ERG Status Is Unrelated to PSA Recurrence in Radically Operated Prostate Cancer in the Absence of Antihormonal Therapy

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Abstract

Purpose: About 50% of prostate cancers have TMPRSS2–ERG fusions with concurrent ERG overexpression. The aim of this study was to determine whether clinical differences exist between ERG-positive and ERG-negative cancers in surgically treated patients not exposed to antihormonal therapy. A secondary aim was to search for differences between these tumor classes.

Experimental Design: A tissue microarray containing samples from more than 2,800 prostate cancers with clinical data was analyzed for ERG alterations by immunohistochemistry and FISH. Results were compared with tumor phenotype, biochemical recurrence, and molecular features considered important for prostate cancer. The effect of ERG on androgen receptor (AR)-dependent transcription was analyzed in cell lines.

Results: ERG expression was found in 52.4% of 2,805 cancers with a 95% concordance between ERG expression and ERG gene rearrangement detected by FISH. ERG expression was unrelated to clinical outcome and tumor phenotype. Differences in AMACR, Annexin A3, Bcl2, CD10, ALCAM, chromogranin A, epidermal growth factor receptor, HER2, mTOR, p53, and synaptophysin status were significant but minimal in absolute numbers. The most striking difference was found for AR expression, which was markedly higher in ERG-positive cancers. In vitro studies showed ERG-dependent impairment of AR-mediated transcriptional activity.

Conclusions: The striking similarities between these two types of prostate cancers rules out a major impact of ERG on tumor aggressiveness in early, not hormonally treated cancer. The marked difference in AR levels between ERG-positive and -negative cancers supports a systematic difference in potential response to hormonal therapy as previously observed in clinical trials. Clin Cancer Res; 17(18); 5878–88. ©2011 AACR.

Introduction

Approximately 50% of prostate cancers are molecularly characterized by gene fusions linking the androgen-regulated gene TMPRSS2 with transcription factors of the ETS family (1). The androgen-responsive TMPRSS2 gene encodes a transmembrane serine protease of unknown function. In the most common fusion, ERG is fused to 5’-TMPRSS2. Fusion of these genes either occurs through translocation, or more often, through deletion of a 3 Mbp intervening sequence between these 2 genes on chromosome 21 (2–4). As a result of this rearrangement, the expression of ERG becomes androgen regulated and, thus, overexpressed in prostatic epithelium. Several studies have investigated clinical and molecular characteristics of fusion versus nonfusion prostate cancers (reviewed in ref. 5). Studies to date have shown that the rate of fusion-positive prostate cancers is higher in Caucasians (50%–52%) than in African Americans (31.3%) or Japanese (15.9%; refs. 6, 7). Molecular analyses have recently suggested several molecular aberrations that specifically differ between fusion-positive and -negative cancers such as PTEN deletions, which were present in a higher

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percentage of fusion-positive cancers as compared with fusion-negative cancers (8–11). Although such findings suggest significant biological differences, the clinical impact of ETS family gene fusions is still unclear in prostate cancer. Whereas some studies have suggested a worse prognosis of fusion as compared with nonfusion cancers (2, 12–14), other studies either found a favorable prognostic association (15, 16) or did not find any association with clinical outcome (17, 18). These conflicting results may partially be due to differences in cohort size and composition, therapy, and clinical endpoints between different studies (reviewed in ref. 5).

The analysis of TMPRSS2–ERG fusions in prostate cancer has so far been hampered by the need for FISH analysis. Antibodies specifically staining ERG have only recently been described (19, 20). Early results reported a high sensitivity and specificity (>95%) of immunohistochemically detectable ERG expression for the presence of TMPRSS2–ERG gene fusions (19). Because immunohistochemistry (IHC) is much faster, less cumbersome to carry out, and sometimes easier to interpret than FISH, the availability of anti-ERG antibodies now drastically facilitates the assessment of ERG rearrangement status and the evaluation of its diagnostic, prognostic, and predictive impact.

