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***TPRSS2-ERG* Fusion Prostate Cancer Heterogeneity: Clinical and Biologic Implications**

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Abstract

OBJECTIVES—To characterize the clonality of *TPRSS2-ERG* fusion in multifocal prostate cancer.

METHODS—From 80 consecutive radical prostatectomy specimens, we identified 32 cases with multiple spatially separate tumors. In each case we assessed 2–3 tumor foci for *TPRSS2-ERG* fusion using an ERG break-apart interphase fluorescence in-situ hybridization assay.

RESULTS—Individual tumor foci showed homogeneity for fusion status (intrafocal clonal homogeneity). In 59% (19/32) of cases, all foci within a case had the same fusion status (interfocal homogeneity): in 80% (15/19) of these cases no foci had fusion, and in 20% (4/19) all foci had fusion. 41% (13/32) of cases demonstrated heterogeneity for fusion status within a case (interfocal clonal heterogeneity).

CONCLUSIONS—We have demonstrated interfocal heterogeneity and intrafocal homogeneity for *TPRSS2-ERG* fusion in prostate cancer with multiple tumors. These findings support the multiclonal nature of prostate cancer with clinical implications for needle biopsy strategies and the development of urine-based screening tests.

Introduction

Fusion of the androgen-regulated gene *TPRSS2* and the *ETS* transcription factor family members, in particular *ERG* has been described recently as the commonest recurrent genomic alteration in prostate cancer 1, present in nearly half of prostate cancers in PSA-screened hospital-based cohorts 2,3. *TPRSS2-ERG* fusion shows high specificity for

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Disclosure:

The University of Michigan at Ann Arbor and the Brigham and Women's Hospital (Boston) have filed a patent for the FISH assay described in the manuscript. Gen-Probe (San Diego, CA) has licensed the rights to develop a commercial test.

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prostate cancer 4, and is associated with higher tumor stage and tumor-specific death or metastasis 2·3·5. This raises the possibility of using the detection of fusion transcripts in urine 6 and in tissue as diagnostic and prognostic tests. Prostate cancer is commonly multifocal at the time of diagnosis 7, yet to date there has been no systematic fluorescence *in situ* hybridization (FISH)-based study of heterogeneity for *TMPRSS2-ERG* in multifocal prostate cancer. In the current study we report the presence of inter-focal heterogeneity and confirm intra-focal homogeneity for *TMPRSS2-ERG* fusion in multifocal prostate cancer and possible clinical implications.

Materials and Methods

Case selection and pathological analysis

All specimens used in this study were from patients who underwent radical prostatectomy for prostate cancer at Brigham and Women's Hospital, as part of Institutional Review Board-approved study protocols. Eighty consecutive radical prostatectomy specimens performed between January and August 2006 were reviewed to identify 32 cases with multiple, spatially-separate tumor foci. The selected cases had tumor foci on contralateral sides of prostate or, if ipsilateral, separated by at least half of the antero-posterior diameter of the prostate in all sections based on the protocol from Cheng et al. 8. In a minority of cases, the tumor foci were separated by at least 3mm in all sections as a minimum requirement. For each case, two to three foci were analysed including the dominant nodule when possible. The diagnosis of carcinoma was confirmed independently by the study pathologists (MB and SP). A Gleason score and a maximum diameter was recorded for each tumor focus. Demographic and clinico-pathologic features of these cases were obtained from the patients' records and pathological examination of the cases. The mean age at surgery was 57.1yr (range 35–68yr). The mean pre-operative serum PSA was 5.5ng/mL (range 3.2–8.7ng/mL) in the 29 cases for which this information was available. 41% (13/32) of the cases were Gleason score 6, 50% (16/32) were Gleason score 7, and 9% (3/32) were Gleason score 9. 75% of cases were stage pT2 (24/32), and 25% were pT3 (8/32). None of the cases were associated with lymph node metastasis.

