Whole-genome and Transcriptome Sequencing of Prostate Cancer Identify New Genetic Alterations Driving Disease Progression


a Department of Urology, Shanghai Changhai Hospital, Second Military Medical University, Shanghai, China; b Biocenter Oulu, Faculty of Biochemistry and Molecular Medicine, University of Oulu, Oulu, Finland; c BGI-Shenzhen, Shenzhen, China; d China National GeneBank-Shenzhen, BGI-Shenzhen, Shenzhen, China; e Division of Biomedical Statistics and Informatics, Mayo Clinic College of Medicine, Rochester, MN, USA; f State Key Laboratory of Genetic Engineering, School of Life Sciences, Fudan University, Shanghai, China; g Division of Genomics and Bioinformatics, CUHK-BGI Innovation Institute of Trans-Omics, The Chinese University of Hong Kong, Hong Kong, China; h BGI Education Center, University of Chinese Academy of Sciences, Shenzhen, China; i School of Biological Science and Medical Engineering, Southeast University, Nanjing, China; j State Key Laboratory of Bioelectronics, Southeast University, Nanjing, China; k Department of Urology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China; l Department of Urology, The Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China; m Uro-Oncology Research Program, Department of Medicine, Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA; n Vancouver Prostate Centre and Department of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada; o Department of Pathology and Laboratory Medicine, David Geffen School of Medicine, University of California, Los Angeles, CA, USA; p Department of Urology, Changzheng Hospital, Second Military Medical University, Shanghai, China; q Department of Urology, Fudan University Shanghai Cancer Center, Shanghai, China; r Department of Pathology, Shanghai Changzheng Hospital, Second Military Medical University, Shanghai, China; s Program for Personalized Cancer Care, NorthShore University HealthSystem, Evanston, IL, USA; t Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA; u Department of Urology, Guangdong Key Laboratory of Clinical Molecular Medicine and Diagnostics, Guangzhou First People's Hospital, Guangzhou Medical University, Guangzhou, China; v James D. Watson Institute of Genome Sciences, Hangzhou, China; w Department of Biology, University of Copenhagen, Copenhagen, Denmark; x The Nova Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Copenhagen, Denmark; y King Abdulaziz University, Jeddah, Saudi Arabia; z Department of Biochemistry and Molecular Biology, Mayo Clinic College of Medicine, Rochester, MN, USA

1 These authors contributed equally to this study.
2 These authors are co-senior authors.
3 Deceased.
4 Corresponding author. Department of Urology, Shanghai Changhai Hospital, Second Military Medical University, Changzhai Road 168, Yangpu District, Shanghai 200433, China. Tel. +86-13601-607755; Fax: +86-21-3505-0006. E-mail address: sunyhsmmu@126.com (Y. Sun).
1. Introduction

Prostate cancer (PCa) is the second most frequently diagnosed cancer and the fifth leading cause of cancer death in men worldwide [1]. The disparity in PCa incidence and death rate is obvious around the globe. The reported incidence and mortality rate of PCa in Asian countries including China were much lower than in Western nations [2], with an estimated 60,300 new cases and 26,600 deaths in Chinese men in 2015 [3]. However, the PCa incidence rate increased rapidly in China with an annual percentage change of 12.6% since 2000 [3]. These findings not only highlight the complexity of genomic abnormalities in PCa, but also stress an urgent need of genome-wide molecular and genetic profiling of prostate tumors from different ethnic groups including Asian men.

Prostate tumors also show highly variable clinical outcomes. Some patients survive for over 10 yr after diagnosis, but others, particularly those with an aggressive phenotype, only survive for 2–3 yr. Given the dramatic differences in treatment response, great efforts have been made to investigate the genetic and epigenetic heterogeneity and cell signaling defects involved in PCa progression [4]. Many advanced approaches, including target-region sequencing, array-based gene expression, copy number variation (CNV), and whole-genome sequencing of tumor samples, have been taken to portray the genomic landscape in PCa [5–7]. These works have reported several PCa-related genomic alterations, including the most common TMPRSS2-ERG fusion, copy number gains of 8q, copy number losses of 3p, 8p, 10q, 13q, and 17p, as well as complex chains of oncogenic structural DNA rearrangements (chromoplexy). However, the functional consequence of many alterations remains unknown.

Recent exome sequencing of prostate tumors revealed specific genetic alterations in coding regions, leading to the identification of frequent mutations in the genes such as SPOP, FOXA1, TP53, and PTEN [8–10], and other genomic alterations in PIK3CA/B, ZBTB16/PLZF, and AR [11]. More recently, The Cancer Genome Atlas (TCGA) described a comprehensive genomic analysis of 333 PCa patients [12], and the International Cancer Genome Consortium performed genomic profiling of 477 localized, nonindolent prostate tumors [13], mainly in the Caucasian population. Together, genome-wide alterations in PCa have been extensively studied in men from Western populations. In contrast, while the incidence rate of PCa has been rising dramatically in cities such as Hong Kong and Shanghai [3], the landscape of genome alterations in Asian PCa cohorts remains incompletely characterized, becoming a major hurdle for comprehensive understanding of the molecular etiology of this fatal disease.
With the goal to systematically define the genomic alterations specifically occurring in Chinese PCa patients, we performed whole-genome and transcriptome sequencing for 65 tumor and the paired normal tissue samples (discovery cohort) and very deep gene-targeted sequencing for additional 145 tumor-normal pairs (validation cohort). By comparing our data with the TCGA datasets [12], we not only identified common genetic alterations among different ethnic groups such as SPOP, TP53, and PTEN, but also observed different frequencies of genomic alterations specifically associated with Chinese patients, including highly frequent deletion of CHD1 and relatively high mutation rates in androgen receptor (AR) upstream regulator genes including NCOA2. Furthermore, our analysis identified PCDH9 as a novel tumor suppressor gene (TSG) whose deletion is associated with poor survival in metastatic PCa patients. We also identified frequent alterations in axon guidance pathway genes including PLXNA1 whose upregulation and amplification are associated with PCa relapse and survival.

2. Patients and methods

2.1. Patients and samples

Treatment-naive prostate tumor and matched normal tissues were collected from the radical prostatectomy series at Shanghai Changhai Hospital and Fudan University Shanghai Cancer Center. The institutional review boards of both hospitals approved the experimental protocols. Informed consent was obtained from all participants. Hematoxylin and eosin (H&E) slides of frozen human tumor tissues and matched normal tissues were examined by a pathologist and another gynecologic pathologist to confirm histological diagnosis and Gleason score. They also verified the high-density cancer foci (>80%) of the selected tumor tissue, and the contamination free of the normal tissues. The frozen blocks for DNA/RNA extraction were examined by the pathologist as described above, followed by consecutive 10 × 10 μm cut of tumor section. These qualified samples were then used for DNA/RNA isolation. Subsequent sample annotation and preparation were prepared as described [6].

