Heterogeneity of *TMPRSS2* Gene Rearrangements in Multifocal Prostate Adenocarcinoma: Molecular Evidence for an Independent Group of Diseases

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Abstract

Recurrent gene fusions between the androgen-regulated gene *TMPRSS2* and the *ETS* family transcription factors *ERG, ETV1*, and *ETV4* have been identified in the majority of prostate adenocarcinomas (PCA). PCA is often multifocal with histologic heterogeneity of different tumor foci. As *TMPRSS2* is a common 5′ partner of *ETS* gene fusions, we monitored *TMPRSS2* rearrangement by fluorescence *in situ* hybridization (FISH) to study the origin and molecular basis of multifocal PCA heterogeneity. *TMPRSS2* rearrangement was evaluated by FISH on a tissue microarray representing 93 multifocal PCAs from 43 radical prostatectomy resections. Overall, 70% (30 of 43) of the cases showed *TMPRSS2* rearrangement, including 63% through deletion (loss of the 3′ *TMPRSS2* signal), 27% through translocation (split of 5′ and 3′ *TMPRSS2* signals), and 10% through both mechanisms in different tumor foci. Of the 30 *TMPRSS2* rearranged cases, 30% showed concordance in all tumor foci, whereas 70% were discordant in at least one focus. In *TMPRSS2* rearranged cases, the largest (index) tumor was rearranged 83% of the time. Pathologic stage, size, or Gleason grade of the multifocal PCA did not correlate with overall *TMPRSS2* rearrangement. Our results suggest that multifocal PCA is a heterogeneous group of diseases arising from multiple, independent clonal expansions. Understanding this molecular heterogeneity is critical to the future development and utility of diagnostic and prognostic PCA biomarkers. [Cancer Res 2007;67(17):7991–5]

Introduction

Prostate adenocarcinoma (PCA) is often a multifocal disease, generally consisting of a dominant (index) tumor and one or multiple separate smaller tumors (1–4). Multifocal PCA frequently shows histologic heterogeneity among different tumor foci exhibiting different Gleason grades (1, 3). Arora et al. showed that of the 115 PCA prostatectomy specimens, 87% (100) contained two or more widely separated tumors and only 9% of multifocal PCAs had all tumor foci with primary and secondary Gleason grades that were the same as the corresponding overall Gleason grades assigned to the whole specimen (1). Due to this anatomic and histologic heterogeneity, needle biopsies are often not representative of the entire tumor.

The histologic and biological heterogeneity of multifocal PCA suggests that they arise independently within the same gland; however, the molecular basis for this hypothesis is poorly understood. Recently, our group identified gene rearrangements in a majority of PCAs, with the 5′ untranslated region of the androgen-regulated gene *TMPRSS2* (21q22.3) fused to the 3′ region of the oncogenic *ETS* transcription factor family members *ERG* (21q22.2), *ETV1* (7q21.2), or *ETV4* (17q21; refs. 5, 6). We showed that most PCAs harboring gene rearrangements can be identified using a 5′ and 3′ break-apart fluorescence *in situ* hybridization (FISH) strategy, flanking the *TMPRSS2* locus (7). Using this approach, 65% of clinically localized PCAs showed rearrangement of *TMPRSS2*, with the majority (55%) being fused with the *ETS* partner *ERG* (7). In addition, as *TMPRSS2* and *ERG* are located ~3 Mb apart on chromosome 21, the rearrangement between these two partners occurs through either a translocation between chromosome 21's or intrachromosomal deletion (8).

It is currently unknown whether different tumor foci in multifocal PCAs harbor these gene rearrangements uniformly. As this molecular aberration is specific to prostate carcinogenesis, analyzing gene fusion status of multifocal PCA should provide insight into tumor origin and the molecular basis of multifocal PCA heterogeneity. Hence, we sought to investigate *TMPRSS2* gene rearrangement in multifocal PCAs.

