Mol Biol Rep (2014) 41:7463–7470
DOI 10.1007/s11033-014-3636-1

# Role of the functional variant (−652T>G) in the XRCC4 promoter in prostate cancer

**Ning Shao · JiuMing Li · Bin Xu · Yang Wang · XiaoJie Lu · NingHan Feng**

Received: 6 March 2014 / Accepted: 21 July 2014 / Published online: 6 August 2014


**Abstract** Several genes encoding DNA repair molecules have been proposed as cancer-susceptibility genes. Many studies have suggested that SNPs in XRCC4 could be implicated in altering the risk of prostate cancer (PCa). We examined the role of the functional variant (−652T>G) in the XRCC4 promoter in PCa. The transcriptional activity of XRCC4 gene was measured by luciferase assay. We performed real-time PCR/immunohistochemical assay to verify the association between expression level of XRCC4 mRNA/protein and XRCC4 −652T>G polymorphism. In addition, electrophoretic mobility shift assay (EMSA) was used to confirm whether this polymorphism has an effect on binding ability of the transcription factor. We found that the G variant significantly increased the transcription activity of the XRCC4 gene and the binding ability of transcriptional factor GATA-1 to the XRCC4 promoter. Furthermore, the results suggested that the XRCC4 protein and mRNA were overexpressed in individuals who carried the −652G allele compared to carriers of the −652T allele. In addition, the expression of XRCC4 in PCa tissues was lower than in adjacent normal tissues. Our data suggest that the XRCC4 promoter −652G>T polymorphism is functional and may influence genetic susceptibility to prostate cancer. Case–control studies are required to validate our findings in the future.

**Keywords** XRCC4 · EMSA · Prostate cancer

Ning Shao and JiuMing Li authors have contributed equally to the present work and each is considered first author.

**Electronic supplementary material** The online version of this article (doi:10.1007/s11033-014-3636-1) contains supplementary material, which is available to authorized users.

N. Shao · Y. Wang · N. Feng
Department of Urology, Second People's Hospital of Wuxi Affiliated to Nanjing Medical University, 68 Zhongshan Road, Wuxi 214002, China

N. Shao · Y. Wang · X. Lu (✉) · N. Feng (✉)
Translational Medicine Center, Second People's Hospital of Wuxi Affiliated to Nanjing Medical University, 68 Zhongshan Road, Wuxi 214002, China
e-mail: 17419772@sina.com

N. Feng
e-mail: ninghan.feng@njmu.edu.cn

J. Li
Department of Urology, BenQ Medical Center Affiliated to Nanjing Medical University, Nanjing 210029, China

B. Xu
Department of Urology, Nanjing Zhongda Hospital Affiliated to Southeast University, Nanjing, China

## Introduction

Prostate cancer (PCa) is one of the most common cancers in males in western countries. Approximately 238,590 new cases are expected to be diagnosed with 29,720 estimated deaths in the USA in 2013 [1]. In recent years, an increasing incidence of PCa has been found in China, mainly due to population aging and improvement of diagnostic screening. However, the underlying etiology of PCa is poorly understood. The most recognized factors associated with PCa risk include age, cigarette smoking, ethnicity, alcohol consumption and so on. In addition, it was suggested that several genetic polymorphisms could influence an individual's susceptibility to PCa [2, 3].

Previous studies have suggested that the DNA repair system may play a critical role in maintaining the integrity of DNA and preventing various cancers. Reduced DNA repair capacity could increase the susceptibility to

tumorigenesis [4]. In addition, defects in DNA repair system commonly lead to various cancers, such as PCa, bladder cancer, gastric cancer and so on [5–7]. Eukaryotic cells have developed the following two pathways to repair DNA double-strand breaks (DSBs): the homologous recombination and the nonhomologous end-joining (NHEJ) pathways. Up until now, single nucleotide polymorphisms (SNPs) in the NHEJ genes are considered to be associated with altered cancer risk [8, 9].

The gene X-ray cross-complementing group 4 (XRCC4) is a specific member of NHEJ system. Recently, it was suggested that XRCC4 could restore DNA double-strand breaks (BSB) and support V(D)J recombination of transiently introduced substrates in the XR-1 CHO cell line [10, 11]. Previous studies have demonstrated that several SNPs in the XRCC4 gene contribute to the development of various cancers [12–14]. There are different polymorphism sites in the XRCC4 gene located in the 5q13–q14 region. Eight of these polymorphisms, including rs6869366, rs28360071, rs1805377, rs3734091, rs28360317, rs2075685, rs2075686 and rs7727691, are the most extensively studied. Both Mandal and Chang found that rs6869366 polymorphisms were significantly associated with PCa risk [15, 16]. In addition, plots of the pairwise linkage disequilibrium (LD) values for the SNPs and LD structures of the promoter in XRCC4 indicated that rs6869366 and rs2075685 were in a block of strong LD [17–19].

