Characterization of TMPRSS2-ETS Gene Aberrations in Androgen-Independent Metastatic Prostate Cancer

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Abstract

Recent gene fusions between the androgen-regulated gene TMPRSS2 and the ETS transcription factor family members ERG, ETV1, and ETV4 have been identified as a critical event in prostate cancer development. In this study, we characterized the prevalence and diversity of these rearrangements in hormone-refractory metastatic prostate cancer. We used a fluorescence in situ hybridization (FISH) split probe strategy to comprehensively evaluate TMPRSS2-ETS aberrations across 97 nonsusosseous metastatic sites of prostate cancer from 30 rapid autopsies of men who died of androgen-independent disease. Tissue microarrays were constructed representing multiple metastatic sites from each patient, and split signal FISH probes for TMPRSS2, ERG, ETV1, and ETV4 were used to assess for TMPRSS2-ETS rearrangements. In patients exhibiting these aberrations, multiple sites from an individual case harbored the same gene fusion molecular subtype suggesting clonal expansion of disease. The most common prostate cancer gene fusion, TMPRSS2-ERG, can be generated by the mechanism of interstitial deletion (Edel) about 39% to 60% of the time in clinically localized disease. Interestingly, we observed that all of the androgen-independent metastatic prostate cancer sites harboring TMPRSS2-ERG were associated with Edel. These findings suggest that TMPRSS2-ERG with Edel is an aggressive and, in this study, uniformly lethal molecular subtype of prostate cancer associated with androgen-independent disease. [Cancer Res 2008;68(10):3584–90]

Introduction

The majority of prostate cancer deaths are attributed to metastatic dissemination of the primary tumor. Androgen ablation is initially effective in the management of metastatic prostate cancer; however, most patients develop an androgen-independent state that is invariably fatal (1). To elucidate the biology of prostate cancer progression and metastasis, we have developed a “rapid” or “warm” autopsy program at the University of Michigan composed of patients that died of androgen-independent metastatic prostate cancer. Tumors are collected from metastatic sites and the prostate, when present, and thus constitute a valuable resource to study the natural history and evolution of prostate cancer (2, 3).

We recently identified the fusion of the 5′-untranslated region of TMPRSS2 (21q22.3) with the ETS family members ERG (21q22.2), ETV1 (7q21.2), or ETV4 (17q21) in prostate cancer (4, 5) that has subsequently been confirmed by multiple groups (6–9). More recently, we have identified several 5′ fusion partners, supporting the existence of additional molecular subtypes of ETS gene fusions (10). We also showed that most prostate cancers harboring gene rearrangements can be identified using a 5′ and 3′ split probe fluorescence in situ hybridization (FISH) strategy with probes tightly flanking the TMPRSS2, ERG, ETV1, and ETV4 loci (4, 5, 11). Using this approach, 65% of clinically localized prostate cancers showed TMPRSS2 rearrangement, with the majority (55%) being fused to ERG (11). Additionally, as TMPRSS2 and ERG are located ~3 Mb apart on chromosome 21, the rearrangement between them occurs either through a translocation between chromosome 21s or by an interstitial deletion (6, 8). Further, in our recent study of multifocal prostate cancers, the majority (70%) of cases showed heterogeneous TMPRSS2 gene rearrangements between different tumor foci, thus supporting multifocal prostate cancer as a heterogeneous group of diseases (12). Similar observations were reported by Clark and colleagues (13). We have also shown the oncogenic potential of ETS fusions through in vitro and in vivo expression of ETV1 (10) and ERG (14).

It is currently unknown to what extent lethal androgen-independent metastatic prostate cancers exhibit gene rearrangements or whether they harbor them uniformly at different metastatic sites. Hence, we sought to characterize the frequency, mechanism, and significance of TMPRSS2-ETS aberrations in a rapid autopsy cohort of 30 American men who died of androgen-independent metastatic prostate cancer.

Materials and Methods

Rapid autopsy tissue procurement and tissue microarray construction. The autopsies were conducted as previously described (2, 3), and they are referred to as rapid or warm because of the short average interval of 3 h between death of patient and onset of autopsy. A tissue microarray was constructed from 30 rapid autopsies representing all metastatic prostate cancer sites and tumor in the prostate (when present; that is, no prior radical prostatectomy). The rapid autopsy program is approved by the University of Michigan Institutional Review Board and supported by Specialized Program of Research Excellence (National Cancer Institute grant CA 69568).

