Prognostic value of E-26 transformation-specific-related gene in prostate cancer based on immunohistochemistry analysis

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Abstract. E-26 transformation-specific-related gene (ERG) has been implicated in prostate cancer; however, its prognostic role remains unclear. Therefore, the present study aimed to investigate the association of ERG with the prognosis after radical prostatectomy in patients with prostate cancer. Patient data were collected at the Huadong Hospital, affiliated with Fudan University, between January 2016 and March 2020. ERG protein expression was detected using immunohistochemistry. Independent-sample t-tests and χ^2 tests were used to evaluate prostate cancer prognosis depending on ERG levels. The Kaplan-Meier method was used to estimate biochemical failure-free survival (BFFS) and the log-rank test was used to test the distribution. Prognostic factors were determined using Cox regression analysis. The median patient age was 69 years (range, 47-82 years). The median prostate-specific antigen (PSA) and free-PSA levels before treatment were 9.58 ng/ml (range, 0.003-187.400 ng/ml) and 1.13 ng/ml (range, 0.0059-30.6100 ng/ml), respectively. ERG protein expression was positive in 43 (16.6%) and negative in 216 (83.4%) cases. The median follow-up period and BFFS were 30 and 28 months, respectively. There was a significant difference in biochemical recurrence (P=0.017) between patients with positive and negative ERG expression. Patients with positive ERG expression had significantly worse BFFS curves compared with those with negative ERG expression (P=0.0038). In the multivariate Cox regression analysis, positive ERG expression was found to be an independent prognostic factor in patients with prostate cancer who underwent radical prostatectomy (hazard ratio, 4.08; 95% confidence interval, 2.03-8.17; P=0.000074). In conclusion, positive ERG expression is an independent prognostic risk factor for prostate cancer. These findings may be valuable for improvements in the clinical application of ERG immunohistochemistry.

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Abbreviations: AJCC, American Joint Committee on Cancer; BCR, biochemical recurrence; BFFS, biochemical failure-free survival; CI, confidence interval; ERG, ETS-related gene; ETS, E-26 transformation-specific; FPSA, free prostate-specific antigen; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; IHC, immunohistochemistry; TMPRSS2, transmembrane protease serine 2; TNM, tumor, node and metastasis; OS, overall survival; PCR, polymerase chain reaction; PSA, prostate-specific antigen

Key words: prostate cancer, E-26 transformation-specific-related gene, immunohistochemistry, biochemical recurrence, prognosis

Introduction

Prostate cancer is the second most common malignancy in men worldwide and the most commonly diagnosed cancer type in men in developed countries (1). Its incidence rate is the highest in Europe, America and Oceania and the lowest in North Africa and Asia (1). Prostate cancer is the fifth leading cause of cancer-associated mortalities in men worldwide, with the highest mortality rates in the Caribbean, South Africa and Central Africa (1). Furthermore, it is the sixth most common malignancy in men in China, and its morbidity and mortality rates have been increasing recently (2).

Transcription factor E-26 transformation-specific (ETS)-related gene (ERG) is a member of the ETS family (3,4). ETS transcription factors are essential for development and differentiation and are involved in embryogenesis, angiogenesis, hematopoiesis and neural development (5,6). ERG is highly expressed in the embryonic mesoderm and endodermis and plays a key role in the vascular system, urogenital tract and bone development (7,8). To the best of our knowledge, Tomlins *et al* (9) reported for the first time in 2005 that in prostate cancer the transmembrane protease serine 2 (*TMPRSS2*) gene is fused with *ERG*, resulting in overexpression of the ERG protein. Subsequently, studies have been conducted on the roles of the *TMPRSS2-ERG* fusion and ERG protein expression in the pathogenesis, detection, diagnosis and prognosis of prostate cancer (10-15). Sedarsky *et al* (10) reported the frequency of ERG expression in men with prostate cancer from different ethnic groups worldwide. Salagierski and Schalken (11) reported that the *TMPRSS2-ERG* fusion can serve as a diagnostic indicator for prostate cancer. Current research has mainly focused on the detection and diagnostic

value of ERG; however, its prognostic value remains controversial.

Biochemical recurrence (BCR) is a marker of early disease progression in patients after radical prostatectomy. According to some reports, patients with positive expression of fusion genes demonstrate a lower risk of BCR compared with patients with negative expression (12,13). However, other studies have shown no difference in disease prognosis or BCR risk between patients with positive and negative expression of fusion genes (14,15).

There are three main methods for detecting the presence of fusion genes: Reverse transcription polymerase chain reaction (PCR), fluorescence *in situ* hybridization and immunohistochemistry (IHC); among these, IHC is the most popular and convenient. The ERG protein is a routine immunohistochemical marker used for prostate puncture and radical prostatectomy specimens (15). Elucidating the hitherto unknown prognostic value of ERG IHC could provide a valuable reference for prostate cancer prognosis. Therefore, in the present study, IHC was used to detect ERG protein in patients undergoing radical prostatectomy. The relationships among ERG protein levels, clinicopathological data and patient prognosis were examined to further clarify the role of ERG IHC results in the prognosis of prostate cancer.

