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Prostate cancer of transition zone origin lacks *TMPRSS2–ERG* gene fusion

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Recent studies have shown a unique chromosomal rearrangement that leads to the fusion of 5′-transmembrane protein serine proteinase-2 (*TMPRSS2*) with the EST-related gene (*ERG*) in prostate cancer. In this study, we used fluorescence *in situ* hybridization to evaluate *TMPRSS2–ERG* gene fusion in prostate cancer of different zonal origins. Radical prostatectomy specimens with multifocal prostate cancer were obtained from 30 patients who were treated at our institution. Two separate tumor foci in each specimen, one in the peripheral zone and the other in the transition zone, were selected for gene fusion analysis. The selected peripheral zone tumor foci had a mean Gleason score of 6.8 (range, 6–7) and a mean tumor volume of 1.2 cm³ (range, 0.1–4.6 cm³). The selected transition zone tumor foci had a mean Gleason score of 6.7 (range, 5–8) and a mean tumor volume of 4.0 cm³ (range, 0.5–9.0 cm³). *ERG* gene rearrangement was not observed in any transition zone tumors; however, it was found in the peripheral zone tumors in 13 cases (43%). In 10 cases, the rearrangement was associated with the deletion of the 5′-end of *ERG*. In conclusion, we found that *TMPRSS2–ERG* gene fusion is associated with the zonal origin of prostate cancer. This gene fusion is prevalent in prostate cancer arising from the peripheral zone, but is lacking in prostate cancer arising from the transition zone.
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Recent studies have shown that most prostate cancers have a unique chromosomal rearrangement in chromosome 21.[1] This rearrangement is characterized by the fusion of 5′-transmembrane protein serine proteinase-2 (*TMPRSS2*) with an oncogene, EST-related gene (*ERG*). Owing to the presence of androgen receptor-responsive elements in 5′-*TMPRSS2*, the *TMPRSS2–ERG* gene fusion leads to an aberrant function of oncogenic *ERG*, which is believed to play an important role in prostate cancer oncogenesis. *TMPRSS2* can also fuse with other oncogenes, such as *ETV1*, *ETV4*, and *ETV5*, leading to prostate cancer,[1–3] but *TMPRSS2–ERG* gene fusion is the most prevalent rearrangement.

Several studies have suggested that *TMPRSS2–ERG* gene fusion is associated with an aggressive phenotype of prostate cancer. Rajput *et al*[4] reported that *TMPRSS2–ERG* gene fusion was more prevalent in moderately differentiated than in well-differentiated prostate cancer. Mehra *et al*[5] reported that *TMPRSS2–ERG* gene fusion was statistically associated significantly with high pathological stage. Nam *et al*[6] found that *TMPRSS2–ERG* expression was the single most significant predictor of disease relapse after surgery, independent of prostate-specific antigen (PSA) level, tumor grade, and tumor stage. However, Gopalan *et al*[7] recently reported that *TMPRSS2–ERG* gene fusion was not associated with tumor pathological stage, biochemical recurrence, metastasis, or with the overall survival of the patient in a study of more than 500 patients. Therefore, the clinical significance of *TMPRSS2–ERG* gene fusion in prostate cancer remains uncertain.

The prostate is composed of three anatomic zones, namely peripheral zone, transition zone, and central zone.[8–9] Although the majority of prostate cancers arise in the peripheral zone, many prostate cancers also develop in the transition zone. Compared with peripheral zone cancers, transition zone cancers tend to have a large tumor volume, a higher PSA level, and a lower Gleason score, and generally have a more favorable prognosis.[10–13] Some studies have suggested that there are differential expression levels of proliferative genes and oncogenes between transition and peripheral zone tumors, which may contribute to their clinical and biological differences.[14–17] However, to our knowledge, the role of *TMPRSS2–ERG* gene fusion in the zonal origin of

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prostate cancer has not been studied. In this study, we evaluated the *TMPRSS2–ERG* gene fusion in prostate cancers arising from the transition zone in comparison with those arising from the peripheral zone.

