ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Role of ERG, PTEN and KI67 Proteins Expression in Prognosis of Metastatic Prostate Cancer

Eman H. Abdelbary[1], Abdelmonem A. Hegazy[2], Mohamed Abdelgawad[3], and Ragab A. Ahmed[4]

1. Pathology Department, Zagazig University, Egypt.
2. Anatomy and Embryology Department, Zagazig University, Egypt.
3. Clinical Oncology and Nuclear Medicine Department, Zagazig University, Egypt.
4. Urology Department, Faculty of Medicine, Zagazig University, Egypt.

Manuscript Info

Manuscript History:

Received: 12 November 2014
Final Accepted: 26 December 2014
Published Online: January 2015

Key words:

ERG, PTEN, KI67, Immunohistochemistry, Metastatic prostate cancer, Prognosis

Corresponding Author

Eman H. Abdelbary

Abstract

Background: Prostate cancer is a leading cause of cancer-related mortality in men. Despite improvements in early detection of prostate cancer as a result of PSA screening, we still lack molecular markers to effectively distinguish patients with high risk of disease progression from the indolent majority**.**

Aim of the work: To assess the prognostic value of ERG, PTEN and KI67 proteins expression in metastatic prostate cancer patients, as well as their relationship to clinicopathologic features of the disease**.**

Patients and Methods: ERG, PTEN and KI67 proteins expression was assessed immunohistochemically in series of metastatic prostate cancers and their prognostic significance was evaluated.

Results: High ERG score as well as high KI67 labeling index (LI) were strongly associated (P <0.001) with high pre-treatment PSA level, Gleason score >7, advanced tumor stage, metastasis affecting both bone & lymph nodes and biochemical progression. PTEN loss was significantly associated with high pre-treatment PSA level (P <0.001), Gleason score >7 (P =0.002), advanced tumor stage (P =0.015), metastasis affecting both bone & lymph nodes (P =0.002) and biochemical progression (P =0.001). A significant association was found between higher KI67 LI (P <0.001), higher ERG expression score (P =0.005) & PTEN loss (P =0.007) and shorter progression free survival. ERG expression and PTEN loss were significantly associated with KI67 LI (P =0.011 & 0.0.013 respectively), however, no relationship could be proved between both of them (P =0.256). ERG (P =0.025) was the only independent predictor for biochemical progression in multivariate analysis.

Conclusions: ERG and KI67 expressions and PTEN loss have an important prognostic value in metastatic prostate cancer. Using them to differentiate between patients who are at a high or low risk of disease progression may help to identify patients who will benefit the most from treatment.

INTRODUCTION

Prostate cancer (PC) is a leading cause of cancer-related mortality in men. More than 600,000 men are annually diagnosed as having prostate cancer worldwide (1). In spite of the improvements in early detection of

prostatic adenocarcinoma as a result of prostate specific antigen (PSA) screening, biomarkers that can effectively distinguish patients with high risk of disease progression from the indolent majority are still lacking. Identification of molecular biomarkers that contribute to the development of lethal disease has the additional benefit of providing specific targets for therapy and improving patient management (2).

One of the most extensively studied markers in prostate cancer is the fusion involving the promoter of transmembrane protease, serine 2 (TMPRSS2) with coding sequence of erythroblastosis virus E26 (ETS) oncogene homolog (ERG). The majority of ERG rearrangements result in the overexpression of an oncogenic ERG protein that can be used as an accurate surrogate marker for fusion status (3). Several studies have established that ERG gene rearrangements, as detected by fluorescence in situ hybridization (FISH), highly correlate with immunohistochemical ERG protein expression (4,5). Since immunohistochemistry (IHC) is performed more routinely by pathology labs than FISH, it may be more clinically relevant to clarify the prognostic value of ERG expression than ERG rearrangements.

Loss of the PTEN tumor suppressor gene (phosphatase and tensin homologue on chromosome 10) is one of the most common somatic genetic aberrations in prostate cancer and is frequently associated with high risk disease. The evaluation of allelic loss of PTEN by FISH is a complex technique that requires counting of the number of fluorescent signals relative to control signals in interphase cells. Thus, reliable detection of PTEN protein status by IHC could prove to be highly useful for its implementation as a clinically relevant prognostic biomarker. Importantly, the development of such an assay may also detect cases where PTEN inactivation occurs by mechanisms other than genomic deletion. As more therapies targeting various components of the PI3K signaling cascade become available, a reliable assay to determine PTEN status will likely have an important role in clinical care (2,6).

KI67 is an immunohistochemical marker of cell proliferation. As the grading system in prostate cancer (unlike many other cancers) does not consider the proliferation rate of the cells, it is possible that measuring the cell proliferation rate in prostate tumors could provide additional prognostic information. Previous studies have shown that KI-67 IHC measurements can significantly predict prostate cancer outcome (7,8).

Aim of the work

This retrospective study was designed to assess the prognostic value of ERG, KI67 labeling index (LI) and PTEN proteins expression in metastatic prostate cancer patients, as well as their relationship to clinicopathologic features of the disease.

PATIENTS AND METHODS

Patients and tissue selection

For this study, formalin-fixed paraffin-embedded tissue samples were collected retrospectively from 40 prostate cancer patients with lymph node and/or bone metastasis, who underwent transrectal ultrasound-guided prostate biopsy at the Zagazig University Hospitals between 2009 and 2011. Follow-up was carried out in Clinical Oncology and Nuclear Medicine Department of the same institute. It consisted of two assessments every year. At each follow-up, patients had a clinical evaluation and a PSA test.

