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ERG protein expression reflects hormonal treatment response and is associated with Gleason score and prostate cancer specific mortality

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Abstract *Background:* ERG (*ETS regulated gene*) protein expression has been shown to reflect ERG genomic rearrangements in prostate cancer (PCA). However, ERG protein expression prognostic value has not been yet investigated.

Design: ERG protein expression was investigated in a cohort of 312 men with PCA diagnosed in transurethral resection of the prostate.

Results: ERG expression was detected in 76/293 (25.9%) of patients. Overall ERG expression was associated with Gleason score (GS) ($p < 0.0001$), tumour volume ($p = 0.04$) and with cancer specific mortality ($p = 0.15$). Low ERG intensity was significantly associated with higher GS ($p = 0.02$) and marginally with cancer specific mortality ($p = 0.11$). The association with cancer specific mortality was more significant in patients without any hormonal manipulation ($p = 0.02$). Multivariate Cox model using GS, tumour volume and ERG intensity to predict time to cancer specific death yielded a marginally significant effect for high versus low ERG protein expression (hazard ratio (HR) = 0.36; 95% confidence interval (CI): 0.10–1.38; $p = 0.14$) and a non-significant effect for GS >7 (HR = 4.85; 95% CI: 0.48, 48.65;

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$p = 0.18$). Men with ERG expression showed longer free progression time to castration resistant disease compared to men with no ERG expression (mean 11.39 versus 6.1 months, $p = 0.08$).

Conclusion: We report significant association between ERG protein levels and each of GS, progression to castration resistant and cancer specific mortality. High ERG intensity was associated with lower GS, better overall survival and longer free progression times to castration resistant disease. ERG protein levels may have prognostic and therapeutic role in PCA and should be investigated in future studies.

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1. Introduction

The rearrangements between the androgen receptor-regulated gene *TMPRSS2* (21q22.3) and other members of the ETS (*E26*) family member of transcription factor gene, commonly *ERG* (21q22.2), are considered the most common prevalent genetic alteration in prostate cancer (PCA).^{1–5} Both intra and inter-chromosomal genetic rearrangements were found to drive the *ERG* rearrangements in PCA. It is documented that *ERG* gene rearrangements occur in 40–60% in surgical cohorts depending on the various techniques used including fluorescence in situ hybridization (FISH), single nucleotide polymorphism arrays and quantitative polymerase chain reaction (QPCR).^{1,6–9} This is in contrast to a rate of 12–15% in unsuspected or watchful waiting cohorts.^{10–12}

Although *ERG* gene rearrangements linkage to aggressive PCA is controversial^{13–16} it is becoming evident that it plays a significant role in disease progression and could signify potential base to molecularly classify PCA.

Recently, studies suggest that ERG protein expression could be used as a surrogate marker for *ERG* genomic rearrangements documenting remarkable concordance between the two with 86–100% sensitivity and 85–96.5% specificity.^{17–22}

Previous studies do not all document clear association between *ERG* gene rearrangements and Gleason score or clinical outcome. This may be due to the fact that about 10% of PCA shows disassociation between genomic *ERG* gene rearrangements and ERG protein expression. Furthermore, the clinical-pathologic and prognostic values of *ERG* protein expression has not been yet fully investigated. In this study we report for the first time significant association between ERG protein expression, Gleason score, tumour volume and cancer specific mortality in a large cohort of men with PCA.

2. Materials and methods

2.1. Study population and tissue microarray construction

The overall study cohorts consisted of 312 men diagnosed with PCA between 2005 and 2009 by transurethral resection of the prostate (TURP). The first cohort consisted of men diagnosed incidentally from tissues removed for clinical suspicion of benign prostate

hyperplasia ($n = 152$). The second cohort represented men with castration resistant prostate cancer (CRPC) treated by channel TURP to relieve symptomatic obstruction due to locally advanced disease ($n = 160$). Initial treatments for patients included observation, radiotherapy or surgery. Development of castration resistant disease was treated by LH–RH (luteinizing hormone–releasing hormone) agonist as monotherapy. Clinical follow-up information was collected from the Alberta Tumour Registry in regard to overall survival, cancer specific mortality and to the implementation of hormonal treatment. The study cohorts were assembled onto two tissue microarrays (TMAs) with an average of two cores (range 2–6) per patient to a total of 714 cores using a manual tissue arrayer (Beecher Instruments, Silver Spring, MD). Each block was assembled without prior knowledge of any clinical or pathological staging information. All Clinical and pathological data were obtained with approval of the institutional review board at the University of Calgary, Faculty of Medicine, Calgary, Alberta, Canada.

