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ERG immunohistochemistry is not predictive for PSA recurrence, local recurrence or overall survival after radical prostatectomy for prostate cancer

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In prostate cancer genomic rearrangements involving genes encoding ETS transcription factors are commonly present, with *androgen-regulated transmembrane protease, serine 2 (TMPRSS2)-v-ets erythroblastosis virus E26 oncogen homologue (ERG)* gene fusion occurring in 40–70%. Studies on the predictive value of *ERG* rearrangement as detected by *in-situ* hybridization or polymerase chain reaction have resulted in varying outcomes. The objective of this study was to correlate immunohistochemical ERG protein expression with clinico-pathological parameters at radical prostatectomy specimens, and to determine its predictive value for postoperative disease recurrence and progression in a prostate cancer screening cohort. Since androgen receptor is downregulated by ERG in cell lines, we also compared the expression of respective proteins. We selected 481 participants from the European Randomized Study of Screening for Prostate Cancer treated by radical prostatectomy for prostate adenocarcinoma. A tissue microarray was constructed containing representative cores of all prostate cancer specimens as well as 22 xenografts and seven cell lines. Immunohistochemical expression of ERG and androgen receptor was correlated with prostate-specific antigen (PSA), Gleason sum, pT-stage, surgical margins, biochemical recurrence, local recurrence, overall death and disease-specific death. ERG expression was detected in 284 patients (65%). Expression occurred significantly more frequent in patients with PSA $\leq$10 ng/ml ($P=0.024$). There was no significant association between ERG and Gleason sum, pT-stage or surgical margin status. PSA ($P=0.011$), Gleason sum ($P=0.003$), pT-stage ($P=0.001$) and surgical margin status ($P<0.001$) all had independent value for postoperative biochemical recurrence, while positive surgical margin ($P=0.021$) was the only independent predictor for local recurrence. ERG protein expression did not have prognostic value for the clinical end points in uni- and multivariate analyses. A positive correlation existed between ERG and androgen receptor expression in single tissue cores ($P<0.001$). In conclusion, immunohistochemical ERG expression has no predictive value for prostate cancer recurrence or progression after radical prostatectomy. Increasing ERG levels are associated with the upregulation of androgen receptor expression in clinical specimens.
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Genomic rearrangement of *androgen-regulated transmembrane protease, serine 2* (*TMPRSS2*) on 21q22.3 to *v-ets erythroblastosis virus E26 oncogen homologue* (*ERG*) on 21q22.2 is the most common genetic alteration in prostate cancer occurring in 40–70% of tumors.[1] Less frequently, *SLC45A3* and

NDRG1 can serve as *ERG* fusion partners, whereas fusions of other ETS family members *ETV1*, *ETV4* and *ETV5* to over 10 different partner genes are found in approximately 10% of tumors.[2–4]

Various groups have investigated the prognostic relevance of *TMPRSS2-ERG* fusion in clinical prostate cancer with varying outcome. This discordance can be explained by diversity in patient cohorts, pathological specimens, clinical end points and *ERG* fusion detection. *ERG* rearrangement has so far been analyzed using either fluorescence *in-situ* hybridization (FISH) or quantitative polymerase chain reaction (QPCR). Advantageously to routine detection of *ERG* fusion, FISH can give qualitative information on the type of gene fusion, resulting either from translocation or deletion, which is reported to be of clinical relevance.[5–7] Application of FISH, however, needs tissue-dependent optimization and can suffer from difficulty in interpretation, especially when only a few atypical glands are present. On the other hand, both quantitative and qualitative information on gene fusion can be obtained by QPCR. Hermans *et al*[8] demonstrated that *TMPRSS2* (*exon 0*)-*ERG* fusions were associated with less aggressive biological prostate cancer behavior. The disadvantage of QPCR for the detection of *ERG* rearrangement, however, is its requirement of frozen tissue containing a high percentage of prostate cancer cells.

Recently, Park *et al*[9] described an ERG-specific antibody EPR3864, which reactivity showed excellent correlation with *ERG* rearrangement as determined by FISH in prostate cancer paraffin-embedded tissues. Likewise, van Leenders *et al*[10] found a strong correlation between ERG protein expression and *TMPRSS2-ERG* expression using QPCR. Strong immunohistochemical staining was associated with high *ERG* transcript levels in radical prostatectomy specimens, indicating that semiquantitative ERG determination reflected molecular expression levels.[10] Therefore, ERG immunohistochemistry is a simple methodology strongly indicative for the presence of *ERG* rearrangement.