In this study, we utilized a tissue microarray (TMA) composed of tissue samples from 3,261 prostate cancer patients who had undergone radical prostatectomy. The aim of this study was to determine the prognostic significance of ERG status in a homogeneously treated series of prostate cancers that were not exposed to antihormonal therapy. Taking advantage of a comprehensive database on molecular alterations in these tumors, we were able to analyze molecular differences between ERG-positive and -negative prostate cancers.

Translational Relevance

Approximately 50% of prostate cancers are molecularly characterized by gene fusions linking the androgen-regulated gene TMPRSS2 with the transcription factor ERG. As a result of this rearrangement, the expression of ERG becomes androgen regulated and thus overexpressed. Despite several previous studies on TMPRSS2–ERG fusion in prostate cancer, the clinical significance remains controversial. In this study, we show that the ERG status has no influence on the risk of prostate-specific antigen recurrence after radical prostatectomy, indicating that ERG does not affect the course of the disease as long as there is no systemic therapy (especially antihormonal therapy). In addition, our data show a strong association between ERG positivity and high androgen receptor expression levels, which is particularly interesting in the light of recent data suggesting a predictive relevance of ERG status for response to antihormone therapy.

Materials and Methods

Patients

Radical prostatectomy specimens were available from 3,261 patients, treated in the Department of Urology, University Medical Center Hamburg-Eppendorf between 1992 and 2005 (Table 1). Follow-up data were available for 2,891 patients, ranging from 1 to 219 months (mean 72 months). None of the patients received neoadjuvant...
endocrine therapy. Additional (salvage) therapy was initiated in case of a biochemical relapse (BCR). In all patients, prostate-specific antigen (PSA) values were measured quarterly in the first year, followed by biannual measurements in the second and annual measurements after the third year following surgery. Recurrence was defined as a postoperative PSA of 0.2 ng/mL and rising thereafter. The first PSA value above or equal to 0.2 ng/mL was used to define the time of recurrence. Patients without evidence of tumor recurrence were censored at the last follow-up. All prostatectomy specimens were analyzed according to a standard procedure. All prostates were completely paraffin-embedded, including whole-mount sections as previously described (21). All hematoxylin and eosin (H&E) stained histologic sections from all prostatectomy specimens were reviewed and one 0.6-mm thick tissue core was punched out from a representative cancer area and transferred onto a TMA format as described (22). The 3,261 cores were distributed among 7 TMA blocks each containing 129 to 522 tumor samples. From the resultant TMA paraffin blocks, serial histologic sections were prepared. The first set of sections was routinely H&E stained, whereas the second and third sets were stained for AMACR and 34BE12 to detect presence or absence of cancer in each tissue section. A further set of sections was then used for ERG IHC. For internal controls, each TMA block also contained different various control tissues, including normal prostate tissue.

