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Distinct genomic alterations in prostate cancers in Chinese and Western populations suggest alternative pathways of prostate carcinogenesis

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Abstract

Prostate cancer is significantly more common in Western men than Asian men, but the basis for this difference remains unknown. Since 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 frequency of these genomic changes was further evaluated by fluorescence in situ hybridization and immunohistochemistry analyses of tissue microarray samples. These alterations may be key genetic changes underlying the regional/ethnic difference in clinical incidence and may 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.

Keywords

prostate cancer; genomic changes; population; 21q22 deletion; *PTEN* deletion

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Prostate cancer, the most common cancer in Western men, shows a wide variation in the clinical incidence and mortality rates of different geographical regions(1). Despite a recent increase, the prevalence of prostate cancer in Asian countries is 20 times less 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). While 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 courtiers.

Material and methods

Samples

39 Chinese (Table S1, all from China) and 5 UK fresh-frozen prostate cancer samples were collected and stored in ethically approved tissue banks. Case-matched adjacent phenotypically normal tissue was available for 33/39 Chinese samples and all five UK cases. 28 benign prostate hyperplasia (BPH), 168 UK prostate cancer cases (<10% were of Asian and none were of Chinese origin) and 143 Chinese prostate cancer cases (all Chinese ethnicity and from China), were collected for tissue microarray (TMA) studies. For both Chinese and UK patients, the majority were not PSA screened. Ethical approvals were obtained from each local ethics committee.

SNP Array Analysis

The predominant tumor nodule and adjacent normal tissue were identified by histopathologists (YY and DL) on fresh-frozen sections and macrodissected (mini-scalpel) or microdissected (PALM Microlaser Technologies, Bernried, Germany). Only foci with greater than 80% purity of cancer cells were macrodissected. SNP array analysis using Human SNP Array 6.0 (Affymetrix, Buckinghamshire, UK) was performed according to the manufacturer's instructions 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, St Louis, MO) 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 to case-matched normal controls. Unpaired Chinese tumor samples were analyzed against eight normal samples that were representative of the majority germline copy number variations. Within Partek GS, 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 0±0.4 log2 ratio. Regions considered positive by only one software (<5%) were subtle changes and were excluded from the final results.

PCR-based Molecular Copy-number Counting (MCC)

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 (Table S2). For each sample, 88 reactions were performed

in a 96-well plate with 8 non-DNA negative controls The presence or absence of PCR product (207 bp and 189 bp for *ERG*(+) and *ERG*(−) markers respectively) was visualised and scored, using the Diana V1.6 software (Raytest GmbH, Straubenhardt, Germany).

Fluorescent *in situ* Hybridization (FISH) Analysis of TMA samples

Representative BPH and cancer areas were identified on H&E stained sections by histopathologists (DB, GR and LB). TMAs were constructed by taking three cores of 1 mm diameter from each sample using a manual Tissue Arrayer (Beecher Instruments, Sun Prairie, WI).

FISH analysis for *TMPRSS2* and *ERG* rearrangements on TMAs was performed as previously described (9) using two bacterial artificial chromosomes (BACs), RP11-95I21 (5′ *ERG*) and RP11-476D17 (3′ *ERG*), described by Tomlins *et al* (10). BAC DNA was amplified using GenomiPhi amplification V2 kit (GE Healthcare, Bucks, UK) 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 (Maidenhead, UK) containing SpectrumOrange-labeled PTEN BAC probe and a SpectrumGreen-labeled chromosome 10 centromere control probe was used following the manufacturer recommended protocol. FISH slides were scanned using a 40x lens on the Applied Imaging Ariol® System (Applied Imaging, San Jose, CA, USA) 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 ABC (Avidin Biotin Complex) method (Vector ABC kit, Vector Laboratories Inc, Burlingame, CA) was employed for immunostaining with high-pressure cooking antigen retrieval. The mouse monoclonal NCL-PTEN primary antibody (1:150, Novocastra, Newcastle, UK) 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 record.

Statistical Analysis

Differences in continuous data were compared using the Student's t test. Differences in discreet data were compared using a Chi-square 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 summarised in Fig. 1A and Table S3. Common regions of chromosomal copy-number gains were 7 (11/39), 8q (16/39) and 3q (9/39) and losses were 5q15-21.3 (18/39), 6q14.1-22.1 (27/39), 8p (25/39), 13q12.3-31.1 (28/39) and 16q12.1-24.3 (20/39).