The androgen receptor pathway plays an important role in the development and maintenance of the normal prostate, as well as in prostate carcinogenesis.[11,12] Because many ETS fusion partners are androgen-regulated, ERG expression might be related to androgen receptor levels in prostate cancer.[11,12] On the other hand, it has been shown that ERG can downregulate the expression of androgen receptor and its target genes.[13]

In this study, we investigated whether ERG protein expression has predictive value for prostate cancer recurrence and progression after radical prostatectomy in a well-defined screening cohort. In addition, we analyzed the relation between androgen receptor and ERG expression in clinical specimens.

Materials and methods

Patient Information

We selected all men from the European Randomized Study of Screening for Prostate Cancer (ERSPC), Rotterdam section, who had undergone radical prostatectomy for prostate adenocarcinoma in Erasmus Medical Center between 1987 and 2010.[14,15] In this study, men aged between 55 and 74 years were invited for a screening visit every 4 years. Recruitment and randomization started in December 1993 and ended December 1999. Up until May 1997, patients with a serum prostate-specific antigen (PSA) level of ≥ 4.0 ng/ml, an abnormal digital rectal examination and/or abnormal transrectal ultrasound underwent lateralized sextant prostate needle-biopsies. As from May 1997, a biopsy was indicated by a PSA level of ≥ 3.0 ng/ml or abnormal digital rectal examination and/or transrectal ultrasound.

Directly after surgery, radical prostatectomy specimens were transported on ice to the pathology department. After fixation in neutral-buffered formaldehyde, the radical prostatectomy specimens were routinely cut in 4-mm transverse slices with additional perpendicular slicing of the apex and basis to allow optimal evaluation of surgical margins, and totally embedded in paraffin. Hematoxylin/eosin (HE) slides were microscopically evaluated by two board-certified pathologists with expertise in urological pathology (TvdK, GvL). At pathological examination, tumor areas were encircled at the glass slides, and Gleason sum, TNM stage and surgical margins were reported according to the international guidelines.

Clinical follow-up was recorded after each control at our outpatient clinic, and data were transmitted to the central study database. Postoperative biochemical recurrence was defined as an increase in serum PSA after two different measurements, at least 3 months apart. Suspicion on local recurrence was verified by a diagnostic needle-biopsy in each case. Death and death of disease was registered by the physician who last treated the patient. Use of samples for research purposes was approved by the Erasmus Medical Center Medical Ethics Committee according to the Medical Research Involving Human Subjects Act (MEC-2004-261).

Tissue Microarray Construction

Histological slides of all patients ($n = 509$) were retrieved from the pathology archives together with corresponding paraffin blocks containing the largest tumor volume (with tumor of at least 0.5 cm in diameter per paraffin block). In 28 cases, the tumor diameter was less than 0.5 cm, which hampered sampling of three separate cores, or no paraffin tissue was available, resulting in 481 patients to be included. In addition, the following control specimens were selected: normal prostate tissues ($n = 10$) from cystoprostatectomies, urothelial cell

Table 1 Prostate cancer xenografts ($n=22$) and cell lines ($n=7$) included in the tissue microarray, together with molecular *ETS* fusion status, and immunohistochemical expression of ERG and androgen receptor (AR)

Source	*ETS fusion status*	*Immunohistochemistry*	
		ERG	*AR*
Xenograft			
DuCaP	*TMPRSS2:ERG*	+	+
LAPC4	—	−	+
LnCaP	*ETV1* translocation	−	+
PC82	*TMPRSS2:ERG*	+	+
PC133	*TMPRSS2:ERG*[a]	−	−
PC135	—	−	−
PC295	*TMPRSS2:ERG*	+	+
PC310	*TMPRSS2:ERG*	+	+
PC324	*TMPRSS2:ERG*[a]	−	−
PC339	*TMPRSS2:ERG*[a]	−	−
PC346B	—	−	+
PC374	*TMPRSS2:ETV1*	−	+
VCaP	*TMPRSS2:ERG*	+	+
Cell line			
Du145N	—	−	−
LAPC4	—	−	+
LNCaP	*ETV1* translocation	−	+
PC3	—	−	−
PC346C	—	−	+
VCaP	*TMPRSS2:ERG*	+	+
22RV1	—	−	+