Materials and Methods

Study population, clinical data, and prostate sample collection. The study consisted of 43 patients, who underwent radical prostatectomy with or without bilateral pelvic lymphadenectomy between 2004 and 2006 at the University of Michigan. None of the patients received preoperative radiation or androgen deprivation therapy. This study was approved by the Institutional Review Board at the University of Michigan hospital. Fresh prostates removed after surgery were weighed, measured, inked, and fixed in 10% neutral formalin. Seminal vesicles, apex, and base were amputated, and the remaining prostate was serially sectioned at 3-mm to 5-mm intervals perpendicular to the long axis of the gland from the base to apex. Tumor maps were generated by tracking each section and reconstructing them as a whole-mount section (Figs. 1*A* and 2*A*). A cancer was considered spatially separate or multifocal if it was 3 mm or more from the closest cancer in any single section or if it was 4 mm or more from the closest cancer on the adjacent section above or below, as described previously and as outlined in Fig. 1*A* (1, 4). The largest tumor focus was designated as the index tumor (*Index T*), whereas additional tumor foci were labeled as secondary tumor 1 (*Sec T1*, for the second tumor focus from a case) and secondary tumor 2 (*Sec T2*, when three different foci could be identified). Each focus was assigned a primary and secondary grade (9). A tissue microarray representing a total of 93 tumor foci, which included an index tumor and at least one separate secondary tumor, was constructed from these

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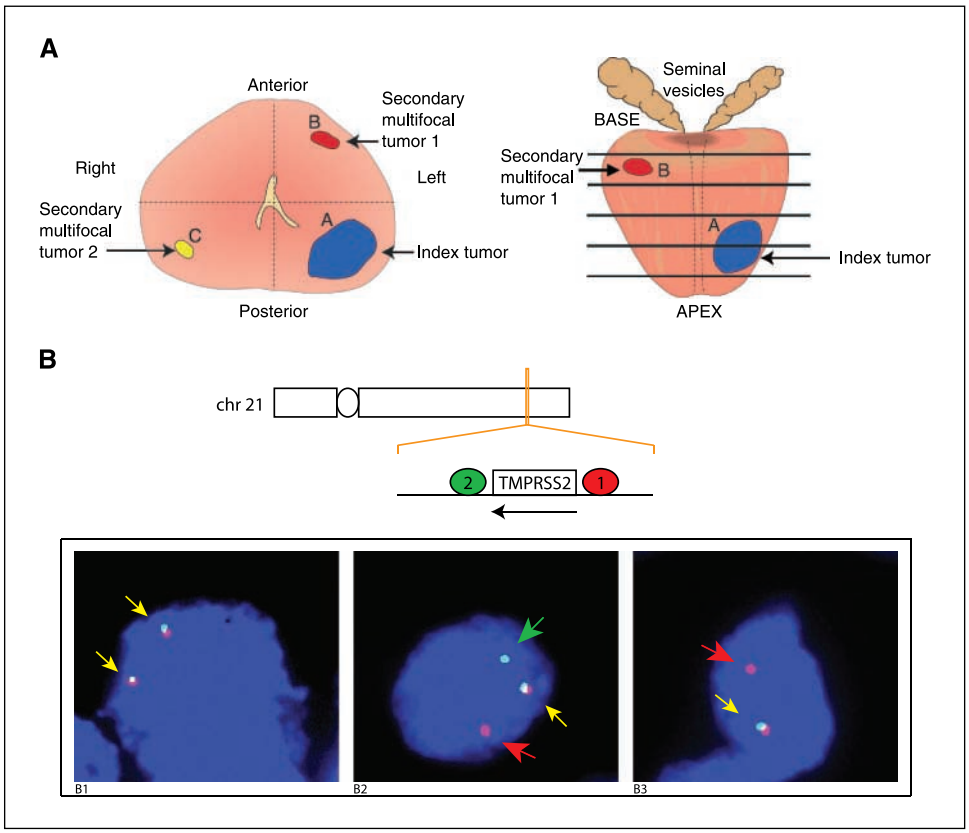