In total, we selected 65 radical prostatectomy specimens with a range of prostate tumor grades and stages from treatment-naive Chinese patients (Supplementary Table 1) for whole-genome and transcriptome sequencing, and obtained DNA and RNA sequencing data from paired tumor and adjacent benign tissues from these 65 patients (Supplementary Table 2). DNA and RNA preparation, and library construction are described in the Supplementary data.

2.2. Data preprocessing and reads mapping

Raw reads of DNA and RNA were filtered using an in-house pipeline based on the following procedure. Reads with sequencing adapters, more than 10% N bases or with low quality were removed. Ribosomal RNAs were removed by aligning the RNA reads to a combined reference sequence of ribosomal RNA from Ensembl, University of California, Santa Cruz, and SILVA database. Clean DNA reads were aligned to the human reference genome hg19 with Burrows-Wheeler Aligner and then processed with SAMTools [14] to remove the polymerase chain reaction duplicates. Clean RNA-sequencing reads were aligned to hg19 genome using Tophat [15]. Supplementary data includes the detailed description of the detection of somatic mutation, structure variation, gene fusion, and CNV, and experimental validation and clustered deleted TSG identification.

2.3. Gene expression quantification

Trapnell et al [16] (http://cufflinks.cbcb.umd.edu/index.html) was used to quantify the gene expression and identify differential gene expression according to the protocol and manual for each patient. Differential gene expression was identified using the threshold of false discovery rate <0.05. For genes differentially expressed in at least one patient, thresholds of fold change ≥2 and p value <0.05 were applied for the other patients with a false discovery rate ≥0.05.

Pathway enrichment and correlation network analyses are described in the Supplementary data.

2.4. Significantly mutated genes analysis

We employed Youn and Simon’s [17] method to predict the significance of gene mutations. The model evaluates both functional impact and mutation prevalence. To evaluate the functional impact, a mutation score was assigned base on BLOSUM80 in the following order: missense < inframe indel < mutation in splice sites < frame shift indel = nonsense. The p value was calculated from the background distribution computed by incorporating different background mutation rates of each sample and the observed mutation across samples.

2.5. Target sequencing of 293 gene panel in validation cohort

Genomic DNA from the 145 paired normal tumor samples of a validation cohort were fragmented to 150–200 bp and subjected to exon capture of 293 PCa relevant genes (Supplementary Table 3) including the 64 significantly mutated genes (SMGs) detected using an in-house pipeline [17] method to predict the significance of gene mutations. The model evaluates both functional impact and mutation prevalence. To evaluate the functional impact, a mutation score was assigned base on BLOSUM80 in the following order: missense < inframe indel < mutation in splice sites < frame shift indel = nonsense. The p value was calculated from the background distribution computed by incorporating different background mutation rates of each sample and the observed mutation across samples.

2.6. Tumor cell biology assays

Cell lines and culture, knockdown and overexpression, proliferation, invasion, migration, blotting, and reporter assay procedures are detailed in the Supplementary data.

2.7. Immunohistochemistry

Tissue microarrays from Shanghai Changhai Hospital (87 cases), Chinese PCa Consortium (419 cases), and Massachusetts General Hospital (213 cases) were used for immunohistochemistry (IHC) staining with the antibodies shown in Supplementary Table 4. The detailed protocol is described in the Supplementary data.

2.8. Animal studies

PC-3 1 × 10⁶ (with shControl and shPLXNA1, respectively), C4-2 (shControl and shPLXNA1, respectively), or DU-145 (vector and PLXNA1-overexpression, respectively), DU-145 (vector and PCDH9-overexpression, respectively) cells were mixed 1:1 with Matrigel (BD Biosciences, San Jose, CA, USA), and injected subcutaneous into nude mice (4–6 mice in each group). No blinding was done. For each group, tumor size was measured every 2 d using a caliper. Subcutaneous tumor volume was calculated as 0.52 × length × width. Mice were sacrificed 40–70 d after injection, and the tumors were dissected. The wet weights of the tumors were determined. Tumors were fixed in 4% formaldehyde and embedded in paraflin. Sections were stained with H&E and other markers by IHC.

All animal experiments were approved by our local animal ethics committee at Second Military Medical University, and were executed in accordance with animal care guidelines.
2.9. Analysis of clinical PCa data sets

Clinical data set, univariate, and multivariate analyses are detailed in the Supplementary data.

2.10. Statistical analysis

Statistical calculations were performed using the SPSS software (version 16.0; SPSS Inc., Chicago, IL, USA) and the statistical package, OriginPro 9 (OriginLab, Northampton, MA, USA). Survival curves were calculated by the Kaplan-Meier method and compared with the Log-rank test and Cox regression analysis.

3. Results

3.1. The genomic alteration landscape in PCa of Chinese men

In this study, tumor-normal paired samples from 65 Chinese PCa patients were analyzed using whole genome sequencing (Supplementary Table 1 and Table 1). These samples were treatment-naive radical prostatectomy specimens. We performed the whole-genome sequencing for three tumor-normal pairs at approximately 96× coverage and for the remaining 62 pairs at approximately 60× coverage. We also performed the whole transcriptome sequencing (RNA-seq) for these patients (Supplementary Table 2).

After mapping reads to the human reference genome, we detected somatic point mutations and somatic insertions and deletions (indels; < 50 bp) that were present in tumors and absent in adjacent normal samples (Supplementary Table 8). We next compared SNVs detected in coding regions with corresponding transcriptome sequencing reads. We classified these variations into four categories according to their expression patterns, as previously reported [22]: expressed, mutant biased, wild-type biased, and silent gene (Fig. 1D). Except for SNVs from a nonexpressed allele or those that were not sufficiently covered by the transcriptome sequencing reads, these sequencing variations showed a high degree of concordance between the genome and transcriptome, with more than 30% of the variations being covered by at least five reads from the transcriptome data.

