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Concurrent nuclear ERG and MYC protein overexpression defines a subset of locally advanced prostate cancer: potential opportunities for synergistic targeted therapeutics

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Abstract

BACKGROUND—Recurrent *ERG* gene fusions, the most common genetic alterations in prostate cancer, drive overexpression of the nuclear transcription factor ERG and are early clonal events in prostate cancer progression. The nuclear transcription factor MYC is also frequently overexpressed in prostate cancer and may play a role in tumor initiation and/or progression. The relationship between nuclear ERG and MYC protein overexpression in prostate cancer, as well as the clinicopathologic characteristics and prognosis of ERG-positive/MYC high tumors, is not well understood.

METHODS—Immunohistochemistry (IHC) for ERG and MYC was performed on formalin-fixed, paraffin-embedded tissue from prostate cancer tissue microarrays (TMAs), and nuclear staining was scored semi-quantitatively (IHC product score range = 0–300). Correlation between nuclear ERG and MYC protein expression and association with clinicopathologic parameters and biochemical recurrence after radical prostatectomy was assessed.

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RESULTS—29.1% of all tumor nodules showed concurrent nuclear ERG and MYC protein overexpression (i.e., ERG-positive/MYC high), including 35.0% of secondary nodules. Overall, there was weak positive correlation between ERG and MYC expression across all tumor nodules ($r_{pb} = 0.149$, $P = 0.045$), although this correlation was strongest in secondary nodules ($r_{pb} = 0.520$, $P = 0.019$). In radical prostatectomy specimens, ERG-positive/MYC high tumors were positively associated with the presence of extraprostatic extension (EPE), relative to all other ERG/MYC expression subgroups, however, there was no significant association between concurrent nuclear ERG and MYC protein overexpression and time to biochemical recurrence.

CONCLUSIONS—Concurrent nuclear ERG and MYC protein overexpression is common in prostate cancer and defines a subset of locally advanced tumors. Recent data indicates that BET bromodomain proteins regulate *ERG* gene fusion and MYC gene expression in prostate cancer, suggesting possible synergistic targeted therapeutics in ERG-positive/MYC high tumors.

Keywords

ERG; MYC; prostate cancer; immunohistochemistry (IHC); BET bromodomain

INTRODUCTION

Recurrent gene fusions involving *ERG* are the most frequent genetic alteration in prostate cancer and result in overexpression of the nuclear transcription factor ERG (1–3).

TMPRSS2-ERG is the most common *ERG* gene fusion in prostate cancer, occurring in approximately 40–50% of tumors (1–3), and when present, this gene fusion represents an early, clonal event in prostate cancer progression (4). The nuclear transcription factor MYC may also play a role in tumor initiation and/or progression (5–7), and MYC protein is frequently overexpressed in prostate cancer (6).

In a variety of human malignancies, MYC gene expression is activated by the BET subfamily of bromodomain-containing chromatin modifying proteins, which may also serve as co-regulators for MYC target gene activation (8–12). Recent data from our group has established a role for BET bromodomain protein-dependent regulation of androgen receptor (AR) signaling in castration-resistant prostate cancer, including transcriptional control of the *TMPRSS2-ERG* gene fusion (13), and other studies have highlighted BET bromodomain-dependent MYC expression in prostate cancer (14). These data suggest that targeted therapeutics with BET bromodomain inhibitors may have a synergistic effect in the subset of prostate cancers that harbor an *ERG* gene fusion and demonstrate MYC overexpression (13–16).

To better understand the clinicopathologic characteristics and prognosis of ERG-positive/MYC high prostate cancer, we sought to evaluate ERG and MYC protein expression by IHC in a large tissue microarray (TMA) cohort of patients with clinically localized prostate cancer.

MATERIALS AND METHODS

This study was approved by the Institutional Review Board at the University of Michigan.

TMA

Outcome TMAs comprised of radical prostatectomy tissue from 200 patients with clinically localized prostate cancer were described previously (17). This cohort consists of patients who underwent radical prostatectomy as monotherapy for prostate cancer between 1995 and 2004 at the University of Michigan Health System (see Supplemental Table 1 for cohort clinicopathologic characteristics); the median clinical follow-up was 2,416 days (range = 42–3,794 days). Similarly, a multifocal prostate cancer TMA comprised of prostate cancer from radical prostatectomy specimens of 27 patients with clinically localized prostate cancer was described previously (4). Briefly, for each patient included in the outcome TMAs, the index nodule was sampled, while for each patient included in the multifocal TMA, the index nodule and up to two multifocal prostate cancer nodules were sampled. Three tissue cores (each 0.6 mm in diameter) were obtained from representative formalin-fixed, paraffin-embedded (FFPE) tissue blocks for each included patient sample.

