromosome 21 that fuses androgen-regulated prostate-specific gene promoter of the transmembrane protease serine 2 (*TMPRSS2*), locus 21q22.3, and the gene *ERG*, locus 21q22.2, a member of the transcription factor erytroblastosis virus E26 transforming sequence family (ETS) (5). Prostate tissue has a strong

androgen dependency and so does PCa, with the signal pathways being mediated by androgen receptors (6). As a result of a *TMPRSS2-ERG* fusion, overexpression of oncogenic transcription factor ERG is driven by androgens through androgen-responsive elements of *TMPRSS2* 5'-untranslated region. Nevertheless, as the disease evolves into castration-resistant PCa, *TMPRSS2-ERG* fusion gene overcomes androgen regulation (7). Fusion gene *TMPRSS2-ERG* appears in early high grade prostatic intraepithelial neoplasia (PIN) and it has been observed that its presence in PCa cells promotes the loss of the tumor supressor gene *PTEN* and other alterations, which in turn lead to the speed up of progression of the disease (8).

In addition to analysis of *TMPRSS2-ERG* fusion gene significance, we also focused on selected microRNAs. MicroRNAs are non-coding RNAs consisting of a small number of nucleotides (~22 nt) that can influence gene expression by interfering with translation or by destabilising target mRNAs. In this way, microRNAs can affect cancerogenesis in both directions; slow the progression by inbibiting oncogen expression or fasten it by down-regulating tumor supressors (9). Many studies have described changes in expression of microRNAs in PCa tissue and their involvement in initiation, progression and the effects of treatment (10). Based on our research of published literature, we chose three tumor-suppressive miRNAs (miR-23b, miR-26a and miR-221) whose levels could be of interest in making prediction of tumor aggressiveness.

The expression level of miR-23b has been observed to be down-regulated in PCa and restoration of the expression inhibited cancer cell proliferation, migration and invasion in PCa cell lines (11). In PCa, miR-23b supresses proto-oncogene Src kinase and its up-regulation shows a strong positive correlation to better prognosis (12). Both miR-26a and miR-221 function as cell proliferation inhibitors, miR-26a inhibits prostate cancer progression by repression of Wnt5a (13) and La-related protein 1 (LARP1) (14). Lower miR-221 expression was associated with a higher risk of recurrence after radical prostatectomy (15).

Furthermore, we included analysis of tumor tissue expression of PSA and prostate cancer antigen 3 (PCA3), formerly referred to as differential display code 3 (DD3). *PCA3* is a long non-coding RNA (lncRNA) beginning to be used as a diagnostic marker of PCa. Recent meta-analysis concluded that urine PCA3 test had acceptable sensitivity and specificity for the diagnosis of PCa (16). There are studies showing association of high PCA3 expression with pathological features of PCa, being predictive of high Gleason score, high-stage and high-volume disease (17, 18). However, less is known about prognostic significance of PCA3 on the basis of the follow-up of patients.

The aim of our study was to evaluate the relationship of *TMPRSS2-ERG* fusion gene status, tumor tissue PSA, PCA3, miR-23b, miR-26a and miR-221 expression levels in combination with routinely used preoperative serum PSA level to the risk of PCa recurrence after radical prostatectomy and, in this way, to predict the aggressivity of the tumor.

Patients and Methods

Patients. Our study group consisted of 108 patients who underwent radical prostatectomy between January 2011 and June 2012 at the Department of Urology of the University Hospital in Pilsen, Czech Republic. All the patients exhibited elevated serum PSA levels or abnormal digital rectal examination. Indication for surgery was confirmed by positive biopsy results, namely the detection of cancerous cells. The median age was 62.9 years (range=43.8-73.9). Clinicopathological data, such as age at the time of surgery, preoperative PSA level, stage of the disease according to the International Union against Cancer (IUCC) and Gleason score are listed in Table I. Approval was obtained from the Institutional Ethics Committee and written informed consent from each patient.

