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 Micro激光 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 using 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 the mean of 20 contiguous probes was significantly outside of the 0 ± 0.4 log 2 ratio. Regions considered positive by only one software (<5%) had subtle changes and were excluded from the final results.

PCR-based molecular copy-number counting

Molecular copy-number counting (MCC) was carried out as previously described (8). Two primer sets ERG(+) and ERG(−) were designed to amplify the undeleted ERG gene region and the region deleted between TMPRSS2 and ERG, respectively (Supplementary Table S2). For each sample, 88 reactions were performed in a 96-well plate with eight non-DNA negative controls. The presence or absence of PCR product [207 bp and 189 bp for ERG(+)] was visualized and scored using the Diana V1.6 software (Raytest GmbH).

Fluorescent in situ hybridization analysis of TMA samples

Representative benign prostate hyperplasia and cancer areas were identified on H&E-stained sections by histopathologists (D.M. Berney, G. Ren, and L. Beltran). TMA samples were constructed by taking three cores of 1-mm diameter from each sample using a manual Tissue Arrayer (Beecher Instruments).

Fluorescent in situ hybridization (FISH) analysis for TMPRSS2 and ERG rearrangements on TMA was performed as previously described (9) using two bacterial artificial chromosomes, RP11-95I21 (5’ ERG) and RP11-476D17 (3’ ERG), described by Tomlins and colleagues (10). Bacterial artificial chromosome DNA was amplified using GenomiPhi amplification V2 kit (GE Healthcare) and labeled with digoxigenin and biotin, respectively, using the BioPrime labeling kit (Invitrogen). For PTEN deletion, the commercial FISH probe, Vysis LSI PTEN (10q23)/CEP10 DUAL Color Probe, from Abbott Molecular containing SpectrumOrange-labeled PTEN bacterial artificial chromosome probe and a SpectrumGreen-labeled chromosome 10 centromere control probe was used following the manufacturer-recommended protocol. FISH slides were scanned using a 40× lens on the Applied Imaging Ariol System (Applied Imaging) with seven 0.5-μm z-stacks. FISH results were analyzed double-blindly. A minimum of 100 cells with clear hybridization signals were counted per core.

Immunohistochemistry

The standard avidin biotin complex method (Vector ABC kit, Vector Laboratories, Inc.) was used for immunostaining with high-pressure cooking antigen retrieval. The mouse monoclonal NCL-PTEN primary antibody (1:150, Novocastra) was used to detect PTEN protein in the TMAs. Both prevalence and intensity of PTEN staining were scored for each core. For each case, the percentage of each intensity score from replicate cores was averaged to give a final PTEN expression result.

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 Fig. 1A and Supplementary 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), 13q (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...
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, 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(−): similar number of ERG(+) and ERG(−) products in the Chinese samples with ERG(−):

### Table 1. MCC confirms ERG deletion in four United Kingdom prostate cancer cases (P9-98) and lack of deletion in four Chinese prostate cancer cases (SH1-SH6)

<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 signals 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 genetic 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.

**Acknowledgments**

We thank I. Bisson, L. Shan, Q. An, and J.B. Cazier for sample collection and technical support.

**Grant Support**

Orchid Cancer Appeal, the UK Medical Research Council and the Cancer Research UK.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received 11/05/2009; revised 04/13/2010; accepted 04/19/2010; published OnlineFirst 06/01/2010.

---

**References**

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


Cancer Res 2010;70:5207-5212. Published OnlineFirst June 1, 2010.

Updated version
Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-09-4074

Supplementary Material
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2010/06/01/0008-5472.CAN-09-4074.DC1

Cited articles
This article cites 20 articles, 5 of which you can access for free at:
http://cancerres.aacrjournals.org/content/70/13/5207.full.html#ref-list-1

Citing articles
This article has been cited by 15 HighWire-hosted articles. Access the articles at:
/content/70/13/5207.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.