Original Article
Triple Aberrant Prostate Cancer (TAPC) - Aggregate role of aberrations in TP53, PTEN and RB1 on ETS gene fusions and prognosis in metastatic castrate resistant prostate cancer

Allison P Watson1,2, Ashraf Shabaneh2,3, Jinhua Wang2,3, Scott M Dehm2,4,5, Arpit Rao1,2, Charles J Ryan1,2

1Division of Hematology, Oncology & Transplantation, Department of Medicine, University of Minnesota, Minneapolis, USA; 2Masonic Cancer Center, Minneapolis MN, USA; 3Institute for Health Informatics, University of Minnesota, Minneapolis, USA; 4Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, USA; 5Department of Urology, University of Minnesota, Minneapolis, USA

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Abstract: Background: Aberrations in TP53, PTEN and RB1 are key drivers of therapy resistance in prostate cancer. Up to 50% of prostate cancers harbor ETS gene rearrangements, a potentially compounding aggressive biological event. Little is known about the impact of aggregate aberrations and gene fusion events in prostate cancer. Methods: Using cBioportal for Cancer Genomics, an open-access resource for exploration of multidimensional cancer genomics data, we integrate whole-exome sequencing, gene expression, and histopathology with longitudinal clinical outcomes. Subsets of prostate tumors with aberrations in all three genes TP53, PTEN and RB1 were identified and correlated with prevalence of gene fusions. Prostate tumors with aberrations in TP53, PTEN, and RB1 were termed “triple aberrant prostate cancer” (TAPC). Results: Of 479 metastatic prostate tumors, 195 (40.7%) were TAPC, versus 21 of 594 (3.5%) of primary prostate tumors. Patients with metastatic TAPC showed a trend toward poorer overall survival than patients harboring 0, 1 or 2 of these aberrations. Twenty-five distinct fusions were identified, all involving ETS transcription factors. Both primary and metastatic prostate cancers with ETS fusions were significantly more likely to be TAPC than those without ETS fusions. Conclusions: This study identified a unique molecular signature consisting of combined aberrations in TP53, PTEN and RB1 that is associated with poorer overall survival, as well as increasing prevalence of ETS gene fusions and differential gene expression patterns favoring aggressive disease and tumor progression. Identification of this subset of patients could inform prognostic decisions and provide a rationale for more aggressive or unique therapeutic approaches.

Keywords: Metastatic castration resistant prostate cancer, TP53, PTEN, RB1, ETS fusion

Introduction

The genomic and clinical landscape of prostate cancer is heterogeneous, with some patients dying of metastatic disease within a couple years of diagnosis, and others living for more than 10 years with organ-confined disease. Several large-scale comprehensive profiling efforts have provided critical new insights into the molecular classification of prostate cancer with the identification of distinct genomic subtypes in primary and metastatic castration-resistant prostate cancer (mCRPC). Subsets of patients with advanced or aggressive disease share clinically relevant and potentially actionable alterations in the tumor or in the germline. Many ongoing prospective clinical trials now seek to enroll patients with defined genomic alterations to more clearly delineate their role in the clinical course of disease. Understanding therapeutically relevant biologic heterogeneity will aid in the development of molecularly targeted agents and a more personalized approach to prostate cancer treatments along the spectrum of disease.

Various genomic and histologic features of prostate cancer have been correlated with adverse features conferring a worse prognosis. Alterations in known, commonly mutated tumor suppressor genes such as TP53, PTEN and RB1 are often implicated in more aggressive
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**Table 1.** Databases utilized for analyses. RNA sequencing expression and DNA mutation data were compiled from several databases including primary and metastatic prostate cancer samples

<table>
<thead>
<tr>
<th>Study</th>
<th>Cancer Stage</th>
<th># of Patients</th>
<th>Total</th>
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<tbody>
<tr>
<td>TCGA (Cell 2015)</td>
<td>Primary</td>
<td>333</td>
<td>594 Primary</td>
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<tr>
<td>MSKCC (PNAS 2014)</td>
<td>Primary</td>
<td>104</td>
<td></td>
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<tr>
<td>MSKCC (Cancer Cell 2010)</td>
<td>Primary</td>
<td>157</td>
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<tr>
<td>SU2C-PCF Dream Team (PNAS 2019)</td>
<td>Metastatic</td>
<td>442</td>
<td>479 Metastatic</td>
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<tr>
<td>MSKCC (Cancer Cell 2010)</td>
<td>Metastatic</td>
<td>37</td>
<td></td>
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<tr>
<td></td>
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<td>1073 total</td>
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Abbreviations: TCGA = The Cancer Genome Atlas program; MSKCC = Memorial Sloan Kettering Cancer Center; SU2C-PCF = Stand Up 2 Cancer - Prostate Cancer Foundation; PNAS = Proceedings of the National Academy of Sciences.

disease and resistance to androgen depletion from early stages of development [1-3]. TP53 mutations may be associated with poor response to androgen receptor (AR) targeted therapy in CRPC [4]. PTEN deletion is associated with increased risks of biochemical recurrence and metastatic progression [5]. RB1 loss is associated with AR overexpression and increased risk of progression to lethal tumor phenotypes [6].

