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# The Association of ERG and c-erbB2 Expressions With Gleason Scoring in Adenocarcinomas of the Prostate

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## ABSTRACT

**BACKGROUND/AIM:** We aimed to determine erythroblast transformation-specific-related gene (ERG) and c-erbB2 expression in patients with adenocarcinoma of the prostate and to investigate the association of these proteins with tumor growth and/or Gleason score, which is the main prognostic marker in these patients.

**MATERIALS AND METHODS:** Radical prostatectomy materials of 59 patients with acinar adenocarcinoma were included in this study. Immunohistochemical analysis for ERG and c-erbB2 was performed. The association of ERG and c-erbB2 expressions with International Society of Urologic Pathologists (ISUP) grade, tumor volume and patient age was investigated.

**RESULTS:** ISUP grade was 1 (equivalent to a Gleason score of 6) in 23 tumors while the rest of the cases were Gleason score >6 tumors. ERG expression was detected in 37.5% of the cases. None of the cases had c-erbB2 expression. There was no significant difference in ERG staining between the low-risk (ISUP 1) and high-risk (ISUP >1) groups (p=0.602). Evaluation of all ISUP groups with the Kruskal–Wallis test showed no significant difference across the groups in terms of ERG expression (p=0.374).

**CONCLUSION:** The present study reflects the ERG expression rate (37.5%) in patients with carcinoma of the prostate in Turkey. Our findings support that ERG overexpression is involved in the pathogenesis but has no association with histological grade in prostate carcinoma.

**Keywords:** Prostate carcinoma, Gleason score, ERG, c-erbB2

## INTRODUCTION

Prostate carcinoma (PCa) is a common malignant tumor of the male genital system and the second most lethal cancer in men.[1] In cancer research, one of the main goals is to identify the mutations responsible for the transformation of a tumor into an aggressive cancer, which may advance locally and lead to distant metastasis. Identifying the mutations is of particular importance in PCa due to the relatively slow course and low mortality of this malignancy.[2]

Studies have shown the fusion of the androgen-induced *TMPRSS2* gene, also located on chromosome 21, to the proto-oncogene, erythroblast transformation-specific (ETS)-related gene (ERG) in 50% of patients with PCa.[3] This fusion results in an increased expression of the ERG protein. Increased ERG expression is thought to be important in tumor proliferation and invasion, acting as a transcription factor for the downregulation of a number of genes, and therefore, thought to be oncogenic. ERG overexpression arising from the *TMPRSS2-ERG* fusion in tissue samples can be reliably detected using immunohistochemistry (IHC).[4,5] Whether ERG overexpression in PCa is a marker of aggressive tumors and, therefore, of poor prognosis remains a matter of debate.[4,6,7] Furthermore, there are studies reporting that ERG expression is more common in western countries compared to Asia, where this rate may be as low as 30%.[3,4]

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C-erbB2 expression has been widely studied in breast, ovarian, and gastric cancers, and improved prognosis has been documented with the use of trastuzumab [the humanized monoclonal anti-human epidermal growth factor receptor 2 (HER2) antibody] in the treatment of breast carcinoma.[8,9] Different rates of c-erbB2 expression have been reported in studies investigating PCa in patients with clinically localized PCa,[10] following neoadjuvant androgen ablation,[11,12] and in high-risk carcinoma of the prostate.[9,12]

The Gleason score is an important classification developed to determine the prognosis of prostate adenocarcinoma; however, the International Society of Urological Pathology (ISUP) recommended stratification of Gleason scores into prognostic groups in 2005. In this regard, tumors with a Gleason score of 6 are considered as low-risk tumors with prognostic grade 1, and those tumors with a Gleason Score >6 as high-risk tumors.[13]

In the present study, we aimed to determine ERG and c-erbB2 expression rates in patients with prostate adenocarcinoma, to investigate the correlation between these proteins and tumor development, and to evaluate the relationship between ERG and c-erbB2 expression in ISUP low- and high-risk groups defined according to the main prognostic marker, i.e. their Gleason score.

