Int J Clin Exp Pathol 2015;8(2):1878-1888
www.ijcep.com /ISSN:1936-2625/IJCEP0003560

Original Article

Nuclear C-MYC expression level is associated with disease progression and potentially predictive of two year overall survival in prostate cancer

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Received November 3, 2014; Accepted December 22, 2014; Epub February 1, 2015; Published February 15, 2015

Abstract: Purpose: Upregulation of nuclear C-MYC protein has been reported to be an early event in prostate cancer (PCa); however, its clinicopathological and prognostic significance remain controversial. We determined the association of nuclear C-MYC protein expression with clinicopathological parameters, prognosis, *ETS-related gene* (ERG) expression, and *TMPRSS2-ERG* status in PCa. Methods: Nuclear C-MYC and ERG expression by immunohistochemistry and *TMPRSS2-ERG* status by triple-color probe fluorescence *in situ* hybridization assay were determined in 50 hormone-naïve PCa patients and 31 radical prostatectomy specimens. Results: Nuclear C-MYC immunostaining was negative, positive, and strong positive in 27.5%, 32.5%, and 40.0% of cases, respectively. C-MYC immunostaining was significantly associated with clinical T stage ($P < 0.001$), distant metastasis at the time of diagnosis ($P < 0.001$) and *TMPRSS2-ERG* status ($P = 0.001$) but not with ERG immunostaining ($P = 0.818$). In the Kaplan-Meier analysis, C-MYC positive cases were found to have worse 2-year OS compared with C-MYC negative cases ($P = 0.027$). However, in the univariate Cox analysis, only *TMPRSS2-ERG* status (hazard ratio [HR] 0.189, 95% CI 0.057-0.629; $P = 0.007$) and distant metastasis (HR 3.545, 95% CI 1.056-11.894; $P = 0.040$) were significantly associated with 2-year OS. After adjusting for these two factors, *TMPRSS2-ERG* status still impacted 2-year OS (HR 0.196, 95% CI 0.049-0.778; $P = 0.020$). Conclusions: Nuclear C-MYC overexpression may be associated with disease progression and potentially predictive of 2-year OS in PCa. This is the first study to demonstrate an association between nuclear C-MYC immunostaining and *TMPRSS2-ERG* status in PCa.

Keywords: C-MYC, ERG, prostate cancer, TMPRSS2-ERG, immunohistochemistry

Introduction

C-MYC amplification has been consistently reported to be correlated with tumor behavior in prostate cancer (PCa) [1-4]. *C-MYC* amplification has also been shown to be a potential marker for disease progression and prognosis in PCa. Elevated *C-MYC* mRNA level has been demonstrated in PCa tissues [5-7] and is associated with biochemical recurrence after radical prostatectomy (RP) [7]. Although nuclear C-MYC protein is also upregulated in PCa, its correlation with clinicopathological parameters, disease progression, and prognosis remain controversial [3, 8-12]. Elevated C-MYC protein identified by immunohistochemistry (IHC) is not necessarily correlated with elevated *C-MYC* mRNA level [8] but is reported to be associated with *C-MYC* amplification [3, 13].

A high prevalence of *TMPRSS2-ETS-related gene* (*ERG*) rearrangement has been reported in PCa [14, 15]. *TMPRSS2-ERG* rearrangement is associated with the androgenic induction of *ETS-related gene* (*ERG*) expression, which has been shown to be a potential marker for PCa diagnosis, stage, and prognosis [16-19]. ERG overexpression has been reported to activate *C-MYC* in PCa, thereby leading to the inhibition of prostate epithelial differentiation [20]. This

finding suggests that *C-MYC* is a critical downstream target of ERG. Although many studies have evaluated ERG and C-MYC protein expression in human PCa, few studies have examined the association of nuclear C-MYC expression with ERG expression and *TMPRSS2-ERG* rearrangement. The objective of our study was to determine the association of nuclear C-MYC protein expression with tumor behavior, ERG expression, and *TMPRSS2-ERG* rearrangement in PCa.

Materials and methods

Patients and tissue samples

This was a single-center retrospective study comprised of 81 patients diagnosed with PCa at Tongji Hospital between 2012 and 2013. PCa diagnosis was based on digital rectal examination, prostate-specific antigen (PSA) level, and Gleason score. All patients signed informed consent documents approved by Tongji Hospital. Prostate tissue specimens (50 diagnostic needle biopsies obtained from hormone-naïve patients and 31 RP specimens) were fixed in 10% buffered formalin, embedded in paraffin, and sliced into 4-μm sections for fluorescence *in situ* hybridization (FISH) and IHC experiments.