Figure 1. *A,* schematic representation of an approach to the analysis of multifocal PCA. Diagrams were constructed by outlining each section and then reconstructing them as a whole-mount section, and tumor maps subsequently were generated. A PCA was considered spatially separate or multifocal if it was 3 mm or more from the closest cancer in any single section or if it was 4 mm or more from the nearest cancer on the adjacent section, above or below. *B,* assay approach to detect *TMPRSS2* gene rearrangement in multifocal PCA. Interphase FISH on formalin-fixed, paraffin-embedded tissues to detect *TMPRSS2* gene rearrangement in PCA. For this assay, the chromosomal location of the gene is indicated (*box*), with the direction of transcription indicated by the arrow. 5′ and 3′ BAC are indicated (*ovals*), with the number identifying the BAC as described below and the color indicating the probe color in the accompanying images. *B1,* two colocalized signals (*yellow arrows*) in a case lacking *TMPRSS2* rearrangement. *B2, TMPRSS2* rearrangement positive case (split positive), as indicated by one pair of split of 5′ and 3′ signals. *B3,* a *TMPRSS2* rearrangement positive case (deletion positive); PCA case showing loss of one green-labeled probe 3′ to *TMPRSS2*. BACs: *1,* RP11-35C4; *2,* RP11-120C17.

prostatectomy specimens. Three cores were taken from each representative tumor focus to construct the tissue microarray as described (7).

***TMPRSS2* gene rearrangement assessment by FISH.** FISH was done as described previously (5–7). Slides were examined using ImagingZ1 microscope (Carl Zeiss) and imaged with a CCD camera (Metafer). FISH signals were scored manually (100× oil immersion) in morphologically intact, nonoverlapping nuclei. A minimum of 50 cancer cells from each tumor focus was recorded. Cases without 50 evaluable cancer cells were reported as insufficient. Cores with very weak or no signals were recorded as insufficiently hybridized. All bacterial artificial chromosomes (BAC) were obtained from BACPAC Resource Center (Oakland, CA). For *TMPRSS2* rearrangement detection, we used RP11-35C4 (5′ to *TMPRSS2*) and RP11-120C17 (3′ to *TMPRSS2*).

A case was considered discordant when one tumor focus showed *TMPRSS2* rearrangement (split or deletion positive; Fig. 1*B*), whereas a second or third tumor focus did not. If a case showed different mechanisms of gene rearrangement (one focus through translocation and another through deletion), the case was considered discordant for gene rearrangement status. Conversely, a case was considered concordant when all the tumor foci in a single case showed a similar mechanism of *TMPRSS2* gene rearrangement, exclusively through a translocation (split) or a deletion. Cases with all the tumor foci negative for *TMPRSS2* gene rearrangement were also labeled as concordant.

Statistical analysis. The relationship between overall *TMPRSS2* rearrangement, pathologic stage, and Gleason score was evaluated by the Pearson χ^2 test of association.

Results and Discussion

As *TMPRSS2* is the most common 5′ partner of the *ETS* family genes (7), we monitored *TMPRSS2* gene rearrangement as a tool to study the origin and molecular basis of multifocal PCA heterogeneity. A normal signal pattern for *TMPRSS2* in 4′,6-diamidino-2-phenylindole–stained nuclei is two pairs of colocalized green and red signals (Fig. 1*B*). A rearrangement was indicated by the break apart of one of the two colocalized signals (without deletion; Fig. 1*B*) or by one pair of colocalized signals and loss of one 3′ probe (green, with deletion; Fig. 1*B*).