## MATERIALS AND METHODS

A total of 59 patients who underwent radical prostatectomy with a diagnosis of acinar adenocarcinoma of the prostate were included in this study. Patients' age, Gleason score (ISUP grade), tumor volume, histopathological results and information on lymph node metastasis were recorded. After retrieving 59 radical prostatectomy materials from the archive, suitable tumor tissues were selected, and IHC analysis for ERG and c-erbB2 was performed with these paraffin blocks. Sections of 4-micron thickness were obtained from 59 formalin-fixed, paraffin-embedded tissues for the IHC assay and positive-charged microscope slides were used to avoid tissue shedding. The sections were placed in an incubator at 60 °C for an hour and deparaffinized with xylene for 15 minutes. The samples were hydrated through a descending-grade series of alcohol and washed in distilled water. The samples were then placed into a BenchMark XT device. ERG (cell marque, RTU, clone EP111, USA) and c-erbB2 (cell marque, RTU, clone EP3, USA) antibodies were applied, and staining was subsequently performed. The preparations stained in the automated staining device were covered using fluid-based covering material. The results were evaluated with an Olympus Bx46 light microscope. Breast carcinoma samples were used as an internal control for c-erbB2 in this study. C-erbB2 was evaluated in line with the scaling method used for c-erbB2 synthesis in breast cancer as per the American Society of Clinical Oncology and College of American Pathologists (ASCO/CAP) 2013 HER2 testing guidelines. According to the scoring criteria we employed in our study, 3+ referred to complete, strong membranous staining in more than 10% of tumor cells; 2+ referred to incomplete, moderate membranous staining in more than 10% of tumor cells; 1+ referred to incomplete, weak membranous staining in more than 10% of tumor cells; and 0 referred to no staining or incomplete, weak membranous staining in less than 10% of the tumor cells.[14,15] The nuclear reactivity of the ERG antibody in endothelial cells was used as an internal control. A 4-step system was utilized to evaluate staining results, where 0 referred to negative, with 1+, 2++ and 3+++ considered as weak, moderate and strong staining, respectively. Staining evaluated as 2++ or 3+++ (moderate and strong) were considered ERG positive. Negative (0) and weak (1+) staining results were considered negative.[16]

An informed consent form was not required for this study as this study is made from archive materials. The study was approved by Tekirdag Namık Kemal University the Non-Interventional Clinical Trials Ethics Committee (protocol no: 2019.222.11.19, date: 19.01.2019).

### Statistical Analysis

The patient demographics and data were analyzed using the SPSS 24 (IBM Corp., Armonk, NY, USA) software. The chi-square test was used to compare variables between the patients in groups, and the Kruskal–Wallis test was used for comparisons across the 4 groups. $P<0.05$ was considered statistically significant.

## RESULTS

The mean age of the 59 patients who had undergone radical prostatectomy was 68.6 years [minimum (min): 54 – maximum (max): 80]. Twenty-three of these cases had tumors with Gleason score 6 (3+3) and prognostic (ISUP) grade 1 (low-risk), while the others had tumors with Gleason score >6 (high-risk). Of the 59 patients, 41 had undergone lymph node dissection and lymph node resection had not been performed in the remaining cases as no metastasis was detected in prostate-specific membrane antigen (PSMA) positron emission tomography/computed tomography (PET−CT). Lymph node metastasis was detected in five of these patients.

ERG expression was observed in 22 (37.5%) of the cases, with three evaluated as 2++ and 19 as 3+++. The distribution of the patients according to the ISUP grading system based on the Gleason score was as follows: 25 patients were ISUP grade 1, 14 patients were ISUP grade 2, 15 patients were ISUP grade 3, and five patients were ISUP grade 4–5. Since the number of patients with ISUP grade 4 and 5 disease was small, these two groups were pooled into a single group for evaluation purposes. ERG expression of the cases by ISUP grades is presented in Table 1.

Based on ISUP grading, tumors with ISUP grade 1 (25 patients) were classified as low-grade and those with ISUP grade 2, 3, 4, or 5 (34 patients) as high-grade. Comparison of ERG staining between the patients in the low- and high-risk groups revealed no statistically significant difference ($p=0.602$). Evaluation of all ISUP groups with the Kruskal–Wallis test showed no significant difference across the groups in terms of ERG expression ($p=0.374$).