Fluorescence in situ hybridization. We used previously documented 5′ and 3′ split probe strategy to detect TMPRSS2, ERG, ETV1, and ETV4 rearrangements (Fig. 1A; ref. 11). Interphase FISH was performed as previously described (4, 5, 11). Slides were examined using an ImagingZ1 microscope (MetaSystems; Carl Zeiss). FISH signals were scored manually (100× oil immersion) in morphologically intact nuclei by two pathologists (R.M. and R.B.S.), and a minimum of 30 cancer cells from each site was recorded. Cancer sites with very weak or no signals were recorded as...
insufficiently hybridized. Only the nonosseous metastatic sites were evaluated during this study; osseous metastatic sites were nonhybridizable due to dense bone structure and hence excluded.

A cancer site was considered positive for a gene aberration when the tumor showed a rearrangement for any of the split probes mentioned above (through split or deletion; Fig. 1B). A metastatic site was labeled as positive for fusion when TMPRSS2 rearrangement (through split or deletion) was concomitant with a rearrangement (through split or deletion) in any of its known 3' partners: ERG, ETV1, or ETV4 (10, 11).

**Results and Discussion**

This University of Michigan rapid autopsy cohort represents patients who died of androgen-independent metastatic prostate cancer (3). Of this group, 28 of the 30 men were initially diagnosed with clinically localized prostate cancer but later developed widely disseminated disease within 5 to 10 years. These patients exhibited progression to lethality despite the use of multiple therapeutic regimens (Supplementary Table S3). In the context of our initial discovery of TMPRSS2-ETS fusions in prostate cancer (4, 5, 11), we sought to perform a detailed characterization of these gene aberrations in androgen-independent metastatic prostate cancer.

Our FISH split probe strategy (Fig. 1A) revealed gene aberrations in 52% of the rapid autopsy cases. Overall, 48%, 37%, 11%, and 4% of the cases were positive for TMPRSS2, ERG, ETV1, and ETV4 split probe assays, respectively (Figs. 1 and 2). As observed in clinically localized disease (6, 7–9, 15), TMPRSS2-ERG fusion was the most common abnormality identified in hormone-refractory metastatic prostate cancer (37%). Additional fusions that were present included TMPRSS2-ETV1 (case 26) and TMPRSS2-ETV4 (case 8). In two cases, ETV1 was rearranged with an unknown partner [cases 4 and 23; the unknown partner in case 23 was recently identified to be C15orf21 in an independent study (10)]. Case 30 was found to be amplified with multiple (seven to nine) copies of ETV1 present in each nuclei. Figure 1B displays representative FISH images of the diversity of aberrations observed in this cohort.

To determine if the same gene rearrangement is shared across metastatic sites in these subjects, we examined 97 tumor foci from different organs as well as the prostate (when present) with the FISH split probe assays. Interestingly, we found that different metastatic cancer foci within an individual patient harbored the same gene aberration (Fig. 2B). Furthermore, the same gene rearrangement exhibited by the metastatic sites was present in tumor in the prostate (when available), as in case 24 (Figs. 2B and 3A). Similarly, cases without TMPRSS2-ETS rearrangements were uniformly negative across all metastatic sites (Fig. 2B). The exception to this was case 13, which harbored a TMPRSS2-ERG rearrangement at metastatic foci within liver, lung, dura, and soft tissue. However, in this patient, metastatic tumor in liver also displayed a deletion of the 5' end of ETV1, suggesting the possibility of a secondary rearrangement acquired during the evolution of metastatic prostate cancer at this site (Fig. 2A and B). Although multifocal prostate cancer may harbor different foci that may be TMPRSS2-ETS positive or negative (12, 13), our study suggests that a single clone evolves from clinically localized disease to seed the metastatic sites with cells harboring the same ETS gene rearrangement (Fig. 3B).