Negative (−) *ETS* fusion status indicates that no fusion of ETS transcription factors has been identified.
[a]*TMPRSS2:ERG* fusion with breakpoint in *TMPRSS2* intron 1, with no known ERG expression.

carcinomas ($n=5$), invasive ductal mammary adenocarcinomas ($n=5$), palliative transurethral resection of the prostate containing hormone-refractory prostate cancer ($n=10$), prostate cancer lymph node metastases ($n=10$) and placenta ($n=1$). In addition, we included seven relevant human prostate cancer cell lines and 22 human prostate cancer xenografts with known *ERG* genomic fusion status (Table 1).[16–18] Cell lines were maintained in appropriate culture media. To facilitate inclusion of cell lines, confluent cell cultures were detached using EDTA, fixed in neutral-buffered formaldehyde, embedded in AGAR 2.5% (Sigma-Aldrich, St Louis, MO, USA), and subsequently in paraffin.

For tissue microarray (TMA) construction, three cylindrical cores (diameter 0.6 mm) were taken from representative areas in the paraffin block and transferred to recipient paraffin blocks (Beecher Microarrayer; Beecher Instruments, Sun Prairie, WI, USA). In total, nine TMA blocks were constructed each including 200 tissue and/or cell line cores.

Immunohistochemistry

Tissue slides (5 μm) were mounted on aminoacetylsilane-coated glass slides (Starfrost, Berlin, Germany), deparaffinized in xylene and dehydrated in ethanol. Endogenous peroxidase activity was blocked by 1% hydrogen peroxide in methanol for 20 min. Microwave (700 W) pretreatment in tris (hydroxymethyl)aminomethane-EDTA (pH 9.0) was performed for 15 min. The slides were incubated with rabbit monoclonal ERG antibody (clone EPR3864; 1:100; Epitomics, Burlingame, CA, USA) and mouse monoclonal anti-androgen receptor (clone F39.4; 1:200; Avris Antibodies, Herford, Germany) overnight at 4°C, followed by chromogenic visualization using the EnVision DAKO kit (Dako, Glostrup, Denmark). After counterstaining with hematoxylin, slides were thoroughly washed, dehydrated, cleared in xylene and mounted in malinol (Chroma-Geselschaft, Körgen, Germany).

Immunohistochemical stainings for ERG and androgen receptor were visually examined as described previously.[10] The intensity of ERG and androgen receptor expression was scored as negative (0; no staining), weak (1+; only visible at high magnification), moderate (2+; visible at low magnification) and strong (3+; striking at low magnification). Nuclear reactivity of the antibody in endothelial cells (ERG) and in stromal cells (androgen receptor) was used as internal control for the staining procedure.[9] In case of staining heterogeneity, the highest level was used for statistical analysis. All cores were scored by two investigators (MH, GvL) in a blinded setting. In a combined session, consensus on expression value was reached in all cases.

Statistics

For statistical analyses, we defined a patient as ERG 'positive' if respective evaluable cores all demonstrated ERG protein expression. If all cores from a patient were negative, we labeled the case as 'negative'. In case expression heterogeneity was present with both ERG-positive and -negative cancer cores taken from one radical prostatectomy, respective case was labeled 'heterogeneous'. The heterogeneous cases were included in the group of 'positive' cases as well as studied as a separate group.

Statistical associations between expression of ERG (as categorical variable) and continuous clinico-pathological parameters (age and PSA at the time of diagnosis) were tested using Student's *t*-test, and with categorical parameters (Gleason sum, pT-stage, surgical margins) using Pearson's χ^2 test. To determine whether ERG expression was predictive for biochemical recurrence, clinical recurrence, overall death or disease-specific death, we used uni- and multivariate Cox regression with stepwise backward entering of covariates. The proportionality assumption for ERG positive *versus* negative cases was visually assessed in Kaplan–Meier curves (not shown). To investigate the statistical association between ERG and androgen receptor expression in the same core, we used Pearson's χ^2 test. A *P*-value

<0.05 was considered significant. All statistics were performed using SPSS 17 (SPSS, Chicago, IL, USA).