Determination of *TMPRSS2-ERG* fusion status

We have described previously the FISH assay for the rearrangement of *TMPRSS2-ERG* 1·3. Briefly, because *TMPRSS2* and *ERG* are so close together on chromosome 21, a break-apart probe system was used. This identifies when the telomeric and centromeric components of *ERG* split apart, and is indirect evidence of *TMPRSS2-ERG* fusion. With this system, a nucleus without *ERG* rearrangement demonstrates 2 pairs of juxtaposed red and green signals, usually forming a yellow merged signal. A nucleus with an *ERG* break-apart (i.e. *TMPRSS2-ERG* fusion through insertion) shows splitting apart of the red and green signal for the translocated *ERG* allele and maintenance of a juxtaposed red-green (yellow) signal pair for the non-translocated *ERG* allele. A nucleus with *TMPRSS2-ERG* fusion through deletion shows loss of one green signal with preservation of the associated red signal and a juxtaposed red-green (yellow) signal pair for the non-translocated *ERG* allele.

Statistics

Examination of the association between clinico-pathological variables and fusion status was assessed by means of the unpaired t-test for continuous variables and by Pearson Chi-Square test for discrete variables. All p-values were considered two-sided. P-value threshold to be considered significant was set at 0.05 (SPSS, Chicago IL).

Results

In the 32 radical prostatectomy specimens studied, the *TMPRSS2-ERG* fusion status was evaluated in 2–3 distinct foci per case to a total of 77 tumor foci: an example is shown in Figure 1A–C and the results are summarized in Figure 1D. Overall, 53% (17/32) of cases had *TMPRSS2-ERG* fusion, consistent with previous studies for PSA-screened prostatectomy cohorts. Heterogeneity for fusion status was seen in 41% (13/32) of cases. Of these, 38% (5/13) had foci of carcinoma with no fusion and foci with fusion by deletion, 31% (4/13) had foci with no fusion and foci with fusion through insertion, 15% (2/13) had foci with fusion through insertion and foci with fusion through deletion, and 15% (2/13) showed 3 distinct foci of carcinoma showing no fusion, fusion through insertion and fusion through deletion. A representative example of a case (# 9) showing inter-focal heterogeneity for *TMPRSS2-ERG* fusion is shown in Figure 1A–C.

In almost all cases, all evaluable tumor cells demonstrated homogeneity for fusion status within a given focus of cancer (intra-focal homogeneity). One exception to this was seen (case #26) whereby a 1.5cm tumor focus showed fusion by deletion and a directly contiguous smaller focus of tumor (0.5cm) showed no fusion: while this may represent clonal divergence within a single tumor, that this represents a “collision” between two independent tumor foci is more in keeping with the findings in the other cases. In 59% (19/32) of cases, all foci had the same fusion status (interfocal homogeneity): 80% (15/19) of these had multiple foci of carcinoma showing no fusion, 10% (2/19) showed multiple foci with fusion by insertion, and 10% (2/19) showed multiple foci with fusion by deletion.

Heterogeneous and homogeneous cases show a similar mean age: 54.7yr and 58.7yr, respectively. The mean pre-operative serum PSA was slightly higher in the cases showing heterogeneity (6.5 vs 4.8 ng/mL), but this was not statistically significant. The association of fusion status with stage was not examined due to the selection criteria for this study. There was no association between the fusion status of a tumor focus and its Gleason score or its size. In 10 of the 13 heterogeneous cases with separate foci of carcinoma showing fusion and no fusion, we were able to assess the status of the major or index nodule: in six cases the major nodule showed fusion (four by deletion, two by translocation), and in four cases the major nodule showed no fusion, while at least one of the smaller foci of tumor showed evidence of fusion.

Comment

It is recognized that prostate cancer is multifocal. Both morphologic and molecular analysis has shown that by the time of cancer diagnosis, more than 80% of prostates harbor multiple separate cancers 7–11. We previously reported clonal homogeneity for *TMPRSS2-ERG* fusion status by FISH in a total of 243/246 cases of prostate cancer in tissue microarrays and confirmed homogeneity in standard tissue sections in 10 of the cases 3·4. Furthermore, when Tomlins et al. 12 studied expression array data on multiple microdissected foci of cancer from individual patients, either all or no foci over expressed ERG and its family members ETV1 and ETV4. One potential confounder is that these studies evaluated multiple areas from one lesion per case.