**Immunohistochemistry**

Freshly cut TMA sections were analyzed in 1 day in one experiment for each antibody. The antibody ERG (clone EPR3864, dilution 1:450; Epitomics) was used for ERG protein detection. Slides were deparaffinized and exposed to heat-induced antigen retrieval for 5 minutes in an autoclave at 121°C at pH 7.8. Bound primary antibody was visualized using the DAKO EnVision Kit (Dako). Only nuclear ERG staining was scored. For each tumor sample, the staining intensity was judged on a scale of 0 to 4. Additional IHC data have previously been generated on a different TMA containing different samples from the identical tumor blocks of the same tumor set by using the following antibodies (pretreatments and dilutions): AMACR (clone 13H4, pH 9, 1:200; Dako), Annexin A3 (ANXA3, clone tgc7, pH 2.5, 1:8,100; TgcBIOMICS; ref. 23), androgen receptor (AR, clone 2F12, pH 9.0, 1:10; Novocastra), Bcl2 (clone 124, pH 9.0, 1:250; Dako), CD10 (MME, clone 56C6, pH 9, 1:50; Novocastra; ref. 24), CD166 (ALCAM, clone MOG/07, pH 7.8, 1:2,700; Novocastra), chromogranin A (CHGA, clone LK2H19, 1:500; Biocare), epidermal growth factor receptor (EGFR; clone 31G7, Promace type XIV, 1:100; Zymed), HER2 (Herceptest; Dako; ref. 25), mTOR (polyclonal, pH 2.0, 1:75; Cell Signaling Technology), p53 (clone DO1, pH 7.8, 1:3,600; Oncogene; ref. 26), PSMA (clone 3E6, pH 6.0, 1:150; Dako; ref. 27), SSTR2 (somatostatin receptor 2, polyclonal, pH 6.0, 1:150; Atlas Antibodies), synaptophysin (clone SY38, pH 9.0, 1:20; Dako). With the exception of some previously published IHC data (23–26), the following scoring system was used: The staining intensity (0, 1+, 2+, and 3+) and the fraction of positive tumor cells were recorded for each tissue spot. A final score was built from these 2 parameters according to the following scores: Negative scores had staining intensity of 0, weak scores had staining intensity of 1+ in 70% or more of tumor cells or staining intensity of 2+ in 30% or more of tumor cells; moderate scores had staining intensity of 1+ in less than 70% of tumor cells, staining intensity of 2+ in less than 30%, and 70% or more of tumor cells or staining intensity of 3+ in 30% or more of tumor cells and strong scores had staining intensity of 2+ in more than 70% of tumor cells or staining intensity of 3+ in more than 30% of tumor cells.

**FISH**

A 2-color ERG break apart FISH probe consisting of 2 BAC clones 1 each at 5'-ERG (spectrum green-labeled RP11-95I21 and RP11-360N24) and the other at 3'-ERG (spectrum orange-labeled RP11-720N21 and RP11-315E22) with approximately a 55-kb genomic gap between the 2 sets was made. One freshly cut 4-μm TMA section from block one of our TMA set containing 522 tumors was used for FISH analysis. The slide was deparaffinized and pretreated enzymatically using a commercial kit (Paraffin Pretreatment Reagent Kit; Vysis) according to the manufacturer’s instructions. For hybridization, the slide was dehydrated in 70%, 85%, and 100% ethanol, air dried, and denaturated for 10 minutes at 72°C in 70% formamide-2X SSC solution. After overnight hybridization at 3°C in a humidified chamber, the slide was washed and counterstained with 0.2 μmol/L 4’,6-diamidino-2-phenylindole in antifade solution. The stained slide was manually interpreted with an epifluorescence microscope. Tumors were defined as “normal” when 2 pairs of overlapping red and green signals were seen per cell nucleus. An ERG translocation was assumed if at least 1 split signal consisting of separate red and green signals was observed per cell nucleus. An interstitial deletion of 5’-ERG sequences was assumed if at least 1 green signal per cell nucleus was lost. Tumors were defined as FISH positive if ERG translocation and/or interstitial deletion were present in at least 60% of the tumor cell nuclei in the corresponding tissue spot.

LPL and c-Myc data were taken from a previous publication (28).

**Cell culture, transfection, and luciferase reporter assays**

The LNCaP (DSMZ), DU-145 (DSMZ), RWPE-1 (American Type Culture Collection) and VCaP (European Collection of Animal Cell Cultures) prostate/prostate cancer cell lines were obtained as indicated, subcultured according to the supplier’s instructions and stocks were frozen at passage 2. Cell line identity verification procedures used can be found at the homepage of the respective supplier. Transfections were done using polyethylene imine (Polysciences).
For the reporter gene assay, $5 \times 10^4$ cells were seeded on 24-well plates for transfection. Expression vectors for AR (PSG5-AR, kindly provided by Dr. Roland Schuele, University of Freiburg, Germany) and ERG (pMSCV-ERG, kindly provided by Dr. Pierre Pandolfi, Boston, MA) were cotransfected with a probasin luciferase reporter (probasin-Luc, kindly provided by Dr. Roland Schuele). Transfection efficiency was normalized by coexpression of Renilla using pCMV-Renilla. Twenty-four hours after transfection, the medium was changed and cells were further maintained in the medium containing charcoal-stripped 10% (vol/vol) FBS with or without 100 nmol/L dihydrotestosterone (DHT) for 24 hours. For depletion experiments, VCaP cells were cotransfected with probasin-Luc and pCMV-Renilla together with endoribonuclease-prepared siRNA (esiRNA) against green fluorescence protein (GFP), AR, and ERG. Cells were lysed and tested using a Dual-Luciferase Assay Kit (Promega) and a Berthold luminometer.