Aside from their individual roles as tumor suppressors, the combination of aberrations in all three TP53, PTEN, and RB1 has been recognized as a molecular signature identifying an aggressive variant of prostate cancer [2, 3]. Prospective comprehensive genomic profiling of metastatic castration-resistant tumors reveals significant enrichment for combined alterations in resistant disease compared with locoregional disease [1, 7]. This suggests a critical role for the combined loss of tumor suppressor function as a driver of aggressive disease biology [8, 9]. Despite the recognition of combined alterations as an overall poor prognostic marker for prostate cancer, little is known about the prevalence of complex and combined aberrations in localized versus metastatic disease or their association with gene fusions.

It is also well established that 50-70% of prostate cancers harbor gene fusions created by a chromosomal rearrangement involving the ETS family of transcription factors and the androgen-regulated gene TMPRSS2 [10, 11]. The TMPRSS2:ERG gene fusion is the most frequent genomic alteration in prostate cancer, which is present in both early and late-stage or castration resistant prostate cancer [11]. Prostate cancers with ERG rearrangements are generally more aggressive and share a distinct histological phenotype [12, 13]. Numerous studies have attempted to elucidate the significance and prognostic value of TMPRSS2:ERG fusion in prostate cancer, but there remains much to be learned. In particular, the co-expression genes and interaction networks have not been fully characterized.

Using open-access resources for interactive exploration of multidimensional cancer genomics data sets [14], including repositories of primary and metastatic prostate cancer studies, we integrate the analysis of whole-exome sequencing, gene expression, and histopathology with longitudinal clinical outcomes including overall survival. We identify a molecular signature consisting of combined aberrations in TP53, PTEN and RB1 that is associated with aggressive disease features and adverse prognosis, as well as an increasing prevalence of ETS gene fusion events and differential gene expression patterns.

**Methods**

**Study population and clinical data collection**

We utilized cBioportal for Cancer Genomics (http://cbioportal.org) to integrate findings from whole-exome sequencing, RNA-seq transcriptome profiling, and histologic analyses of publicly available databases totaling 1073 samples composed of both primary and metastatic prostate cancer linked with longitudinal clinical outcomes and overall survival. The samples in this dataset were combined clinical trial data including 594 patients with primary prostate cancer [15-17] and 479 patients with metastatic prostate cancer [17, 18] (Table 1). We analyzed the genomic aberrations in three genes of particular interest: TP53, PTEN and RB1 using the collected datasets. The genomic aberra-
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tions in this analysis included missense and truncating mutations, copy deletions and copy gains. The prevalence of gene fusion events was analyzed using the larger database of mCRPC samples [18]. A diagram outlining the general study schema is shown in Supplementary Figure 1.

Bioinformatics analyses

The primary clinical variable for this analysis was overall survival in mCRPC, which was obtained from the Stand Up 2 Cancer - Prostate Cancer Foundation (SU2C-PCF) Dream Team study [18]. Overall survival time and copy number variation (CNV) status of the same sample ID were merged, and a Kaplan-Meier survival curve was plotted using R package (ver 3.4.3) “survival” (3.1-8) and “survminer” packages (version 0.4.6). The patient sub-group classes were defined by all four possible aberration categories, including no genetic aberrations, and 1, 2, and 3 gene aberrations.

Differential transcriptomic gene expression and gene fusion analyses were performed on the SU2C-PCF Dream Team [18] metastatic prostate cancer dataset. The same analysis was applied on the primary prostate cancer dataset obtained through The Cancer Genomic Atlas program [15]. Given the high prevalence of ERG fusions compared to others, we calculated the correlation of ERG fusion events with the aggregate gene aberrations in TP53, PTEN and RB1. Circo plots were generated to visualize the presence of gene fusions of each patient subgroup using FusionHub Circo Views tool.