2.2. ERG protein expression by immunohistochemistry (IHC)

Briefly, 4 μm thick sections from formalin-fixed paraffin-embedded tissue blocks were stained with Ventana autostainer. Prior to staining, heat induced antigen retrieval was carried out by vegetable steamer in sodium citrate antigen retrieval buffer (10 mM pH 6.0) for 40 min, and then cooling down to room temperature for about 20 min. The slides were incubated for 60 min at 37 C with ERG rabbit monoclonal antibody (Epitomics, clone EPR 3864) at 1:50 dilution. A Ventana iView DAB detection kit (Ventana Tucson, Ariz, United States of America (USA)) was used for HRP (horseradish peroxidase) detection and counter stain.

2.3. Pathological analysis

All TMA cores diagnoses were confirmed by the two study pathologists (L.H.T. and T.A.B.) on the initial slides to verify the histological diagnosis. Gleason scoring was assessed according to the 2005 International Society of Urological Pathology (ISUP) criteria.²³ For each patient, the two predominant patterns were

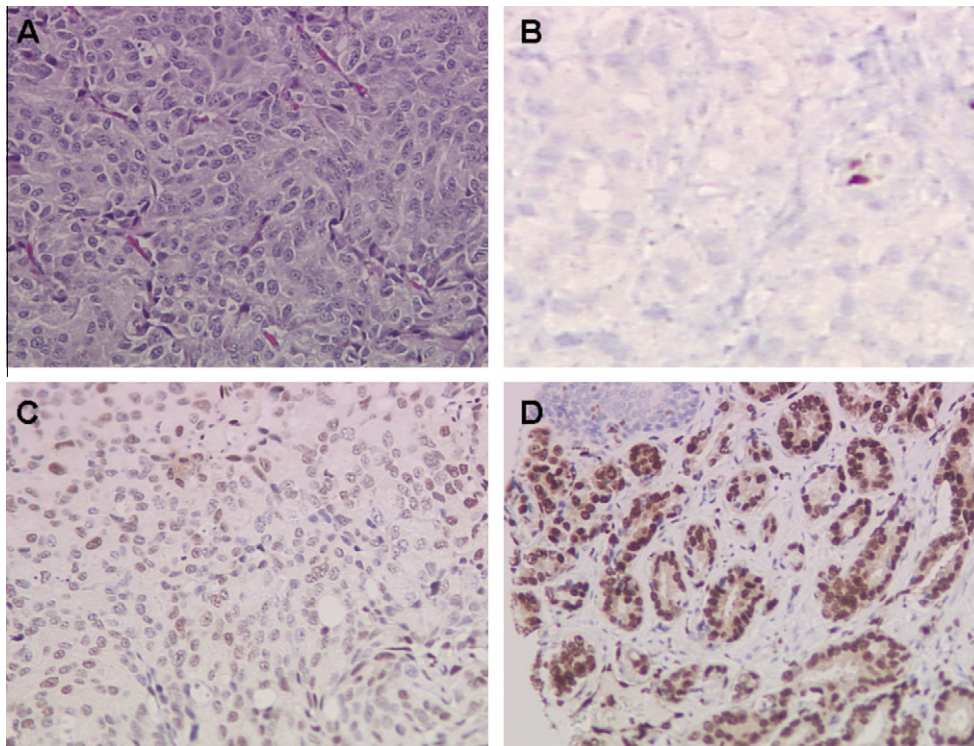


Fig. 1. Representative images of ERG immunohistochemistry in prostate cancer. (A) H & E of Gleason pattern 5 (score 10), (B) ERG negative expression in Gleason score 10 with positive endothelial cells as control, (C) ERG weak expression of Gleason score 10, and (D) ERG high expression in Gleason score 6.

Table 1
Combined study cohorts demographics.

