ERG Rearrangement Is Associated with Prostate Cancer-Related Death in Chinese Prostate Cancer Patients

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Abstract

Recently, ETS-related gene (ERG) gene rearrangements, phosphatase tensin homologue (PTEN) deletions and EGFR family aberrations were characterized as potential biomarkers for prostate cancer (PCa) patient management. Although ERG gene rearrangement has been identified in approximately 50% of localized prostate cancers in western countries, the prognostic significance of this critical molecular event remains unknown in Chinese patients. Using fluorescence in situ hybridization (FISH) and immunohistochemistry, we evaluated ERG, PTEN and EGFR family aberrations in a cohort of 224 Chinese prostate cancer patients diagnosed in transurethral resection of the prostate (TUR-P). Overall, ERG rearrangement was detected in 23.2% (44/190) cases, of which 54.5% (24/44) showed deletion of the 5' end of ERG. PTEN deletion was identified in 10.8% (19/176) cases. Amplification of EGFR and HER2 genes was present in 1.1% (2/178) and 5.8% (10/173) of cases, respectively. Significant correlation between ERG rearrangement and PTEN deletion was identified in this cohort. EGFR and HER2 aberrations occurred more frequently in PCas without ERG rearrangement than in those with ERG rearrangement, although this did not reach statistical significance. Overall, ERG rearrangement was associated with pre-operative PSA values (P = 0.038) and cancer-related death (P = 0.02), but not with the age, clinical T stage, Gleason score, or Ki-67 labeling index (LI). Notably, multivariate analysis including known prognostic markers revealed ERG rearrangement was an independent prognostic factor (P = 0.022). Additionally, ERG rearrangement status was helpful to identify patients with poor prognosis from PCa group with low Ki-67 LI. In summary, we reported that ERG rearrangement status allows stratification of PCa patients into different survival categories.

Introduction

Prostate cancer (PCa) is a heterogeneous disease with a variable natural history [1,2]. It is estimated that only a small fraction of patients suffers from potential life-threatening disease that requires aggressive treatment. Currently, the established prognostic factors (Gleason score, pathological stage and serum prostate-specific antigen (PSA)) cannot precisely distinguish clinically aggressive patients from potentially indolent cases [2,3]. Thus, novel prognostic biomarkers are urgently needed for PCa patient management.

Recently, recurrent gene fusions involving the ETS family of transcription factors, ERF,ETV1, ETV4, ETV5 and ELK3, fused to androgen-regulated gene TMPRSS2 or other upstream partners, have been identified in the majority of PCAs in western countries [5–8]. Among these aberrations, ERF rearrangement, which mostly results from TMPRSS2-ERG fusion, is the most prevalent and occurs in approximately 50% of localized PCAs [8]. As TMPRSS2 and ERF are located ~3 Mb apart on chromosome 21, the rearrangement between them occurs either through insertion or by an interstitial deletion (EDel) [6]. TMPRSS2-ERG fusion leads to over-expression of ERG, which may play a critical role in PCa development [8]. To date, the prognostic significance of ERG rearrangement in PCa remains controversial. Although several studies have indicated that ERG rearrangement confers a worse prognosis [9–12], others found either a favorable prognostic association[13–17] or no association with clinical outcome [18–20]. Of note, most of these data are from Caucasian patients in western countries. Although the emerging data suggested the distinct prevalence of ERG rearrangement in PCa among different ethnic groups [21,22], survival analysis of ERG aberrations is rare in Asian populations.

PTEN (phosphatase and tensin homolog deleted on chromosome 10) is a key tumor suppressor gene in PCa [23]. Deletion of the PTEN occurs in 20–70% of PCAs and has been linked to rapid tumor progression and early recurrence [24]. Previously, we and
others reported the significant association between \textit{PTEN} deletion and \textit{ERG} rearrangement both in localized and metastatic PCs [25]. Recent clinical data have suggested that \textit{PTEN} deletion and \textit{ERG} rearrangement could be used for prognostic stratification of PCa patients [26].

The epidermal growth factor receptor (\textit{EGFR}) and \textit{HER2} belong to the \textit{EGFR} family and are known to regulate cell proliferation, differentiation, angiogenesis, and survival. Amplification and over-expression of \textit{EGFR} and \textit{HER2} have been described in PCs and associated with cancer progression, poor prognosis or development of androgen independence [27]. Yet so far, the link between \textit{ERG} rearrangement and genetic aberrations of \textit{EGFR} and \textit{HER2} remains unclear.

