ERG expression is associated with increased risk of biochemical relapse following radical prostatectomy in early onset prostate cancer

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ERG expression is associated with increased risk of biochemical relapse following radical prostatectomy in early onset prostate cancer

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Abstract

Purpose ERG expression has been proposed to signify molecular subtype of PCA. However, its significance in early onset prostate cancer (PCA) is not characterized.

Materials and methods ERG protein expression was investigated in a cohort of 121 men diagnosed with localized PCA at ≤50 years of age with a mean follow-up time of 65.7 months. ERG was correlated to patients’ outcome and clinical-pathological parameters using univariate and multivariate analysis.

Results ERG expression was detected in 76/118 (64.4 %) analyzable patients’ samples and showed interfocal heterogeneity (differences between foci) in 17/118 (14.4 %) patients. There was significant association between ERG expression and Gleason score (\( p = 0.022 \)), but not with any other clinical-pathologic parameter, including pre-surgical PSA levels, tumor volume, pathological stage, surgical margin or lymph-vascular invasion. ERG had significant effect on the rate of biochemical relapse following radical prostatectomy, with ERG positive patients showing higher relapse rates vs. ERG negative patients (\( p = 0.007 \)). However, considering time till biochemical relapse post-radical prostatectomy, ERG expression showed positive insignificant trends (\( p = 0.071 \)). Notably, and of great significance, in this cohort of early onset disease, none of the ERG negative PCA patients exhibited biochemical relapse.

Conclusion The study results suggest that ERG expression may be of added prognostic value in localized prostate cancer in patients with early onset PCA. However, the issue of ERG interfocal heterogeneity observed may require the evaluation of several tumor foci to assess ERG status per case. Incorporating ERG status into existing nomograms may be of added prognostic value in patients with early onset PCA.

Keywords Prostate cancer · Early onset disease · Young patients · ERG protein expression · Prognostic value · Biochemical relapse

Introduction

Prostate cancer (PCA) is the most common cancer and the second leading cause of cancer deaths in the western world [1]. It is well known that age is a risk factor for PCA [2]. However, a subset of PCA diagnosed early in life in men 50 years of age or younger ("early-onset PCA") accounts for about 2 % of all PCA [3]. A few studies have reported, although with some inconsistencies, differences in
clinicopathological characteristics between early-onset PCA and “classical” cases of PCAs (“elderly-onset” PCA) diagnosed in 60–80 year-old men [4–8]. The early- and elderly-onset PCAs also likely differ in their patho-mechanism and molecular changes for specific pathways associated with disease progression. Noticeably it has recently suggested that early-onset PCAs, harbour specific androgen driven somatic alteration including higher frequency of androgen-regulated ETS gene fusion such as TMPRSS2-ERG relative to elderly-onset PCA [3, 9].

The rearrangements between the androgen receptor-regulated gene TMPRSS2 (21q22.3) and members of the ETS family member of transcription factor gene, most commonly ERG (21q22.2), are among the most common genetic alteration detected in prostate cancer [10–12]. ERG gene rearrangements have been detected in approximately 50 % of radical prostatectomy PCA specimens of PSA-screened, predominantly Caucasian cohorts compared to approximately 12–15 % incidence in watchful waiting or unsuspected cancer diagnosed by TURP, which is likely reflective of the zonal origin of PCA tumors (i.e. peripheral vs. transitional zone) [13–16]. Recently, ERG protein expression assessed by immunohistochemical staining has been shown to highly correlate with the ERG gene rearrangement status in PCA [17, 18].

Previous studies investigating the prognostic significance of ERG gene rearrangements and expression in elderly-onset PCA have revealed mixed results [15, 19–28]. The present report is the first study designed specifically to investigate the potential prognostic value of ERG expression in early-onset PCA in radical prostatectomy specimens from patients under 50 years of age at diagnosis in relation to clinicopathologic variables and clinical outcome.