Materials and methods

Clinical data. The present retrospective study included 338 patients with prostate cancer who underwent radical prostatectomy at the Huadong Hospital, affiliated with Fudan University (Shanghai, China), between January 2016 and March 2020. Patient data were obtained through their medical records. Of the 338 cases, 22 were excluded because ERG, 33 were excluded because they were not followed up, 24 were excluded because of missing PSA, free prostate-specific antigen (FPSA) and Ki-67 data; finally, 259 cases were analyzed in total (Fig. 1). The patient ages ranged from 47 to 82 years, with a median age of 69 years. Paraffin-embedded sections of all surgical specimens were prepared according to standard procedures and were reviewed independently by two senior pathologists. These sections were graded using the Gleason scoring system (16), and clinical staging was performed according to the 2017 American Joint Committee on Cancer (AJCC) tumor, node and metastasis (TNM) staging system (17) to determine the involvement of the surgical margins in tumors and the proportion of tumors. Simultaneously, the patients needed a monthly PSA review within 6 months after radical surgery and PSA and other related examinations every 3 months. The primary follow-up endpoint was BCR (a PSA value of >0.2 ng/ml for two consecutive measurements) and the secondary endpoint was death.

Immunohistochemical reagents, methods and judgment of results. A rabbit monoclonal antibody against human ERG (the primary antibody) was purchased from Fuzhou Maixin Biotech Co., Ltd. (cat. no. RMA-0748). A peroxidase-labeled polymer conjugated to goat anti-mouse and goat anti-rabbit immunoglobulins (secondary antibody) were purchased from DAKO (EnVision two-step staining kit; cat. no. GK500705; Agilent Technologies, Inc.). IHC was used to detect the expression of ERG protein in prostate cancer cells. Both lesions were stained in bilateral cases. The EnVision two-step staining kit (DAKO; Agilent Technologies, Inc.) was used for IHC analysis. The prostate cancer tissues were fixed using 10% neutral buffered formalin for 24 h at room temperature. Paraffin-embedded tissues were dissected at a thickness of 4 μm and dewaxed. Antigen retrieval was performed with Tris-EDTA buffer (pH 9.0) for 20 min in a microwave and then allowed to cool down at room temperature for other 20 min. The sections were washed three times with phosphate-buffered saline for 3 min each time. Endogenous peroxidase was blocked with 0.3% hydrogen peroxide in phosphate-buffered saline at room temperature for 15 min. The sections were then incubated with primary antibodies (diluted at 1:200) for 60 min at room temperature, incubated with secondary antibody (not diluted) for 45 min at room temperature, treated with diaminobenzidine color, counterstained with hematoxylin at room temperature for 4 min and tablet sealed. Each step was performed as per the kit manufacturer's instructions (18). The results of ERG IHC were observed by the light microscope (Olympus Corporation). Positive ERG expression was indicated by medium- to strong-brown staining in the nucleus (Fig. 2).

Statistical analyses. When the total sample size was >40, the lowest expected count of the analyzed contingency table was >1 and the expected count in <20% of the cells of the analyzed contingency table was ≤5, the χ^2 test was used to compare categorical variables. When the expected count could not meet the assumptions of using χ^2 test, the Fisher's test was used. Finally, the χ^2 test was used to compare surgical margins, tumor percentage and staining of Ki-67, FPSA or BCR between ERG-positive and -negative cases. The Fisher's test was used to compare the Gleason score, TNM stages, age and PSA group between ERG-positive and -negative cases. An independent-samples t-test was used to compare the biochemical failure-free survival (BFFS) (the period of survival before BCR after radical prostatectomy) and overall survival (OS) between the two groups of patients. The Kaplan-Meier method was used to estimate BFFS and the log-rank test was used to evaluate the distribution. Univariate and multivariate Cox regression analyses were used to evaluate prognostic factors; a=0.05 and P<0.05 was considered to indicate a statistically significant difference. All data were analyzed using SPSS 26.0 software (IBM Corp.) and R4.0.4 software (R Development Core Team; http://www.R-project.org).

Results

Clinicopathological features in patients undergoing radical prostatectomy. Among the specimens from 259 patients, 43 (16.6%) were ERG-positive and 216 (83.4%) were ERG-negative. The patient ages ranged from 47 to 82 years, with a median age of 69 years, and 30 patients (11.6%) were aged ≤60 years. The PSA levels ranged from 0.003 to 187.400 ng/ml before treatment, and the median PSA level was 9.58 ng/ml. Before treatment, the FPSA levels ranged from 0.0059 to 30.6100 ng/ml, and the median FPSA level was 1.13 ng/ml. In total, 18 patients (6.9%), 162 patients