Clinical data and follow-up information were collected retrospectively from the archives of Urology and Clinical Oncology & Nuclear Medicine Departments. Histopaothologic characteristics were confirmed by blinded review of the original pathology slides using the 2011 consensus guidelines. Biochemical progression was defined as rise of serum PSA level of 2 ng/mL or more above the baseline PSA.

The study complied with the guidelines of the local ethics committee.

Immunohistochemistry

Immunohistochemical staining was carried out using the streptavidin–biotin immunoperoxidase technique. Sections of 3–5 μm thickness were cut from formalin-fixed, paraffin-embedded blocks, mounted on positively charged slides, deparaffinized in xylene, and rehydrated in graded alcohol. Sections were boiled in citrate buffer (pH 6.0) for 20 min and then washed in PBS (pH 7.3). Thereafter, blocking of endogenous peroxidase activity with 6% H2O2 in methanol was carried out. The slides were then incubated overnight with monoclonal antibodies: ERG (1:200 dilution, sc-353 from Santa Cruz, USA), PTEN (MMAC1 Ab-4, clone 17.A, Thermo scientific, MA, USA) and KI67 (clone MIB-1, DAko, Carpinteria, CA, USA). Incubation with a secondary antibody and product visualization were performed with diaminobenzidine substrate (Lab Vision Corporation, Fermont, USA) as the

chromogen. The slides were finally counterstained with Mayer's hematoxylin and washed with distilled water and PBS.

To prevent antigen degradation, sections were stored at 4°C before immunohistochemical analysis. Endothelial cells, normal prostatic acini and tonsils were used as positive control to confirm the specificity of staining with ERG, PTEN and KI67 respectively. Negative controls were made with primary antibody replaced by PBS. Positive and negative control slides were included within each batch of slides.

Interpretation of immunohistochemistry

For ERG IHC, we evaluated for the percentage of nuclei stained and expression intensity using a 4-point scale (0–3). The highest intensity score present for each case was recorded as the final intensity score and the percentage of the cancer nuclei with that expression intensity was also noted. The composite score was calculated by multiplying the percentage of cells stained (0–100%) by the intensity level. This created a score (0–300) which was recorded for each case. Sections with no immunoreactions were considered negative, while sections with nuclear staining were positive (5).

For PTEN IHC, a negative sample was regarded as having no staining in either the cytoplasmic or nuclear cellular compartment; for positive samples, only cytoplasmic staining was scored (9). The PTEN IHC status was interpreted as positive or negative, defined as 10% tumor cells staining positive.

When evaluating KI67 LI, the percentage of positive cells was estimated as the proportion of KI-67 stained malignant cells. The KI67 labeling index was low if 0–10% of cells were stained and high if more than 10% of cells were stained (8).

Statistical analysis

Continuous variables were expressed as the median (range), and the categorical variables were expressed as a number (percentage). Continuous variables were checked for normality by using Kolmogorov-Smirnov test. Data found to be non-normally distributed were analyzed using the Mann-Whitney U (MW) test for two groups & Kruskal-Wallis H (KW) test for three groups. Percent of categorical variables were compared using the Pearson's Chi-square (χ^2) test. Association between expression of KI67 LI, ERG and PTEN were done by McNemar (χ^2) test for paired data with exact correction was done if number of discordant pairs was fewer than 20, while strength of relationship between these nominal data were determined by computing Contingency Coefficient (C) with (+) sign was indicator for direct relationship & (-) sign was indication for inverse relationship, also values near to 1 was indicator for strong relationship & values near 0 was indicator for weak relationship. To determine whether KI67 LI, ERG and PTEN were predictive for biochemical progression, we used uni- and multivariate Cox regression with stepwise backward entering of covariates. The proportionality assumption for positive versus negative cases was visually assessed in Kaplan–Meier curves. A P value <0.05 was considered significant. All statistics were performed using SPSS 22.0 for windows (SPSS Inc., Chicago, IL, USA) & MedCalc 13 for windows (MedCalc Software bvba).

RESULTS

Clinicopathological results

A total of 40 archival tissues from patients with metastatic prostate cancer were included in this study. The mean age ± SD was 64.97 years ± 6.26, (range: 52-80 y); and the mean PSA value ± SD was 21.3 ± 8.39, (range: 9-44 ng/ml). The prostatic carcinoma cases were histologically classified into 27 cases of Gleason score ≤ 7 and 13 cases of Gleason score > 7. Twenty one cases (52.5%) were classified as tumor stage 2, 16 cases (40%) as stage 3 and 3 cases (7.5%) were stage 4. Distant metastasis was detected in lymph node in 9 cases (22.5%), bone in 21 cases (52.5%) and both in 10 cases (25%). Biochemical progression was noted in 12 patients (30%).

Association of K167 LI, ERG & PTEN proteins expression with patients' clinicopathological features

