# ERG Protein Expression and Gene Rearrangements Are Present at Lower Rates in Metastatic and Locally Advanced Castration-resistant Prostate Cancer Compared to Localized Disease

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**OBJECTIVE** To compare ERG expression and gene rearrangements rates in metastatic and castration-resistant prostate cancer (CRPC) to localized disease as *ERG* is the most common genetic event in early prostate cancer (PCa) with potential prognostic and therapeutic implications.

**METHODS** We evaluated ERG protein expression in 344 patients with PCa in 3 cohorts including localized, metastatic, and castration-resistant disease using immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH).

**RESULTS** ERG protein expression was detected exclusively in the neoplastic epithelium and was found in 6.8% and 46.3% of high-grade prostatic intraepithelial neoplasia (HGPIN) and localized PCa, respectively. In metastatic and locally advanced CRPC, ERG expression was significantly lower, occurring at 36.1% and 37.2%, respectively. In PCa with foamy gland morphology, ERG protein expression was detected in only 18.6% compared with reported rates of about 42%-48% in acinar PCa. Moreover, ERG protein expression and gene rearrangements showed an overall consistency rate of 90.6% ($P$ <.0001). The consistency rate was 100% both in benign glands and HGPIN, and 96.1% in localized PCa. However, it was significantly lower at 76.9% and 85% in node metastatic and CRPC, respectively ($P$ <.0001).

**CONCLUSION** ERG protein expression is restricted to neoplastic prostatic epithelium and is present at lower rates in metastatic and CRPC compared to localized PCa. IHC and FISH concordance rates were significantly lower in node metastatic and CRPC compared to localized PCa, which may suggest different biological and therapeutic implications. The lower rate of ERG protein expression in foamy gland PCa may suggest potential differences for this pattern of PCa at the molecular level. UROLOGY 82: 394–399, 2013. © 2013 Elsevier Inc.

The fusion of androgen receptor-regulated gene *TMPRSS2* (21q22.3) to a transformation-specific transcription factor gene, *ERG* (21q22.2), has been reported as the most prevalent genetic alteration in prostate cancer (PCa). Other gene rearrangements involving ETS family members including *ETV1*, *ETV4*, and *ETV5* are much less common.[1-4] As a consequence of this rearrangement, the 5′ partner of the *ERG* gene is placed under the control of the androgen-regulated *TMPRSS2* gene causing increased ERG expression.[1] *ERG* gene rearrangements were reported in 40%-60% of localized PCa in surgical cohorts, depending on the type of detection technique used, such as fluorescence in situ hybridization (FISH), single nucleotide polymorphism arrays, and quantitative polymerase chain reaction.[1,5] The rate of *ERG* rearrangements in high-grade prostatic intraepithelial neoplasia (HGPIN) was reported to be at 10%-20% and, noticeably, the *ERG* rearranged HGPIN shared the same fusion pattern with the corresponding cancer.[6] Some studies reported that *ERG* gene rearrangement is associated with aggressive cancer, metastasis, or cancer-related death.[7-9] However, these results were

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contrasted by other studies, which document absence of association with adverse outcomes or even better prognosis when *ERG* gene rearrangement is present.[10,11]

Recently, several studies examined the ERG protein expression in localized PCa, utilizing an ERG antibody that became available and that was thought to be reflective of *ERG* gene rearrangements.[12-14] These recent studies documented a remarkable concordance (sensitivity 86%-100%, specificity 85%-96.5%) between ERG protein expression evaluated by immunohistochemistry (IHC) and *ERG* gene rearrangements status by FISH, quantitative polymerase chain reaction, or branched-chain deoxyribonucleic acid technique. However, the majority of these studies focused only on evaluating ERG protein expression in localized PCa with 1 study comparing the rates in castration-resistant prostate cancer (CRPC) and distant metastasis.[15] The status of ERG protein expression was not addressed in the setting of lymph node metastatic or in specific PCa subtypes. This characterization is important, given the potential application of ERG IHC as a prognostic and an ancillary tool in surgical prostate pathology.

In the present study, we evaluated ERG protein expression in localized, lymph node metastatic, and CRPC cohorts, including also cases of foamy type PCa. We also evaluated the concordance between ERG protein expression by IHC and *ERG* gene rearrangements status by FISH in these settings.