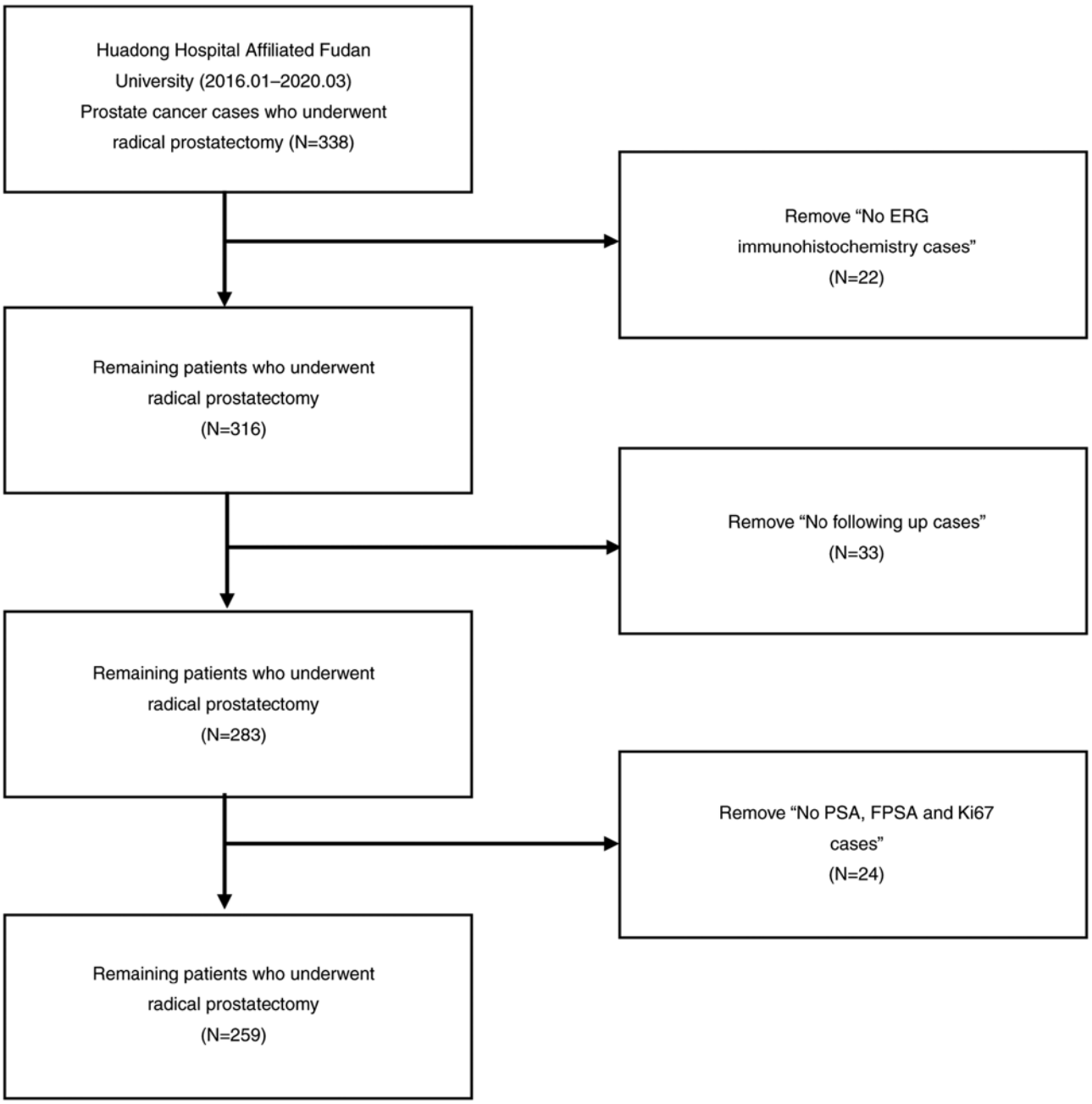

Figure 1. Flowchart of patient selection. ERG, ETS-related gene; PSA, prostate-specific antigen; FPSA, free prostate-specific antigen.

(62.5%), 29 patients (11.2%), 49 patients (18.9%) and 1 patient (0.4%) had Gleason scores of 6, 7, 8, 9 and 10, respectively. According to the 2017 AJCC TNM staging system, there were 7 cases of T1 (2.7%), 130 cases of T2 (50.2%), 115 cases of T3 (44.4%), 7 cases of T4 (2.7%), 237 cases of N0 (91.5%), 12 cases of N1 (4.6%) and 10 cases of Nx (3.9%) stages. There were 255 cases of M0 (98.5%) and 4 cases of M1 (1.5%) stages. The minimum tumor proportion of the radical resection specimens was 1.0%, the maximum was 95% and the median was 30%. Patients were followed up until December 2020; the longest follow-up time was 60 months, the shortest was 10 months and the median was 30 months. During the follow-up, BCR occurred in 48 patients (18.5%), four patients died (1.5%) and five were lost to follow-up after BCR. The mean BFFS was 28.6 months.

Comparison of clinicopathological features between patients with positive and negative ERG

IHC results. Patients were classified according to age as ≤60 and >60 years old; tumor percentage as ≤25, 25-50, 50-75 and >75%; Ki-67-positive staining as ≤5 and >5%; PSA-positive staining as ≤10, 10-20, 20-100 and >100 ng/ml; and FPSA-positive staining as ≤1, 1-4 and >4 ng/ml. Patients with ERG-positive or -negative prostate surgical specimens were compared for the Gleason scores, TNM stages, surgical margins, ages, tumor percentages, Ki-67, PSA, FPSA, BCR, BFFS and OS. Analysis using the independent sample t-test, χ^2 test and Fisher's test revealed no significant differences in the abovementioned indicators, except for BCR, between the two groups of patients (P>0.05; Table I); there was a significant difference in the distribution of BCR (P=0.017) between the groups (Table I).