The Ki-67 LI is a classical proliferation marker and has been found to be a predictor of outcome for PCa patients treated with radical prostatectomy [28,29] or radiotherapy. Ki-67 has emerged as one of the global predictive markers of treatment outcome in PCa patients.

The aim of the current study was to investigate whether \textit{ERG} rearrangement was associated with a more aggressive phenotype of PCa. Herein, we systematically characterized the frequency and prognostic significance of \textit{ERG} rearrangement in a large cohort of Chinese PCa patients (n = 200). We further determined whether the \textit{ERG} rearrangement can be utilized as a prognostic indicator and provide additional value in prognostic analysis. Additionally, the relationship of \textit{ERG} gene rearrangement with other molecular markers, including \textit{PTEN} deletion and genetic aberrations of \textit{EGFR} and \textit{HER2}, was also investigated.

**Materials and Methods**

**Patients**

A total of 224 PCa patients who underwent tumor resection by transurethral resection of prostate (TUR-P) were included in our study. The tumor samples were obtained from Qilu Hospital of Shandong University (Jinan, China), The Affiliated Hospital of Qingdao University (Qingdao, China) and Liaocheng General Hospital (Liaocheng, China) between 2003 and 2011. All of these patients were hospitalized due to symptoms of lower tract urinary obstruction. Eighty-five PCa patients in the current study had transrectal ultrasound-guided prostate biopsy and 63.5% (54/85) cases had peripheral zone cancer that extended into transition zone. None of the patients received preoperative radiation or androgen deprivation therapy. Anti-androgen flutamide therapy was followed after surgery and follow-up data were available for 190 patients, ranging from 3 to 147 months (mean 47 months). Because the number of non-PCa deaths (n = 17) was limited in this cohort, the prostate cancer-related death approached the all-cause mortality. The clinical and pathological characteristics of 190 PCa cases in our cohort are summarized in Table 1. Three tissue microarrays (TMAs) were assembled using a manual tissue arrayer; for each case, two cores (1.0 mm in diameter) were taken from each representative tumor focus and morphology were arrayed; for each case, two cores (1.0 mm in diameter) were taken from each representative tumor focus and morphology were arrayed. A \textit{4 \mu m} section form each TMA was stained with H&E to verify the presence of tumor in PCa cases. Detailed clinical and pathological profile were obtained from medical records and maintained on a secure relational database with TMA data. Informed written consents were obtained from the PCa patients and this study was approved by the Institutional Review Board at the school of medicine of Shandong University and local ethics.

| Parameters | Count | Percentage (%) |
|------------|-------|----------------|
| Age(years) |       |                |
| < 60       | 29    | 15             |
| 60–69      | 41    | 22             |
| \(\geq70\) | 120   | 63             |
| Gleason scores |       |                |
| < 7        | 26    | 14             |
| \(\geq7\)  | 70    | 37             |
| > 7        | 94    | 49             |
| cT         |       |                |
| \(\leqT2\) | 138   | 73             |
| T3         | 30    | 16             |
| T4         | 22    | 11             |
| Preoperative PSA levels(ng/ml) |       |                |
| \(\leq4\)  | 22    | 12             |
| 4–10       | 27    | 14             |
| 10–20      | 25    | 13             |
| >20        | 116   | 61             |
| Distant metastasis at diagnosis |       |                |
| No         | 150   | 79             |
| Yes        | 40    | 21             |

Fluorescence in situ Hybridization (FISH) was performed to detect \textit{ERG} rearrangement [25]. Bacterial artificial chromosomes (BACs) were obtained from the BACPAC Resource Center (Oakland, CA), and probes RP11-95I21 (5’ to \textit{ERG}) and RP11-476D17 (3’ to \textit{ERG}) were prepared as described [25]. The integrity and correct localization of all probes were verified by hybridization to metaphase spreads of normal peripheral lymphocytes. To detect \textit{PTEN} deletion, the commercially available DNA probes for cytoband 10q23 (Spectrum Orange \textit{PTEN} locus-specific probe) and region 10p11.1–q11.1 (Spectrum Green centromere of chromosome 10 probe) (LSI \textit{PTEN}/CEP 10; Vysis Inc. Des Plaines, IL, USA) for chromosome identification were utilized. The \textit{PTEN} genomic probe spans 368 kb and starts 166 kb from 5’ end of the gene and extends 98 kb past the 3’ end of the gene. Assessment of \textit{EGFR} and \textit{HER2} gene aberrations was performed using the GLP \textit{EGFR}/CSP 7 probe and GLP \textit{HER2}/CSP17, respectively (GP Medical Technologies, Beijing, China).