We also identified 8159 somatic structural variations (SVs) at base-pair resolution (Supplementary Table 8). These SVs comprised 1286 interchromosomal translocations, 2472 intrachromosomal translocations, 2769 deletions, 1627 insertions, and five inversions (Fig. 1B). A total of 4651 events (57.0%) affected coding regions, of which 2274 (27.1%) were chromosomal translocations. We also detected somatic CNVs for each tumor-normal tissue pair in our PCa cohort (Fig. 1E). A total of 125 gene fusions were identified by integrative analysis of whole-genome and whole-transcriptome sequencing data. Four of these gene fusions were recurrent in our cohort, including ETS fusions (6/65 [9.2%], including 4 TMPRSS2-ERG), TCC6-MIPOL1 (4/65 [6.2%]), BPTF-LRRC37A3 (3/65 [4.6%]), and WWVP2-NFAT5 (2/65 [3.1%]; Supplementary Table 9). The prevalence of ETS family gene fusions (6/65) in our cohort (Fig. 1F) is much lower than that in Caucasian cohort (approximately 50%) as reported previously [23,24]. We confirmed the low prevalence of TMPRSS2-ERG fusion in our cohort by fluorescent in situ hybridization and breakpoint polymerase chain reaction followed by Sanger sequencing (Supplementary Table 9).
Figs. 3A–C). Low frequency of ETS family gene fusions has been reported in other cohorts of Asian PCa patients[25–30]. Thus, our data further confirm that low frequency of ETS fusion represents a salient genetic characteristic of PCa in Asian men.

3.2. Highly frequent deletion of the CHD1 gene

Previous studies in cell culture showed that the chromatin modifier CHD1 is required for the genesis of ERG fusions [31]. This finding provides a plausible explanation for a previous finding in PCa patient samples where CHD1 deletion (CHD1−) almost always coexists with ETS fusion negative (ETS−) [9]. In our discovery cohort, we not only showed that CHD1 deletion and ETS fusion are almost mutually exclusive (Fig. 1F; p = 0.0012, permutation test), but most importantly, we found that CHD1 loss/deletion is approximately in 31% (20/65) of prostate tumors in Chinese men examined (Fig. 1F) and that the deletion rate is almost two times higher than in TCGA patients (majority of them are Caucasian men, CHD1, 54/333 [16%]) [12]. In contrast, only approximately 6% of PCa in Chinese patients had ERG fusions (Supplementary Table 9). Thus, our data not only provide first-hand evidence that the CHD1 gene is frequently deleted in Chinese men, but further extend the previous finding in Caucasian patients that CHD1 deletion inversely correlates with ERG fusions in PCa. Next, we performed mutation co-occurrence and mutual exclusion analysis for important prostate relevant genetic alterations. We confirmed that CHD1 loss/deletion significantly co-occurs with SPOP mutation (Supplementary Fig. 4). We also observed CHD1 loss/deletion significantly co-occurs with SPOPL loss/deletion, PCDH9 loss/deletion in our dataset, and significant co-occurrence of PCDH9 and RB1 loss/deletion.
In addition, we validated these correlations by analyzing the TCGA published dataset [12]. The large number of patients in TCGA published dataset enable us to discover more genetic alterations correlated with CHD1 loss/deletion (Supplementary Fig. 4). We found in the TCGA published dataset that CHD1 loss/deletion also significantly co-occurs with loss/deletion of APC and RB1, respectively, and FOXA1 mutations, as well as mutual exclusive with mutation/loss/deletion of TP53 and PTEN, respectively.

3.3. Detection of mutations in AR upstream regulator genes in Chinese patients

Next, we identified a total of 64 SMGs in our discovery cohort (Supplementary Table 10). To validate this finding and explore more potential driver genes in PCa of Chinese patients, we focused on a set of 293 PCA relevant genes (Supplementary Table 3), which include these 64 SMGs and others reported previously [8,9]. We then performed targeted deep sequencing (approximately 243 x) for the 293 genes using a customized capture array in a validation cohort of 145 matched tumor-normal pairs (Supplementary Tables 11 and 12).

While our analysis revealed that most of the SMGs, including SPOP, TP53, ATM, PTEN, and CTNNB1 were commonly mutated in our cohorts of Chinese men and the TCGA cohort of Caucasian patients (Fig. 2A, Supplementary Table 13), we found that alteration frequencies in chromatin remodeling and histone modification genes such as MLL3 (13/210 [6.19%]) and transcription coregulator genes such as FOXA1 (13/210 [6.19%]) and NCOR2 (7/210 [3.33%]) were relatively higher in our discovery and validation cohort of clinically localized PCa compared those in the TCGA dataset (Fig. 2A, Supplementary Fig. 6). Among the frequently mutated genes, NCOR2 was found mutated in our cohort (3.33%), but not in the TCGA cohort (Fig. 2A). NCOR2 encodes a transcriptional coregulator existed in a protein complex containing histone deacetylases that modifies chromatin structure and serves as a transcriptional corepressor for the nuclear receptor superfamily of transcription factors [32] including AR in PCa. We also observed frequent NCOR2 expression alterations in our discovery cohort of PCa (Supplementary Table 7). Furthermore, we found that steroidogenic enzyme genes such as CYP11B1 and HSD14B4 were mutated in our cohorts, but little or not in TCGA patients (Fig. 2A). In contrast, we found no apparent alterations of AR in our cohort (Fig. 2B). Thus,
Fig. 3 – Association between PCDH9 deletion and its expression, and prostate cancer (PCA) aggressiveness. (A) Landscape of copy number alteration in Chinese cohort of PCA. (B) Statistically significant copy number alterations were shown on 13q21.31-q21.33 region. The G-score was calculated using GISTIC algorithm. Genomic positions of tumor suppressor genes (TSGs) within PCDH9-DACH1-KLF5-LECT1-OLFM4 cluster were determined. (C,D) PCDH9 expression in prostate tissue samples with different somatic copy number alteration status. Note that PCDH9 deletion was significantly associated with its downregulation in PCA samples. PCDH9_wt, samples with wild type PCDH9; PCDH9_loss, samples with PCDH9 loss; PCDH9_deletion, samples with...
while there were no detectable alterations in the AR gene itself, we found that the mutation rate in AR upstream modulator genes was relatively higher in PCs in Chinese men in comparison to that in Caucasian patients.