FISH

The FISH probe used for detecting *TMPRSS2-ERG* status was purchased from Kreatech (cat. no. KBI-10726; Kreatech Diagnostics, Amsterdam, the Netherlands). This triple-color probe is optimized to detect the deletion between *TMPRSS2* and *ERG* at 21q22 associated with *TMPRSS2-ERG* fusion and translocations involving the *TMPRSS2* region such as ETV1 t(7; 21) and ETV4 t(17; 21) [21]. Tissue sections were heated at 56°C for 6 h, deparaffinized in xylene, rehydrated through a graded ethanol series, and heated at 90°C in a double distilled water bath for 30 min. After cooling at room temperature, sections were digested with protease K at 37°C for 15-18 min. The sections were washed 2 times with 2 × saline sodium citrate, refixed in 10% formaldehyde for 10 min, and dehydrated through a graded ethanol series. The sections were incubated with the FISH probe and denatured by heating at 80°C for 5 min in a steamer. Sections were hybridized by heating at 37°C overnight in a steamer. After a quick wash, the sections were counterstained with 4',6-diamidino-2-phenylindole to visualize nuclei and observed under a fluorescent microscope. The sections were reviewed and pathologically diagnosed by two independent pathologists. Sections with diagnostic disagreement between the reviewers were re-reviewed until a consensus was reached.

IHC

Sections were routinely processed as follows: deparaffinized in xylene, rehydrated through a graded ethanol series, subjected to heat-induced antigen retrieval in a steamer, and incubated in 3% H_2O_2 to block endogenous peroxidase. The sections were incubated overnight at 4°C with mouse anti-human C-MYC monoclonal antibody (cat. no. MAB-0185; clone 9E10.3; MaxVision, Fuzhou Maixin Biotechnology Co. Ltd., Fuzhou, China) and rabbit anti-human ERG monoclonal antibody (cat. no. ZA-0545; clone EP111; Beijing ZSBIO Biotechnology Co. Ltd., Beijing, China). Sections incubated with phosphate-buffered saline (PBS) alone were used as a negative control. Immunostaining was developed using the MaxVision HRP-Polymer anti-mouse/rabbit IHC Kit (cat. no. KIT-5010; Fuzhou Maixin Biotechnology Co. Ltd.) and a 3,3'-diaminobenzidine (DAB) detection kit (cat. no. DAB-0031; MaxVision, Fuzhou Maixin Biotechnology Co. Ltd.). The slides were washed with 0.1 mM PBS and then incubated with horseradish peroxidase-polymer anti-mouse/rabbit antibody (MaxVision) for 20 min at room temperature. After washing with PBS, the sections were incubated with DAB at room temperature for 5 min. All slides were processed by the same pathologist.

PCa cells were identified by hematoxylin-eosin staining. Immunohistochemical staining for nuclear C-MYC in PCa cells was evaluated using quick score (QS), a double-grading system [22, 23]. The QS represents the sum of a proportional score (PS) and intensity score (IS). The PS was calculated as the ratio of C-MYC immunopositive tumor cells to the total number of tumor cells. The PS was classified as follows: 0, none; 1, 1%-30%; 2, 31%-60%; and 3, 61%-100%. The IS was classified as follows: 0, no immunostaining at high magnification; 1, immunostaining only visible at high magnification; 2, immunostaining readily visible at low magnification; and 3, immunostaining strongly visible at low magnification. Cases in which all tumor foci expressed nuclear ERG protein were considered positive; all other cases were considered negative. Consistent with the literature

Table 1. Clinicopathological characteristics of 81 Chinese prostate cancer patients

Parameters	
Median follow up period for survivors (months, range)	21 (12~31)
Median age (years, range)	68 (49~94)
Sample sources	
Hormone-naïve needle biopsies, n (%)	50 (61.7)
Tumor tissues after RP, n (%)	31 (38.3)
Median PSA at the time of diagnosis (ng/ml)	67.270 (0.009~999.999)
PSA at the time of diagnosis (ng/ml), n (%)	
< 10	11 (13.6)
10~20	10 (12.3)
> 20	60 (74.1)
Gleason score, n (%)	
< 7	13 (16.0)
7	23 (28.4)
> 7	45 (55.6)
Clinical T stage, n (%)	
T1	7 (8.7)
T2	27 (33.3)
T3	27 (33.3)
T4	18 (22.2)
x	2 (2.5)
Lymph node metastasis at the time of diagnosis, n (%)	
Negative	47 (58.0)
Positive	25 (30.9)
x	9 (11.1)
Distant metastasis at the time of diagnosis, n (%)	
Negative	43 (53.1)
Positive	30 (37.0)
x	8 (9.9)

PSA, prostate-specific antigen; RP, radical prostatectomy; x, cannot be evaluated.