Immunohistochemistry

IHC was performed on TMA sections as described previously (6,18), using primary antibodies against ERG (Ventana Medical Systems, EPR3864, predilute; Tucson, AZ, USA) and MYC (Epitomics, clone Y69, 1:200 dilution; Burlingame, CA, USA). For each evaluable TMA core, IHC was scored semi-quantitatively by two study pathologists (A.M.U. and R.M.), based on nuclear staining intensity (0–3; i.e., negative, weak, moderate, or strong) and percentage of positive tumor cells (0–100), and an IHC product score was calculated (range = 0–300). For a given patient and tumor nodule, IHC product scores were averaged across evaluable TMA cores.

Statistical Methods

All statistical analyses were performed using R (version 3.0.2). For all TMAs, correlation between ERG and MYC expression was assessed by calculating the point-biserial correlation coefficient (r_{pb}). For all analyses, ERG expression was dichotomized as “positive” (IHC product score > 0) or negative (IHC product score = 0). When assessing its correlation with ERG, MYC expression was treated as a continuous variable (IHC product score = 0–300); for all other analyses, MYC expression was dichotomized as “high” (IHC product score ≥ 46.67) or “low” (IHC product score < 46.67) using the median IHC product score of all tumors. For the outcome TMAs, association between ERG and/or MYC expression and specific clinicopathologic features was evaluated by Fisher’s exact test or the Student’s t-test (for categorical and continuous data, respectively). The relationship between ERG/MYC expression subgroups and biochemical recurrence-free survival after radical prostatectomy [as defined by prostate-specific antigen (PSA) relapse] was examined using the “survival” package (19) in R. The probability of biochemical recurrence-free survival was calculated using the Kaplan-Meier product limit method, and the log-rank test was used to compare the survival curves. Finally, Cox proportional hazard univariate and multivariate regression models were used to calculate the hazard ratio and associated 95% confidence intervals for biochemical recurrence of ERG/MYC expression subgroups; multivariate models included adjustment for specific clinical variables, including Gleason score (GS), seminal vesicle invasion (SVI), and extraprostatic extension (EPE).

RESULTS

Across all TMAs, prostate cancer tissue from 164 (72.2%) patients was evaluable for both ERG and MYC expression, including 138 (69.0%) patients from the outcome TMAs and 26 (96.3%) patients from the multifocal TMA. For the multifocal TMA, a total of 44 (69.8%) tumor nodules were evaluable for both ERG and MYC expression, including 24 (88.9%) index nodules and 20 (55.6%) secondary nodules. Overall, ERG expression was positive in 96 (52.7%) tumor nodules, including 86 (53.1%) index nodules and 10 (50.0%) secondary nodules. MYC expression varied tremendously across all tumor nodules (median IHC product score = 49.2, range = 0–270), including secondary tumor nodules (median IHC product score = 46.7, range = 17.5–270). Overall, there was weak positive correlation between ERG and MYC expression across all tumor nodules ($r_{pb} = 0.149$, $P = 0.045$), although this correlation was strongest in secondary nodules ($r_{pb} = 0.520$, $P = 0.019$) and not statistically significant in index nodules alone ($r_{pb} = 0.084$, $P = 0.282$) (see Table 1 for details). A significant subset of ERG-positive tumor nodules, however, demonstrated high MYC expression (as defined as greater than the median IHC product score) (see Figures 1 and 2); indeed, ERG-positive/MYC high tumors accounted for 29.1% (53/182) of all tumor nodules, including 28.4% (46/162) of index nodules and 35.0% (7/20) of secondary nodules.

In our outcome TMA cohort, MYC high tumors were positively associated with the presence of EPE ($P = 0.004$) and high pathologic stage [≥ 3 (Path1992 and Path1997); $P = 0.002$]; no other examined clinicopathologic parameters were significantly associated with high MYC expression (see Supplemental Table 2 for details). Similarly, ERG-positive tumors were positively associated with the presence of EPE ($P = 0.020$) and high pathologic stage [≥ 3 (Path1992 and Path1997); $P = 0.035$]; in addition, these tumors were positively associated with large tumor size (>1.5 cm; $P = 0.035$), low gland weight (< 50 g; $P = 0.007$), and positive DRE (clinical T2 disease; $P = 0.018$) (see Supplemental Table 3 for details). Relative to all other ERG/MYC expression subgroups, ERG-positive/MYC high tumors were positively associated with the presence of EPE ($P = 0.001$) and high pathologic stage [≥ 3 (Path1992 and Path1997); $P = 0.001$]; in addition, these tumors were positively associated with low gland weight (< 50 g; $P = 0.029$) (see Table 2 for details).