Results

Patient Characteristics

The mean age of the prostatectomy patients was 64.76 years (range 55.4–75.1 years). The Gleason sum was <7 in 265 (55%), 7 in 188 (39%) and >7 in 28 (6%) cases, respectively. In total 343 (71%) tumors were organ-confined (pT2) and 138 (29%) cases showed extra-prostatic expansion (pT3/4). Surgical margins were positive in 119 (25%) cases. The mean follow-up of our cohort was 107.3 months. The clinico-pathological characteristics are summarized in Table 2.

Table 2 Clinico-pathological and follow-up information of prostate cancer patients treated by radical prostatectomy

Clinico-pathological parameter	*Mean (median; range) or* n *(%)*
Age (years)	64.76 (65.4; 55.4–75.1)
PSA level (ng/ml)	
Total	7.23 (5.2; 0.3–125.2)
≤10 ng/ml	418 (87%)
>10 ng/ml	62 (13%)
Unknown	1 (0%)
Follow-up (months)	107.3 (112.2; 0.00–203.8)
Gleason sum	
<7	265 (55%)
7	188 (39%)
3+4	153 (32%)
4+3	35 (7%)
>7	28 (6%)
pT-stage (TNM 2002)	
T2	343 (71%)
T3a	93 (19%)
T3b	17 (4%)
T4	28 (6%)
Lymph node metastasis	
Yes	1 (0%)
No	480 (100%)
Surgical margins	
Positive	119 (25%)
Negative	362 (75%)
Biochemical recurrence	
Yes	110 (23%)
No	371 (77%)
Local recurrence	
Yes	21 (4%)
No	460 (96%)
Overall death	
Yes	81 (17%)
No	399 (83%)
Unknown	1 (0%)
Death from prostate cancer	
Yes	9 (11%)
No	57 (70%)
Unknown	15 (19%)

ERG Expression

In 44 prostate cancer patients, no tumor was identified in any of the tissue cores. Immunohistochemical analysis of the remaining prostate cancer patients ($n=437$) demonstrated nuclear ERG expression in 239 cases (55%) and negative staining in 153 cases (35%). In 45 cases (10%), heterogeneous expression was observed, meaning that both areas with and without ERG expression were present within the same tumor. In all cases, endothelial cells were positive (1%, 2+; 99%, 3+), indicating that the immunohistochemical procedure was efficient and reliable. Different ERG and corresponding androgen receptor expression patterns are depicted in Figure 1.

In 49 patients (10%), high-grade prostate intraepithelial neoplasia was present in the tissue cores, of which 18 cases (37%) demonstrated ERG expression, 29 (59%) were negative and two (4%) heterogeneous. Normal prostate epithelial glands were negative in all cases, except in one (0%, intensity 2+).

Six of nine (67%) evaluable prostate cancer lymph node metastases showed ERG expression, while three were negative (33%); for one patient, no tumor was present in the three cores. In castration-resistant prostate cancer treated by palliative transurethral resection, ERG was uniformly expressed in four patients (40%), with heterogeneous expression in three (30%) and negative staining in three patients (30%).

To validate ERG immunohistochemistry, we also included a large series of human prostate cancer cell lines and xenografts with known *ERG* fusion status in the TMA. Nuclear ERG protein expression was found in VCaP, which has a known *TMPRSS2-ERG* fusion,[16] while other cell lines without *ERG* fusion were negative for ERG staining (Table 1). From the 22 xenografts analyzed, VCaP, DuCaP, PC82, PC295 and PC310 showed uniform ERG expression, while the other 17 were negative. As shown in Table 1, ERG immunohistochemistry was concordant with *TMPRSS2-ERG* fusion status. The control tissues derived from urothelial cell carcinoma, breast adenocarcinoma and placenta were all negative for ERG.

