Lineage Relationship of Gleason Patterns in Gleason Score 7 Prostate Cancer

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Abstract

Gleason score 7 (GS7) prostate cancer [tumors with both Gleason patterns 3 (GP3) and 4 (GP4)] portends a significantly more aggressive tumor than Gleason score 6 (GS6). It is, therefore, critical to understand the molecular relationship of adjacent GP3 and GP4 tumor cell populations and relate molecular abnormalities to disease progression. To decipher molecular relatedness, we used laser capture microdissection (LCM) and whole-genome amplification (WGA) to separately collect and amplify DNA from adjacent GP3 and GP4 cell populations from 14 cases of GS7 prostate cancer. We then carried out massively parallel mate-pair next generation sequencing (NGS) to examine the landscape of large chromosomal alterations. We identified four to 115 DNA breakpoints in GP3 and 17 to 480 in GP4. Our findings indicate that while GP3 and GP4 from the same tumor each possess unique breakpoints, they also share identical ones, indicating a common origin. Approximately 300 chromosomal breakpoints were localized to the regions affected in at least two tumors, whereas more than 3,000 were unique within the set of 14 tumors. TMPRSS2–ERG was the most recurrent rearrangement present in eight cases, in both GP3 and GP4. PTEN rearrangements were found in five of eight TMPRSS2–ERG fusion-positive cases in both GP3 and GP4. Hierarchical clustering analysis revealed that GP3 has greater breakpoint similarity to its partner GP4 compared with GP3 from different patients. We show evidence that LCM, WGA, and NGS of adjacent tumor regions provide an important tool in deciphering lineage relationships and discovering chromosomal alterations associated with tumor progression.

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Introduction

The elucidation of causal DNA rearrangements is critical to the understanding of cancer development and progression, and clinically relevant in regards to prognosis and treatment. Although, the precursor lesions for prostate cancer are not completely defined (1, 2), significant progress has been made in identifying the molecular events underlying initiation and progression using genomic and functional studies. The most frequent chromosomal rearrangement in prostate cancer, the TMPRSS2–ERG gene fusion, is considered to be an early event in prostate tumorigenesis. The significance of TMPRSS2–ERG fusion protein as a prognostic or predictive marker has been controversial (3–5), but recent evidence suggests that ERG status defines unique subtypes of prostate cancer (6). Other molecular changes, such as deregulation of PTEN have also been implicated in prostate cancer (7–12). Despite the discovery of some recurrent DNA rearrangements, association of these changes with clinical and pathologic features, particularly with tumor grade, has been limited.

Morphologic heterogeneity in prostate cancer, assessed with Gleason pattern (GP) and the summation score (GS; refs. 13, 14), is strongly related to prognosis. Gleason score 6 (GS6) prostate cancer is either cured by treatment or needs no treatment at all (15, 16). In up to 30% of cases, the finding of GS6 on needle biopsy specimen is associated with insignificant cancer. In contrast, men with Gleason score 7 (GS7) and higher have significant prostate cancer, and are at increased risk of cancer progression. Despite the close association of GP to tumor behavior, the relationship of morphologic heterogeneity and molecular heterogeneity has not been elucidated. While there is a possibility that GP3 and GP4 regions in GS7 prostate cancer are of different origin, it is generally accepted that GP4 adjacent to GP3 are related and that GP3 progresses to GP4, which shows more aggressive behavior. However, the transition supposition has only been inferred by determining the ERG status using FISH or immunostaining. In addition, there is a poor understanding of genomic differences between GP3 that progresses and GP3 that does not.

Several studies have addressed the issue of tumor heterogeneity in prostate cancer by comparing changes in multiple separate tumor nodules within a prostate gland (17–21). Assessment of the occurrence of the TMPRSS2–ERG fusion by
FISH in multifocal prostate cancer show that 40% to 50% of foci are discordant in regards to the presence or absence of the fusion, but within any one focus the absence or presence of the fusion is consistent (18–21). These studies support the notion that multifocal primary prostate cancers are composed of multiple clones. A study of metastatic prostate cancer using high-resolution genome-wide single polymorphism and copy number survey approach has shown the lineage relationship of metastases to the primary tumor (22).