Characteristic	Statistic	Value
Surgery age (years)	<i>N</i>	312
	Mean	76.5 years
	Range	50.7–93.5 years
Total Gleason score	<i>N</i>	310
	Mean (SD)	7.71(1.52)
	Range	5–10
Categorical Gleason score	GS = 5–6	100
	GS = 7	49
	GS = 8–10	159
Tumour volume*	≤5%	50
	>5%	172
Follow-up time (in months)	<i>N</i>	309
	Mean	23.45 months
	Range	1.5–100.2 months
ERG expression	<i>N</i> total	293
Unsuspected cohort	ERG positive (%)	22/136* (16.1%)
Castration resistant cohort	ERG positive (%)	54/157* (34.4%)

* Not all cases have available information.

sampled and included on the TMAs for analysis. ERG protein expression was assessed semi-quantitatively using 3-tiered system (negative; low and high). Cases with either low or high intensities were considered positive based on previous correlation with *ERG* gene rearrange-

ment as detected by FISH (data not shown). The ERG antibody was consistently strongly expressed in endothelial cells, which acted as internal control for expression and intensity level. Fig. 1 shows examples of negative, low and high ERG expressions.

Table 2
Association of ERG protein expression and Gleason score in the combined study cohorts.

Gleason score	ERG negative	ERG positive	Total (%)	<i>p</i> -Value*
GS 6	75 (91.5%)	7 (8.5%)	82(100%)	
With 7				0.009
With >7				<0.0001
GS 7	32 (68.1%)	15 (31.9%)	47 (100%)	
With 6				0.009
With >7				0.964
GS >7	104 (66.2%)	53 (33.8%)	157 (100%)	
With 6				<0.0001
With 7				0.964

* Overall *p* value between the three groups ($p < 0.0001$).

2.4. Statistical analysis

Patient characteristics were presented as frequencies and percentages for categorical variables, and as means and ranges for continuous variables. Chi-square tests were used to test for associations between ERG protein expression and Gleason score, as well as tumour volume. The Kaplan–Meier approach along with the log-rank test was used for the survival analyses to test the association between ERG expression and prostate cancer related death. In all statistical tests a *p* value < 0.05 was considered significant.

3. Result

3.1. The study population

Mean patients' age was 76.5 years (range 50.7–93.5 years) with average follow-up time of 23.4 months (range 1.5–100.2 months). 119/312 (38.14%) of patients received hormonal therapy due to disease progression. A total of 293/312 (93.9%) patient's samples were available for assessment. Table 1 demonstrates patients' demographics of the study cohort. We sought to investigate ERG expression in the two cohorts separately due to differences in cohort's design.

3.2. ERG expression in the incidental prostate cancer cohort

Overall ERG expression was detected in 22/136 (16.1%), with 5/22 (22.8%) of patients showing low ERG intensity versus 17/22 (77.2%) showing high ERG intensity. There were no significant statistical differences between patients with ERG expression versus those with no expression in regard to cancer specific mortality ($p = 0.4$). The differences between high and low ERG expressions could not be calculated as all cases were censored. There was a trend for ERG to be expressed in higher tumour volumes, 6/41 (14.6%) in pTa versus 14/52 (26.9%) in pTb ($p = 0.3$). We observed significant association between ERG expression and

higher Gleason score. In this cohort, ERG expression was detected in 8/81 (9.8%) of GS5-6 versus 14/45 (31.1%) being detected in GS7 ($p < 0.03$).

3.3. ERG expression in the castration resistant prostate cancer cohort

ERG expression was present in 54/157 (34.4%) of patients, with 31/54 (57.4%) and 23/54 (42.6%) of patients demonstrating low and high ERG expressions respectively. In this cohort 40/117 (34.1%) of ERG expressions were detected in pT1b versus 0% in pT1a. However, in this cohort only two cases were of pTa stage. We also noticed that higher GS (9–10) tumours tended to express lower intensity of ERG 28/138 (20.2%) versus 3/22 (13.6%) in GS (7–8). While higher intensity showed the opposite results being detected in higher incidence in lower grade tumours. As in this cohort, we had only one patient with GS7 and none with GS<7, we could not investigate ERG association to Gleason score.

3.4. ERG expression in relation to Gleason score and tumour volume in the combined cohorts

To better investigate ERG association to GS and tumour volume, we grouped the two cohorts to allow for a wide range for tumour volume distribution and different Gleason scores. Overall ERG protein expression was detected in 76/293 (25.9%) patients with 8/91 (8.7%), 14/46 (30.4%) and 51/149 (34.2%) being detected in Gleason score 6, 7 and 8–10, respectively. Moreover, there was statistical significance between ERG expression in Gleason score 6 versus 7 and >7 ($p < 0.0001$) and between ERG expression and tumour stage (pT1a versus pT1b) as reflected by tumour volume ($\leq 5\%$ versus $> 5\%$) ($p = 0.04$) (Table 2 and Fig. 2A and B).