**TMPRSS2-ERG** gene rearrangement in metastatic disease is similar to what has been observed in clinically localized disease (6–9, 11). In our University of Michigan cohort, we observed TMPRSS2-ERG gene rearrangements in 54% of clinically localized prostate cancer (11) and 37% in metastatic disease. In clinically confined disease, 39% of TMPRSS2-ERG gene fusions are through intrachromosomal deletion (Edel), whereas 61% are not (and thus presumably caused by translocation of chromosome 21s). By contrast, 100% of androgen-independent prostate cancers harboring the TMPRSS2-ERG gene fusion (10 out 10 subjects and 29 of 29 metastatic sites) exhibit Edel (Fig. 2).

Taken together, this is the first study that comprehensively characterizes TMPRSS2-ETS aberrations in end-stage, androgen-independent metastatic prostate cancer. Based on our split probe strategy (11), we found that 52% of the cases in this cohort harbored TMPRSS2-ETS rearrangements. This frequency (52%) is similar to the 65% frequency of gene aberrations reported by our group for clinically localized prostate cancer (11) and in agreement with other reports in U.S. prostatectomy cohorts (9, 16). This indicates that the high frequency of TMPRSS2-ETS aberrations in clinically localized prostate cancer is maintained during progression to an androgen-independent metastatic state. Overall, TMPRSS2 was rearranged in 48% of the cases (Fig. 2). Gene fusions between 5’ TMPRSS2 and 3’ ERG were the most frequent form (37%) of TMPRSS2-ETS rearrangement in androgen-independent prostate cancer, similar to the 40% to 55% previously reported in clinically localized prostate cancers (6, 9, 11, 17). The low frequency of TMPRSS2-ETV1 (3%) and TMPRSS2-ETV4 (3%) is also comparable with previous reports (11, 16). As TMPRSS2 and ERG are in close proximity (3 Mb) on chromosome 21, interstitial deletion between these two genes is a common mechanism of TMPRSS2-ERG fusion in prostate cancer (6, 8, 18). In our previous study of clinically localized prostate cancers and TMPRSS2-ERG rearrangements in American men treated with radical prostatectomy, 39% were fused through deletion of 5’ end of ERG (11). Likewise, Perner and colleagues (6) showed intronic deletions between ERG and TMPRSS2 resulting in TMPRSS2-ERG fusion in 60% of clinically localized cancers and 41% of hormone-naive lymph node metastasis. Despite comparable frequencies of TMPRSS2-ERG rearrangement in end-stage, androgen-independent prostate cancer and clinically localized and hormone-naive prostate cancers, the mechanism of rearrangement between them is strikingly different. In comparison with clinically

