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ORIGINAL ARTICLE

Immunohistochemical expression of the ERG gene in prostatic adenocarcinoma and its relationship with clinicopathological parameters

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Abstract

Introduction: This study aimed to determine the immunohistochemical expression of the ERG gene in prostatic adenocarcinoma and to evaluate the relationships between ERG expression and clinicopathological parameters. Design: Cross-sectional study. Setting: Pathology department of a tertiary hospital. *Materials and Methods:* The prostatectomy materials of 122 patients diagnosed with prostate adenocarcinoma between 2004 and 2017 in Zonguldak Bülent Ecevit University Faculty of Medicine, Department of Pathology were included in the study. Main Outcome Measures: Clinical data were obtained from patient files and macroscopic data were obtained from surgery and pathology reports. ERG expression, age, prostate-specific antigen levels, Gleason pattern and score, Gleason grade, and pathological stage were recorded. *Results:* The mean age of the patients was 62.66 ± 5.81 years and overall preoperative PSA was 10.40 ± 8.88 ng/ml. ERG was positive in 52.46% of the patients. PSA levels were similar in ERG positive and negative samples ($p = 0.935$). There was no significant relationship between Gleason score and ERG positivity ($p = 0.197$). ERG expression did not change with regard to age groups ($p = 0.441$) or tumour stage ($p = 0.371$). *Conclusion:* This study shows that the frequency of ERG positivity was high in our patients and that ERG positivity was not associated with clinical and pathological features, such as PSA levels, Gleason score, age and pathological stage.

Keywords: ERG expression, Gleason score, Prostate cancer, Prostate specific antigen

INTRODUCTION

Prostate cancer (PCa) is the second most common cancer (after lung cancer) in men throughout the world and also in Turkey, and it ranks 5th in cancer-related deaths.[1] However, incidence and mortality rates vary greatly between countries and regions.[2] Worldwide, it was estimated that there would be 1,300,000 new cases of PCa and 359,000 PCa-related deaths in 2018.[3] PCa is a heterogeneous disease and the mechanisms leading to it are poorly understood.[4] The different rates of PCa in different parts of the world indicate that genetic factors play a role in its aetiology. In addition, familial predisposition, environmental factors and diet are also reported to have a role in its aetiology.[5,6]

One of the many genes described in prostate carcinogenesis is the *E-26* transformation-specific (ETS [erythroblast transformation-specific]) related gene (ERG), which encodes a protein, also called ERG, acts as a transcription factor that regulates genes involved in proliferation, differentiation, embryonic development, angiogenesis and inflammation. It has become the most studied gene in PCa during the last few years.[7,8] ERG is the most common oncogenic gene in the pathogenesis of PCa and plays a role in vasculogenesis, angiogenesis, haematopoiesis and bone development[9] as well as many important cellular processes such as regulation of differentiation, apoptosis and proliferation.[10-12] It is thought that ERG fusion protein stops maturation in early stem cells in the prostate and initiates carcinogenesis.[13] ERG

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fusion in PCa was first discovered in 2005 by Tomlins *et al*.[14] In the aetiology of PCa, fusion of *TMPRSS2* ("transmembrane protease, serine 2 gen) gene to the proto-oncogene ERG is an important oncogenic trigger and is seen in up to 60%, especially in western societies.[15] Also, the ERG gene is alternatively spliced; of particular interest is its cassette exon 7b. Higher exon 7b inclusion rates are associated with increased cell proliferation and advanced prostate cancer.[16] ERG expression can be reliably determined by immunohistochemical methods.[17] There is a strong correlation between sensitivity and specificity between immunohistochemistry and fluorescence in situ hybridization (FISH) for ERG in prostate tissue.[18] Since benign prostatic tissue and stromal cells are not stained with ERG, immunohistochemical detection of ERG expression increases the accuracy of PCa diagnosis.

Currently, there is controversy regarding the role of ERG oncoprotein resulting from *TMPRSS2-ERG* fusion in the development and progression of PCa and its relationship with prognostic factors. In some studies, a relationship was found between ERG and low-grade Pca[19,20], while in others it was associated with a high Gleason score.[21-23] There are studies suggesting that ERG positivity is unrelated to the Gleason score, high-grade tumour and metastasis.[24] Studies evaluating the relationship between ERG and preoperative total PSA (tPSA) values and prognosis have also failed to determine a conclusive role for ERG.[14,20,25]

As such, there is a need to investigate the role of ERG in the development and progression of PCa and to determine its possible relationships with clinicopathological factors. The aim of this study was to measure ERG expression via immunohistochemical methods in prostatic adenocarcinoma and to evaluate the relationships between the ERG expression and the clinicopathological characteristics of patients with PCa.