Clinico-Pathological Correlations

The relation of ERG immunohistochemical expression and clinico-pathological parameters is depicted in Table 3. Expression of ERG occurred significantly more frequent in patients with PSA ≤10 ng/ml ($P=0.024$). There was no statistically significant relation between ERG immunohistochemistry and

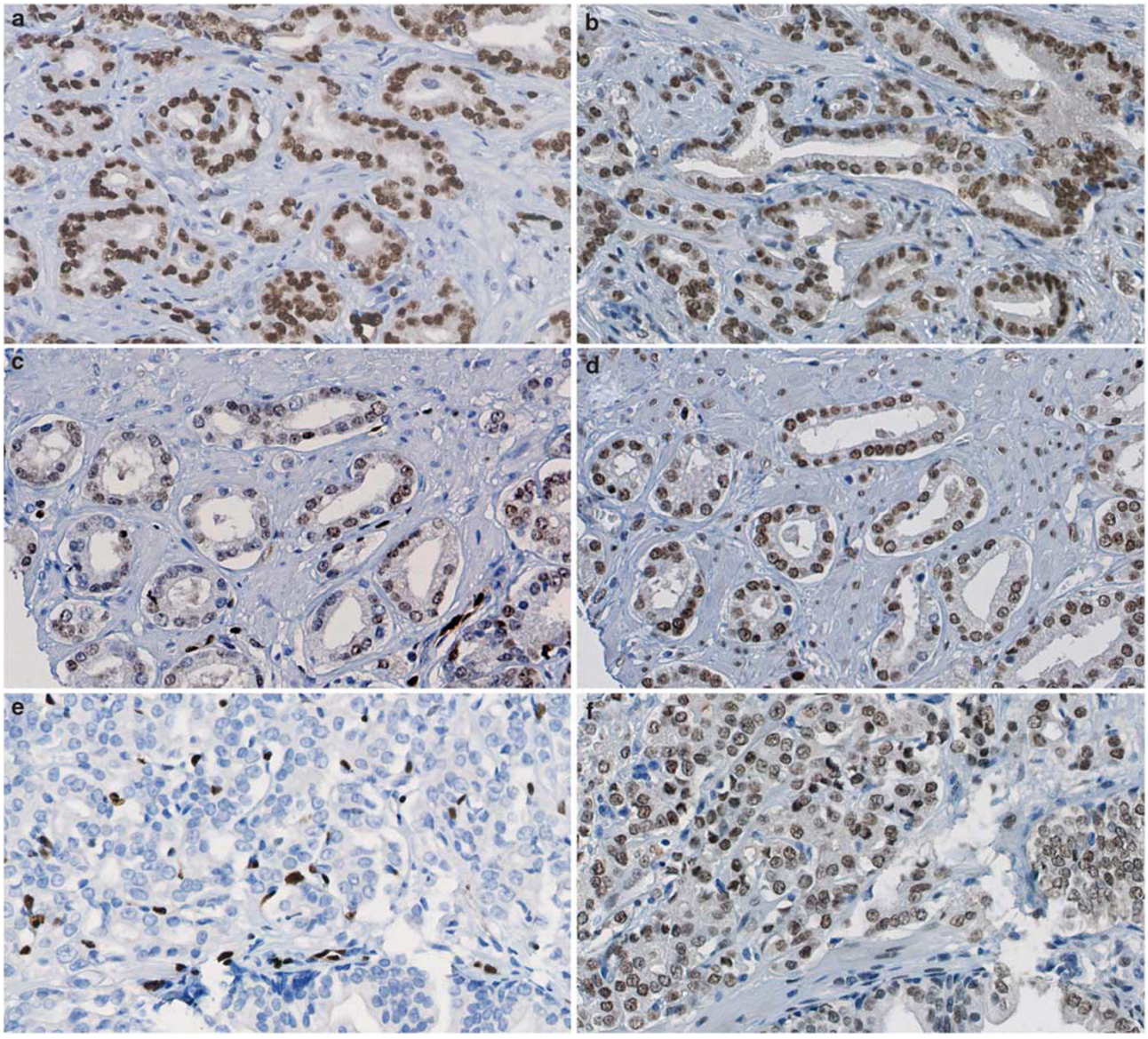

Figure 1 Immunohistochemical expression of ERG (left column) and androgen receptor (AR) (right column) in corresponding prostate cancer specimens. (**a**) ERG intensity 3+; (**b**) AR intensity 2+; (**c**) ERG intensity 1+; (**d**) AR intensity 2+; (**e**) ERG negative; and (**f**) AR intensity 2+. In panels **a**, **c** and **e**, endothelial cells with intensity 3+ serve as internal controls. Original magnification ×400.