A total of 93 tumor foci from 43 radical prostatectomy specimens were analyzed. In 36 specimens, two tumor foci and, in the remaining seven cases, three tumor foci could be evaluated for *TMPRSS2* rearrangement. Overall, 30 of 43 cases (70%) showed *TMPRSS2* rearrangement within at least one tumor focus, whereas the remaining 13 of 43 (30%) tumors lacked *TMPRSS2* gene rearrangement in all tumor foci. We observed *TMPRSS2* rearrangement in 27% of the cases through translocation (split of 5′ and 3′ *TMPRSS2* signals; Fig. 1*B*), 63% through deletion (loss of one 3′ *TMPRSS2* signal; Fig. 1*B*), and 10% through both mechanisms in separate tumor foci. In the 30 rearranged cases, uniform gene rearrangement mechanism was seen in 9 of 30 cases (30%). Rearrangement in the index tumor was seen in 25 of 30 cases (83%), whereas in 5 of 30 cases (17%) the rearrangement was seen only in secondary tumors. Overall, 21 of 43 cases (49%) were concordant for *TMPRSS2* gene rearrangement mechanism or concordant by lack of *TMPRSS2* rearrangement in all foci. Although heterogeneity for *TMPRSS2* rearrangement between individual tumor foci was common, individual tumor cells within each tumor focus were homogeneous for *TMPRSS2* rearrangements, suggesting that individual tumor foci develop through clonal expansion. Table 1 summarizes the *TMPRSS2* gene rearrangement status and its assessment in 93 tumor foci from 43 cases with their relevant clinical and pathologic characteristics. Overall *TMPRSS2* rearrangement status was not significantly associated with pathologic stage ($P = 0.98$), Gleason grade ($P = 0.93$), or tumor size ($P = 0.13$). Figure 2*B* represents a heat map of *TMPRSS2* rearrangement status between different tumor foci across 43 cases in this cohort.

Although the multifocality and morphologic heterogeneity of PCA is well recognized (1, 3, 4), until the findings of this study it was unclear whether this represented a single disease or multiple independent diseases of distinct clonal origin. The molecular basis of multifocal PCA has been explored using comparative analyses of an allelotype, the marker of genetic linkage on specific chromosomal regions, based on multiple microsatellite loci in different areas of a given tumor (2, 10–13). This procedure identifies regions or polymorphic markers likely to harbor genes altered in prostate carcinogenesis (2, 10, 13). However, the interpretation of patterns of such allelic imbalance between different foci of PCA remains complex and depends on the tissue purity and analysis of multiple loci, which are usually considered critical for this type of analysis. Based on this approach, some studies have provided evidence suggestive of a genetic relationship (13), whereas other studies do not suggest common clonality of multifocal PCAs (2, 10, 12).

Recently, our group identified recurrent gene fusions between the androgen-regulated gene *TMPRSS2* and the oncogenic *ETS* family transcription factors as a crucial molecular event in the majority of PCAs (5, 6). As this molecular abnormality is specific to prostate carcinogenesis, in this study we monitored *TMPRSS2* gene rearrangement as a tool to study the origin and molecular basis of multifocal PCA heterogeneity (7).

As previously shown by our group and others, this study confirms that a majority of PCAs harbor *TMPRSS2-ETS* family gene rearrangements (6–8, 14), suggesting an important role in prostate cancer development and progression. The 70% frequency of *TMPRSS2* gene rearrangement seen in these cases is similar to the 65% frequency we recently reported in a cohort of clinically localized PCAs in American men treated with radical prostatectomy (7). Interestingly, when different tumor foci of individual cases were compared for the *TMPRSS2* gene rearrangement status, only 9 of 30 cases (30%) showed similar gene fusions across foci,

