Priority Report

Distinct Genomic Alterations in Prostate Cancers in Chinese and Western Populations Suggest Alternative Pathways of Prostate Carcinogenesis

Xueying Mao1,2, Yongwei Yu4, Lara K. Boyd1, Guoping Ren1,5, Dongmei Lin1,6, Tracy Chaplin2, Sakunthala C. Kudahetti1, Elzbieta Stankiewicz1, Liyan Xue1, Luis Beltran3, Manu Gupta2, R. Tim D. Oliver2, Nick R. Lemoine1, Daniel M. Berney1, Bryan D. Young2, and Yong-Jie Lu1,2

Abstract

Prostate cancer is significantly more common in Western men than in Asian men, but the basis for this difference remains unknown. Because genomic studies of Asian prostate cancer are very limited, we used a genome-wide approach to reveal the genomic alterations in Chinese prostate cancers. We found a significant reduction in the frequency of certain somatic genomic changes that are commonly found in Western prostate cancers, including the 21q22.2-22.3 deletion, which involves the TMPRSS2:ERG fusion gene, and 10q deletion, which causes PTEN inactivation. Array results were confirmed by PCR-based molecular copy-number counting in selected samples. The different frequencies of these genomic changes were further evaluated by fluorescent in situ hybridization and immunohistochemistry analyses of tissue microarray samples. These alterations might be key genetic changes underlying the regional/ethnic difference in clinical incidence and might be induced by specific environmental and/or genetic risk factors that Western men are exposed to. Our findings suggest that tumors arise in Western and Chinese populations by alternative pathogenetic mechanisms. Cancer Res; 70(13): 5207–12. ©2010 AACR.

Introduction

Prostate cancer, the most common cancer in Western men, shows a wide variation in the clinical incidence and mortality rates of different geographic regions (1). Despite a recent increase, the prevalence of prostate cancer in Asian countries is 20 times lower than in Western countries (1, 2). Numerous studies have been carried out to understand the factors contributing to this difference. However, each of the currently identified risk factors is associated with only a modest increase in the incidence of prostate cancer and the real contribution of such factors is still debatable (2, 3). Carcinogenesis is associated with multiple somatic genomic alterations (4, 5). Although the genomic alterations in prostate cancer cells from Western populations are well studied, data from Asian samples are limited. To determine the similarities and/or differences in genomic alterations in prostate cancer samples from high- and low-incidence populations, we analyzed the genomic alterations in Chinese prostate cancer (low incidence) and found specific genomic differences in cancers from China and Western countries.

Materials and Methods

Samples

Thirty-nine samples from China (Supplementary Table S1) and five fresh-frozen prostate cancer samples from the United Kingdom were collected and stored in ethically approved tissue banks. Case-matched adjacent phenotypically normal tissue was available for 33 of 39 Chinese samples and for all 5 cases from the United Kingdom. Twenty-eight benign prostate hyperplasia, 168 prostate cancer cases from the United Kingdom (<10% were of Asian and none were of Chinese origin), and 143 Chinese prostate cancer cases (all of Chinese ethnicity and from China) were collected for tissue microarray (TMA) studies. The majority of patients from China or the United Kingdom were not screened for prostate-specific antigen. Ethical approval was obtained from each local ethics committee.
Single nucleotide polymorphism array analysis

The predominant tumor nodule and adjacent normal tissue were identified by histopathologists (Y. Yu and D. Lin) on fresh-frozen sections and macrodissected (mini-scalpel) or microdissected (PALM Microdissection Technologies). Only foci with >80% purity of cancer cells were macrodissected. Single nucleotide polymorphism (SNP) array analysis using Human SNP Array 6.0 (Affymetrix) was performed according to the instructions of the manufacturer and scanned with a Genechip Scanner 3000 7G (Affymetrix) using Affymetrix Genechip Operating Software. Hybridization data were filtered with the same operating software to exclude weakly hybridized SNPs.