3.4. Identification of highly frequent deletion of the adhesion gene PCDH9

To identify additional PCs relevant genes, we next assessed broader DNA copy number changes in our cohort. Overall, we found very few large regions with significant gain in copy numbers, except for the known amplifications at chromosome 8q (8q13, 8q21, 8q22, 8q23 and 8q24) [6,24,33], a region harboring oncogenes such as MYC (8q24.21), PVT1 (8q24.21), and NCOA2 (8q13.3; Fig. 3A, Supplementary Table 14). In contrast, we found frequent large deletions in several genomic regions, including chromosome 5q (eg, 5q21 with CHD1, RGMB, and 5q22.1—q22.2 with APC), 6q, 8p, 10q (10q23.31 with PTEN), 13q (13q14.2 with RB1), and 16q (Fig. 3A, Supplementary Fig. 5), which is consistent with the findings observed previously [6,24,33]. The deletion of chromosome 2q (2q14.3, 2q22.3) was also reported by Taylor et al [6]. Notably, compared with the CNV profiling from Taylor et al [6] and Ross-Adams et al [24] there was no significant deletion of chromosome 21q (21q22) in our cohort. Chromosome 21q (21q22) was frequently deleted in PCs, the deletion of which would lead to the formation of TMPRSS2-ERG fusion [6,23,24]. This indicated that the low frequency of TMPRSS2-ERG fusion in our cohort might be induced by the lack of interstitial deletion of the intervening genomic region between ERG and TMPRSS2 genes on chromosome 21 (deletion).

In addition, we also observed novel frequent large chromosomal deletions in our cohort, which include an approximately 8.6-Mb deletion [24/65 [36.9%]] at 2q22.1-q22.3 containing 12 genes such as SPOPL, an approximately 8-Mb deletion (31/65 [47.7%]) at 13q21.31-q21.33 harboring 10 genes such as PCDH9, and an approximately 0.15-Mb deletion (21/65 [32.3%]) at 16q24.1 encompassing six genes such as FOXL1, FOXC2, and FOX1 (Supplementary Table 14). We next performed integrative analysis of data from somatic copy-number alteration, corresponding gene expression, and somatic mutations in our cohort, and built a pipeline to identify putative clustered deleted TSGs, which is defined as a cluster of deleted genes located in close proximity or neighboring genes in the same large deletion region. In total, we identified five high-confidence clustered deleted TSGs within seven large copy number deletion regions (Supplementary Tables 15–17).

We next focused on PCDH9, as it was the most frequently deleted gene in comparison to others in the large deletion region at 13q21.31-q21.33 (Fig. 3B). In addition, PCDH9 was differentially expressed (rank sum test p < 0.05) in the 65 tumor samples compared with that in the matched normal tissues, whereas RB1 expression showed no difference (p = 0.481; Supplementary Fig. 5B). PCDH9 deletion was significantly correlated with its reduced expression within the same set of tumors (p = 1.74 × 10⁻⁻, t test; Fig. 3C). This concordance was also observed in additional clinical dataset of PCs (Fig. 3D, Supplementary Fig. 7A) [6,12]. Interestingly, we noticed that PCDH9 was downregulated in a subset of tumors without PCDH9 loss/deletion (p = 7.62 × 10⁻¹⁰, t test; Fig. 3C), despite the observation that the expression level of PCDH9 was much lower in tumors with PCDH9 loss than the ones without copy number loss (p = 7.13 × 10⁻⁴, two-tailed t test; Fig. 3C), suggesting that mechanisms other than genomic deletion may also contribute to the reduced expression of PCDH9 in prostate tumors.

3.5. PCDH9 is a TSG with prognostic potential in PCs

PCDH9 is a member of the protocadherin family and functions in cell-cell adhesion, neural projection, and synapse formation [34]. Recent evidence showed that loss of PCDH9 expression was associated with higher histological grade and poor prognosis in glioma [35], implying a tumor suppressor role for PCDH9 in cancer. To determine whether this is the case in PCs, we investigated PCDH9 messenger RNA (mRNA) expression across several independent clinical data sets [6,9,36–39]. The results demonstrated that PCDH9 expression was dramatically downregulated during progression of PCs to the advanced/metastatic stage in multiple cohorts of data sets (Fig. 3E–G, Supplementary Figs. 7B–H). Moreover, PCDH9 downregulation markedly correlated with elevated levels of prostate-specific antigen and high clinical stage of prostate tumors (Fig. 3H, Supplementary Fig. 8). Furthermore, the time to biochemical relapse was significantly shorter in the group of PCs patients with lower PCDH9 expression (Fig. 3I, Supplementary Figs. 9A and 9B), though this association was not observed in additional three PCa datasets by querying the camcAPP, a user-friendly web interface [40], probably due to biological heterogeneity in different patient cohorts. This analysis also showed that PCDH9 was a better predictor than any other individual gene within this region in two clinical PCa datasets [39,41] (Supplementary Table 18).

Given that PCDH9 loss/deletion correlates with PCDH9 downregulation (Fig. 3B–D) and that PCDH9 downregu-
tion is detected in advanced tumors (Fig. 3C–D, Supplementary Fig. 7), we explored whether PCDH9 loss/deletion directly correlates with PCA aggressiveness. This analysis revealed that PCDH9 loss/deletion indeed showed to be associated with PCA recurrence [6,34] (Fig. 3, Supplementary Fig. 9C). Moreover, PCDH9 loss/deletion frequently occurred in metastatic prostate tumors and castration-resistant PCa (CRPC) patients (Fig. 3K, Supplementary Figs. 9D and 9E), and significantly associated with decreased overall survival of metastatic PCA patients (Fig. 3L, Supplementary Figs. 9F and 9G), indicative of the aggressive nature of tumors with PCDH9 copy number loss/deletion. Taken together, these results suggest a prognostic value of PCDH9 loss/deletion and downregulation in PCa and strongly imply a tumor suppressor role of PCDH9 in PCa development and progression.

We next examined whether PCDH9 plays a causal role in prostate oncogenesis. We demonstrated that transient knockdown of PCDH9 by small interfering RNA led to increased cell proliferation, migration, and invasion in PCa cell lines LNCaP and DU145, and immortalized prostatic epithelial cell line RWPE-1 (Fig. 4A–C, Supplementary Figs. 10A–E). Accordingly, ectopic expression of PCDH9 significantly attenuated Pca cell proliferation, migration, and invasion (Fig. 4D, Supplementary Fig. 10F). In addition, we observed that knockdown of PCDH9 highly promoted colony formation in RWPE-1 cells (Supplementary Fig. 11). We further evaluated the effect of PCDH9 on tumor growth in vivo using subcutaneous transplantation of DU145 cells with stably overexpressed PCDH9 (DU145-PCDH9) or control (DU145-pReceiver) into nude mice. We found markedly reduced tumor volume and weight in the mice transplanted with DU145-PCDH9 (Fig. 4E and 4F). H&E staining revealed prostate tumors formed in mice (Fig. 4G). IHC analysis showed apparent overexpression of PCDH9 and reduction of proliferation marker Ki-67 [42] in the xenograft tumors derived from DU145-PCDH9 cells (Fig. 4G, Supplementary Fig. 12), suggesting that inhibited growth of PCDH9 overexpression tumors in vivo is due at least in part to reduced cell proliferation.