Furthermore, we made a bioinformatics analysis of the promoter region by using a computer algorithm (AliBaba2). The results suggested that XRCC4 −652T>G polymorphism may affect the binding affinity of the core sequence with GATA-1 (Fig. S1). Therefore, we performed the study to investigate the role of the functional variant (rs2075685, −652T>G) in the XRCC4 promoter in the development of PCa. In addition, luciferase assay, immunohistochemistry and electrophoretic mobility shift assay (EMSA) were all used to test the function of the variant.

## Materials and methods

### Study subjects

21 cases of PCa tissues were obtained from the Department of Urology at the Second People's Hospital of Wuxi Affiliated to Nanjing Medical University. The mean age of these patients was 67 years (range 58–75). These included 12 cases of tissues obtained from early stage patients that had been detected in prostate-specific antigen (PSA) screening and who had undergone transrectal systematic ultrasound-guided needle biopsies. Nine cases of tissues obtained from advanced PCa patients who received transurethral prostatic resection (TURP) due to urinary retention. None of them had accepted any radiology or chemotherapy before. We used PSA staining to confirm portion of tumor tissue. In our study, only samples with >60 % tumor content could be adopted. The ethics approval was obtained from the ethics committees of Nanjing Medical University. In addition, the patients joined this study with informed consents. Genotyping was performed by GenScript biotechnology Nanjing Co. Ltd. Tumor grade was evaluated by the Gleason scoring system [20]. Disease stage was determined by imaging examination, radionucleotide bone scans and pathological findings.

### Cell culture

Two human prostate cancer cell lines (DU145 and PC3) were purchased from the Shanghai Cell Bank, Chinese Academy of Sciences. DU145 and PC3 were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10 % fetal bovine serum (FBS, Sijiqing Biological Engineering Materials) and streptomycin/penicillin at 37 °C, in an atmosphere of 5 % $CO_2$.

### Construction of reporter plasmids

We then explored whether this polymorphism had an effect on its gene expression in vitro. Through amplifying the XRCC4 promoter region from subjects that carried T allele (−652TT), we completed the T allelic reporter constructs. The amplified fragments were 333 bp. The T constructs included artificial Kpn I and Bgl II enzyme restriction sites and contained a forward primer of 5′-ATTGGTACCGAGAATAAGGTGGAAGGG-3′ and a reverse primer of 5′-GGAAGATCTGTCCGAGGAAGACTGCTG-3′. The pGL3 basic vector (Promega, Madison, WI) and the above-prepared fragment were cleaved with the Kpn I and Bgl II enzymes together. Both of them were then ligated by T4 DNA ligase (New England BioLabs). After that, we used the QuikChange sitedirected mutagenesis kit and completed the G allelic reporter constructs by site-directed mutagenesis. At last, we confirmed the allele, the orientation and integrity of each insert of both constructs by sequencing.

### Transient transfections and luciferase assays

The luciferase assays were similar as the reference described elsewhere [3]. Both of cells (PC3 and DU145) were seeded ($1.0 \times 10^6$) into 24-well plates. After that, we transfected each well with 652T or the 652G allelic reporter constructs by using Lipofectamin 2000 (Invitrogen) according to the manufacturer's protocol. pRL-SV40, which contained the Renilla luciferase gene, were cotransfected with the above plasmids as an internal standard. We used the pGL3-basic vector without an insert as a negative control. Then, we collected the cells after 48 h

and prepared cell lysates according to the manufacture's instruction. At last, we used Dual-Luciferase Reporter Assay System (Promega) to measure luciferase activity and normalize against the activity of the Renilla luciferase gene. For each plasmid construct, independent triplicate experiments were performed.

### Electrophoretic mobility shift assay (EMSA)

To examine whether the T652G polymorphisms had an effect on protein binding, synthetic double-stranded oligonucleotides 5′-ttaagagaataaggtggaa-3′ and 5′-ttaagagaagaaggtggaa-3′ were labeled with biotin corresponding to the −652T and −652G sequence from the XRCC4 promoter region. EMSA were uesd with the LightShift Chemiluminescent electrophoretic mobility shift assay/Gel-Shift kit (Beyotime). Each gel shift reaction (10 μl) composed of 1 μl 3′-end labeled probe, 2 μl nuclear extract prepared from Du145 cells, 2 μl EMSA/Gel-Shift binding buffer (5×) including poly(deoxyinosinic–deoxycytidylic acid) and 5 μl nuclease-free water. For the negative control reaction (10 μl), a total of 1 μl 3′-end labeled probe were combined with 2 μl EMSA/Gel-Shift binding buffer (5×) and 7 μl nuclease-free water. For the probe or mutated probe cold competition reaction (10 μl), 1 μl labeled probe combined with 1 μl 100-fold molar excess of unlabeled probe or 1 μl 100-fold molar excess of unlabeled mutated probe, 2 μl nuclear extract prepared from Du145 cells, 2 μl EMSA/Gel-Shift binding buffer (5×) and 4 μl nuclease-free water. For each supershift reaction (10 μL), 1 μl labeled probe was combined with 1 μl GATA-1 antibodies, 2 μl nuclear extract prepared from Du145 cells, 2 μl EMSA/Gel-Shift binding buffer (5X) and 4 μl nuclease-free water.