Finally, there was no significant association between ERG and/or MYC protein overexpression and time to biochemical recurrence after radical prostatectomy (as determined by serum PSA levels) in our outcome TMA cohort (data not shown; see Tables 2 and 3 and Supplemental Tables 2–4 for details), although in multivariate analysis, SVI and EPE were still significant prognostic factors for biochemical recurrence in ERG-positive/MYC high tumors (see Table 3 for details).

DISCUSSION

ERG gene fusions and MYC protein overexpression are common oncogenic alterations in prostate cancer (1–3,6). In this study, we examined nuclear ERG and MYC protein overexpression in two large prostate cancer outcome TMA cohorts, as well as a multifocal prostate cancer TMA cohort. Concurrent nuclear ERG and MYC protein overexpression was frequently detected, including nearly one-third of index and secondary nodules. Overall,

weak positive correlation between ERG and MYC expression was identified across all tumor nodules, with relatively stronger correlation in secondary nodules. In radical prostatectomy specimens, both ERG and MYC protein overexpression were independently associated with locally advanced disease, and compared to all other ERG/MYC expression subgroups, ERG-positive/MYC high tumors more frequently demonstrated EPE; there was no significant association, however, between ERG and/or MYC protein overexpression and time to biochemical recurrence.

Early *in vitro* experiments with ERG-positive prostate cancer cell lines demonstrated that the ERG oncoprotein is recruited to the MYC gene promoter, where it activates MYC gene expression and represses prostatic epithelial differentiation (20). The relationship between nuclear ERG and MYC protein overexpression in human prostate cancer tissues, however, has not been extensively explored. Recently, in a large TMA cohort with prostate cancer tissue from more than 500 patients, *Ayala et al.* described moderate positive correlation between ERG and MYC protein overexpression (21). Our data from over 150 unique patients support the finding that nuclear ERG and MYC protein overexpression is positively correlated in prostate cancer, but overall, we observed only relatively weak correlation. Interestingly, in our TMA cohorts, correlation between ERG and MYC protein overexpression was stronger in secondary than index nodules – although the overall number of secondary nodules was relatively low. Regardless, the reason for the observed difference in ERG and MYC protein overexpression in index and secondary nodules is unclear; given that multifocal prostate cancer results from independent clonal events (4), it is possible ERG and MYC protein overexpression are regulated by distinct molecular mechanisms in index and secondary nodules. Additional focused study of the relationship between nuclear ERG and MYC protein overexpression in multifocal prostate cancer is warranted.

The clinicopathologic characteristics and prognostic significance of nuclear ERG protein overexpression in prostate cancer have been studied extensively over the past decade (22–26); in general, ERG-positive tumors are associated with lower pre-operative PSA and higher pathologic stage at radical prostatectomy, without significant association with GS or clinical outcome (i.e., biochemical recurrence, distant metastasis, or prostate cancer-specific mortality). In contrast, the clinicopathologic features and prognostic significance of nuclear MYC protein overexpression in prostate cancer have not been well characterized. Low MYC protein expression has been reported as an independent poor prognostic factor in high-risk prostate cancer patients who received adjuvant chemotherapy after radical prostatectomy (27), however, in a large TMA study with prostate cancer tissue from more than 200 patients, *Fromont et al.* reported that MYC gene amplification but not MYC protein expression was significant associated with biochemical recurrence after radical prostatectomy (28). Our data indicate that both nuclear ERG and MYC protein overexpression are independently associated with locally advanced prostate cancer, although neither is associated with biochemical recurrence.

We also examined the clinicopathologic parameters and prognostic significance of concurrent nuclear ERG and MYC protein overexpression in prostate cancer, a subgroup of tumors that heretofore have not been extensively characterized. We found that nearly one-third of tumor nodules showed concurrent nuclear ERG and MYC protein overexpression.

Although ERG-positive/MYC high tumors did not show significant differences in pre-operative PSA levels or index nodule GS, relative to all other ERG/MYC expression groups, these tumors demonstrated more frequent EPE, indicating that ERG and MYC protein overexpression defines a subset of locally advanced prostate cancer. Despite this strong association with EPE – a known poor prognostic factor for biochemical recurrence, there was no significant association with biochemical recurrence for ERG-positive/MYC high tumors.