Statistical analyses

Differential gene expression analysis was performed using the Limma® package on the dataset from the SU2C-PCF Dream Team [18] study of metastatic prostate cancer. Differentially expressed gene markers for each class were selected based on adjusted p-values (p<0.01). Marker gene expression values were z-score transformed and visualized in a heatmap using the ComplexHeatmap® package. Highly differentially expressed gene sets were analyzed using the ENRICHR® database and tools to determine affected cellular pathways associated with triple aberrant status (defined as aberrations in TP53, PTEN and RB1). The significance of gene aberration prevalence between ETS fusion positive patients and ETS fusion negative patients was determined by chi-squared test.

Study validation

As a means of validating the association between triple aberrant status with ETS fusion events and survival, we utilized a small subset of tumors from a study including 50 lethal, heavily pre-treated mCRPCs obtained at autopsy [19]. The same study schema was applied to this study dataset, including clinical variables such as overall survival and determination of the prevalence of ETS fusion events.

Results

Combined aberrations in TP53, PTEN and RB1 were more commonly observed in mCRPC than primary prostate cancer

Integrating data from several studies, including 594 patients with primary prostate cancer and 479 patients with metastatic prostate cancer, we evaluated the prevalence of aberrations in TP53, PTEN and RB1. We denoted the aggregate presence of aberrations in all three genes as “triple aberrant prostate cancer” (TAPC), and correlated this with disease state. Of the 479 metastatic prostate tumors analyzed, 194 (40.5%) were TAPC, versus 21 of 594 (3.5%) of primary prostate tumors. Of the three queried genetic aberrations, PTEN was most highly dysregulated in TAPC samples, prevalent in 20.5% of primary tumors and 63.9% of metastatic tumors (P = 1.81E-19). TP53 and RB1 were also highly attenuated in TAPC (P = 3.17E-21 and P = 7.41E-24, respectively).

Triple aberrant status is associated with poor overall survival in metastatic prostate cancer

Kaplan-Meier curves were created using overall survival data from 137 patients with metastatic disease. Overall survival data was integrated with the presence of aberrations in TP53, PTEN and RB1. The presence and combination of aberrations in these genes was associated with poorer overall survival in metastatic prostate cancer. In the metastatic cohort, patients with TAPC experience a trend towards shorter overall survival than patients harboring 0, 1, or 2 of these alterations (P = 0.12, Figure 1A). Consistent with prior observations [20], any TP53
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Figure 1. A. Patients with metastatic TAPC demonstrate a trend toward poorer overall survival (OS) versus those with non-TAPC. Kaplan-Meier curves were created using OS data from 137 patients with metastatic castration resistant prostate cancer. OS data was integrated with the presence of combined aberrations in TP53, PTEN and RB1 and displayed as TAPC (all 3 aberrations, n = 47) versus Non-TAPC (0, 1, or 2 aberrations, n = 83). B. Patients with mCRPC and TP53 aberration (TP53 mut, n = 84) demonstrate poorer OS versus TP53 wild-type tumors (TP53 wt, n = 47).

alteration was associated with a shorter survival compared with those without such an aberration (P = 0.0036, Figure 1B).

Presence of gene fusions is associated with increased prevalence of triple aberrant status in both primary and metastatic prostate cancer

Data regarding gene fusions was available for 330 of the 444 patients with metastatic prostate cancer from the SU2C-PCF Dream Team study [18]. We identified subsets of patients with aberrations in TP53, PTEN and RB1, and the prevalence of gene fusions was associated with the presence of a single aberration, or a combination of aberrations in two or more of these genes. We found that in all metastatic prostate cancer, gene fusions were detected in 173 of the 330 (52.4%). A significant association was detected in the prevalence of TAPC between ETS fusion positive versus ETS fusion negative tumors (Figure 2A). Over 56% of ETS fusion positive tumors were classified as TAPC, compared to 34% of ETS fusion negative tumors (P = 9.66E-06). Of the queried genetic aberrations in this study, the presence of TP53 mutation was the most strongly associated with gene fusions in these patients overall.

The correlation of increased prevalence of TAPC status in tumors with ETS fusion events was also found in primary prostate tumors (Figure 2B). To validate these findings in an outside study database, we utilized a small subset of mCRPC tumors from the Michigan Study [19]. This confirmed that TAPC status was more common in prostate tumors with ETS fusion events versus those without (Supplementary Figure 3).