To further understand the role of PCDH9 in PCa, we sought to identify potential downstream target genes of PCDH9. We thus performed genome-wide expression profiling of DU145-PCDH9 and DU145-pReceiver cells (Fig. 4H, Supplementary Table 19). We found that overexpression of PCDH9 downregulated oncogenic drivers of PCa such as MYB and STEAP1, and the highly prostate-specific HOXB13, and the cancer stem cell marker ALDH1A1, and upregulated tumor suppressors including FOXO4, EPHB2, and PBX1, and the epithelial-mesenchymal transition (EMT) marker CDH1 (Fig. 4H, Supplementary Figs. 13A and 13B). We also used the classic PCa cell line C4-2 to validate PCDH9 affected genes, which is consistent with the results from DU145 (Supplementary Fig. 13B). Intriguingly, querying several clinical PCA data sets [6,12,36,38], we observed inverse correlations between PCDH9 and MYB, STEAP1, or HOXB13 expression, and positive correlations between PCDH9 and FOXO4, EPHB2, or PBX1 (Figs. 13C–H, Supplementary Table 19), indicating that PCDH9 may impact these gene expression in the clinical setting. Together, these data suggest that PCDH9 plays a tumor suppressor role in PCa by suppressing oncogenic and enhancing tumor suppressive pathways.

3.6. Frequently altered axon guidance pathway genes in PCa

We next investigated genes with genomic alterations (somatic mutations, copy number, and structural variations) and changed expression in known and novel pathways that impact PCa progression and aggressiveness. Consistent with previous studies [5], in our cohort we also found frequent alterations in genes present in phosphoinositol 3-kinase, retinoblastoma protein, RAS/RAF, and AR signaling pathways (Fig. 5A, Supplementary Table 20) that are known to be important in PCa [4]. To identify novel progression pathways in PCa, we mapped genes affected by SNVs, indels, CNVs, and SVs to canonical pathways and performed enrichment analysis for genes with different variation types. Notably, the axon guidance pathway genes were consistently found to be significantly altered by different variation types, particularly by SNVs and SVs (p < 0.01, hypergeometric test; Fig. 5B, Supplementary Table 21). We analyzed genetic data from an independent cohort of PCa [18] and found that the axon guidance pathway was also significantly (p < 0.01, hypergeometric test) altered with SV-disrupted genes (Supplementary Table 21). These analyses suggest a novel link between frequent somatic alterations in axon guidance pathway genes and the pathogenesis of PCa, and a likely role of axon guidance pathway in PCa development.

The axon guidance pathway consists of semaphorins, slits, netrins, and ephrins that were originally characterized as the guidance of axons during embryonic development. Recently, this pathway has been found to modulate cancer cell growth, survival, invasion, and angiogenesis [43,44]; however, somatic alterations in axon guidance pathway genes are not fully understood in PCa. Here we found frequent SV alterations in ROBO1 (15.38% [10/65] of patients) and SLIT2 (6.15% [4/65] of patients) involved in SLIT-ROBO signaling (Supplementary Table 22), which are similar to the mutation frequency observed previously in pancreatic cancer [43], suggesting that aberrant SLIT-ROBO signaling may be common in cancer. To examine the related gene expression of these genomic alterations, we next integrated genome rearrangement events in axon guidance pathway genes with corresponding RNA-seq data in our study cohort (Supplementary Fig. 14A). This analysis revealed that low expression of SLIT2 was markedly correlated with the prevalence of SVs in four tumors. Particularly in the CH33 tumor, we observed a high number of RNA-seq reads in the left region of the breakpoint and less reads in the right region. Low expression of SLIT2 protein in tumors was confirmed by IHC (Supplementary Fig. 14B). Given that SLIT2 copy numbers in tumor and normal tissues of sample CH33 were highly accordant, abnormal expression of SLIT2 was likely to be caused by intrachromosomal translocation. Together, whether SV alterations
Fig. 4 – In vitro and in vivo functional validation of PCDH9 as a tumor suppressor in prostate cancer. (A) Cell proliferation was measured by CCK-8 assay (absorbance at 450 nm) in the prostate cell line DU145 in given time points. (B) Quantitation of relative invasion for the cells transfected with control and the efficient small interfering RNAs (siRNAs) against PCDH9. (C) Quantitation of relative migration for the cells transfected with control and the efficient siRNAs against PCDH9. (D) Attenuation of PCa cell proliferation, invasion and migration by ectopic overexpression of PCDH9. (A–D) All experiments were performed in triplicate. Error bars, standard deviation. Results were statistically evaluated with two-tailed t-test. (E) Subcutaneous xenograft assay showed reduced tumor growth of DU145 cells stably expressing PCDH9 compared with control group \( (n=6 \text{ mice per group}; \text{two-tailed } t\text{-test}). \) (F) Scatter plot showed tumor weights from individual mice in each group \( (n=6 \text{ mice per group}; \text{two-tailed } t\text{-test}). \) (G) Hematoxylin and eosin (H&E) and immunohistochemistry analysis of Ki-67, PCDH9 expression in DU145 xenograft tumors. Representative images from six independent samples are shown. Original magnification, 400×; scale bars: 20 μm. (H) Heat map shows selected differential expression genes identified by genome-wide transcriptional profiling of DU145 cells stably expressing PCDH9 (DU145P) and control vector (DU145M) with three replicates, respectively. The color code represents log2 transformed R/G fold ratios.

OD = optical density

\* \( p < 0.05. \)

\** \( p < 0.01. \)

\*** \( p < 0.001. \)
in SLIT-ROBO signaling impacted prostate carcinogenesis through disrupting gene expression warrants further investigation.