The unlabeled probe was preincubated for 10 min at room temperature with nuclear extracts prior to addition of the labeled probe for competition assays. For supershift assays, 1 μL GATA-1 antibodies was incubated with nuclear extracts for 0.5 h at 4 °C. Then, an additional incubation for 0.5 h at room temperature with a labeled probe was needed. After that, samples were separated on a native nondenaturing 4.5 % polyacrylamide gel and then transferred to an anylon membrane. At last, chemiluminescent reaction with the stabilized Streptavidin-horseradish peroxidase conjugate was used to detect the positions of the biotin-labeled probe in the membrane according to the manufacturer's instructions.

### Real-time analysis of XRCC4 mRNA

TRIzol reagent (Molecular Research Center) was used to isolate total RNA from tissues. By using oligo primer and SuperscriptII (Invitrogen, CA, USA), 2 μg aliquot of total RNA from each specimen was reverse transcribed into single-strand cDNA. Then, the ABI Prism 7000 sequence detection system (Applied Biosystems) was used to perform relative gene expression quantitation for XRCC4, with β-actin as an internal reference gene. For XRCC4, the primers were 5′-TGGACTGGGACAGTTTCTGA-3′ and 5′-CTGCTCCTGACAACAATGCT-3′ and for β-actin were 5′-GGCGGCACCACCATGTACCCT-3′ and 5′-AGGGGCCGGACTCGTCATACT-3′. For PCR reaction mixture (final volume 20 μL), 0.1 μmol/L each primer and 1× SYBR Premix EX-*Taq* premix reagent were combined with 50 ng cDNA. Cycling conditions were 95 °C for 2 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. The expression of individual XRCC4 was measured relative to expression of β-actin. At last, all experiments were performed in a blinded fashion, with laboratory persons unaware of the genotyping results.

### Immunohistochemistry

10 % buffered formalin was used to fix each PCa tissues. The archival paraffin-embedded tissue blocks were cut into 4-μm thick sections and mounted on slides coated with silane. Immunohistochemical staining was performed according to the manufacturer's protocols. Three high-power fields (HPFs, 100× or 200) were selected randomly in each specimen after immunohistochemical staining for XRCC4. After that, 100 cells were counted in each field. For each sample, the average percentage of positive cells from the three HPFs were calculated. Expression levels of XRCC4 were assessed semiquantitatively as follows. Percentage of positive cells (PP): 0, negative staining; 1, ≤10 % of PCa cells positive; 2, 11–50 % of PCa cells positive; 3, 51–80 % of PCa cells positive; 4, >80 % of PCa cells positive. In addition, staining intensity (SI) was determined as follows: 0 for no staining, 1 for slight staining, 3 for strong staining, and 2 for staining between 1 and 3. Immunoreactive score (IRS) = SI*PP [21].

### Statistical analysis

Lewontin's standardized coefficient D' and the linkage disequilibrium (LD) coefficient r2 were used to eaxamine LD among the polymorphisms [22]. Haploview program was then used to estimate the pairwise LD between markers and partition haplotype blocks. The method of LD confidence interval was used to define LD blocks [23]. When the one-sided upper 95 % CI boundary on D' is >0.98 with the lower boundary >0.7, the pair of SNPs is defined as a strong LD SNPs. The statistical analyses of comparing the expression levels of XRCC4 between 652T and 652G PCa tissues were calculated using the Mann–Whitney test. The difference in levels of luciferase reporter