## MATERIAL AND METHODS

### Study Population and Tissue Microarray Construction

This hospital-based study consisted of 2 cohorts. The first cohort included 121 consecutive cases with localized PCa who were treated by retropubic radical prostatectomy, including 24 patients specifically chosen as regional lymph node metastases cases. The second cohort consisted of 223 patients with CRPC (ie, men with locally advanced and castration-resistant tumors). Their tissue material was obtained by transurethral resection of the prostate. A total of 4 tissue microarray (TMA) blocks were constructed, 2 from localized and node metastatic PCa and 2 from locally advanced CRPC belonging to 344 patients. Prostate samples were embedded in 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. Two to 6 cores (average 2.5) 0.6 mm in diameter were taken from paraffin-embedded tissue blocks of each sample for a total of 1314 cores including: benign prostate tissue (n = 143), HGPIN (n = 40), localized PCa (n = 493), lymph node metastatic PCa (n = 58), and CRPC (n = 580). After construction, 4 μm sections were cut and stained with hematoxylin and eosin on the initial slides to verify the histological diagnosis. All clinical and pathological data were captured with approval of the Ethics Review Board at the University of Calgary-Faculty of Medicine, Calgary, Alberta, Canada.

### *ERG* Gene Rearrangement Status by FISH

We used the break-apart FISH assay to indirectly assess for the *ERG* gene rearrangements, as previously described.[16,17] In brief, 2 probes differentially labeled were designed to span the telomeric and centromeric neighboring regions of the ERG locus. This break-apart probe allows differentiation between *ERG* rearrangement through insertion vs an intronic deletion and no gene rearrangement regardless of the specific 5′ partner being fused to ERG. The samples were analyzed under a 60X oil immersion objective using an Olympus BX-51 fluorescence microscope equipped with appropriate filters, a device camera (Olympus, Center Valley, PA), and the CytoVision FISH imaging and capturing software (Applied Imaging, San Jose, CA). For each focus, we scored at least 100 nuclei. For *ERG* rearrangements, positive cases had 100% of the nuclei rearranged.

### ERG Protein Expression by Immunohistochemistry

We used ERG rabbit monoclonal antibody (Epitomics, clone EPR 3864) at 1:50 dilution. In brief, 4-μm thick sections from formalin-fixed paraffin-embedded tissue blocks were stained with a Ventana autostainer. Before the staining, heat-induced antigen retrieval was carried out by a vegetable steamer in sodium citrate antigen retrieval buffer (10 mM pH 6.0) for 40 minutes, followed by cooling down to room temperature for about 20 minutes. Slides were incubated for 60 minutes at 37°C with the ERG antibody and a Ventana iView DAB detection kit (Ventana, Tucson, AZ) was used for horseradish peroxidase detection and counterstain. Negative control was performed by omitting the primary antibody and substituting it with normal mouse 1/200 prediluted serum (Ventana).

### Pathological Analysis

All TMA cores were assigned a diagnosis (ie, benign, HGPIN, or PCa) by 2 pathologists (L.H.T. and T.A.B.). For each sample, at least 1 available core was evaluated for hematoxylin and eosin, IHC, and FISH. Protein expression was assessed semiquantitatively and without prior knowledge of the clinical information by evaluating the intensity of the expression using a 3-tiered system (0 negative, 1 weak, and 2 strong), as previously described.[7] We defined all levels of staining intensity (weak and strong) as positive and reflective of the *ERG* gene rearrangements. Of note, when positive signal was detected, it was uniformly present in more than 90% the cells. In all cases, ERG IHC was strongly detected in normal endothelial cells acting as positive internal control.

### Statistical Analysis

The ERG expression and rearrangements were presented as frequencies and percentages for each diagnostic category and as means and ranges for continuous variables. Chi-square tests were used to test for associations between ERG protein expression and *ERG* gene rearrangements and Gleason score (IBM SPSS v. 19 was used for the analysis). In all statistical tests, a $P$ value $<.05$ was considered significant.