Interphase FISH was performed as previously described [25,30]. Slides were examined using an ImagingZ1 microscope (Carl Zeiss, Oberkochen, Germany). FISH signals were scored manually (100 x oil immersion) in morphologically intact and non-overlapping nuclei by two pathologists (B.H., and M.Q.), and a minimum of 50 cancer cells from each site were recorded. Cancer sites with very weak or no signals were recorded as insufficiently hybridized. Cases lacking tumor tissue in all two cores were excluded.

To validate deletion of \textit{PTEN} and amplification of \textit{EGFR} and \textit{HER2}, we utilized a previously documented method with minor modification [25,31]. Briefly, based on hybridization in five control cores (data not shown), hemizygous deletion of \textit{PTEN} gene
was termed as >50% nuclei (mean±3 standard deviations in non-neoplastic controls) containing either one signal of locus probe and ≥2 signals of reference probe (absolute deletion), or two signals of locus probe and ≥4 signals of reference probe (relative deletion). Homozygous deletion of PTEN was exhibited by the concurrent lack of the both PTEN locus signals and the presence of control signals in >30% of cells. Specimens were considered amplified for EGFR when >10% of tumor cells displayed either EGFR: CEP 7 ratio >2 or countless tight clusters of signals of the locus probe (3–5 copies). EGFR copy number gain was defined as a low copy number increase due to chromosome 7 polysomy. Similarly, specimens were considered amplified for HER2 when >10% of tumor cells displayed either HER2: CEP 17 ratio >2 or countless tight clusters of signals of the locus probe (3–5 copies). HER2 copy number gains were defined as a low copy number increase due to chromosome 17 polysomy. Representative FISH images of ERG rearrangement were shown in Figure 1. Figure 2A and 2B demonstrated representative cases with PTEN deletion as well as HER2 amplification.

**Immunohistochemistry**

Immunohistochemistry (IHC) for PTEN, EGFR and HER2 was performed using a polymer-based method (EnvisionTM +Dual Link System-HRP). Sources and dilutions of primary antibodies were as follows: anti-PTEN (Cell signaling, 1:100), anti-EGFR (DAKO, 1:500), anti-HER2 (DAKO, 1:500) and anti-Ki67 antibody (DAKO, 1:100). Sections from TMA (4 μm) were deparaffinized and prepared by successive passages through xylene and grade concentration of ethanol as routine procedure, then antigens were retrieved by pressure cooker using a citrate buffer(0.01 M), for 8 minutes 120°C. Endogenous peroxidase activity was blocked by incubation with 0.3% hydrogen peroxide solution for 15 min. The tissue sections were incubated overnight at 4°C with primary antibodies. After a washing in PBS, the sections were treated with EnvisionTM +Dual Link System-HRP reagent at room temperature for 30 min. 3, 3’-Diaminobenzidine tetrahydrochloride was used as the chromogen for 3 minutes and the tissue sections were counterstained with haematoxylin.

The immunostaining of EGFR and HER2 was semiquantitatively evaluated based on intensity of membrane reactivity following the original DAKO Herceptest criteria with a threshold.

![Image of FISH probe design and representative images of ERG rearrangement](https://example.com/figure1.png)

**Figure 1. FISH probe design and representative images of ERG rearrangement.** (A) Schematic map of 'TMPRSS2' and 'ERG' position on 21q22.2–22.3. T and C orientate toward the telomeric and centromeric regions, respectively. BACs located 5’ and 3’ to ERG were used as probes for interphase FISH. Chromosomal coordinates are from the March 2006 build of the human genome using the UCSC Genome Browser. The TMPRSS2 and ERG loci are separated by approximately 3 Mb. (B) FISH was performed using BACs as indicated with the corresponding fluorescent label on formalin-fixed paraffin-embedded tissue sections for break-apart FISH of the ERG gene. (B & E), ERG rearrangement negative case, as indicated by two pairs of co-localized green and red signals. (C & F), ERG rearrangement positive (translocation) case showed one pair of split 5’ and 3’ signals. (D & G), ERG rearrangement positive (with deletion) case showed loss of one green labeled probe 5’ to ERG.