By comparison to data reported previously for Western prostate cancers (4, 11-13), we have identified genomic alterations common to 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 (27/39) and 13q12.3-31.1 (28/39) deletions detected in this study is greater than that previously reported for Western cancers (4, 12, 13). Tumour suppressor genes *RB, BRCA2* and *KLF5* are located at 13q12.3-31.1 and candidate tumour 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 21q22.2-22.3.3 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 over-expression 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 1/39 Chinese samples. In prostate cancer, *PTEN* is commonly inactivated and chromosome deletion account for the majority of PTEN loss-of-function cases (17). Despite occurring in about 40% of prostate cancer samples (12, 17, 18), deletion at 10q *PTEN* region was observed in only 3/39 Chinese cases (Fig. 1A and Table S3). Using the same methods, we analyzed five UK cancer samples and detected 3/5 and 4/5, respectively, for deletions at 10q23 and 21q22.2-22.3 (Fig. S1 and Table S4). Although the number of samples is small, the high frequency of 10q23 and 21q22.2-22.3 deletions detected in our UK samples indicates that our method is 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 UK samples 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*(−):*ERG*(+) ratios range from 0.90 to 1.04. Consistent with the 21q22.2-22.3 deletion detected by the SNP microarrays, a reduced number of *ERG*(−) products were observed in the UK samples with *ERG*(−):*ERG*(+) range from 0.40 to 0.73 (Table 1 and 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. BPH samples were used as controls. While *ERG* status was consistent in replicate cores, in some cases, *PTEN* deletion was only detect in one or two cores from a case. We considered cases to be PTEN deletion cases if *PTEN* signal was lost in any cores. We found that *ERG* 5′ region was deleted in 29.7% (46/155) and the 5′ and 3′ BAC signals were split in 11.6% (18/155) of UK samples (Table S5 and S7), but only in 5.4% (5/93) and 2.1% (2/93) of Chinese samples, respectively (Table S6 and S7). Representative FISH images are shown in Fig. 2. While the frequency of *ERG* rearrangements in UK samples is similar to that previously published for Western samples (12, 15, 16), they were observed less frequently in the Chinese samples ($P<0.001$, Table S7). Consistent with our SNP array results, the *PTEN* genomic region was deleted in 42.3% (66/156) of UK samples (Table S5 and S7), but only in 14.3% (12/84) of Chinese samples (Table S6 and S7), which is statistically significant ($P<0.001$). Representative FISH images are shown in Fig. 2. As PTEN can 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% (111/159) of UK samples (Table S5 and S7), but only in 34% (31/91) of Chinese samples (Table S6 and S7). This difference is statistically significant (P<0.001, Table S7). Representative images are shown in Fig. 2. *PTEN* deletion accounts for more than half of cases where PTEN expression is reduced and in most cases, deletion resulted in PTEN underexpression (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 than UK cases (P<0.001 for both, 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 co-existence of *TMPRSS2:ERG* fusion with both *PTEN* deletion and inactivation in the UK samples (P<0.001 and =0.01 respectively) (Table S8). It is, therefore, interesting to find that *ERG* and *PTEN* abnormality are the predominant genomic differences between Western and Chinese samples; supporting the co-function of *ERG* over-expression and *PTEN* inactivation in prostate cancer development and implying that Western men may 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 may 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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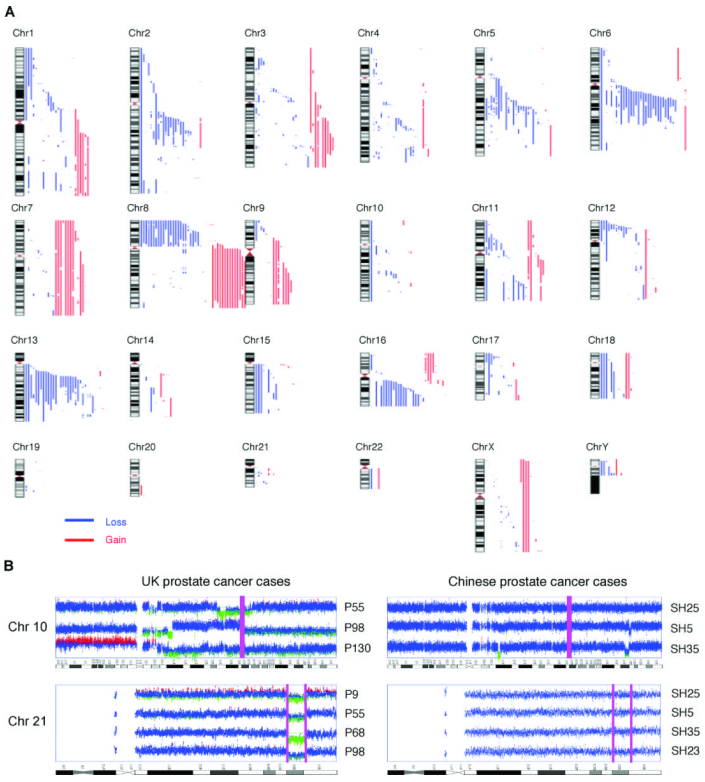