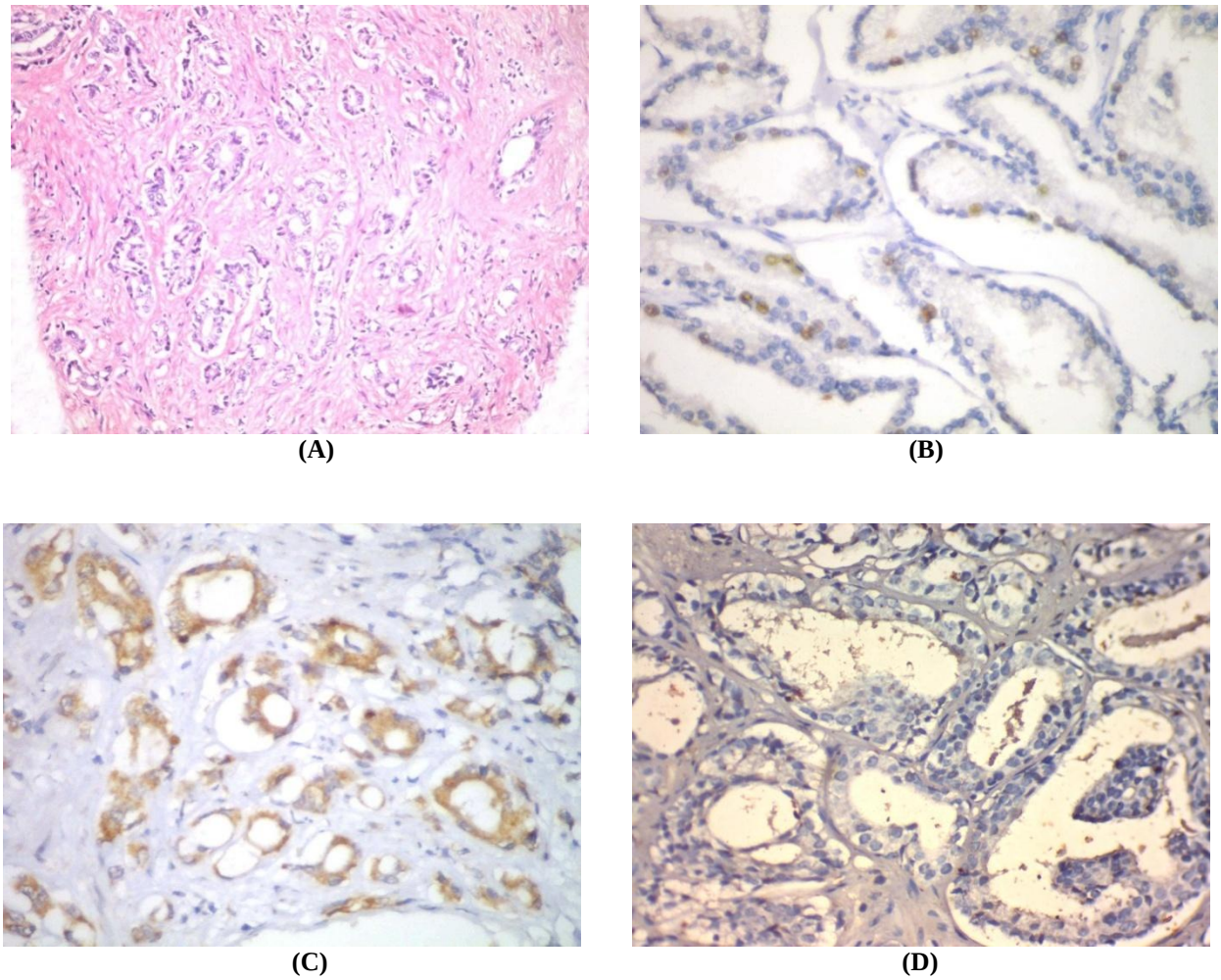
High ERG score as well as high KI67 LI were strongly associated (P <0.001) with high pre-treatment PSA level, Gleason score >7 (Figs. 1,2), advanced tumor stage, metastasis affecting both bone & lymph nodes and biochemical progression, (Table 1).

PTEN loss was significantly associated with high pre-treatment PSA level (P <0.001), Gleason score >7 (P =0.002) (Figs. 1,2), advanced tumor stage (P =0.015), metastasis affecting both bone & lymph nodes (P =0.002) and biochemical progression (P =0.001), (Table 1).

Table (1): Association of K167 LI, ERG & PTEN proteins expression with patients' clinicopathological features in metastatic prostate cancer

Characteristics	No	KI67 LI			ERG		PTEN		
		Low (n=29)	High (n=11)	p*	Median (Range)	p§	-ve (n=14)	+ve (n=26)	p*
Age (years)									
≤ 65 years	22 (55)	16 (55.2)	6 (54.5)	0.972	45 (0 – 270)	0.749	9 (64.3)	13 (50)	0.386
> 65 years	18 (45)	13 (44.8)	5 (45.5)		37.5 (0 – 240)		5 (35.7)	13 (50)	
PSA (ng/ml)									
≤ 20 ng/ml	22 (55)	22 (75.9)	0 (0)	<0.001	0 (0 – 120)	<0.001	2 (14.3)	20 (76.9)	<0.001
> 20 ng/ml	18 (45)	7 (24.1)	11 (100)		180 (0 – 270)		12 (85.7)	6 (23.1)	
Gleason score									
≤ 7	27 (67.5)	25 (86.2)	2 (18.2)	<0.001	0 (0 – 240)	<0.001	5 (35.7)	22 (84.6)	0.002
> 7	13 (32.5)	4 (13.8)	9 (81.8)		180 (90 – 270)		9 (64.3)	4 (15.4)	
Tumor stage (pT)									
T2	21 (52.5)	21 (72.4)	0 (0)	<0.001	0 (0 – 140)	<0.001	3 (21.4)	18 (69.2)	0.015
T3	16 (40)	7 (24.1)	9 (81.8)		165 (0 – 255)		9 (64.3)	7 (26.9)	
T4	3 (7.5)	1 (3.4)	2 (18.2)		240 (180 – 270)		2 (14.3)	1 (3.8)	
Distant Metastasis (M)									
M1a	9 (22.5)	8 (27.6)	1 (9.1)	<0.001	0 (0 – 160)	<0.001	1 (7.1)	8 (30.8)	0.002
M1b	21 (52.5)	19 (65.5)	2 (18.2)		0 (0 – 180)		5 (35.7)	16 (61.5)	
M1a & b	10 (25)	2 (6.9)	8 (72.7)		232.5 (140 – 270)		8 (57.1)	2 (7.7)	
Biochemical progression									
No progression	28 (70)	26 (89.7)	2 (18.2)	<0.001	0 (0 – 170)	<0.001	5 (35.7)	23 (88.5)	0.001
Progression	12 (30)	3 (10.3)	9 (81.8)		225 (0 – 270)		9 (64.3)	3 (11.5)	
Biochemical progression free time									
≤ 24 months	22 (55)	14 (48.3)	8 (72.7)	0.165	60 (0 – 270)	0.269	8 (57.1)	14 (53.8)	0.842
> 24 months	18 (45)	15 (51.7)	3 (27.3)		37.5 (0 – 240)		6 (42.9)	12 (46.2)	