Regardless, the relatively high frequency of concurrent nuclear ERG and MYC protein overexpression suggests a potential role for synergistic targeted therapeutics with BET bromodomain inhibitors in ERG-positive/MYC high prostate cancer (13–16). BET bromodomain proteins (BRD2, BRD3, BRD4, and BRDT) are epigenetic modulators that function as transcriptional co-factors to coordinate gene expression (29). In prostate cancer, BET bromodomain proteins interact with the N-terminal portion of AR and facilitate recruitment to and transcriptional regulation of target gene loci, including *TMPRSS2-ERG* (13); these chromatin modifying proteins also regulate *MYC* gene expression (8–12), possibly in part via recruitment of ETS family proteins (including ERG) to specific *MYC* enhancer regions (13). These data indicate that ERG-positive/MYC high prostate cancer may be particularly sensitive to targeted therapeutics with BET bromodomain inhibitors, as they may simultaneously inhibit *ERG* gene fusion and *MYC* gene expression in these tumors (in addition to global AR signaling blockade).

Pre-clinical *in vitro* and *in vivo* prostate cancer models have shown promising response to BET bromodomain inhibitors (13,14), and early phase clinical trials investigating the utility of these molecules for treatment of patients with advanced solid tumors (including castration-resistant prostate cancer) are currently ongoing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Concurrent nuclear ERG and MYC protein overexpression in a subset of clinically localized prostate cancer

(A–C) MYC IHC demonstrates moderate to strong nuclear expression in a subset of ERG-positive prostate cancer (D–F), including low (A, B, D, E) and high (C, F) grade tumors. Overall, nuclear ERG and MYC protein expression shows weak positive correlation ($r_{pb} = 0.149$; P -value = 0.045) in clinically localized prostate cancer (see Table 1 for details).

Figure 2. Nuclear ERG and MYC protein overexpression in multifocal prostate cancer
 MYC (top panels) and ERG (bottom panels) IHC in the index nodule (red) and one secondary nodule (green) of three patients with multifocal prostate cancer. For patients A and B, both the index and secondary nodules are ERG-positive and show high MYC expression. For patient C, the index nodule is ERG-positive and shows high MYC expression, while the secondary nodule is ERG-negative and demonstrates low MYC expression. Nuclear ERG and MYC protein expression shows moderate positive correlation ($r_{pb} = 0.520$; P -value = 0.019) in secondary nodules (see Table 1 for details).

Table 1

Correlation of ERG and MYC protein expression in prostate cancer.

	ERG IHC Status	<i>n</i>	MYC IHC Product Score				<i>r_{pb}</i>	<i>P</i> -value
			Mean	Median	Range	95% CI		
All tumors	All	182	57.6	49.2	0–270	50.9–64.3	0.149	0.045
	Positive	96	64.0	59.2	0–270	54.3–73.9		
	Negative	86	50.4	43.33	0–270	41.6–59.3		
Index nodules	All	162	56.2	49.2	0–270	49.5–62.9	0.084	0.282
	Positive	86	59.6	57.5	0–200	50.5–68.7		
	Negative	76	52.3	45.8	0–270	42.4–62.2		
Secondary nodules	All	20	69.0	46.7	17.5–270	38.5–99.5	0.520	0.019
	Positive	10	102.0	82.5	20–270	45.4–158.6		
	Negative	10	36.0	31.3	17.5–60	24.3–47.7		

r_{pb} = point-biserial correlation coefficient

Table 2

Association of ERG and MYC expression with clinicopathologic parameters.