Gleason sum, pT-stage or surgical margin status. PSA at the time of diagnosis ($P=0.011$), Gleason sum ($P=0.003$), pT-stage ($P=0.001$) and surgical margin status ($P<0.001$) all had independent predictive value for postoperative biochemical recurrence in multivariate analysis (Table 4), while positive surgical margin ($P=0.021$) was the only independent predictor for local recurrence (Table 5). ERG protein expression did neither have a prognostic value for biochemical nor local recurrence after radical prostatectomy in uni- and multivariate analysis. While age ($P<0.001$) and PSA ($P=0.001$) both were independently predictive for overall death, pathological parameters and ERG immunohistochemistry were not (Table 6). Serum PSA ($P=0.022$) was the only independent prognostic factor for disease-specific death, with Gleason sum ($P=0.052$) and surgical margin status ($P=0.070$) approaching significance (data not shown). The low number of events ($n=9$), however, limits the power of this analysis. Since multiple end points were tested, *P*-values should be interpreted cautiously. If patients with heterogeneous expression ($n=45$) were analyzed as a separate group, we neither were able to find a significant correlation between ERG and follow-up parameters (data not shown).

Androgen Receptor Expression

To compare androgen receptor and ERG expression, we investigated both proteins in the same tissue

cores. Of the 969 evaluable cores, androgen receptor expression was weak (1+) in 96 (10%), moderate (2+) in 861 (89%) and strong (3+) in 78 (8%) cases. As depicted in Figure 2, a positive correlation existed between the level of nuclear androgen receptor and ERG, with increased androgen receptor expression at higher ERG levels ($P<0.001$). The androgen receptor expression level of the ERG-negative cases was not statistically different from cases with moderate (2+) ERG expression ($P=0.32$).

Table 3 Correlation of immunohistochemical ERG expression with clinico-pathological parameters at radical prostatectomy

Parameter	*ERG expression*		P-*value*
	Negative	*Positive*	
Age (years) (mean; range)	64.4 (63.7–65.1)	64.5 (64.0–65.0)	0.824
PSA level (ng/ml)			
≤10	125 (82%)	253 (89%)	0.024
>10	28 (18%)	30 (11%)	
Gleason sum			
<7	72 (47%)	164 (58%)	0.069
7	66 (43%)	107 (38%)	
3+4	54 (35%)	88 (31%)	
4+3	12 (8%)	19 (7%)	
>7	15 (10%)	13 (4%)	
pT-stage			
pT2	115 (75%)	191 (67%)	0.185
pT3a	22 (14%)	65 (23%)	
pT3b	7 (5%)	10 (4%)	
pT4	9 (6%)	18 (6%)	
Surgical margin			
Positive	39 (26%)	72 (25%)	0.975
Negative	114 (74%)	212 (75%)	

Discussion

Since its discovery in 2005, several groups have investigated the clinical significance of *TMPRSS2-ERG* fusion for prostate cancer behavior with variable outcome.[8,19–23] This variability might be due to differences in study cohorts, clinical end points and methodologies for detecting gene fusion. For example, RT-PCR-detected *TMPRSS2-ERG* fusion was found in more than 70% of patients surgically treated by radical prostatectomy for prostate cancer.[24] In other studies, *TMPRSS2-ERG* fusion detected by FISH was present in only 15–30% of prostate cancer diagnosed at transurethral resection for benign prostate hyperplasia.[6,25] While prostate cancer at diagnostic prostate needle-biopsies showed *TMPRSS2-ERG* fusion in 46% by FISH,[26] our group recently found nuclear ERG overexpression in 61% using immunohistochemistry.[10]

The description of a novel ERG antibody in 2010 highly facilitates detection of *ERG* genomic fusions in clinical specimens.[9] Nuclear staining by the

Table 4 Predictive value of ERG expression for postoperative biochemical recurrence