Tissue samples and RNA extraction. The FFPE (formalin-fixed, paraffin-embedded) tissue samples were used for RNA extraction as we described previously (19, 20). Briefly, areas selected for analysis highlighted by pathologist (either tumor tissue or adjacent normal prostate tissue) were manually macrodisected from 15-μm thick FFPE tissue sections prepared with microtome (Leica RM 2135; Leica Biosystems, Nussloch, Germany). Total RNA was isolated using the RNeasy FFPE Kit (Qiagen, GmbH, Hilden, Germany) according to the protocol of the manufacturer. The concentration of the isolated RNA was measured with NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA); in case of lower concentration than 15 ng/μl the isolation was repeated.

Quantitative estimation of TMPRSS2-ERG, PSA and PCA3 expression. Reverse transcription (RT) was performed from 250 ng of total RNA with Superscript III Reverse Transcriptase (Life Technologies, Carlsbad, CA, USA) and random hexamers as primers. Quantitative estimation of *TMPRSS2-ERG*, *PSA* and *PCA3* transcripts was performed by real-time polymerase chain reaction (PCR) method with UPL probes (Universal ProbeLibrary; Roche, Mannheim, Germany) on Stratagene Mx3005P apparatus (Agilent Technologies, Santa Clara, CA, USA). The sequences of primers for *TMPRSS2-ERG* fusion gene (forward primer 5'-TAGGC GCGAGCTAAGCAG-3' targeting *TMPRSS2* exon 1 and reverse primer 5'-GTCCATAGTCGCTGGAGGAG-3' targeting ERG exon 4) were previously designed and validated by other researchers (21, 22). We found the appropriate UPL probe (probe #1) for this pair of primers by ProbeFinder Software (Roche, Mannheim, Germany). The sequences of primers and corresponding UPL probes for *PSA* mRNA (forward primer 5'-GTGCTTGTGG CCTCTCGT-3', reverse primer 5'-CAGCAAGATCACGCTTTTGT-3', probe #44) and *PCA3* lncRNA (forward primer 5'-TGGGAAGGACCTGAT GATACA-3', reverse primer 5'-TGTGTGGCCTCAGA TGGTAA-3', probe #66) were generated by ProbeFinder Software. The PCR reactions were carried out in 96-well plates in a volume of 20 μl containing 1.0 μl of RT product, 2.0 μl of each primer and 2.0 μl of UPL probe and FastStart TaqMan Probe Master reaction mix (Roche, Mannheim, Germany). The reaction conditions were initial

Table I. *Clinicopathological characteristics of patients with prostate cancer (n=108).*

Characteristics	Number of patients	%
Age (years)		
<55	13	12.0
55-60	48	44.5
>60	47	43.5
Preoperative PSA (ng/ml)		
<4.0	5	4.6
4.0-10.0	58	53.7
>10.0	45	41.7
Clinical stage		
I	16	14.8
II	63	58.3
III	29	26.9
Gleason score		
6	37	34.2
7	54	50.0
8	11	10.2
9	6	5.6

PSA, Prostate-specific antigen.

Table III. *Relation between TMPRSS2-ERG fusion gene status and time to recurrence (Cox model).*

TMPRSS2-ERG status	Number of patients		HR	95% CI	*p*-Value
	+	–			
Mere presence	34	74	1.52	0.48-4.80	0.4739
High expression	13	95	6.14	1.95-19.39	0.0020*

HR, Hazard ratio; CI, confidence interval. *$p<0.05$.

denaturation at 95˚C for 10 min, followed by 50 cycles of 95˚C for 10 s and 60˚C for 30 s. The expression levels were normalized to total RNA and PSA (*PCA3*/*PSA* ratio). All samples were assessed in technical duplicates. If Ct values obtained from technical duplicates were in discrepancy, the sample assessment was repeated.