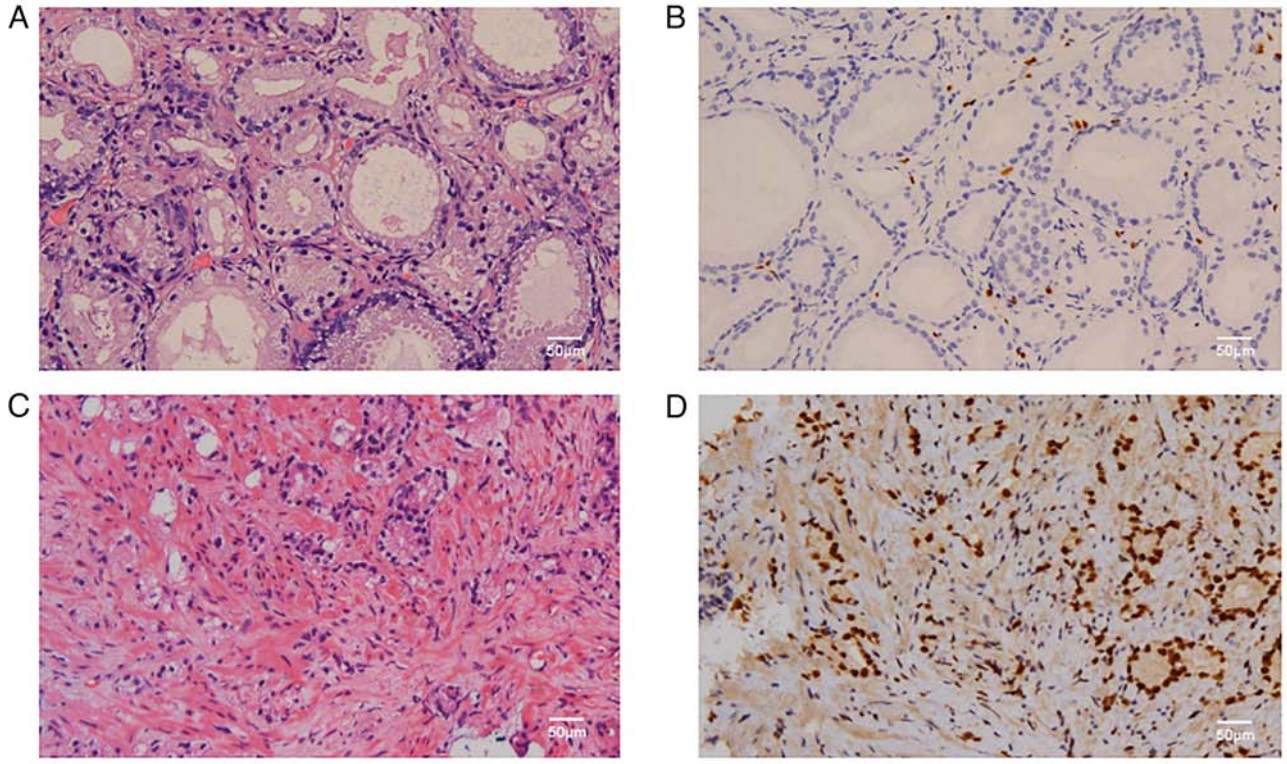

Figure 2. ERG immunohistochemistry. Immunohistochemical staining results for ERG protein. (A) HE staining for prostate cancer tissues (magnification, x200). (B) Negative ERG immunohistochemistry (magnification, x200): No staining of prostate cancer nuclei, with endothelial cell staining as an internal control. (C) HE staining for prostate cancer tissues (magnification, x200). (D) Positive ERG immunohistochemistry (magnification, x200): Nuclei of prostate cancer cells are stained brown. ERG, ETS-related gene; HE, hematoxylin-eosin.

BFFS evaluation in patients undergoing radical prostatectomy. BFFS in patients who showed positive and negative results for ERG was estimated using the Kaplan-Meier method, and a BFFS curve was generated; the difference in BFFS curves between these two groups of patients was significant (P=0.0038; Fig. 3) and the distribution was tested using the log-rank test. Patients with ERG-positive status had a worse BFFS compared with those with ERG-negative status. In addition, OS was estimated using the Kaplan-Meier method. There was no significant difference in the OS curves between these two groups of patients (Fig. 4).

BFFS was estimated in patients with different Gleason scores, positive and negative surgical margins, tumor proportions, Ki-67 scores and FPSA values using the Kaplan-Meier method. BFFS curves were generated, and the distribution was tested using the log-rank test. The difference in Gleason scores among the BFFS curves was significant (P=0.0021; Fig. 5A). The lower the Gleason score, the higher the BFFS and the lower the susceptibility to BCR. The difference in tumor proportions among the BFFS curves was significant (P=0.008; Fig. 5B). The BFFS in patients with tumor proportions between 50 and 70% was lower compared with that of in patients with other tumor proportions; these patients were more prone to BCR. The difference in BFFS curves between patients with positive and negative surgical margins was significant (P=0.03; Fig. 5C). The BFFS in patients with negative surgical margins was higher compared with that in patients with positive surgical margins, and patients with positive surgical margins were more prone to BCR. The differences in BFFS curves among the Ki-67 groups were significant (P=0.013; Fig. 5D). The BFFS of Ki-67 ≤5% cases was higher compared with that of Ki-67>5% cases, and patients with Ki-67>5% were more prone to BCR. The differences in BFFS curves among the FPSA groups were statistically significant (P=0.032; Fig. 5E). The BFFS in FPSA >4 ng/ml cases was lower compared with that in other cases. The difference in BFFS curves among the N stages groups was significant (P=0.0052; Fig. 5F). The BFFS of N1 stage cases was lower compared with that of cases with other N stages.

