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Original Article

ERG positive prostatic cancer may show a more angiogenetic phenotype

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ABSTRACT

In Western populations, roughly half of prostatic carcinoma (PC) cases show translocations involving the ETS transcription factor family genes and overexpression of the ERG transcription factor. ETS transcription factor family is known to be involved in angiogenesis. Microvascular density (MVD) influences the prognosis of various solid tumors, yet its significance in PC remains unclear. The aim of the study was to analyze microvascular density in prostatic adenocarcinoma in relationship to ERG expression. This is a retrospective study of 112 cases whose aim is to compare MVD in ERG-positive and negative cases and to test its relationship with other pathological parameters. Immunohistochemical stains for ERG and CD31 were done using standard methods, using tissue microarrays. The cases were classified as ERG-positive and negative and the CD31-positive vessels were counted. Fifty-one cases (45.54%) were positive for ERG, and the average MVD was 111.81 vessels/mm^2 (standard deviation, SD 54.20) for the entire group, 101.30 vessels/mm^2 (SD 50.60) for ERG-negative and 124.38 vessels/mm^2 (SD 56.17) for ERG-positive cases. There was only a slight tendency for higher MVD in higher grades and stages of cancer. None of other parameters, such as patients' age, lymphovascular invasion, presence of intraductal carcinoma or positive surgical margin, showed a significant relationship to MVD. This shows for the first time that ERG expression and MVD may have a significant relationship in prostatic adenocarcinoma.

Introduction

Prostatic carcinoma (PC) is an extremely important disease because of its high prevalence, morbidity and mortality [12]. Recently it has been shown that a subset of PCs are related to translocations involving the ERG gene and overexpression of the ERG protein product [10,26]. ERG-positive and ERG-negative cases may differ in prognosis [25,31] although this relationship may differ between populations. ETS family genes are involved in vascular development and formation of new vessels (neoangiogenesis) [18,20]. Neoangiogenesis constitutes an essential step in cancer development, and the number of vessels is known to influence prognosis of some cancers [11,32]. In the case of PC, however, this is a controversial issue [22].

The aim of the study is to compare microvascular density in ERG-positive and negative cases, and to test its relationship with the stage, grade and other pathological parameters.

Materials and methods

The material for the study consisted of unselected prostatectomy specimens from the files of the Pathology Department. The slides were reviewed by an urological pathologist, and the cases were reclassified according to the International Society of Urological Pathology (ISUP) modification of the Gleason system and staged according to TNM 7th edition [5,7,9]. Lymphovascular invasion and status of surgical margins were also reevaluated. From each case, one section containing well preserved cancer tissue, with morphology predominant in the tumor was chosen. On the slide, the region of interest containing carcinoma tissue was marked and then copied to the surface of a paraffin block. For the tissue microarray (TMA) production, a manual device (Histopathology Inc., Budapest, Hungary) was used. From the region marked as

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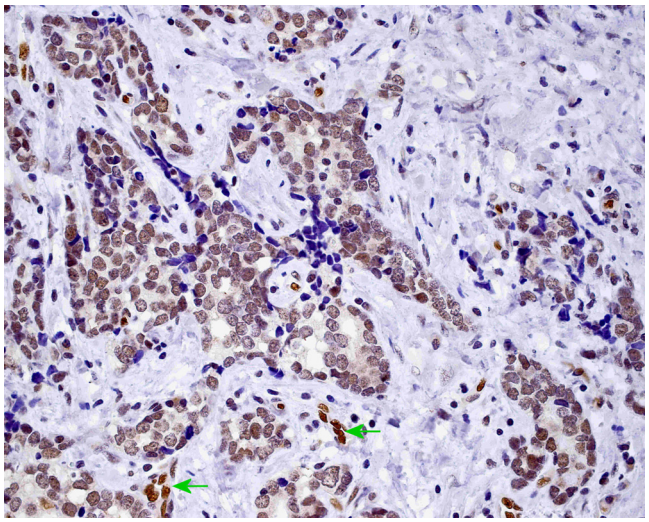

Fig. 1. Strong nuclear reaction for ERG in prostatic adenocarcinoma. Reaction in the endothelial cells (arrows) serve as positive control. Immunohistochemistry, original magnification 400×.

cancer on each paraffin block, two 2 mm cores were obtained and transferred onto a recipient block. The case numbers with respective location in the TMA were noted on an Excel (Microsoft Inc., Redmond, USA) spreadsheet. The upper-left corner of TMA was left empty to allow proper orientation of the resulting slides. From the TMA paraffin blocks, 2 μm sections were prepared and stained with hematoxylin–eosin and immunohistochemistry. Hematoxylin–eosin slides were used to control the quality of tissue selection and to determine Gleason score of specific spots. Immunohistochemistry was done in the routine manner. Briefly, antigen retrieval was performed by heating in a citrate buffer for 20 min. A rabbit monoclonal anti-ERG antibody (clone EPR3864, Abcam PLC, Cambridge, UK) was used in 1:200 dilution. A mouse monoclonal anti-CD31 antibody (clone JC70A, DAKO A/S, Glostrup, Denmark) was used in 1:20 dilution. LabVision detection system (Thermo Fisher Scientific, Waltham, USA) was used. The slides were counterstained with hematoxylin and coverslipped. Results of the ERG staining were scored as positive when unequivocal nuclear staining was present (Fig. 1). Very faint nuclear and any cytoplasmic reactions were ignored. For CD31 immunohistochemistry, all the positive structures (Fig. 2) in a single core were counted by one of