While IHC c-erbB2 staining was positive in the control tissues, no staining was detected in the tumor cells in any of the cases. Figure 1 shows one of the PCa cases and samples of ERG and c-erbB2 staining.

The patients stratified according to their age by decade and their corresponding ERG expressions are shown in Table 2. There was no statistically significant difference between the patients' age and their ERG expression ($p=0.165$).

The mean tumor volume of the cases was 22.25±19% (min: 1–max: 80). The mean tumor volume was 18.6±2.4% in the 37 ERG negative patients, and 28.3±5% in the 22 ERG positive patients. We found no significant difference in tumor volume between ERG positive and negative patients ($p=0.06$). Mean tumor volume according to the age distribution of the patients is presented in Table 3. No statistically

significant difference was noted between the patients' age and their tumor volume (p=0.1).

While three of the five patients with lymph node metastasis had ERG expression, there was no ERG expression in the other two patients.

## DISCUSSION

In the present study, we detected ERG positivity in 37.5% of those patients diagnosed with PCa. This ratio is consistent with the average PCa rates reported in the USA and Asian countries. Furthermore, to the best of our knowledge, this is the first published study to investigate ERG expression in patients with PCa in Turkey. However, we found no statistically significant difference in ERG staining between low- and high-grade PCa cases. Our findings support the notion that ERG overexpression is involved in the pathogenesis, although ERG expression has no correlation with the histological grade in PCa. None of the cases included in the present study had c-erbB2 expression.

ERG, the ETS-related gene, is a transcription factor from the erythroblastosis virus E26 oncogene family located on chromosome 21.[17] Members of the ETS family are known to play key roles in embryonic development, cell proliferation, differentiation, angiogenesis, inflammation, and apoptosis. ERG is expressed in endothelial tissues, hematopoietic cells, renal cells and cells of the urogenital system. The protein encoded by this gene is required for

**Table 1. ERG expression of the cases by ISUP grades**

| | ERG | | Total |
|---|---|---|---|
| | Negative | Positive | |
| ISUP 1 | 14 | 11 | 25 |
| ISUP 2 | 10 | 4 | 14 |
| ISUP 3 | 7 | 8 | 15 |
| ISUP 4–5 | 2 | 3 | 5 |
| Total | 33 | 26 | 59 |

ERG: erythroblast transformation-specific (ETS)-related gene, ISUP: International Society of Urological Pathology.

**Table 2. Age distribution of patients and ERG staining by age**

| | Negative | Positive | Total | Rate of positivity, % |
|---|---|---|---|---|
| 50–59 | 4 | 0 | 4 | 0 |
| 60–69 | 16 | 14 | 30 | 48 |
| 70–79 | 15 | 8 | 23 | 35 |
| 80–89 | 2 | 0 | 2 | 0 |
| Total | 37 | 22 | 59 | 37.5 |

ERG: erythroblast transformation-specific (ETS)-related gene.

**Table 3. Mean tumor volume according to the age distribution of patients**

| | Tumor volume |
|---|---|
| 50–59 | 20.7 |
| 60–69 | 28.1 |
| 70–79 | 15.9 |
| 80–89 | 10 |
| Mean | 22.25 |