Univariate and multivariate Cox regression analyses in patients undergoing radical prostatectomy. Univariate Cox regression analysis was performed for ERG IHC, PSA, FPSA, age, Gleason score, surgical margins, tumor percentage, Ki-67 and TNM stages in patients undergoing radical prostatectomy. Multivariate Cox regression analysis was performed based on results of the univariate Cox regression analysis. In the univariate Cox regression analysis, positive IHC staining of ERG [hazard ratio (HR), 2.48; 95% confidence interval (CI), 1.32-4.66; P=0.005], FPSA >4 ng/ml (HR, 2.84; 95% CI, 1.14-7.05; P=0.025), positive surgical margin (HR, 1.91; 95% CI, 1.06-3.43; P=0.030), tumor proportion of 50-75% (HR, 3.06; 95% CI, 1.43-6.53; P=0.004), Ki-67 scores >5% (HR, 2.16; 95% CI, 1.16-4.02; P=0.016) and N1 stage (HR, 3.39; 95% CI, 1.43-8.03; P=0.006) were risk factors for patients undergoing radical prostatectomy (Table II). In the multivariate Cox regression analysis, results of positive IHC staining of ERG were observed (HR, 4.08; 95% CI, 2.03-8.17; P=0.000074). Gleason scores of 8 (HR, 5.23; 95% CI, 1.01-27.15; P=0.049) and 10 (HR, 18.45; 95% CI, 1.58-216.20;

Table I. Comparison of clinical patient characteristics with ERG protein expression.

Variables	Group	Overall	ERG-negative	ERG-positive	P-value
N		259	216	43	
ERG (%)	Negative	216 (83.4)	216 (100.0)	0 (0.0)	<0.001[a]
	Positive	43 (16.6)	0 (0.0)	43 (100.0)	
GS (%)	6	18 (6.9)	13 (6.0)	5 (11.6)	0.375
	7	162 (62.5)	133 (61.6)	29 (67.4)	
	8	29 (11.2)	27 (12.5)	2 (4.7)	
	9	49 (18.9)	42 (19.4)	7 (16.3)	
	10	1 (0.4)	1 (0.5)	0 (0.0)	
T (%)	T1	7 (2.7)	6 (2.8)	1 (2.3)	0.807
	T2	130 (50.2)	109 (50.5)	21 (48.8)	
	T3	115 (44.4)	96 (44.4)	19 (44.2)	
	T4	7 (2.7)	5 (2.3)	2 (4.7)	
N (%)	N0	237 (91.5)	198 (91.7)	39 (90.7)	0.899
	N1	12 (4.6)	10 (4.6)	2 (4.7)	
	Nx	10 (3.9)	8 (3.7)	2 (4.7)	
M (%)	M0	255 (98.5)	212 (98.1)	43 (100.0)	1.000
	M1	4 (1.5)	4 (1.9)	0 (0.0)	
Margins (%)	Negative	183 (70.7)	153 (70.8)	30 (69.8)	1.000
	Positive	76 (29.3)	63 (29.2)	13 (30.2)	
Age (%)	≤60 years	30 (11.6)	22 (10.2)	8 (18.6)	0.122
	>60 years	229 (88.4)	194 (89.8)	35 (81.4)	
Tumor percent (%)	≤25%	124 (47.9)	101 (46.8)	23 (53.5)	0.446
	25-50%	67 (25.9)	60 (27.8)	7 (16.3)	
	50-75%	28 (10.8)	22 (10.2)	6 (14.0)	
	>75%	40 (15.4)	33 (15.3)	7 (16.3)	
Ki-67 group (%)	≤5%	219 (84.6)	184 (85.2)	35 (81.4)	0.691
	>5%	40 (15.4)	32 (14.8)	8 (18.6)	
PSA group (%)	≤10 ng/ml	134 (51.7)	113 (52.3)	21 (48.8)	0.240
	10-20 ng/ml	77 (29.7)	67 (31.0)	10 (23.3)	
	20-100 ng/ml	45 (17.4)	34 (15.7)	11 (25.6)	
	>100 ng/ml	3 (1.2)	2 (0.9)	1 (2.3)	
FPSA group (%)	≤1 ng/ml	115 (44.4)	100 (46.3)	15 (34.9)	0.232
	1-4 ng/ml	126 (48.6)	103 (47.7)	23 (53.5)	
	>4 ng/ml	18 (6.9)	13 (6.0)	5 (11.6)	
BCR (%)	0	211 (81.5)	182 (84.3)	29 (67.4)	0.017[a]
	1	48 (18.5)	34 (15.7)	14 (32.6)	
BFFS, months [mean (SD)]		28.6 (15.1)	29.0 (15.2)	26.7 (14.6)	0.378
OS, months [mean (SD)]		32.6 (13.5)	32.4 (14.1)	33.6 (9.9)	0.592

[a]P<0.05. ERG, ETS-related gene; GS, Gleason score; OS, overall survival; BFFS, biochemical failure-free survival; PSA, prostate-specific antigen.

P=0.020) were independent prognostic factors for these patients (Table II).