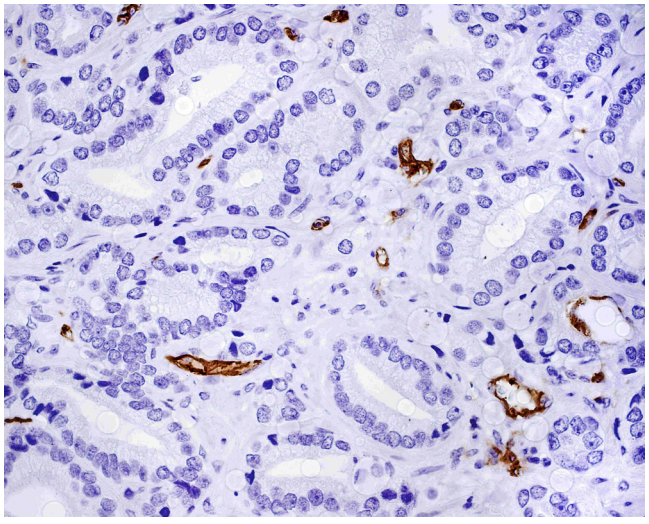

Fig. 2. Vascular profiles with endothelia stained for CD31 in prostatic adenocarcinoma. Immunohistochemistry, original magnification 400×.

Table 1
The frequency of Gleason scores.

Gleason score	*N*	%
3 + 3	46	41.07
3 + 4	41	36.61
3 + 5	2	1.79
4 + 3	13	11.61
4 + 4	3	2.68
4 + 5	4	3.57
5 + 3	1	0.89
5 + 4	2	1.79

the authors (AS) who was blinded to the ERG staining results and other pathological data. The results were expressed as the number of vascular profiles per 1 mm^2 and collected in the Excel spreadsheet containing the case numbers. Statistics were calculated with Statistica 10 (StatSoft Inc., Tulsa, USA). Student's-*t*, Mann–Whitney *U*, Kruskall–Wallis ANOVA tests were used when appropriate. Correlations were calculated by Spearman's method. The significance level was set to 0.05.

Results

The material under study consisted of 112 patients, aged 42–78 years, with a mean of 62.21, median 62 and a standard deviation (SD) of 6.42. Frequency of the Gleason grades are shown in Table 1. In the individual cores, Gleason pattern 3 was present in 74 cases (66.07%), pattern 4 in 36 cases (32.14%) and pattern 5 in 2 cases (1.79%). Stage pT2 was in 44 cases (39.29%), pT3 in 66 cases (58.92%) and pT4 in 2 cases (1.79%). In 1 case (0.89%) there were lymph node metastases. Positive surgical margins were present in 50 cases (44.64%). Lymphovascular invasion was present in 38 cases (34.23%) and intraductal carcinoma component in 3 cases (2.68%). 51 cases (45.54%) were positive for ERG. The average MVD for all cases was 111.81/mm^2 (range 4.46–283.30, SD 54.20). The average for ERG-negative cases was 101.30 while for ERG-positive was 124.38 (Fig. 3) which was statistically significant ($p < 0.024$). It was observed that there was a tendency for MVD to increase with Gleason grades (110.02 for pattern 3; 114.26 for pattern 4; 133.77 for pattern 5), however this was not statistically significant (even between the most prevalent patterns 3 and 4). There was slightly higher MVD in the two pT4 cases (120.08 versus 111.59 for <pT4 cases), yet the difference in MVD between pT2 and pT3 was

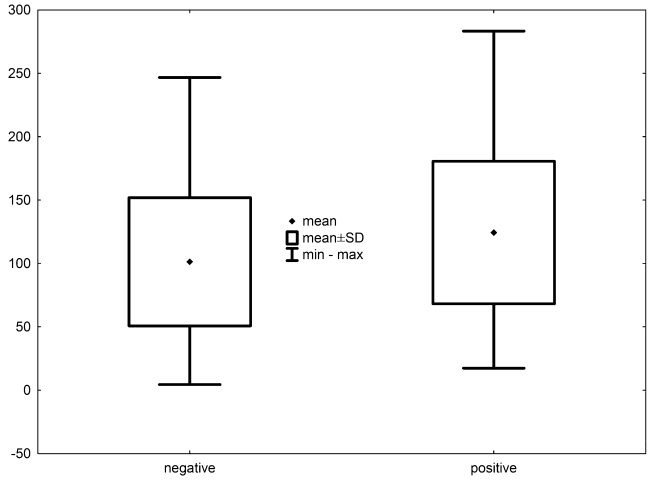

Fig. 3. Microvascular density in ERG-negative (left) and ERG-positive (right) prostatic carcinoma cases. Central point is arithmetic mean, box mean ± standard deviation, whisker range of the values. The difference between two groups is statistically significant ($p < 0.024$).