Quantitative estimation of microRNAs. A quantitative estimation of miR-23b, miR-26a and miR-221 was performed by a RT real-time PCR method using TaqMan® MicroRNA Assays (Applied Biosystems, manufactured by Roche, Branchburg, NJ, USA). We used *RNU6B* (U6snRNA) as a normalizer. The Ct values were corrected using calibrators to eliminate differences between individual runs of the Stratagene Mx3005P Real-Time PCR apparatus (Agilent Technologies, Santa Clara, CA, USA). The results are presented as normalized values as a ratio of the number of copies of the given gene to that of the reference gene. To obtain gene expression data we used the ΔΔCt approach ($2^{-\Delta\Delta Ct}$ algorithm).

Table II. *TMPRSS2-ERG fusion gene status in relationship to the stage of the disease and Gleason score.*

TMPRSS2-ERG	Presence detected		High expression	
all (n=108)	34	31.5%	13	12.0%
Clinical stage				
I (n=16)	7	43.8%	1	6.3%
II (n=63)	13	20.6%	6	9.5%
III (n=29)	14	48.3%	6	20.7%
Gleason score				
6 (n=37)	8	21.6%	3	8.1%
7 (n=54)	17	31.5%	7	13.0%
8 (n=11)	4	36.4%	2	18.2%
9 (n=6)	5	83.3%	1	16.7%

Quantitative estimation of blood serum PSA protein. Preoperative blood samples were taken from the cubital vein before any procedures involving the prostate manipulation, collected in VACUETTE® blood collection tubes (Greiner Bio-One, Kremsmünster, Austria). The serum was separated by centrifugation at 1700 × *g* for 10 minutes and PSA determined using the UniCel DxI 800 chemiluminescent immunoassay system (Beckman Coulter, Brea, CA, USA) as described previously (23).

Statistical analysis. SAS version 9.3 statistical software (SAS Institute Inc., Cary, NC, USA) was used for all statistical calculations. The results with $p<0.05$ were considered statistically significant. Non-parametric two-sided Wilcoxon signed-rank test was used for comparing the two groups (tumor tissue and normal prostate tissue) and Pearson's Chi-squared test for evaluating categorical data. Evaluation of prognostic significance (the relation of markers to time to recurrence) was performed as analysis of maximum likelihood estimates (Cox regression hazard model); the Kaplan-Meier survival distribution functions were generated for markers significant in Cox model.

Results

We have previously analyzed the *TMPRSS2-ERG* fusion gene status by the RT-qPCR method with UPL probes quantifying mRNA transcripts, so we were able to classify cases not only into categories (fusion gene present/absent) but according to the expression level as well. We detected presence of the *TMPRSS2-ERG* fusion in 34 out of 108 samples (31.5%). Nevertheless, in about two thirds of positive cases it was a late amplification with cycle threshold (Ct) above 40. Therefore, we decided to distinguish a category of high level of *TMPRSS2-ERG* fusion transcript expression (Ct≤40.0) in which 13 out of 108 samples (12.0 % fell within). We did not record statistically significant differences of the presence or high expression of *TMPRSS2-ERG* fusion gene in relationship to the stage of the disease, but there were differences related to Gleason