Twenty-five distinct gene fusion events were identified in this analysis, all involving ETS transcription factors (Supplementary Table 1). The most common ETS rearrangement involved ERG, which was present in 134 of the 174 gene fusions (77.0%). The remainder of the gene fusion events identified in this study consisted of ETV1 (12.6%), ETV4 (6.9%), and ETV5 (1.7%), ETV11 (1.1%) and FL1 (0.6%). Of the 330 metastatic prostate cancer samples with fusion data, the TMPRSS2:ERG fusion was detected in 8 patients without gene aberrations in the
queried genes, and in 12, 26 and 82 patients with 1, 2 and 3 gene aberrations, respectively. Overall survival did not differ significantly in triple aberrant cases with gene fusions versus triple aberrant cases without gene fusions.

**Gene expression patterns associated with triple aberrant status favor a more aggressive clinical phenotype**

Circo fusion plots were generated to illustrate gene fusion events in metastatic prostate cancer samples and correlated with the presence of genetic aberrations in TP53, PTEN and RB1 (Figure 3). The circo plots demonstrated that prostate cancers harboring ETS fusion events also had multiple aberrations within TAPC markers, with two aberrations possibly being driven by the most possible types of ETS fusions. TAPC fusion drivers also had a very rich profile of possible fusions, while single and no aberration profiles had very limited driver possibilities.

Differential gene expression analysis revealed that many genetic markers associated with cancer cell apoptosis were highly down regulated in TAPC. For example, Plekstrin Homology Like Domain Family A Member 3 (PHLD3A), a known tumor suppressor regulated by p53 [21], was significantly down-regulated in TAPC (P = 2E-15). TAPC samples also demonstrated significantly decreased expression of the Transforming Growth Factor Beta (TGFB) marker genes compared to non-TAPC samples. Consistent with prior studies suggesting that attenuation of TGFB receptor 2 (TGFB2) expression plays a role in prostate cancer progression [22], we also found that attenuation of TGFBR2 (P = 1.01E-8) was highly apparent in TAPC samples, as was TGFB1 suppression (P = 2.47E-07) (Figure 4).

Functional analysis of these marker genes using ENRICHR© databases and tools [23, 24] demonstrated associations with carcinogenic associated molecular pathways. Gene markers associated with cancer progression showed significant enrichment within the KEGG (ver 2019) human pathway dataset: BioCarta (ver 2016) and GO Biological Process (ver 2013) gene sets suggested differentiation in apoptosis associated pathways (Supplementary Figure 2).

**Discussion**

This study evaluated the molecular signature of prostate cancer along the spectrum of disease, with particular attention to the most commonly altered tumor suppressor genes. We propose, based on prior data, that aberrations in TP53, PTEN and RB1, individually and in aggregate, serve as markers of more aggressive disease. In this study, we found that over 40% of patients with metastatic prostate cancer were classifiable as TAPC, versus only 3.5% of primary prostate cancer. Additionally, while our results did not reach statistical significance, patients with metastatic TAPC demonstrated a trend toward poorer OS. This study also revealed the novel finding of increased prevalence of TAPC in advanced prostate cancer with ETS fusions versus those without ETS fusions. The trend was apparent in primary prostate cancer as well,
but the prevalence of TAPC is much less in early disease states because most early prostate cancers demonstrate fewer aberrations in the queried genes. These data are consistent with an evolutionary model of selective pressure for molecularly and phenotypically aggressive or resistant prostate cancer cells, leading to increased prevalence of genetic aberrations with advanced disease.

Fusions of TMPRSS2, an androgen-regulated promoter, with members of the ETS family of transcription factors are common and initiating events in prostate cancer carcinogenesis. The most common fusion is TMPRSS2:ERG, present in 40-50% of prostate tumors [25]. Our study identified 25 distinct gene fusion events in 52% of the metastatic castration resistant prostate tumors. TMPRSS2 was the most frequent 5' partner identified in this study, and most commonly involved ERG, but also ETV1, ETV4, ETV11, ETV5, and FL1 at much lower frequencies - consistent with prior studies [26]. When stratified by aberrations in TP53, PTEN

Figure 3. Circo fusion plots were generated to illustrate gene fusion events in metastatic prostate cancer samples and correlated with the presence of genetic aberrations in TP53, PTEN and RB1.
and RB1, the prevalence of ETS fusions was higher. This finding was also validated in an outside study (Supplemental Figure 3). These data indicate that ETS fusion positive prostate cancers have higher likelihood to become TAPC than ETS fusion negative prostate cancers. As ETS fusions are known to be the first genomic hit in prostate cancer development and progression [13, 27], it is likely that the increased prevalence of gene fusions in TAPC is likely the inciting event rather than aggregate genetic aberrations preceding generation of ETS fusions.