Materials and methods

Case Selection and Pathological Evaluation

We retrospectively searched our pathology file at The University of Texas M. D. Anderson Cancer Center (Houston, TX, USA) from 2001 to 2008, and selected 30 patients with multifocal prostate cancer in radical prostatectomy specimens on the basis of two criteria, namely (1) there were at least two independent tumor foci, as described earlier[18] and (2) one tumor focus was located in the peripheral zone, and the other one was in the transition zone. When several tumor foci were present, the largest tumor focus from each zone was selected for the analysis. No patient had undergone radiation, hormone therapy, or transurethral prostatic resection before radical prostatectomy. Each tumor focus was graded according to the Gleason grading system. The volume of each tumor focus was calculated using a formula described earlier.[18] The demographic and clinicopathologic features of the patients were obtained from medical records and from pathological examination of specimens.

Determination of *TMPRSS–ERG* Gene Fusion

TMPRSS2–ERG gene fusion was evaluated using break-apart fluorescence *in situ* hybridization (FISH). The break-apart probes, consisting of a rhodamine-labeled 5′-*ERG* probe (*BAC RP11-95I21*) and a fluorescein isothiocyanate-labeled 3′-*ERG* probe (*BAC RP11-476D17*), were obtained from the Children's Hospital of Oakland Research Institute (Oakland, CA, USA). Tissue pretreatment was performed using the Paraffin Pretreatment Kit I (Vysis, Des Plaines, IL, USA), and hybridization and washing were performed using Vysis hybridization reagents, following the manufacturer's protocols. We used the break-apart approach, resulting in two pairs of co-localized green and red signals in cells, with no rearrangement of *ERG*. In cells with *TMPRSS2–ERG* gene fusion, only one pair of co-localized green and red signals was maintained; the other broke into one green signal and one red signal. In cells with *TMPRSS2–ERG* gene fusion associated with the deletion of intervening DNA, one pair of co-localized green and red signals and one green signal were present—the 5′-region (red) signal was lost. The specificity and quality of the probes were confirmed by hybridization to the metaphase spread of the normal peripheral lymphocytes. A mean of 100 cells was evaluated per tumor focus.

Table 1 Summary of pathological features and *TMPRSS2–ERG* gene fusion status in peripheral zone and transition zone tumor foci

Case No.	*PZ focus*			*TZ focus*		
	GS	*Vol. (cm³)*	*Fusion status*	*GS*	*Vol. (cm³)*	*Fusion status*
1	3+4	0.8	Pos. with no del.	3+3	0.8	Neg.
2	3+4	1.9	Pos. with del.	2+3	3.8	Neg.
3	3+4	3.0	Pos. with del.	3+4	2.8	Neg.
4	3+4	4.6[a]	Pos. with del.	3+4	7.2	Neg.
5	3+4	0.8	Pos. with del.	3+2	2.4	Neg.
6	3+4	0.5	Pos. with del.	3+2	1.7[b]	Neg.
7	3+4	2.2	Neg.	3+4	1.7	Neg.
8	3+3	1.4	Pos. with del.	3+3	2.4	Neg.
9	4+3	1.8	Pos. with no del.	3+4	4.9[b]	Neg.
10	3+4	0.4	Neg.	3+4	12.8[a,b]	Neg.
11	3+3	0.4	Neg.	3+3	6.0	Neg.
12	3+4	1.6	Neg.	3+3	2.4	Neg.
13	3+3	0.7	Neg.	3+4	4.8	Neg.
14	3+4	1.1	Pos. with no del.	3+3	0.6	Neg.
15	3+4	2.7	Neg.	3+4	6.7	Neg.
16	3+4	0.6	Neg.	3+4	0.5	Neg.
17	3+4	2.8[a]	Neg.	3+4	2.5	Neg.
18	3+4	0.5	Neg.	3+4	3.6	Neg.
19	3+3	0.8	Neg.	3+4	5.0	Neg.
20	3+4	0.3	Pos. with del.	4+3	2.9	Neg.
21	3+3	1.6	Neg.	4+3	9.0[b]	Neg.
22	3+4	1.2	Pos. with del.	4+3	6.0	Neg.
23	4+3	1.2	Neg.	3+4	4.8	Neg.
24	3+4	0.2	Pos. with del.	3+3	4.8	Neg.
25	3+4	0.4	Neg.	4+3	6.0[a]	Neg.
26	3+3	0.1	Pos. with del.	3+5	4.7[b]	Neg.
27	3+4	0.1	Neg.	4+4	1.4[a,b]	Neg.
28	4+3	0.8	Neg.	4+3	3.8[a,b]	Neg.
29	4+3	1.4	Neg.	3+5	3.1	Neg.
30	3+3	0.1	Neg.	4+3	1.8[a,b]	Neg.

del., deletion; GS, Gleason score; neg., negative; pos., positive; PZ, peripheral zone; TZ, transition zone; vol., volume.
[a]Extraprostatic extension.
[b]Tumor involving margin of resection.