Materials and methods

Study population and tissue microarray construction

The study cohort consisted of 121 patients who were treated by retro-pubic radical prostatectomy for localized prostate cancer between 2000 and 2005 with a mean follow-up of 65.7 months. Clinical and pathological data were obtained with approval from the University of Calgary institutional review board. Biochemical relapse was defined as a rise of serum PSA (prostate-specific antigen) levels of >0.2 ng/ml at two separate occasions following undetectable levels post radical prostatectomy. Gleason scoring was assessed according to the 2005 ISUP criteria [29]. Prostate samples were embedded on two tissue microarrays (TMA) blocks using a manual tissue arrayer (Beecher Instruments, Silver Spring, MD). Each block was assembled without prior knowledge of any clinical or pathological staging information. An average of three blocks per case were sampled per patient with four PCA and two benign cores on average per case. The two predominant Gleason patterns of each case were sampled and included on the TMA blocks for analysis. A total of 704 cores (479 cancer and 225 benign) 0.6 mm in diameter were targeted for sampling. After construction, 4 µm sections were cut and stained with haematoxylin and eosin to verify the histological diagnosis.

ERG protein expression by immunohistochemistry (IHC)

Briefly, 4 µm thick sections from formalin-fixed paraffin-embedded tissue blocks were stained with Ventana auto-stainer. Prior to the 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 cooled down to room temperature for about 20 min. The slides were incubated for 60 min at 37 °C with ERG rabbit monoclonal antibody (Biocare Medical, clone 9FY) at 1:50 dilution. A Ventana iView DAB detection kit (Ventana Tucson, Ariz, USA) was used for horseradish peroxidase detection and counter stain. To confirm antibody specificity, a second set of TMA slides were stained with a second ERG antibody (Epitomics, clone EPR 3864) at 1:50 dilution.

Pathological analysis

All TMA cores diagnoses were confirmed by the two study pathologists (KCH and TAB). ERG protein expression was assessed as negative or positive, based on previous correlation with ERG gene rearrangement as detected by fluorescent in situ hybridization break-apart probe (data not shown). The ERG antibody was consistently strongly expressed in endothelial cells, which acted as internal control. Figure 1 shows examples of negative and positive ERG expression in benign and malignant prostatic glands.

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, surgical margin, tumour volume, lymph-vascular invasion and pathological stage. The Kaplan–Meier approach along with the log-rank test was used to test the association between ERG expression and biochemical PSA relapse. In all statistical tests a p value <0.05 was considered significant.
Results

Clinical and pathological demographics of the study cohort

The original cohort consisted of 121 patients. Two patients received neoadjuvant hormonal therapy due to high Gleason score (GS 9 and 10) and one patient with inadequate tumor volume for sampling (<1 %) were excluded from the analysis. A total of 118/121 patients’ samples were available for analysis who had undergone radical prostatectomy for localized PCA. Mean patients’ age in this cohort was 47.0 years (range 36.2–49.9 years) with an average follow-up time of 65.7 months. The majority of cases [116/118 (98.3 %)] in this cohort were of Gleason score 6 and 7 and more than 95 % were organ confined with ~75 % demonstrating negative surgical margins. Overall, lymph-vascular invasion was noted in 5.3 % (6/114) of samples. Table 1 demonstrates overall patients’ demographics of the study cohort. All patients included in this analysis underwent radical prostatectomy as initial monotherapy.

In this cohort, complete follow-up data related to PSA biochemical relapse was available for 85 patients. Overall 11.8 % (10/85) of patients demonstrated biochemical PSA relapse over the study follow up period and three deaths recorded, but none were cancer-specific mortalities (one none cancer death, one due to metastatic melanoma and one due to leukemia). No bony or lymph node metastasis were detected in any of the patients with available bone metastasis or lymph node status (0/91).

Expression of ERG protein by anti-ERG monoclonal antibody in PCA

The two anti-ERG antibodies showed significant intensity correlation (Pearson correlation of 0.945; p < 0.0001).