We observed comparable rates for the distribution of low and high ERG intensities with 34/73 (46.6%) patients demonstrating low ERG intensity versus 39/73 (53.4%) patients with high ERG intensity. Using this criterion, there was significant inverse relationship between ERG intensity levels and Gleason score with weaker intensity

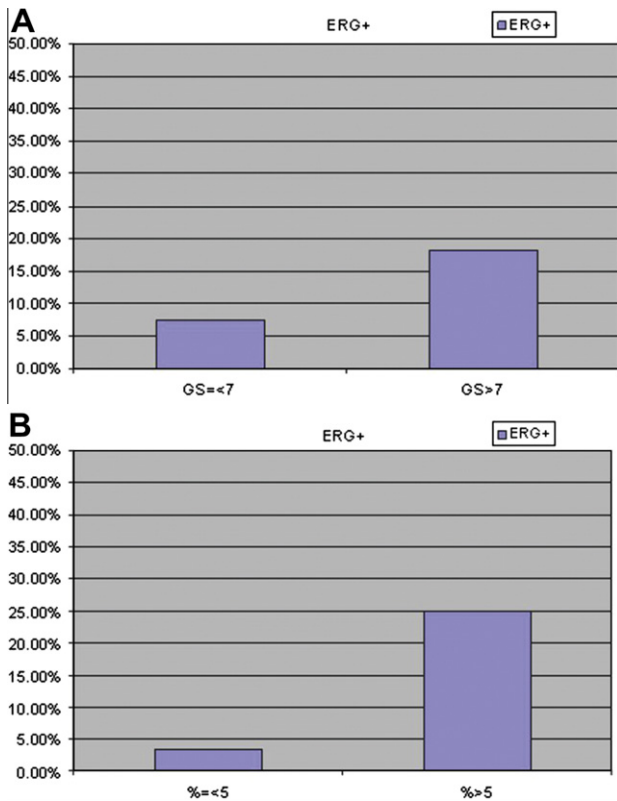


Fig. 2. Graphs showing relation of ERG expression to (A) Gleason score and (B) Tumour volume.

of ERG occurring more frequently in higher Gleason score tumours and higher intensity ERG occurring in lower Gleason score tumours ($p = 0.02$).

All the above analyses remained statistically significant when cases with prior hormonal manipulation were removed (not shown). Table 3 and Fig. 3A and B demonstrate the relationship between ERG intensity level and each of Gleason score and tumour volume.

3.5. ERG protein expression in relation to cancer specific mortality

To investigate the significance of ERG expression in patients' overall survival and as ERG levels are affected by androgen manipulation, we removed patients who

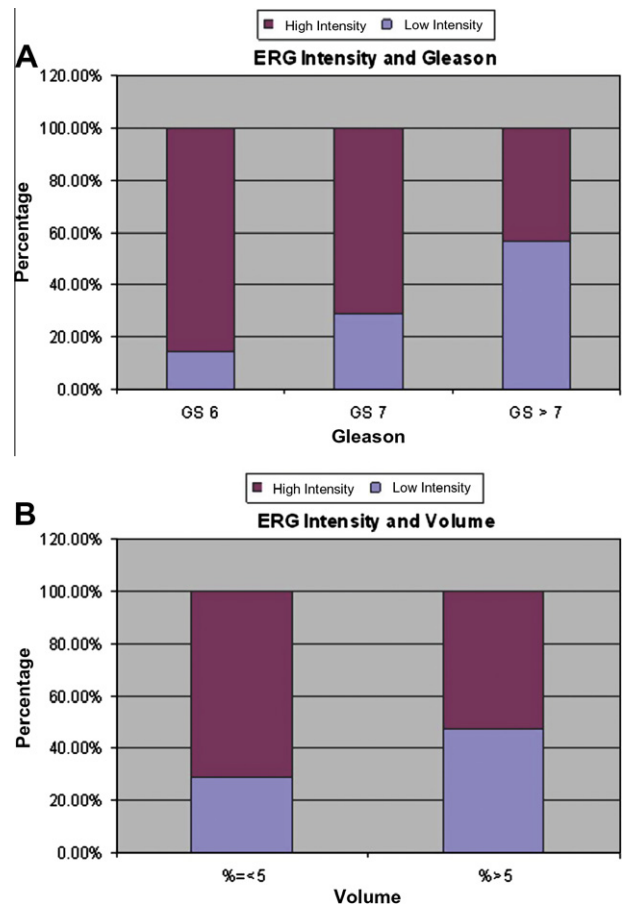


Fig. 3. Graphs showing relation of ERG intensity levels (low versus high) to (A) Gleason score and (B) Tumour volume.