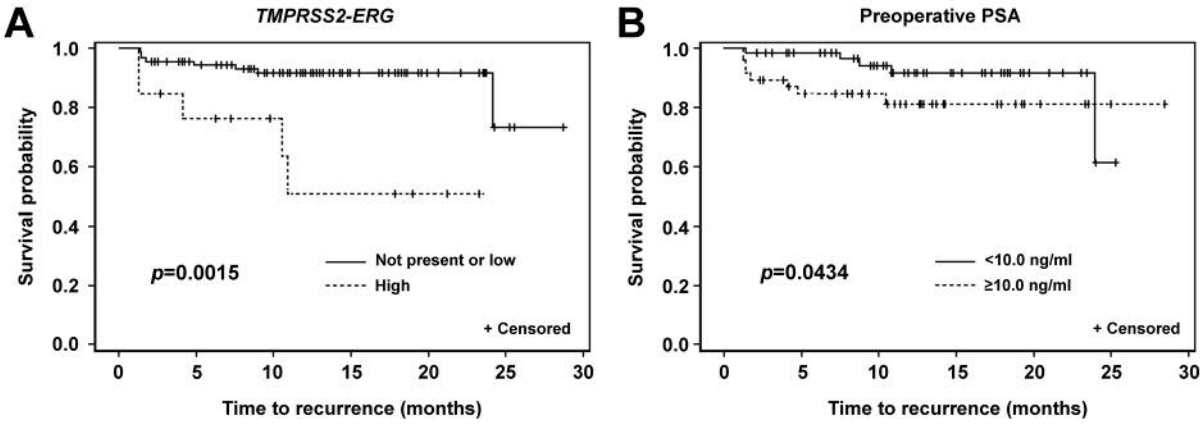

Figure 1. *Relation of TMPRSS2-ERG fusion transcript expression (A) and preoperative serum PSA level (B) to time to recurrence (Kaplan-Meier curves).*

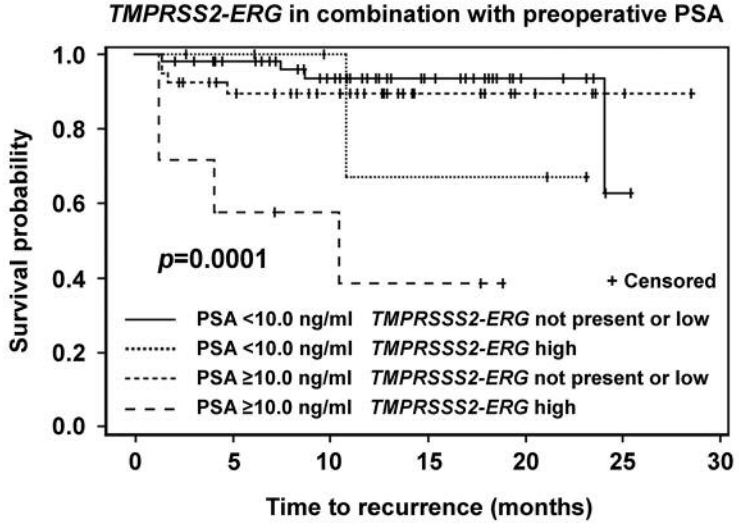

Figure 2. *Relation of TMPRSS2-ERG fusion transcript expression in combination with preoperative serum PSA level to time to recurrence (Kaplan-Meier curves). A combination of high PSA level and high TMPRSS2-ERG expression was associated with the shortest time to recurrence.*

score (Pearson's Chi-squared test, p=0.0259). Higher Gleason score was associated with higher percentage of *TMPRSS2-ERG* positivity (Table II).

The *TMPRSS2-ERG* fusion gene status was also evaluated in relation to time to recurrence (disease-free interval (DFI)). The mere presence of *TMPRSS2-ERG* had no relation to DFI; however, we found significantly shorter DFI in patients with high expression of *TMPRSS2-ERG* in the carcinoma tissue (p=0.0020, Cox model, analysis of maximum likelihood estimates); details in Table III. Preoperative PSA level, the currently available biomarker for PCa, was also evaluated by Cox model and its high levels proved to be marker of shorter DFI (p=0.0153). There was no correlation between preoperative PSA level and *TMPRSS2-ERG* fusion gene status. Kaplan-Meier survival curves were generated for both *TMPRSS2-ERG* fusion gene status (Figure 1A) and preoperative PSA (Figure 1B). Kaplan-Meier survival curves were also computed for the combinations of *TMPRSS2-ERG* status and preoperative serum PSA level (Figure 2). The category of patients with high preoperative PSA level splits according to the *TMPRSS2-ERG* status into groups with different prognosis; those with high expression of *TMPRSS2-ERG* have shorter DFI.