Although, it is accepted that multiple separate tumors within the prostate gland arise from different clones, there is no data regarding a common origin of different GPs within a unifocal dominant tumor. Indeed, no study has conclusively shown whether cells within a single tumor harbor identical molecular alterations. For instance, the TMPRSS2–ERG fusion can occur at a number of breakpoints that indicate a different clonal origin, so that identification of the fusion by FISH or by immunoperoxidase staining in different GPs within the same tumor does not conclusively prove a common origin as these studies are not specific to any breakpoint. Moreover, molecular changes in the context of morphologic heterogeneity within a tumor, specifically related to the differences within GPs, have not been analyzed. It is unclear whether molecular events characteristic of aggressive GP4 are present in the associated GP3 in GS7 cancer. It is also unknown whether the chromosomal changes in the associated GP3 in GS7 cancer are more similar to GP3 from other patients or more similar to its associated GP4. In this study, we identified patients with a unifocal GP7 prostate cancer to determine the extent of variability in chromosomal rearrangements between different GPs within the same tumor and to determine whether these tumors exhibited a common origin. To investigate lineage relationships between adjacent GPs, we carried out laser capture microdissection (LCM), whole-genome amplification (WGA), and massively parallel mate-pair next generation sequencing (NGS), and compared molecular alterations in GP3 and GP4 within the same tumor and between tumors from different patients.

Materials and Methods

Isolation of prostate GP3 and GP4 DNA, mate-pair library construction and sequencing

Fourteen cases of fresh-frozen GS7 prostate cancer composed of GP3 adjacent to GP4 were selected for study. The tumors were the dominant tumor nodules within each gland. In each case, 10 μm unstained sections were cut from fresh-frozen blocks of tissue of patient with prostate cancer collected from a radical prostatectomy specimen. LCM (Arcturus) was used to separately isolate cells specific to the GP3 and GP4 populations minimizing contamination of adjacent cells (Fig. 1A). Histologically normal glands were additionally collected from same slides at distances minimizing the chances of contaminating with the tumor tissues. The cells were lysed directly on the cap, and whole-genome DNA sequencing was carried out as previously described (23). A mate-pair library was prepared using the Mate-pair Library Prep Kit (Illumina), following the manufacturer’s recommendations. Briefly, 10 μg of WGA DNA was fragmented using Covaris sonication to 4 and

Figure 1. An example of GS7 prostate cancer specimen used for LCM. A, hematoxylin and eosin staining. B, microdissected areas corresponding to pattern GP3 (C) and GP4 (D), light images.
5 kb, fragments and mate-pair libraries were prepared as previously described and sequenced at one or 2 libraries per lane on the Illumina GAII or HiSeq platforms, respectively (23).

**Bioinformatics analysis**

A set of algorithms developed to detect unsuspected large chromosomal aberrations was used (24, 25). The read-to-reference–genome–mapping algorithm (24) was modified to map both mate-pair reads across the whole genome as recently described (25). We used the protocol that allowed to sequence the ends of large fragments of genomic DNA (2.5–5KB), thus "effectively covering" breakpoints about 15× on the average. However, the coverage at the nucleotide level was usually less than 5×, precluding us from making specific comments regarding mutation calls (Supplementary Fig. S1). Breakpoints covered by at least 3 mate-pairs in each sample were collected for further analysis.

Agglomerative hierarchical clustering was used to quantify phylogenetic relationships between samples. A distance matrix was built to capture genetic distance between all possible pairs. A distance between 2 samples was defined as 1.1 raised to the power of the lesser number of the unique abnormalities multiplied by 0.1, minus the number of shared abnormalities multiplied by 2. Clustering used the "hcust" function in R package using the McQuitty Similarity Analysis method.