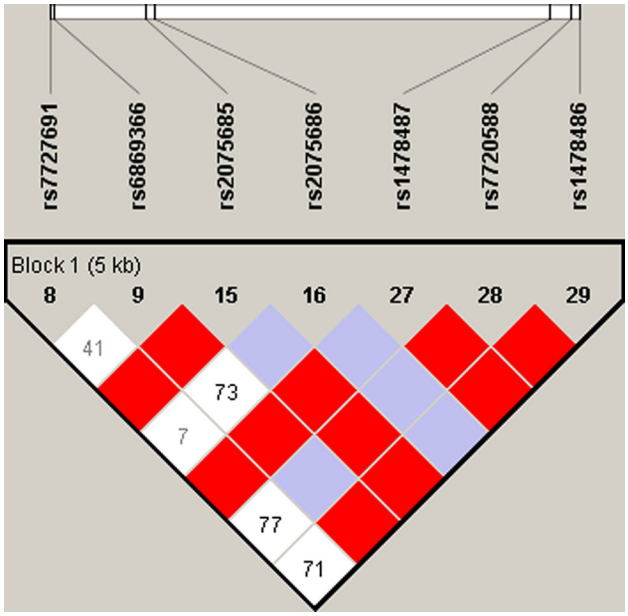



**Fig. 1** Graphical representation of the SNP locations and LD structure of the promoter in XRCC4 in chinese

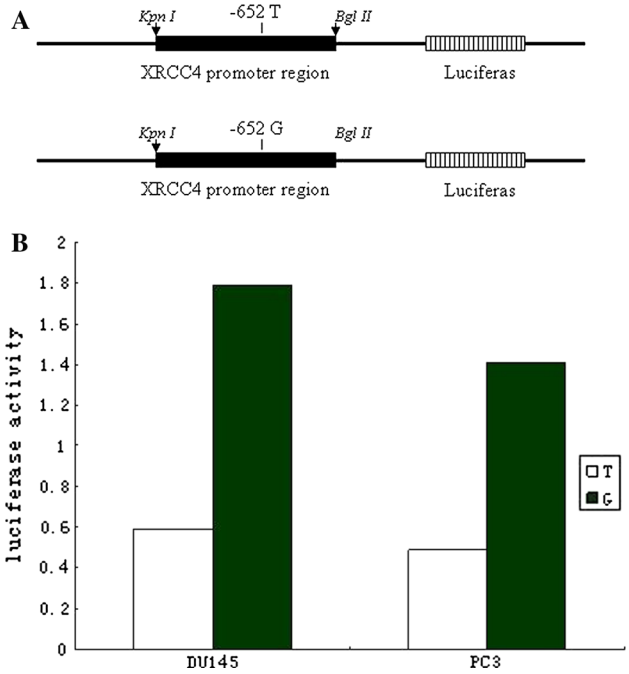



**Fig. 2** XRCC4 reporter gene constructs for the XRCC4 promoter and luciferase expression of the constructed promoter in different cell lines. **a** Schematic drawing of the reporter gene constructs containing a 333 bp XRCC4 promoter region; the only difference between the two constructs was a G or T at the −652T>G polymorphic site. **b** Luciferase activity of the two XRCC4 promoter constructs in two cell lines: DU145 and PC3. The luciferase activity of each construct was normalized against the internal control of Renilla luciferase. Columns, mean from two independent experiments; bars, $p < 0.01$ for both comparisons of each cell line between the activities of the reporter gene constructs

gene expression between different constructs was examined by Student's *t* test. The SPSS software, version 16.0 (SPSS, Inc.) was used to perform all statistical analyses through two-sided p values.

## Results

### Haplotype block structure

Figure 1 shows plots of the pairwise linkage disequilibrium (LD) values for the SNPs and LD structures of the promoter in XRCC4 in Chinese. The LD plot indicated that rs7727691, rs6869366, rs2075685 were in a block of strong LD (size = 5 kb, Fig. 1.)

### Effects of the XRCC4 −652T>G polymorphism on the transcriptional activity

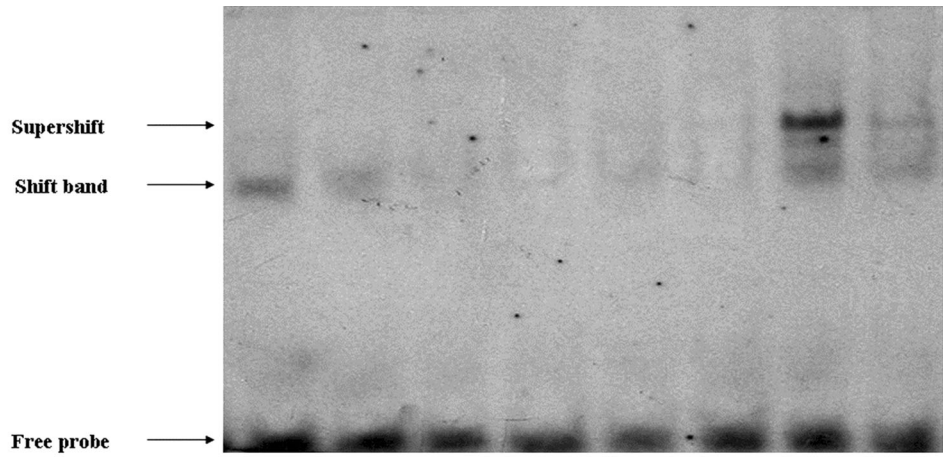
We constructed two luciferase reporter vectors (pGL3) to test the allele-specific effect of the XRCC4 −652T>G variants on the promoter activity. The reporter constructs contained a T or G allele at the −652 polymorphic site and spanned the 333 bp of the XRCC4 promoter region (Fig. 2a). Then, they were used to transiently transfect he DU145 and PC3 cells.

As shown in Fig. 2b, the vectors with the G allele had significantly higher luciferase activities, compared with the T allele in PC3 and Du145 cell lines ($p < 0.01$ for all). These results indicated that the −652G allele in the XRCC4 promoter region had an increased transcriptional activity compared with −652T allele.

### Identification of the GATA-1-binding region and allele-specific effects of the XRCC4 promoter

The EMSA was performed to confirm whether −652T>G polymorphism has an effect on binding ability of the transcription factor by analyzing the binding of oligo probes containing either −652T or −652G alleles to nuclear proteins extracted from the Du145 cells.