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of 10% immunopositive cells. The scoring system was described elsewhere [27]. Evaluation of PTEN was based on the cytoplasmic staining intensity; the tumors were divided into three categories as previously described [25]. Grade 2 showed increased or equal staining intensity compared to the corresponding normal tissue; grade 1 had decreased staining intensity, and grade 0 demonstrated complete absence of staining. The Ki-67 labeling index (LI) was defined as the fraction of tumor cells showing any nuclear Ki-67 immunoreactivity and was considered high if 10% or more of the tumor nuclei were stained. For this purpose, 100–200 tumor cells were analyzed for each case. Representative immunohistochemical images of Ki-67 were shown in Figure 2C.

Statistical Analysis

Statistical analyses were carried out using the Statistical Package for Social Sciences, version 19.0 (SPSS), with a significance level of 0.05 (two-tailed probability). Pearson’s χ² test and Fisher’s exact test were used to evaluate the associations between ERG rearrangement and clinicopathologic variables as well as other molecular aberrations. Kaplan-Meier analysis was utilized to assess the prognostic value of ERG rearrangement in PCa patients. The prognostic value of ERG rearrangement was further determined in univariable and multivariable analysis, including PSA values at diagnosis, Gleason score, clinical tumor stage, distant metastasis, Ki-67 LI and EGFR family gene aberrations.

Results

Frequency of ERG Rearrangement, PTEN Deletion and EGFR Family Aberrations

Overall, ERG was rearranged in 23.2% (44/190) of Chinese PCa patients, of which 54.5% (24/44) demonstrated deletion of the 5’ end of ERG. Interestingly, two out of these 24 cases demonstrated two copies of the 3’-ERG signals, suggesting the duplication of ERG rearrangement. PTEN deletion was identified in 10.8% (19/176) of cases, with hemizygous and homozygous deletions present in 12 of 19 (63.2%) and 7 of 19 (36.8%) cases, respectively. Amplification of HER2 was identified in 10 of 173 (5.8%) tumors and polysomy of chromosome 17 was noted in 41 of 173 (23.8%) cases. By contrast, only 2 of 178 (1.1%) cases showed amplification of EGFR with polysomy of chromosome 7 being present in 18 of 178 (10.1%) tumors.

Relationships between ERG Rearrangement and Clinicopathologic Variables

ERG gene rearrangement was significantly associated with preoperative PSA levels in PCa patients (P = 0.038) (Table 2). The incidence of ERG rearrangement was significantly lower in patients with Low PSA level (<4 ng/ml) compared with those having medium or high PSA levels. However, no significant correlation
Association of ERG Rearrangement with Other Molecular Markers

As deletion of PTEN and amplifications of EGFR and HER2 are relevant genomic aberrations in PCAs, we next explored the association of ERG rearrangement with these molecular events in our cohort. As shown in Table 2, the ERG rearrangement was present in approximately 63.2% (12/19) of PCa patients with PTEN deletion (hemizygous or homozygous). Likewise, PTEN deletion occurred more frequently in cases that harbored ERG rearrangement (30.8%, 12/39) as compared with those ERG rearrangement negative cases (5.1%, 7/137). Overall, a significant association between PTEN deletion and ERG rearrangement was observed in Chinese PCa cohort (P = 0.0005). Of note, 46/152 (25.2%) PCa cases revealed decreased PTEN protein expression by immunohistochemistry. Concordance between PTEN deletion status and PTEN protein expression was also identified in our cohort (data not shown).

Amplification of EGFR was identified only in two PCa cases, both of which were negative for ERG rearrangement. Similarly, 9 out of 10 (90.0%) PCa cases with HER2 amplification were absent for ERG rearrangement. ERG rearrangement was more often present in PCa cases without HER2 amplification (34/163, 20.9%) than in HER2-amplified tumors (1/10, 10.0%) (P = 0.149).

Immunohistochemical overexpressions of EGFR and HER2 were identified in 17.6% (31/176) and 6.0% (11/181) of cases, respectively. HER2 protein overexpression was significantly correlated to amplification of HER2 (P < 0.01). However, there was no correlation between EGFR protein expression and gene amplification (data not shown); ERG rearrangement was neither associated with EGFR nor HER2 protein expression.

Survival Analysis of ERG Rearrangement in Relation to Cancer-related Death

To determine whether the presence of ERG rearrangement was a prognostic factor for PCa, we compared cancer-related death rates between patients with or without ERG rearrangement. On the basis of the Kaplan-Meier survival estimates, the group of patients with ERG rearrangement had a much greater rate of mortality than patients who lacked the gene rearrangement (P = 0.02) (Figure 3).