[17, 24], we observed strong diffuse (IS, 2~3) ERG immunostaining in tumor foci in most positive cases. A few cases contained both ERG-positive and ERG-negative tumor foci. These cases were termed ERG heterogeneous and included in the ERG positive group. Blood vessel staining was used as an internal control. Two independent pathologists reviewed the immunostaining results. Cases with disagreement were reanalyzed until a consensus was reached.

Statistical analysis

Patient characteristics, FISH and IHC results were compared using Fisher's exact and Pearson chi-square tests. Overall survival (OS) was calculated from the date of first treatment to the date of death from any cause or last follow-up. OS was evaluated using the Kaplan-Meier method and compared using the log-rank test. Cox analysis was used to investigate the association of clinicopathological parameters (PSA level at the time of diagnosis, Gleason score, clinical T stage, lymph node metastasis, and distant metastasis), *TMPRSS2-ERG* status, and C-MYC immunostaining with OS. Two-tailed *P* values < 0.05 were considered statistically significant. All statistical analyses were performed using SPSS 12.0 software (SPSS, Chicago, IL, USA).

Results

Patient characteristics

Eighty-one formalin-fixed paraffin-embedded samples, representing the spectrum of the disease, were included in the study. The clinicopathological characteristics of the patients are shown in **Table 1**. Median PSA at the time of diagnosis was 67.270 ng/mL (range, 0.009~999.999 ng/mL). PSA at the time of diagnosis was < 10 ng/mL in 13.6% (11/81) of patients, 10~20 ng/mL in 12.3% (10/81) of patients, and > 20 ng/mL in 74.1% (60/81) of patients. Gleason score was > 7 in 55.6% (45/81) of patients and 7 in 28.4% (23/81) of patients. At the time of diagnosis, 55.6% (45/81), 30.9% (25/81), and 37.0% (30/81) of patients had clinical T3/4 stage disease, lymph node metastasis, and distant metastasis, respectively.

Relationship of nuclear C-MYC immunostaining to ERG immunostaining and TMPRSS2-ERG status

IHC and FISH results are shown in **Table 2**. ERG and C-MYC were both expressed in the nucleus (**Figure 1**). ERG and C-MYC protein expression

Table 2. Association of C-MYC immunostaining with ERG expression and *TMPRSS2-ERG* status

	No. of pts (%)	C-MYC			*P*	ERG		*P*
		Negative (QS = 0)	Positive (QS = 1~3)	Strong positive (QS = 4~6)		Negative	Positive	
TMPRSS2-ERG status	76	20 (26.3)	26 (34.2)	30 (39.5)	0.001	43 (56.6)	33 (43.4)	0.001
Negative	21 (27.6)	2 (10.0)	11 (42.3)	8 (26.6)	Ref	18 (41.9)	3 (9.1)	Ref
Del (21q22) *TMPRSS2-ERG* Fusion	7 (9.2)	0	2 (7.7)	5 (16.7)	0.277	0	7 (21.2)	<0.001
Break *TMPRSS2*	26 (34.2)	15 (75.0)	6 (23.1)	5 (16.7)	0.003	13 (30.2)	13 (39.4)	0.014
Break *ERG*	22 (29.0)	3 (15.0)	7 (26.9)	12 (14.0)	0.393	12 (27.9)	10 (30.3)	0.045
ERG	79	22 (27.9)	26 (32.9)	31 (39.2)	–			
Negative	44 (55.7)	11 (50.0)	15 (57.7)	18 (58.1)	Ref	–	–	–
Positive	35 (44.3)	11 (50.0)	11 (42.3)	13 (41.9)	0.818	–	–	–

QS, quick score.