As shown in Fig. 3, a specific DNA/nuclear protein complex (a shifted band) was generated by the −652G. In addition, the levels of the protein complex generated by the −652G allele probes were significantly higher than those generated by the 652T allele probes (lanes 1 and 2). However, the shifted band was abolished by both 100-fold unlabeled 652G and 100-fold unlabeled 652T probes (lanes 3–6). Moreover, anti-GATA-1 antibodies caused a supershift band of the biotin-labeled probe/nuclear protein (lane 7 and 8). Besides, the levels of supershift band caused by the 652G allele probes were significantly higher than those




| lane | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Nuclear extract | + | + | + | + | + | + | + | + |
| Labeled -652G probe | + | - | + | - | + | - | + | - |
| Labeled -652T probe | - | + | - | + | - | + | - | + |
| Anti-GATA-1 | - | - | - | - | - | - | + | + |
| Cold unlabeled -652G probe (100X) | - | - | + | - | - | + | - | - |
| Cold unlabeled -652T probe (100X) | - | - | - | + | + | - | - | - |

**Fig. 3** Analysis of transcription factor binding sites in the XRCC4 promoter region containing the −652T>G polymorphism. Electrophoreticmobility shift assay (EMSA) with biotin-labeled either −652T or G probe and Du145 cell nuclear extracts. *Lanes* 1 and 2, mobilities of the labeled probes with nuclear extracts in the absence of competitor. *Lanes* 3, 4, 5 and 6, a specific nuclear protein binding can be completely abolished both by 100-fold unlabeled −652T or G probes. *Lanes* 7 and 8, Super shift assays incubating with anti-GATA-1 antibody showed a supershifted protein complex

caused by the −652T allele probes. It further suggests that the GATA-1 was the transcription factor that binds the promoter region containing the −652G allele. In addition, as a negative control, no band could be found with only labeled −652G or −652T probe and without nuclear extract (Fig. S2).

In summary, these results suggest that the T652G polymorphism in the XRCC4 promoter sits in the core of the GATA-1 binding motif, and the T to G substitution enhances the affinity of GATA-1 to this region in the XRCC4 promoter, possibly leading an increased expression level of the XRCC4.

## Effects of XRCC4 −652T>G SNP on XRCC4 mRNA levels

Real-time PCR quantization of XRCC4 mRNA in individual PCa tissues was performed to examine the effect of −652T>G SNP on XRCC4 expression. It was found that subjects with the −652TG genotypes had significantly higher XRCC4 mRNA levels than those with the −652TT genotype ($t = 2.290$, $p = 0.034$), as shown in Fig. 4.

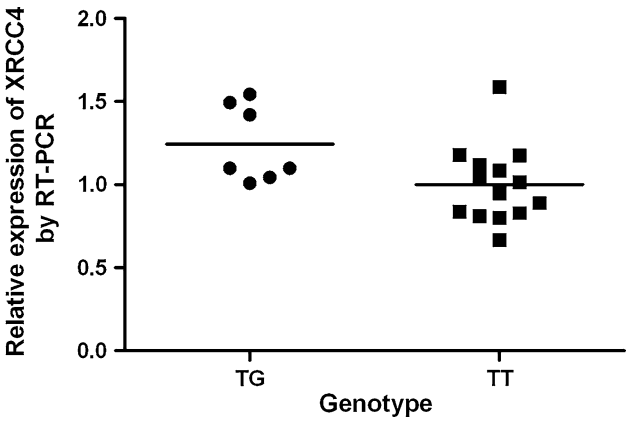



**Fig. 4** XRCC4 mRNA expression level in PCa tissue. Expression level among the −652TG genotype was significantly higher than those with the −652TT genotype ($p < 0.05$)

## Association of XRCC4 −652T>G SNP with expression levels of XRCC4

To confirm the results of luciferase assays and real-time PCR, we performed the immunohistochemical assay to verify the