We also observed frequent gain/amplifications (eg, PLXNA1, PLXNA3, PLXNB2, and PLXNB3) or deletions (eg, DPYS12 [also termed collapsing response mediator protein-2, CRMP2] and FYN) in semaphorin signaling pathway genes in about 80% of tumors (Fig. 5C, Supplementary Table 22). To assess potential functional impact of these alterations in axon guidance pathway (especially semaphorin signaling) in our cohort, we thus performed a weighted gene expression correlation network analysis [45] of the dysregulated genes in axon guidance pathway and phosphoinositide 3-kinase/AKT signaling pathways. This analysis revealed that genes in the semaphorin signaling pathway showed coexpression pattern genes in the phosphoinositide 3-kinase/AKT pathway and AR signaling (Supplementary Fig. 15A), indicating the potential biological cooperation of these pathways in PCa. Moreover, it has been reported that activation of FYN kinase leads to phosphorylation of CRMP2 by CDK5, which primes further phosphorylation of CRMP2 by GSK3β and reduces affinity of CRMP2 for tubulin heterodimers, microtubule growth, and cell collapse [46,47]. Thus, it can be postulated that loss of FYN may lead to activation of CRMP2 and axon growth, but further deletion of CRMP2 may prohibit
microtubule growth, resulting in loss of cell polarity, and cell dedifferentiation (Fig. 5D).

3.7 High frequent PLXNA1 amplification in PCa

To evaluate the potential role of axon guide pathways in PCa, we focused on PLXNA1, an amplified upstream activator of the pathway (Fig. 5C, Supplementary Fig. 15B). We found that PLXNA1 knockdown attenuated the expression of AR target genes (Supplementary Fig. 15C), and androgen-induced AR transcriptional activity measured by luciferase assays (Supplementary Fig. 15D) in the tested PCa cell lines. We also observed that knockdown of PLXNA1 inhibited nerve growth factor-induced AKT phosphorylation, while overexpression of PLXNA1 promoted AKT phosphorylation (Supplementary Fig. 15E). In addition, we noted that there was very scant basal expression of PLXNA1 protein in two tested AR-positive CRPC cell lines relative to PC3, an AR-negative CRPC cell model (Supplementary Fig. 15D). Consistent with this, we observed an increased expression of PLXNA1 in LNCaP treated with AR antagonist MDV3100 (Supplementary Fig. 16A). Moreover, the analysis of a large cohort of PCa dataset revealed a negative correlation between AR and PLXNA1 expression (Supplementary Fig. 16B). In two additional independent large patient cohorts [9,48], we observed higher mRNA levels of PLXNA1 in neuroendocrine CRPC with attenuated AR expression and signaling than that in adenocarcinomas CRPC (Supplementary Fig. 16C and 16D), suggesting an association between AR signaling, transcriptional activity and PLXNA1 expression. Consistent with this, we observed a strong AR binding at PLXNA1 in PCa cells VCaP upon the stimulation of AR signaling in a publicly available AR ChIP-seq data [49] (Supplementary Fig. 16E). Together, these studies further support potential cross-talk between these pathways. Given the known roles of AKT and AR signaling in PCa development [4] and axon guidance pathway in many types of tumors [44], our results imply a previously uncharacterized function of semaphorin signaling gene PLXNA1 in PCa.

Notably, PLXNA1 was one of the most frequently altered semaphorin signaling genes with alterations in 23% of our samples by gain/amplification and upregulation (Supplementary Table 22). Accordingly, PLXNA1 amplification was markedly associated with its increased expression (Fig. 6A and 6B) in two independent clinical data sets [12,50], which may lead to altered activity of PLXNA1 in PCa cells. Thus, we decided to investigate the functional roles of PLXNA1 in PCa.

3.8 PLXNA1 alterations impact PCa progression and prognosis

To characterize the role of PLXNA1 in PCa, we first examined the effect of PLXNA1 knockdown on cellular phenotypes (Supplementary Fig. 17A). We observed that depletion of PLXNA1 greatly attenuated the proliferation, invasion and migration of all tested PCa cell lines (Fig. 6C–E, Supplementary Figs. 17B–E). Consistently, ectopic overexpression of PLXNA1 strikingly promoted the proliferation, invasion and migration of DU145 cells (Fig. 6F, Supplementary Fig. 17F), in line with observed high expression of PLXNA1 in aggressive PCa cell lines, including ARCaPM and LNCaP[RANKL] (Fig. 6G). Both cell lines exhibit mesenchymal features with a high metastatic propensity to bone and soft tissue (Fig. 6G), and was therefore used to evaluate the effect of PLXNA1 knockdown on aggressive phenotypes of PCa cells. Given that EMT, stemness, and neuroendocrine phenotypes are associated with aggressive metastatic CRPC [4], we asked whether PLXNA1 knockdown affects the expression of markers associated with these aggressive phenotypes. Strikingly, PLXNA1 knockdown in LNCaP[RANKL] and ARCaPM cells resulted in reduced expression of the mesenchymal biomarkers vimentin, N-cadherin and fibronectin, and upregulation of E-cadherin, an epithelial marker (Fig. 6H). Moreover, we observed suppression of stem cell markers CD44, CD133, CD49f, OCT4, NANOG, SOX2, and LIN28B, and neuroendocrine markers CgA, SYP, and FOXA2 (Fig. 6H). Consistently, EMT, stemness, and neuroendocrine phenotypes were enhanced by ectopic overexpression of PLXNA1 in tested PCa cell lines (Supplementary Fig. 17G).

We further evaluated the effect of PLXNA1 knockdown on tumor growth in vivo using xenograft mouse model. We found greatly decreased tumor volume and weight in the mice transplanted with short hairpin RNA (shRNA)-mediated PLXNA1 knockdown cells (Fig. 6I). Consistent results were obtained when overexpression of PLXNA1 significantly promoted the growth of xenograft tumors (Fig. 6I). Histopathological analysis of PLXNA1 knockdown tumors confirmed a marked suppression of tumor cell proliferation as assessed by Ki-67 immunostaining (Fig. 6J). Moreover, compared with controls, PLXNA1 knockdown tumors indicated reversal of EMT phenotype, characterized by increased E-cadherin staining, reduced expression of N-cadherin, vimentin and fibronectin, and repression of neuroendocrine phenotype in PCa indicated by weaker immunostaining of CgA and Syp (Fig. 6J), which is consistent with our observations in PCa cells with aggressive properties (Fig. 6G). Collectively, these data have established the importance of PLXNA1 for PCa cell growth and metastasis, and suggest a link between high PLXNA1 expression and prostate tumor aggressive phenotype, implying that highly frequent PLXNA1 alterations observed in our study cohort (Fig. 5C, Supplementary Table 22) might impact PCa progression and prognosis in a diagnostic setting.