M1a: Non regional LN metastasis; M1b: Bone metastasis; Qualitative data are presented as number (%); Quantative data are presented as median (range) as they are non-normally distributed; * Chi-square test; § Mann Whitney U test for two groups, Kruskal-Walls H test for three groups ; $p < 0.05$ is significant.



(A) **(B)**

(C) **(D)**

Figure (1): Prostate cancer with Gleason score (3+4) showing: (A) Infiltrating small glands of irregular shape and spacing (H&E x200); (B) Focal strong nuclear ERG immunoreactivity (IHC x400); (C) Strong cytoplasmic and scattered nuclear PTEN immunoreactivity (IHC x400); (D) Low Ki67 LI (IHC x400).

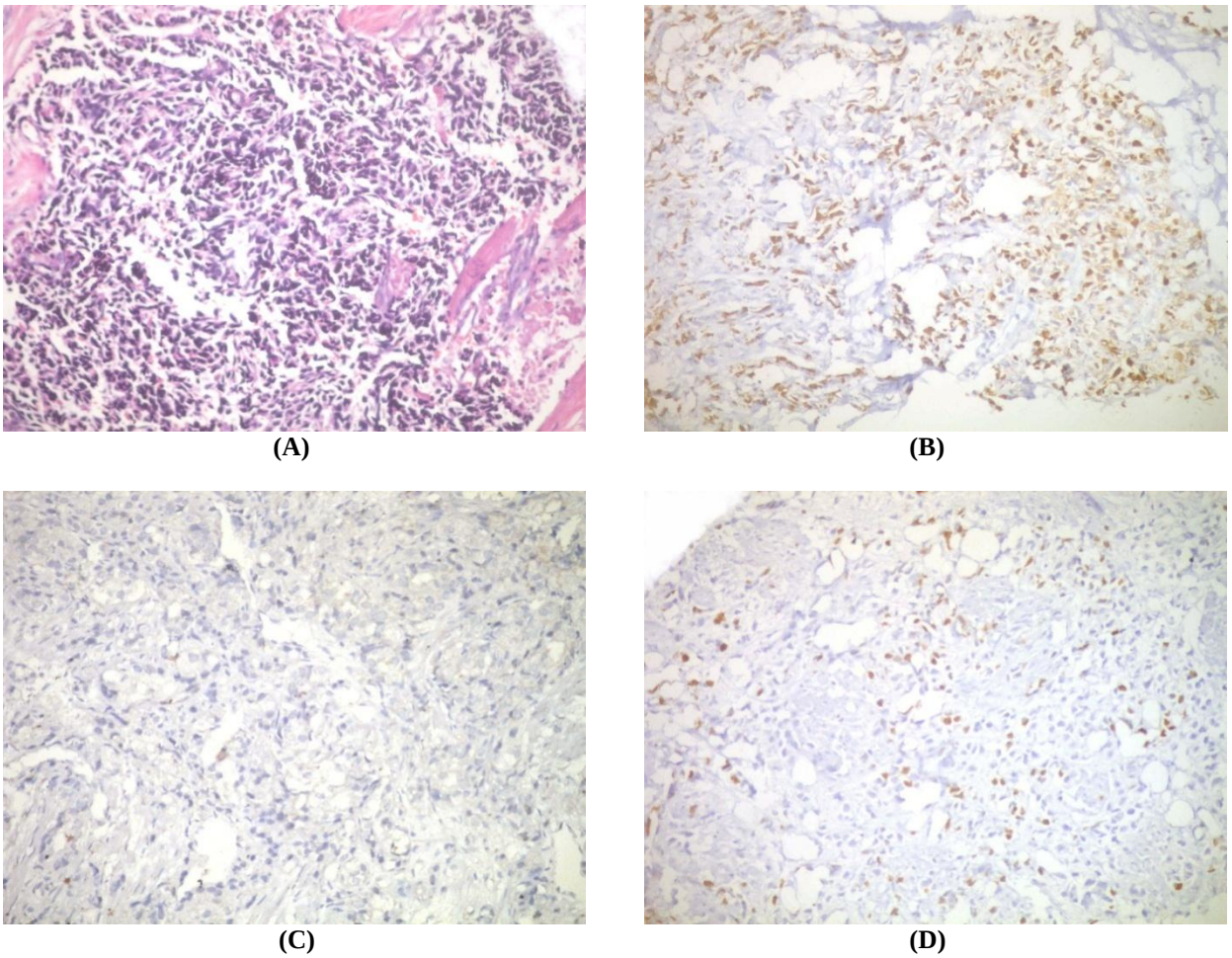


Figure (2): Prostate cancer with Gleason score (5+4) showing: (A) Cords of malignant cells (H&E x200); (B) Diffuse strong nuclear ERG immunoreactivity (IHC x200); (C) Negative PTEN immunoreactivity (IHC x200); (D) High KI67 LI (IHC x200).

Association of K167 LI, ERG & PTEN proteins expression with biochemical progression and biochemical progression free survival

55% of ERG-positive, 81.8% of high KI67LI and 64.3% of PTEN-negative cases were complicated by biochemical progression within 3 years. Conversely, only 5% of ERG-negative, 10.3% of low KI67 LI and 11.5% of PTEN-positive cases showed progressive disease.