To analyze whether the breakpoints correlate with binding sites for ERG, androgen receptor (AR), or cluster within open or closed chromatin, we used published ChIP-Seq data (26) for the AR, ERG, RNA polymerase II binding sites and trimethylated histone H3K4, trimethylated histone H3K36, trimethylated histone H3K9, and trimethylated histone H3K27 chromatin marks. Data were downloaded from Gene Expression Omnibus (27) Intervals of ≤50 kb (as in ref. 12) surrounding corresponding sets of ChIP-Seq peaks were used to estimate deletion or enrichment of the breakpoints. Significance of enrichment or deletion of observed breakpoints compared with background was calculated according to the binomial distribution.

**Validation by PCR and Sanger sequencing**

PCR was conducted on WGA DNA using combinations of breakpoint-specific primers (sequences are listed in Supplemental Table S1) using the HotStar Taq DNA Polymerase Kit (Qiagen), the Long template PCR Kit (Roche), or EasyA High Fidelity polymerase (Stratagene) on a GeneAmp PCR System 9700 (Applied Biosystems). Amplification products were visualized on a 0.8% to 1% agarose gel, single bands were excised, DNA was extracted, and Sanger sequenced using the appropriate primers on a 3730 DNA Analyzer (Applied Biosystems). A mixed pool Genomic DNA sample was used as a normal DNA control (Promega).

**Immunostaining**

Sections of 5 μm tissue sections were incubated in EDTA-containing antigen retrieval buffer for 30 minutes, followed by application of primary antibody (ERG clone 9FY, Biocare) at 1:25 dilution for 32 minutes at 37 C. Immunohistochemical staining was completed on the Ventana Benchmark XT automated platform using standard reagents.

**FISH**

FISH studies were conducted on formalin-fixed, paraffin-embedded tissue using a probe designed to assess PTEN status. Bacterial artificial chromosomes RP11-165M8, RP11-380G5, RP11-793O2, and RP11-348E17 selected using publicly available database (28) were obtained from Invitrogen. DNA was isolated following a standard protocol using a Qiagen Plasmid Purification Kit (Qiagen). The extracted DNA was fluoroentially labeled in Spectrum Orange-dUTP via nick translation (Abbott Molecular) and was tested on normal male blood metaphases. PCR was conducted to confirm the clones covered the regions of interest. Centromere 10 status was assessed using a commercially available probe (Abbott Molecular). Tissue sections 5-μm thick were deparaffinized in a 90°C oven, immersed twice in xylene and twice in 100% ethanol, dried, the region to be probed marked, and then microwaved in 10 mmol/L citric acid for 10 minutes. The slides were subsequently pretreated with 2x saline-sodium citrate buffer for 5 minutes, digested for 40 minutes with DigestALL (Invitrogen), and washed. Following dehydration and drying, the slides were denatured at 80°C for 5 minutes and hybridized at 37°C overnight in a humidified incubator. DAPI1 (10%; Abbott Molecular) was used for counterstaining.

**Results**

To identify genetic similarities and differences that occur in GP3 associated with GP4, we used LCM to collect cells within GP3 and adjacent GP4 in 14 cases of fresh-frozen prostate cancer. Figure 1 shows a representative example of the hematoxylin and eosin-stained region of GP3 and GP4 tumor in the frozen tissue samples (Fig. 1A), together with the section image after LCM (Fig. 1B) and the captured GP3 and GP4 cells engrafted on the LCM caps (Fig. 1C and D, respectively). The DNA was amplified directly from the engrafted cells (23) and massively parallel mate-pair library sequencing was carried out. Binary algorithms developed in our group (24, 25) were used to analyze the data and detect large chromosomal abnormalities including translocations, deletions, and insertions. The discovered breakpoints were localized to the genes as well as intragenic regions. Interchromosomal translocations and intrachromosomal rearrangements including amplifications, deletions, or inversions were equally represented in GP3 and GP4 within the same tumors. Clusters of breakpoints encompassing areas of a few megabases were also observed in several samples (16/28 samples), suggesting consequences of chromothripsis, a recently described phenomenon (29–31). In 7 cases, both GP3 and GP4 showed similar patterns for chromothripsis, whereas 2 cases showed chromothripsis only in GP4.

