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Biomarkers Associated with Tumor Heterogeneity in Prostate Cancer[1,2]

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Abstract

BACKGROUND: Prostate cancers exhibit intratumor heterogeneity (ITH), like other cancer types. The ITH may affect diverse phenotypes such as treatment response, drug resistance, and clinical outcomes. It is crucial to consider ITH to understand tumorigenesis. *METHODS:* Genomic and transcriptomic profiles of prostate cancer patients were investigated to determine which markers are correlated with the degree of tumor heterogeneity. In addition, the correlation between the immune activity and clonality of tumors was examined. *RESULTS:* Tumor heterogeneity across all prostate cancer samples was variable. However, ITH events were dependent on genomic and clinical features. Interestingly, prostate-specific antigen score increased in tumors with multiple subclones, indicating high-grade tumor heterogeneity. On the other hand, CD8-positive T-cell activation decreased in highly heterogeneous tumors. Intriguingly, *PTEN* deletion was prominently enriched in high heterogeneity groups, with a strong association with heterozygous loss. Expression of major genes including *PTEN*, *CDC42EP5*, *RNLS*, *GP2*, *NETO2*, and *AMPD3* was closely related to tumor heterogeneity in association with *PTEN* deletion. *CONCLUSIONS:* In prostate cancer, ITH, a potential factor affecting tumor progression, is associated with *PTEN* deletion and cytotoxic T cell inactivation.

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Introduction

In many industrialized nations, prostate adenocarcinoma is one of the most common malignant diseases in men [1]. Prostate cancer (PC) is considered clinically heterogeneous. Some prostate cancers are indolent and localized, while others are aggressive and easily spread to other parts of the body. Therefore, it is necessary to understand key features related to tumor progression and invasiveness. Many cases of prostate cancer are multifocal; most radical prostatectomy specimens harbor morphologically and clonally distinct tumor foci [2–4]. Studies of metastatic tumors from primary PC have suggested that all of those tumors evolved from one clone, as they share a significant portion of genetic alterations [5,6]. The characteristics and diversity of a clone may explain the aggressiveness of prostate cancer.

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Therefore, it is important to perform a comprehensive genomic and transcriptomic characterization of the primary cancer lesion to understand the biology of the tumor and the factors associated with tumor progression. However, major factors that lead to tumor heterogeneity during prostate cancer progression are still not clear. Recently, next-generation sequencing provided a molecular portrait of genomic alterations. Furthermore, intratumor heterogeneity (ITH) has been inferred by clonality analysis [7]. In various types of cancer, the characterization of clonal heterogeneity may provide useful information for predicting patient prognosis and treatment response.

Herein, we investigated factors that are associated with ITH of prostate cancer through comprehensive genomic and transcriptomic analysis. We also investigated genomic alterations, altered pathways, and clinical features as the indicators of high ITH.

Materials and Methods

Dataset Collection

Genomic and transcriptomic alterations including somatic mutations, copy number alterations, and gene fusions were collected from The Cancer Genome Atlas Research Network (The Cancer Genome Atlas Research Network 2015) [8]. Gene expression data from RNA-seq were obtained from GDAC Firehose (http://gdac.broadinstitute.org). A total of 85 samples with clonality information were used to determine the association between genomic alteration and expression profile.

Gene Expression Analysis

Differentially expressed genes (DEGs) were identified using DESeq R package (www.huber.embl.de/users/anders/DESeq/). Significant DEGs by both ≥2-fold change and adjusted P value <.05 were chosen. In order to identify overrepresented functions of an interesting group, gene set enrichment test was performed using Gene Set Enrichment Analysis (software.broadinstitute.org/gsea/), based on REACTOME pathway database in the Molecular Signatures Database (MSigDB). To estimate the fractions of immune-associated cell types including CD8-positive T cells, CIBERSORT was applied using RNA-seq expression profiles [9]. It can infer relative proportions of each immune cell types using gene expression profiles.

Clonality and Tumor Purity Information

Clonality information was obtained from a pan-cancer analysis of the ITH, measured using PyClone and EXPANDS tools [7]. The number of subclones ranged from one to eight and represented the ITH level. In order to categorize high and low ITH, we defined a tumor as an oligoclone when there were one or two subclones; otherwise, the tumor was defined as a multiclone [10]. Tumor purity information was collected from pan-cancer analysis of the tumor purity [11]. In brief, the purity levels were arbitrarily chosen from multiple estimators. The consensus purity estimation method is the median value for estimators after normalization.

Statistical Analysis

The significance of clinical outcomes of the selected genes was plotted using Kaplan-Meier survival analysis using the survival package in R (http://CRAN.R-project.org/package=survival). Log-rank test was used for survival analysis. Fisher's exact test was used for the statistical analysis of ITH and genomic mutations. $P < .05$ was considered statistically significant. Information gain (IG) was used to select the informative features for discriminating cancers with high clonality. IG for tumor samples D and a feature a is defined as [12]:

$$IG(D, a) = Entropy(D) - \sum_{v \in value(a)} \frac{D_v}{D} Entropy(D_v)$$

where value(a) is the set of all possible values for feature a and D_v is the subset of D which the feature a has value v.

Results

Genomic Profiles According to Degree of ITH

A total of 85 patients having clonality information with prostate cancer were evaluated. Patient characteristics according to clonality are shown in Table 1. Here, an oligoclone had one or two subclones, and a multiclone had more than two subclones. Prostate-specific antigen (PSA) is one of the major markers used to diagnose prostate cancer. In the prostate cancer cohort, PSA level (n = 472) was significantly correlated with clinical outcome (Supplementary Figure S1). Although the association between tumor heterogeneity and level of PSA is not prominent, high level of PSA (>1.5) was more frequently observed in the group with multiclone, indicating high ITH (Figures 1 and 2*A*). In the oligoclone group, only two patients had a high level of PSA, while there were seven such patients in the multiclone group. Furthermore, the average level of PSA was 0.28 in the oligoclone group and 1.67 in the multiclone group (Table 1). The analysis suggested that the level of tumor progression or invasiveness is substantially associated with ITH and PSA levels.

Several factors such as average PSA level, tumor mutation burden (TMB), and CD8 scores are associated with the number of clones (Figure 2). The data demonstrated that the level of PSA increased as the number of clones increased. TMB also increased slightly when the number of clones increased. On the other hand, the activation score of CD8 generally declined with the accumulation of clones. These results were not likely affected by tumor purity estimated by four different kinds of measurements including immunohistochemistry, as tumor purities of samples in PC were not different according to number of subclones (Supplementary Table S1).

Moreover, we measured the activation degree of immune cells adjacent to cancer cells using decomposition of RNA-sequencing data. When comparing the immune profiling based on tumor heterogeneity, the activation score of T cell (CD8+) showed slight differences (P = .05) between tumors with oligoclones and those with multiclones (Supplementary Figure S2). On average, patients who had high heterogeneity showed a lower immune activation score of T cell compared to those with low heterogeneity. Generally, cancer cells are known to develop immunosuppression or avoidance [13]. Our analysis indicated that the immune avoidance mechanism works better by reducing the activation of T cells in multiclonal than oligoclonal prostate cancer.

Association of PTEN Deletion and ITH

We also analyzed the association between ITH and mutational profiles, including somatic mutations and copy number alterations (CNAs), from whole-exome sequencing data. While most alterations did not showed any difference in degree of heterogeneity, there were substantial deviations in *PTEN* CNA (Figure 3*A*). To observe the genomic factors that can lead to a separation of clonality, information gain (IG) was adopted, and *PTEN* CNA among many factors showed