Furthermore, we evaluted the expression levels of microRNAs and found miR-23b, miR-26a and miR-221 to be down-regulated in tumor tissue compared to normal prostate

tissue (p=0.0031, p=0.0022, p<0.0001, respectively). We found no correlation of the expression of these microRNAs to clinical stage, Gleason score, *TMPRSS2-ERG* status or prognosis.

There was no significant difference in the expression of *PSA* mRNA in tumor tissue compared to normal prostate tissue. *PCA3* was significantly overexpressed in tumor tissue compared to normal prostate tissue for both ways of expression values normalization, to total RNA (p<0.0001) and as a *PCA3*/*PSA* ratio (p<0.0001). We found no correlation of the expression of either PSA or PCA3 to clinical stage, Gleason score, *TMPRSS2-ERG* status or prognosis.

The expression of miR-23b, miR-26a and miR-221 had no relation to *TMPRSS2-ERG* status and DFI. We recorded significantly lower expression of miR-221 in tumor tissue compared to normal prostate tissue (p=0.0005).

Discussion

It is known that some prostate carcinomas may not cause any trouble or only progress very slowly. Microscopic foci of prostate cancer are frequently randomly found in autopsies of men over the age of 50 who died from other reasons (24). Due to the progress in PCa screening, a rising number of men are diagnosed with PCa; nevertheless, not in all cases immediate radical treatment is necessary. There is an increasing need to distinguish tumors that are rapidly progressing to spare patients with slowly growing tumors the strain of overtreatment. To identify those patients with tumors that tend to progress, we used a panel of genes whose expression is significantly involved in PCa carcinogenesis (*TMPRSS2-ERG*, *PCA3*, miR-23b, miR-26a and miR-221) (12, 14, 25). We assessed their expression on RNA level that allowed their quantification using RT real time PCR. We combined these markers with serum PSA, as a routinely used marker of high senzitivity for detection of PCa, but with a limited prognostic value.

As the most promising indicator of worse outcome, we have revealed a combination of high level of preoperative serum PSA and a presence of high level expression of *TMPRSS2-ERG* fusion gene in tumor tissue. Despite of limitation in specificity, serum PSA is one of the most useful biomarkers in oncology and still has not lost its potential that is evident from currently developing concept of prostate health index (Phi) (23). Therefore, the identification of new biomarkers that can be used alone or in combination with PSA is welcome to improve distinguishing the aggressive from the indolent ones (26).

The *TMPRSS2-ERG* fusion was identified to be the most common gene rearrangement in PCa; published data report its prevalence approximately 50% in PSA screened localized PCa (27). In our cohort, we detected presence of the *TMPRSS2-ERG* fusion in 31.5% of PCa samples. The mere presence of the fusion gene in our cohort had no relation to prognosis. The meta-analysis of Pettersson *et al*. concluded that *TMPRSS2-ERG* or *ERG* overexpression did not strongly predict recurrence or mortality among men treated with radical prostatectomy; the cohort of this meta-analysis included patients from Europe, North America and Asia (28). It appears, however, that the levels of *TMPRSS2-ERG*, not merely its presence, have the dicisive effect on progression (29). Similarly, in our cohort, we found significantly shorter time to biochemical recurence in patients with high expression of *TMPRSS2-ERG* transcript.

TMPRSS2-ERG fusion gene presence can be detected non-invasivelly from samples of urine. Stephan *et al*. in their work focused on diagnostics of PCa and concluded that *PCA3* and Phi were superior to the other parameters and further stated that the advantage of *TMPRSS2-ERG* might be seen in subgroups of aggressive PCa (30).

MicroRNAs, whose expression was assessed (miR-23b, miR-26a and miR-221) in our study, were chosen on the base of described role in pathogenesis of PCa (12, 14, 25). Nevertheless, we did not record any prognostic significance. We observed lower expression of miR-221 in PCa tumor tissue. This finding supports tumor suppressor role of miR-221 in PCa, which was described in several previously published studies (15, 31, 32). However, some other studies (33, 34) indicate up-regulation of miR-221 in PCa.