The number of genomic breakpoints (i.e., junctions of the rearranged chromosomal regions) ranged from 10 to 180 per tumor between the 28 GP3 and GP4 samples, and significant variation was observed between adjacent GP3 and GP4 within the same tumor (Fig. 2A). Evaluation of genomic breakpoints showed that recurrent events were uncommon between patient tumors (Fig. 2B), but were common between adjacent GP3 and GP4 within the same tumor. Specifically, within the 14 patient tissues, 176 chromosomal breakpoints were shared by...
adjacent GP3 and GP4 pairs, whereas more than 1,000 events were unique to individual GS7 tumors (Fig. 2).

As expected, ERG was the most frequently rearranged gene, present in 8 of the 14 cases (Fig. 2B). Deletion of PTEN and FOXP1, 2 genes often altered in prostate cancer (12, 32–35), were next most common, present in 4 and 3 cases, respectively (Fig. 2B). In addition, recurrent alterations involving genes that have not been previously implicated in prostate cancer were observed. Among these were vacuolar sorting protein 13BB (VPS13B), transcription factor SP3, integral membrane protein triadin (TRDN), and cAMP-specific phosphodiesterase 4D (PDE4D; Fig. 2B). Interestingly, breakpoints at the SP3 loci in 2 cases were present in both GP3 and GP4, green squares indicate that alteration is present only in GP3, and red squares indicate that alteration is present only in GP4. PR indicates a case number.

Figure 2. Commonality and differences in chromosomal alterations in paired GP3 and GP4 in 14 patients with prostate cancer. A, a graphical representation of the extent of similarity of subpopulations of cells from the same patient in terms of large chromosomal abnormalities. The number of alterations is shown on the y-axis, and the cases are depicted on the x-axis. B, molecular intertumoral and intratumoral heterogeneity in 14 patients with prostate cancer. Each row represents a gene locus, and each column is GS7 prostate cancer case. Blue squares indicate that alteration is present in both GP3 and GP4, green squares indicate that alteration is present only in GP3, and red squares indicate that alteration is present only in GP4. PR indicates a case number.

Figure 3. Phylogenetic relationships between the tumors and between GPs within the tumor. Agglomerative hierarchical clustering was used to quantify relationships between samples. A distance matrix was built to capture genetic distance between all possible pairs.
rarrangements play a role in early steps of tumorigenesis, whereas others play a greater role in disease progression. We also compared genes that harbored breakpoints, affected in 2 or more prostate tumors in our dataset, to the list of genes available in cosmic Census database (36) that are accepted as important “drivers” in tumorigenesis (Supplementary Table S2). A total of 9 genes recurrently affected in our 14 patients with GS7 prostate cancer have been previously annotated as “drivers” in cosmic database. These included ERG–TMPRSS2 fusion, PTEN deletion, immunoglobulin kappa locus rearrangements (IGK@), FOXP1 translocation, ALK translocation, transcription factor 12 translocation (TCF12), p53 deletion, and musashi homologue 2 (MSI2) translocation (Supplementary Table S2A). Four more genes (Supplementary Table S2B) are likely to have similar functional importance as their close homologs listed as “drivers” in cosmic database. We consider the rest of the genes shown in Fig. 2 as potential novel contributors (drivers) to tumorigenesis in prostate cancer, whereas their specific role is to be identified in future studies.

In each of the GP3 and GP4 pairs from the 14 cases studied, a set of shared recombination events was predicted by our algorithm (Fig. 2A). This observation is consistent with the notion that the adjacent GP share a common origin. The number of shared rearrangements, however, varied extensively between the GP within each tumor and did not correlate with the total numbers of abnormalities in each tumor. Likewise, the number of breakpoints between ERG-positive and ERG-negative cases did not significantly differ (Fig. 2A). Interestingly, 3 cases PR12, PR27, and PR28, presented little commonality between GP3 and GP4, raising the possibility that the adjacent patterns in these cases may have developed independently (Fig. 2A). The majority of cases showed a significant number of common breakpoints between GP3 and GP4 within a tumor, indicating a common origin. In 3 cases, PR11, PR12, and PR28, no breakpoints unique to GP3 were identified (Fig. 2A). Cases PR6, PR24, and PR26 showed just a small number of unique GP3 events. In contrast, significant numbers of unique chromosomal rearrangements were observed in the GP4 in each case.
case, with PR22 and PR27 showing a high number of abnormalities specific to GP4. Only case PR10 presented more unique breakpoints in the GP3 as compared with GP4 (Fig. 2A).