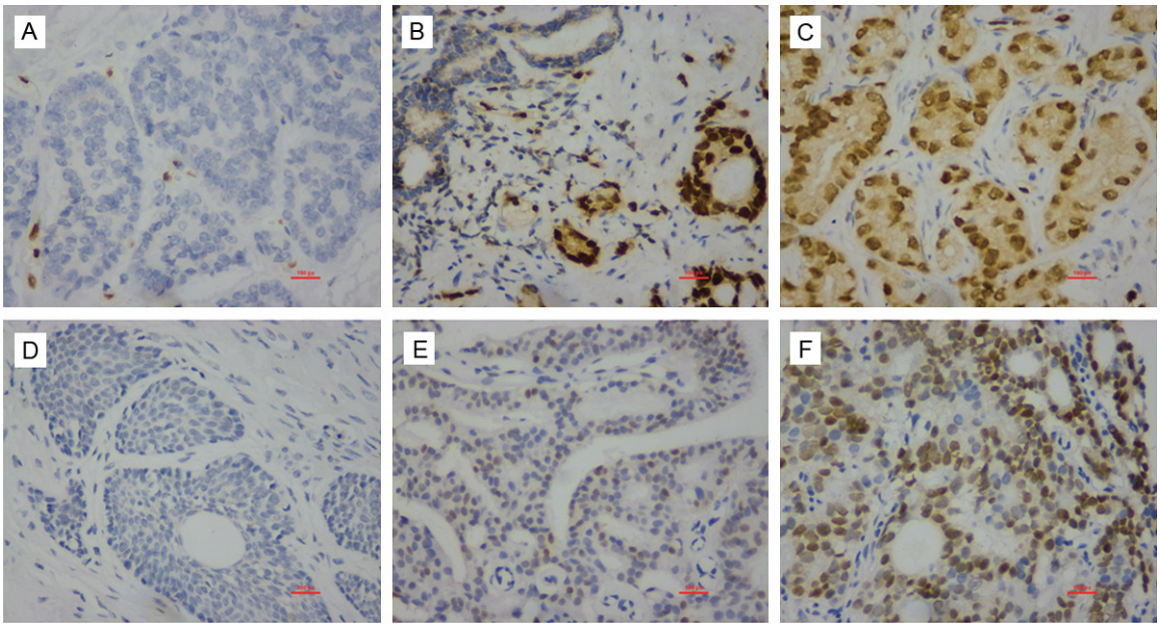

Figure 1. Representative ERG negative (A), ERG heterogeneous (B), ERG positive (C), C-MYC negative (D; QS = 0), C-MYC positive (E; QS = 1~3), and C-MYC strong positive (F; QS = 4~6) immunostaining (400 ×). QS, quick score.

were successfully evaluated in 79 and 80 cases, respectively. C-MYC immunostaining was divided into three groups according to the QS: negative, 0; positive, 1~3; and strong positive, 4~6. The percentage of negative, positive, and strong positive cases was 27.5%, 32.5%, and 40.0%, respectively, thus nuclear C-MYC protein was expressed in 72.5% of the cases. ERG positive immunostaining was present in 44.3% of cases, in which 10 cases were ERG heterogeneous. *TMPRSS2-ERG* status was successfully evaluated in 76 cases and classified into four groups: *TMPRSS2-ERG* negative, Del (21q22) *TMPRSS2-ERG* Fusion, Break *TMPRSS2*, and Break *ERG* (**Figure 2**). The percentage of cases in the *TMPRSS2-ERG* negative, Del (21q22) *TMPRSS2-ERG* Fusion, Break *TMPRSS2*, and Break *ERG* groups was 27.6%, 9.2%, 34.2%, and 29.0%, respectively.

In the Pearson chi-square analysis, C-MYC and ERG protein expression identified by IHC were not correlated ($P = 0.818$). However, nuclear C-MYC protein expression was significantly correlated with *TMPRSS2-ERG* status ($P = 0.001$). The frequency of C-MYC negative immunostaining was higher in the Break *TMPRSS2* group than in the *TMPRSS2-ERG* negative group ($P = 0.003$). A significant correlation was also found between *TMPRSS2-ERG* status and ERG immunostaining ($P = 0.001$). Compared with *TMPRSS2-ERG* negative cases, Del (21q22) *TMPRSS2-ERG* Fusion ($P < 0.001$), Break *TMPRSS2* ($P = 0.014$) and Break *ERG* ($P =$