ERG protein expression was detected in 76/118 patients (64.4 %) with 17/118 (14.4 %) patients showing different ERG status (negative vs. positive) between different tumor foci (interfocal heterogeneity). To investigate ERG relation to Gleason score, we characterized ERG expression based on Gleason sum of individual TMA cores sampled. Grouping individual PCA TMA cores into GS <7 vs. 7

Table 1 Demographics of the study population cohort investigated for ERG expression

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) (mean; range)</td>
<td>47.0 (36.2–49.9)</td>
</tr>
<tr>
<td>Gleason summary</td>
<td></td>
</tr>
<tr>
<td>&lt;7</td>
<td>62 (52.5 %)</td>
</tr>
<tr>
<td>7</td>
<td>54 (45.8 %)</td>
</tr>
<tr>
<td>3 + 4</td>
<td>45 (39.1 %)</td>
</tr>
<tr>
<td>4 + 3</td>
<td>9 (7.6 %)</td>
</tr>
<tr>
<td>&gt;7</td>
<td>2 (1.7 %)</td>
</tr>
<tr>
<td>pT-stage</td>
<td></td>
</tr>
<tr>
<td>pT2</td>
<td>113 (95.8 %)</td>
</tr>
<tr>
<td>pT3</td>
<td>5 (4.2 %)</td>
</tr>
<tr>
<td>Surgical margin</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>88 (74.6 %)</td>
</tr>
<tr>
<td>Positive</td>
<td>30 (25.4 %)</td>
</tr>
<tr>
<td>Lymph-vascular invasion</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>108 (94.7 %)</td>
</tr>
<tr>
<td>Positive</td>
<td>6 (5.3 %)</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>89 (100 %)</td>
</tr>
<tr>
<td>Present</td>
<td>0</td>
</tr>
<tr>
<td>Bone metastasis</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>91 (100 %)</td>
</tr>
<tr>
<td>Present</td>
<td>0</td>
</tr>
<tr>
<td>Hormonal treatment</td>
<td></td>
</tr>
<tr>
<td>Neoadjuvant</td>
<td>0</td>
</tr>
<tr>
<td>Adjuvant</td>
<td>0 (0.00 %)</td>
</tr>
<tr>
<td>PSA biochemical relapse</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>75 (88.2 %)</td>
</tr>
<tr>
<td>Positive</td>
<td>10 (11.8 %)</td>
</tr>
<tr>
<td>PCA specific mortality/total deaths</td>
<td>0/3</td>
</tr>
</tbody>
</table>
(3 + 4 vs. 4 + 3), there was significant positive association between ERG expression and GS ($p = 0.032$) (Fig. 2a). However, when comparing PCA TMA cores as GS $\leq 7$ vs. $7 > 7$, there was an inverse relationship between ERG expression and GS of tumors in the TMA cores with GS $\leq 7$ ($p = 0.022$) (Fig. 2b).

Expression of ERG in relation to clinical-pathological parameters

When investigating relations between ERG expression and pathological parameters other than GS, there was no significant association between ERG expression and any other parameter analyzed including mean tumor volume ($p = 0.866$), pathological disease stage ($p = 0.457$), surgical margin status ($p = 0.886$) and lymph-vascular invasion ($p = 0.926$). In addition, there was no significant association between ERG expression and tumour volume. Specifically, the mean tumour volume of ERG expressing PCA was 14.9 cm$^3$ and that of non-ERG expressing PCA was 15.1 cm$^3$ ($p = 0.866$). ERG positive tumors tended to show higher none significant mean value of serum PSA compared to ERG negative tumors (5.21 vs. 4.77 ng/ml) ($p = 0.44$). Similarly, there were no significant differences between ERG positive and negative tumors in relation to mean or median PSA density (0.23 vs. 0.20 cc/ng/ml; $p = 0.53$) and (0.16 vs. 0.13 cc/ng/ml; $p = 0.13$), respectively. ERG expression was present in 72/113 (63.7 %) patients with pT2 versus 4/5 (80.0 %) patients with pT3 ($p = 0.457$). When analyzed against surgical margin status, ERG expression was present in 19/29 (65.5 %) PCA with positive surgical margin versus 57/89 (64.0 %) PCA with negative surgical margin ($p = 0.886$). Overall, lymph-vascular invasion was noted in 5 % (6/114) of patients in this population cohort, with 4/6 detected in ERG positive vs. 2/6 detected in ERG negative tumors. However, this was not statistically significant ($p = 0.926$). Table 2 illustrates patients’ clinical-pathological demographics of the study cohort in relation to ERG expression status.