## RESULTS

### Expression of ERG Protein in Benign and Neoplastic Prostate Epithelium of Localized, Metastatic, and CRPC

ERG protein positivity was confined to the nuclei of the prostatic secretory epithelium. Strong staining intensity was also consistently detected in the endothelial cells and the vascular endothelium which acted as an optimal

internal positive control. The staining intensity varied in the neoplastic prostatic glands. We did not observe a distinct ERG expression in the lymphocytes, as described in one previous study,[18] but only observed faint and focal ERG staining. ERG protein expression was present in: 6.8% HGPIN (2/29), 46.3% localized PCa (190/410), 36.1% metastatic PCa (13/36), and 37.2% CRPC (181/486) cases. In this study, we observed a significant difference between the incidence of ERG protein expression in the localized vs metastatic and CRPC ($P = .002$). The difference was also significant at the genomic level assessed by FISH, with 43.1% (147/341) of localized PCa cases demonstrating *ERG* rearrangement vs only 14.6% (6/41) of metastatic PCa and 31.8% (140/440) of CRPC ($P < .0001$). No significant differences in ERG expression were observed between metastatic and CRPC at the protein and genomic levels ($P > .05$). As expected, and consistent with the earlier results of genomic *ERG* rearrangements, none of the benign prostate glands expressed ERG protein, including the normal prostatic glands adjacent to the ERG-positive malignant epithelium (Fig. 1A). Only 2 HGPIN cores belonging to 2 patients were positive for ERG protein expression; the corresponding PCas from the same patients also showed ERG expression (Fig. 1B). In those cases, the positive nuclear ERG expression was noticed exclusively in the altered HGPIN cells, whereas the adjacent benign cells were ERG-negative (Fig. 1C-D). We also observed a high concordance rate of ERG protein expression between the metastatic nodal PCa and the primary tumor (95.2%, 20/21). The only discordant case that was negative in the primary PCa, but was positive in the metastatic PCa, demonstrated weak and heterogeneous ERG protein expression in the whole tissue section. When examined, the *ERG* rearrangement of this case also was concordant with protein expression in both metastatic nodal PCa and the primary tumor.

In our localized PCa cohort, we had 86 foamy gland adenocarcinoma cores belonging to 30 patients. Although this study was not specifically designed to investigate the ERG expression in the different subtypes or morphologic patterns of PCa, we noticed that foamy gland (xanthomatous) adenocarcinoma showed lower ERG positivity of 18.6% (16/86) compared to the acinar (usual type) adenocarcinoma. Only 9 of 30 cases were pure foamy gland adenocarcinoma, whereas the other 21 of 30 cases were admixed with acinar adenocarcinoma. In these 21 cases in which both tumor components, foamy and acinar, were concomitantly available for analysis, 90.5% (19/21) showed concordance of the ERG protein expression for the same patient (data not shown) (Fig. 1E,F). *ERG* gene rearrangements also were observed in foamy gland adenocarcinoma. All the 86 cores had consistent ERG status on protein and genomic levels, although 2 cases showed disaccord ERG protein expression between foamy type and acinar type in the same patient. In addition, our samples also included 6 cores with abundant extracellular mucin (mucinous adenocarcinoma) belonging to 4 patients and 2 additional cores with pseudohyperplastic features (pseudohyperplastic adenocarcinoma). We found that 83% (5/6) mucinous adenocarcinoma cores were positive for ERG protein (Fig. 1G), but the 2 pseudohyperplastic adenocarcinomas were negative (Fig. 1H).

### Consistency of ERG Protein Expression and Genomic *ERG* Gene Rearrangements

We performed a comparative evaluation between the ERG protein expression detected by IHC and *ERG* gene rearrangements detected by FISH. The overall consistency rate between ERG protein and *ERG* gene rearrangements in patients in which both cores were available was 90.6% (714/788) in cores by IHC and FISH belonging to 284 patients (both IHC+/FISH+ and IHC−/FISH−). The ERG protein expression demonstrated a sensitivity of 93% (confidence interval [CI] 0.98%-0.96%) and specificity of 90% (CI 0.87%-0.92%) with a positive predictive value of 80% (CI 76%-85%) and a negative predictive value of 96% (CI 95%-98%). We also investigated the consistency between ERG FISH/IHC in each diagnostic category separately. The consistency rate was 100% in both benign prostatic glands and HGPIN, 96.1% (270/281) in localized PCa, 76.9% (20/26) in metastatic PCa, and 85% (323/380) in CRPC. Significant differences in the consistency rates were observed between localized PCa vs metastatic PCa and localized vs CRPC (both $P < .0001$, Table 1). Of the 74 cores showing discrepancy between ERG protein and genomic *ERG* rearrangements, 24.3% (18/74) showed rearrangement only by FISH and 75.7% (56/74) cores showed ERG protein expression only by IHC. Of the cores showing only genomic *ERG* rearrangement but negative protein expression (FISH+/IHC−), 61.1% (11/18) demonstrated *ERG* rearrangements through insertion (ie, translocation), whereas 38.9% (7/18) demonstrated rearrangement through deletion (Table 2).