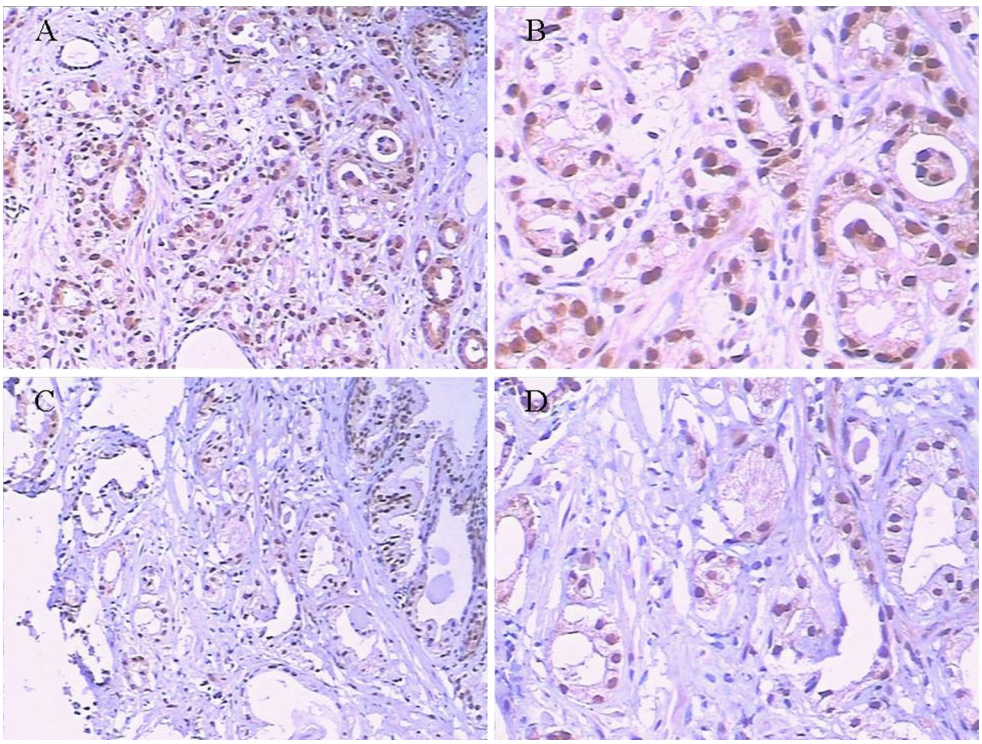



**Fig. 5** Immunohistochemical analysis of XRCC4 protein expression levels in PCa tissues. Immunohistochemical staining in PCa tissues from individuals who carried TG genotype (**a** ×100, **b** ×200). Immunohistochemical staining in PCa tissues from individuals who carried TT genotype (**c** ×100, **d** ×100)

association between the expression level of XRCC4 protein and XRCC4 −652T>G polymorphism. We collected 21 tumor tissues. Of these, 7 cases carried the −652TG genotype and the remaining 14 cases carried the −652TT genotype. There were no tumor tissues of who carried the −652GG genotype due to few corresponding PCa patients.

Immunostaining signals of XRCC4 were distributed evenly. XRCC4 staining was positive in tumor cells and normal prostate epithelial cells. Almost >90 % of PCa cells were positive and strong staining in PCa tissues of patients carrying the −652TG genotype was noted. Our analysis showed that the expression of XRCC4 in PCa tissues of patients carrying the −652TG genotype were moderately higher than those carrying the −652TT genotype. (Mann–Whitney test, $Z = -2.428$, $p = 0.015$, Fig. 5.) In addition, the expression of XRCC4 in PCa tissues were lower than in adjacent normal tissues ($z = -3.093$, $p = 0.002$, Table 1). However, there were no significant differences between the expression of XRCC4 and stages of PCa or Gleason scores. ($Z = -0.165$, $p = 0.869$; $Z = -1.114$, $p = 0.265$, respectively, Table 1).

**Table 1** Expression of XRCC4 (IHC) and clinicopathological characteristics in Chinese patients with PCa

| Group | N | IRS ± SD | p | Z |
|---|---|---|---|---|
| XRCC4 −652TG | 7 | 12 ± 0 | | |
| XRCC4 −652TT | 14 | 9.43 ± 2.409 | 0.015 | −2.428 |
| PCa tissues | 21 | 10.29 ± 2.305 | | |
| Adjacent normal tissues | 21 | 12 ± 0 | 0.002 | −3.093 |
| Gleason <7 | 2 | 12 ± 0 | | |
| Gleason ≥7 | 19 | 10.11 ± 2.355 | 0.265 | −1.114 |
| Localized | 12 | 10.33 ± 2.535 | | |
| Advanced | 9 | 10.22 ± 2.108 | 0.869 | −0.165 |

Localized: $T_{1–2}N_0M_0$; Advanced: $T_{3–4}NxMx$ or $TxN_1Mx$ or $TxNxM_1$
Clinical staging according to the international tumor-node-metastasis (TNM) system for prostate cancer

## Discussion

Over the past years, multiple studies have suggested that polymorphic variations in humans may be responsible for interindividual differences in susceptibility to various cancers. Previous studies have found that genes in DNA repair pathways could play an important role in the development of tumors. XRCC4, a member of DNA repair genes, has been studied extensively regarding its relationship with human cancers such as urothelial bladder cancer, lung cancer, gastric cancer and so on [8, 24–27]. Among them, rs6869366, rs1805377 and rs2075685 polymorphisms were the most extensively studied. However, two

studies that investigated XRCC4 polymorphisms in PCa did not investigated rs2075685 polymorphism [14, 15].

In the present study, we observed a significant association between the rs2075685 (−652T>G) polymorphism and transcriptional activity of XRCC4 in vitro. In addition, the results showed that the T to G substitution of this polymorphism significantly increased the transcription activity of the XRCC4 gene. It was also found that the T to G substitution of this polymorphism significantly enhanced the binding affinity of the transcriptional activator GATA-1 in vitro. Besides, subjects with the −652TG genotypes had higher XRCC4 mRNA levels than those with the −652TT genotype. Furthermore, the results suggested that the XRCC4 protein were overexpressed in individuals who carried the −652G allele in vivo. In addition, the expression of XRCC4 in PCa tissues were lower than in adjacent normal tissues. As a result, the XRCC4 rs2075685 (−652T>G) polymorphism could be considered a functional SNP both in vitro and in vivo.