ERG protein expression in relation to patient’s clinical outcome

When investigating the prognostic value of ERG expression to clinical patient’s outcome and since there was no

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Distribution of ERG negative and positive in relation to patients’ clinical-pathological demographics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ERG negative (42 patients)</td>
</tr>
<tr>
<td>Age (years) (mean; range)</td>
<td>47.5 (36.2–49.7)</td>
</tr>
<tr>
<td>Gleason summary</td>
<td></td>
</tr>
<tr>
<td>$&lt;7$</td>
<td>25 (59.5 %)</td>
</tr>
<tr>
<td>7</td>
<td>16 (38.1 %)</td>
</tr>
<tr>
<td>3 + 4</td>
<td>14 (33.3 %)</td>
</tr>
<tr>
<td>4 + 3</td>
<td>2 (4.8 %)</td>
</tr>
<tr>
<td>$&gt;7$</td>
<td>1 (2.4 %)</td>
</tr>
<tr>
<td>pT-stage</td>
<td></td>
</tr>
<tr>
<td>pT2</td>
<td>41 (97.6 %)</td>
</tr>
<tr>
<td>pT3</td>
<td>1 (2.4 %)</td>
</tr>
<tr>
<td>Surgical margin</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>31 (73.8 %)</td>
</tr>
<tr>
<td>Positive</td>
<td>11 (26.2 %)</td>
</tr>
<tr>
<td>Lymph-vascular invasion</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>38 (95.0 %)</td>
</tr>
<tr>
<td>Present</td>
<td>2 (5.0 %)</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td>Not available</td>
</tr>
<tr>
<td>Absent</td>
<td>32 (100 %)</td>
</tr>
<tr>
<td>Present</td>
<td>0</td>
</tr>
<tr>
<td>PSA relapse</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>33 (100 %)</td>
</tr>
<tr>
<td>Positive</td>
<td>0 (0 %)</td>
</tr>
</tbody>
</table>
cancer-specific mortality or development of bony metastasis in the study cohort during the study follow-up time (average 65.7 months), we investigated the potential association between ERG expression and post surgical serum PSA levels reflective of biochemical relapse after radical prostatectomy. 85/118 of patients with samples available for analysis in this cohort had combined ERG and PSA biochemical relapse data. ERG positive tumors were significantly associated with PSA biochemical relapse following radical prostatectomy compared to ERG negative tumors ($p = 0.007$). However, when considering time till PSA biochemical relapse (81/85 patients with time till PSA relapse were available for analysis using the Kaplan–Meier method), ERG positive expression was marginally associated with higher rates of post-radical prostatectomy biochemical relapse ($p = 0.071$) (Fig. 3). Similar results were obtained when patients with either extreme of Gleason scores (three patients with Gleason score $<6$ or 1 patient with Gleason score $>7$) were removed from the study cohort ($p = 0.078$; data not shown). Notably, in this cohort, there was no biochemical relapse in any of the ERG negative PCA patients (0/33) vs. 19.2 % (10/52) ERG positive tumors (Fig. 3). Multivariate Cox regression model analysis with ERG as a variable was not applicable here since there are no PSA biochemical recurrence events occurring in ERG negative patients. In addition, none of the patients developed bone metastasis or cancer specific mortality over the follow up time period, so we could not correlate ERG expression to the development of either endpoint. Notably, GS had no significant prognostic value in this young patients cohort when we tested the association between GS ($<7$ vs. $3 + 4$ vs. $4 + 3$) and biochemical PSA relapse. But positive trends were observed with higher GS ($p = 0.37$) (Fig. 4).