Results

The mean age of patients in this study was 59 years (range, 46–71 years). Twenty-four were white, and six were black. The overall Gleason scores in radical prostatectomy specimens had a mean of 7.0 (range, 6–8). There were a mean of 3.3 (range, 2–5) tumor foci in the specimens. Although the tumor was confined to the prostate in 23 cases, it extended into the extraprostatic adipose tissue in 7 cases (Table 1). In all cases, the tumor did not invade the seminal vesicles. In eight cases, the tumors involved the margin of resection. In addition, tumor metastasis to the lymph node was present in 1 of the 22 patients who underwent pelvic lymph node dissection.

Two separate tumor foci were selected for analysis from each radical prostatectomy specimen (Figure 1a). Prostatic adenocarcinoma in the transition zone typically showed large irregular glands lined by tall columnar cells with pale-to-clear cytoplasm and basally located nuclei (Figure 1b), whereas prostatic adenocarcinoma in the peripheral zone was characterized by small round glands lined by cuboidal

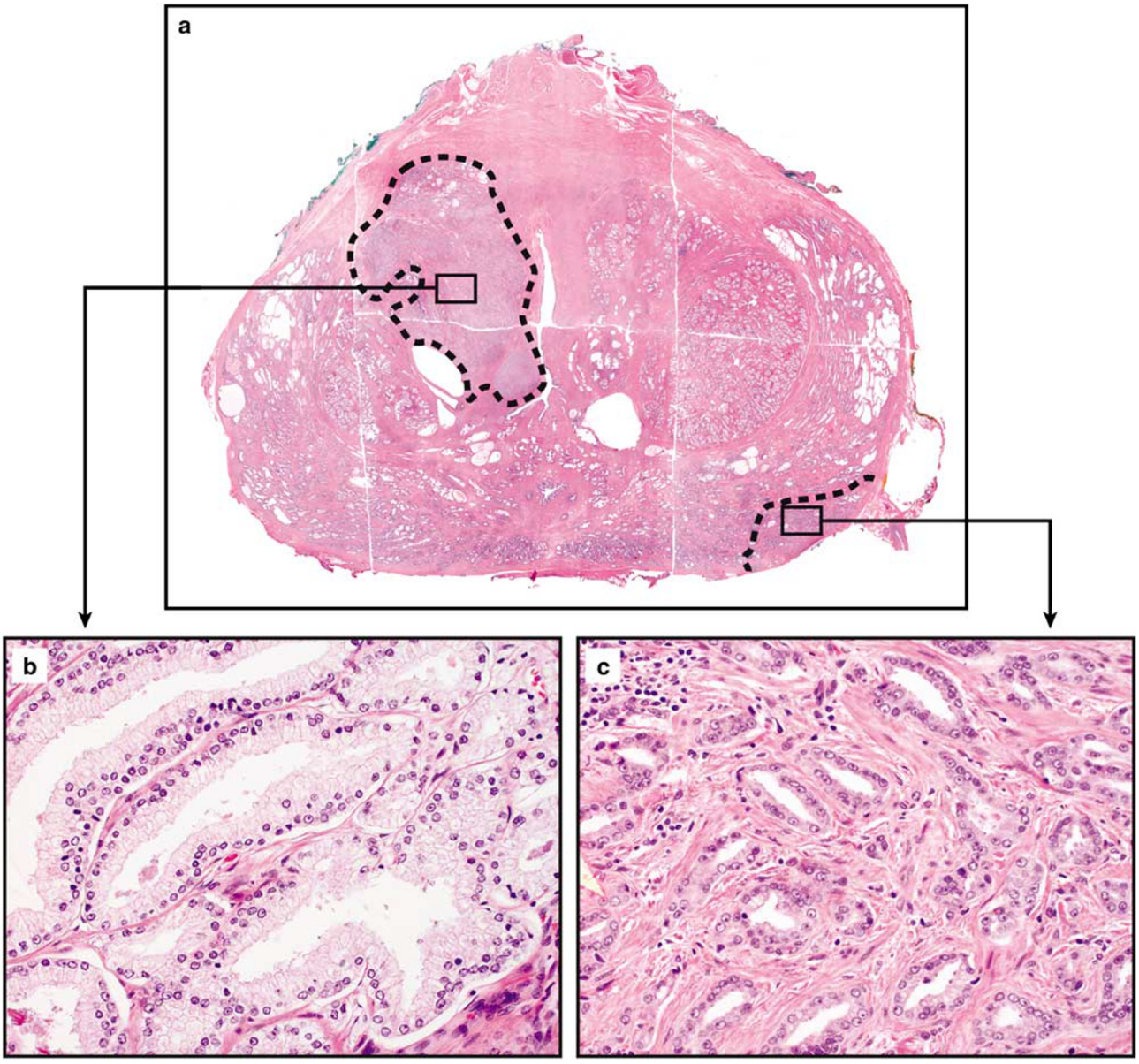

Figure 1 Prostatic adenocarcinoma of different zonal origins. (**a**) A transverse section through the mid-prostate shows two separate foci of prostatic adenocarcinoma, one in the left transition zone and the other in the right posterolateral peripheral zone. (**b**) Prostatic adenocarcinoma in the transition zone shows large irregular glands lined by tall