ORIGINAL ARTICLE

A high-density tissue microarray from patients with clinically localized prostate cancer reveals ERG and TATI exclusivity in tumor cells

G Lippolis¹, A Edsjö², U-H Stenman³ and A Bjartell¹

BACKGROUND: Prostate cancer (PCa) is characterized by high tumor heterogeneity. In 2005, the fusion between the androgen-regulated gene TMPRSS2 and members of the ETS family was discovered in prostate cancer. In particular, fusion of TMPRSS2 with ERG was found in approximately 50% of prostate cancers and considered as an early event in the onset of the disease. The prognostic value of this fusion is still contradictory. Bioinformatics showed that overexpression of SPINK1 gene in a subset of fusion-gene-negative prostate cancers was associated with a poor prognosis. In theory, overexpression of the tumor-associated trypsin inhibitor (TATI) protein encoded by SPINK1 in fusion-gene-negative tumor cells opens the way to selected treatments for genotypically different cases. However, their expression has never been assessed at the cellular level in the same tissue samples.

METHODS: As ERG expression has been shown to be a surrogate of fusion gene occurrence in prostate cancer, we have used double immunohistochemical staining to assess expression of ERG and TATI on a large tissue microarray comprising 4177 cases of localized prostate cancer.

RESULTS: We did not detect any co-expression of ERG and TATI in the same cancer cells, which confirms previous suggestions from in silico studies. ERG was associated with Gleason score (GS), surgical margins and pathological stage, but had no prognostic value in this cohort. TATI was weakly associated with pathological stage but had no significant association with outcome.

CONCLUSIONS: We here provide a morphological basis for ERG and TATI exclusivity in prostate cancer cells. Future therapies should be based on a combination of different targets in order to eradicate tumor cells with gene fusions and cells expressing other tumor-associated antigens. Further studies are needed to understand why ERG and TATI are not co-expressed in the same prostatic tumor cells.

Keywords: ERG; TATI; SPINK1; immunohistochemistry; radical prostatectomy

INTRODUCTION

Prostate cancer (PCa) is the second most frequently diagnosed cancer, the sixth cause of cancer death in males worldwide and the most common cancer in developed countries.¹ At the time of diagnosis, PCa is often multifocal and highly heterogeneous, leading to difficulty in accurately determining the prognosis and the most appropriate form of therapy.² The disease development can range from slow-growing and localized tumors to rapidly growing and highly metastatic tumors. As a result, there is a need to find biomarkers that can identify aggressive forms of the disease. Thus far, this approach has not produced any widely used clinical tests to accurately predict the progression of the disease; however, many studies have cast light on its biological features.³⁵

PCa, like many other malignancies, is characterized by mutations in genes that promote (oncogenes) or protect against cancer (tumor suppressors). These genetic abnormalities include point mutations and chromosomal aberrations (gain, losses, rearrangements).⁶ In 2005, TMPRSS2:ETS family gene fusions were discovered in PCa.⁷ By using cancer outlier profile analysis, members of the ETS family were found to be overexpressed in a subset of PCa types, with ERG being the most common fusion partner. This fusion seems to occur in approximately 50% of PCas⁸ and since TMPRSS2 is an androgen-regulated gene, this leads to androgen-regulated overexpression of the oncoprotein ERG. It seems to be an early event in the onset of PCa, but results from various studies on its prognostic value are contradicting. Rajput et al.⁹ found that the ERG fusion gene was more frequent in moderately to poorly differentiated PCas than in well-differentiated tumors. Perner et al.¹⁰ found a significant association between TMPRSS2:ERG fusions via deletion and higher tumor stage as well as the presence of metastatic disease involving pelvic lymph nodes. Additionally, Fine et al.¹¹ described an association between the TMPRSS2-ERG gene fusion and low Gleason score. However, others have reported no association with outcome in patients treated by prostatectomy,¹² or no association with other clinicopathological parameters.¹³

Among patients not harboring ETS rearrangements, Tomlins et al.,¹⁴ using the cancer outlier profile analysis bioinformatics method, identified SPINK1 as an outlier highly expressed in a subset of cases. Furthermore, this subset of high SPINK1-expressing tumors was associated with an increased risk for biochemical recurrence. Subsequent studies have also investigated the association of tumor-associated trypsin inhibitor (TATI) protein corresponding to the SPINK1 gene) with clinicopathological