**Discussion**

This study reports the potential prognostic significance of ERG protein expression in early-onset PCA, specifically in patients under 50 years of age at diagnosis. The majority of previous reports on ERG gene fusion or ERG protein expression in PCA have focussed on elderly patient
population with a mean age around 65 years. In those studies, the significance of ERG expression to patients’ outcome are mixed with some showing association with adverse outcome while others documenting no association to even some suggesting better prognosis [15, 22–28]. This is likely reflective of the cohorts studied (localized surgical cohorts vs. population based and advanced/castrate resistant cohorts), in addition to the endpoint being investigated (biochemical relapse vs. disease progression vs. cancer specific mortality). Furthermore, the heterogeneity status of ERG expression in different tumor foci may play significant role, if it is not accounted for.

There is currently no specific study documenting clinical and prognostic significance of ERG expression in early-onset PCA despite recent studies demonstrating significantly higher frequency of TMPRSS2-ERG gene fusion and ERG protein expression in early-onset PCA compared to elderly-onset disease [3, 9]. To our knowledge, this is the first study to investigate and document potential prognostic significance of ERG expression in early-onset prostate cancer using a cohort of radical prostatectomy patients with localized PCA diagnosed <50 years of age (mean patient age of 47 years; range 36.2–49.9 years). In this cohort, the frequency of ERG expression was 64.4 % which is similar to that reported previously for radical prostatectomy tumours in patients of ≤55 years of age [9] and higher than the approximately 50 % reported in general surgical cohorts.

Here in, we documented a significant association between ERG expression and higher rates of biochemical relapse post-radical prostatectomy (p = 0.007). However, the association was at marginal significance (p = 0.071) when assessing time till PSA relapse, likely reflective of the smaller number of patients with documented date of biochemical PSA status (81 patients). This may be reflective of the early and usually aggressive management given to younger patients so that the outcome for these patients is usually favourable. Of note, the entire biochemical relapse cases occurred in ERG positive PCA (19.2 %) with none occurring in ERG negative PCA (0/33). Notably, GS had no significant prognostic value to biochemical PSA relapse in this cohort. This may be due to the composition of this specific cohort as the majority of patients were of GS ≤7 with organ confined disease and negative surgical margins. This reflects that in such young population, the prognosis is relatively good with only few patients exhibiting aggressive behaviour. As none of the ERG negative patients demonstrated PSA biochemical recurrences compared to ~20 % of ERG positive patients, ERG status may therefore provide additional prognostic information beyond those obtained by clinical and pathological assessment and could be of added value to physicians in overall treatment plan for such patients. However, this potential prognostic role must be interpreted with caution, as although the issue of heterogeneity is beyond our study objectives and our study was not designed to specifically investigate heterogeneous ERG expression, we found 14.4 % of patients within this cohort to show different ERG status between multiple foci analyzed. The percentage observed here is less than a previous report of 41 % [30]. However, it is still significant and likely to affect the potential implementation of ERG as a tissue biomarker, at least requiring the evaluation of multiple different tumor foci.

Lastly, in this cohort, ERG expression showed positive trends to increased GS in the subgroup of patients with GS of ≤7, while showing inverse trends in patients with higher GS of >7. This confirm that ERG expression is more frequent in intermediate GS (GS 6 & 7) as reported earlier by our group [25] and also supports its early role in disease process and that it may be biologically more relevant in early onset prostate cancer as it is now documented to occur at higher frequency in young patients compared to elder patients population with PCA [3, 9].

Conclusion

Utilizing an early-onset localized radical prostatectomy cohort diagnosed at less than 50 years of age, we demonstrate for the first time a possible adverse prognostic significance of ERG expression in predicting PCA biochemical relapse in this young patient population. Despite the smaller number of patients with documented time till biochemical relapse, which represented a limitation of our study, of significant interest is that none of the biochemical relapse occurred in ERG negative PCA patients, suggesting that ERG expression status may have added prognostic value in this subgroup of patients when added to preoperative nomograms. However, should ERG be considered as a tissue based biomarker of clinical relevance, it will likely require the evaluation of several tumor foci within individual patients due to the heterogeneity of ERG expression observed between different tumor foci.

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Conflict of interest The authors have no conflict of interest to declare in this study.

References