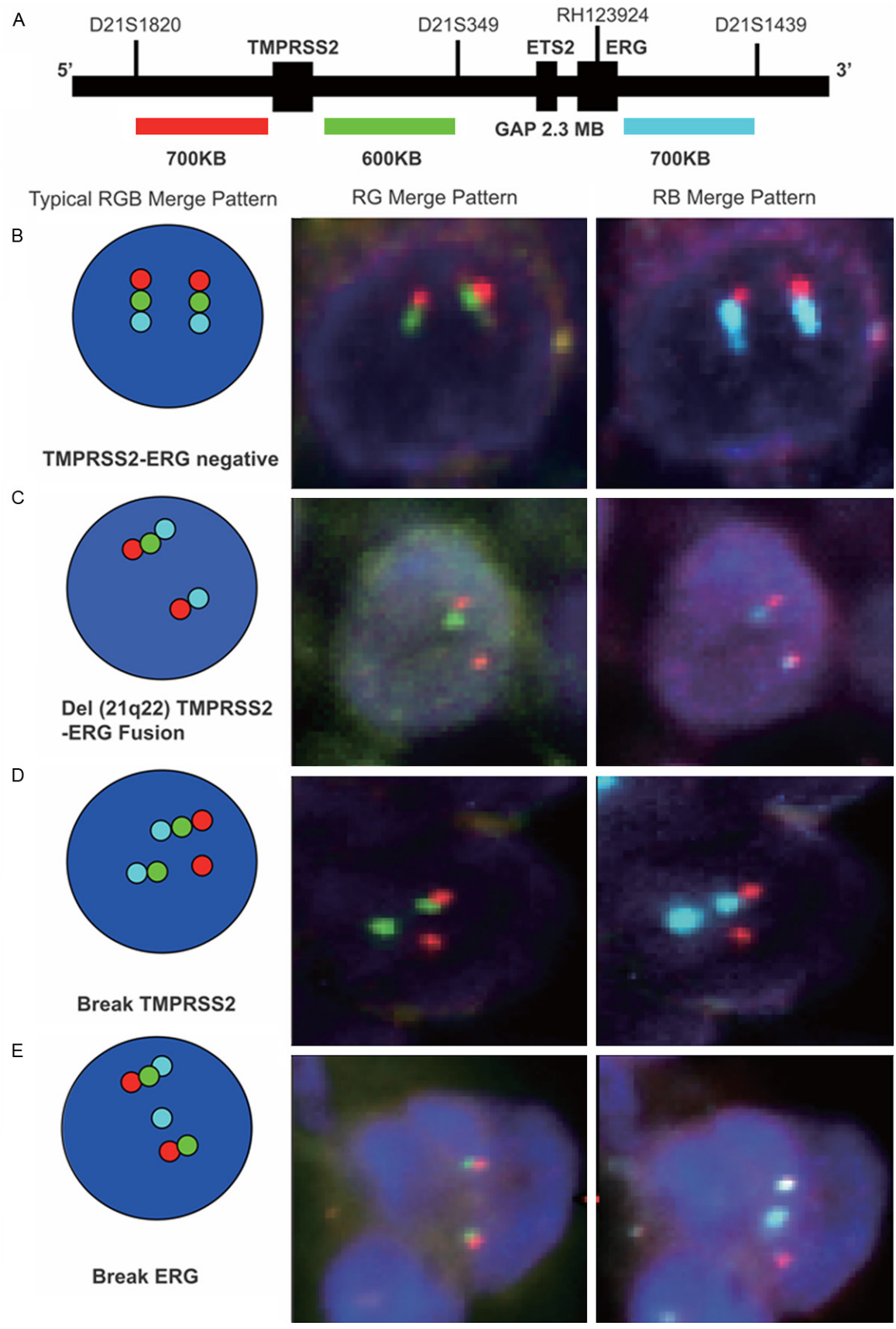
A
D21S1820
TMPRSS2
D21S349
RH123924
ETS2
ERG
D21S1439
5'
3'
GAP 2.3 MB
700KB
600KB
700KB
Typical RGB Merge Pattern
RG Merge Pattern
RB Merge Pattern
B
TMPRSS2-ERG negative
C
Del (21q22) TMPRSS2
-ERG Fusion
D
Break TMPRSS2
E
Break ERG

Figure 2. A. Schematic map of '*TMPRSS2*' and '*ERG*' gene region on 21q22. Red, blue and green signal represents the distal *TMPRSS2*, proximal *TMPRSS2* and proximal *ERG* gene region, respectively. B-E. Represent four different *TMPRSS2-ERG* patterns identified in this study.

0.045) were significantly associated with ERG overexpression.

Relationship between nuclear C-MYC immunostaining and clinicopathological parameters

The results of the statistical analysis for the relationship between clinicopathological parameters and nuclear C-MYC immunostaining are shown in **Table 3**. Nuclear C-MYC staining was significantly correlated with clinical T stage ($P < 0.001$). The percentage of C-MYC strong positive (QS = 4~6) cases with clinical T1, T2, T3, and T4 stage disease was 14.3%, 22.2%, 38.5%, and 83.3%, respectively. Distant metastasis at the time of diagnosis was also significantly correlated with nuclear C-MYC expression ($P < 0.001$). The C-MYC strong positive rate was 70.0% in distant metastasis positive cases and 20.9% in distant metastasis negative cases. TMPRSS2-ERG status was significantly associated with distant metastasis at the time of diagnosis ($P = 0.018$; **Table 3**). Patients with TMPRSS2-ERG negative and Break ERG partners had a similar distant metastasis rate (47.4% vs. 45.5%). However, distant metastasis occurred more frequently in patients with Del (21q22) TMPRSS2-ERG Fusion than in patients with Break TMPRSS2 (71.4% vs. 13.6%). ERG staining, C-MYC immunostaining, and TMPRSS2-ERG status were not significantly associated with any other clinicopathological parameters.