Therefore, to systematically investigate the question of the heterogeneity of *TMPRSS2-ERG* fusion, we used FISH to study 32 prostatectomies with distantly separated tumors. Using annotated sections of prostate, we were able to accurately map tumor foci equivalent to a whole-mount approach 13. We found that in virtually all cases, a tumor focus was homogeneous for fusion status. This is consistent with our prior studies 3·4 and is in keeping with the previous suggestion that *TMPRSS2-ERG* fusion is an early event in prostate cancer.

We noted that fusion by insertion and fusion by deletion occurred as disjoint events, suggesting that these are distinct clonal mechanisms for achieving *TMPRSS2-ERG* fusion. Interestingly, we found a high rate (41%) of interfocal heterogeneity for fusion status in our cases. This is higher than expected from the studies cited above, and is likely related to sampling. In this study we emphasized assaying multiple foci in each case, whereas in tissue microarray construction, generally the emphasis is on including multiple samples from the dominant nodule. It is difficult to infer from our study the absolute prevalence of heterogeneity for *TMPRSS2-ERG* fusion in hospital-based prostatectomy cohorts, given that we studied higher stage cases (at least pT2c) in order to maximize the chance of detecting heterogeneity. In addition, many prostates contain more than 3 foci: a systematic study of all distinct tumor foci within a prostate would most likely increase the percentage of heterogeneous cases. However, this current study definitively documents clonal diversity of prostate cancer lesions for the first time using the *TMPRSS2-ERG* in situ probes. Fusions between *TMPRSS2* and *ETS* family members other than *ERG* are uncommon² and we did not assay for them in this study. We anticipate that evidence for further heterogeneity will be found when these distinct fusion events are studied.

We did not see a correlation between the focus Gleason score and its fusion status. Nor did we see a correlation between focus fusion status and focus size. In the heterogeneous cases in which we could assess the index or dominant nodule and in which there were foci of tumor with and without fusion, in 40% of cases the dominant nodule showed no fusion.

Emerging data suggests that *TMPRSS2-ERG* fusion prostate cancer is more aggressive than fusion negative prostate cancer^{3,5,14}. The finding that *TMPRSS2-ERG* fusion demonstrates heterogeneity and that current biopsy strategies do not systematically sample all tumor nodules, it is likely that prostate cancers with fusion could be missed at time of biopsy. Therefore the development of a non-invasive diagnostic test for fusion transcripts in urine should help to exclude missing *TMPRSS2-ERG* fusion prostate cancer clones even if they are not the dominant nodule⁶. In the future, it is likely that a positive urine test would be followed by biopsy, and that if cancer is present, confirmatory FISH would be performed on the tissue specimen. Our finding of interfocal heterogeneity for *TMPRSS2-ERG* fusion has direct relevance in the context of a urine test result that is positive for fusion and a subsequent prostate biopsy with cancer that is negative for fusion: the implication of interfocal heterogeneity being that there is an unsampled tumor focus in the prostate. Indeed, given the potential role of *TMPRSS2-ERG* fusion as a prognostic marker for aggressive disease, saturation biopsies might be indicated to accurately assess a patient's risk for disease progression.

Conclusions

TMPRSS2-ERG fusion is a clonal molecular event that occurs early in prostate cancer development. We demonstrate for the first time that *TMPRSS2-ERG* fusion can be used to document prostate cancer heterogeneity. Given the potential diagnostic and prognostic significance of *TMPRSS2-ERG* fusion, these findings also have implications for the development of clinical tests.

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Figure 1. Representative example of a heterogeneous case (Case #9) and a summary of the results for all cases

A–C: H&E sections from the anterior half of prostate with two spatially separate foci of cancer showing no fusion (A and B) and fusion by insertion (A and C). The insert in B shows a nucleus with two yellow signals (juxtaposed red and green signals) indicating that there is no fusion. The insert in C shows one yellow signal and split red and green signals, indicating that fusion through insertion has taken place.

D: Piechart summary of the results of the study with cases showing interfocal homogeneity in light grey (fusion) and dark grey (no fusion) and cases showing interfocal heterogeneity in light red (fusion and no fusion) and dark red (clonally distinct fusion).