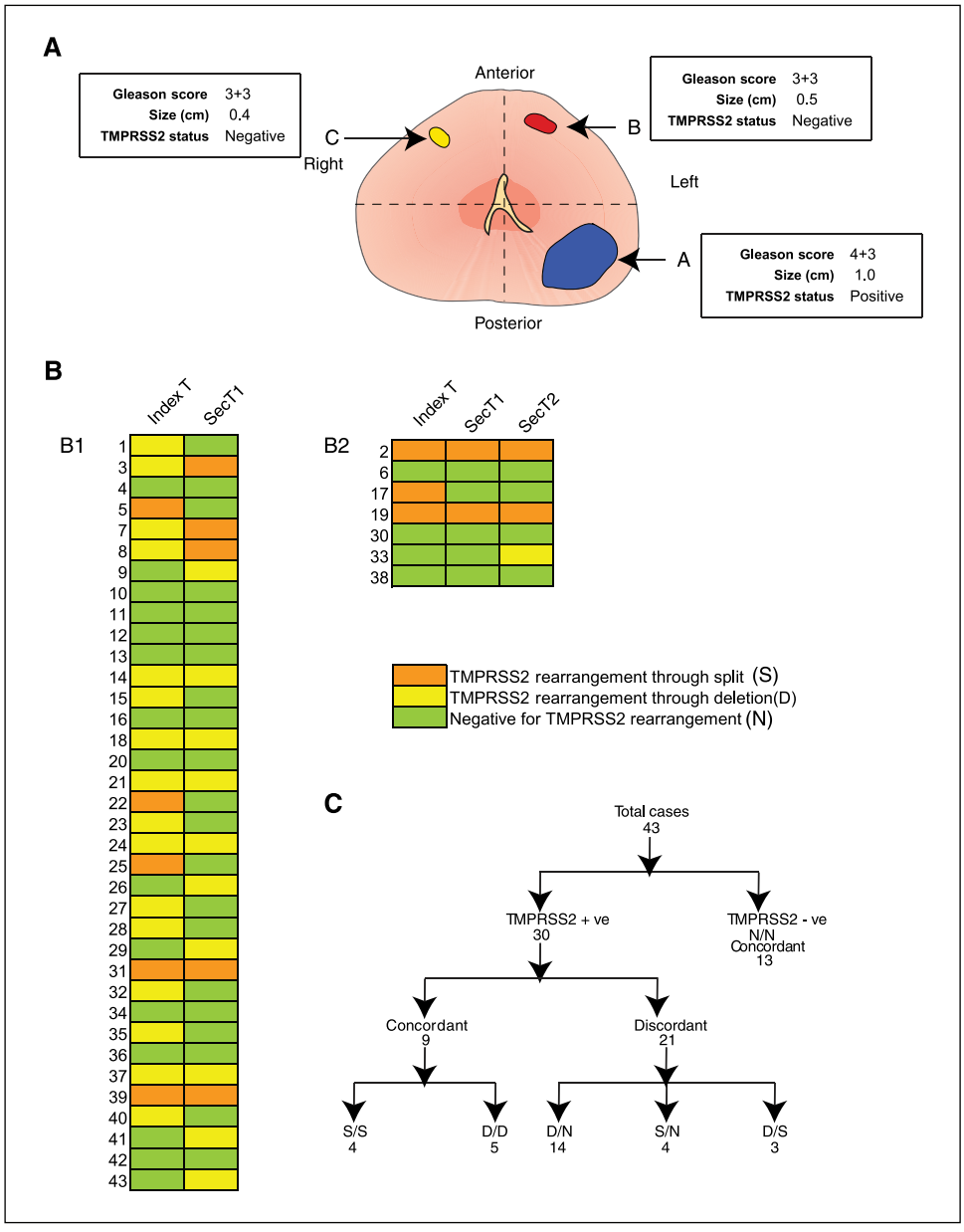

Figure 2. *A,* representative reconstructed map of the prostate sections in case 17. Index tumor from this case is represented as *A*, and two secondary tumors in the same prostate as *B* (secondary tumor 1) and *C* (secondary tumor 2); relative location of the three tumor foci. A summary of Gleason score, tumor size, and *TMPRSS2* rearrangement status for each of these foci is presented in the boxes. *B,* comparison of *TMPRSS2* rearrangement status of different tumor foci in 93 multifocal tumors from 43 PCA cases. The *TMPRSS2* gene aberration status of each tumor focus is indicated individually in the form of a heat map using a color schema. *B1,* gene rearrangement status of cases with two tumor foci; index tumor (*Index T*) with one additional tumor focus called secondary tumor 1 (*Sec T1*). *B2,* the cases where an index tumor was accompanied by two additional secondary tumors, secondary tumor 1 and secondary tumor 2 (*Sec T2*). *C,* flow chart demonstrating summary of *TMPRSS2* rearrangement status in 93 multifocal tumors from 43 PCA cases. *N,* negative for *TMPRSS2* rearrangement; *S,* split of 5′ and 3′ ends of *TMPRSS2*; *D,* deletion of 3′ end of *TMPRSS2*; *N/N,* all tumor foci negative for TMPRSS2 rearrangement; *D/D,* all foci deleted; *S/S,* all foci split for *TMPRSS2*; *S/N,* one focus split and other remaining negative; *S/D,* one focus split and other deleted for *TMPRSS2*; *D/N,* one focus deleted and other focus negative for *TMPRSS2* rearrangement.