¹Division of Urological Cancers, Department of Clinical Sciences, Skåne University Hospital, Malmö, Lund University, Malmö, Sweden; ²Department of Clinical Pathology and Cytology, Sahlgrenska University Hospital, Gothenburg, Sweden and ³Department of Clinical Chemistry, Helsinki University Central Hospital, Helsinki, Finland. Correspondence: Dr A Bjartell, Department of Urology, Skåne University Hospital, Malmö SE 205 02, Sweden. E-mail: Anders.Bjartell@med.lu.se

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variables. Leinonen et al. showed that in a cohort of patients primarily treated with endocrine therapy, TATI-positive cases had shorter progression-free survival, with TATI falling out as an independent prognostic factor. No association with other clinico-pathological variables was observed. The possibility of TATI-positive tumors being selectively targeted by antibodies for therapeutic purposes was demonstrated in an in vitro study showing decreased proliferation, invasion and intravasation upon TATI inhibition. The relationship of TATI with other potential biomarkers has also been investigated in castration-resistant PCa. Using consecutive tissue sections and different staining techniques (fluorescence in situ hybridization and traditional immunohistochemistry, IHC), it was reported that overexpression of TATI occurred in PTEN-deleted tumors, none of which showed androgen receptor amplification. Based on bioinformatic analyses, it was suggested that SPINK1 and TMPRSS2:ERG expression were mutually exclusive in prostatic tumors. However, to our knowledge, no studies have yet compared the protein expression of TATI and ERG in the same tissue sections. It has recently been shown that ERG staining is highly sensitive and specific as a surrogate marker for TMPRSS2: ERG gene fusion. Therefore, co-occurrence of TATI expression and TMPRSS2:ERG gene fusion can now be analyzed at the protein level, as conventional IHC can be used instead of fluorescence in situ hybridization to evaluate fusion gene status on tissue sections.

The aim of our study was to investigate for possibly the first time the co-occurrence of TATI and ERG in the same tissue sections by using IHC with double staining in order to determine if they are in fact expressed in different cell populations. This would further support the clinical attempts to selectively treat patients with genotypically different PCa. We have used a large tissue microarray (TMA) consisting of 4177 samples from clinically localized PCa patients who underwent radical prostatectomy.

**MATERIALS AND METHODS**

**Patients**

Tissue specimens from primary prostatic tumors were collected from 4177 patients who underwent open radical prostatectomy at the Department of Urology, University Medical Center Hamburg-Eppendorf between 1992 and 2005. Clinicopathological features included pre-operative PSA level, pathological stage (pT) as defined by the American Joint Committee on Cancer in 2002, pathological Gleason score (GS), lymph node involvement (N), surgical margins status (SMS), and, if available, also time to occurrence of metastasis (Table 1). Biochemical recurrence was defined as an increase of postoperative PSA to 0.2 ng ml\(^{-1}\) with a confirmatory value. In total, 913 cores were considered benign and 693 were considered malignant. ERG and TATI staining were specifically performed for each block. The initial number of patients included was 4177 and the design of the experiment was set to have one core from each patient. Statistical analysis was performed using SPSS (v.20, IBM, Chicago, IL, USA). Kaplan–Meier and log-rank test were used to evaluate the relationship between protein expression and BCR or metastatic disease. Crosstabs were used to show the relationship between protein expression and clinico-pathological characteristics, and \(\chi^2\) test or the Fisher’s exact test was used to assess the significance of differences.

**RESULTS**

**Immunostaining**

The initial number of patients included was 4177 and the design of the experiment was set to have one core from each patient. Ninety-nine cores were considered benign and 693 were damaged or missing and therefore excluded from analysis. ERG was found to be expressed in 41.7% of the cancer cases.
with intensity scores of $+1$ in 13.9%, $+2$ in 20.4% and $+3$ in 7.4%. Staining was found in the nuclei of cancer cells (Figure 1) and in some areas of prostatic intraepithelial neoplasia (PIN). As expected, endothelial cells and macrophages also stained positive for ERG in both benign and malignant areas. We did not observe expression of ERG in any of the benign epithelial structures. Expression of TATI in tumor cells was observed in 5.2% of the cores (175/3385) with the following distribution: $+1$ in 2.2%, $+2$ in 1.9% and $+3$ in 1.0%. As previously demonstrated, TATI protein was exclusively localized in the cytoplasm of epithelial cells (Figure 1). A very weak immunostaining for TATI was often found in the cytoplasm of benign luminal epithelial cells.