had hormonal treatment prior to tissue sample acquisition. We assessed the association of ERG to overall survival in the subgroup of patients not being subjected to any hormonal treatment ($n = 177$). In this subgroup of patients, ERG expression was marginally associated with higher rates of cancer specific mortality ($p = 0.15$) (Fig. 4A). Within this subgroup of ERG positive patients, patients with high ERG intensity showed significant overall survival advantage compared to patients with low ERG intensity ($n = 37$, $p = 0.02$) (Fig. 4B). This selective advantage to high ERG intensity was also

Table 3

Association of ERG level of protein expression (low and high) to Gleason score in the combined study cohorts.

Gleason score	Low ERG intensity (expression)	High ERG intensity (expression)	Total (%)	<i>p</i> -Value*
GS 6	1 (14.3%)	6 (85.7%)	7 (100%)	
With 7				0.469
With >7				0.035
GS 7	4 (28.6%)	10 (71.4%)	14 (100%)	
With 6				0.469
With >7				0.061
GS >7	29 (56.9%)	22 (43.1%)	51 (100%)	
With 6				0.035
With 7				0.061

* Overall p value between the three groups ($p < 0.03$).

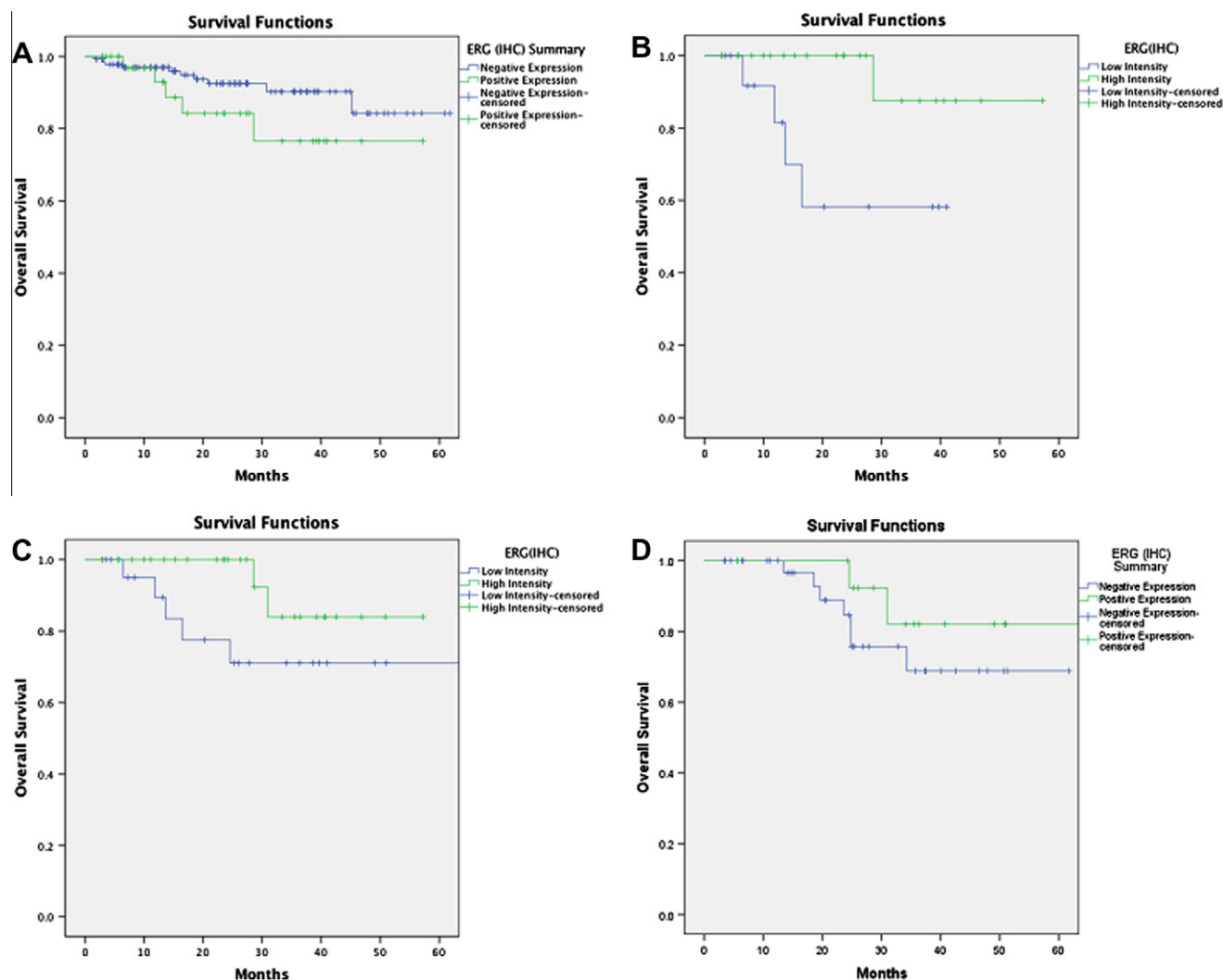


Fig. 4. Kaplan Meier survival curves. (A) Overall survival of prostate cancer (PCA) patients with ERG positive, and negative tumours not subjected to hormonal treatment ($n = 177$, $p = 0.15$). (B) Overall survival difference of High versus low ERG expression in a subgroup of patients not subjected to hormonal therapy ($n = 37$, $p = 0.02$). (C) Overall survival difference of High versus low ERG intensity in the overall PCA cohort not subjected to prior hormonal therapy, regardless of type of treatment implemented ($n = 53$, $p = 0.11$). (D) Overall survival of ERG expression in the CRPC subgroup treated by LH–RH agonist ($n = 54$, $p = 0.31$).

noted in the overall study cohorts, who were not subjected to prior hormonal treatments and regardless of the type of treatment being implemented ($n = 53$, $p = 0.11$) (Fig. 4C). A multivariate Cox model using Gleason score, tumour volume and intensity of ERG protein expression to predict time to cancer-specific death yielded a marginally significant effect for high versus low ERG protein expression (HR = 0.36; 95% CI: 0.10–1.38; $p = 0.14$) and a non-significant effect for Gleason score greater than 7 (HR = 4.85; 95% CI: 0.48, 48.65; $p = 0.18$) (Table 4).