**Total RNA extraction and real-time quantitative PCR**

Total RNA from cells was extracted using Trizol and RNeasy system (Macherey-Nagel). RNA was reverse transcribed using the High-Capacity cDNA Archive Kit (Applied Biosystems). Real-time reverse transcriptase PCR (RT-PCR) was done as described previously (29). For all other genes, Assay-on-Demand primer/probe sets supplied by Applied Biosystems were used (Assay IDs are available upon request). Relative expression was calculated by normalization to a selected housekeeper mRNA [glyceraldehyde-3-phosphate (GAPDH)] by the DDC$_{\text{t}}$ method (30).

**Statistics**

Statistical calculations were done with JMP statistical software (Version 8.0, SAS Institute). Contingency tables were calculated with the $\chi^2$-test and Fisher’s exact test to analyze differences between groups. Survival curves were calculated by the Kaplan–Meier method and compared with the Log-rank test.

**Results**

**Technical issues**

As in all TMA studies, a fraction of the cases were noninformative due to complete lack of tissue samples, absence of unequivocal cancer tissue, or noninterpretable FISH signals. The percentage of noninterpretable samples was 14.0% for ERG immunostaining in this study.
Immunohistochemistry
A positive ERG immunostaining could be observed in 52.4% (1,469/2,805) of cases. As previously described, small vessels staining could be used as internal controls for all samples (31). The staining was always nuclear and was observed in invasive cancer, in high-grade prostatic intraepithelial neoplasia (PIN) as well as in a subset of lymphocytes. Representative images are given in Figure 1A–C. The ERG staining intensity was considered 1+ in 18.2%, 2+ in 33.4%, 3+ in 42.9%, and 4+ in 5.5% of the positive cases. Because there was no significant difference between ERG staining intensity and ERG gene rearrangement as detected by FISH as well as other parameters, ERG immunostaining intensity 1 to 4+ were combined as "positive" in subsequent statistical analyses (data not shown).

ERG expression by immunohistochemistry versus TMPRSS2–ERG fusion by FISH
A subset of 453 cancers was successfully analyzed by FISH on consecutive sections to those used for ERG IHC. There was an overall concordance of 95.8% between FISH and IHC. Two hundred thirty of 247 IHC ERG-positive cancers showed ERG gene rearrangements by FISH (93.1%), whereas ERG gene rearrangements were only seen in 2 of 206 cancers (1.0%) with a negative ERG IHC result.

Table 2. ERG expression and tumor phenotype

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<th>ERG positive (%)</th>
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Relationship with different molecular markers
The majority of previously examined immunohistochemical stainings and FISH findings differed significantly between fusion-positive and fusion-negative prostate cancers. These include the expression of AR, AMACR, Annexin A3, Bcl2, CD10, CD166, chromogranin A, EGFR, HER2 and mTOR, p53, and synaptophysin. No significant differences between fusion and nonfusion type cancers were found for c-Myc amplification, SSTR2 expression, and LPL deletion. The results are summarized in Figure 3, which shows that the absolute differences between fusion and nonfusion cancers were mostly low, despite highly significant P values. The strongest difference was found for AR expression, which was considered strong in 77.2% of ERG positive but in only 57.7% of ERG-negative cancers (Fig. 4). AR immunostaining was unrelated to the risk of BCR (P = 0.5606, Fig. 2B). Hence, the combination of AR and ERG immunostaining was also unrelated to the risk of BCR (P = 0.0975, Fig. 2C).