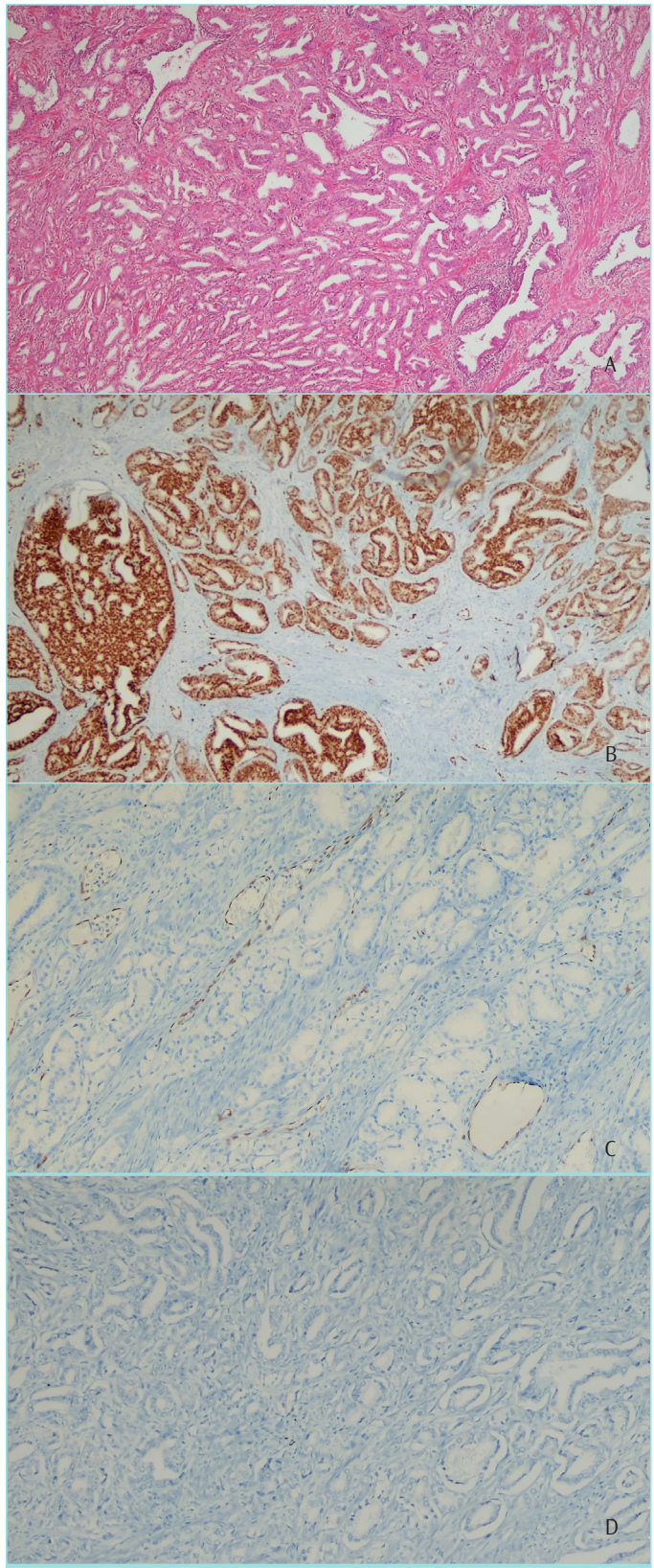


**Figure 1.** A) Prostate carcinoma Gleason score 6(3+3), H&E, B) ERG staining C) ERG no staining D) c-erbB2 no staining.

H&E: Hematoxylin and eosin stain, ERG: erythroblast transformation-specific (ETS)-related gene.

the induction of hematopoiesis, maturation of megakaryocytic cells, vascular cell remodeling, and subendothelial adhesion of platelets.[18] We aimed to determine the ERG expression rate in PCa patients in Turkey and to determine the role of ERG in the pathogenesis and the difference between histological grades.

After Tomlins et al.[3] described the TMPRSS2-ERG fusion in PCa in 2005, several studies investigated the frequency of ERG expression and its effect on prognosis across different populations and ethnic groups. The prevalence of ERG expression varies between 50%–70% in the Western PCa population[19,20] while this rate has been reported to be about 30% in Asian populations.[16] Tan et al.[2] investigated ERG expression by means of IHC in 80 PCa cases in Malaysia and detected ERG expression in 46% of their cohort. They found no correlation between ERG expression and tumor grade or stage. However, they reported higher rates of ERG expression in younger patients (<60 years) (p=0.01). With an expression rate of 69%, they also found significantly higher ERG expression in Indian patients compared to those from Malaysia. Aldaoud et al.[16] reported an ERG expression rate of 33.2% in patients with PCa in the Arab population, adding that ERG expression was associated with PSA level. However, it had no correlation with histological grade or patient age. In our study, we did not find a statistically significant difference between age and ERG expression (p=0.165). In the largest PCa series investigated to date, with 633 cases from a single center in China, Nie et al.[21] reported an ERG expression rate of 16.3%, demonstrating high levels of ERG expression in cases with ISUP grade 1 disease but found no significant relationship with patient age. Chaux et al.[22] compared ERG expression with IHC and ERG fusion in their study in the USA, showing TMPRSS2-ERG fusion with fluorescent *in situ* hybridization (FISH) in 45.7% of the PCa cases and ERG expression with IHC in 45.0%. Similar to other reports in the literature, their study showed that IHC analysis of ERG may be a good surrogate marker for TMPRSS2-ERG rearrangement.