Interestingly, we identified areas showing transition from benign to PIN and malignant epithelium with ERG expression as the markers of transition (Figure 2). TATI was not found to be overexpressed in any of these areas. Representative immunostainings for ERG and TATI is shown in Figure 1.

Expression of ERG and TATI to predict the outcome after radical prostatectomy
We also investigated if expression of ERG or TATI could predict BCR or metastatic events. Kaplan–Meier curves were built on dichotomization where expression of ERG and TATI was either positive or negative. Neither ERG nor TATI predicted BCR (log rank
Mantel–Cox), $P = 0.689$ and $P = 0.447$, respectively, Figure 3) or development of metastatic disease (log rank (Mantel–Cox), $P = 0.681$ and $P = 0.530$, respectively Figure 4). In a univariate Cox regression model, ERG and TATI intensity as a continuous or as a dichotomized variable was not a significant predictor of BCR or of metastatic disease.

**DISCUSSION**

Previous studies18,19,23 have shown that ERG expression analyzed by IHC is strongly correlated with ERG gene fusion as detected by fluorescence in situ hybridization analysis. Here we applied IHC of ERG on a high-density TMA ($n = 4177$) in order to explore a
It also extends the TMA used in a previous study by Minner et al. The observed expression of ERG (positive in 41.7%) and TATI (positive in 5.7%) is in accordance with results from previous publications, although the frequency is slightly lower. This can be related to the fact that in the TMA used, only one core was available from each patient. If we assume that ERG IHC is a good surrogate marker for TMPRSS2:ERG fusion in PCa, our present results favor the view that the occurrence of this gene fusion in PIN is an early event in tumor development. Our data also confirm the findings reported by Furusato et al., who observed the presence of ERG in PIN and found a strong concordance of ERG-positive foci in PIN with ERG-positive carcinoma. However, our results do not display its usefulness as a prognostic biomarker as previously suggested.

Our data show that ERG did not predict the course of the disease in radical prostatectomy-treated patients, since it was neither related to BCR nor related to metastatic onset. ERG positivity was significantly associated with pT stage, SMS and GS but not with N stage or with the preoperative PSA value. Even if the expression of ERG was significantly different in the groups with various GSs (≤6, 3 + 4, 4 + 3, ≥8) and pT stages (pT2, pT3, pT4), there was no clear linear trend. ERG seemed to be more often expressed in tumors with pT3 stage and a Gleason score of 3 + 4 than in other stages and grades, as previously described. As for the association with SMS, it must be interpreted with caution until it has been confirmed in subsequent studies. Of note, our data set is larger than earlier-described ones and seems to exclude the use of ERG staining for stratification of patients for the risk of relapse.

TATI has previously been shown to identify a subgroup with more aggressive cancer. Our data show a significant association (P = 0.0496) with pT stage, but the association is weak and it is difficult to draw conclusions. No association was found with BCR or metastatic event.

In silico data from studies on different PCa cohorts have suggested that SPINK1/TATI and TMPRSS2:ERG are expressed in a mutually exclusive manner. In this study, we aim to clarify if this pattern of expression is observed at the protein level. Our presented data seem to show that ERG and TATI are expressed in separate tumor cell populations and that further studies are needed to elucidate the underlying tumor biology. Another confirming observation is that expression of ERG-positive cells may indicate a transition from benign to PIN or from PIN to malignancy as illustrated in Figure 2.

In conclusion, by using immunohistochemical double staining, we showed that ERG and TATI are exclusively expressed in separate tumor cell populations. However, in this setting, neither ERG nor TATI was a useful predictor of outcome in PCa patients undergoing radical prostatectomy. The results provide a morphological basis for future PCa therapy using a combination of different targets in order to eradicate tumor cells expressing different markers. Further studies are needed to elucidate why ERG and TATI are not co-expressed in the same prostatic tumor cells.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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