In conclusion, a combination of high preoperative serum PSA and high expression of *TMPRSS2-ERG* could be promising in distinguishing those tumors that are aggressive and together with patient status make it possible to decide if radical resection is beneficial.

Conflicts of Interest

The Authors declared they have no conflicts of interest.

Acknowledgements

This study was supported by the grant of Ministry of Health of the Czech Republic - Conceptual Development of Research Organization (Faculty Hospital in Pilsen - FNPl, 00669806) and the project of Faculty of Medicine in Pilsen SVV-2016-260283.

References

1 Attard G, Parker C, Eeles RA, Schröder F, Tomlins SA, Tannock I, Drake CG and de Bono JS: Prostate cancer. Lancet Lond Engl *387*: 70-82, 2016.

2 Storås AH, Sanda MG, Boronat OG, Chang P, Patil D, Crociani C, Suarez JF, Cvancarova M, Loge JH and Fosså SD: Erectile dysfunction and sexual problems two to three years after prostatectomy among american, norwegian, and spanish patients. Clin Genitourin Cancer *14*: e265-273, 2016.

3 Dahl S, Loge JH, Berge V, Dahl AA, Cvancarova M and Fosså SD: Influence of radical prostatectomy for prostate cancer on work status and working life 3 years after surgery. J Cancer Surviv Res Pract *9*: 172-179, 2015.

4 Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun X-W, Varambally S, Cao X, Tchinda J, Kuefer R, Lee C, Montie JE, Shah RB, Pienta KJ, Rubin MA and Chinnaiyan AM: Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science *310*: 644-648, 2005.

5 Rubin MA and Chinnaiyan AM: Bioinformatics approach leads to the discovery of the TMPRSS2:ETS gene fusion in prostate cancer. Lab Investig J Tech Methods Pathol *86*: 1099-1102, 2006.

6 Shafi AA, Yen AE and Weigel NL: Androgen receptors in hormone-dependent and castration-resistant prostate cancer. Pharmacol Ther *140*: 223-238, 2013.

7 Cai C, Wang H, Xu Y, Chen S and Balk SP: Reactivation of androgen receptor-regulated TMPRSS2:ERG gene expression in castration-resistant prostate cancer. Cancer Res *69*: 6027-6032, 2009.

8 Carver BS, Tran J, Gopalan A, Chen Z, Shaikh S, Carracedo A, Alimonti A, Nardella C, Varmeh S, Scardino PT, Cordon-Cardo C, Gerald W and Pandolfi PP: Aberrant ERG expression cooperates with loss of PTEN to promote cancer progression in the prostate. Nat Genet *41*: 619-624, 2009.

9 Hagan JP and Croce CM: MicroRNAs in carcinogenesis. Cytogenet Genome Res *118*: 252-259, 2007.

10 Bertoli G, Cava C and Castiglioni I: MicroRNAs as Biomarkers for Diagnosis, Prognosis and Theranostics in Prostate Cancer. Int J Mol Sci 17, 2016.

11 Goto Y, Kojima S, Nishikawa R, Enokida H, Chiyomaru T, Kinoshita T, Nakagawa M, Naya Y, Ichikawa T and Seki N: The microRNA-23b/27b/24-1 cluster is a disease progression marker and tumor suppressor in prostate cancer. Oncotarget *5*: 7748-7759, 2014.

12 Majid S, Dar AA, Saini S, Arora S, Shahryari V, Zaman MS, Chang I, Yamamura S, Tanaka Y, Deng G and Dahiya R: miR-23b represses proto-oncogene Src kinase and functions as methylation-silenced tumor suppressor with diagnostic and prognostic significance in prostate cancer. Cancer Res *72*: 6435-6446, 2012.