The data published in the literature reflect varying rates of ERG expression across different populations. Our study was conducted in the Turkish population and our positivity rate is comparable to that reported in Malaysia, Arab countries and the USA. Higher rates have been reported in European and Indian populations. Consistent with the other findings in the literature, we did not find a significant relationship between ERG expression and Gleason score or age.

The c-erbB2 (HER2/neu) oncogene is a transmembrane tyrosine kinase receptor gene localized on chromosome 17. Using the mitogen-activated protein kinase and phosphatidylinositol 3' kinase (MAPK and PI3K) molecular signaling pathways, which are known to be involved in PCa, c-erbB2 plays an important role in the growth, differentiation and motility of cancer cells.[23] We applied c-erbB2 to PCa cases with the hypothesis that c-erbB2, which is involved in tumor differentiation and motility, would be expressed differently in different ISUP grades. Thus, we aimed to establish therapeutic targets as in breast cancer.

Bansal et al.[9] investigated c-erbB2 expression by means of IHC in 41 patients with PCa of different types and histological grades, reporting a c-erbB2 expression score of 3 in 14.6% of their cases and 2 in 4.9% (requiring confirmation with FISH), with negative results (i.e. no c-erbB2 expression) reported in 80.5% of their cohort. Other studies in the literature have reported varying rates of c-erbB2 expression with IHC in PCa, such as 62%,[24] 29%,[25] 10%[26] and 37%[27]. Mutlu et al.[15] investigated c-erbB2, AR and CD117 expression in 80 cases with prostate adenocarcinoma and 20 cases with benign prostate tissue in Turkey, reporting c-erbB2 expression in 15 cases (18.75%) with PCa. This rate was found to be 35% in the metastatic group.

In our study, we did not detect c-erbB2 expression in any of 59 cases with prostate carcinoma. All cases were analyzed with an internal control and no c-erbB2 expression was found in the normal prostate tissues and tumor cells included in our sample. We believe this may be related to the clone we used in our study. Similar to cases with breast carcinoma, therapeutic targeted treatments may be developed for PCa patients with c-erbB2 expression.

### Limitations of the Study

A limitation of our study is the small number of cases and the data of only one center. More meaningful results that will reflect Turkey's average can be obtained with multicenter studies. Another limitation is that c-erbB2 expression should be confirmed by more ideal methods such as ISH.

## CONCLUSION

The present study reflects the ERG expression rate (37.5%) in patients with carcinoma of the prostate in Turkey. This ratio is consistent with the average PCa rates reported in the USA and Asian countries. However, we found no statistically significant difference in ERG staining between low- and high-grade PCa cases. Although our study included only a limited number of cases, which may be a sample that is too small to reflect the average situation in Turkey, our findings on ERG expression in PCa appear comparable to the data reported in Asian and European populations. Our findings support the notion that ERG overexpression is involved in the pathogenesis, although ERG expression has no correlation with the histological grade in PCa.

## MAIN POINTS

- ERG expression was detected in 37.5% of the prostate carcinoma cases.
- None of the cases had c-erbB2 expression.
- There was no significant difference in ERG staining between the low-risk (ISUP 1) and high-risk (ISUP >1) groups (p=0.602).

### ETHICS

**Ethics Committee Approval:** The study was approved by Tekirdag Namık Kemal University the Non-Interventional Clinical Trials Ethics Committee (protocol no: 2019.222.11.19, date: 19.01.2019).

**Informed Consent:** An informed consent form was not required for this study as this study is made from archive materials.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Concept: K.D., S.K., Design: K.D., S.K., Materials: K.D., S.K., Literature Search: K.D., S.K., Writing: K.D., S.K., Critical Review: K.D., S.K.

**DISCLOSURES**

**Conflict of Interest:** No conflict of interest was declared by the authors.

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