A high degree of commonality between adjacent GP3 and GP4 was also supported by independent agglomerative hierarchical clustering analysis (Fig. 3). This analysis showed that the GP3 is more closely associated with its associated GP4 than with GP3 from other patient’s tumors. As expected, the GP3 and GP4 in PR12, PR28, and PR27 were the least related, as reflected by their height scores in the dendrogram (Fig. 3).

Conventional PCR followed by Sanger sequencing was used to identify the exact structure of the chromosomal fusions predicted by our bioinformatics algorithm. We first validated ERG fusion events for which mate-pair reads were always observed in adjacent GP3 and GP4 (Figs. 2 and 4A), and in these cases, GP3 and GP4 shared the exact breakpoints for TMPRSS2–ERG, indicating a common origin (Fig. 4 and Supplementary Fig. S2). Importantly, DNA from the adjacent histologically normal tissue (AN) did not contain the ERG fusion events. Both GPs in each tumor showed strong ERG protein expression (Fig. 4C and Supplementary Fig. S2). The specific position of the breakpoint within the ERG gene varied between cases, but localized within in the commonly reported intron 2 or 3 regions (Fig. 4A). Similarly, each position of the TMPRSS2 breakpoint predicted the association of the promoter and resultant androgen responsive expression of the resultant ERG fusion gene product. On the basis of comparison of TMPRSS2–ERG gene fusion status in GS7 cases and adjacent histologically normal tissue (AN), we postulate that TMPRSS2–ERG gene fusion is a strong marker of lineage relationship for adjacent GPs.

Previously, it has been reported that AR and ERG protein cooperate to facilitate the formation of DNA double-strand breaks (37, 38). In addition, breakpoints were shown enriched near open chromatin, AR, and ERG DNA-binding sites in the setting of the TMPRSS2–ERG fusion, but inversely correlated with these regions in tumors lacking ETS fusions (12). Therefore, we investigated whether the breakpoints in our set of cases correlated with binding sites for ERG, AR, or cluster within open or closed chromatin. By using published ChIP-Seq data (26) for the AR, ERG, RNA polymerase II binding sites and histone modifications, marks for open and closed chromatin, we examined their association with our breakpoint data. A significant enrichment for AR and ERG-binding sites in the vicinity of the breakpoints in half of ERG-positive cases was observed (Supplementary Fig. 3SA and S3B). In case, PR21 G4 depletion for both AR and ERG CHIP-Seq peaks was found. ERG-negative cases did not show a consistent trend. With the exception of GP4 in PR27, in which a depletion for each of the 3 binding sites was found, no significant correlation was observed for any CHIP-Seq peaks in ERG-negative cases.

Trimethylated histone H3K4 and trimethylated histone H3K36 (Supplementary Fig. 3SD), marks of open chromatin, were enriched in the majority of ERG-positive cases with a significant P value. A few ERG-negative cases have also shown an enrichment for H3K4me3, in contrast with the previously

| Table 1. Occurrence of validated breakpoints across different Gleason patterns |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Case PR28      | Case PR12      | Case PR27      |
| AN  | G3  | G4  | AN  | G3  | G4  | AN  | G3  | G4  |
| Event Genes involved | mate-pairs | Present | Event Genes involved | mate-pairs | Present | Event Genes involved | mate-pairs | Present |
| Chr 21 deletion ERG–TMPRSS2 | n/a | 8  | 20 | n/a | 0  | 20 | n/a | 0  | 16 |
| Chr8–Chr11 KCNK9–CLMP | n/a | 1  | 14 | n/a | 0  | 8  | n/a | 1  | 22 |
| Chr12–Chr22 CACNA1C–ZNRF3 | n/a | 0  | 27 | n/a | 0  | 11 | n/a | 10 | 9  |
| Chr8–Chr6 TUBB | n/a | 10 | 9  | +  | +  | +  | +  | +  | +  |
| Chr8–Chr6 TUBB | n/a | 10 | 9  | +  | +  | +  | +  | +  | +  |
| Chr6 deletion TRDN | n/a | 8  | 0  | +  | –  | –  | –  | –  | –  |

Abbreviation: n/a, not tested
*Two breakpoints representing balanced translocation.
published data (12). Only distribution of breakpoints in ERG-negative case PR27 GP4 positively correlated with closed chromatin marks H3K9me3 and H3K27me3 (Supplementary Fig. 3S3E) and negatively correlated with marks of the open chromatin (Supplementary Fig. 3D).