Figure 1.

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 Chromosome 10 and 21 in UK 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 UK but not Chinese samples. In each SNP intensity plot, the middle horizontal line represents a log2 ratio of 0 compared with normal controls. The bottom and upper lines represent log2 ratios of −1 and +1, respectively.

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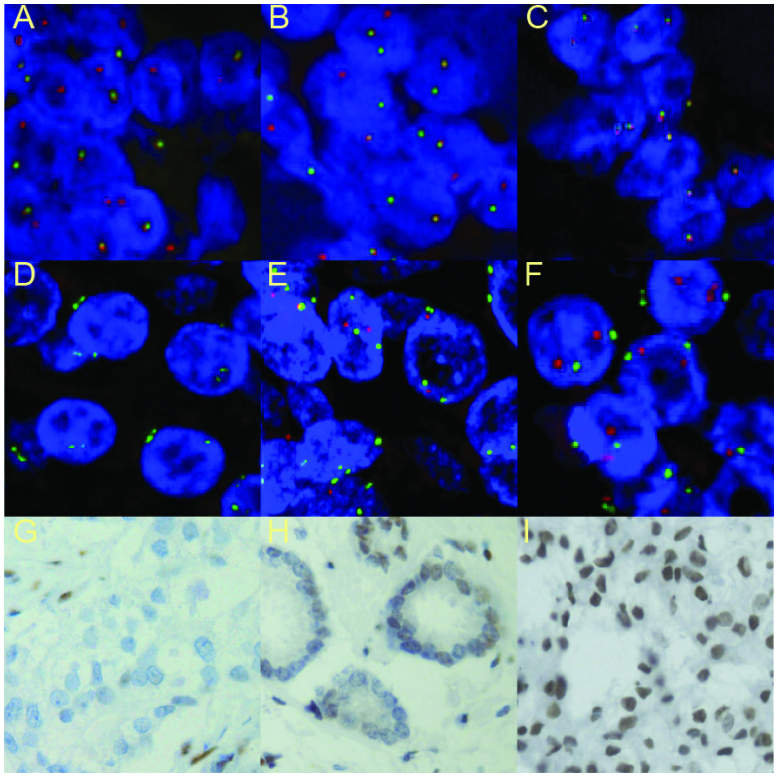

Figure 2.

Representative *ERG* and *PTEN* FISH images. **A-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 UK case with *ERG* deletion (**A**), a UK case with *ERG* split signals (**B**) and a Chinese case with no *ERG* deletion (**C**). **D-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 UK samples and no *PTEN* loss in a Chinese sample (**F**). **G-I**: Immunohistochemistry analysis shows loss of PTEN expression in cancer cells and positive stromal cells in a UK *PTEN* homozygous deletion case (**G**), lack of PTEN expression in a proportion of cancer cells from a UK partial *PTEN* deletion case (**H**) and strong PTEN expression (+++) in a Chinese sample without *PTEN* deletion (**I**).

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Table 1

Molecular copy-number counting confirms *ERG* deletion in four UK prostate cancer cases (P9-98) and lack of deletion in four Chinese prostate cancer cases (SH1-SH6).

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