ERG rearrangement status was shown to be a significant prognostic predictor of prostate cancer-related survival [HR (95% CI): 3.368 (1.261–8.955), P = 0.015] in univariate analysis (Table 3). PSA values at diagnosis (P = 0.009), Gleason score (P < 0.001), clinical tumor stage (P = 0.011), distant metastasis (P = 0.006), Ki-67 LI (P = 0.002), EGFR amplification (P = 0.023), and HER2 amplification (P = 0.001) were also significantly related to cancer-related survival in univariate analysis. Notably, in a multivariate analysis that included known prognostic markers, ERG rearrangement status remained a significant predictor (P = 0.022) with a hazard ratio of 2.099 (95% CI: 1.112–3.962) (Table 3).

Prognostic Relevance of ERG Rearrangement and Ki-67 LI

We next determined whether combining markers further improved prognostic value. Since Ki-67 is a known strong prognosticator in PCa and has independent predictive value for cancer-related survival in our cohort, we directly compared the prognostic effects of ERG rearrangement and Ki-67 LI in combination. For this analysis, we grouped all cancers according to their ERG status (not rearranged vs. rearranged) and the Ki-67 Label index status (LI <10% vs LI >10%). Cox regression analyses were therefore conducted using the group with low Ki-
67 LI and no ERG aberration as the reference. As shown in Figure 4, the largest group, which comprised those who had no ERG rearrangement and low Ki-67 LI, had a greater cancer-related survival when compared with the three other groups. Notably, the subset of patients with ERG rearrangement and high Ki-67 LI had the worst cancer-related survival.

We further determined whether ERG rearrangement status could be utilized in improving risk stratification of PCa patients with low Ki-67 LI. Kaplan-Meier analysis showed that ERG rearrangement status was a prognostic factor in the group of patients with low Ki-67 LI (P = 0.019) (Figure 5A). The median survival of PCa patients with and without ERG rearrangement was 69 and 89 months, respectively. However, ERG rearrangement status lost its predictive value of outcome in those with high Ki-67 LI (Figure 5B). By contrast, ERG rearrangement status was not helpful in identifying high-risk PCa patients with low Gleason score (data not shown).

**Discussion**

This is one of the largest series of PCa patients (n>200) reported so far in China analyzing ERG rearrangement. Our cohort comprises men treated with TUR-P and all of the study patients had symptoms of lower tract urinary obstruction, therefore representing a select subgroup of clinically recognized PCas. The patients with incidental PCas were excluded from our study. Although more and more PSA-screed PCa patients have been identified in western countries, there are limited data regarding the clinical phenotype or natural history of PCa. Of note, our cohort included a subset of patients with high grade PCas. This differed from most Western patients who were found to have PCa due to PSA screening and were often treated with radical prostatectomy.

**Table 3.** Univariate and multivariate analysis of variables associated with survival in PCa patients.

| Parameter                  | Univariate analysis | Multivariate analysis |
|----------------------------|---------------------|-----------------------|
|                            | HR(95%CI)   | P     | HR(95%CI)   | P     |
| age(years)*                | 0.588 (0.328–1.053) | 0.074 | –           | –     |
| Pre-PSA                    | 0.601 (0.410–0.880) | 0.009 | Non significance |   |
| Gleason score              | 2.297 (1.455–3.625) | <0.001 | 4.680 (2.020–10.483) | <0.001 |
| Clinical tumor stage       | 2.011 (1.177–3.435) | 0.011 | Non significance |   |
| Metastasis                 | 2.106 (1.240–3.577) | 0.006 | 2.897 (1.236–6.789) | 0.014 |
| Ki-67                      | 2.592 (1.435–4.682) | 0.002 | 2.641 (1.084–6.435) | 0.019 |
| HER2 amplification         | 6.687 (2.253–19.844) | 0.001 | Non significance |   |
| HER2 IHC                    | 3.240 (0.998–10.527) | 0.05  | Non significance |   |
| EGFR amplification         | 5.255 (1.259–21.929) | 0.023 | Non significance |   |
| ERG rearrangement           | 3.368 (1.261–8.955) | 0.015 | 2.099 (1.112–3.962) | 0.022 |

HR = hazard ratio; CI = confidence interval; PSA = prostate-specific antigen.