No association was observed between the level of ERG intensity and the *ERG* gene rearrangement mechanism (translocation vs deletion, $P > .5$).

### ERG Protein Expression in Relation to the Gleason Score in Localized Prostate Cancer

We also investigated the ERG consistency rates for the localized PCa cohort by FISH and IHC in relationship to Gleason score of the individual TMA cores. We observed consistency rates between ERG protein expression and genomic *ERG* gene rearrangements in 95.9% (211/220) of Gleason score 6, 100% (30/30) of Gleason score 7, and 93.5% (29/31) of Gleason score 8-10. Although, in this study, no statistical differences were found between the rates of ERG protein expression and genomic *ERG* rearrangements, we noted that tumors with higher Gleason scores showed a trend toward weaker ERG protein expression compared with lower Gleason score tumors (data not shown).

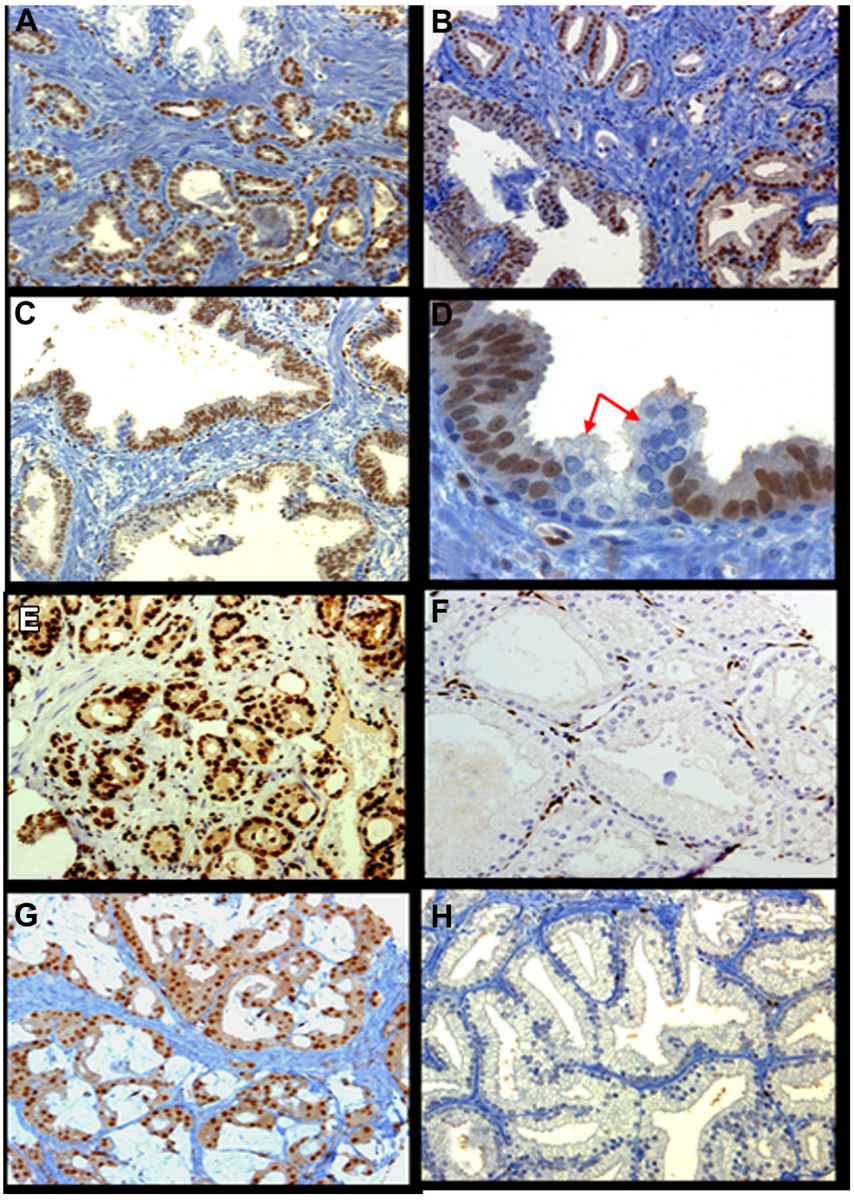


**Figure 1.** ERG protein expression in prostate cancer (×20). **(A)** Cancer cells show a strong nuclear ERG expression, whereas the adjacent benign glands are negative. **(B)** Both cancer cells and high-grade prostatic intraepithelial neoplasia (PIN) are strongly positive for ERG in the same core. **(C-D)** Low- and high-power views of high-grade prostatic intraepithelial neoplasia (HGPIN) showing moderate ERG expression. The arrow indicates normal epithelial cells that are negative for ERG expression, whereas surrounding prostatic intraepithelial neoplasia cells are positive. **(E-F)** Prostate cancer (PCa) from the same patient shows positive ERG in acinar PCa, but negative foamy gland PCa, respectively. **(G)** ERG-positive expression in mucinous carcinoma. **(H)** Absence of ERG expression in pseudohyperplastic PCa. (Color version available online.)

## DISCUSSION

Several studies have investigated the role of *ERG* gene rearrangement in PCa since its initial characterization as the most common genetic alteration in PCa in 2005.[1] Recently, a few studies have attempted to investigate the role of ERG protein expression as a tool for the diagnosis of minimal PCa and as a surrogate marker for genomic *ERG* gene rearrangements, besides its prognostic value.[13,18-21] Herein, we investigated the differences in ERG expression in localized, lymph node metastatic, and advanced CRPC. We also assessed the possibility of utilizing ERG protein expression by IHC as a surrogate marker for genomic *ERG* rearrangements by FISH in the same settings.