To assess clinical impact of PLXNA1 on human PCa progression, we first examined potential correlation between PLXNA1 expression and disease severity. We found that PLXNA1 expression is greatly upregulated upon PCa progression to metastatic stage (Fig. 7A, Supplementary Fig. 18A–D) in multiple clinical data sets [9,37,51]. Furthermore, PLXNA1 upregulation is significantly correlated with elevated prostate-specific antigen level and high clinical stage of PCa patients [6,52] (Supplementary Fig. 18E–G). Immunostaining of PLXNA1 in an independent cohort of 87 primary PCa samples showed that increased PLXNA1 expression was associated with high Gleason score, advanced tumor stage, and early biochemical recurrence after prostatectomy (Fig. 7B). The latter observation indicates poorer prognosis of PCa patients with higher levels of PLXNA1. Consistent with this finding, by querying from five independent clinical PCa
Fig. 6 – Functional validation of PLXNA1 in prostate cancer cell growth and tumor progression. (A,B) A significant correlation between PLXNA1 amplification and overexpression was observed in two independent cohorts of human prostate cancer samples. Mann-Whitney U-tests were performed to evaluate statistical significance for the comparisons between groups. (C) Decreased cell proliferation was observed in the tested prostate cell line PC3 via CCK-8 assay at the indicated time point. (D) Quantitation of cell invasion following transfection with nontargeting small interfering RNA (siRNA) or siRNAs against PLXNA1. (E) Quantitation of cell migration following transfection with non-targeting siRNA or PLXNA1 siRNA. (F) Ectopic
tumor growth and progression, and worse prognosis of the
including amplification and overexpression could confer
Altogether, our results indicate that
PLXNA1
and CRPC patients[6] (Fig. 7E, Supplementary Fig.18M), and
copy number gain was highly frequent in metastatic PCa
PLXNA1
er
correlates with PCa aggressiveness, we investigated wheth-
PLXNA1
cohorts (Fig. 7C and 7D, Supplementary Table 23). We also
recurrence, metastasis-free and overall survival in both
prognostic variable of PCa for predicting biochemical
analyses suggest that PLXNA1 is a new biomarker that
associations not only with biochemical recurrence but also
with metastasis-free and overall survival (Fig. 7C and 7D).
Notably, multivariate and univariate regression analyses
revealed that PLXNA1 upregulation was an independent
prognostic variable of PCa for predicting biochemical recurrence,
metastasis-free and overall survival in both
cohorts (Fig. 7C and 7D, Supplementary Fig. 19). Together, these
analyses suggest that PLXNA1 is a new biomarker that
distinguishes aggressive disease.
Finally, given that PLXNA1 gain/amplification correlates
with its expression and that PLXNA1 expression in turn correlates with PCa aggressiveness, we investigated wheth-
er PLXNA1 copy gain directly correlates with relapse status
of patients with PCa. This analysis revealed that PLXNA1
copy number gain was highly frequent in metastatic PCa and
and CRPC patients [6] (Fig. 7E, Supplementary Fig. 18M), and
in primary PCa tumor samples with near 50% rate of
biochemical relapse by querying the cameAPP database
[24,40] (Supplementary Fig. 18N). In addition, our analysis
of a TCGA cohort of PCa showed that PCa patients with copy
number gain of PLXNA1 had significantly higher risk of
biochemical recurrence than the patients having tumors with no copy number changes of PLXNA1 [38] (Fig. 7F).
Altogether, our results indicate that PLXNA1 alterations
including amplification and overexpression could confer
tumor growth and progression, and worse prognosis of the
patients with PCa.

4. Discussion
Emerging evidence indicates that there are remarkable
disparities in PCa epidemiology among different ethnic
groups [28,53]. However, the underlying molecular mecha-
nisms remain largely unknown. Notably, recent studies
consistently show that the incidence of ETS family gene
fusions is much lower (ranging from 8% to 21%) in PCa in
Asian men compared with the prevalence of approximately
50% in Caucasian patients from Western countries
[26–30]. In agreement with these findings, we detected a
low prevalence (9.2%) of ETS fusions in our cohort. Previous
studies demonstrate that the tumor suppressor CHD1 is
required for ERG rearrangement in PCa [30] and that CHD1
deletion is almost always correlated with ETS-negative PCa
[9]. Compared with the frequency (16%) of CHD1 deletion in
the TCGA dataset [12], we observed a much higher
prevalence (31%) of CHD1 deletion in the Chinese cohort
we studied. Thus, our discovery of the inverse correlation of
CHD1 deletion and ETS fusions between Chinese and
Caucasian populations provides a plausible explanation as
to why the rate of ETS fusions is lower in Chinese PCa
patients.
A recent study found that the parasympathetic nervous
system and sympathetic nervous system played key roles in
triggering PCa and influencing metastasis [54]. Nerves of
the sympathetic nervous system and parasympathetic
nervous system promote tumor growth by producing
norepinephrine and acetylcholine, which activate a signal-
ning pathway within stromal cells in tumor microenviron-
ment [54]. However, the mechanism by which nervous
system directly affects PCa cell growth remains largely
unknown. Axon guidance pathways are critical for neural
development. It has been shown previously that activation
of PLXNA2 by semaphorin 3A leads to activation of the
FYN-CDK5 pathways, which not only causes reduced
microtubule growth in the distal end of axon and growth
cone collapse by inducing GSK3β-mediated phosphoryla-
tion of CRMP2 and lost affinity of CRMP2 for tubulin
heterodimers, but also promotes inhibition of the RAC-PAK
pathway [46]. Notably, we found that both FYN and CRMP2
genes were frequently deleted in our cohort and TCGA
patients. These findings suggest that coordinated deletion
of FYN and CRMP2 signaling may lead to a loss of cell
polymer and dedifferentiation of prostatic cells, and further