columnar cells with pale-to-clear cytoplasm and basally located nuclei. (**c**) Prostatic adenocarcinoma in the peripheral zone shows small round glands lined by cuboidal cells with amphophilic cytoplasm and centrally located nuclei.

cells with amphophilic cytoplasm and centrally located nuclei (Figure 1c). The selected peripheral zone tumor foci had a mean Gleason score of 6.8 (range, 6–7) and a mean tumor volume of 1.2 cm^3 (range, 0.1–4.6 cm^3). The selected transition zone tumor foci had a mean Gleason score of 6.7 (range, 5–8) and a mean tumor volume of 4.0 cm^3 (range, 0.5–9.0 cm^3). In 24 cases, the selected transition zone tumor focus was larger than the peripheral zone tumor focus.

TMPRSS2–ERG gene fusion was evaluated by FISH in both peripheral zone and transition zone tumor foci. In all cases, transition zone tumors showed normal signal patterns for *ERG*, with no gene rearrangement; the pattern was characterized by two pairs of co-localized green and red signals (Figure 2a). In 13 cases (43%), peripheral zone tumors showed *ERG* gene rearrangement, which was characterized by the break apart of one of the two co-localized signals. In 10 cases, *ERG* gene rearrangement was associated with the deletion of the 5′-end of *ERG* (red signal) (Figure 2b); the rearrangement was not associated with the deletion in the remaining three cases (Figure 2c).

A total of 24 patients were followed-up for a mean of 34.3 months (range, 8–82 months) after radical