Polymorphisms represent most common variations in a DNA sequence, which may alter the activity of the encoded gene. In recent years, SNPs have been considered the main approach to describe interindividual differences and the implications for cancer predisposition. XRCC4 plays an important role in the repair of DNA DSB. The DNA repair system is needed to maintain the genetic integrity of all tissues. Deficiencies in the DNA repair system may result in chromosomal aberrations, which in turn cause cell malfunctioning, cell death and tumorigenesis. One of the most deleterious DNA damaging types is DSB, which is repaired in eukaryotes by the following two major pathways: homologous recombination (HR) and NHEJ. XRCC4 forms a complex with DNA ligase IV, and this complex is essential for the repair of all double-strand DNA breaks by the NHEJ pathway in eukaryotes [28–30]. As XRCC4 is a member of DNA repair genes, SNPs in the promoter region of XRCC4 may influence its functions. Therefore, SNPs of XRCC4 and environmental carcinogens may have some joint effects and change the susceptibility to cancers significantly.

Taking our results into account, rs2075685 (−652T>G) polymorphism could be considered a functional SNP. We hypothesized that the T to G substitution of this polymorphism may overexpress XRCC4 and possibly affect susceptibility to PCa. This was supported by the results of our luciferase assays, EMSA and immunohistochemistry experiments. Together, our study suggest that the −652G variant allele is a protective factor for PCa. However, the associations between rs2075685 (−652T>G) polymorphisms and PCa risk requires well-designed genotyping experiments with large sample sizes to validate.

The protective role of XRCC4 −652G genetic variants in PCa we found indicated that rs2075685 (−652T>G) polymorphisms is an important SNP and worthy of further study. However, our study may have certain limitations because of its design. The current sample size is too small to perform genotyping experiments. Selection bias and/or systematic errors may have occurred because the fields were randomly selected in each specimen during immunohistochemistry. Other limitations may be related to the fact that the present study was restricted to a Chinese Han population. Because the role of SNPs in cancer risk may vary with ethnicity, prospective studies with larger numbers of participants from other ethnicities are warranted.

In conclusions, we observed a significant association between the rs2075685 (−652T>G) polymorphism and transcriptional activity of XRCC4 in vitro. We also found that the G variant increased the binding ability of transcriptional factor GATA-1 to the XRCC4 promoter. Furthermore, the results suggest that the XRCC4 protein and mRNA was overexpressed in individuals who carried the −652G allele. In addition, the expression of XRCC4 in PCa tissues was lower than in adjacent normal tissues. Therefore, the XRCC4 promoter −652G>T polymorphism is functional and may influence genetic susceptibility to prostate cancer. Well-designed case–control studies are needed to validate our findings in the future.

**Acknowledgments** This study was supported by National Natural Science Foundation of China (Grant No. 81272831, 81202034), Jiangsu Province's Outstanding Medical Academic Leader program (RC201178), Six-Kinds-of-Top-Talent Program of Jiangsu Province (2011) and Science Foundation of Benq Medical Center (SRD20100004).

**Conflict of interest** The authors declare no conflict of interest.

## References

1. Siegel R, Naishadham D, Jemal A (2012) Cancer statistics, 2012. CA Cancer J Clin 62(1):10–29
2. Wang B, Wang D, Zhang D, Li A, Liu D, Liu H et al (2010) Pro variant of TP53 Arg72Pro contributes to esophageal squamous cell carcinoma risk: evidence from a meta-analysis. Eur J Cancer Prev 19(4):299–307
3. Shao N, Wang Y, Lu K, Jiang WY, Li Q, Wang N et al (2012) Role of the functional MKK4 promoter variant (−1304T>G) in a decreased risk of prostate cancer: case-control study and meta-analysis. J Cancer Res Clin Oncol 138(9):1531–1539
4. Hsieh YY, Bau DT, Chang CC, Tsai CH, Chen CP, Tsai FJ (2008) XRCC4 codon 247*A and XRCC4 promoter −1394*T related genotypes but not XRCC4 intron 3 gene polymorphism are associated with higher susceptibility for endometriosis. Mol Reprod Dev 75(5):946–951
5. Tomescu D, Kavanagh G, Ha T, Campbell H, Melton DW (2001) Nucleotide excision repair gene XPD polymorphisms and genetic predisposition to melanoma. Carcinogenesis 22(3):403–408
6. Bau DT, Wu HC, Chiu CF, Lin CC, Hsu CM, Wang CL et al (2007) Association of XPD polymorphisms with prostate cancer in Taiwanese patients. Anticancer Res 27(4C):2893–2896