Signal intensity data from SNP arrays were analyzed using Partek Genomics Suite (Partek Incorporated) and our own GOLF (V2.2.10) software (6, 7). The signal intensity ratio between test and normal samples was plotted for DNA copy number alterations. Ensembl Genome Browser Build 36.2 was applied to define the genomic regions. For paired samples, tumor data were compared with case-matched normal controls. Unpaired Chinese tumor samples were analyzed against eight normal samples that were representative of the majority of germ line copy number variations. Utilizing the Partek Genomics Suite, the copy number workflow was used with default parameters. With GOLF, chromosomal gain/loss events were considered if the signal intensity ratio of 0 ± 0.4 log 2 ratio. Regions considered positive by only the mean of 20 contiguous probes was significantly outside gain/loss events were considered if the signal intensity ratio used with default parameters. With GOLF, chromosomal gain/loss events were considered if the signal intensity ratio of 0 ± 0.4 log 2 ratio. Regions considered positive by only the mean of 20 contiguous probes was significantly outside gain/loss events were considered if the signal intensity ratio used with default parameters. For paired samples, tumor data were compared with case-matched normal controls. Unpaired Chinese tumor samples were analyzed against eight normal samples that were representative of the majority of germ line copy number variations. Utilizing the Partek Genomics Suite, the copy number workflow was used with default parameters. With GOLF, chromosomal gain/loss events were considered if the signal intensity ratio of 0 ± 0.4 log 2 ratio. Regions considered positive by only the mean of 20 contiguous probes was significantly outside gain/loss events were considered if the signal intensity ratio used with default parameters.

Statistical analysis

Differences in continuous data were compared using Student’s t test. Differences in discreet data were compared using a χ² test.

Results and Discussion

The genome-wide alterations in Chinese prostate cancer and the differences from Western cases

Thirty-nine Chinese prostate cancer samples were analyzed using Affymetrix SNP Array 6.0 high-density microarrays and microarray data have been deposited in the Gene Expression Omnibus (accession no. GSE18333). This study generated a high-resolution genomic alteration map for Chinese prostate cancer, which we hope will facilitate the genetic study of prostate cancer in this population. The genomic copy number changes observed in the Chinese prostate cancer samples are summarized in Table S3. Common regions of chromosomal copy number gains were 7 (11 of 39), 8q (16 of 39), and 3q (9 of 39) and losses were 5q15-21.3 (18 of 39), 6q14.1-22.1 (27 of 39), 8p (25 of 39), 13q12.3-31.1 (28 of 39), and 16q12.1-24.3 (20 of 39).

By comparison with data reported previously for Western prostate cancers (4, 11–13), we have identified genomic alterations common in both populations, including gains of 7 and 8q, and losses of 6q14.1-22.1, 8p, 13q12.3-31.1, and 16q12.1-24.3. However, the frequency of 6q14.1-22.1 (27 of 39) and 13q12.3-31.1 (28 of 39) deletions detected in this study were greater than that previously reported for Western cancers (4, 12, 13). Tumor suppressor genes RB, BRCA2, and KLF5 are located at 13q12.3-31.1 and candidate tumor suppressor genes at 6q14.1-22.1 have been suggested but not yet confirmed (4, 14). In Western populations, 6q15 and 6q21 deletions characterize certain subgroups of prostate cancer (4, 14). The roles of these genes
Genomic Difference in Chinese and Western Prostate Cancers

Figure 1. Lack of 10q23 and 21q22.2-22.3 deletions in Chinese prostate cancer detected by SNP array analysis. A, summary of SNP Array 6.0 results of 39 Chinese prostate cancer cases. Red and blue bars on the right of each chromosome represent regions of copy number gains and losses, respectively. B, SNP intensity profile of chromosomes 10 and 21 in U.K. and Chinese samples. The purple bar indicates the location of PTEN (Chr 10), ERG (Chr 21, left bar), and TMPRSS2 (Chr 21, right bar), respectively, which mark the regions deleted in the U.K. but not in Chinese samples. In each SNP intensity plot, the middle horizontal line represents a log 2 ratio of 0 compared with normal controls. The bottom and upper lines represent log 2 ratios of −1 and +1, respectively.

at 6q14.1-22.1 and 13q12.3-31.1 in Chinese prostate cancer development should therefore be investigated.