Influence of ERG expression on AR-mediated transactivation in prostate cancer cell lines
Given the strong association between ERG and AR expression in our IHC data, the relationship between these parameters was analyzed in cell lines. Among the different cell lines tested, VCaP was the only cell line which resembled the prostate cancers in terms of AR and ERG expression. Specifically, the effect of ERG on AR-dependent transcription was further analyzed in ERG-negative LNCaP cells and ERG-positive VCaP cells (Fig. 5, Panel A). After transfection, cells were incubated with or without dihydrotestosterone (DHT), and the AR-mediated transcriptional activity was
monitored using the AR-dependent probasin promoter chemoluminescence assay. In LNCaP cells, a marked induction of AR-mediated transcriptional activity was observed after exposure to 100 nmol/L DHT. The probasin luminescence was strongly reduced in cells coexpressing AR and ERG as compared with AR or ERG alone (Fig. 5, Panel B). Overexpression of AR and ERG showed no effect on the activity of the GAPDH promoter (data not shown). To extend these data, we conducted depletion experiments using an esiRNA-based approach in VCaP cells. VCaP is the only available prostate carcinoma cell line that harbors a TMPRSS2-ERG translocation and overexpresses AR (Fig. 5, Panel C). Transfection of VCaP cells with esiRNA directed against AR resulted in abrogation of DHT-induced probasin luminescence. Inversely, depletion of ERG enhanced the probasin luminescence about 3-fold as compared with the controls. Overall, our data show the functional link between AR and ERG expression in VCaP cells and further indicate that ERG modulates the transcription of AR-dependent genes.

Discussion

The analysis of 2,805 prostate cancers showed ERG expression in 52.4%. This is in the range of previous studies, which found ERG expression in 48% (31), and 28% (32) by IHC, in 50%–60% by quantitative RT-PCR (33, 34). Also, TMPRSS2–ERG gene fusion by FISH has been described in 47% to 55% of prostate cancers (1, 7, 35, 36). In our study, ERG expression analyzed by IHC was strongly associated with the presence of ERG gene rearrangement detected by FISH at a concordance of 95.8%. This figure corresponds to recent data by Park and colleagues (31) describing a sensitivity of 95.7% and a specificity of 96.5% of IHC for the identification of TMPRSS2–ERG fusions. Some of the few cases with ERG expression in the absence of a FISH detected fusion may be due to other molecular changes. Rare ERG fusions have also been found with other androgen-dependent genes such as SLC45A3 (37) and NDRG1 (38). We assume that the two cases with ERG rearrangement in the absence of detectable ERG expression represent IHC failures caused by, for example, suboptimal tissue fixation, a preanalytical problem that cannot always be avoided (39). It is also possible that ERG expression is halted in some fusion-positive prostate cancers through specific molecular mechanisms, such as inactivation of the AR pathway. It is noteworthy, however, that ERG expression was never seen in nonneoplastic prostate epithelium, either in this study or that of Park and colleagues (31). Given this specificity of ERG expression for neoplastic prostate epithelium, that is, prostate cancer and high-grade PIN, ERG immunostaining may indeed represent a highly diagnostic marker as previously suggested (31), albeit in 50% of the cases. On the basis of current data, it seems possible, that ERG-positive atypical small acinar proliferation may be judged as cancer in the future.