Relationship of nuclear C-MYC immunostaining to OS

The median follow-up period for surviving patients was 21 months (range, 12-31 months). Five patients were dead at the last follow-up. Two-year OS (Kaplan-Meier estimate) in the nuclear C-MYC negative, positive (QS = 1~3), and strong positive (QS = 4~6) groups was 100%, 48.0 ± 22.9%, and 65.0 ± 12.8%, respectively (**Table 4**). In the Kaplan-Meier analysis, 2-year OS was significantly lower in the nuclear C-MYC positive and strong positive cases than in the C-MYC negative cases ($P = 0.022$ and $P = 0.026$, respectively). However, in the univariate Cox analysis, nuclear C-MYC immunostaining was not significantly correlated with 2-year OS (hazard ratio [HR] 0.021, 95% CI 0.000-7.990; $P = 0.203$), but *TMPRSS2-ERG* status (HR 0.189, 95% CI 0.057-0.629; $P = 0.007$) and distant metastasis (HR 3.545, 95% CI 1.056–11.894; $P = 0.040$) were significantly associated with 2-year OS (**Table 5**). After adjusting for these two factors, only *TMPRSS2-ERG* status still impacted 2-year OS (HR 0.196, 95% CI 0.049–0.778; $P = 0.020$).

Discussion

Our study showed that nuclear C-MYC protein expression detected by IHC is significantly associated with advanced clinical T stage, distant metastasis at the time of diagnosis, and 2-year OS estimated by the Kaplan-Meier method. These findings indicate that C-MYC might contribute to disease progression and predict early mortality in PCa. We found that nuclear C-MYC protein expression is not significantly correlated with ERG protein expression but is strongly correlated with *TMPRSS2-ERG* status. To our knowledge, this is the first study to determine the association of nuclear C-MYC protein expression with ERG expression and *TMPRSS2-ERG* rearrangement in human PCa.

Prior studies have reported that 8q24 gain at the *C-MYC* locus is a common recurrent aberration in PCa [1, 25, 26]. Studies have consistently demonstrated that *C-MYC* amplification detected by FISH or array comparative genomic hybridization is associated with tumor behavior in PCa [1-3]. Furthermore, *C-MYC* amplification has been shown to be a potential marker for PCa progression and prognosis [1-3]. *C-MYC* mRNA level has also been found to be markedly increased in PCa tissues [5-7]. Although elevated *C-MYC* mRNA level was found not to be correlated with clinicohistological parameters, it is predictive of biochemical recurrence after RP [7]. C-MYC protein expression detected by IHC has been shown not to be associated with *C-MYC* mRNA level [8] but strongly correlated with *C-MYC* amplification [3, 13]. Many studies have demonstrated the upregulation of nuclear C-MYC protein in PCa; however, to date, the relationship between nuclear C-MYC protein expression detected by IHC and prostate tumor behavior remains conflicting. Antonarakis et al. [10] established an immunohistochemical signature to predict prognosis in PCa patients receiving docetaxel after RP. Nuclear C-MYC protein expression, a component of this signa-