Kaplan–Meier survival curve analysis for ERG expression, KI67 LI and PTEN expression revealed significant association between higher ERG expression score ($P = 0.005$), higher KI67 LI ($P < 0.001$) & PTEN loss ($P = 0.007$) and shorter progression free survival (Table 2, Fig. 3).

Table (2): Biochemical progression & Biochemical progression free survival in all studied metastatic prostate cancer patients (N =40)

Characteristics	All (N=40)	KI67 LI			ERG			PTEN		
		Low (n=29)	High (n=11)	p	-ve (n=20)	+ve (n=20)		-ve (n=14)	+ve (n=26)	p*
Number of progressive cases	12 (30)	3 (10.3)	9 (81.8)	<0.001*	1 (5)	11 (55)	0.001*	9 (64.3)	3 (11.5)	0.001*
Median follow-up (months)	24	26	23	---	24	24	---	24	24	---
Median progression free survival (months)	NR	NR	24		NR	30		24	NR	
1 year progression free survival (%)	100%	100%	100%	<0.001§	100%	100%	0.005§	100%	100%	0.007§
2 year progression free survival (%)	72.2	91.8%	32.7%		100%	52%		48.2%	90.6%	
3 year progression free survival (%)	52.1%	85.7%	0%		88.9%	29.2%		19.3%	83.1%	
Hazard Ratio	---	8.75		---	10.27		---	0.201		---
95% CI of HR	---	2.39 – 32.06		---	3.31 – 31.8		---	0.062 – 0.646		---

Qualitative data are presented as number(%); -ve ERG: score 0, +ve ERG: score > 0; NR denote not reached yet.; * Chi-square test.; § Logrank test. ; CI: Confidence interval; HR; Hazard ratio; P < 0.05 is significant.

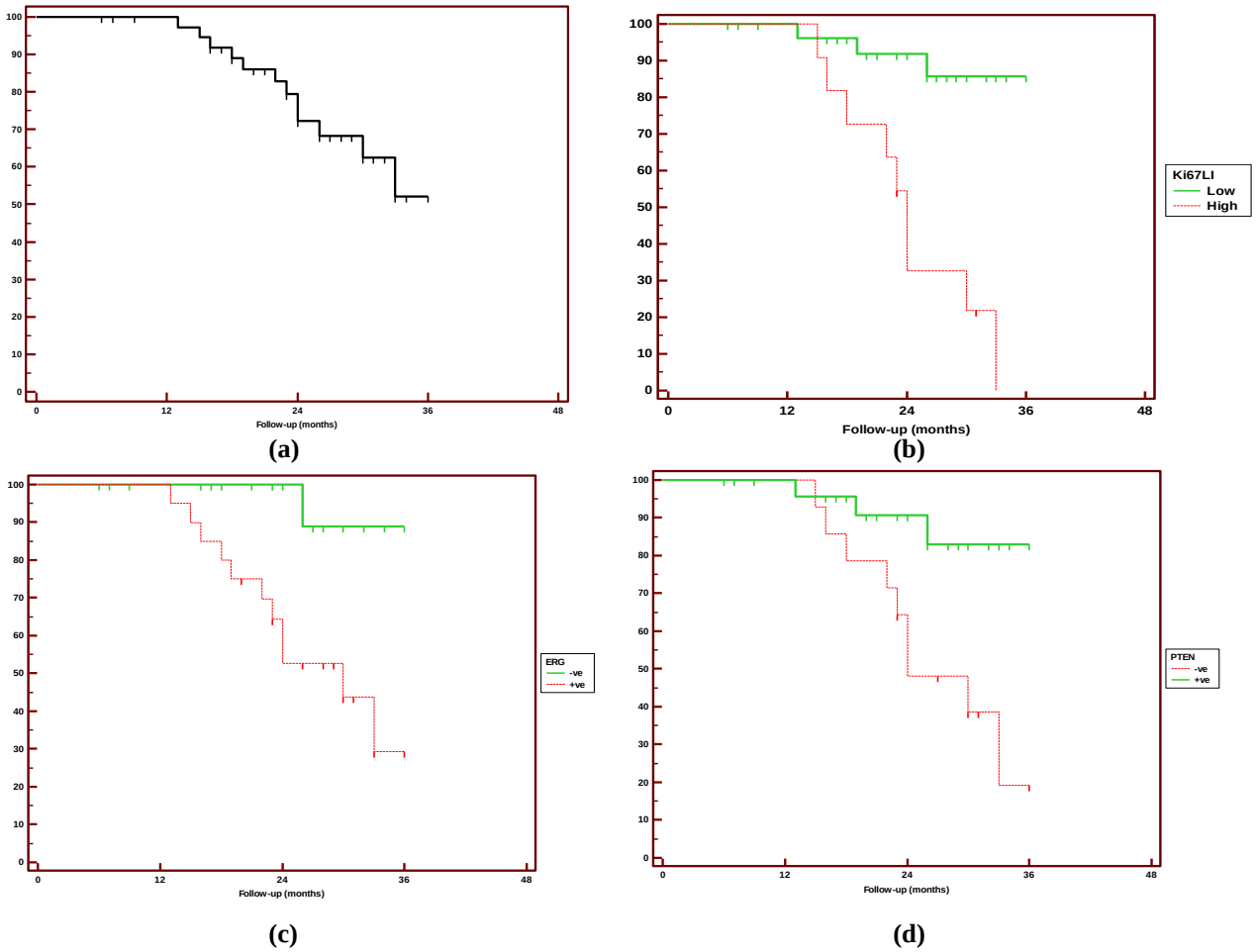


Figure (3): Kaplan-Meier progression free survival curve: (a) In all studied metastatic prostate cancer patients (N=40); (b) Stratified according to KI67 LI; (c) Stratified according to ERG; (d) Stratified according to PTEN.