3.6. ERG protein expression relation to progression to castration resistant disease

Several previous studies have reported significant association between ERG and the levels of androgen receptor, which may have prognostic implication.^{24–26} However, to

our knowledge, none of the previous studies have documented significant prognostic association between ERG protein levels and prostate cancer progression to castrate resistant disease. Herein, we sought to investigate existing relationship between ERG expression and the development of castration resistant disease and overall survival. Fifty four patients demonstrated disease progression following PCA diagnosis and were subjected to LH–RH agonist as anti androgen monotherapy. ERG expression was present in 16/54 (29.6%). Patients with ERG expression had longer free progression time to castration resistant disease compared to patients with no ERG expression [mean progression time; 11.39 ± 4.4 months versus 6.1 ± 0.51 months, respectively] ($p = 0.08$). In this subgroup of patients subjected to hormonal manipulation, patients with ERG expression showed marginal lower cancer specific mortality rates versus patients with no ERG expression, suggesting better survival advantage

Table 4
Cox regression model: using Gleason summary, % tumour volume & ERG expression.

Analysis of maximum likelihood estimates								
Parameter	DF	Parameter estimates	Standard error	Chi-square	Pr > ChiSq	Hazard ratio	95% hazard ratio confidence limits	
GS >7 versus ≤7	1	1.57947	1.17610	1.8036	0.1793	4.852	0.484	48.646
% volume >5% versus ≤5%	1	−0.20319	1.17137	0.0301	0.8623	0.816	0.082	8.106
ERG high versus low expression	1	11.01439	0.68269	2.2065	0.1374	0.363	0.095	1.383

in hormonally treated patients with ERG expression ($n = 54$, $p = 0.31$) (Fig. 4D).