Our data show that the ERG status is unrelated to the clinical disease course in radically operated prostate cancers. There was no discernable difference with respect to PSA relapse between 1,192 ERG-positive and 1,086 ERG-negative cancers. Although ERG immunostaining was statistically associated with tumor stage ($P = 0.0003$) and Gleason grade ($P < 0.0001$), there was no clear trend despite these $P$ values and the absolute numbers differed only little. Previous studies investigating the possible clinical/prognostic relevance of ERG expression have described conflicting data. Studies analyzing between 59 and 445
cases with FISH or RT-PCR have reported fusion-positive cancers to be associated with either poor prognosis or more aggressive cancer (2, 12, 40, 41). In contrast, Saramaki and colleagues (15) have analyzed 253 cases with FISH and found fusion-positive cancers to be significantly associated with longer progression-free survival. In line with our previous collected molecular findings obtained by FISH and IHC using various methods according to specific properties of the analyzed gene/protein. Overall the data show that for the majority of analyzed parameters, the differences are rather small, however. These data show that for the majority of analyzed gene/protein. Overall the differences are statistically significant. The color code varies between the analyzed parameter: AMACR, CD166, chromogranin A (CHA), EGFR, mTOR, p53, SSTR2, synaptophysin (SYN), low (dark grey), moderate (light grey), strong (medium grey). Bcl2: low (dark grey), high (light grey), CD10: apical (dark grey), cytoplasmatic (light grey), mixed staining (medium grey). C-Myc: gene polysomy (dark grey), gene gain (light grey), gene amplification (medium grey). HER2 immunostaining: 1+, 2+, and 3+. Combined. Lipoprotein lipase (LPL): gene loss. Annexin A3 (ANX3): weak (dark grey), moderate (light grey), and strong (medium grey).

For most of these parameters, the absolute differences were rather small, however. These data show that pathway alterations involving these genes are neither essential nor exclusive for either one of these 2 main prostate cancer subgroups. Molecular analyses have recently suggested several molecular aberrations that specifically

study, several other investigations failed to detect a prognostic difference between fusion-negative and fusion-positive cancers in cohorts of 521 (18) and 214 (17) cancers. Our study on 2,891 cancers with clinical follow-up data used PSA recurrence as its clinical endpoint. We thus believe that our data strongly exclude a significant difference in the biological behavior of ERG-positive and ERG-negative cancers in the absence of antihormonal or other systemic therapy.

It was the secondary aim of this study to determine whether differences exist in molecular features that were previously discussed as potentially relevant for prostate cancer between fusion-positive and fusion-negative cancers. For this purpose, ERG data were compared with a variety of molecular features that were available from previous studies (23–27). Our analysis showed statistically significant differences for most analyzed features that were previously analyzed on our TMA, including AR, AMACR, Annexin A3, Bcl2, CD10, CD166, chromogranin A, EGFR, HER2, and mTOR, p53, and synaptophysin. For most of these parameters, the absolute differences were rather small, however. These data show that pathway alterations involving these genes are neither essential nor exclusive for either one of these 2 main prostate cancer subgroups. Molecular analyses have recently suggested several molecular aberrations that specifically

Figure 3. Molecular findings in ERG-positive and ERG-negative tumors. The figure describes previously collected molecular findings obtained by FISH and IHC using various methods according to specific properties of the analyzed gene/protein. Overall the data show that for the majority of analyzed parameters, the difference between ERG-positive and ERG-negative tumors is rather low in absolute numbers but statistically significant. The color code varies between the analyzed parameter: AMACR, CD166, chromogranin A (CHA), EGFR, mTOR, p53, SSTR2, synaptophysin (SYN), low (dark grey), moderate (light grey), strong (medium grey). Bcl2: low (dark grey), high (light grey), CD10: apical (dark grey), cytoplasmatic (light grey), mixed staining (medium grey). C-Myc: gene polysomy (dark grey), gene gain (light grey), gene amplification (medium grey). HER2 immunostaining: 1+, 2+, and 3+. Combined. Lipoprotein lipase (LPL): gene loss. Annexin A3 (ANX3): weak (dark grey), moderate (light grey), and strong (medium grey).

Figure 4. Comparison of androgen receptor (AR) expression levels in ERG-positive and ERG-negative prostate cancer. AR expression was considered strong in 77.2% of ERG-positive cancers but in only 57.7% of ERG-negative cancers.