Association between K167 LI, ERG & PTEN proteins expression in metastatic prostate cancer

Both ERG expression and PTEN loss were significantly associated with KI67 LI (P =0.011 & 0.0.013 respectively), however, no relationship could be proved between both of them (P =0.256), (Table 3).

Table (3): Association & correlation between KI67LI, ERG and PTEN expression

	KI67 LI			ERG			PTEN		
	low (n=29)	High (n=11)	Stat	-ve (n=20)	+ve (n=20)	Stat	-ve (n=14)	+ve (n=26)	Stat
KI67 LI									
Low (n=29)				20 (100)	9 (45)	χ^2=6.400 C=+0.489 p=0.011	3 (21.4)	26 (100)	χ^2=6.081 C=+0.615 p=0.013
High (n=11)		----		0 (0)	11 (55)		11 (78.6)	0 (0)	
ERG									
-ve (n=20)	20 (69)	0 (0)	χ^2=6.400 C=+0.489 p=0.011		----		3 (21.4)	17 (65.4)	χ^2=1.285 C=+0.344 p=0.256
+ve (n=20)	9 (31)	11 (100)					11 (78.6)	9 (34.6)	
PTEN									
-ve (n=14)	3 (10.3)	11 (100)	χ^2=6.081 C=+0.615 p=0.013	3 (15)	11 (55)	χ^2=1.285 C=+0.344 p=0.256		----	
+ve (n=26)	26 (89.7)	0 (0)		17 (85)	9 (45)				

Stat: Analytic statistics for association & correlation between pairs; Qualitative data are presented as number(%); χ^2: McNemar test; C: Contingency Coefficient; P < 0.05 is significant.

Predictive of value of KI67LI, ERG and PTEN expression for biochemical recurrence in metastatic prostate cancer patients

PSA (P <0.001), Gleason score (P =0.007), pT-stage (P <0.001), M1 (a or b) (P <0.001), KI67 LI (P =0.001), ERG (P >0.001) and PTEN (P =0.008), all had independent predictive value for biochemical progression in univariate analysis, however, ERG (P =0.025) was the only independent predictor for biochemical progression in multivariate analysis, (Table 4).

Table (4): Predictive of value of KI67LI, ERG and PTEN expression for biochemical progression in metastatic prostate cancer patients

	Univariate analysis				Multivariate analysis			
	β	HR (95%CI)		p	β	HR (95%CI)		p
Age (years)	- 0.028	0.972	(0.868 – 1 .087)	0.625	- 0.113	0.892	(0.728 – 1.095)	0.279
PSA (ng/ml)	0.304	1.355	(1.167 – 1.574)	<0.001	0.335	1.395	(0.993 – 1.961)	0.056
Gleason score	0.782	2.187	(1.231 – 3.884)	0.007	- 0.246	0.781	(0.237 – 2.570)	0.686
Tumor stage	2.184	8.882	(3.236–24.377)	<0.001	1.131	3.100	(0.533 – 18.007)	0.209
M1 (a or b)	1.986	7.287	(2.284–23.251)	<0.001	- 1.458	0.232	(0.037 – 1.655)	0.147
KI67 LI	2.204	9.069	(2.447–33.605)	0.001	3.807	1.336	(0.000 – 7.210)	0.975
ERG	0.020	1.020	(1.009 – 1.031)	<0.001	0.024	1.025	(1.003 – 1.047)	0.025
PTEN	- 1.609	0.200	(0.054 – 0.735)	0.008	4.719	1.551	(0.000 – 1.764)	0.969

β: Regression coefficient; CI: Confidence interval; HR: Hazard ratio; Overall model fit: chi square=41.186, d.f=8, P < 0.05 is significant.

DISCUSSION

The search for accurate biomarkers in prostate cancer is critical for evolution of proper management of prostate cancer. This study evaluated three leading molecular biomarkers (ERG, KI67 and PTEN) for their contribution to prognosis of metastatic prostatic prostate cancer.

Several studies have attempted to evaluate ERG as a prognostic indicator of some risk factors including Gleason score , tumor stage and serum PSA level and the results have been variable (5,10-14). Our results showed

that high ERG score was strongly associated (P <0.001) with high pre-treatment PSA level, Gleason score >7, advanced tumor stage and bone/nodal metastasis.

ERG overexpression in prostate cell culture models was shown to increase cell proliferation and invasiveness (15), probably due to the binding of ERG fusion protein to CXCR4 and Urokinase Plasminogen Activator (PLAU) and MMP3 promoters with subsequent upregulation of their cell surface proteins expression promoting cell invasiveness and aggressive behavior (16,17).

Sun et al. (16) demonstrated that ERG was recruited to PSA enhancer and to the prostein promoter upstream EST element, showing direct interaction between ERG and PSA expression.

Although KI67 is the most commonly studied immunohistochemical marker in prostate cancer, yet only few studies have evaluated its role in predicting disease progression, especially in metastatic prostate cancer. Similar to previous studies (8,18), we found that high KI67 LI were strongly associated (P <0.001) with adverse clinicopathological features such as PSA >20ng/ml, Gleason score >7, advanced tumor stage and metastasis affecting both bone & lymph nodes.