7. Chiu CF, Wang CH, Wang CL, Lin CC, Hsu NY, Weng JR et al (2008) A novel single nucleotide polymorphism in XRCC4 gene is associated with gastric cancer susceptibility in Taiwan. Ann Surg Oncol 15(2):514–518
8. Yu H, Zhao H, Wang LE, Han Y, Chen WV, Amos CI et al (2011) An analysis of single nucleotide polymorphisms of 125 DNA repair genes in the Texas genome-wide association study of lung cancer with a replication for the XRCC4 SNPs. DNA Repair (Amst) 10(4):398–407
9. Helleday T, Lo J, van Gent DC, Engelward BP (2007) DNA double-strand break repair: from mechanistic understanding to cancer treatment. DNA Repair (Amst) 6(7):923–935
10. Mari PO, Florea BI, Persengiev SP, Verkaik NS, Bruggenwirth HT, Modesti M et al (2006) Dynamic assembly of end-joining complexes requires interaction between Ku70/80 and XRCC4. Proc Natl Acad Sci USA 103(49):18597–18602
11. van Heemst D, Brugmans L, Verkaik NS, van Gent DC (2004) End-joining of blunt DNA double-strand breaks in mammalian fibroblasts is precise and requires DNA-PK and XRCC4. DNA Repair (Amst) 3(1):43–50
12. Berwick M, Vineis P (2000) RESPONSE re: markers of DNA repair and susceptibility to cancer in humans: an epidemiologic review. J Natl Cancer Inst 92(18):1537
13. Yin M, Liao Z, Liu Z, Wang LE, O'Reilly M, Gomez D et al (2011) Genetic variants of the nonhomologous end joining gene LIG4 and severe radiation pneumonitis in nonsmall cell lung cancer patients treated with definitive radiotherapy. Cancer 18(2):528–535
14. Mittal RD, Gangwar R, Mandal RK, Srivastava P, Ahirwar DK (2012) Gene variants of XRCC4 and XRCC3 and their association with risk for urothelial bladder cancer. Mol Biol Rep 39(2):1667–1675
15. Chang CH, Chiu CF, Wu HC, Tseng HC, Wang CH, Lin CC et al (2008) Significant association of XRCC4 single nucleotide polymorphisms with prostate cancer susceptibility in Taiwanese males. Mol Med Rep 1(4):525–530
16. Mandal RK, Kapoor R, Mittal RD (2010) Polymorphic variants of DNA repair gene XRCC3 and XRCC7 and risk of prostate cancer: a study from North Indian population. DNA Cell Biol 29(11):669–674
17. Bau DT, Yang MD, Tsou YA, Lin SS, Wu CN, Hsieh HH et al (2010) Colorectal cancer and genetic polymorphism of DNA double-strand break repair gene XRCC4 in Taiwan. Anticancer Res 30(7):2727–2730
18. Shim HJ, Yun JY, Hwang JE, Bae WK, Cho SH, Lee JH et al (2010) BRCA1 and XRCC1 polymorphisms associated with survival in advanced gastric cancer treated with taxane and cisplatin. Cancer Sci 101(5):1247–1254
19. Gomes BC, Silva SN, Azevedo AP, Manita I, Gil OM, Ferreira TC et al (2010) The role of common variants of non-homologous end-joining repair genes XRCC4, LIG4 and Ku80 in thyroid cancer risk. Oncol Rep 24(4):1079–1085
20. Gleason DF, Mellinger GT (1974) Prediction of prognosis for prostatic adenocarcinoma by combined histological grading and clinical staging. J Urol 111(1):58–64
21. Remmele W, Stegner HE (1987) Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue. Pathologe. 8(3):138–140
22. Lewontin RC (1988) On measures of gametic disequilibrium. Genetics 120(3):849–852
23. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B et al (2002) The structure of haplotype blocks in the human genome. Science 296(5576):2225–2229
24. Hsu NY, Wang HC, Wang CH, Chang CL, Chiu CF, Lee HZ et al (2009) Lung cancer susceptibility and genetic polymorphism of DNA repair gene XRCC4 in Taiwan. Cancer Biomark 5(4):159–165
25. Tseng RC, Hsieh FJ, Shih CM, Hsu HS, Chen CY, Wang YC (2009) Lung cancer susceptibility and prognosis associated with polymorphisms in the nonhomologous end-joining pathway genes: a multiple genotype-phenotype study. Cancer 115(13):2939–2948
26. Figueroa JD, Malats N, Rothman N, Real FX, Silverman D, Kogevinas M et al (2007) Evaluation of genetic variation in the double-strand break repair pathway and bladder cancer risk. Carcinogenesis 28(8):1788–1793
27. Chang CH, Chang CL, Tsai CW, Wu HC, Chiu CF, Wang RF et al (2009) Significant association of an XRCC4 single nucleotide polymorphism with bladder cancer susceptibility in Taiwan. Anticancer Res 29(5):1777–1782
28. Weterings E, van Gent DC (2004) The mechanism of non-homologous end-joining: a synopsis of synapsis. DNA Repair (Amst) 3(11):1425–1435
29. Lees-Miller SP, Meek K (2003) Repair of DNA double strand breaks by non-homologous end joining. Biochimie 85(11):1161–1173
30. Ahnesorg P, Smith P, Jackson SP (2006) XLF interacts with the XRCC4-DNA ligase IV complex to promote DNA nonhomologous end-joining. Cell 124(2):301–313