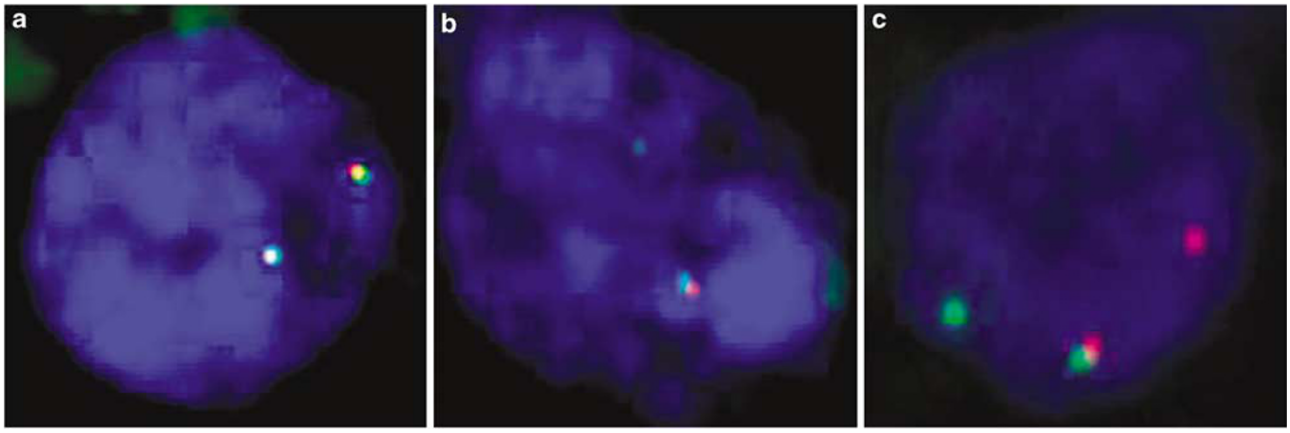

Figure 2 *ERG* gene rearrangement in prostatic adenocarcinoma of different zones. (**a**) Prostatic adenocarcinoma in the transition shows no *ERG* gene rearrangement. (**b**) Prostatic adenocarcinoma in the peripheral zone shows *ERG* gene rearrangement with the deletion of the 3′-region (red signal). (**c**) Prostatic adenocarcinoma in the peripheral zone shows *ERG* gene rearrangement with no deletion.

prostatectomy; the remaining 6 patients were followed-up for <6 months. Twenty-two of the 24 patients were alive without evidence of disease. Of the remaining two patients, one experienced recurrent increase in PSA levels 21 months after surgery; the other patient presented with tumor metastasis to a lymph node at the time of surgery and subsequently received hormonal therapy with undetectable PSA 26 months after surgery. *ERG* gene rearrangement was not found in the studied tumor foci of either of the two patients.

Discussion

The zonal anatomy of the prostate was first described by McNeal.[8,9] Using a three-dimensional model, McNeal divided the prostate into three anatomic zones, namely the peripheral zone, transition zone, and central zone. The peripheral zone forms the bulk of the posterior, lateral, and apical regions of the prostate, and accounts for 75% of the gland. The transition zone surrounds the proximal prostatic urethra and accounts for 5% of the gland in the absence of benign prostatic hyperplasia. The central zone forms a cone-shaped structure that constitutes the majority of the base of the prostate and accounts for 20% of the gland. The prostate is covered anteriorly by the anterior fibromuscular stroma. This zonal anatomy model of the prostate has been widely accepted.

Most (75%) prostate cancers develop in the peripheral zone, but some (20%) arise from the transition zone.[10–13] The zonal origin of prostate cancer is largely determined on the basis of McNeal's model. The transition zone boundary, a thin layer of compressed fibromuscular tissue extending in an arc from the dorsal urethra to the anterior fibromuscular stroma, can sometimes be appreciated on examination with a low-magnification lens.[9] In addition, histological features may help to establish the zonal origin of prostate cancer in small biopsy specimens.[10] Transition zone prostate cancers often show large irregular glands with tall columnar cells, pale-to-clear cytoplasm, and basally located nuclei. In contrast, peripheral zone prostate cancers are characterized by small round glands with cuboidal cells, amphophilic cytoplasm, and centrally located nuclei.

However, one should be cautious in using the histological features to determine the zonal origin of prostate cancer. Garcia *et al*[19] recently studied the so-called 'transitional-zone look', which is characterized by prostatic glands of variable sizes with tall columnar cells showing basally oriented nuclei and clear to pale pink cytoplasm, in different zonal origins of prostate cancer. They found that only half of transition zone cancers showed an extensive (>50%) 'transition-zone look', although the 'transition-zone look' was more frequent in transition zone cancers than in peripheral zone cancers. Furthermore, they also observed a non-focal (>25%) 'transition-zone look' in up to 35% of peripheral zone cancers. Therefore, the histological features alone are insufficient to determine the zonal origin of prostate cancer, especially in limited samples, such as prostate needle biopsy. In this study, we used the McNeal's zonal anatomy model to determine the zonal origin of prostate cancer.