The tumor suppressor PTEN is a critical regulator of growth factors and inhibitor of PI3K. Loss of PTEN is frequently observed in prostate cancer, resulting in the deregulation of cell survival, growth, and proliferation. Our findings revealed that PTEN loss was significantly associated with high pre-treatment PSA level (P <0.001), Gleason score >7 (P =0.002), advanced tumor stage (P =0.015) and metastasis affecting both bone & lymph nodes (P =0.002). These findings agreed with previous work (2, 19,20).

The acquisition of the TMPRSS2:ERG fusion and concomitant PTEN deletion at an earlier phase in prostatic oncogenesis appear to be an additional determinant of the phenotype that govern a more aggressive tumor phenotype through aberrant regulation of downstream target genes that alter normal cellular activity with epithelial-mesenchymal transition, increased invasiveness, cell proliferation and cellular migration (21,22).

In our study, Kaplan-Meier survival curve analysis revealed significant association between ERG protein expression and shorter progression free survival (P =0.005). Similarly, several studies reported that TMPRSS2-ERG fusions confers a worse prognosis (10,23), whereas others have not (13). Discrepancies in the reported significance of ERG in patient prognosis could be related to the difference in patient race, detection method and the end point of the study (i.e. biochemical recurrence or patient survival). It is well known that ERG protein expression is the active form of the gene product, which makes it a better and more accurate method in documenting its prognostic significance. TMPRSS2-ERG gene fusion has been proved to be directly associated with stromal changes predicting worse prognosis of prostate cancer, which indicates that the more aggressive phenotype of ERG positive tumors is caused by changes in the stroma (14).

We found that cases with PTEN loss were complicated by early biochemical progression in survival curve analysis (P =0.007). It has been strongly associated with prostate cancer progression in several previous studies (2,20,21,23). PTEN plays an important role in modulating PI3K pathway by catalyzing degradation of PIP3 generated by PI3K. PIP3 activates protein kinase AKT which modulates a number of downstream targets which play an important role in apoptosis and cell cycle progression, so it can be targeted by emerging modalities inhibiting PI3K or PI3K and mTOR.

Higher KI67 LI was associated with shorter progression free survival in our cases (P < 0.001). It was proved to be associated with poor outcome of prostate cancer in previous studies (18,20,24). This could be explained by the fact that high KI67 LI is directly associated with high Gleason scores and advanced tumor stages and consequently worse outcome.

Both ERG expression and PTEN loss were significantly associated with higher KI67 LI (P =0.011 & 0.0.013 respectively), however, no relationship could be proved between both of them (P =0.256). Cuzick et al. (6) reported an inverse relationship between PTEN and KI67. Both Hagglof et al. (14) and Qi et al. (25) found a significant association between ERG and KI67. In contrast to our findings regarding ERG relationship to PTEN, Barwick et al. (23) and Qi et al. (25) reported attenuation of PTEN in TMPRSS2-ERG fusion-positive tumors.

In univariate analysis, high Gleason score and clinical parameters of aggressive disease such as PSA, pT-stage, distant metastasis as well as KI67 LI, ERG protein expression and PTEN loss had independent predictive value for biochemical progression. However in multivariate analysis, ERG immunoreactivity was the only independent predictor for biochemical progression. These findings indicate that clinicopathological features and KI67 LI/PTEN expression play a role as independent predictor for biochemical progression only if the patient had negative ERG expression (score 0), once the patients had positive ERG expression (score >0), the biochemical progression relayed on level of ERG expression only regardless of other clinicopathological features as well as KI67 LI/PTEN expression features

Similarly, Nam et al. (10) reported that ERG gene fusion was the strongest predictor of disease recurrence. Moreover, concomitant PTEN loss and ERG fusion were proved as statistically independent predictors of biochemical recurrence and worse prognosis (21,26,27). KI67 has been widely utilized as a prognostic biomarker in malignancy including prostate cancer. Its independent predictive value for prostate cancer outcome was proved by Qi et al. (25).

We demonstrated that not only ERG expression was predictive for clinical outcome of patients, but also a higher ERG score was associated with aggressive disease and advanced clinical course, the same situation as high KI67 LI and PTEN loss. Hence, the use of ERG, PTEN and KI67 proteins expression yielded highly significant prognostic factors that could be used to segregate patients into high- and low- risk groups regarding biochemical progression.

CONCLUSIONS

Our findings indicate that ERG and KI67 expressions and PTEN loss have an important predictive value in metastatic prostate cancer. Using them to differentiate between patients who are at a high or low risk of disease progression may help to identify patients who will benefit the most from treatment. The value of these markers in targeted therapy will require a standardized clinical trial with larger sample and longer follow up period.

Abbreviations

PC: Prostate cancer, PSA: Prostatic specific antigen, IHC: Immunohistochemistry, FISH: Fluorescence in situ hybridization, ERG: ETS-related gene protein, ETS: Erythroblast transformation- specific, PI3K: Phosphoinositide-3 kinase, PTEN: Phosphatase and tensin homologue, KI67 LI: KI67 labeling index, CXCR4: Chemokine (c-x-c motif) receptor 4, MMP3: Matrix metalloproteinase 3, PIP3: Phosphatidyl inositol triphosphate.

Authors' contributions

All authors: Provision of study materials, design, collection, tissue processing techniques and assembly of data; EA: Examination and histologic grading of the pathologic specimens; AH: Sharing in identification of the normal tissues and statistical analysis of results; MA and RA evaluate the clinical profiles of the patients; EA wrote the manuscript; AH, MA and RA critically revised the manuscript; All authors have read and approved the final manuscript.

Acknowledgments

There is no funding and no conflict of interest in this article.