13 Zhao S, Ye X, Xiao L, Lian X, Feng Y, Li F and Li L: MiR-26a inhibits prostate cancer progression by repression of Wnt5a. Tumour Biol J Int Soc Oncodevelopmental Biol Med *35*: 9725-9733, 2014.

14 Kato M, Goto Y, Matsushita R, Kurozumi A, Fukumoto I, Nishikawa R, Sakamoto S, Enokida H, Nakagawa M, Ichikawa T and Seki N: MicroRNA-26a/b directly regulate La-related protein 1 and inhibit cancer cell invasion in prostate cancer. Int J Oncol *47*: 710-718, 2015.

15 Zheng Q, Peskoe SB, Ribas J, Rafiqi F, Kudrolli T, Meeker AK, De Marzo AM, Platz EA and Lupold SE: Investigation of miR-21, miR-141, and miR-221 expression levels in prostate adenocarcinoma for associated risk of recurrence after radical prostatectomy. The Prostate *74*: 1655-1662, 2014.

16 Cui Y, Cao W, Li Q, Shen H, Liu C, Deng J, Xu J and Shao Q: Evaluation of prostate cancer antigen 3 for detecting prostate cancer: a systematic review and meta-analysis. Sci Rep *6*: 25776, 2016.

17 Ferro M, Lucarelli G, Bruzzese D, Perdonà S, Mazzarella C, Perruolo G, Marino A, Cosimato V, Giorgio E, Tagliamonte V, Bottero D, De Cobelli O and Terracciano D: Improving the prediction of pathologic outcomes in patients undergoing radical prostatectomy: the value of prostate cancer antigen 3 (PCA3), prostate health index (phi) and sarcosine. Anticancer Res *35*: 1017-1023, 2015.

18 Cantiello F, Russo GI, Ferro M, Cicione A, Cimino S, Favilla V, Perdonà S, Bottero D, Terracciano D, De Cobelli O, Morgia G and Damiano R: Prognostic accuracy of Prostate Health Index and urinary Prostate Cancer Antigen 3 in predicting pathologic features after radical prostatectomy. Urol Oncol *33*: 163.e15-23, 2015.

19 Kalfert D, Pesta M, Kulda V, Topolcan O, Ryska A, Celakovsky P, Laco J and Ludvikova M: MicroRNA profile in site-specific head and neck squamous cell cancer. Anticancer Res *35*: 2455-2463, 2015.

20 Smid D, Kulda V, Srbecka K, Kubackova D, Dolezal J, Daum O, Kucera R, Topolcan O, Treska V, Skalicky T and Pesta M: Tissue microRNAs as predictive markers for gastric cancer patients undergoing palliative chemotherapy. Int J Oncol *48*: 2693-2703, 2016.

21 Kan Z, Jaiswal BS, Stinson J, Janakiraman V, Bhatt D, Stern HM, Yue P, Haverty PM, Bourgon R, Zheng J, Moorhead M, Chaudhuri S, Tomsho LP, Peters BA, Pujara K, Cordes S, Davis DP, Carlton VEH, Yuan W, Li L, Wang W, Eigenbrot C, Kaminker JS, Eberhard DA, Waring P, Schuster SC, Modrusan Z, Zhang Z, Stokoe D, de Sauvage FJ, Faham M and Seshagiri S: Diverse somatic mutation patterns and pathway alterations in human cancers. Nature *466*: 869-873, 2010.

22 Danila DC, Anand A, Sung CC, Heller G, Leversha MA, Cao L, Lilja H, Molina A, Sawyers CL, Fleisher M and Scher HI: TMPRSS2-ERG status in circulating tumor cells as a predictive biomarker of sensitivity in castration-resistant prostate cancer patients treated with abiraterone acetate. Eur Urol *60*: 897-904, 2011.

23 Fuchsova R, Topolcan O, Windrichova J, Hora M, Dolejsova O, Pecen L, Kasik P, Novak J, Casova M and Smejkal J: PHI in the early detection of prostate cancer. Anticancer Res *35*: 4855-4857, 2015.