To better understand the relationship of GP3 and GP4 in the cases that showed little or no commonality by mate-pair analysis (Fig. 3B), we validated a set of selected breakpoints (Table 1). In PR28, the identical TMPRSS2–ERG fusion and an event involving chromosomes 8 and 11 was validated in both the GP3 and GP4 but was not present in AN (Fig. 4B and Fig. 5A, respectively). The STARD13 gene on chromosome 13 was validated in GP3, GP4, and AN of case PR12 (Fig. 5B). Similar findings were shown for the chromosome 4 and 13 rearrangement involving PDS5B in this case (Fig. 5B). For both breakpoints, more product was observed in GP4 than in the associated GP3 and AN, suggesting that a smaller fraction of cells in the latter 2 populations harbor the alteration. Because the cells from each population were isolated by LCM and DNA was processed independently, the chance of cross-contamination was very unlikely. In addition, for a subset of rearrangements, we have conducted a semiquantitative PCR with varying number of cycles (Supplementary Fig. S4). There was a correlation between the amount of the amplified product and the number of cycles, indicating that a different proportion of cells within samples harbor the alteration.

Individual rearrangements in case PR27 showed various distributions (Fig. 5C). Interestingly, a few breakpoints in PR27 were present only in GP3, a few only in GP4, and several were detected in GP3, GP4, as well as adjacent histologically normal. As no events were identified shared by GP3 and GP4 exclusively, this raised a possibility that they represent different clones of the common predecessor. We also compared the relationship between GP3 and GP4 in 2 cases, PR10 and PR11 that had a large number of shared breakpoints by mate-pair analysis (Fig. 2A). A validation of selected alterations revealed their presence in both GP3 and GP4 and their absence in histologically normal tissue (AN) correlating with mate-pair analysis (Fig. 5D). The shown deletion in chromosome 1 resulted in a fusion between the first exon of LPAR3 gene, coding for a G protein-coupled receptor family member highly expressed in normal prostate, and zinc finger factor ZZZ3 gene (Fig. 5D). Unlike cases in which GP3 and GP4 did not cluster well together, GPs showing more commonality by the mate-pair analysis had lineage relationship.

To show heterogeneity/homogeneity of the cell population for a given alteration within a single GP area, we conducted FISH analysis. A specific probe for PTEN was used to compare the signal in adjacent histologically normal cells, GP3 and GP4 (Fig. 6). Consistent with our validation results using PCR and Sanger sequencing (Fig. 6A), cells in both GP3 and GP4 showed heterozygous deletion of PTEN, whereas adjacent histologically normal cells harbored intact signals for both PTEN alleles (Fig. 6D). Because cells from histologically normal tissue, GP3 and GP4 for genomic analysis of alterations were collected individually using LCM and processed separately, we concluded that the presence of certain rearrangements in adjacent to tumor histologically normal cells is a true observation and not a result of DNA contamination.

Discussion
This is the first study to examine the chromosomal alterations in adjacent GPs within the same tumor using a unique approach enabling interrogation of small tumor samples with

Figure 5. Validation analysis of selected breakpoints identified by mate-pair in the selected GS7 cases. A–C, representative gel images of validated breakpoints between adjacent GP3 and GP4 patterns. GN is normal genomic DNA, AN is adjacent histologically normal tissue, collected independently, and GP3 and GP4 are adjacent GPs. D, validation of a deletion on chromosome 1 identified in PR11, which is classified as a case with greater commonality between GP3 and GP4. Designations are as described in A–C. A chromatogram illustrating sequence analysis and a schematic of the putative fusion gene are also shown.
great specificity. Our findings indicate that while GP3 and GP4 from the same tumor each possess unique breakpoints, they also share identical ones, indicating a common origin. In addition, defining these lineage relationships allows a better understanding of tumor progression.