ERG protein expression was detected exclusively in the neoplastic cells of PCa and rarely in the HGPIN, in agreement with earlier reports.[18,22] Most importantly, we did not observe any staining in the benign epithelium, contrary to some previous studies suggesting weak expression in benign glands adjacent to PCa foci, even though we utilized the same antibody as most of the previous reports.[12,13,23] This may reflect differences in staining/concentration protocols, but it also underlies the fact that ERG is specific for the neoplastic prostatic cells.

**Table 1.** Consistency of ERG protein expression with *ERG* gene rearrangement in prostate cancer progression

| Prostate Sample | Available Cores | Consistent | | Inconsistent FISH/IHC | Consistency* |
|---|---|---|---|---|---|
| | | FISH+/IHC+ | FISH−/IHC− | | |
| Benign | 79 | N/A | N/A | 0 | 100% (79/79) |
| HGPIN | 22 | 2/22 (9%) | 20/22 (91%) | 0 | 100% (22/22) |
| Localized PCa | 281 | 126/281 (45%) | 144/281 (51%) | 11 | 96.1% (270/281) |
| CRPC | 380 | 98/380 (26%) | 225/380 (59%) | 57 | 85.0% (323/380) |
| Lymph node metastatic PCa | 26 | 3/26 (12%) | 17/26 (65%) | 6 | 76.9% (20/26) |
| Total | 788 | 227/788 (29%) | 386/788 (49%) | 74 | 90.6% (714/788) |

CRPC, castration-resistant prostate cancer; FISH, fluorescence in situ hybridization; HGPIN, high-grade prostatic intraepithelial neoplasia; IHC, immunohistochemistry; PCa, prostate cancer.

* Significant difference was observed between localized vs lymph node metastatic and CRPC ($P < .0001$, chi-square test).

**Table 2.** Inconsistency of ERG immunohistochemistry and fluorescence in situ hybridization in prostate cancer

| Variables | FISH+/IHC− | FISH−/IHC+ | Total |
|---|---|---|---|
| Localized PCa | 0 (0%) | 11 (100%) | 11 |
| CRPC | 16 (28.1%)<br>T: 9, D: 7 | 41 (71.9%) | 57 |
| Lymph node metastatic PCa | 2 (33.3%)<br>T: 2, D: 0 | 4 (66.7%) | 6 |
| Total | 18 (24.3%)<br>T: 11, D: 7 | 56 (75.7%) | 74 |

D, deletion; T, translocation; other abbreviations as in Table 1.