Discussion

Several studies have investigated the prognostic role of ERG IHC in prostate cancer worldwide; however, the findings have been inconsistent (12-15). In the present study, patients with ERG IHC-positive status had a higher BCR and worse BFFS compared with patients with ERG IHC-negative status after radical prostatectomy. According to the multivariate Cox regression analysis, the ERG-positive status was an independent prognostic factor for patients undergoing radical prostatectomy. Overall, the reason for the differences between the results of the current study and those of previous studies may be that the specimens used in the present study were all derived from radical prostatectomy cases and all patients enrolled were from China. Prostate cancer has multiple foci

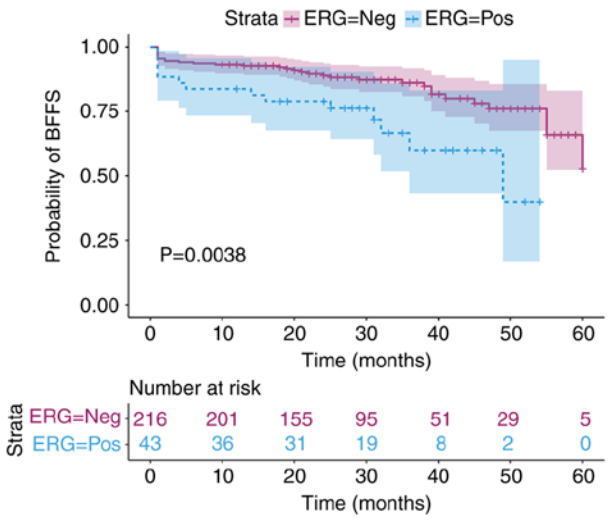

Figure 3. Biochemical failure-free survival curves of ERG-positive and -negative cases after radical prostatectomy. The shaded area corresponds to the 95% confidence interval; the table below is the risk exposure table. P=0.0038. ERG, ETS-related gene; Neg, negative; Pos, positive; BFFS, biochemical failure-free survival.

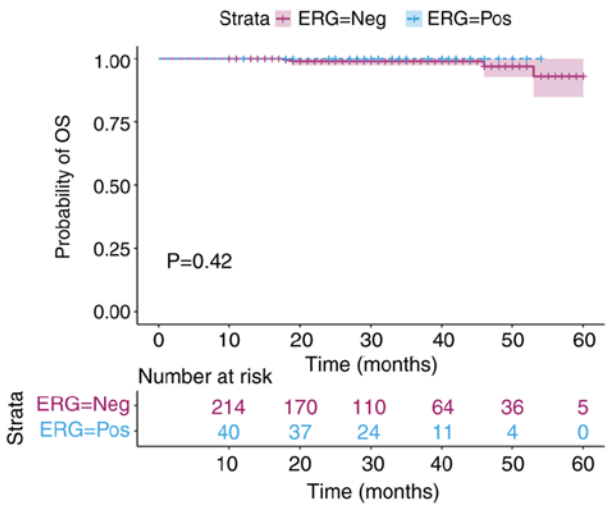

Figure 4. OS curves of ERG-positive and -negative cases after radical prostatectomy. The shaded area corresponds to the 95% confidence interval; the table below is the risk exposure table. P=0.42. ERG, ETS-related gene; OS, overall survival; Neg, negative; Pos, positive.

and the biopsy specimens cannot represent the whole cancer. Furthermore, there exist differences in gene mutations between patients with prostate cancer of different races/ethnicities. Nevertheless, the current findings confirmed the prognostic value of ERG IHC in prostate cancer. Therefore, more aggressive treatment strategies should be adopted for patients with positive ERG IHC results, and comprehensive perioperative treatment should be administered to patients undergoing radical prostatectomy.

In the current study, there were no differences in the Gleason score, TNM stages, surgical margins, age, tumor percentage, Ki-67, PSA and FPSA between patients with positive and negative ERG IHC. The Kaplan-Meier method and Cox regression analysis were used to estimate the prognosis in patients using ERG IHC, PSA, FPSA, age, Gleason score, surgical margins, tumor percentage, Ki-67 and TNM stages; results confirmed that ERG IHC, Gleason score, tumor proportion, surgical margins and Ki-67 were among the factors affecting prostate cancer prognosis. Among these, positive ERG IHC status and Gleason scores of 8 and 10 were independent prognostic factors for prostate cancer.

The *TMPRSS2-ERG* fusion gene has been studied widely and is a common molecular occurrence in high-grade intraepithelial neoplasia of the prostate as well as prostate cancer (19). It induces intraepithelial neoplasia in normal prostate cells in transgenic mice but does not transform into invasive carcinoma; when accompanied by phosphatase and tensin homolog loss, aggressive cancer may develop (20). Therefore, fusion genes may play important roles in prostate cancer development and have significant clinical value for the diagnosis and prognosis of prostate cancer. The *TMPRSS2-ERG* fusion leads to ERG protein overexpression. One study reports that *ERG* silencing leads to cell cycle arrest in prostate cancer cells (21); this is consistent with the report that lowering ERG protein expression reduces the proliferation and migration of prostate cancer cells (22). Both studies suggest that ERG proteins play an important role in prostate cancer. The functions of *ERG* among prostate cancer-related genes are attracting attention worldwide, with increasing studies on this topic. ERG IHC has become a common detection tool used for prostate biopsy and radical prostatectomy specimens (23). The positivity rate of ERG IHC in the present study was 16.6%, slightly lower compared with the average level of 20% in Asia and notably lower compared with the average of 50% in Europe and America (24); these differences may be caused by differences in races/ethnicities. The genomics of prostate cancer in the population in Asia differs from that in the population in Europe and America, such as the presence of *TMPRSS2-ERG*, *BRCA2* and *FOXA1* (24).