4. Discussion

The study is the first to report significant association between ERG protein expression and patient's prognosis reflected as either overall survival or the development of CRPC. The study also documents significant association with Gleason score and tumour volume (stage). It has been documented that *ERG* gene rearrangements are associated with increased transcript levels of ERG.¹⁰ Moreover, ERG immunohistochemistry has been documented to be reflective of genomic *ERG* gene rearrangements.^{21,22,29} There are also several recent reports documenting its potential usefulness as a tool aiding in the pathological diagnosis of prostate needle biopsies.^{17,27,28} However, although ERG protein expression was investigated in cohorts of localized PCA,^{24,30} to date, there are no studies investigating its association in unsuspected or castration resistant disease. Our study is the first to document association of ERG protein expression with Gleason score, higher tumour volume and disease progression relative to castration resistant disease and patients' overall survival.

ERG protein expression was detected in 16.1% and 34.4% of the men in the unsuspected and the castration resistant cohorts, respectively. This is consistent with recent report documenting differences in the incidence of *ERG* gene rearrangements relative to cohorts' types and tumour location.³¹ The difference in ERG incidence observed here points towards potential implication of ERG in CRPC, and reflects that ERG plays a more significant and prognostic role in the castration resistant disease compared to localized or hormone naive metastatic disease. Although not related directly to the current study, we investigated *ERG* gene rearrangements by FISH and ERG protein expression by IHC separately in a subset of patients ($n = 160$). We observed a concordance rate of 88.0% (data not shown) supporting ERG IHC as a suitable surrogate to *ERG* gene rearrangements.^{18,19,21} Based on these findings, it is documented that a subset of PCA tumours (~10%) shows discordance between genomic *ERG* and expression of ERG. This may explain in part the failure of earlier reports to document significant prognostic value for ERG. Another possible reason for the conflicting results

published could be related to the type of the cohorts being investigated, as most studies documenting significant association were reflective of the natural history of disease progression while those unable to show significant association were more representing surgical cohorts with active PSA screening programs.^{32,33}

It is well known that ERG protein expression is the active form of the gene product and hence could be a better method in documenting any prognostic significance. In the current study, we document for the first time association between overall ERG protein expression as assessed by immunohistochemistry, Gleason score and tumour volume. More important, we show inverse relation between higher Gleason score and the level of ERG intensity, with higher Gleason score tumours expressing lower ERG intensity. This finding is important in view of recent reports suggesting potential application of ERG IHC in diagnostic needle biopsy.

ERG positive tumours were associated with higher rates of cancer specific mortality, in line with previous reports assessing genomic *ERG* rearrangements or copy numbers.^{10,34} However, herein, we document for the first time significant prognostic differences between tumours with low and high ERG intensities (high versus low ERG protein). High intensity tumours were at better selective advantage (even when considering GS and tumour volume) in the context of hormonal manipulation as they were associated with better prognosis in castration resistant patients. This was even more significant in patients without any hormonal manipulation. This suggest better prognosis in the high intensity group and may be contradicting previous reports suggesting that increased *ERG* copy numbers are associated with worse prognosis.³⁴ However, this could be partially explained by the fact that ERG intensity levels are inversely correlated to higher Gleason score and higher tumour volumes which means that lower intensity tumours are aligned to poorly differentiated cancers and hence the worse outcome. The last observation was even more significant in patients not subjected to any hormonal treatment suggesting an existing relationship between ERG and hormonal treatments.

The association between ERG expression and hormonal manipulation is further supported by our results of better overall survival in patients with ERG expression versus those with no ERG expression. In our

cohort, patients with ERG expression demonstrated longer time free progression to castration resistant disease compared to patients with no ERG expression suggesting again that ERG positive tumours may be at selective advantage to hormonal manipulation and more responsive to hormonal treatments compared to ERG negative as suggested by earlier reports.³⁵

In conclusion, we report significant association between ERG protein expression, and each of Gleason score, tumour volume and patients' overall survival. Patients with ERG positive tumours seem to be at higher risk of dying from prostate cancer. However, high intensity ERG tumours seem to be at better selective advantage and more responsive to hormonal manipulation with better overall survival compared to low intensity ERG tumours. Studies incorporating ERG status in clinical trials could be beneficial for stratifying patients into different prognostic and therapeutic responsive groups.

Conflict of interest statement

None declared.

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