Only 2 of 29 HGPIN lesions (6.8%) expressed ERG protein, which was also observed in the corresponding PCa in these patients. All 27 remaining ERG-negative HGPIN lesions showed no ERG expression in the matching PCa samples comparable to earlier reports.[6] Interestingly, we observed another ERG-positive tumor focus in 1 of ERG-negative HGPIN cases that implied the heterogeneity of ERG protein expression. Furthermore, we documented high consistency rate of ERG expression in the primary and the node metastatic PCa. But whether ERG is implicated as a driver for PCa metastasis still needs further investigation. On the other hand, it is worth noting that, in this study, in which multifocal primary disease with different ERG status was noted between different foci (2 patients with both positive and negative foci), the lymph node metastatic focus was always positive for ERG expression. This result is supportive of previous observations of consistency between primary and lymph node ERG status in which multifocal disease with different ERG status is noted.[24,25]

Although we did not observe differences in the rate of ERG consistency between IHC and FISH in relation to Gleason score, in contrast to earlier studies, we document significantly lower incidence and concordance rates of ERG protein expression and *ERG* gene rearrangements in metastatic PCa and CRPC, which is in line with the lower ERG incidence in metastatic PCa compared to local CRPC reported by Scheble et al.[15] This highlights the possibility of altering the ERG protein expression during disease progression and potentially through therapeutic hormonal interventions. This later observation may restrict the use of IHC as a surrogate for *ERG* rearrangement in metastatic and CRPC. The significant disassociation between genomic *ERG* rearrangements and protein expression in metastatic and CRPC over localized cancer may have potential prognostic implication and may explain the different therapeutic response for those patients in comparison to those with no such dissociation, as recent data from our group documented prognostic disadvantage for ERG-positive tumors over those that are ERG-negative, but clinical advantage for those treated by LHRH (lupron).[7] This, however, still needs to be investigated in further studies. It is also noteworthy to document our observation of lower ERG intensity in higher Gleason score compared to lower Gleason score, which needs further confirmation in larger studies. We only investigated Gleason score in this study, as not all outcomes were available. The association with pathological parameters is beyond this study aims and is currently being analyzed in subsequent studies.

Finally, in regard to PCa subtypes, we detected a lower incidence of ERG protein expression in foamy gland adenocarcinoma. The similar result was observed by Han et al,[26] in which they found a relatively low occurrence rate (29%) of *ERG* rearrangement by FISH in foamy gland carcinoma. This, however, is lower than the rate of positive *ERG* rearrangements documented by Mosquera et al[27] using FISH. These differences could be related to the particular cohorts studied or the methods used for interpretation. We also noticed a nonsignificant trend toward weaker ERG intensity in the foamy gland component compared to the acinic PCa component when both were present, as observed by Furusato et al.[12] The lower rates of ERG expression in foamy gland PCa may suggest a distinct molecular mechanism of carcinogenesis, which requires further investigation.

Antibody-based ERG protein detection methods indicate that both *ERG* gene rearrangements and consequent protein product levels correspond significantly. Herein, ERG protein expression exhibited an overall sensitivity of 93% and specificity of 90%, which is similar to recently published studies.[12,18] However, in contrast with most previous reports, we included a wide range of prostatic lesions (benign, HGPIN, localized, metastatic, and CRPC) and we documented a significant difference in the incidence and consistency rates of ERG protein and *ERG* gene rearrangements in different subsets. Based on previous reports, *ERG* rearrangement drives the ERG protein

expression. However, some cases in our cohorts, as well as in other studies, showed no ERG protein expression despite the presence of *ERG* rearrangement. The reasons for this discrepancy are not fully understood, but may stem from either technical or biological issues, suggesting that mechanisms other than *ERG* rearrangement may be responsible for the ERG protein overexpression.

It is worth mentioning that PCa is not the only neoplasm that expresses the ERG protein. Recently, Miettinen et al[28] investigated the ERG protein expression in a large number of epithelial and nonepithelial neoplasms. They observed ERG protein expression in nearly all benign and malignant vascular endothelial cells. Meanwhile, about half of PCa cases (45.4%) showed ERG expression and rare cases of epithelial and hematological malignancies; mainly blastic extramedullary myeloid tumors also expressed ERG.

Whether ERG is of any prognostic or therapeutic significance also remains a question that needs further clarification, as some earlier reports suggested that tumors with *ERG* gene rearrangements or protein expression have prognostic implication and may show better response for abiraterone or hormonal manipulation.[7,29]

## CONCLUSION

In summary, the lower incidence of *ERG* oncogenetic events in lymph node metastatic and CRPC may have significant biological and prognostic implications. The relative high consistency between ERG protein expression and genomic *ERG* rearrangement suggests that IHC can be a useful alternative for detecting ERG status mainly in localized PCa, but may not be as reliable in metastatic or CRPC. Moreover, hormonal treatments may interfere and affect the level of ERG protein expression. Finally, the lower incidence of ERG protein expression in foamy gland adenocarcinoma suggests a distinct mechanism of carcinogenesis.

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