Surprisingly, in the Chinese samples, we found low frequencies of two common genomic changes observed in Western samples: deletion of 21q22.2-22.3.3 between the ERG and TMPRSS2 genes and deletion of 10q23, including the PTEN gene locus. TMPRSS2:ERG is the most common gene fusion event in human cancers, occurring in ~50% of prostate cancers (4, 5, 10–16), and leads to the overexpression of ERG from the TMPRSS2 promoter. Deletion of 21q22.2-22.3,
between TMPRSS2 and ERG, accounts for more than half of the TMPRSS2:ERG fusion cases (12, 15, 16). However, this deletion was observed in only 1 of 39 Chinese samples. In prostate cancer, PTEN is commonly inactivated and chromosome deletion accounts for the majority of PTEN loss-of-function cases (17). Despite occurring in ~40% of prostate cancer samples (12, 17, 18), deletion at 10q PTEN region was observed in only 3 of 39 Chinese cases (Fig. 1A and Supplementary Table S3). Using the same methods, we analyzed five cancer samples from the United Kingdom and detected three of five cases with deletions at 10q23 and four of five cases with deletions at 21q22.2-22.3 (Supplementary Fig. S1 and Supplementary Table S4). Although the number of samples was small, the high frequency of 10q23 and 21q22.2-22.3 deletions detected in our samples from the United Kingdom indicates that our method was sufficient to detect these genomic alterations. Representative images of chromosomes 10 and 21 are shown in Fig. 1B.

**Confirmation of SNP array results using MCC analysis**

To confirm our SNP microarray results, the genomic copy numbers at 21q22.2-22.3 between TMPRSS2 and ERG were determined by MCC in four samples from the United Kingdom with the 21q22.2-22.3 deletion, and four Chinese samples without this deletion. Using two sets of MCC primers, ERG(+) and ERG(−), we detected a similar number of ERG(+) and ERG(−) products in the Chinese samples with ERG(−):

<table>
<thead>
<tr>
<th>Sample</th>
<th>ERG(+) No.</th>
<th>ERG(−) No.</th>
<th>Ratio ERG(−):ERG(+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P9</td>
<td>39</td>
<td>20</td>
<td>0.51</td>
</tr>
<tr>
<td>P55</td>
<td>30</td>
<td>12</td>
<td>0.40</td>
</tr>
<tr>
<td>P68</td>
<td>55</td>
<td>40</td>
<td>0.73</td>
</tr>
<tr>
<td>P98</td>
<td>34</td>
<td>21</td>
<td>0.62</td>
</tr>
<tr>
<td>SH1</td>
<td>31</td>
<td>28</td>
<td>0.90</td>
</tr>
<tr>
<td>SH3</td>
<td>47</td>
<td>48</td>
<td>1.02</td>
</tr>
<tr>
<td>SH5</td>
<td>79</td>
<td>82</td>
<td>1.04</td>
</tr>
<tr>
<td>SH6</td>
<td>65</td>
<td>67</td>
<td>1.03</td>
</tr>
</tbody>
</table>

**Figure 2.** Representative ERG and PTEN FISH images. A to C, FISH analysis of ERG gene status using probes for ERG 3’ undeleted region (red signal) and ERG 5’ deleted region (green signal) shows a U.K. case with ERG deletion (A), a U.K. case with ERG split signals (B), and a Chinese case with no ERG deletion (C). D to F, the detection of PTEN copy number using probes for the PTEN locus (red signal) and chromosome 10 centromere (green signal) shows homozygous (D) and heterozygous (E) PTEN loss in U.K. samples and no PTEN loss in a Chinese sample (F). G to I, immunohistochemistry analysis shows loss of PTEN expression in cancer cells and positive stromal cells in a U.K. PTEN homozygous deletion case (G), lack of PTEN expression in a proportion of cancer cells from a U.K. partial PTEN deletion case (H), and strong PTEN expression (+++) in a Chinese sample without PTEN deletion (I).
with the 21q22.2-22.3 deletion detected by the SNP microarrays, a reduced number of \( ERG(-) \) products was observed in the samples from the United Kingdom with \( ERG(-) \); \( ERG(+) \) ranging from 0.40 to 0.73 (Table 1; Supplementary Fig. S2).