Table 3. Correlation of clinicopathological parameters with C-MYC immunostaining

Parameters	C-MYC				*TMPRSS2-ERG* status					ERG		
	Negative (QS = 0)	Positive (QS = 1~3)	Strong positive (QS = 4~6)	*P*	Negative	Del (21q22) *TMPRSS2-ERG* fusion	Break *TMPRSS2*	Break *ERG*	*p*	Negative	Positive	*P*
Gleason score	22	26	32	0.414	21	7	26	22	0.235	44	35	0.278
< 7	3 (13.6)	6 (23.1)	4 (12.5)		3 (14.2)	1 (14.3)	5 (19.2)	1 (4.5)		4 (9.1)	7 (20.0)	
7	9 (40.9)	7 (26.9)	7 (21.9)		9 (42.9)	1 (14.3)	9 (34.6)	4 (18.2)		12 (27.3)	11 (31.4)	
> 7	10 (45.5)	13 (50.0)	21 (65.6)		9 (42.9)	5 (71.4)	12 (46.2)	17 (77.3)		28 (63.6)	17 (48.6)	
PSA (ng/ml)	22	26	32	0.259	21	7	26	22	0.105	44	35	0.369
< 10	6 (27.3)	3 (11.5)	2 (6.3)		1 (4.8)	1 (14.3)	8 (30.8)	2 (9.1)		5 (11.4)	6 (17.1)	
10~20	3 (13.6)	3 (11.5)	4 (12.5)		1 (4.8)	0	4 (15.4)	3 (13.6)		3 (6.8)	5 (14.3)	
> 20	13 (59.1)	20 (77.0)	26 (81.2)		19 (90.4)	6 (85.7)	14 (53.8)	17 (77.3)		36 (81.8)	24 (68.6)	
Clinical T-stage	20	26	32	<0.001	21	7	26	22	0.478	43	34	0.050
T1	0	6 (23.1)	1 (3.1)		3 (14.3)	0	3 (11.5)	1 (4.5)		3 (7.0)	4 (11.8)	
T2	13 (65.0)	8 (30.8)	6 (18.8)		5 (23.8)	4 (57.1)	9 (34.6)	6 (27.3)		10 (23.3)	17 (50.0)	
T3	6 (30.0)	10 (38.5)	10 (31.2)		9 (42.9)	0	9 (34.6)	9 (40.9)		17 (39.5)	8 (23.5)	
T4	1 (5.0)	2 (7.6)	15 (46.9)		4 (19.0)	3 (42.9)	5 (19.3)	6 (27.3)		13 (30.2)	5 (14.7)	
Lymph node metastasis	20	23	29	0.312	14	6	25	22	0.285	37	35	0.286
Negative	15 (75.0)	16 (69.6)	16 (55.2)		10 (71.4)	4 (66.7)	19 (76.0)	11 (50.0)		22 (59.5)	25 (71.4)	
Positive	5 (25.0)	7 (30.4)	13 (44.8)		4 (28.6)	2 (33.3)	6 (24.0)	11 (50.0)		15 (40.5)	10 (28.6)	
Distant Metastasis at the time of diagnosis	19	24	30	< 0.001	19	7	22	22	0.018	38	35	0.256
Negative	17 (89.5)	17 (70.8)	9 (30.0)		10 (52.6)	2 (28.6)	19 (86.4)	12 (54.5)		20 (52.6)	23 (65.7)	
Positive	2 (10.5)	7 (29.2)	21 (70.0)		9 (47.4)	5 (71.4)	3 (13.6)	10 (45.5)		18 (47.4)	12 (34.3)	

PSA, prostate-specific antigen; QS, quick score.

Table 4. Two-year OS probability estimated by Kaplan-Meier analysis

Parameter	No. of pts	2-year OS	*P*
C-MYC			0.027
Negative (QS = 0)	22	100.000	Ref
Positive (QS = 1~3)	26	0.480 ± 0.229	0.022
Strong positive (QS = 4~6)	32	0.650 ± 0.128	0.026
TMPRSS2-ERG status			0.019
Negative	21	0.774 ± 0.119	Ref
Del (21q22) *TMPRSS2-ERG* fusion	7	0.400 ± 0.219	0.037
Break *TMPRSS2*	26	0.892 ± 0.072	0.455
Break *ERG*	22	0.756 ± 0.135	0.981
ERG			–
Negative	44	0.805 ± 0.080	Ref
Positive	35	0.602 ± 0.137	0.414

ture, was significantly associated with progression-free survival in both the univariate and multivariate analyses. Fromont et al. [3] found that 32% of 287 PCa cases had positive nuclear C-MYC immunostaining, with rates ranging from 10% to 95%. In their study, nuclear C-MYC immunostaining increased with Gleason score but was not correlated with disease progression. Reis et al. [11] reported that the positive immunostaining rate of nuclear C-MYC was 40.2%, 57.3%, and 51.5% in localized PCa, PCa metastatic to lymph nodes, and PCa metastatic to bone, respectively; however, nuclear C-MYC immunostaining was not correlated with disease progression or tumor behavior. Gurel et al. [8], using a novel anti-MYC antibody, suggested that C-MYC protein upregulation is a prevalent and early event in PCa. In their study, 81.6% of cancer lesions were considered nuclear C-MYC positive; however, nuclear C-MYC positivity was not significantly correlated with clinicopathological characteristics or disease recurrence after RP. Differences in disease stage and antibody source might have contributed to the conflicting findings regarding the clinicopathological and prognostic role of nuclear C-MYC protein in PCa in previous studies and the present study.

Many studies have investigated C-MYC and ERG protein expression in PCa, but few studies have focused on the relationship between these two proteins. A recent study demonstrated that ERG overexpression activates *C-MYC* to block prostate epithelial differentiation in cell culture models, animal models, and human prostate tumors [20]. This finding suggests a critical link between *C-MYC* and ERG in PCa. Quantitative real-time polymerase chain reaction analysis has suggested a significant association between *C-MYC* and *ERG* mRNA expression [7]. To our knowledge, the present study is the first to investigate the relationship between ERG and C-MYC protein expression detected by IHC. Unfortunately, we did not confirm the association between ERG and nuclear C-MYC immunostaining in human PCa tissues.