**Figure 1.** FISH probe design and representative TMPRSS2-ETS chromosomal aberrations detected in androgen-independent metastatic prostate cancer. A, for all assays, the chromosomal location of the gene is indicated (boxes), with the direction of transcription indicated by the arrow. The 5' and 3' bacterial artificial chromosomes (BAC) are indicated in ovals, with the number identifying each respective BAC and the color indicating the probe color in the accompanying images. B, representative FISH images of the various TMPRSS2-ETS aberrations observed in this study. Green and red arrows, individual signals; yellow arrows, colocализed signals in 4',6-diamidino-2-phenylindole–stained nuclei. B1, colocázized signals in metastatic prostate cancer cells in a case lacking TMPRSS2 rearrangement. B2, TMPRSS2 rearrangement positive case with Edel, as indicated by split 5' and 3' signals. B3, TMPRSS2 rearrangement positive case with Edel, as indicated by one pair of colocализed signals (yellow arrow) and the loss of one red-labeled probe 3' to TMPRSS2. B4, colocализed signals (yellow arrows) in a representative case lacking ERG rearrangement. B5, an ERG rearrangement positive case with Edel showing loss of one red-labeled probe 5' to ERG. B6, ETV1 rearrangement positive case without deletion, as indicated by split 5' and 3' signals (green and red arrows). B7, ETV1 rearrangement positive case with deletion exhibiting loss of one red-labeled probe 5' to ETV1. B8, ETV1 amplification shown by multiple (seven to nine) copies of colocализed signals in case 30. B9, a case showing rearrangement of ETV4, as indicated by break-apart probes. Numbered BACs are as follows: 1, RP11-35SC4 (5' to TMPRSS2); 2, RP11-120C17 (5' to TMPRSS2); 3, RP11-9521 (5' to ERG); 4, RP11-478D17 (3' to ERG); 5, RP11-46L15 (5' to ETV1); 6, RP11-124L22 (5' to ETV1); 7, RP11-100E5 (5' to ETV4); and 8, RP11-436J4 (3' to ETV4).
Figure 2. Diversity of TMPRSS2 and ETS rearrangements in androgen-independent metastatic prostate cancer. A, heat map representation of the TMPRSS2 and ETS aberrations in metastatic prostate cancers characterized in each subject. **, prostate cancer tissues could not be procured at the time of autopsy because of previous radical prostatectomy and hence were not available for evaluation. No cancer focus was hybridizable for FISH probes in cases 9, 27, and 29. ****, case 13, which harbored a TMPRSS2-ERG rearrangement at metastatic foci within liver, lung, dura, and soft tissue liver, also showed a deletion of 5' end of ETV1 in metastatic tumor within liver (presumably a nonspecific secondary aberration). Color legend signifies respective aberrations. B, heat map representation of TMPRSS2 and ETS rearrangement status of the metastatic sites and residual tumor in the prostate (when present) as evaluated in this cohort. C, table of results for rearrangements in TMPRSS2, ERG, ETV1, and ETV4 as detected by the assays shown in Fig. 1. In this cohort, 27 of 30 cases were evaluable for at least one assay in various metastatic prostate cancer (PCA) sites in this cohort, and the number of evaluable cases for each assay is indicated. The percentage (of evaluable cases for that assay) and number of cases with rearrangements for each assay are listed. For TMPRSS2, ERG, ETV1, and ETV4, the percentage (of rearrangement positive cases) and number of cases with assays consistent with intrachromosomal deletion between TMPRSS2 and ERG are given. The bottom panel contains a comparison of similar assays in a clinically localized prostate cancer cohort we previously evaluated (11). The number and percentage of cases with rearrangements for each assay are given, as well as the number and percentage of TMPRSS2 and ERG rearrangement positive cases with interstitial deletion.
Figure 3. The clonal nature of TMPRSS2-ETS aberrations in the spread of metastatic prostate cancer. A, representative case 24 of metastatic prostate cancer highlighting the clonal nature of cells harboring TMPRSS2-ETS gene fusions. In this case, the subject harbored TMPRSS2-ERG with Edel in the prostate and metastatic sites in the liver, lung, dura, and lymph node [representative FISH images from each site are shown; using the multicolor FISH probe strategy, a nucleus with an ERG deletion shows replacement of one juxtaposed red and green signal pair with a single green signal representing loss of the 5' end (red probe)]. B, this schematic depicts that although multifocal prostate cancer might harbor TMPRSS2-ETS gene fusion–positive nodes and gene fusion–negative nodes, only one “clone” or molecular subtype will progress and result in androgen-independent metastatic disease.
localized cancer and hormone-naive metastases, ERG rearrangement in androgen-independent metastatic prostate cancers occurred exclusively through intrachromosomal deletion (100%). This suggests that prostate cancers with ERG fusion due to 5′ deletion harbor an aggressive molecular subtype that is susceptible to higher recurrence, evolves into an androgen-independent state, and eventually progresses to metastasis. Other studies have documented the association of ERG fusions with a poor outcome (6, 18–20). Perner and colleagues (6) reported a significant association between clinically localized and hormone-naively locally advanced prostate cancers with TMPRSS2-ERG rearrangement through deletions and higher tumor stage and the presence of positive pelvic lymph nodes. Similarly, Attard and colleagues (18) showed that patients with 5′ ERG deletion had a significantly worse cause-specific and overall survival. In this study, the presence of an identical ERG 5′ deletion in primary cancer within the prostate (when available) and all the metastatic sites from same individual strongly indicates that this molecular aberration occurred at the stage of localized disease before progression to a metastatic state (case 24, Fig. 3A). Future studies will investigate the mechanism behind a preferential selection of ERG-deleted (Edel) clinically localized prostate cancers to progress into an androgen-independent phenotype.

This study shows and characterizes a common gene rearrangement underlying localized prostate cancer and multiple androgen-independent metastatic foci in an individual patient (Figs. 2B and 3A). Our results showed that a unique clone of malignant cells in the prostate disseminated to seed different metastatic sites. Further, an aberrant clonal phenotype, acquired by clinically localized cancer, maintained its unique identity as the cancer metastasized, as shown by the consistent presence of TMPRSS2-ETS throughout metastatic foci. Thus, it is apparent that malignant cells from a single focus undergo clonal expansion and systemic dissemination to give rise to metastatic deposits in advanced prostate cancer. Such aberrations are presumably a primary initiating event, not secondarily accrued, because they are uniform across metastatic cancer foci. These findings support previous studies that suggested that untreated metastatic tumors contain the bulk of chromosomal alterations necessary for recurrence and eventual spread during antihormonal treatment (21).