**Frequency of \( ERG \) rearrangements and \( PTEN \) deletion/inactivation evaluated using TMAs**

We evaluated the genomic differences between Western and Chinese prostate cancers using FISH analysis of TMAs containing a separate set of formalin-fixed, paraffin-embedded samples from the two populations. Benign prostate hyperplasia samples were used as controls. Although \( ERG \) status was consistent in replicate cores, in some cases, \( PTEN \) deletion was only detected in one or two cores from a case. We considered cases to be \( PTEN \) deletion cases if \( PTEN \) signal was lost in any of the cores. We found that the \( ERG \) 5’ region was deleted in 29.7% (46 of 155) and the 5’ and 3’ bacterial artificial chromosome sequences were split in 11.6% (18 of 155) of samples from the United Kingdom (Supplementary Table S5 and S7), but only in 5.4% (5 of 93) and 2.1% (2 of 93) of Chinese samples, respectively (Supplementary Table S6 and S7). Representative FISH images are shown in Fig. 2. Although the frequency of \( ERG \) rearrangements in samples from the United Kingdom were similar to that previously published for Western samples (12, 15, 16), they were observed less frequently in the Chinese samples (\( P < 0.001 \), Supplementary Table S7). Consistent with our SNP array results, the \( PTEN \) genomic region was deleted in 42.3% (66 of 156) of samples from the United Kingdom (Supplementary Table S5 and S7), but in only 14.3% (12 of 84) of Chinese samples (Supplementary Table S6 and S7), which is statistically significant (\( P < 0.001 \)). Representative FISH images are shown in Fig. 2. As \( PTEN \) could also be inactivated by mutation and DNA methylation, we determined the frequency of \( PTEN \) inactivation using immunohistochemistry on the same TMA samples. Low-level (− or +) expression of \( PTEN \) was detected in 69.8% (111 of 159) of samples from the United Kingdom (Supplementary Tables S5 and S7), but in only 34% (51 of 91) of Chinese samples (Supplementary Tables S6 and S7). This difference is statistically significant (\( P < 0.001 \); Supplementary Table S7). Representative images are shown in Fig. 2. \( PTEN \) deletion accounts for more than half of the cases in which \( PTEN \) expression is reduced, and in most cases, deletion resulted in \( PTEN \) underexpression (Supplementary Table S5).

High Gleason score is generally associated with an increased frequency of \( TMPRSS2:ERG \) fusion events and \( PTEN \) abnormalities (13, 16–18). Although the average Gleason score and age were significantly higher for the Chinese cases compared with the cases from the United Kingdom (\( P < 0.001 \) for both, Supplementary Table S7), Gleason score and age were not significantly correlated with \( ERG \) rearrangements or \( PTEN \) deletion/inactivation in either sample group (\( P > 0.05 \) for all correlations), and therefore, cannot account for the difference in the frequency of these genetic alterations.

It has recently been reported that the \( TMPRSS2:ERG \) fusion cooperates with \( PTEN \) inactivation to drive prostate cancer development (18–20). We also observed the coexistence of \( TMPRSS2:ERG \) fusion with both \( PTEN \) deletion and inactivation in samples from the United Kingdom (\( P < 0.001 \) and \( = 0.01 \), respectively; Supplementary Table S8). It is therefore interesting to find that \( ERG \) and \( PTEN \) abnormalities are the predominant genomic differences between Western and Chinese samples; supporting the cofunction of \( ERG \) overexpression and \( PTEN \) inactivation in prostate cancer development and implying that Western men might be exposed to a currently unknown causative factor(s) for these specific genetic alterations.

To summarize, we have used SNP array analysis to reveal, for the first time, high-resolution genomic alterations in Chinese prostate cancers. In doing so, we have identified key differences in the somatic genomic alterations in prostate cancers from two different risk populations. These genetic differences might underlie the regional/ethnic difference in clinical incidence and suggest different pathways of prostate carcinogenesis in these populations. Based on the nature of these genomic alterations, specific studies can be designed to accelerate the identification of causative factors, and thus, the mechanisms underlying these genomic alterations and the development of the disease.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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