*not included in multivariate analysis.
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**Figure 3.** Kaplan-Meier survival curves for PCa patients with and without ERG rearrangement. The cancer-related survival rates were compared between patients with and without ERG rearrangement using the log-rank test. doi:10.1371/journal.pone.0084959.g003
Overall, the frequency of ERG rearrangement was 23.2% in our cohort and this was comparable with that previously reported by Mao et al [21] and Ren et al [32] in Chinese PCa patients. In consistent with these findings, Kimura et al [17] and Lee et al [33]

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**Figure 4. Kaplan-Meier curves illustrating cancer related survival among PCa patients.** The patients were stratified by ERG rearrangement and Ki-67 LI in combination and log-rank test was performed.
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**Figure 5. Kaplan-Meier survival analysis of PCa patients in relation to ERG rearrangement status.** (A) low Ki-67 LI (<10%) subgroup, (B) high Ki-67 LI (≥10%) subgroup.
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Ki-67 is a well-known proliferation marker [41–43]. By contrast, different effects on proliferation and invasion association between study. In line with Antonarakis’s report [40], no significant biomarker in malignancy including PCa. Its independent predictive value for PCa related survival has been confirmed in our study. In line with Antonarakis’s report [40], no significant association between ERG rearrangement and Ki-67 LI status was identified. One explanation is that ERG rearrangement has different effects on proliferation and invasion in vitro, respectively, Ki-67 is a well-known proliferation marker [41–43]. By contrast, in 2008, Tomlins et al reported that alternation of ERG gene expression significantly affect invasion in vitro but has no effect on cellular proliferation [41]. However, when stratifying for Ki-67 status, ERG rearrangement was a prognostic factor for cancer-related survival only in PCa patients with low Ki-67 LI. A major clinical challenge in PCa management is the inability to readily distinguish indolent from aggressive tumors in patients who present with low Gleason grade, low tumor volume or low Ki-67 LI. Our data suggested that determination of ERG rearrangement status could be helpful in stratification of PCa patients with low Ki-67 LI into different survival categories.

Although gene fusion is a key molecular event in PCa development and TMPRSS2-ERG fusion may induce high grade prostatic neoplasia (HGPIN), it is not sufficient to generate a fully transformed phenotype in vitro and in vivo [41,44]. Several independent groups have suggested ERG may cooperate with other genetic aberrations to promote PCa development and progression, such as PTEN haploinsufficiency, enhanced androgen receptor (AR) signaling, overexpression of SOX9 and aberrant phosphoinositide 3-kinase (PI3K) pathway [43–47]. Most recently, TMPRSS2-ERG was shown to mediate Epithelial to Mesenchymal Transition (EMT) through the induction of WNT signaling pathway via FZD4 as well as ZEB1/ZEB2 axis [48,49]. Previously, we and others have suggested the significant association between PTEN deletion and ERG rearrangement both in localized and metastatic PCAs in western countries. In this study, we confirmed significant association between PTEN deletion and ERG rearrangement in Chinese PCa cohort (P = 0.0008). Thus our data highlighted a possible cooperative role of both ERG and PTEN aberrations in a subset of Chinese PCa cases.

Genetic aberrations of HER2 and EGFR were associated with advanced-stages disease, metastasis and shorter survival in PCa progression. Previous studies have shown the rarity of EGFR/HER2 amplifications in PCa. Schlomm et al [30] reported that amplification of EGFR was present only in 6 of 2,446 PCa cases (0.25%). Similarly, Baek et al [51] found no amplification of the EGFR or HER2 genes in 66 PCa specimens. In our cohort, amplification of HER2 was present in 5.8% of Chinese PCa cases. Although not reaching statistic significance, ERG rearrangement seemed to be more often present in PCa cases without HER2 amplification than in HER2-amplified tumors. Therefore, HER2 genetic aberration might play a role in a subset of Chinese PCa patients without ERG rearrangement.

It should be noted that a small proportion of tumors showing ERG arrangement may harbor a fusion between ERG and genes other than TMPRSS2, including SLC45A3 or NDRG1. On the other hand, it has been suggested that cancers harboring gene fusions occurring by deletion have worse prognosis than those occurring by translocation. However, we did not find significant associations between ERG rearrangement by translocation or positive by deletion cancers and outcomes in Chinese PCa patients.

In total, for the first time, we reported that ERG rearrangement was associated with cancer-related death in Chinese PCa patients. Determination of ERG rearrangement status allows stratification of PCa patients into different survival categories.

Author Contributions
Conceived and designed the experiments: BH. Performed the experiments: MQ XY FZ TL YL. Analyzed the data: MQ XS. Contributed reagents/materials/analysis tools: XY YL JZ HY YR XQ XS. Wrote the paper: MQ BH.
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