MATERIALS AND METHODS

Defining the Working Group

In this study, the prostatectomy materials of all patients diagnosed with PCa between 2004-2017 in the Department of Pathology of Zonguldak Bulent Ecevit University Faculty of Medicine were included (n = 122). Clinical data were obtained from patient files and macroscopic data were obtained from surgery and pathology reports. Cases that did not contain sufficient tumour tissue without histopathological and immunochemical evaluation were excluded. Age, preoperative tPSA values, stage, Gleason grade and Gleason grade groups were included in the evaluation. Patients were classified according to their age (younger or older than 65 years of age) with regard to the criteria of the World Health Organization (WHO).[26]

Histomorphological Evaluation

Hematoxylin-eosin stained archive sections prepared from paraffin blocks of radical prostatectomy materials fixed in 10% formalin solution were examined microscopically. Each case was re-evaluated by two researchers according to the WHO 2016 Gleason scoring system and paraffin blocks with the highest volume of primary Gleason rating were selected.

Immunohistochemical Method

Four-micrometer thickness sections were prepared from the paraffin blocks with the highest volume of primary Gleason degree, and the samples were taken on positively charged slides. After sections were deparaffinised at 70 degrees for 30 minutes in an oven, the primary antibody ERG (Ventana, anti-ERG antibody, clone EPR3864, rabbit monoclonal) staining was performed in a BenchMark Ultra fully automated immunohistochemical staining device (Ventana Medical Systems Inc, Tuscon, AZ, USA). The stained sections were passed through 70% alcohol and sealed with a xylene-based sealer. It was then boiled in a microwave oven with 10 mM / L, pH 6.0 citrate buffer solution, and cooled for 20 min at normal room temperature. Sections were blocked for endogenous peroxidase. A ready-to-use (diluted) monoclonal antibody (Ventana, anti-ERG antibody, clone EPR3864, rabbit monoclonal) was used for ERG.

Immunohistochemical Evaluation

ERG stained sections were examined using Nikon Eclipse Ci-L (Nikon Instruments Inc., Melville, NY, USA) light microscope. Spleen sections (vascular endothelium) were used as positive control tissue. In addition, lymphocytes and vascular endothelium in the prostate tissue were evaluated as a positive internal control and benign prostate glands as a negative internal control. ERG nuclear staining was evaluated qualitatively and quantitatively. In the evaluation of staining intensity, the absence of staining was scored as (0), weak (1+), moderate (2+) and strong (3+). The scores obtained were multiplied

by the percentage of cells (0-100) indicating the staining intensity and an H score ranging from 0 to 300 was calculated. Any nuclear staining positivity (H-score> 0) was accepted as an indicator of ERG expression. Later, the results were categorised as 0–100 points (low), 101–200 points (medium), and >200 points (high) ERG expression, according to H score.[27]

Ethical approval

The study was approved by the ethics committee of Zonguldak Bulent Ecevit University, Faculty of Medicine. Informed consent was obtained from all individual participants included in the study.

Statistical analysis

Data were analysed using the SPSS 25 statistical package program. For the normality check, the Shapiro Wilk test was used. Data are given as mean ± standard deviation or median (interquartile range (IQR)) for continuous variables according to the normality of distribution and frequency (percentage) for categorical variables. The comparison of tPSA levels according to ERG status was performed with the Mann-Whitney U test or Kruskal Wallis test, depending on the number of groups analysed. Age, Gleason score and distribution of pathological stages according to ERG status were analysed by Chi-square tests. Statistical significance level was accepted as $p < 0.05$.

RESULTS

The mean age of the 122 patients was 62.66 ± 5.81 (min 47, max 80). Preoperative tPSA values were 10.40 ± 8.88 ng /ml. ERG was positive in 52.46% (n = 64) of the patients. The median (IQR) ERG H-scores were 65.50 (0.00 – 171.00). Data for other clinicopathologic variables are summarised in Table 1. Microscopic images of 1+, 2+ and 3+ stained samples according to ERG H-score categories are shown in Figure 1, 2 and 3.