With the increasing prevalence of TAPC alterations in advanced disease states that harbor ETS fusions, we hypothesized that there would be unique gene expression patterns in prostate tumors without ETS fusions or TAPC aberrations versus those with one or more aberrations in these genes. Gene expression patterns in these tumors suggest trends in gene pathways that are known to promote an aggressive tumor phenotype and tumor progression, which are much more heavily associated with TAPC samples. Similarly, we observed down regulation of apoptotic genes more commonly in TAPC samples. This was demonstrated by both individual gene markers and gene sets enrichment analysis.

A similar prior study identified the frequency and distribution of monoallelic/biallelic TP53, RB1 and PTEN alterations by OncoPanel sequencing of tumor tissue from prostate cancer across the disease spectrum, noting combined aberrations in all three genes in 12% (5 of 43) of metastatic castration sensitive prostate cancer samples and 50% (24 of 48) of metastatic castration resistant prostate cancer samples [3]. This is consistent with an evolutionary model of selective pressure for molecularly and phenotypically aggressive or resistant prostate cancer cells, leading to increased prevalence of genetic aberrations with advanced disease. Notably, complex aberrancies are more common in tumors with ETS fusions, highlighting the high degree of genetic instability inherent in such tumors. The slightly different percentage of TAPC identified in our study may be attributed to larger sample sizes, differences in detection profiling platforms or differing definitions of genetic aberrations used in each study. We utilized a high variation of genomic aberration types, and as such, classified patients with very different molecular profiles into one group termed TAPC.

There are several inherent limitations to this study. One limitation to using genomic databases includes the presence of confounding factors such as tumor heterogeneity and subsequent sampling bias in tissue collection. Additionally, the purity of the DNA and RNA collected in these studies cannot be definitively assessed. Several similar studies have been
done recently using the same publicly available databases, and results must be interpreted with caution as clinical data is often limited, especially in terms of prior and ongoing treatment regimens. We attempted to validate these findings utilizing outside studies including similar patient characteristics.

This study identified a unique molecular signature consisting of combined aberrations in TP53, PTEN and RB1 that is associated with poorer overall survival, as well as an increasing prevalence of ETS gene fusion events and differential gene expression patterns that favor aggressive disease features and tumor progression. By combining the molecular signature created by aggregate aberrations in tumor suppressor genes with androgen-regulated gene fusions, it is possible that this particular cohort of patients may represent a subset with worse overall prognosis, potentially providing a stronger rationale for more aggressive treatment. This may also provide a foundation for a biologically defined classification of prostate cancer that would allow clinicians an opportunity to select patients for unique therapeutic approaches.

Acknowledgements

All authors conceived of the presented idea. AW and AS contributed equally to this manuscript. AW wrote the manuscript with input from all other authors. AS designed the computational framework and analyzed the data. JW and SD contributed to the interpretation of the results. AR provided critical feedback and helped shape the research and analysis. CR supervised the entire project. All authors discussed the results and contributed to the final manuscript.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Charles J Ryan, Division of Hematology, Oncology & Transplantation, University of Minnesota, 420 Delaware Street SE, MMC 480, Minneapolis, MN 55455, USA. E-mail: ryancc@umn.edu

References


Supplementary Figure 1. Schematic showing generalized study design. We collected biological and clinical data from multiple studies based on prostate cancer stage at diagnosis (primary versus metastatic) and ample sample size for statistical analysis. TAPC status was determined using CNA of the three queried genes (\textit{TP53}, \textit{PTEN} and \textit{RB1}). We also analyzed biological and clinical outcomes based on the available data.
Supplementary Figure 2. Integration of TAPC-associated marker genes with the ENRICHR® database demonstrated notable trends in apoptotic and cancer-associated pathways.
Supplementary Table 1. Gene fusions detected in metastatic prostate cancer samples

<table>
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<tr>
<th>Gene Fusions</th>
<th># of gene aberrations</th>
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<tr>
<td></td>
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<tr>
<td>TMPRSS2-ERG</td>
<td>8</td>
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<tr>
<td>SLC45A3-ETV1</td>
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<tr>
<td>TMPRSS2-ETV4</td>
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<tr>
<td>ACPP-ETV1</td>
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Twenty-five distinct gene fusion products were identified in this analysis, all involving ETS transcription factors.

Supplementary Figure 3. Metastatic prostate cancer tumors with ETS fusions are more likely to be TAPC, a validation from an outside study (Michigan 2012).