Table 1. Summary of clinicopathologic features and *TMPRSS2* rearrangement status in 93 multifocal PCAs from 43 radical prostatectomy cases

Case no.	Index tumor			Secondary tumor 1			Secondary tumor 2			P stage
	Size (cm)	GS	TMPRSS2 status	Size (cm)	GS	TMPRSS2 status	Size (cm)	GS	TMPRSS2 status	
1	0.8	3 + 5	Deletion positive	0.8	3 + 3	Negative				T2
2	1.4	4 + 3	Split positive	0.7	3 + 3	Split positive	0.5	3 + 3	Split positive	T3b
3	1.7	4 + 3	Deletion positive	1.0	3 + 4	Split positive				T3a
4	2.3	4 + 4	Negative	0.7	3 + 3	Negative				T2
5	0.9	4 + 4	Split positive	0.4	3 + 3	Negative				T3b
6	0.7	3 + 3	Negative	0.4	3 + 3	Negative	0.4	3 + 3	Negative	T2
7	1.8	3 + 3	Deletion positive	1.7	3 + 3	Split positive				T2
8	1.0	3 + 4	Deletion positive	0.6	3 + 3	Split positive				T2
9	1.3	3 + 4	Negative	1.1	3 + 3	Deletion positive				T3a
10	1.2	4 + 5	Negative	0.7	3 + 4	Negative				T3a
11	0.7	3 + 3	Negative	0.4	3 + 3	Negative				T2
12	1.5	3 + 4	Negative	0.5	3 + 3	Negative				T2
13	1.2	3 + 3	Negative	1.1	3 + 4	Negative				T2
14	1.3	3 + 4	Deletion positive	0.8	4 + 3 + 5	Deletion positive				T3a
15	1.2	4 + 3	Deletion positive	1.0	3 + 4	Negative				T3a
16	1.1	4 + 5	Negative	0.8	3 + 4	Negative				T2
17	1.0	4 + 3	Split positive	0.5	3 + 3	Negative	0.4	3 + 3	Negative	T2
18	1.5	4 + 3	Deletion positive	0.6	3 + 3	Deletion positive				T2
19	1.7	3 + 5	Split positive	0.7	3 + 4	Split positive	0.3	3 + 3	Split positive	T3a
20	2.0	4 + 4	Negative	1.0	3 + 3	Negative				T3a
21	2.5	3 + 3	Deletion positive	0.6	3 + 3	Deletion positive				T2
22	1.2	4 + 3	Split positive	0.6	3 + 3	Negative				T2
23	1.1	3 + 3	Deletion positive	0.7	3 + 4	Negative				T2
24	2.0	4 + 5	Deletion positive	0.5	3 + 3	Deletion positive				T2
25	1.5	3 + 4	Split positive	1.2	3 + 3	Negative				T2
26	0.5	3 + 3	Negative	0.4	3 + 4	Deletion positive				T2
27	2.0	3 + 3	Deletion positive	0.5	3 + 3	Negative				T2
28	1.6	3 + 4 + 5	Deletion positive	0.7	3 + 3	Negative				T3a
29	1.2	3 + 3	Negative	1.2	3 + 3	Deletion positive				T2
30	1.1	3 + 4	Negative	0.7	4 + 5	Negative	0.6	3 + 3	Negative	T2
31	0.9	3 + 4	Split positive	0.4	3 + 3	Split positive				T3a
32	1.6	3 + 4	Deletion positive	1.5	3 + 3	Negative				T2
33	2.2	3 + 3	Negative	1.0	3 + 3	Negative	1.0	3 + 3	Deletion positive	T2
34	1.0	3 + 3	Negative	1.0	3 + 3	Negative				T2
35	1.6	3 + 3	Deletion positive	1.3	3 + 3	Negative				T2
36	1.4	4 + 5	Negative	0.3	3 + 3	Negative				T3a
37	0.4	3 + 3	Deletion positive	0.3	3 + 3	Deletion positive				T2
38	1.0	5 + 3	Negative	0.3	3 + 3	Negative	0.3	3 + 3	Negative	T3a
39	1.8	3 + 4	Split positive	1.0	3 + 3	Split positive				T2
40	2.0	3 + 4	Deletion positive	1.2	3 + 3	Negative				T3a
41	1.9	3 + 4	Negative	0.7	3 + 3	Deletion positive				T2
42	1.2	4 + 4	Negative	0.6	3 + 3	Negative				T3a
43	1.1	3 + 3	Negative	0.9	3 + 3	Deletion positive				T2