In the ERG H-score categories, the distribution was similar according to age groups ($p = 0.977$), Gleason score groups ($p = 0.749$), tumour stages ($p = 0.335$), and. Finally, there were again no statistically significant relationships between PSA values and ERG-H score ($p = 0.400$) (Table 2).

DISCUSSION

In our study, the percentage of patients with ERG positivity obtained by immunohistochemical methods was found to be 52.46%. This ratio is in the range of the results of previous articles. According to the results obtained in the related studies in the literature, immunohistochemical ERG positivity varies between 10.2% and 68.7%.[21,24,28,29] The highest positivity rate (68.7%) is from a research performed by Font Tello *et al*. in 78 PCa cases using both immunohistochemistry and qRT-PCR methods.[24] In addition to immunohistochemical methods, FISH and PCR methods were used to detect ERG. In papers using the FISH method, ERG positivity varies between 16.9% and 58.7%.[19,30-32] On the other hand, the PCR technique showed that ERG positivity ranged from 42.3% to 84%.[23,33,34]

ERG expression rates determined in the literature vary in a wide range. This variability may be attributed primarily to the difference between selected ERG detection methods. Although researchers prefer to detect ERG expression mostly by immunohistochemical methods, as mentioned before, some researchers have used methods such as FISH and PCR.[19,23,30-34] However, it has been reported that ERG expression can be reliably detected with immunohistochemical methods[17], and there is a strong correlation between sensitivity and specificity between immunohistochemistry and FISH in prostate tissue.[18]

The ethnic characteristics of the population in which the research was conducted and the differences between the methods employed by researchers and laboratories could also lead to varying levels of positive results. Indeed, in an investigation conducted in Turkey, Yilmaz and colleagues found that ERG was positive in 46.5% of the radical prostatectomy samples they analysed.[35] The fact that the ERG positivity rate found in our study is close to the rate in this paper may be important in terms of differences between countries and may form the basis for similar studies in the future.

One of our important objectives in this article was to investigate the relationship between ERG positivity and clinical parameters. In many studies, age was evaluated as one of the parameters that could affect ERG positivity and various comparisons have been reported. In our research, no significant relationship was found between ERG positivity and age ($p = 0.441$). There are many investigations in the literature reporting results in the same direction as our findings.[27,35,36] However, in some studies in which ERG was found to be associated with age,

Table 1: Clinicopathologic features of prostate cancer patients (n = 122)

Age	62.66 ± 5.81
Preoperative Total PSA (ng/ml)	10.40 ± 8.88
Gleason pattern and score	
3+3:6	65 (53.28%)
3+4:7	36 (29.5%)
4+3:7	11 (9.02%)
3+5:8	2 (1.64%)
4+4:8	2 (1.64%)
4+5:9	4 (3.28%)
5+4:9	2 (1.64%)
Gleason Group	
Grade 1	65 (53.28%)
Grade 2	36 (29.5%)
Grade 3	11 (9.02%)
Grade 4	4 (3.28%)
Grade 5	6 (4.92%)
Stage	
pT2	83 (68.03%)
pT3a	19 (15.57%)
pT3b	12 (9.84%)
pT4	8 (6.56%)
ERG expression	
Negative	58 (47.54%)
Positive	64 (52.46%)
ERG H-Score	65.50 (0.00 – 171.00)
ERG H-Score Category	
Low	70 (57.38%)
Medium	30 (24.59%)
High	22 (18.03%)

Data are given as mean ± standard deviation or median (1st quartile – 3rd quartile) for continuous variables according to normality of distribution, and as frequency (percentage) for categorical variables.

it was observed that the mean age of patients that were ERG negative was higher.[37] On the other hand, there are studies showing that ERG positivity is associated with lower age.[38] The conflicting results in the literature demonstrate that further studies are needed to clearly reveal the relationship between ERG and age (if any). It is also apparent that researchers must take into account the impact of other clinical factors in this relationship; therefore, larger case groups that better represent the Pca population are required.