Parameter	Cutoff	ERG+/MYC high vs. ERG+/MYC low				ERG+/MYC high vs. ERG-/MYC high				ERG+/MYC high vs. ERG-/MYC low				ERG+/MYC high vs. all others combined			
		E+/MYC ^{hi} (n=39)	E+/MYC ^{lo} (n=34)	P- value	E+/MYC ^{hi} (n=39)	E-/MYC ^{hi} (n=29)	P- value	E+/MYC ^{hi} (n=39)	E-/MYC ^{lo} (n=36)	P- value	E+/MYC ^{hi} (n=39)	Others (n=99)	P- value				
Age	60 y	24 (61.5%)	15 (44.1%)	0.163	24 (61.5%)	14 (48.3%)	0.328	24 (61.5%)	21 (58.3%)	0.817	24 (61.5%)	50 (50.5%)	0.261				
	>60 y	15 (38.5%)	19 (55.9%)		15 (38.5%)	15 (51.7%)		15 (38.5%)	15 (41.7%)		15 (38.5%)	49 (49.5%)					
Pre-op PSA	7 ng/mL	19 (48.7%)	16 (47.1%)	1.000	19 (48.7%)	19 (65.5%)	0.219	19 (48.7%)	23 (63.9%)	0.246	19 (48.7%)	58 (58.6%)	0.343				
	>7 ng/mL	20 (51.3%)	18 (52.9%)		20 (51.3%)	10 (34.5%)		20 (51.3%)	13 (36.1%)		20 (51.3%)	41 (41.4%)					
Tumor size	1.5 cm	17 (43.6%)	13 (38.2%)	0.812	17 (43.6%)	19 (67.9%)	0.081	17 (43.6%)	20 (55.6%)	0.359	17 (43.6%)	52 (53.1%)	0.348				
	>1.5 cm	22 (56.4%)	21 (61.8%)		22 (56.4%)	9 (32.1%)		22 (56.4%)	16 (44.4%)		22 (56.4%)	46 (46.9%)					
Gland weight	50 g	31 (79.5%)	24 (70.6%)	0.424	31 (79.5%)	18 (62.1%)	0.172	31 (79.5%)	16 (44.4%)	0.002	31 (79.5%)	58 (58.6%)	0.029				
	>50 g	8 (20.5%)	10 (29.4%)		8 (20.5%)	11 (37.9%)		8 (20.5%)	20 (55.6%)		8 (20.5%)	41 (41.4%)					
Gleason score	6	11 (28.2%)	7 (20.6%)	0.581	11 (28.2%)	4 (13.8%)	0.230	11 (28.2%)	13 (36.1%)	0.802	11 (28.2%)	24 (24.2%)	0.657				
	7	24 (61.5%)	24 (70.6%)		24 (61.5%)	22 (75.9%)		24 (61.5%)	22 (61.1%)		24 (61.5%)	68 (68.7%)					
	8	4 (10.3%)	3 (8.8%)		4 (10.3%)	3 (10.3%)		4 (10.3%)	1 (2.8%)		4 (10.3%)	7 (7.1%)					
Surgical margin	Negative	29 (74.4%)	20 (58.8%)	0.213	29 (74.4%)	21 (72.4%)	1.000	29 (74.4%)	24 (66.7%)	0.612	29 (74.4%)	65 (65.7%)	0.418				
	Positive	10 (25.6%)	14 (41.2%)		10 (25.6%)	8 (27.6%)		10 (25.6%)	12 (33.3%)		10 (25.6%)	34 (34.3%)					
EPE	No	21 (53.8%)	27 (79.4%)	0.027	21 (53.8%)	22 (75.9%)	0.078	21 (53.8%)	33 (91.7%)	<0.001	21 (53.8%)	82 (82.8%)	0.001				
	Yes	18 (46.2%)	7 (20.6%)		18 (46.2%)	7 (24.1%)		18 (46.2%)	3 (8.3%)		18 (46.2%)	17 (17.2%)					
SVI	No	35 (89.7%)	32 (94.1%)	0.679	35 (89.7%)	28 (96.6%)	0.384	35 (89.7%)	35 (97.2%)	0.360	35 (89.7%)	95 (96%)	0.222				
	Yes	4 (10.3%)	2 (5.9%)		4 (10.3%)	1 (3.4%)		4 (10.3%)	1 (2.8%)		4 (10.3%)	4 (4%)					
N-stage	pNX/N0	36 (94.7%)	30 (90.9%)	0.658	36 (94.7%)	27 (96.4%)	1.000	36 (94.7%)	31 (86.1%)	0.255	36 (94.7%)	88 (90.7%)	0.728				
	pN1	2 (5.3%)	3 (9.1%)		2 (5.3%)	1 (3.6%)		2 (5.3%)	5 (13.9%)		2 (5.3%)	9 (9.3%)					
DRE	T1	21 (53.8%)	20 (58.8%)	0.814	21 (53.8%)	18 (62.1%)	0.621	21 (53.8%)	32 (88.9%)	0.001	21 (53.8%)	70 (70.7%)	0.073				
	T2	18 (46.2%)	14 (41.2%)		18 (46.2%)	11 (37.9%)		18 (46.2%)	4 (11.1%)		18 (46.2%)	29 (29.3%)					