Parameter	*Univariate analysis*		*Multivariate analysis*	
	HR (95% CI)	P-*value*	*HR (95% CI)*	P-*value*
Age (years)	1.06 (1.01–1.10)	0.018	1.03 (0.98–1.09)	0.204
PSA	1.03 (1.02–1.04)	<0.001	1.01 (1.00–1.03)	0.011
Gleason sum	2.03 (1.65–2.51)	<0.001	1.45 (1.13–1.85)	0.003
pT-stage	1.81 (1.55–2.13)	<0.001	1.39 (1.14–1.70)	0.001
Surgical margin	3.10 (2.13–4.51)	<0.001	2.23 (1.46–3.42)	<0.001
ERG expression	1.12 (0.74–1.71)	0.584	1.18 (0.77–1.81)	0.452

HR = hazard ratio; CI = confidence interval; PSA = prostate-specific antigen.

Table 5 Predictive value of ERG expression for postoperative local recurrence

Parameter	*Univariate analysis*		*Multivariate analysis*	
	HR (95% CI)	P-*value*	*HR (95% CI)*	P-*value*
Age (years)	0.96 (0.87–1.06)	0.446	0.92 (0.83–1.02)	0.108
PSA	1.03 (1.01–1.05)	0.002	1.02 (1.00–1.04)	0.138
Gleason sum	1.76 (1.10–2.81)	0.019	1.42 (0.84–2.39)	0.188
pT-stage	1.56 (1.07–2.26)	0.021	1.05 (0.64–1.71)	0.853
Surgical margin	4.18 (1.76–9.93)	0.001	3.24 (1.19–8.81)	0.021
ERG expression	0.88 (0.35–2.23)	0.782	0.93 (0.36–2.39)	0.872

HR = hazard ratio; CI = confidence interval; PSA = prostate-specific antigen.

Table 6 Predictive value of ERG expression for overall death

Parameter	*Univariate analysis*		*Multivariate analysis*	
	HR (95% CI)	P-*value*	*HR (95% CI)*	P-*value*
Age (years)	1.11 (1.05–1.17)	<0.001	1.13 (1.06–1.20)	<0.001
PSA	1.02 (1.00–1.03)	0.017	1.03 (1.01–1.04)	0.001
Gleason sum	1.13 (0.87–1.48)	0.349	1.09 (0.81–1.46)	0.587
pT-stage	0.99 (0.78–1.27)	0.951	0.76 (0.55–1.03)	0.080
Surgical margin	1.21 (0.75–1.94)	0.440	1.14 (0.68–1.91)	0.630
ERG expression	1.33 (0.80–2.23)	0.272	1.51 (0.89–2.57)	0.125

HR = hazard ratio; CI = confidence interval; PSA = prostate-specific antigen.

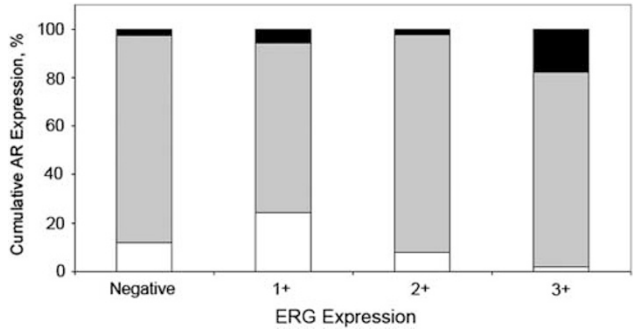

Figure 2 Cumulative bar chart showing the associations of nuclear ERG and androgen receptor expression. For androgen receptor, weak (1+) expression is depicted in *white*, moderate (2+) expression in *gray* and strong (3+) expression in *black*.

EPR3864 antibody showed excellent concordance with the presence of *TMPRSS2-ERG* fusion detected by FISH or QPCR.[9,10] Although crossreactivity of the EPR3864 antibody with ETS family member FLI-1 has been reported by the manufacturer, we demonstrated lack of FLI-1 expression in prostate cancer, indicating that the antibody actually detects ERG protein in this disease.[10] In this study, we found concordance of nuclear ERG expression and *TMPRSS2-ERG* fusion in seven human prostate cancer cell lines and 22 xenografts, with exception of xenografts PC133, PC324 and PC339, which have breakpoint in intron 1 of *TMPRSS2*.[16–18]