Prostate cancer is usually a multifocal disease with considerable histologic, biologic, and molecular variations between different tumor foci (22). In our recent evaluation of multifocal prostate cancers, the majority of cases (70%) had heterogeneous TMPRSS2 gene rearrangements between different tumor foci suggesting independent clonal expansion (12). The histologic, immunophenotypic, and molecular heterogeneity is persistent when prostate cancer progresses to an androgen-independent phenotype (3). Despite the known heterogeneity of primary and advanced prostate cancers, we remarkably show identical TMPRSS2-ETS aberrations in all the metastatic samples and primary prostate cancer (when available) within a single individual patient. This strongly suggests that, although primary nodes of multifocal prostate cancers arise through separate clonal expansion, metastatic cancer arises through clonal expansion of malignant cells from a unique primary focus capable of dissemination. Our findings indicate that if a gene fusion–positive cancer focus spreads from the prostate, the same clone is preferentially seeded across various organs to produce identical gene fusion characteristics. Similarly, all the metastatic foci are uniformly negative for these aberrations in TMPRSS2-ETS–negative primary prostate cancers (Fig. 3A and B).

Further, to investigate the relationship between androgen receptor (AR) and TMPRSS2-ETS aberrations, we examined AR immunohistochemical staining status for multiple sites of these 30 rapid autopsy cases (as shown in Supplementary Table S1). We did not observe any significant correlation between AR median staining and TMPRSS2-ETS or TMPRSS2-ERG rearrangements. Similar to AR immunohistochemical data, TMPRSS2-ETS and TMPRSS2-ERG aberrations also did not correlate with AR mRNA expression (Supplementary Table S2). Hierarchical clustering of direct AR target genes yields similar patterns for cases with and without TMPRSS2-ETS rearrangements (Supplementary Fig. S1A). This suggests that alternate mechanisms may be in play to maintain TMPRSS2-ETS expression in hormone-refractory prostate cancer. In a recent study, Hermans and colleagues (23) identified cases of androgen-independent metastatic prostate cancer that harbored TMPRSS2-ERG fusions at the genomic level [as identified by array comparative genomic hybridization (aCGH)] but did not express TMPRSS2-ERG fusion transcripts, leading them to suggest that ETS gene fusions are highly relevant in androgen-dependent metastatic cancer but are bypassed in androgen-independent disease. Our results suggest that bypass of gene fusions may occur in some cases; however, many androgen-independent metastatic prostate cancers with TMPRSS2-ETS fusion as evidenced by aCGH or FISH strongly express fusion transcripts. For example, in our original report (4), both the index TMPRSS2-ERG (MET28; case 28 in the present cohort) and TMPRSS2-ETV1 (MET26; case 26 in the present cohort) cases were androgen-independent cancers that strongly overexpressed ERG or ETV1, respectively.

In summary, TMPRSS2-ETS rearrangements in end-stage, androgen-independent prostate cancers provide strong evidence that ERG fusion through deletion represents an aggressive molecular subtype of prostate cancer with a high susceptibility to evolve into androgen-independent metastatic state. This study provides compelling evidence that metastatic prostate cancer arises through clonal expansion of a single focus of primary prostate cancer. Insight into the biology of these rearrangements will allow novel therapeutic regimens to be tailored to a susceptible molecular subtype of poor outcome cases.

Disclosure of Potential Conflicts of Interest

The University of Michigan has filed a patent on ETS gene rearrangements in prostate cancer, on which R. Mehra, S.A. Tomlins, M.A. Rubin, and A.M. Chinnaiyan are coinventors, and the diagnostic field of use has been licensed to Gen-Probe Inc. Gen-Probe has not played a role in the design and conduct of the study, in the collection, analysis, or interpretation of the data, or in the preparation, review, or approval of the article. A.M. Chinnaiyan serves as a consultant to Gen-Probe Inc. The other authors disclosed no potential conflicts of interest.

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References

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