Parameter	Cutoff	ERG+/MYC high vs. ERG-/MYC low			ERG+/MYC high vs. ERG-/MYC high			ERG+/MYC high vs. ERG-/MYC low			ERG+/MYC high vs. all others combined		
		E+/MYC ^{hi} (n=39)	E+/MYC ^{lo} (n=34)	P- value	E-/MYC ^{hi} (n=39)	E-/MYC ^{hi} (n=29)	P- value	E-/MYC ^{hi} (n=39)	E-/MYC ^{lo} (n=36)	P- value	E+/MYC ^{hi} (n=39)	Others (n=99)	P- value
Race	Black	3 (7.7%)	3 (8.8%)	1.000	3 (7.7%)	3 (10.3%)	1.000	3 (7.7%)	8 (22.2%)	0.188	3 (7.7%)	14 (14.1%)	0.557
	White	31 (79.5%)	28 (82.4%)		31 (79.5%)	24 (82.8%)		31 (79.5%)	27 (75%)		31 (79.5%)	79 (79.8%)	
	Other	5 (12.8%)	3 (8.8%)		5 (12.8%)	2 (6.9%)		5 (12.8%)	1 (2.8%)		5 (12.8%)	6 (6.1%)	
Biochemical recurrence	No	24 (61.5%)	23 (67.6%)	0.631	24 (61.5%)	19 (65.5%)	0.803	24 (61.5%)	23 (63.9%)	1.000	24 (61.5%)	65 (65.7%)	0.695
	Yes	15 (38.5%)	11 (32.4%)		15 (38.5%)	10 (34.5%)		15 (38.5%)	13 (36.1%)		15 (38.5%)	34 (34.3%)	
Multifocal	No	7 (17.9%)	9 (26.5%)	0.410	7 (17.9%)	6 (21.4%)	0.762	7 (17.9%)	9 (26.5%)	0.410	7 (17.9%)	24 (25%)	0.499
	Yes	32 (82.1%)	25 (73.5%)		32 (82.1%)	22 (78.6%)		32 (82.1%)	25 (73.5%)		32 (82.1%)	72 (75%)	
Path1992	<3	21 (53.8%)	27 (79.4%)	0.027	21 (53.8%)	21 (72.4%)	0.138	21 (53.8%)	33 (91.7%)	<0.001	21 (53.8%)	81 (81.8%)	0.001
	3	18 (46.2%)	7 (20.6%)		18 (46.2%)	8 (27.6%)		18 (46.2%)	3 (8.3%)		18 (46.2%)	18 (18.2%)	
Path1997	<3	21 (53.8%)	27 (79.4%)	0.027	21 (53.8%)	21 (72.4%)	0.138	21 (53.8%)	33 (91.7%)	<0.001	21 (53.8%)	81 (81.8%)	0.001
	3	18 (46.2%)	7 (20.6%)		18 (46.2%)	8 (27.6%)		18 (46.2%)	3 (8.3%)		18 (46.2%)	18 (18.2%)	

EPE = Extraprostatic extension, SVI = seminal vesicle invasion, DRE = digital rectal examination

Table 3

Multivariate Cox regression analysis for association of ERG and MYC expression with biochemical recurrence.

Parameter	ERG+/MYC high vs. ERG+/MYC low			ERG+/MYC high vs. ERG-/MYC high			ERG+/MYC high vs. all others combined		
	HR	95% CI	P-value	HR	95% CI	P-value	HR	95% CI	P-value
ERG+/MYC high (vs. other)	1.414	(0.619, 3.273)	0.419	0.672	(0.273, 1.652)	0.386	0.430	(0.170, 1.085)	0.074
GS (7 vs. 6)	3.620	(0.821, 15.956)	0.089	2.504	(0.538, 11.649)	0.242	1.430	(0.502, 4.070)	0.503
GS (8 vs 6)	3.358	(0.489, 23.043)	0.218	1.771	(0.250, 12.529)	0.567	1.342	(0.272, 6.625)	0.718
SVI (+ vs. -)	2.435	(0.713, 8.312)	0.155	4.537	(1.451, 14.188)	0.009	3.275	(1.012, 10.594)	0.048
EPE (+ vs. -)	5.780	(2.125, 15.722)	<0.001	3.094	(1.202, 7.962)	0.192	5.431	(1.917, 15.385)	0.001
							3.936	(2.029, 7.637)	<0.001

HR = hazard ratio, CI = confidence interval, GS = Gleason score, SVI = seminal vesicle invasion, EPE = extraprostatic extension