In our study, we compared Gleason score, tumour stage and PSA levels, which are currently the most widely used criteria for PCa, in order to define ERG expression and prognostic risk groups and to guide treatment. In this investigation, there was no significant difference between the groups in terms of ERG expression. Similar to our article, no significant relationship was found between Gleason score and ERG positivity in the majority of studies in the literature.[23,30,34,37,39,40] On the other hand, in some studies, a significant correlation was found between low[19,22,38] or high [21,24,25,27,31,41] Gleason score and ERG positivity. When we evaluate our findings together with the previous researches, it is quite difficult to clearly determine the relationship between ERG positivity and Gleason score. While a significant number of studies support our findings, others have demonstrated associations with a low Gleason score or a high Gleason score.

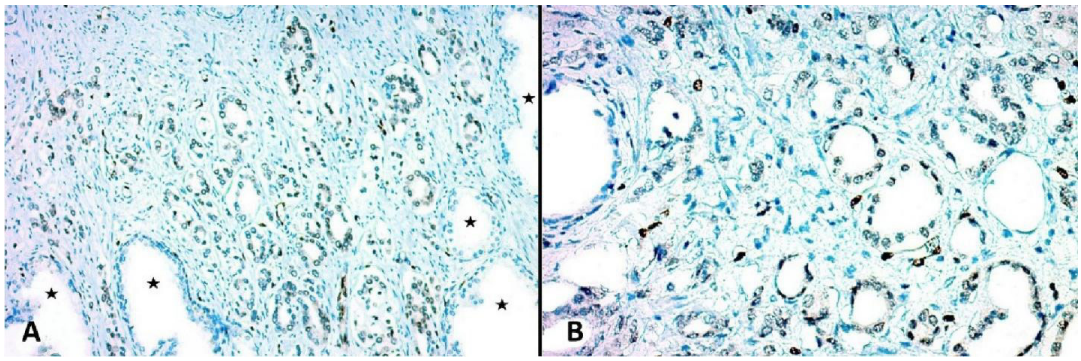

FIG. 1: 1+ staining immunohistochemically according to ERG H-Score categories. A: Benign prostate glands (stars) (100X) used as a negative internal control. B: ERG staining (200X) in tumour cells at larger magnification.

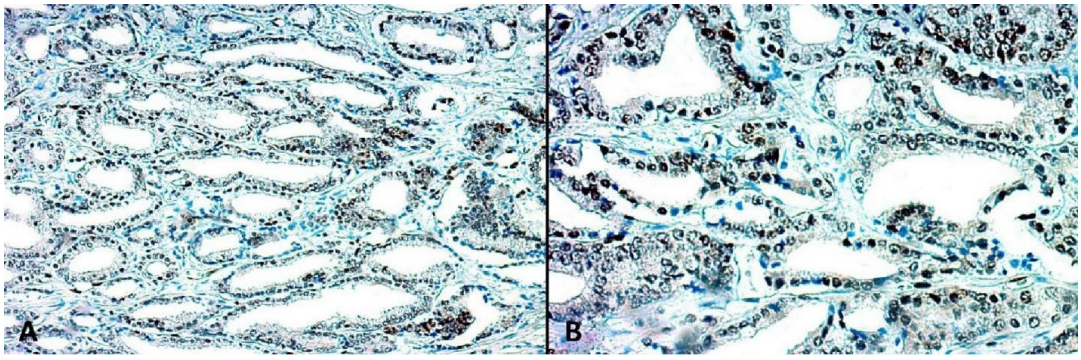

FIG. 2: 2+ staining immunohistochemically according to ERG H-Score categories. (A, 100X; B, 200X)