24 Stangelberger A, Waldert M and Djavan B: Prostate cancer in elderly men. Rev Urol *10*: 111-119, 2008.

25 Kneitz B, Krebs M, Kalogirou C, Schubert M, Joniau S, van Poppel H, Lerut E, Kneitz S, Scholz CJ, Ströbel P, Gessler M, Riedmiller H and Spahn M: Survival in patients with high-risk prostate cancer is predicted by miR-221, which regulates proliferation, apoptosis, and invasion of prostate cancer cells by inhibiting IRF2 and SOCS3. Cancer Res *74*: 2591-2603, 2014.

26 Sanguedolce F, Cormio A, Brunelli M, D'Amuri A, Carrieri G, Bufo P and Cormio L: Urine TMPRSS2: ERG fusion transcript as a biomarker for prostate cancer: Literature Review. Clin Genitourin Cancer *14*: 117-121, 2016.

27 Tomlins SA, Bjartell A, Chinnaiyan AM, Jenster G, Nam RK, Rubin MA and Schalken JA: ETS gene fusions in prostate cancer: from discovery to daily clinical practice. Eur Urol *56*: 275-286, 2009.

28 Pettersson A, Graff RE, Bauer SR, Pitt MJ, Lis RT, Stack EC, Martin NE, Kunz L, Penney KL, Ligon AH, Suppan C, Flavin R, Sesso HD, Rider JR, Sweeney C, Stampfer MJ, Fiorentino M, Kantoff PW, Sanda MG, Giovannucci EL, Ding EL, Loda M and Mucci LA: The TMPRSS2:ERG rearrangement, ERG expression, and prostate cancer outcomes: a cohort study and meta-analysis. Cancer Epidemiol Biomark Prev Publ Am Assoc Cancer Res Cosponsored Am Soc Prev Oncol *21*: 1497-1509, 2012.

29 Font-Tello A, Juanpere N, de Muga S, Lorenzo M, Lorente JA, Fumado L, Serrano L, Serrano S, Lloreta J and Hernández S: Association of ERG and TMPRSS2-ERG with grade, stage, and prognosis of prostate cancer is dependent on their expression levels. The Prostate *75*: 1216-1226, 2015.

30 Stephan C, Jung K, Semjonow A, Schulze-Forster K, Cammann H, Hu X, Meyer H-A, Bögemann M, Miller K and Friedersdorff F: Comparative assessment of urinary prostate cancer antigen 3 and TMPRSS2:ERG gene fusion with the serum [-2]proprostate-specific antigen-based prostate health index for detection of prostate cancer. Clin Chem *59*: 280-288, 2013.
31 Guzel E, Karatas OF, Semercioz A, Ekici S, Aykan S, Yentur S, Creighton CJ, Ittmann M and Ozen M: Identification of microRNAs differentially expressed in prostatic secretions of patients with prostate cancer. Int J Cancer *136*: 875-879, 2015.
32 Xuan H, Xue W, Pan J, Sha J, Dong B and Huang Y: Downregulation of miR-221, -30d, and -15a contributes to pathogenesis of prostate cancer by targeting Bmi-1. Biochemistry (Mosc) *80*: 276-283, 2015.
33 Sun T, Yang M, Chen S, Balk S, Pomerantz M, Hsieh C-L, Brown M, Lee G-SM and Kantoff PW: The altered expression of MiR-221/-222 and MiR-23b/-27b is associated with the development of human castration resistant prostate cancer. The Prostate *72*: 1093-1103, 2012.
34 Song C, Chen H, Wang T, Zhang W, Ru G and Lang J: Expression profile analysis of microRNAs in prostate cancer by next-generation sequencing. The Prostate *75*: 500-516, 2015.

Received July 1, 2016
Revised August 17, 2016
Accepted August 18, 2016