Abbreviations: GS, Gleason score; P stage, pathologic stage; T2, organ confined cancer; T3a, extraprostatic extension; T3b, seminal vesicle invasion.

demonstrating remarkable heterogeneity between multiple tumor foci. Of 21 discordant multifocal *TMPRSS2* rearranged cases (70%), the mechanism of heterogeneity between different tumor foci was through either the presence or absence of *TMPRSS2* rearrangement, or the presence of *TMPRSS2* rearrangement through translocation in one tumor focus and deletion in another focus. The mechanism of *TMPRSS2* gene rearrangement through translocation or deletion has been shown previously to be potentially significant (8). Perner et al. reported a significant association between tumors with *TMPRSS2-ERG* rearrangement through deletions and higher tumor stage and the presence of positive pelvic lymph nodes when compared with cancers without *TMPRSS2-ERG* fusions (8). This molecular heterogeneity is likely to occur in the process of tumor origin and/or progression, and therefore, our results suggest that this genetic heterogeneity may be an underlying molecular mechanism for diverse clinical and morphologic manifestations of PCAs. In multifocal PCA, the index (or the largest) tumor focus is usually considered to be biologically most relevant or significant (1, 4, 9). Our results indicate that an index tumor is usually (83%) representative of this molecular

abnormality; however, in 17% of the cases, *TMPRSS2* rearrangement was seen in only secondary tumors. Therefore, this result also suggests that, in a small proportion of cases, the secondary tumors can also have significant alterations that may be biologically significant. Therefore, the size of a PCA and its degree of histologic differentiation may not always reflect the extent of its genetic alterations. Although *TMPRSS2* rearrangement status monitors all reported *ETS* rearrangements, other molecular mechanisms may play an important role in foci lacking *TMPRSS2* rearrangement. We hypothesize that uncharacterized 5′ partners to *ETS* family genes or additional new 5′ and 3′ partners may be important in tumor development. Future work will focus on identifying other novel rearrangement that may have biological significance.

With the widespread use of serum prostate-specific antigen screening, over 90% of PCAs diagnosed in American men are clinically localized (15). These clinically localized PCAs are biologically diverse, ranging from clinically indolent tumors to a subset of aggressive tumors with potential for recurrence and metastasis (16, 17). Biomarkers that can accurately identify and stratify these prognostic subsets of clinically localized PCAs currently remain the field of major investigation. Interestingly, it is noted that no single biomarker or group of biomarkers that can successfully diagnose or predict disease recurrence 100% of the time has yet been identified. Although many previous studies, including recent ones, have suggested the distinct molecular subtypes of gene fusions as potential prognostic biomarkers, these studies, by definition, have ignored the heterogeneity of multifocal PCA (7, 8, 18, 19). Therefore, as shown earlier by our group in androgen-independent prostate cancers, the current study further emphasizes that understanding this heterogeneity is the key to the development of such future diagnostic or prognostic molecular biomarkers, specifically in small needle biopsy samples (20).

In summary, this report uses *TMPRSS2* rearrangement status to provide compelling evidence that suggests diverse molecular origins of multifocal PCA. Our findings show that a significant proportion of multifocal PCAs is a heterogeneous group of diseases that arise from multiple, independent clonal expansions. Understanding this heterogeneity is critical to the future development of diagnostic or prognostic biomarkers.

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