The fusion of *TMPRSS2* and *ERG* results from long-term exposure to androgen, increased androgen receptor activity and inhibition of the protein PIWIL1, which prevents DNA double-strand breaks (25). Thus, the *TMPRSS2-ERG* fusion gene is a unique molecular marker for prostate cancer and this finding is of great significance for prostate cancer diagnosis. Nguyen *et al* (26) proposed that the urine-based detection of the *TMPRSS2-ERG* fusion gene can be used as a marker for prostate cancer diagnosis with good specificity and sensitivity, providing a new non-invasive test for diagnosis of prostate cancer. Lin *et al* (27) previously reported that urine *TMPRSS2-ERG* levels after digital rectal examination are associated with higher tumor volumes and Gleason scores in subsequent prostate biopsies.

High-grade intraepithelial prostate tumors containing the *TMPRSS2-ERG* fusion gene are easily transformed into prostate cancer (28), revealing the prognostic value of the *TMPRSS2-ERG* fusion gene in high-grade intraepithelial neoplasia of the prostate. Active surveillance of patients with high-grade intraepithelial neoplasia of the prostate and positive IHC staining of ERG in prostate biopsy pathology is therefore necessary.

The present study has several limitations. First, this was a single-center study conducted at the Huadong Hospital, affiliated with Fudan University. The majority of the participants were Asian and the sample size was small, which

Table II. Univariate and multivariate Cox regression analyses.

	Univariate analysis		Multivariate analysis	
Parameters	HR (95% CI)	P-value	HR (95% CI)	P-value
ERG		0.009[a]		
Negative	Ref		Ref	
Positive	2.48 (1.32-4.66)	0.005[a]	4.08 (2.03-8.17)	0.000074[a]
PSA group, ng/ml		0.335		
≤10	Ref			
10-20	1.48 (0.78-2.84)	0.232		
20-100	1.59 (0.74-3.40)	0.231		
>100	5.18 (0.68-39.20)	0.111		
FPSA group, ng/ml		0.074		
≤1	Ref			
1-4	0.85 (0.46-1.57)	0.607		
>4	2.84 (1.14-7.05)	0.025[a]		
Age group, years		0.699		
≤60	Ref			
>60	1.20 (0.47-3.03)	0.705		
Total GS		0.009[a]		
6	Ref		Ref	
7	1.05 (0.25-4.52)	0.944	1.40 (0.31-6.26)	0.661
8	3.13 (0.69-14.30)	0.141	5.23 (1.01-27.15)	0.049[a]
9	2.80 (0.63-12.30)	0.175	2.83 (0.51-15.57)	0.232
10	8.56 (0.77-95.00)	0.081	18.45 (1.58-216.20)	0.020[a]
Margins		0.035[a]		
Negative	Ref		Ref	
Positive	1.91 (1.06-3.43)	0.030[a]	1.56 (0.78-3.11)	0.204
Tumor percent, %		0.032[a]		
≤25	Ref		Ref	
25-50	0.81 (0.38-1.71)	0.581	0.74 (0.34-1.62)	0.450
50-75	3.06 (1.43-6.53)	0.004[a]	1.65 (0.69-3.94)	0.262
>75	0.96 (0.39-2.39)	0.931	0.59 (0.21-1.67)	0.317
Ki-67, %		0.023[a]		
≤5	Ref		Ref	
>5	2.16 (1.16-4.02)	0.016[a]	1.50 (0.74-3.06)	0.262
T		0.052		
T1	Ref			
T2	20000000 (0-Inf)	0.997		
T3	36100000 (0-Inf)	0.996		
T4	40500000 (0-Inf)	0.996		
N		0.010 [a]		
N0	Ref		Ref	
N1	3.39 (1.43-8.03)	0.006[a]	2.18 (0.80-5.96)	0.129
Nx	$3.34x10^{-8}$ (0-Inf)	0.997	$4.86x10^{-8}$ (0-Inf)	0.995
M		0.464		
M0	Ref			
M1	2.32 (0.32-17.10)	0.408		

[a]P<0.05. BCR, biochemical recurrence; CI, confidence interval; ERG, ETS-related gene; GS, Gleason score; HR, hazard ratio; PSA, prostate-specific antigen; inf, Infinity.