overexpression of PLXNA1 dramatically promoted PCa cell proliferation, invasion and migration. (C-F) All experiments were performed in triplicate.
Error bars, standard deviation. Results were statistically examined by two-tailed t-test. (G) Immunohistochemistry analysis of PLXNA1 expression in
human prostate cancer cell lines ARCaPM and LNCaPRANK. PLXNA1 expression in murine xenograft tumors induced by injecting these cells either
subcutaneously or orthotopically and bone metastases. (H) Reversal of EMT and decreased expression of the stem cell marker and neuroendocrine
phenotypes in LNCaPRANK and ARCaPM following siRNA-mediated knockdown of PLXNA1. The quantitative reverse transcription-polymerase chain
reaction results were shown as mean ± standard deviation. of triplicate values for each sample. (I) Growth curves for subcutaneous xenografts derived
from C4-2 (n = 6 mice per group) and PC-3 cells (n = 6 mice per group) stably expressing a short hairpin RNA (shRNA) against PLXNA1 (shPLXNA1) or
scrambled shRNA (shControl). Bottom panel indicates growth curves for subcutaneous xenografts (n = 4 mice per group) derived from DU145 with
stable expression of PLXNA1 or control vector. Scatter plots (right) show tumor weights from individual animals in each group. (J) Hematoxylin and
eosin staining and immunohistochemistry analysis of Ki-67, PLXNA1, E-cadherin, N-cadherin, vimentin, fibronectin, SYN, NSE, and CgA expression in
PC-3 cell-derived xenograft tumors. Representative images from six separate tumors are shown. Original magnification, 400×; scale bar 20 μm.
OD = optical density; TCGA = The Cancer Genome Atlas.
* p < 0.05.
** p < 0.01.
*** p < 0.001.
Fig. 7 – PLXNA1 upregulation in human prostate cancer (PCa) correlates with tumor progression and the risk of biochemical relapse and reduced survival. (A) PLXNA1 messenger RNA expression is strikingly upregulated in metastatic prostate tumors from a clinical PCa dataset. (B) Association of PLXNA1 overexpression with Gleason grade, tumor stage, and biochemical recurrence. The *p* values were assessed using chi-square test. Kaplan-Meier curves show the risk of biochemical recurrence after radical prostatectomy for the patient groups stratified by PLXNA1 protein expression levels determined by immunostaining. The *p* value was determined using log-rank test. (C,D) Kaplan-Meier analysis of the risk of biochemical recurrence and reduced progression/metastasis-free and overall survival in prostate tumors with high or low protein levels of PLXNA1 in two independent cohorts of PCa cases. (C,D) The *p* values were assessed by a log-rank test and Cox regression analysis, respectively. (C) Numbers of patients in each category were
investigation of this concept is warranted. Additionally, our weighted gene expression correlation network analysis showed that amplification of PLXNA1 and deletion of the FYN-CRMP2 signaling axis correlates with deregulation of the PI3K/AKT and AR pathways, activation of which was validated in PLXNA1-overexpressed PCa cells.

Interestingly, our analyses revealed potential involvement of axon guidance pathway in PCA progression. We identified frequent and diverse somatic aberrations in genetic components of the axon guidance pathway. Our experimental evidence from gene and protein expression, in vitro and in vivo assessments indicate that axon guidance pathway gene PLXNA1 is involved in PCA aggressiveness. Patients with higher level of PLXNA1 expression in PCa are at markedly higher risk of having biochemical relapse and decreased metastasis-free and overall survival in multiple independent cohorts. Given that invasion- and metastasis-inhibiting and antiangiogenic agents such as anti-PLXNB1 and Uncl-Sema3E (binds to PLXND1) antibodies have been reported [55], PLXNA1 is expressed on the cell surface and may therefore be easily targeted by these agents. Further studies will aim to define PLXNA1 as a potential marker to risk-stratify patients with PCa and to investigate PLXNA1 protein as an effective therapeutic target to treat advanced PCa.

5. Conclusions

In summary, we report the first comprehensive mutational landscape of PCa in Chinese men, which will provide an invaluable resource for in depth comparison of genomic alterations in PCa across ethnic groups. Our integrated analysis of whole-genome and transcriptome data from the same patient reveals potential disease relevant mutations and copy number alterations in AR pathway genes, cell adhesion molecules such as PCDH9, and axon guidance pathway genes such as amplification of PLXNA1 and deletion of FYN and CRMP2. While further functional assessment of these genetic alterations is warranted, our findings contribute to the understanding, prediction, prognosis, and precise treatment of PCa in men with vast ethnic disparity.

Author contributions: Yinghao Sun had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Sun, Ren, Wei, Hou.

Acquisition of data: Ren, Wei, Liu, Wang, Yin, Gao, Xu, Ye, Xu, Gao, Zhou, Yang, Hou, Zhang, Zhu, Qin, Shao, Pang, Huang, Sun.


Drafting of the manuscript: Ren, Wei, Liu, Hou, Huang.

Critical revision of the manuscript for important intellectual content: Ren, Wei, Liu, Wang, Wu, Zhong, Huang, Sun.

Statistical analysis: Ren, Wei, Wang, Liu, Xu, Huang.

Obtaining funding: Ren, Wei, Yang, Wang, Sun.

Administrative, technical, or material support: Ren, Wei, Liu, Hou, Huang, Zhang, Peng, Zhou, Zhang, Su.

Supervision: Ren, Wei, Xu, Li, Zhang, Wang, Yang, Wang, Huang, Sun.

Other: None.

Financial disclosures: Yinghao Sun certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: None.

Funding/Support and role of the sponsor: This work was supported by the Program for Changjiang Scholars and Innovative Research Team in University scheme of the Ministry of Education of China (NO.JRT111, Sun); National Basic Research Program of China (2012CB518300, Sun; NO.2011CB80902, 2011CB80903, Wang); National High Technology Research and Development Program of China (NO.2012AA02A201, Zhang); National Natural Science Foundation of China (81472397, Ren); Shanghai Pujiang Program (12PJD008, Ren); PCa Foundation Young Investigator Award (Ren); Shanghai Municipal Health and Family Planning Commission Outstanding Young Investigator (XYQ2013077, Ren); Shanghai Municipal Education Commission (Ren); Guangdong Innovative Research Team Program (NO. 2009010016, Yang); Academy of Finland (284618 & 279760, Wei), University of Oulu Strategic funds, Jane and Aatos Erkko Foundation, and Finnish Cancer Foundation grants (Wei).

Acknowledgments: This manuscript is dedicated to the memory of our wonderful colleague, the excellent research scientist, Prof. Changjun Yin in recognition of his immense contributions to Chinese Urology as well as this project. We thank Dr. Laurie Goodman with the help in revision and editing of the manuscript, Professor Michael Dean and Professor Qiang Pan-Hammarström for valuable suggestions. We thank all the lab members for helpful discussion. Whole Genome and whole transcriptome sequencing data has been deposited in The European Genome-phenome Archive (EGAS00001000868).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eurouro.2017.08.027.

References