REFERENCES

1) Parkin DM, Bray F, Ferlay J, et al. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74–108.
2) Lotan TL, Gurel B, Sutcliffe S, et al. PTEN protein loss by immunostaining: analytic validation and prognostic indicator for a high risk surgical cohort of prostate cancer patients. Clin Cancer Res 2011;17:6563–6573.
3) Braun M, Goltz D, Shaikhibrahim Z, et al. ERG protein expression and genomic rearrangement status in primary and metastatic prostate cancer—A comparative study of two monoclonal antibodies. Prostate Cancer Prostatic Dis. 2012;15(2):165–169.
4) Xue L, Mao X, Ren G, et al. Chinese and Western prostate cancers show alternate pathogenetic pathways in association with ERG status. Am J Cancer Res. 2012;2(6):736–744.
5) Spencer SE, Johnston RB, Gordon RR, et al. Prognostic value of ERG oncoprotein in prostate cancer recurrence and cause-specific mortality. Prostate. 2013;73(9): 905–912.
6) Cuzick J, Yang ZH, Fisher G, et al. Prognostic value of PTEN loss in men with conservatively managed localised prostate cancer. British Journal of Cancer. 2013;108:2582-2589.
7) Kristiansen G. Diagnostic and prognostic molecular biomarkers for prostate cancer. Histopathology 2012; 60(1):125–141.
8) Fisher G, Yang ZH, Kudahetti S, et al. Prognostic value of Ki-67 for prostate cancer death in a conservatively managed cohort. British Journal of Cancer 2013;108:271-277.
9) Sangale Z, Prass C, Carlson A, et al. A robust immunohistochemical assay for detecting PTEN expression in human tumors. Appl Immunohistochem Mol Morphol 2011;19(2):173–183.
10) Nam RK, Sugar L, Yang W, et al. Expression of the TMPRSS2:ERG fusion gene predicts cancer recurrence after surgery for localised prostate cancer. Br J Cancer 2007; 97(12):1690–1695.

11) Bismar TA, Dolph M, Teng L, et al. ERG protein expression reflects hormonal treatment response and is associated with Gleason score and prostate cancer specific mortality. European Journal of Cancer 2012;48: 538– 546.
12) Suh JH, Park JW, Lee C, et al**.** ERG Immunohistochemistry and clinicopathologic characteristics in Korean prostate adenocarcinoma patients. Korean Journal of Pathology 2012;46:423-428.
13) Eguchi, FC, Faria EF, Neto CS, et al. The role of TMPRSS2:ERG in molecular stratification of PCa and its association with tumor aggressiveness: a study in Brazilian patients. Sci. Rep. 2014;4(5640):1-5.
14) Hägglöf C, Hammarsten P, Strömvall K, et al. TMPRSS2-ERG expression predicts prostate cancer survival and associates with stromal biomarkers. PLoS ONE. 2014;9(2):e86824.
15) Klezovitch O, Risk M, Coleman I, et al. A causal role for ERG in neoplastic transformation of prostate epithelium. Proc Natl Acad Sci USA 2008;105:2105-2110.
16) Sun C, Dobi A, Mohamed A, et al. TMPRSS2- ERG fusion, a common genomic alteration in prostate cancer activates C-MYC and abrogates prostate epithelial differentiation. Oncogene 2008;27(40):5348-5353.
17) Cai J, Singareddy R, Kropinski A, et al. Androgens Induce Functional CXCR4 through ERG Factor Expression in TMPRSS2-ERG Fusion-Positive Prostate Cancer Cells. Transl Oncol 2010;3:195-203.
18) Zellweger T, Gunther S, Zlobec I, et al. Tumour growth fraction measured by immunohistochemical staining of KI67 is an independent prognostic factor in preoperative prostate biopsies with small-volume or low-grade prostate cancer. International Journal of Cancer 2009;124 (9):2116–2123.
19) Krohn A, Diedler T, Burkhardt L, et al. Genomic deletion of PTEN is associated with tumor progression and early PSA recurrence in ERG fusion-positive and fusion-negative prostate cancer. American Journal of Pathology, 2012;181(2):401-412.
20) Cuzick J, Berney DM, Fisher G, et al. Prognostic value of a cell cycle progression signature for prostate cancer death in a conservatively managed needle biopsy cohort. Br J Cancer. 2012;106:1095–1099.
21) Yoshimoto M, Joshua AM, Cunha IW, et al. Absence of TMPRSS2:ERG fusions and PTEN losses in prostate cancer is associated with a favorable outcome. Mod Pathol 2008; 21:1451–1460.
22) John JS, Powell K, Conley-LaComb MK, et al. TMPRSS2-ERG fusion gene expression in prostate tumor cells and its clinical and biological significance in prostate cancer progression. J Cancer Sci Ther 2012;4 (4):94-101.
23) Barwick BG, Abramovitz M, Kodani M, et al. Prostate cancer genes associated with TMPRSS2–ERG gene fusion and prognostic of biochemical recurrence in multiple cohorts. British Journal of Cancer 2010;102:570 – 576.
24) Berney DM, Gopalan A, Kudahetti S, et al. Ki-67 and outcome in clinically localized prostate cancer: analysis of conservatively treated prostate cancer patients from the Trans-Atlantic Prostate Group study. Br J Cancer 2009;100(6): 888–893.
25) Qi M, Yang X, Zhang F, et al. ERG rearrangement is associated with prostate cancer-related death in Chinese prostate cancer patients. PLoS ONE 2014; 9(2): e84959.
26) Carver BS, Tran J, Gopalan A, et al. Aberrant ERG expression cooperates with loss of PTEN to promote cancer progression in the prostate. Nat Genet 2009;41: 619-624.
27) Reid AH, Attard G, Ambroisine L, et al. Molecular characterisation of ERG, ETV1 and PTEN gene loci identifies patients at low and high risk of death from prostate cancer. Br J Cancer 2010; 102:678-684.