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Comparison of androgen receptor (AR) expression levels in ERG-positive and ERG-negative prostate cancer. AR expression was considered strong in 77.2% of ERG-positive cancers but in only 57.7% of ERG-negative cancers.
differ between fusion-positive and fusion-negative cancers, such as PTEN deletions, which were found in a higher percentage of fusion-positive cancers as compared with fusion-negative cancers (8–11). Carver and colleagues (9) found in 14 of 15 ERG FISH-positive samples reduced or absent PTEN expression compared with 13 of 25 ERG FISH-negative samples. Accordingly, King and colleagues (8) found in 14 of 57 ERG FISH-positive samples a PTEN loss compared with 3 of 64 ERG FISH-negative samples. Expression screening studies had also described a number of molecular differences between fusion-positive and fusion-negative prostate cancer (42–44). However, most of the latter studies suggest only comparatively small changes in the expression levels of most individual differentially expressed genes. We have calculated the linear fold changes from the data reported by Yu and colleagues (47) showing a significantly reduced expression of AR as well as repression of AR-regulated genes (45, 46). It is therefore not surprising that the strongest difference between fusion-positive and fusion-negative cancers was found for AR expression in this study. This observation prompted us to further study the functional consequences of ERG–AR interaction. Downregulation of the AR-dependent probasin promoter under the influence of ectopically induced ERG in LNCaP cells showed that ERG could impair the expression of AR-mediated genes. This finding is consistent with the data reported by Yu and colleagues (47) showing a significantly reduced expression of AR as well as repression of AR-dependent promoters, such as those of TMPRSS2 and KLK3 in ERG-overexpressing prostate cell lines. It is possible that significantly elevated levels of AR in ERG-expressing cancer cells in vivo, as shown by our immunohistochemical experiments, may reflect a compensatory receptor upregulation as a result of decreased levels of 1 or several AR-regulated genes. A potential crosstalk between ERG rearrangement and AR signaling was also suggested by Rickmann and colleagues (48). Depending on the level of androgen signaling, they found that ERG exerts dual activities on the expression of Trefoil factor 3 (TFE3), a differentially regulated gene with respect to ERG rearrangement.
Our data describing a strong association between ERG positivity and high AR expression levels is particularly interesting in the light of recent data suggesting a predictive relevance of ERG status for response to antiandrogen therapy. Attard and colleagues (49) showed that men with androgen-resistant cancers had a higher maximal prostate-specific response when treated with the antiandrogen abiraterone acetate if the tumors were ERG-positive compared with ERG-negative tumors. This is also in line with a recent study by Karnes and colleagues (50), which showed that patients with ERG-positive tumors showed a more significant treatment effect in response to adjuvant androgen deprivation than patients with ERG-negative tumors. This could be due to the fact that ERG-positive tumors are particularly dependent on a functional AR because ERG can only be overexpressed in the presence of AR. The lack of prognostic significance of ERG expression in our study using BCR as an endpoint does not rule out a predictive value of ERG for antiandrogen therapy. In prostatectomy patients, antiandrogen therapy will only be applied after BCR, which represents the clinical endpoint of our study.

Tumor heterogeneity is a major issue in tumor biology. Moreover, in case of prostate cancer more than 90% of affected prostate glands contain multiple independent cancers (31, 52). As a consequence, heterogeneous molecular findings will often remain undetected in TMA studies, especially in case of multifocal or large cancer. Despite of this, earlier TMA studies have shown, that 1 core per tumor is sufficient for finding associations between molecular markers and clinicopathologic parameters in prostate cancer, at least if the TMAs contain large numbers of patient samples (23, 25–27, 53). Attempts to better represent prostate cancer in TMAs by taking up to 10 different cores from 1 selected donor block may not sufficiently improve representativity because the median number of tumor-containing blocks ranges between 10 and 11 in our laboratory (unpublished data).

In summary, our data show that TMPRSS2–ERG fusion is unrelated to prostate cancer phenotype and that differences in many important molecular features are little between fusion-positive and fusion-negative cancers. The lacking influence of the ERG status on the risk of PSA recurrence after radical prostatectomy indicates that ERG does not affect the course of the disease as long as there is no systemic therapy. Upregulation of AR in fusion-positive cancers could however argue for a specific response type to hormone therapy as suggested in some early studies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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