We identified ERG expression in 65% of prostate cancer patients treated by radical prostatectomy. The prevalence of ERG overexpression is well in line with our earlier study on ERG immunohistochemistry in 61% of prostate cancer diagnosed on needle-biopsies and earlier studies on *TMPRSS2-ERG* fusion in prostate cancer.[10] One-tenth of prostate cancer revealed heterogeneity of ERG expression between different tissue cores taken from the same tumor, which corresponds with other studies on genomic fusion heterogeneity in prostate cancer.[10,27]

Correlation of ERG immunohistochemistry with clinico-pathological parameters in this population of 481 prostate cancer patients revealed that expression of ERG occurred significantly more frequent in patients with PSA $\leq$10 ng/ml ($P = 0.024$). There was no statistically significant relation between ERG immunohistochemistry and age, Gleason sum, pT-stage or surgical margin status. While an association of ERG fusion with low Gleason sum was identified in other prostate cancer cohorts,[28,29] others did not.[30] Using logistic regression, we did not find a statistically significant predictive value of immunohistochemical ERG expression on radical prostatectomy with subsequent biochemical or clinical recurrence, overall death or disease-specific death. Although predictive value of *TMPRSS2-ERG* fusion for biochemical recurrence and death has been reported by some studies,[6,7,31,32] ERG fusion with deletion and duplication of the 5′ end (Edel and 2+Edel) has specifically been associated with worse outcome.[6,7,31,32] Obviously, immunohistochemistry is not able to identify the genetic aberration leading to ERG overexpression. It remains to be elucidated whether *TMPRSS2-ERG* fusion by deletion or duplication is causally related to worse outcome, or merely reflect general genetic imbalance.

As transcription of the *ERG* fusion partner *TMPRSS2* is regulated by androgen receptor, it is hypothesized that androgen receptor and *ERG* signaling are functionally related. Such a mechanism was proposed because androgen receptor signaling induced TOP2B-mediated double-strands DNA breaks with *de novo TMPRSS2-ERG* fusion in LAPC4 and LNCaP cells.[33] On the other hand, ERG inhibits androgen receptor signaling by reducing receptor expression and activity, together with the induction of repressive epigenetic programs.[13] In this study, we investigated the relationship between the ERG and androgen receptor pathways as reflected by the respective protein expression in clinical specimens. Our results showed that increased androgen receptor expression is significantly correlated with higher ERG levels in single cores. Moreover, androgen receptor expression levels in ERG-negative cases were statistically similar to those in cases with moderate ERG expression. To avoid scoring artifacts, we used endothelial and stromal cells as internal controls for ERG and androgen receptor, respectively, in each case. These findings suggest that there is no repressive effect of ERG signaling on androgen receptor expression in clinical specimens. The fact

that androgen receptor expression in ERG-negative cores was similar to its expression in moderate ERG-positive cases furthermore reflects a more complex relation of both important pathways.

This study including 481 patients is one of the largest study cohorts on ERG prognostic value until now and included the well-defined European Randomized Study of Screening for Prostate Cancer population. This screening study has shown that PSA-based screening reduced the rate of death from prostate cancer.[15] Part of the screening protocol is pathological revision of all radical prostatectomy specimens by a pathologist with expertise in urological pathology. In addition, the protocol ensures uniformity in tumor detection, and monitoring of relevant clinical, pathological and follow-up parameters.

While ERG immunohistochemistry is easy to perform and interpret, it does not reveal qualitative information on the *ERG* genomic fusion. It cannot distinguish whether fusion to *TMPRSS2* or another fusion partner causes ERG overexpression.[9,34,35] Likewise, with immunohistochemistry no distinction can be made between genomic deletion, translocation or duplication leading to ERG overexpression. While immunohistochemical analysis was performed by visual semiquantitative scoring by two independent researchers and not by automated imaging, we have previously shown that semiquantitative scoring correlated with quantitative ERG mRNA levels.[9]

Conclusions

Immunohistochemical ERG expression in a well-defined screening cohort of 481 prostate cancer patients revealed no statistical relationship between ERG expression and tumor recurrence or death. Therefore, we conclude that immunohistochemical ERG expression does not have a role in the prognostic stratification of prostate cancer patients after radical prostatectomy.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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