Interestingly, we found that C-MYC protein expression is strongly correlated with *TMPRSS2-ERG* status in PCa. Our study is the first to demonstrate this association. This finding suggests that Break *TMPRSS2* is significantly associated with C-MYC negative immunostaining. Our specimens were obtained from a Chinese patient cohort. Therefore, it is difficult to find evidence in the literature to support our findings as *TMPRSS2-ERG* fusion is the dominant *ERG* fusion pattern in Western countries. Furthermore, no study, to our knowledge, has directly investigated the relationship of C-MYC protein expression to *TMPRSS2-ERG* status in human PCa. One study [27] did evaluate the association of *TMPRSS2-ERG* status with 8q gain in 200 diagnostic prostate needle biopsies; however, no significant correlation was found. Unfortunately, this study did not examine the relationship between *TMPRSS2-ERG* status and *C-MYC* amplification. Future studies are needed to confirm the association of nuclear C-MYC protein expression with *TMPRSS2-ERG* status in PCa.

The present study used a novel commercial tricolor FISH probe to detect rearrangements associated with TMPRSS2 and ERG genes. This tricolor probe not only readily detects the TMPRSS2-ERG fusion generated by interstitial deletion/insertion but also possible TMPRSS2 and ERG rearrangements with other partners [21, 28, 29]. TMPRSS2-ERG fusion by Del (21q22) was observed in 9.2% of cases in the present study. Using the same tricolor probe, Ribeiro et al. [28] identified TMPRSS2-ERG fusion by insertion in 44.0% of PCa cases. However, we did not observe this fusion in the

Table 5. Univariate and multivariate analysis of variables associated with survival in PCa

	Univariate analysis		Multivariate analysis*	
Parameters	HR (95% CI)	*p*	HR (95% CI)	*p*
Age	1.003 (0.946-1.065)	0.910	–	
PSA at the time of diagnosis	1.729 (0.548-5.459)	0.350	–	
Gleason score	1.723 (0.556-5.341)	0.346	–	
Clinical T stage	1.362 (0.683-2.716)	0.380	–	
Lymph node metastasis at the time of diagnosis	1.741 (0.560-5.418)	0.338	–	
Distant metastasis at the time of diagnosis	3.545 (1.056-11.894)	0.040	0.532 (0.124-2.289)	0.397
C-MYC staining	0.021 (0.000-7.990)	0.203	–	
ERG staining	1.682 (0.532-5.313)	0.376	–	
TMPRSS2-ERG status	0.189 (0.057-0.629)	0.007	0.196 (0.049-0.778)	0.020

*: only significant factors identified by univariate analysis are included in multivariate analysis. HR, hazard ratio; CI, confidence interval; PSA, prostate-specific antigen.

present study. Consistent with our findings, Serra et al. [29] also did not detect this TMPRSS2-ERG fusion. Interestingly, a high frequency of Break TMPRSS2 was found in both the present study and Serra et al. study [29] (38.2% and 40.5%, respectively) and might correspond to TMPRSS2 rearrangements with other genes. However, it is possible that unknown TMPRSS2 and ERG gene fusion mechanisms exist. For example, we found that Break TMPRSS2 was significantly associated with ERG overexpression. In the present study, 29% of cases showed Break ERG partner, which was not observed in the Ribeiro et al. [28] and Serra et al. [29] studies. It is possible that rearrangement mechanisms differ among ethnic groups. Furthermore, sample selection bias and the number of cases analyzed might also influence the predominant rearrangements observed. In the present study, we found that Del (21q22) TMPRSS2-ERG fusion was associated with 2-year OS. However, 2-year OS in the Break TMPRSS2 and Break ERG groups was similar to that in the TMPRSS2-ERG negative group. In contrast, FitzGerald et al. [30] found that median survival time did not differ according to TMPRSS2-ERG fusion type. Thus, further studies are needed to confirm the association of TMPRSS2-ERG fusions with survival in PCa.

In summary, our study findings suggest that nuclear C-MYC protein expression is associated with disease progression and predictive of 2-year OS in PCa. Furthermore, C-MYC immunostaining is not significantly correlated with ERG expression but is strongly associated with *TMPRSS2-ERG* status.

Acknowledgements

We thank the following pathologists for evaluating the FISH results: Ying Wang, Heng Zhang, Qinlu Li, and Chao Zhang (Chromosome Working Group from Laboratory of Hematology, Tongji Hospital). This study was supported by the National Natural Science Foundation of China (grant number 81302218) and Hubei Natural Science Foundation (grant number 2013-CFB175).

Disclosure of conflict of interest

None.

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