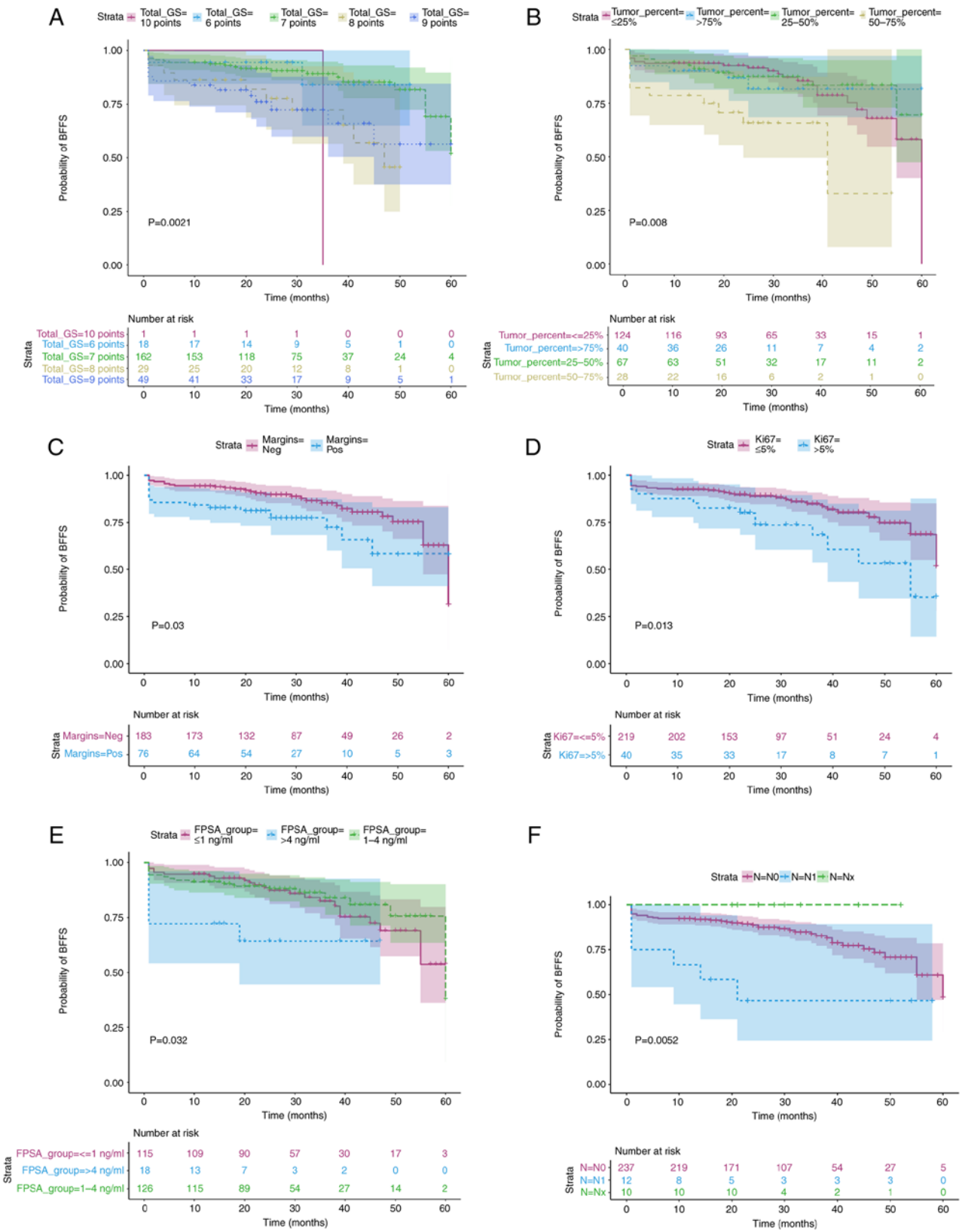

Figure 5. BFFS curves for GS, tumor proportions, surgical margins, FPSA, Ki-67 and N stages after radical prostatectomy. The shaded area corresponds to the 95% confidence interval; the table below is the risk exposure table. (A) BFFS curves for Gleason scores, P=0.0021. (B) BFFS curves for tumor proportions, P=0.008. (C) BFFS curves for surgical margins, P=0.03. (D) BFFS curves for Ki-67, P=0.013. (E) BFFS curves for FPSA, P=0.032. (F) BFFS curves for N stages, P=0.0052. ERG, ETS-related gene; FPSA, free prostate-specific antigen; IHC, immunohistochemistry; BFFS, biochemical failure-free survival; GS, Gleason Score; N, node.

could have caused selection bias and systematic errors in the study. Performing multi-center studies and expanding the sample size and other sample data would help strengthen the credibility of the results. Most of the samples included

are ERG negative. The difference between the number of ERG-positive samples and the number of ERG-negative samples is unavoidable, because the average positivity rate of ERG IHC is 20% in Asia. Second, this was a retrospective study, having several limitations when compared with a prospective study, and there may be interfering factors that lack credibility and have not been considered. Third, the IHC method used in this study was qualitative, and its application in the detection of ERG expression has certain limitations. In future, prostate cancer specimens could be divided according to the proportion of tumor cells stained by ERG IHC (low, intermediate and high). Quantitative methods such as western blotting and qPCR might also yield more convincing results.

As precision medicine has become mainstream, molecular detection has become a common clinical approach (29). For instance, breast cancer can be classified into subtypes based on the estrogen receptor and human epidermal growth factor receptor 2 (HER2) (30). Estrogen receptors and HER2 can predict tumor progression and help in deciding optimal breast cancer treatments (30). Similarly, the *TMPRSS2-ERG* fusion gene is commonly and uniquely found in prostate cancer cases; however, whether it can act as a potential indicator for prostate cancer typing requires further study.

The present study identified differences in BCR between patients with positive and negative ERG IHC results. Patients with ERG IHC-positive status had a worse prognosis and were more prone to BCR compared with those with ERG IHC-negative status. ERG IHC positivity is thus an independent risk factor for predicting postoperative BCR in prostate cancer. ERG IHC is expected to become a prognostic indicator of prostate cancer, and its clinical application has been further improved. In conclusion, the present study revealed that patients with positive ERG IHC status were prone to BCR after radical prostatectomy and that positive ERG expression was an independent prognostic risk factor for prostate cancer.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ZS, YZ, JT and RW designed and investigated the trial. YZ, JT and RW analyzed and interpreted the data and wrote the manuscript. HX, ZC, LX and HD collected and analyzed the data. YZ, JT, RW, HX, ZC, LX and HD reviewed and revised the manuscript. ZS, YZ, JT and RW confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study, which involved human participants, was reviewed and approved by the Ethics Committee of the Huadong Hospital, affiliated with Fudan University (approval no. 20220050). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Since this study was a retrospective cohort study, an informed consent waiver was applied.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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