Most studies have found that transition zone prostate cancer has a more favorable clinical course than peripheral zone prostate cancer.[10–13] Although transition zone tumors often present with a larger volume and a higher PSA level than peripheral zone tumors, they tend to have a lower Gleason score. Shannon *et al*[11] found that transition zone tumors were more likely to be organ-confined than peripheral zone tumors. Greene *et al*[12] reported that transition zone tumors not only have a lower rate of extraprostatic extension but also a significantly lower rate of biochemical recurrence than peripheral zone cancers of the same tumor volume and grade.

Furthermore, Noguchi *et al*[13] found that the 5-year biochemical cure rate for transition zone tumors was 72% compared with 49% for peripheral zone tumors.

Several factors may contribute to the indolent clinical course of transition zone tumors. Anatomically, transition zone tumors are confined posterolaterally by the peripheral zone and anteriorly by the anterior fibromuscular stroma. Therefore, transition zone tumors are less likely to extend out of the prostate than peripheral zone tumors. Gene expression profiling using cDNA microarray techniques have shown that hundreds of genes have different expression levels in transition zone and peripheral zone tumors.[14,15] Compared with peripheral zone tumors, transition zone tumors have a low proliferation rate and low microvessel density.[16] In addition, transition zone tumors also express lower levels of *p53* and *bcl-2* than peripheral zone tumors.[16,17] These intrinsic biological difference between transition zone and peripheral zone tumors may also contribute to their different clinical behaviors.

Most prostate cancers are multifocal. We earlier analyzed radical prostatectomy specimens through whole-mount processing and found that 83% (149 of 180) of prostate cancers had at least 2 tumor foci.[18] Furthermore, we found that the tumor foci of both peripheral zone and transition zone origins were present in 52% of the cases. Recently, Arora *et al*[20] found that 87% of prostate cancers were multifocal in the radical prostatectomy specimens, and only 9% of multifocal prostate cancers had all the tumor foci with the same primary and secondary Gleason grades. Cheng *et al*[21] analyzed microsatellite DNA alterations from separate tumor foci in the same prostate. Fifteen of 18 cases had discordant patterns of allelic loss in separate tumor foci. The histological and biological heterogeneity of multifocal tumors suggests that they arise independently within the same prostate gland.

Interestingly, *TMPRSS2–ERG* gene fusion also shows significant heterogenicity in multifocal prostate cancers. Barry *et al*[22] reported that 13 of the 32 (41%) radical prostatectomy specimens with multifocal prostate cancer showed discordant *TMPRSS2–ERG* gene fusion. Furthermore, in the specimens with a positive *TMPRSS2–ERG* fusion, 76% of the cases showed discordance in at least one focus. Mehra *et al*[23] also reported that 20 of the 43 (49%) multifocal prostate cancers had the discordant *TMPRSS2–ERG* gene fusion status. In our studies, 13 of the 30 (43%) specimens showed the discordant *TMPRSS–ERG* gene fusion status. The heterogenicity of *TMPRSS2–ERG* gene fusion suggests that multifocal prostate cancer may arise from multiple independent clonal expansions.

Our study has some potential limitations. Owing to the small number of specimens, we could not show an association of *TMPRSS2–ERG* gene fusion with pathological stage, tumor volume, or Gleason score in peripheral zone tumors. Furthermore, *TMPRSS2–ERG* gene fusion was not investigated in all tumor foci in the radical prostatectomy specimen. However, the aim of our study was to investigate the association between *TMPRSS2–ERG* gene fusion and the zonal origin of prostate cancer. The association of *TMPRSS2–ERG* gene fusion with pathological stage and Gleason score has been evaluated in several large studies.[4,5] Similarly, the heterogenicity of *TMPRSS2–ERG* gene fusion in multifocal prostate cancer has also been investigated.[22,23] Nonetheless, further validation in large cohort studies is still needed.

In summary, we showed that *TMPRSS2–ERG* gene fusion was associated with the zonal origin of prostate cancer. This fusion was highly prevalent in prostate cancer arising from the peripheral zone, but was generally lacking in prostate cancer arising from the transition zone. The lack of *TMPRSS2–ERG* gene fusion in transition zone prostate cancer, suggests that there are genetic and biologic differences in prostate cancer of different zonal origins.

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Conflict of interest

The authors declare no conflict of interest.

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