Characterization of lineage (i.e., descent from a common precursor) relationships between concurrent GP3 and GP4 in prostate cancer in the past has been hampered by the difficulty in separately obtaining pathologically well-characterized cell populations in sufficient quantity. Although large-scale sequencing efforts have already been applied to prostate cancer, none of these studies has focused on progression from GP3 to GP4. Whole-genome sequencing of several cancers revealed that solid tumors harbor many chromosomal rearrangements and thousands of single nucleotide variations (SNV). We have used both strategies to investigate lineage relationships between phenotypically different parts of a tumor from a same individual by enumerating common alterations that are not found in normal distant tissue. We found that using analysis of chromosomal rearrangements helps to elucidate lineage relationships in more specific manner than SNVs. SNVs are known to be present not only in tumors, but also in normal tissue and normal peripheral blood. This requires an additional profiling of blood and normal tissue adjacent and distant to the tumor to decipher somatic tumorigenic changes. In contrast, chromosomal rearrangements, related to tumorigenicity, are usually found in the tumor but not in blood. Therefore, in our efforts to show tumoral lineage, we took advantage of sequencing mate-pair libraries to identify unique abnormalities between different cell populations in the same tumor.

We analyzed 14 cases of GS7 prostate cancer and found a significant heterogeneity in GPs between different patient's tumors in agreement with previous reports (11,12). Recurrent chromosomal alterations were not common and much less frequent than chromosomal changes unique to a specific tumor. Similar observations were made in a recent study by Berger and colleagues (12) where complete sequencing of 7 cases of GS7 cancer showed numerous chromosomal changes as well as point mutations. However, point mutations within GP3 and GP4 were not examined in the Berger study. Our findings indicate that GS7 tumors show significant
heterogeneity within GP3 and GP4, yet share identical genomic alterations, indicating a common origin. Moreover, we show that in the majority of cases, GP3 shares more similarities with its partner GP4 than GP3 from other patients' tumors (Fig. 2 and 3). Some of the molecular changes, such as loss of PTEN, were evident in GP3 as well as GP4, indicating that genomic changes associated with tumor aggressiveness occur before the morphologic transition from GP3 to GP4.

On the basis of our molecular analyses of associated GPs, we propose a model to explain the role of chromosomal rearrangements in cancer initiation and progression (Fig. 7). In the first scenario, a subpopulation of cells with identical molecular alterations within heterogeneous GP3 gives rise to GP4 in which cells acquire more changes as the tumor progresses (Fig. 7A). PR28 and PR11 (Figs. 4B and 5D, respectively) represent examples of this scenario. Alternatively, GP3 and GP4 might arise from different clones by using different subsets of molecular changes (Fig. 7B). Finally, a specific subset of molecular rearrangements in adjacent nonneoplastic tissue leads to transformation into GP3, which does not progress and becomes a "dead end" (Fig. 7C). A different subset of rearrangements gives rise to GP3, which progresses into GP4, such as in PR27 (Fig. 5C).

Although tumor heterogeneity within and between cancers poses a number of problems related to treatment and person-alized medicine (39), the application NGS in conjunction with careful selection subset of relevant population of cells for interrogation seems to be a useful tool for identification of lineage relationship and recurrent molecular events within heterogeneous tumors (40). Such knowledge can be of great value in stratifying "indolent" from "significant" prostate cancers and can help to improve patient management, particularly when applied to needle biopsy specimens that sample only a very small portion of tumor.

In conclusion, we used LCM, massively parallel NGS and a unique bioinformatics algorithm to better define the genomic landscape within and between GS7 prostate cancer. In most cases, the GPs within each tumor had a common origin and were more similar to each other than GPs in other patient's tumors. Future work will help to determine the use of discovered molecular markers in the diagnosis of "indolent" and "significant" prostate cancers.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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References


36. http://www.sanger.ac.uk/genetics/CGP/Census/


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