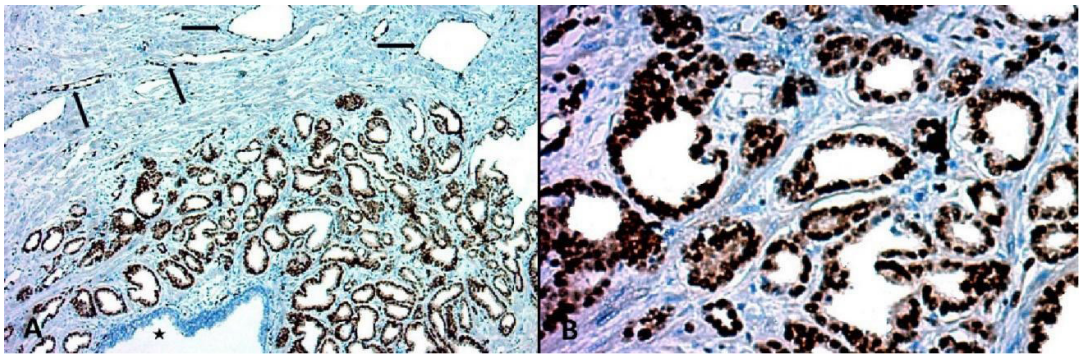

FIG. 3: 3+ staining immunohistochemically according to ERG H-Score categories. A: Benign prostate gland used as a negative internal control (asterisk) and vascular endothelium (arrows) used as a positive internal control (arrows) (50X). B: ERG staining (200X) in tumour cells at larger magnification.

When the literature is reviewed, a great proportion of studies demonstrate a lack of relationship between tumour stage and ERG expression,[22,23,28,32,34,36-38,40] whereas some others have shown marginal to moderate relationships between higher tumour stage and ERG positivity.[21,24,25,30,35,41] In an investigation conducted by Petterson *et al*., ERG level was associated with high tumour stage, but it was not associated with mortality or prognosis.[42] In our paper, no correlation was found between tumour stage and ERG positivity.

There are many articles reporting increased ERG positivity in high-grade tumours, and this

Table 2: Age, PSA, gleason score and stage distribution according to ERG expression

	ERG expression		*p* value
	Negative (n = 58)	Positive (n = 64)	
Age			
≤ 65	36 (62.07%)	45 (70.31%)	0.441
> 65	22 (37.93%)	19 (29.69%)	
Preoperative PSA (ng/ml)	7.73 (6.00 – 12.00)	7.61 (6.00 – 12.00)	0.935
Gleason Score			
6	34 (58.62%)	31 (48.44%)	0.197
7 (3 + 4)	12 (20.69%)	24 (37.50%)	
7 (4 + 3)	7 (12.07%)	4 (6.25%)	
≥ 8	5 (8.62%)	5 (7.81%)	
Stage			
pT2	42 (72.41%)	41 (64.06%)	0.371
pT3a	6 (10.34%)	13 (20.31%)	
pT3b	7 (12.07%)	5 (7.81%)	
pT4	3 (5.17%)	5 (7.81%)	

Data are given as median (1st quartile – 3rd quartile) for continuous variables, and as frequency (percentage) for categorical variables.

raises the question of whether ERG is considered as a prognostic factor. In studies evaluating the prognosis of ERG-positive patients, it is difficult to make a clear decision on the subject. Some of the researches suggest that there is a significant relationship between poor prognosis and ERG,[32,42] while others suggest that there is no such relationship.[28,44] When the sample sizes in the investigations and the methods of detecting ERG positivity are evaluated together, we believe the results show that it would be erroneous to claim that ERG positivity is a definite prognostic factor in PCa with current evidence.

In studies examining the relationship between PSA levels and ERG positivity, similar to previous comparisons, the results are rather various. In our study, no significant correlation was found between PSA levels and ERG positivity. Although there is no relationship between PSA levels and ERG in most of the studies,[23,42,45] those that have shown relationships between PSA levels and ERG expression have reported conflicting findings.[28,36,38,40,41,46] As with other clinical parameters, the relationship between PSA level and ERG positivity is not fully understood. The fact that the literature has reached different conclusions can be explained by the differences in population, the manner in which samples were obtained, ERG detection methods and differences in sample numbers.

The most important limitation of our study is that ERG gene analyzes were not performed. Although immunohistochemical methods significantly correlate with gene expressions, it is an important limitation to evaluate the effect of such parts as exon 7b. Also, the fact that the investigation does not include long-term follow-ups is another important limitation.

CONCLUSION

In summary, no statistically significant relationship was found between ERG and primary parameters, such as age, PSA levels, Gleason score and tumour stage, in this study. In the literature, the results obtained from studies comparing similar clinicopathological parameters with ERG show very apparent conflicts. In many researches, including this study, the biological relationship between ERG and clinicopathological parameters has not been fully established. The inability of different investigations to reach common conclusions raises questions about the potential of ERG to provide new diagnostic and therapeutic opportunities. However, due to the limited number of cases studied in different centers, these possible relationships (if any) should be clearly demonstrated. In this respect, it may be useful to diversify subgroups of clinicopathological parameters and to investigate specific populations.

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